Scientific Committee on Consumer Products

SCCP

OPINION ON

Kojic acid

The SCCP adopted this opinion at its 17th plenary of 30 September 2008

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCP

Questions concerning the safety of consumer products (non-food products intended for the consumer).
In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

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1. BACKGROUND

Kojic acid, the chemical name 5-hydroxy-2-hydroxymethyl-4-pyrone, is used in cosmetic products as a skin whitening or depigmenting agent.

Kojic dipalmitate is mentioned in the Inventory of Cosmetic Ingredients, but derivative esters of Kojic acids are also used. The substance is listed as an emollient, whereas Kojic acid itself is listed as an antioxidant.

The Commission received a request from a Member State for a safety evaluation of the use of Kojic acid.

The data provided are from the IARC monographs, volume 79.

2. TERMS OF REFERENCE

1. *Does the SCCP consider the use of Kojic acid as a skin whitening or depigmenting agent in cosmetic products safe for the consumer?*

2. *Does the SCCP foresee any other concerns to the safe use of Kojic acid?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Kojic acid (INCI)

3.1.1.2. Chemical names

5-Hydroxy-2-(hydroxymethyl)-4H-pyrane-4-one
4H-Pyran-4-one, 5-hydroxy-2-(hydroxymethyl)-
5-Hydroxy-2-hydroxymethyl-4-pyrone
2-hydroxymethyl-5-hydroxy-4-pyrone

3.1.1.3. Trade names and abbreviations

AEC Kojic Acid
Kojic Acid SL
Kojissan TQ
Melanobleach-K
OriStar KA
ROTA KA

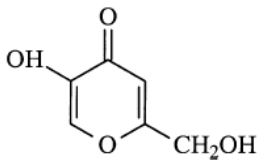
Trade name Mixtures:

Dermawhite HS
Phytoclar
Rice extract "COS"
Vegewhite

3.1.1.4. CAS / EINECS number

CAS: 501-30-4
EINECS: 207-922-4

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Empirical formula: $C_6 H_6 O_4$

3.1.2. Physical form

White to light yellow crystalline powder

3.1.3. Molecular weight

Molecular weight: 142.12

3.1.4. Purity, composition and substance codes

Purity: > 97% (Ref.: 2)
98 – 102% (batch 8A44)

3.1.5. Impurities / accompanying contaminants

Impurities may include heavy metals (10 mg/kg max., not specified) and arsenic (4 mg/kg max.)

Ref.: 45

Sample P005464:

Arsenic:	≤ 2 ppm
Chloride:	≤ 50 ppm
Heavy metals:	≤ 10 ppm
Sulfate:	≤ 120 ppm
Aflatoxins	< 1.08 ppb

Ref.: 101

3.1.6. Solubility

Soluble in water (43.85 g/l); acetone, ethyl acetate and pyridine

Ref.: 45

Slightly soluble in ethanol, insoluble in diethyl ether, chloroform or benzene

Ref.: 101

3.1.7. Partition coefficient (Log P_{ow})

No data submitted

3.1.8. Additional physical and chemical specifications

Appearance:	odourless, slightly bitter taste
Melting point:	155 °C
pH:	4.7 (1 w/v% in water)
p*Ka* value:	7.90 and 8.03
UV absorption:	λmax 270 nm (solvent: water)

Characterization by UV spectrum; IR spectrum; NMR spectrum; Mass spectrum; HPLC chromatogram

Ref.: 101

Quantitation methods by Spectrophotometry, Thin-layer chromatography, Gas chromatography, High-performance liquid chromatography, growth inhibition of *Bacillus thuringiensis*, enzyme-linked immunosorbent assay (ELISA) have been developed.

Review in ref. 71

3.1.9. Stability

No data submitted

General Comments on physico-chemical characterisation

- No data were provided on Log P_{ow}
- No data were provided on the stability of Kojic acid in the test solutions and in the marketed product.
- The purity of the test substance was not reported in several tests.

3.2 Function and use

Kojic acid is used as a skin lightning agent in cosmetic products in use concentrations of 1%. It is used in leave-on creams, which are generally applied to the face, but can also be used on the hands.

Comment
The SCCP is aware of products on the market containing Kojic acid at concentrations higher than 1%.

Kojic acid is a fungal metabolite commonly produced by many species of *Aspergillus*, *Acetobacter*, and *Penicillium*. It has been shown to act as a competitive and reversible inhibitor of animal and plant polyphenol oxidases, i.e. tyrosinase that catalyzes the conversion of tyrosine to melanin via 3,4-dihydroxyphenylalanine and dopaquinone. Kojic acid inhibits melanosis by interfering with the uptake of oxygen required for enzymatic browning. Spectrophotometric and chromatographic methods demonstrated that Kojic acid was capable of reducing *o*-quinones to diphenols to prevent the final pigment (melanin) from forming. It is widely used as a skin-lightening agent in cosmetics (concentration 2-4%) or dermatological preparations because of its slow and reversible competitive inhibition of tyrosinase.

Kojic acid might have the property of an insecticide due to its inhibitory effect on tyrosinase as well as its ability to interact with *o*-quinones of catecholamines, thus preventing the sclerotization process.

Because of these inhibitory properties on a variety of oxidases, Kojic acid has been commercially used in Japan for many years as a food additive in fresh vegetables, crabs and shrimps in order to maintain their freshness (antioxidant) and to inhibit discoloration, as a preservative, as an antioxidant for fats and oils, in the preparation of derivative esters (i.e. Kojic oleate, Kojic stearate), in adhesives, in chelate-forming resins and as a plant growth-regulating agent to increase production, early maturing and increase sweetness. Kojic acid has been used in flavourings at 0.2% to add lustre, to prevent discolouration on vegetables at 1.0%, in flour production at 0.1%, in meat production at 0.2%, in syrup at 0.05%.
Ref.: 71, 40, 44

Kojic acid possesses weak antimicrobial properties and is active against several common bacterial strains at dilutions of 1:1,000 to 1:2,000.

Ref.: 51

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Study 1

Guideline:	/
Species/strain:	CFLP mice
Group size:	2 males and 2 females per group (range finding screen) 5 males and 5 females per group (main experiment)
Test substance:	Kojic acid in 0.5% methylcellulose
Batch:	/
Purity:	/
Dose:	1000, 4000 and 16 000 mg/kg bw (range finding screen) 4000, 6400, 10000 and 16000 mg/kg bw (main experiment)
Observ. Period:	14 days
GLP:	/

Mice (body weight 21 - 27 g) were treated with single doses of the test substance (40%, w/v) dissolved in 0.5% methylcellulose by oral intubation at dosage volumes of 10 to 40 ml/kg bw. The control group received the vehicle alone (40 ml/kg). Animals were observed for mortality and toxic effects for a total of 14 days. All mice were examined macroscopically when they had died or at the end of the observation period.

Results
Results of the range finding test indicated that the LD_{50} was in the range of 4000 to 16000 mg/kg bw. In the main experiment lethargy, piloerection, abnormal body carriage, ataxia and depressed respiration rate were observed shortly after dosing. These signs were accompanied by gasping amongst mice treated at 6400 mg/kg bw. Bodyweight increases of rats treated at 16000 mg/kg bw were slightly depressed during the first week. Recovery of survivors was apparently complete within four days of dosing. Autopsy revealed congestion of the lungs and pallor of the liver, kidneys and spleen in animals died after treatment. The LD_{50} and its 95% confidence limits were calculated to be 5100 (3900 – 6700) mg/kg bw.

Ref.: 4

Study 2

Guideline:	/
Species/strain:	CFY rats
Group size:	2 males and 2 females per group (range finding screen) 5 males and 5 females per group (main experiment)
Test substance:	Kojic acid in 1% methylcellulose
Batch:	/
Purity:	/
Dose:	1000, 4000 and 16 000 mg/kg bw (range finding screen) 1000, 1600, 2500 and 4000 mg/kg bw (main experiment)
Observ. Period:	14 days
GLP:	/

Rats (body weight 102 - 123 g) were treated with single doses of the test substance (40%, w/v) dissolved in 1% methylcellulose by oral intubation at dosage volumes of 2.5 to 10 ml/kg bw. The control group received the vehicle alone (10 ml/kg). Animals were observed

for mortality and toxic effects for a total of 14 days. All rats were examined macroscopically when they had died or at the end of observation period.

Results
Results of the range finding test indicated that the LD_{50} was in the range of 1000 to 4000 mg/kg bw. In the main experiment lethargy, piloerection, ataxia, depressed respiration rate and loss of righting reflex were observed shortly after dosing. These signs were accompanied by increased salivation and body tremors in rats treated above 1000 mg/kg bw, increased lacrimation and diuresis in rats at 1600 mg/kg bw and by convulsions prior to death in rats at 2500 and 4000 mg/kg bw. Bodyweight increases of rats treated at 1600 mg/kg bw were slightly depressed during the first week. Recovery of survivors was apparently complete within seven days of dosing. Autopsy revealed congestion of the lungs and pallor of the liver, kidneys and spleen in animals died after treatment. The LD_{50} and its 95% confidence limits were calculated to be 1800 (1500 – 2000) mg/kg bw.

Ref.: 2

Study 3

Guideline:	OECD 401, 1987
Species/strain:	Wistar rats
Group size:	5 males and 5 females per group
Test substance:	Kojic acid in 0.5% methylcellulose
Batch:	8A44
Purity:	/
Dose:	2000 mg/kg bw
Observ. Period:	14 days
GLP:	in compliance

5 male rats (body weight 167 ± 4 g) and 5 female rats (body weight 140 ± 4 g) were treated with single doses of the test substance dissolved in 0.5% methylcellulose by gavage (10 ml/kg). The control group received the vehicle alone. Animals were observed for mortality and toxic effects frequently during the hours following administration of the test substance and once daily thereafter for a total of 14 days. Animals were weighed before administration of test substance (day 0), and on day 1, 8 and 15. At the end of the observation period animals were sacrificed and autopsied.

Results
In the treated group sedation or hypoactivity, dyspnea and lateral recumbency were observed in all animals on day 1. One female was found dead 6 hours after treatment. Recovery was complete on day 2 in the other animals. The body weight gain of the surviving animals of the treated group was similar to that of the control group. No abnormalities were observed at necropsy. In the control group no clinical signs and no death occurred. The LD_{50} of the test substance administered to rats by the oral route was > 2000 mg/kg bw.

Ref.: 3

3.3.1.2. Acute dermal toxicity

Guideline:	OECD 402, 1987
Species/strain:	Wistar rats
Group size:	5 males and 5 females per group
Test substance:	Kojic acid (test substance 53758)
Batch:	8A44
Purity:	/
Dose:	2000 mg/kg bw
Observ. Period:	14 days

GLP: in compliance

Rats (body weight 274 ± 16 g for males and 205 ±9 g for females) were treated with single doses of the test substance in its original form at a dose of 2000 mg/kg bw. The test substance was placed on a gauze pad pre-moistened with 2 ml of water and then applied to an area of the skin representing approximately 10% of the body surface. The test site was then covered by a semi-occlusive dressing for 24 hours. The control animals received 2 ml of purified water under the same experimental conditions. Clinical signs, mortality and body weight gain were checked for a period of 14 days following treatment. All animals were subjected to necropsy.

Results
No mortality, clinical signs, cutaneous reactions or apparent abnormalities at necropsy were observed. The general behaviour of the animals was not affected by the treatment with the test substance. Body weight gain was reduced slightly between day 1 and day 8 in treated animals compared to the control animals. This effect was attributed to the test procedure. The LD_{50} was higher than 2000 mg/kg bw.

Ref.: 5

3.3.1.3. Acute intra-peritoneal toxicity

Study 1

Guideline:	/
Species/strain:	CFLP mice
Group size:	2 males and 2 females per group (range finding screen) 5 males and 5 females per group (main experiment)
Test substance:	Kojic acid in 0.5% methylcellulose
Batch:	/
Purity:	/
Dose:	1000, 4000 and 16 000 mg/kg bw (range finding screen) 1600, 2500, 4000, 6400, and 10000 mg/kg bw (main experiment)
Observ. Period:	14 days
GLP:	/

Mice (body weight 20 - 28 g) were treated with single doses of the test substance (40%, w/v) dissolved in 0.5% methylcellulose by intraperitoneal injection at dosage volumes of 4 to 25 ml/kg bw. The control group received the vehicle alone (25 ml/kg). Animals were observed for mortality and toxic effects for a total of 14 days. All mice were examined macroscopically when they had died or at the end of observation period.

Results
Results of the range finding test indicated that the LD_{50} was in the range of 1000 to 4000 mg/kg bw. In the main experiment lethargy, piloerection, ataxia and depressed respiration rate were observed shortly after dosing. Gasping was also observed amongst mice treated at 2500 mg/kg bw. Recovery of survivors was apparently complete within two days of dosing. Autopsy revealed pallor of the liver, haemorrhage of the lungs and injection of the blood vessels of the abnormal viscera in animals died after treatment. The LD_{50} and its 95% confidence limits were calculated to be 2600 (2200 – 3000) mg/kg bw.

Ref.: 6

Study 2

Guideline:	/
Species/strain:	CFY rats
Group size:	2 males and 2 females per group (range finding screen)

	5 males and 5 females per group (main experiment)
Test substance:	Kojic acid in 1% methylcellulose
Batch:	/
Purity:	/
Dose:	1000, 4000 and 16 000 mg/kg bw (range finding screen) 1000, 1600, 2500 and 4000 mg/kg bw (main experiment)
Observ. Period:	14 days
GLP:	/

Rats (body weight 100-136 g) were treated with single doses of the test substance (40%, w/v) dissolved in 1% methylcellulose by intraperitoneal injection at dosage volumes of 2.5 to 10 ml/kg bw. The control group received the vehicle alone (10 ml/kg). Animals were observed for mortality and toxic effects for a total of 14 days. All rats were examined macroscopically when they had died or at the end of observation period.

Results
Results of the range finding test indicated that the LD_{50} was in the range of 1000 to 4000 mg/kg bw. In the main experiment lethargy, piloerection, ataxia, abnormal body carriage and depressed respiration rate were observed shortly after dosing. These signs were accompanied by increased salivation, diuresis, coarse body tremors, gasping and convulsions prior to death in rats treated above 1000 mg/kg bw. Coarse body tremors and convulsions were also observed in rats at 1000 mg/kg bw. One female of the 1000 mg/kg bw group developed persisting paralysis of the hind limp on day three. Bodyweight increases of male rats treated at 1600 mg/kg bw were slightly depressed during the first week. Recovery of survivors was apparently complete within five days of dosing. Autopsy revealed haemorrhage of the lungs, pallor of the liver and injection of the blood vessels of the abnormal viscera as well as opacities of one or both eyes in animals died after treatment. The LD_{50} and its 95% confidence limits were calculated to be 2400 (2000 – 3000) mg/kg bw.

Ref.: 7

3.3.1.4. Acute subcutaneous toxicity

Study 1

Guideline:	/
Species/strain:	CFLP mice
Group size:	2 males and 2 females per group (range finding screen) 5 males and 5 females per group (main experiment)
Test substance:	Kojic acid in 0.5% methylcellulose
Batch:	/
Purity:	/
Dose:	1000, 4000 and 16 000 mg/kg bw (range finding screen) 1600, 2500, 4000, 6400, 10000 and 16000 mg/kg bw (main experiment)
Observ. Period:	14 days
GLP:	/

Mice (body weight 20 - 32 g) were treated with single doses of the test substance (40%, w/v) dissolved in 0.5% methylcellulose by subcutaneous injection at dosage volumes of 4 to 40 ml/kg bw. The control group received the vehicle alone (40 ml/kg). Animals were observed for mortality and toxic effects for a total of 14 days. All mice were examined macroscopically when they had died or at the end of the observation period.

Results
Results of the range finding test indicated that the LD_{50} was in the range of 4000 to 16000 mg/kg bw. In the main experiment lethargy, piloerection, ataxia, depressed respiration rate

gasping and abnormal body carriage were observed shortly after dosing. These signs were accompanied by coarse body tremors amongst mice treated above 2500 mg/kg bw. Haemorrhage at the site of injection was observed immediately after dosing time in all mice. Recovery of survivors was apparently complete within four days of dosing. Autopsy revealed pallor of the liver, and haemorrhage of the lungs injection in animals died after treatment. The LD_{50} and its 95% confidence limits were calculated to be 2700 (1900 – 3900) mg/kg bw.

Ref.: 8

Study 2

Guideline:	/
Species/strain:	CFY rats
Group size:	2 males and 2 females per group (range finding screen) 5 males and 5 females per group (main experiment) 5 males and 5 females (investigation of opacities)
Test substance:	Kojic acid in 1% methylcellulose
Batch:	/
Purity:	/
Dose:	1000, 4000 and 16 000 mg/kg bw (range finding screen) 1000, 1600, 2500, 4000, 6400 and 10000 mg/kg bw (main experiment) 4000 mg/kg bw (additional group, investigation of opacities)
Observ. Period:	14 days
GLP:	/

Rats (body weight 103-157 g) were treated with single doses of the test substance (40%, w/v) dissolved in 1% methylcellulose by subcutanous injection at dosage volumes of 2.5 to 25 ml/kg bw. The control group received the vehicle alone (25 ml/kg). Animals were observed for mortality and toxic effects for a total of 14 days. All rats were examined macroscopically when they had died or at the end of the observation period. Eyes were investigated by Keeler indirect ophthalmoscope 2.5 hours after dosing.

Results
Results of the range finding test indicated that the LD_{50} was in the range of 4000 to 16000 mg/kg bw. In the main experiment lethargy, piloerection, diuresis, abnormal body carriage and depressed respiration rate were observed shortly after dosing. These signs were accompanied by ataxia and convulsion amongst rats treated at 2500 mg/kg bw and above and by tremors amongst rats treated at 6400 mg/kg bw and above. Recovery of survivors was apparently complete within six days of dosing. Bodyweight increases of male rats treated at 2500 and 4000 mg/kg bw and of the remaining females at 4000 mg/kg bw were slightly depressed during the first week. Autopsy revealed haemorrhage of the lungs, pallor of the liver and haemorrhage at the injection site. Opacity of one or both eyes was observed in 19 of 39 mortalities. In the additional group investigated for effects on the eyes, evidence of lenticular opacities was observed in both eyes of two male rats. Drying and clouding of the cornea occurred in five rats together with swelling of the cornea in one male and one female rat. One male died before examination could be performed.

The LD_{50} and its 95% confidence limits were calculated to be 2600 (2000 – 3200) mg/kg bw.

Ref.: 9

3.3.1.5. Acute intravenous toxicity

No data submitted

3.3.1.6. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline: /
Species/strain: Albino Rabbit
Group size: 6 animals
Test substance: Kojic Acid
Batch: 8224
Purity: /
Dose: 0.5 g under occlusive conditions
GLP: /

A suspension of 0.5 g test substance in 0.5 ml water was applied under occlusive conditions to the right, abraded and the left, intact skin flanks of six rabbits. After 24 h patches were removed and the skin was evaluated for erythema and oedema. Additionally the skin was investigated 48 h after the end of the exposure period.

Results
No erythema or oedema occurred in the test performed. Kojic acid was not considered to be an irritant to rabbit skin.

Ref.: 10

3.3.2.2. Mucous membrane irritation

Guideline: /
Species/strain: rabbit (1st experiment)
Angora rabbit (2nd experiment)
Group size: 3 animals (preliminary test)
5 animals (1st experiment)
2 males, 2 females (2nd experiment)
9 animals (2nd experiment, supplementary test)
Test substance: Kojic Acid
Batch: /
Purity: /
Dose: 3%, 0.05 ml
GLP: /

In the preliminary test, 0.05 ml of 3% Kojic acid aqueous solution was instilled in the right eye of three rabbits (mean body weight: 2.8 kg); after 1, 3, 6, and 48 hours observation was carried out without washing of the eyes.

In the first experiment 0.05 ml of 3% Kojic acid aqueous solution was instilled in the left eye of five rabbits (mean body weight: 2.7 kg). Eyes were examined without washing after 0.5, 1, 6, 24, 48, and 72 hours.

In the second experiment, performed for the purpose of accuracy, 3% Kojic acid in aqueous solution was tested in two male and two female Angola rabbits (mean body weight: 2.37 kg). In a supplementary test the substance was given to one eye of 9 Angola rabbits.

Results

In the preliminary test and in the first experiment, 3% Kojic acid aqueous solution caused no eye disturbances. In the second experiment mild transient hyperemia was observed in 2 of 4 animals. No other inflammatory changes or corneal disturbances were observed. Eye irritability was reported to be very weak. In the supplementary test no specific response was observed for up to 72 hours.

Ref.: 16

3.3.3. Skin sensitisation

Buehler test

Guideline:	OECD 406, 1992
Species/strain:	guinea pigs
Group size:	5 females and 5 males (control group) 10 females and 10 males (treated group)
Test substance:	Kojic acid
Batch:	8A44
Purity:	/
Concentration:	Induction: 30% test substance (w/w) in corn oil (treated groups) Challenge: 30% test substance (w/w) in corn oil (all groups)
GLP:	in compliance

During a 2-week induction period animals of the treated group received three topical applications of the test substance. Application sites were covered by an occlusive dressing for 6 hours on each occasion. Animals of the control group received the vehicle under the same experimental conditions. After a rest of 14 days all groups were challenged by a topical application of the test substance to the posterior right flank. The non-treated left flank served as control and received the vehicle. Test substance and vehicle were maintained under an occlusive dressing for 6 hours. Skin reactions were evaluated 24 and 48 hours after removal of the dressing.

Results
During the induction period, very slight or well defined skin reactions were observed in a few animals of the treated group. After challenge, no cutaneous reactions were observed in the control group. In the treated group, a very slight erythema (grade 1) was noted in one female and a well-defined erythema (grade 2) was recorded in another female at the 24- and the 48 h reading. Both animals showed cutaneous reactions during the induction period. The positive control, 0.5% 2,4-Dinitrochlorobenzene, induced positive reactions in 33% of treated animals (3/10).

Ref.: 13

Comment
Two out of 20 animals showed a positive reaction, indicating a sensitising potential of the substance.

In a further Guinea pig study (cited in reference 4), Kojic acid was considered to be non-sensitising.

Ref.: AR1

Human data

Patch testing with 220 female patients with suspected cosmetic-related contact dermatitis was performed. Of the 220 patients, 8 used at least 1 skin care product containing Kojic acid, 5 of whom reacted to Kojic acid as well as to one or more of their own products containing 1% Kojic acid but not to their other products not containing it, and 3 of whom were negative to Kojic acid and all their own products. Patch testing with Kojic acid in the

remaining group of 212 patients who had not previously used skin care products with Kojic acid was negative without exception. The 5 Kojic acid sensitive patients, aged 34 to 58 years, developed facial dermatitis 1-12 months after starting application of cosmetic products with Kojic acid.

Ref.: AR12

A 30 year old woman who developed an eczematous eruption after use of a cream formulation containing 3% Kojic acid showed strong positive reactions in a patch test with Kojic acid at 1 and 5%.

Ref.: A2

Conclusion
Kojic acid is sensitising in humans.

3.3.4. Dermal / percutaneous absorption

Study 1

Guideline:	OECD 428 (draft 2000)
Species:	human, 4 donors, 2 skin samples/donor
Tissue:	dermatomed skin, thickness: 396 ± 32 µm
Groups:	eight replicates
Test substance:	[^{14}C] Kojic acid
Batch:	[^{14}C] Kojic acid: CFQ 12878
Purity:	radiochemical purity 99%
Dose level:	1.045 ± 0.010% (w/w) in formulation 657926
Exposure time:	16 hours
GLP:	in compliance

The dermal absorption/percutaneous penetration of [^{14}C] Kojic acid in a formulation was studied on human dermatomed skin from 4 donors. Skin samples (2 per donor) were mounted on static diffusion cells with an area of 2 cm^2 during a period of 16 hours. Test substance was applied in a concentration of 1.045 ± 0.010% (w/w) in formulation 657926 in an amount of 2.00 ± 0.16 mg/cm^2 corresponding to 20.61 ± 1.68 µg $_{eq}$/cm^2 [^{14}C] Kojic acid. Before application of the formulation, the integrity of each skin sample was checked by measuring Trans-Epidermal Water Loss (TEWL). Sixteen hours after application remaining formulation was removed from the skin surface. For mass balancing amounts of [^{14}C] Kojic acid and/or [^{14}C] metabolites were analysed by liquid scintillation in the skin excess, the stratum corneum (5 to 15 strips), the epidermis, dermis and in the receptor fluid. The receptor fluid used was physiological buffered saline.

Results
The quantities of test substance detected are shown in the following table. Both the amount measured in epidermis and dermis as well as the amount in the receptor fluid were taken as systemically available. Total recovery was 96.41± 4.82 of the applied dose. The amount of bio-available test substance was 3.58 ± 2.56 µg $_{eq}$/cm² [^{14}C] Kojic acid. A maximum of 7.28 µg $_{eq}$/cm² [^{14}C] Kojic acid (30.71% of dose applied) was detected in epidermis + dermis + receptor fluid.

ANALYSED SAMPLE	Formulation 657926 [^{14}C] n = 8	
	[µg $_{eq}$/cm²]	[%]
Skin rinsing	15.52 ± 1.43	75.77 ± 9.30
Adsorption (stratum corneum)	0.76 ± 0.48	3.65 ± 2.22
Absorption (epidermis/dermis)	1.93 ± 1.07	9.17 ± 4.31

Penetration (receptor fluid)	1.65 ± 1.49	7.81 ± 6.79
Bio-available	3.58 ± 2.56	16.98 ± 11.10
Total recovery		96.41 ± 4.82

Ref.: AR3

Comment
The composition of formulation 657926 given in the dossier was not legible. Since, deviating from the SCCP Notes of Guidance only 8 samples were investigated, the maximum value for bioavailability of 7.28 µg $_{eq}$/cm² [^{14}C] Kojic acid could be used for MOS calculation.

Study 2

Guideline: /
Species: human
Groups: 6 healthy postmenopausal Japanese women
Test substance: Kojic acid
Batch: /
Purity: /
Dose levels: 1% in cream formulation, 500 mg
Exposure time: 1 day
GLP: /

Percutaneous absorption was studied in an open, uncontrolled, single application study. 500 mg of a cream formulation containing 1% Kojic acid was applied once to the entire surface of facial skin (left and right cheeks) of six healthy postmenopausal Japanese women. The area of the application site was wiped with gauze wetted with lukewarm water and dried before application. The test substance was applied with a finger of the investigator. Medical and physical examination, a standard electrocardiogram as well as laboratory tests were performed before and 24 hours after application. Plasma concentrations of Kojic acid were measured (HPLC, limit of quantitation: 100 ng/ml) before, 0.5, 1, 1.5, 3, 6, 12, and 24 hours after application.

Results
Kojic acid was detected in plasma of all patients at one or more blood collection times. Mean C_{max} was 1.54 ng/ml and the mean AUC $_{0\text{-}24}$ h was 19.4 ng/ml x hr. Detection limit was 1 ng/ml. There were no abnormal laboratory test values that were judged to be clinically important abnormalities in this study.

It was considered that the potential dermal transfer of Kojic acid to blood seems very low. There were thought to be no problems regarding the safety since no adverse events were observed.

Ref.: 100

Comment
The composition of the cream formulation used in this study was not given. Individual data on medical and physical examinations are missing. The application area is rather small for measuring dermal penetration into blood.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

Rabbits, dermal application

Guideline:	/
Species/strain:	New Zealand White strain rabbits
Group size:	5 males and 5 females/group
Test substance:	Kojic Acid in 1% aqueous methylcellulose
Batch:	780213, 8224, 8313
Purity:	/
Dose levels:	0, 0.65, 6.5 or 65% w/v 0, 13, 130, 1300 mg/kg bw/day Dermal application
Exposure period:	30 days
GLP:	/

Rabbits (2.0 to 2.5 kg bw) were treated with Kojic acid at 0.65, 6.5, and 65% w/v suspensions in 1% aqueous methylcellulose daily for 30 consecutive days. The control group received the vehicle alone. The appropriate test materials were spread evenly over the abraded mid-dorsal region of each rabbit at a constant dosage volume of 2 ml/kg/day. The treatment site was covered for 6 hours each day with gauze. Subsequently the test substance was removed with warm water. Animals were observed daily for local effects, clinical signs and mortality. Body weight and food consumption were recorded weekly. Blood samples for haematological and biochemical parameters were taken prior to treatment and in the control and in the highest dose group prior to termination additionally. Animals were killed after the end of treatment period for autopsy and histopathology.

Results
Slight dermal reactions were observed in all rabbits. However, effects were more persistent in animals treated with Kojic acid. For several rabbits erythematous papules and abscesses were reported. Bacteriological investigation of 2 animals revealed an infection with *Staphylococcus aureus*. One female of the 13 mg/kg bw/day group and one male of the 130 mg/kg bw/day group were found dead and one male of the control group was sacrificed in extremis. Lesions of lung and liver and lesions of kidney and brain, respectively, were considered to be factors possibly contributing to the death of these animals. Statistically significant changes compared to controls were reported for MCHC (mean corpuscul haemoglobin concentration), MCV (mean cell volume) and A/G ration for the highest dose group. In the lowest dose group the pituitary weight was significantly increased. Ophthalmoscopic investigation revealed changes in the eyes in one control animal, one animal of the lowest dose group, 3 animals of the 130 mg/kg bw/day group and 3 animals of the highest dose group. Plaques in aorta were reported in one control male, 3 males and 1 female in the 13 mg/kg bw/day group, one male and one female in the 130 mg/kg bw/day group and in 4 males of the 1300 mg/kg bw/day group. Pale kidneys were reported for all treated groups.

Ref.: 11

Comment
Effects on skin and eyes can not be evaluated due to bacteriological infection of animals. Haematological and biochemical parameters after the treatment period were only investigated for the highest dose group. No conclusions on dose-dependency of statistically significant changes can be obtained therefore.

Rats, dermal application

Guideline:	OECD 410
Species/strain:	Wistar Hannover rats
Group size:	10 females and 10 males/group (low- and intermediate dose) 16 females and 16 males/group (control and high dose group)
Test substance:	Kojic Acid in 0.5% aqueous methylcellulose
Batch:	8B49
Purity:	/
Dose levels:	0, 100, 300, 1000 mg/kg bw/day Dermal application
Exposure period:	28 days
Recovery:	2 weeks
GLP:	in compliance

Rats were randomly allocated to three treated and one control groups of 16 males and 16 females (control and high dose-level groups) and 10 males and 10 females (low and intermediate dose-level groups). Dose levels were selected following results of a 7-day preliminary tolerance study performed on the same species at 2000 mg/kg bw/day. In the main study the first six males and females of the control and high dose-level groups were kept at the end of the treatment period for a two-week treatment-free period. Treated animals received the test substance, daily, by cutaneous route, for four weeks, at the dose-levels of 100, 300 and 1000 mg/kg/day. The test substance preparations were administered as suspensions in the vehicle, 0.5% aqueous methylcellulose solution (w/w). Control animals received the vehicle alone in the same way. Test and control formulations were applied to the dorsum uniformly over an area which was approximately 10% of the total body surface area. The animals were checked daily for mortality and clinical signs. The food consumption and body weight of the animals were measured once a week. Complete haematology, blood biochemistry investigations and urinalysis were performed at the end of the treatment period, in the first 10 animals of control and high dose-level groups and in all animals of the low or intermediate dose-level groups. White blood cell and lymphocytes counts were also determined on the first six surviving animals of control and high dose-level groups at the end of the treatment-free period. Blood levels for T_3, T_4 and TSH were not measured. At the end of the treatment or treatment-free period, the animals were killed. Representative organs were weighed and the animals were submitted to a detailed macroscopic post-mortem examination. A microscopic examination was performed on designated tissues for animals of control and high dose-level group at the end of the treatment period.

Results
No death occurred during the study and no relevant clinical signs were observed. No topical effects which could be attributed to treatment were observed. Overall body weight gains or final body weights were similar in control and treated groups and food consumption was unaffected by treatment. No thyroid weights were recorded for the treatment period. However, after the recovery period thyroid weights were slightly increased in females compared to controls. Statistically significant lower group mean values for total white blood cell count and for lymphocytes count were observed at the end of the treatment period in males and females given 300 or 1000 mg/kg/day. This was only partially reversed at the end of the treatment-free period for animals which were treated at the high dose-level. For the low dose level recovery was not investigated. Values for monocytes, erythrocytes and inorganic phosphorus were decreased in males of the highest dose group. In the urine neither qualitative nor quantitative changes were observed at the end of the treatment or treatment-free period. Lower absolute and relative spleen weights were observed in females given 1000 mg/kg bw/day. Because there were no histopathological changes observed in the spleen, the significance of the splenic weight changes was uncertain. No treatment-related macroscopic or microscopic post-mortem findings were noted at the end of the treatment period.

Conclusion
Based on the changes observed in lymphocytes and white blood cell counts, the No Observed Adverse Effect Level (NOAEL) was established at 100 mg/kg/day.

Ref.: 19

Rats, oral, diet

Guideline:	/
Species/strain:	F344 rats
Group size:	9 males/group (Experiment 1) 8 males or 8 females/group (Experiment 2) 8 males/group (Experiment 3)
Test substance:	Kojic Acid
Batch:	/
Purity:	/
Dose levels:	Experiment 1: 0, 0.008, 0.03, 0.125, 0.5, or 2% in diet Average daily intake calculated: 0, 5.85, 23.8, 95.3, 393.6, 1387.3 mg/kg bw/day Experiment 2 and 3: 0 or 2% in diet
Exposure period:	28 days
GLP:	/

Experiment 1
Groups of nine animals received 0 (control), 0.008; 0.03, 0.125, 0.5 or 2.0% Kojic acid containing diet for 4 weeks. Twenty-four hours before the end of the experiment, four animals in each group received 0.2 ml/100 g bw Na ^{125}I at a concentration of 2.5 x10^5 c.p.m./ml (0.1 M) in saline. Animals were killed and the thyroids were dissected, weighed and investigated for ^{125} I uptake. The remaining five animals in each group were killed on the same day for hormone determination. The thyroid glands were removed and fixed for sectioning. Sections were stained with hematoxylin and eosin for histopathological assessment.

Experiment 2
Male and female rats were divided into eight and four groups, respectively, each consisting of eight animals, and given 0 (control) or 2.0% Kojic acid containing diet. Groups were killed at weeks 1, 2, 3 and 4 for males and at weeks 2 and 4 for females. Half of the animals served for investigation of ^{125} I uptake and the other half for hormonal and histological examinations.

Experiment 3
Male rats were divided into six groups, each consisting of eight animals, and given 0 (control) and 2.0% Kojic acid containing diet for 4 weeks. At the end of this treatment period, Kojic acid diet was replaced with control basal diet for 0, 6, 12, 24, 48 hours. Groups were then killed and examined as in experiments 1 and 2, except that ^{125} I was injected 12 h before death.

Results
Experiment 1
Diet containing ≥ 0.125% of Kojic acid increased thyroid weight in a dose-dependent manner. The weight in the 2.0% group reached nine times the control value. ^{125}I uptake into the thyroid was more sensitive to Kojic acid treatment, being significantly suppressed at 0.03%. Organic ^{125}I formation was, however, interrupted only in the highest dose group. Serum T_3, T_4 and TSH level were also only affected in the 2.0% group.

Experiment 2
Thyroid weight increased linearly from 11 to 98 mg during 4 weeks treatment with 2% Kojic acid in males while the increase was significant but less prominent in females, from 7.5 to 40 mg. Suppression of ^{125}I uptake in the thyroid glands was also time dependent. In males, it started to decrease after 1 week feeding of Kojic acid and reached only approximately 2% of the control at week 3, when organic ^{125}I formation was significantly decreased by 50% compared to controls. In females, however, the effects were far less significant, only 20% suppression of ^{125}I uptake was noted at week 4. Both, serum T_3, and T_4 level decreased to minimum levels after 2 weeks of Kojic acid treatment and recovered thereafter, although remaining lower than the control levels in both sexes. Serum TSH level started to increase at week 1 and reached a maximum at weeks 2-3.

Experiment 3
Organic ^{125}I formation returned to normal after 6 hours, ^{125}I uptake per unit thyroid weight rose to 70% of the control level within 24 hours. T_3 and T_4 were 47 and 34% of control levels after 4 weeks feeding of Kojic acid diet. They increased to normal within 48 hours after return to standard diet, high levels of TSH decreased to normal within 24 hours.

Ref.: 30

Comment
From this study a NOAEL of approximately 23.8 mg/kg bw/day can be derived with respect to thyroid weight, and a NOAEL of approximately 5.85 mg/kg bw/day with respect to iodine uptake.

Rats, oral, diet

Guideline:	/
Species/strain:	F344 rats
Group size:	8 males/group
Test substance:	Kojic Acid
Batch:	/
Purity:	/
Dose levels:	0, 0.008, 0.03, 0.125, 0.5, 2.0% in diet Average daily intake calculated: 0, 5.85, 23.8, 95.3, 393.6, 1387.3 mg/kg bw/day
Exposure period:	4 weeks
GLP:	/

Male F344 rats received basal diet containing the test substance or basal diet alone for 4 weeks. After the end of treatment period blood samples were taken from 5 animals for hormone analysis and animals were autopsied. Histopathological examination of thyroid and pituitary tissues was performed. The remaining animals were sacrificed for measurement of ^{125}I uptake and its organification in the thyroid. Therefore rats were injected ip with 0.4 ml of 0.1 M $Na^{125}I$ in saline 24 hours before sacrifice.

Results
There were no significant intergroup differences in the final body weights. Absolute and relative thyroid weights were increased significantly in the groups who received 0.5 and 2% Kojic acid. For pituitary and liver relative weights differed compared to the control. ^{125}I uptake decreased in a dose-dependent manner from 0.03% Kojic acid on. In addition, significant reduction of organic formation of iodine and serum T_3 and T_4 levels were observed in the 2% Kojic acid group along with pronounced elevation of TSH. Histopathologically, decreased colloid in the thyroid follicles and follicular cell hyperthrophy in the thyroid were apparent at high incidences in the groups given 0.03% Kojic acid or more. In addition, thyroid capsular fibrosis was evident in all rats of the 2% Kojic acid group. In quantitative morphometric analysis the ratio of the area of follicular epithelial cells

to the area of the colloids in a unit area was significantly increased in groups treated with 0.03% Kojic acid and above.

Ref.: 31

Comment
On the base of histopathological findings and altered ^{125}I uptake a NOAEL of approximately 6 mg/kg bw/day can be derived.

Rat, oral, gavage

Guideline:	/
Species/strain:	F344 rats
Group size:	10 males/group
Test substance:	Kojic Acid in 0.5% carboxymethylcellulose
Batch:	/
Purity:	/
Dose levels:	0, 4, 15, 62.5, 250, 1000 mg/kg bw/day at volumes of 5 ml/kg bw
Exposure period:	28 days
GLP:	/

Male F344 rats received Kojic acid in doses of 4, 15, 62.5, 250, 1000 mg/kg bw/day at volumes of 5 ml/kg bw by gavage for 28 consecutive days. Clinical signs of animals were checked twice daily. Body weights, food and water consumption were determined twice a week. Necropsy was performed, thyroid weights were recorded and histopathological examination was performed. The uptake of iodine and the iodination were determined before the onset of administration, and at weeks 1, 2, 3, and 4 of administration for 5 animals per group. Blood samples for hormone analysis were collected 24 hours after final administration. Pharmacokinetic parameters were determined after single oral administration of ^{14}C-Kojic acid (10 µCi/100 g, corresponding to 100 mg/kg bw/day). Blood samples were collected 10, 30 minutes and 1, 3, 6, and 24 hours after administration.

Results
At 1000 mg/kg bw/day rats showed a decrease in motility, inhibition of body weight gain, and a decrease in food consumption. A significant increase in absolute and relative thyroid weight and hypertrophy of epithelial cells of the thyroid gland follicles were observed at every time point investigated. In addition the uptake of radioactive iodine form blood into the thyroid gland was enhanced significantly and the TCA-precipitable radioactive iodine in the thyroid gland increased in those rats. Although serum T_4 concentration was low in rats treated with 1000 mg/kg bw/day, no changes in TSH concentration were observed. None of these changes were found in the other groups except for a significant decrease in T_3 level in week 1 at 250 mg/kg bw/day. Absorption of Kojic acid was rapid. T_{max} of blood concentrations of radioactivity was 1.0 ± 0.0 hours with C_{max} of 25.07 ± 4.56 µg eq/ml. $T_{1/2}$ was 4.8 ± 0.3 hours. Elimination was nearly complete within 24 hours. $AUC_{0\text{-}24\ h}$ was calculated to be 101.54 ± 19.35 µg eq/ml.

Ref.: 32

Comment
A NOAEL of 62.5 mg/kg bw/day can be derived from this study. C_{max} was 25.07 ± 4.56 µg eq/ml and $AUC_{0\text{-}24\ h}$ was calculated to be 101.54 ± 19.35 µg eq/ml.

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Guideline:	/
Species/strain:	SD rats

Group size:	20 males/group
Test substance:	Kojic Acid
Batch:	/
Purity:	/
Dose levels:	0, 250, 500, 1000, 2000, 3000 mg/kg bw/day in 1% aqueous solution of carboxymethylcellulose (0.5 ml/100 g bw) by gavage
Exposure period:	13 weeks
Recovery:	4 weeks
GLP:	/

Consecutive administration for 13 weeks at dose levels of 0, 250, 500, 1000, 2000, 3000 mg/kg bw/day was performed in male rats, followed by 4 weeks of no dosing. Dose selection was performed according to results of a preliminary study with male and female rats (10/group), which received 0, 1000, 1500, 2000, 2500 mg/kg bw/day. After confirmation of the absence of sex differences the main experiment was conducted with males only. Animals were sacrificed at 4, 13, and 17 weeks of administration (5, 10, and 5 animals, respectively) for autopsy, haematological and serobiochemical examinations as well as for urinalysis. Blood levels for T_3, T_4 and TSH were not measured and thyroid weights were not recorded in this study. For autopsy, those animals which were poor in weight gain in the treated groups were selected. Groups were excluded from further examination when deaths exceeded the number of animals to be sacrificed. Animals were weighed daily and observed for behaviour and symptoms. Food and water intakes were measured once a week. Dead animals were autopsied at time of death.

Results
In the highest dose group, all animals died within the first tree weeks of treatment. In the 2000 mg/kg bw/day group 11 animals died during the treatment period and in the 1000 mg/kg bw/day group one animals died in week three. Observations reported were strong sedation and tonic or clonic spasms in the groups treated with 500 mg/kg bw/day and above and bleeding from eyes, ablepsy, exophthalmos, hematuria, epistaxis and vomiting in the groups treated with 1000 mg/kg bw/day and above. Autopsy performed in animals which died as well as at the end of the 4th, 13th, and 17th week revealed bleeding, pyoid substance and sclerosis in the lung, digestive tract congestion and adrenal atrophy. Significant decreases of body weight gain occurred in the groups, which received 500 mg/kg bw/day and above, which persisted during the recovery period. Changes in biochemical parameters included a decrease in GLU in the 2000 mg/kg bw/day group at the end of week 13 as well as an increase in GOT at the end of the 13th and 17th week in the 1000 and 2000 mg/kg bw/day group. No statistically significant differences in haematological parameters were reported. Urinalysis revealed protein and occult blood in urine in some of the treated animals but no dose-dependency was observed. Decrease in urinary pH values was observed in the high dose groups. During treatment period statistically significant decreases in absolute organ weights were reported for liver (250 mg/kg bw/day and above) heart, kidney (500 mg/kg bw/day and above), thymus, spleen (1000 mg/kg bw/day and above), lungs, adrenal gland, and testes (2000 mg/kg bw/day). Changes in relative organ weights occurred in lungs, liver, kidney and testes (500 mg/kg bw/day and above), spleen (1000 mg/kg bw/day) and adrenal gland (2000 mg/kg bw/day). In the 250 mg/kg bw/day group one animal showed congestion, perivascular cell infiltration and granulation in the kidney.

Ref.: 17

Comment
In all groups pulmonary lesions were noted and primary inflammation of bronchial mucosa in the control group. This effect was referred to incorrect administration. A LOAEL of 250 mg/kg bw/day can be derived from this study.

3.3.5.3. Chronic (6 months) toxicity

Guideline:	/
Species/strain:	SLC-SD rats
Group size:	10 males/group
Test substance:	Kojic Acid
Batch:	/
Purity:	/
Dose levels:	0, 125, 250, 500, 1000 mg/kg bw/day in 1% aqueous solution of carboxymethylcellulose (0.5 ml/100 g bw) by gavage
Exposure period:	26 weeks
Recovery:	5 weeks (groups: 250, 500, 1000 mg/kg bw/day, 10 animals/group)
GLP:	/

Male rats (110 – 140 g bw) were given 0, 125, 250, 500 or 1000 mg Kojic acid/kg bw/day in 1% aqueous solution of carboxymethylcellulose (0.5 ml/100 g bw) by gavage for 26 consecutive weeks. The control group was given the vehicle alone. These dose levels were selected on the basis of a previous 21-day oral toxicity study in rats and known data of a 13-week study. Treated animals were observed for abnormalities in health conditions and behaviour daily. Body weight, feed consumption and water intake were determined twice a week until 13 weeks after the initial administration and once a week thereafter. Two days before necropsy performed 26 weeks after the initial administration and two days before necropsy performed after the end of the recovery period, urine, accumulated for 16 hours was examined. Haematological and serobiochemical tests were performed before necropsy. Blood levels for T_3, T_4 and TSH were not measured. Animals were killed and subjected to macroscopic examination, selected organs were weighed, and organs/tissues were preserved. Microscopic examination was performed.

Results
There were no substance related deaths. Two animals in the highest dose groups died because of injuries resulted from treatment. In the groups receiving 250 mg/kg bw/day and more, excitation and subsequent sedation were observed for two and three hours after administration of Kojic acid. In the groups receiving 500 mg/kg and more, there were also some cases accompanied by exophthalmos and salivation. Suppression of body weight gain was reported in groups receiving 250 mg/kg bw/day Kojic acid and above. As to the feed consumption and water intake, in the groups treated with 500 mg/kg and above a temporary decrease of feed consumption and increase of water intake was observed. Decrease of the urine volume was observed in the two highest dose groups and at 1000 mg/kg bw/day a decrease of urinary pH was reported. Statistically significant haematological and biochemical differences reported include an increase in creatinine in the 250 and 500 mg/kg bw/day groups; an increase in ALP values in the 500 and 1000 mg/kg bw/day groups and increases in GOT, GPT, bilirubin, relative amount of monocytes as well as decreases in number of erythrocytes, haematocrit and haemoglobin in the highest dose group. These changes were not observed at the end of recovery period. Relative weights for several organs were statistically different from controls in the dose groups received 250 mg/kg bw/day and above. Decrease in absolute organ weights were reported for the heart in the dose groups treated with 500 mg/kg bw/day and above and for the spleen in the 500 mg/kg bw/day group only. Absolute organ weight increased in the adrenals in the dose groups treated with 500 mg/kg bw/day and above. Thyroid weights were increased significantly at 500 and 1000 mg/kg bw/day. In two cases of the 1000 mg/kg bw/day dose group vacuolation of anterior cells of the pituary gland was observed to a slightly greater degree compared to the control group. However, theses changes were reported not to be caused by Kojic acid.

It was estimated that the no effect level of Kojic acid is 125 mg/kg bw/day when administered orally to male rats over a period of 26 weeks.

Ref.: 18

Comment
Studies on repeated dose, subchronic and chronic oral toxicity were performed with male rats only.

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

Study 1

Guidelines:	/
Species/Strain:	*Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537
Replicates:	Duplicates in a single test
Test substance:	Kojic acid
Solvent:	Distilled water
Batch:	8427
Purity:	/
Concentrations:	500, 1000 2000, 4000 µg/plate, with and without metabolic activation
Treatment:	Plate incorporation method with 48 h incubation time
GLP:	/
Date:	May 1980

The Ames-test was performed with the bacterial tester strains *Salmonella typhimurium* TA 98, TA 100, TA 1535 and TA 1537 with and without S9-mix. Liver S9 fraction from PCB-induced rats was used as exogenous metabolic activation system. The test substance was tested at four concentrations in the range of 500 to 4000 µg/plate. Tests were performed in duplicates. N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG), AF2, 9-aminoacridine, and 2-aminoanthracene served as positive controls.

Results
Dose dependent increases in the number of mutant colonies were observed at doses of 1000 – 2000 µg/ml and above in the strains TA 100, TA 1535 and TA 98 with and without metabolic activation.

Conclusion
Under the experimental conditions used Kojic acid was mutagenic in the gene mutation tests in bacteria both in the absence and the presence of S9 metabolic activation.

Ref.: 21

Comment
The test is performed before the implementations of OECD guidelines. The description of the test is rather poor. Purity is not mentioned. The test has only limited value.

Study 2

Guideline:	OECD 471, 1994
Species/strain:	*Salmonella typhimurium* TA 1535, TA 1537, TA 98, TA 100, TA 102
Replicates:	Triplicates, three independent tests
Test substance:	53758
Solvent:	Distilled water
Batch:	R63462 (Summary) or RG3462 (B/General information)
Purity:	/

Concentrations: 0, 30, 100, 300, 1000, 3000, 5000 µg/plate, with and without metabolic activation
Treatment: Agar incorporation test and plate incorporation method with 48 h incubation time
GLP: in compliance
Date: January 1997

The test substance was evaluated in independent experiments in the absence and presence of metabolic activation prepared from the livers of rats given Aroclor 1254 (S9 mix). Test concentrations were based on the results in a toxicity test with all strains. Toxicity was evaluated on the basis of microscopic examination of the background lawn. The agar incorporation assay (experiment 1) and the plate incorporation method (experiment 2) were used. In the 3rd experiment only TA 1537 was tested without metabolic activation. Known mutagens (sodium acide, 2-anthramine, 9-amino-acridine, 2-nitrofluorene, mitomycin C and benzo[a]pyrene) were used as positive controls, and cultures treated with distilled water (solvent) were used as negative controls. Three plates per treatment condition were used.

Results
The test substance was not toxic in all strains tested with and without metabolic activation. Mutant rate increased dose-dependently except for the strain TA 1537 without metabolic activation in experiment 1. However, effects were weak in strains TA 1535, and TA 98 with and without metabolic activation as well as in TA 102 with metabolic activation. Most pronounced effects were reported for TA 100. In experiment 2 a dose dependent increase in the number of revertants was seen in all strains tested. In experiment 3 the positive results for TA 1537 were confirmed.

Conclusion
Under the experimental conditions used Kojic acid was mutagenic in the gene mutation tests in bacteria both in the absence and the presence of S9 metabolic activation.

Ref.: 93

Comment
No clarification found if test substance 53758 is Kojic acid. The batch no is reported as R63462 in the Summary and as RG3462 in B/General information. Since also the purity of the test compound is unclear, the test is of limited value.

Study 3

Guidelines: /
Species/strain: *Salmonella typhimurium* TA 98, TA 100
Replicates: Duplicates in two independent experiments
Test substance: Kojic acid, aqueous solution
Solvent: Distilled water
Batch: /
Purity: /
Concentrations: 100, 250, 500, 750, 1000, 2000, 4000, 6000 µg/plate, with and without metabolic activation
Treatment: Plate incorporation assay and pre-incubation assay with 20 minute incubation
GLP: /
Date: Before May 1991

Mutagenicity of Kojic acid was determined using the standard plate-incorporation and the preincubation assay. Assays were performed in duplicate runs, each with 4 plates per dose in the presence or absence of liver S9 mix prepared from rats pre-treated with Aroclor 1254. Toxicity was evaluated on the basis of microscopic examination of the background

lawn. As positive control 2-aminofluorene was used for both strains in the presence of S9 mix, methylmethane sulfonate for strain TA 100 and 2-nitrofluorene for strain TA 98 in the absence of S9 mix.

Results
In the absence of S9 mix Kojic acid was toxic for TA 98 at 1000 µg/plate and above. For TA 98 Kojic acid was mutagenic at concentrations of 100 µg/plate and above without metabolic activation and at concentrations of 2000 µg/plate and above with metabolic activation. For the tester strain TA 100 mutagenicity was reported at concentrations of 1000 µg/plate and above without metabolic activation and at concentrations of 2000 µg/plate and above with metabolic activation. Results of the two methods used were comparable.

Conclusion
Under the experimental conditions used Kojic acid was mutagenic in strains TA 98 and TA 100 of this gene mutation test in bacteria both in the absence and the presence of S9 metabolic activation.

Ref.: A3

Comment
Data are from a paper from the open literature. Since the purity of Kojic acid and the batch number are not mentioned and the solvent is unclear, the test is of limited value.

Study 4

Guidelines:	OECD 471
Species/strain:	*Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537 *Escherichia coli* WP2 uvrA
Replicates:	Triplicates, two independent tests
Test substance:	Kojic acid
Solvent:	DMSO
Batch:	8 A 44
Purity:	100%
Concentrations:	0, 33, 100, 333, 1000, 2500, 5000 µg/plate, with and without metabolic activation
Treatment:	Plate incorporation method with 48 h incubation time
GLP:	in compliance
Date:	May – October 1998

Kojic acid was investigated for the induction of gene mutations in *Salmonella typhimurium* and *Escherichia coli* (Ames test). Liver S9 fraction from phenobarbital/β-naphthoflavone induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-experiment for toxicity with all strains. Toxicity was evaluated on the basis of reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn. The data of the pre-experiment were reported as part of the main experiment 1. Kojic acid was tested up to the prescribed maximum concentration of 5000 µg/plate using the direct plate incorporation method. Known mutagens (2-aminoanthracene, 4-nitro-o-phenylene-diamine) were used as positive controls, and cultures treated with solvents were used as negative controls.

Results
Plates incubated with the test substance showed normal background growth up to 5000 µg/plate with and without metabolic activation. In both experiments a dose dependent increase in revertant colony numbers was observed following treatment in the presence and absence of metabolic activation in all strains tested except for TA 1537.

Conclusion

Under the experimental conditions used Kojic acid was mutagenic in the gene mutation tests in bacteria both in the absence and the presence of S9 metabolic activation.

Ref.: 22

Study 5

Guidelines:	OECD 471
Species/strain:	*Salmonella typhimurium* TA 98 and TA 100
Replicates:	Triplicates, two independent tests
Test substance:	Kojic acid
Solvent:	DMSO (TA 100 and TA 98 with S9 mix) Deionised water (TA 98 without S9 mix))
Batch:	1 B 123
Purity:	considered 100%
Concentrations:	0, 3, 10, 33, 100, 333, 1000 µg/plate without metabolic activation 0, 33, 100, 333, 1000, 2500, 5000 µg/plate with metabolic activation
Treatment:	Plate incorporation method with 48 h incubation time
GLP:	in compliance
Date:	May – November 2001

Kojic acid was investigated for the induction of gene mutations in *Salmonella typhimurium* (Ames test). Liver S9 fraction from phenobarbital/β-naphthoflavone induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-experiment for toxicity with all strains. Toxicity was evaluated on the basis of reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn. The data of the pre-experiment with metabolic activation were reported as part of the main experiment 1. Kojic acid was tested up to the prescribed maximum concentration of 5000 µg/plate using the direct plate incorporation method. Known mutagens (2-aminoanthracene, 4-nitro-o-phenylene-diamine) were used as positive controls, and cultures treated with solvents were used as negative controls.

Results
In the absence of metabolic activation, toxicity limited the concentration range at 1000 µg/plate (for strain TA 100). Furthermore DMSO as solvent control showed irreproducible erratic toxic effects in TA 98 without metabolic activation. Although the solubility of the test substance is lower in water than in DMSO, water was found to be acceptable for these treatment groups. No substantial and reproducible increase in revertant colony numbers of any of the two tester strains was observed following treatment with Kojic acid at the dose levels tested, neither in the presence nor absence of metabolic activation. Reference mutagens showed a distinct increase of revertant colonies.

Ref.: 89

Conclusion
Under the experimental conditions used Kojic acid was not mutagenic in the gene mutation tests in bacteria both in the absence and the presence of S9 metabolic activation.

Study 6

Guidelines:	/
Species/strain:	*Salmonella typhimurium* TA 98, TA 100
Replicates:	/
Test substance:	Kojic acid (commercially obtained)
Solvent:	Water or DMSO
Batch:	/
Purity:	/

Concentrations: 10, 100, 1000, 10000 µg/plate, with and without metabolic activation
Treatment: /
GLP: /
Date: Before January 1979

The Ames-test was performed with the bacterial tester strains *Salmonella typhimurium* TA 98, and TA 100, with and without phenobarbital induced rat liver enzymes (S9-mix). Quercetin, sterigmatocystein and benzo[a]pyrene served as a positive control.

Results
A dose dependent increase in revertant colony numbers was observed following treatment with Kojic acid for strain TA 100 but not for TA 98 both neither in the presence nor in the absence of metabolic activation.

Conclusion
Under the experimental conditions used Kojic acid was mutagenic in strain TA 100 of this gene mutation tests in bacteria both in the absence and the presence of S9 metabolic activation.

Ref.: 59

Comment
The test is performed before the implementations of OECD guidelines. Data are from a paper from the open literature describing the results of a number of different compounds. The test protocol is poorly described. Raw data are lacking. Purity of Kojic acid and the batch number are not mentioned. The solvent is unclear. The test is of no value.

Study 7

Guidelines: /
Species/strain: *Salmonella typhimurium* TA 98 and TA 100
Replicates: /
Test substance: Kojic acid (commercially obtained)
Solvent: /
Batch: /
Purity: /
Concentration: /
Treatment: /
GLP: /
Date: Before July 1987

The description of the test is very poor. The study is not relevant in the scope of this evaluation.

Ref.: AR2

SOS Chromotest

Guidelines: /
Species/strain: *Escherichia coli* K12
Replicates: /
Test substance: Kojic acid (commercially obtained)
Solvent: Water
Batch: /
Purity: /
Concentrations: 1-2 mg/100 µl with and without metabolic activation
Treatment: /

GLP: /
Date: Before 1986

An SOS chromotest was conducted with Kojic acid. No further details on the methods are given in the paper.

Results
Kojic acid was negative with and without S9 mix.

Conclusion
Under the experimental conditions used Kojic acid was not mutagenic in the SOS chromotest both in the absence and the presence of S9 metabolic activation.

Ref.: 83

Comment
Data are from a paper from the open literature describing the results of a number of different compounds. The test protocol is not described. The test is of no value.

In vitro Gene Mutation Test in Mammalian Cells (*hprt* locus)

Guideline:	/
Species/strain:	Chinese hamster V79 cells
Replicates:	/
Test substance:	Kojic acid, commercial
Solvent:	Culture medium
Batch:	87122
Purity:	/
Concentrations:	0, 30, 100, 300, 1000, 3000 and 10000 µg/ml
Treatment:	16 h treatment, expression period 6 days and selection period of 12 days
GLP:	/
Date:	September – November 1981

Kojic acid was assayed for gene mutations in V79 cells without S9 metabolic activation. Test concentrations were based on the results of a survival test. After treatment cells were washed and successively cultured by 2-day intervals for 3 times. Subsequently cells were inoculated for a survival test or for mutation test. Culture period was 6 days for survival and 12 days for mutation tests (with addition of 10 µg/ml 6-thioguanine). Ethyl methanesulfonate was used as positive control.

Results
No increase in the mutant rate in accordance with the increase of Kojic acid concentration and no statistical significance between the control group and the treated group was observed.

Conclusion
Under the experimental conditions used Kojic acid was not mutagenic in this gene mutation test in V79 cells.

Ref.: 23

Comment
Test was performed without metabolic activation only. Purity of Kojic acid was not mentioned.

In Vitro* gene mutation assay with Mouse Lymphoma cells (*hprt*-locus*)

Guideline:	OECD 476, 1997
Species/strain:	Mouse lymphoma cell line L5178Y/TK$^{+/-}$
Replicates:	Duplicates, two independent tests
Test substance:	Kojic acid
Solvent:	Deionised water
Batch:	1402
Purity:	> 97%
Concentrations:	0, 300, 600, 900, 1200, 1421 µg/ml with and without metabolic activation Treatment: 3 h treatment without and with S9 mix; expression period 7 days and selection period of 13-14 days
GLP:	in compliance
Date:	November 2001 – May 2002

The assay was performed in two independent experiments in the presence and absence of Aroclor 1254 induced rat liver post-mitochondrial fraction (S9-mix). The test substance was dissolved in deionised water. Test concentrations were chosen on the basis of the results of a pre-test measuring relative survival. Highest concentration tested was 1421 µg/ml which is approximately the prescribed maximum concentration of 10 mM. The solubility limits of Kojic acid were reported to be at least 27 mg/ml for water and greater than 279 µg/ml for tissue culture medium, respectively. No details on precipitation were given. Experiment 2 in the absence of S9-mix had to be repeated due to low post-treatment cell counts in all cultures believed to be due to technical error. Negative and positive controls were in accordance with the OECD guideline.

Results
No marked toxicity was observed at any dose level. The highest dose tested in the range finding experiment yielded 97% relative survival in the absence and 115% relative survival in the presence of S9 mix. A statistically significant increase in mutant frequency was observed following treatment with Kojic acid at the lowest dose level (300 µg/ml) tested in the presence of S9 mix in experiment 2. However, there was no evidence of a dose-related response in this experiment and no other statistically significant increases in mutant frequency were observed at any dose level tested in the absence or presence of S9-mix in experiment 1 and 2. The effect was therefore considered to be incidental and of no biological significance. Positive control substances (4-nitroquinoline in the absence and benzo[a]pyrene in the presence of S9-mix) induced a clear increase in mutations. High sporadically heterogeneity values observed for mutation and for viability were not believed to have prejudiced the validity of the study.

Conclusion
Under the experimental conditions used Kojic acid did not induce an increase in the mutation frequency at the *hprt* locus of mouse lymphoma cells and, consequently, is not mutagenic in mouse lymphoma cells in vitro.

Ref.: 91

***In vitro* chromosome aberration test**

Study 1

Guideline:	/
Species/strain:	Chinese hamster ovary (CHO-K1) cells
Replicates:	Duplicates
Test substance:	Kojic Acid, commercial

Solvent:	M-199 culture medium
Batch:	/
Purity:	/
Concentrations:	1.5 – 12 mg/ml in M-199 for cytotoxicity 3, 4.5, and 6 mg/ml with and without metabolic activation
Treatment::	2 h treatment with 24 h recovery without and with S9.
GLP:	/
Date:	Before May 1991

Exponentially growing cells were incubated for 2 hours with Kojic acid with and without S9 mix. After incubation cells were washed and incubated for another 24 hours in fresh medium. Colchicine (0.1 µg/ml) was added for the last 3 hours of culture. Cells were fixed onto slides and air dried. For scoring chromosomal aberrations slides were stained in 4% Giemsa. At least 100 metaphases were scored for each dose. Positive controls included triethylenemelamin (0.25 µg/ml without S9-mix) and cyclophosphamide (5 µg/ml with S9-mix).

Results
Kojic acid at doses of 9 mg/ml and above was cytotoxic and a TC_{50} of 10.86 ± 3.86 mg/ml was determined based on the loss of cellular proteins. Kojic acid caused a dose-related increase in the percentage aberrant CHO cells in the presence and absence of S9 mix. All types of aberrations occurred in the tests except for rings which were reported in tests with metabolic activation only.

Conclusion
Under the experimental conditions used Kojic acid induced an increase in the percentage aberrant cells and, consequently, is mutagenic (clastogenic) in CHO cells in vitro.

Ref: AR14, A3

Comment
Data are from a paper from the open literature. In a proper chromosome aberration test the results have to be reported as an increase in the number (or percentage) of aberrant cells excluding gaps. It is unclear whether in the percentage aberrant cells as reported in this paper gaps are included or not. Since also the purity of Kojic acid, the batch number is not mentioned and the solvent is unclear, the test is of limited value.

Study 2

Guideline:	OECD 473, 1997	
Species/strain:	Chinese hamster V79 cells	
Replicates:	Duplicates, two experiments	
Test substance:	Kojic Acid	
Solvent:	Deionised water	
Batch:	1 B 123	
Purity:	Considered 100%	
Concentrations:	Experiment 1:	355, 710, 1420 µg/ml without and with S9-mix
	Experiment 2:	250, 500, 1000 µg/ml without S9-mix
		355, 710 1420 µg/ml with S9-mix
Treatment:	Experiment 1:	4 hours with 14 h recovery without and with S9-mix
	Experiment 2:	18 or 28 h without recovery without S9-mix
		4 hours with 24 h recovery with S9-mix
GLP:	in compliance	
Date:	May 2001 – January 2002	

Kojic acid has been investigated in the absence and presence of metabolic activation for the induction of chromosomal aberrations in V79 cells. Dose selection of the cytogenetic

experiments was performed considering the toxicity data and the occurrence of precipitation. In a range finding pre-test cells were scored for cytotoxicity in a concentration range from 11.1 to 1420 µg/ml. Cells were treated for 4 h or 18/28 h (experiment 2 without S9-mix). Harvest time was 18 or 28 h (experiment 2) after the beginning of treatment. Liver S9 fraction from phenobarbitone/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Per culture 100 metaphase plates were scored for structural chromosome aberrations. Known clastogens in the presence (cyclophosphamide) or absence of S9 mix (ethylmethane sulfonate), and untreated solvent cultures were used as positive or negative controls, respectively.

Results
No precipitation and no relevant influence of the test substance on pH value or osmolarity were observed. In the pre-test no toxic effects occurred after 4 hours treatment in the absence and presence of S9-mix. 24 hours continuous treatment with 710 µg/ml and above showed toxic effects (number of cells < 50% of solvent control). In addition in experiment 1 and 2 more or less dose dependent reductions in cell numbers were observed except for experiment 1 with S9-mix. The number of cells never fell below 50% of solvent control. Reduction of mitotic indices was observed only in experiment 2. The number of cells with aberrations increased statistical significantly after 18 hours (250 and 1000 µg/ml) and 28 hours (1000 µg/ml) of exposure.

Conclusion
Under the experimental conditions used Kojic acid induced an increase in the number of aberrant cells and, consequently, is mutagenic (clastogenic) in V79 cells in vitro.

Ref: 90

Comment
The authors concluded that the positive response may be related to cytotoxicity, but that a weak clastogenic effect could not be excluded.

In vitro sister chromatid exchange test

Guideline:	/
Species/strain:	Chinese hamster ovary (CHO-K1) cells
Replicates:	Duplicates, 3 experiments which were pooled
Test substance:	Kojic Acid, commercial
Solvent:	M-199 culture medium
Batch:	/
Purity:	/
Concentrations:	1.5 – 12 mg/ml in M-199 for cytotoxicity 3, 4.5, and 6 mg/ml with and without metabolic activation
Treatment:	2 h treatment with 24 h recovery without and with S9-mix
GLP:	/
Date:	Before May 1991

Exponentially growing cells were incubated for 2 hours with Kojic acid with and without S9-mix. After incubation cells were washed and incubated for another 24 hours in fresh medium containing 5-bromodeoxyuridine (3 µg/ml). Colchicine (0.1µg/ml) was added for the last 3 hours of culture. Cells were fixed onto slides and air dried. A modified fluorescence technique was used for differential staining of sister chromatids At least 30 metaphases were scored for each dose. Positive controls included MMS (0.03 µg/ml without S9-mix) and cyclophosphamide (5 µg/ml with S9-mix).

Results
Kojic acid at doses of 9 mg/ml and above was cytotoxic and a TC_{50} of 10.86 ± 3.86 mg/ml was determined based on the loss of cellular proteins. Kojic acid caused a dose-related and

statistically significant of SCE frequencies in CHO cells in the presence and absence of S9-mix. Binding of Kojic acid to constituents of the S9-mix, however, was reported and related to a decrease of the availability of the test substance to interfere with the genome.

Conclusion
Under the experimental conditions used Kojic acid induced an increase in the number of SCE per metaphase and, consequently, is genotoxic in CHO cells in vitro.

Ref.: A3

Comment
Data are from a paper from the open literature. Since the purity of Kojic acid and the batch number are not mentioned and the solvent is unclear, the test is of limited value.

Micronucleus test *in vitro*

Guidelines:	/
Cells:	SVK14 (keratinocytes), Hep G2
Test substance:	Kojic acid
Solvent:	DMSO
Batch:	6K32
Purity:	/
Concentrations:	SVK14 (with and without metabolic induction): 3 hours treatment: 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000 µg/ml 24 hours treatment: 500, 1000, 1500, 2000, 2500, 3000, 4000, 5000, 6000, 7000, 8000 µg/ml Hep G2 (without metabolic induction): 3 and 24 hours treatment: 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000 µg/ml
Treatment:	SVK4: 3 h with 48 or 72 h recovery without and with S9-mix 24 h with 48 or 72 h recovery without and with S9-mix Hep G2 3 h with 30 h recovery 24 h with 30 h recovery
GLP:	/
Date:	July and August 1997

Kojic acid has been investigated in the absence and presence of metabolic activation for the induction of micronuclei in SVK4 (with and without S9 mix) and Hep G2 cells (without S9-mix). Treatment periods were 3 or 24 h; harvest times 48 or 72 h (SVK4 cells) or 30 h (Hep G2 cells). Incubation was partly in the presence of cytochalasin B (at a final concentration of 3 µg/ml). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Negative and positive controls were cyclophosphamide and MMS.

Results
Cytotoxic effects were reported for SVK14 cells at concentrations of 3000 µg/ml and above after 24 hours exposure and for Hep G2 cells at concentrations of 5000 µg/ml and above after 24 hours treatment. Mutagenic effects were reported only for Hep G2 cells after 3 hours of exposure to 7000 and 8000 µg/ml. Effects were reported to be related to cytotoxicity, however, survival rate was 97% and 100% for theses concentrations of Kojic acid.

Conclusion
Under the experimental conditions used Kojic acid did not induced an increase in the number of micronucleated SVK14 cells and, consequently, is not genotoxic in SVK14 cells in vitro. Since the relevance of positive mutagenic effects found exclusively in Hep G2 cells is

unclear, the result of this in vitro micronucleus test for Hep G2 cells has to be considered inconclusive.

Ref.: 54

Summary: Mutagenicity / Genotoxicity *in vitro*

Strain / Cell type	Concentration	S9	Result
Bacterial gene mutation assay			
Salmonella typhimurium			
TA 98, TA 100, TA 1535, TA 1538	500 to 4000 µg/plate	+ / -	weak mutagenic *
TA 98, TA 100, TA 102, TA 1535, TA 1537	30 to 5000 µg/plate	+ / -	mutagenic *
TA 98, TA 100	100 to 6000 µg/plate	+ / -	mutagenic *
TA 98, TA 100, TA 1535, TA 1537	33 to 5000 µg/plate	+ / -	mutagenic
TA 98, TA 100	33 to 5000 µg/plate	+	negative
TA 98, TA 100	3 to 1000 µg/plate	-	negative
TA 98, TA 100	10 to 10000 µg/plate	+ / -	mutagenic in TA 100 **
E. coli			
WP2 uvrA	33 to 5000 µg/plate	+ / -	mutagenic *
K12	1-2 mg/100 µl	+ / -	negative **
Mutation induction test in mammalian cells			
Chinese hamster V 79 cells	30 to 10000 µg/ml	-	negative
***In Vitro* Mouse Lymphoma assay (*hprt* locus)**			
Mouse lymphoma cell line	300 to 1421 µg/ml	+ / -	negative
***In vitro* chromosome aberration**			
Chinese hamster ovary (CHO) cells	3 to 6 mg/ml	+ / -	mutagenic *
Chinese hamster V79 cells	355 to 1420 µg/ml (4 h)	+ / -	negative
	250 to 1000 µg/ml (18 and 28 h)	-	weak clastogenic
Sister chromatide exchange			
Chinese hamster ovary (CHO) cells	3 to 6 mg/ml	+ / -	mutagenic *
Micronucleus test *in vitro*			
SVK14 (keratinocytes)	1000 to 8000 µg/ml (3 h)	+ / -	negative
	500 to 8000 µg/ml (24 h)	+ / -	negative
Hep G2	1000 to 8000 µg/ml (3 and 24 h)	-	uncertain

* Relevance of this test is unclear
** Test of no value

3.3.6.2. Mutagenicity / Genotoxicity *in vivo*

Comet assay

Guideline:	/
Species:	Wistar rats
Group sizes:	5 males/group
Test substance:	Kojic acid
Solvent:	0.5% aqueous solution of Cremophor
Batch:	3115
Purity:	100,6 %
Dose levels:	0, 1000 and 2000 mg/kg bw in 0.5% aqueous solution of Cremophor at 20 ml/kg oral administration (gavage)
Route:	Oral, two application 21 h apart
Sacrifice times:	3 h after the last dose
GLP:	in compliance
Date:	April – December 2004

Kojic acid was tested in an in vivo comet assay with liver, stomach and colon cells of male Wistar rats. Ethylmethanesulfonate (300 mg/kg bw as single oral dose) served as positive control. Dose selection was based on clinical symptoms observed in a pilot study Male rats received two oral doses of the test substance at 2000 mg/kg bw or 1000 mg/kg bw, respectively, 21 hours apart, administered in a 0.5% aqueous solution of Cremophor at 20 ml/kg. Animals were sacrificed three hours after the second treatment. Intact liver cells were prepared via in situ perfusion; intact stomach cells with trypsine and intact colon cells with DTT and proteinase K. Organ cells were subjected to the comet assay procedure according to Singh et al. (Exp. Cell Res. 175, 184-191, 1988) with minor modifications.

Results
In the pilot study animals showed symptoms of toxicity after treatment with Kojic acid at both doses. Clinical symptoms were roughened fur, strongly semi-anaesthetised state, and strongly reduced motility. In the main experiment animals showed symptoms of toxicity at 2000 mg/kg bw indicating systemic availability of the test substance following oral gavage. No compound related cytotoxicity was observed in cells of liver, stomach and colon after isolation. Treatment with Kojic acid did not result in a biologically relevant increase of the mean tail length.

Conclusion:
In the comet assay with liver, stomach and colon cells, Kojic acid was not genotoxic.

Ref.: 98

Dominant lethal test

Guideline:	/
Species:	BDF_1 mice
Group sizes:	30 animals/group, positive control: 20 animals
Test substance:	Kojic acid
Solvent:	1% sodium carboxymethylcellulose
Batch:	87122
Purity:	/
Dose levels:	0, 350 and 700 mg/kg bw
Route:	Oral administration (gavage) for 5 consecutive days
Sacrifice times:	Females were autopsied 13 days after copulation
GLP:	/
Date:	September – November 1981

In a preliminary test mice were administered orally with aqueous solutions of the test substance in 1% sodium carboxymethylcellulose at 0.5 ml/25 g bw for five consecutive days. Ten administration groups from 0 to 2000 mg/kg bw/day were chosen and each dose group consisted of 10 males. Animals were observed for one week after the last administration of the test substance. Animals treated with Kojic acid showed sensitivity to sound. In the groups treated with 354 mg/kg bw or more slight exophthalmos, arrest of locomotive activity and abdominal position extending forelimb forward were reported. A dose-depending reduction in body weight gain was observed. The highest dose without mortality was 707 mg/kg bw/day. The LD_{50} was calculated to be 1031.2 mg/kg bw/day (894.9 – 1189.1 mg/kg bw). At the end of the observation period, alive animals were sacrificed and autopsied. No statistically significant differences of testes weight between treated groups and controls were seen. No gross abnormalities were detected.

A preliminary mating test was performed under similar conditions with doses of 250, 354, 500, and 707 mg/kg bw/day; groups consisted of five males. Each male was mated with an untreated female after exposure period. Females were autopsied 13 days after copulation, and pregnancy was detected in all of the females. No apparent reduction in the number of live foetuses was noted.

In the main experiment, each male mouse from each group was mated with a single female after the end of administration period. Mating was continued for 56 days by exchanging an unmated female every 4 days. The mated females were sacrificed and autopsied about 13 days after the mating started. Each female mouse was examined for the number of successful pregnancy, corpora lutea, implantation as well as alive and dead foetuses. As positive control 20 males were orally administered with 500 mg of 7,12-dimethylbenz(a)anthracene dissolved in olive oil per kg bw.

Results
The number of pregnant females in treated groups was comparable to those of the negative control. No induced dominant lethality was observed for both concentrations tested in all mating periods, except for the 700 mg/kg/day group at the 37 – 40 day mating period, were post implantation losses were slightly but statistically significant decreased (5.1%). Apparent induction of dominant lethals (> 15%) was observed in several mating periods with two peaks at the 5-8 day (61.54%) and the 41-44 day (45.45%) mating periods after treatment with the positive control.

Conclusion
Kojic acid was considered not to induce dominant lethality under the conditions tested.
Ref.: 25, AR13

Bone marrow micronucleus test in mice

Study 1

Guideline:	OECD 474
Species:	NMRI mice
Group sizes:	6 females and 6 males/group
Test substance:	Kojic acid
Batch:	1B123
Purity:	Considered 100%
Dose levels:	187.5, 375, 750 mg/kg bw
Route:	i.p.
Vehicle:	1% CMC
Sacrifice times:	24 and 48 h (highest dose only) after treatment
GLP:	in compliance
Date:	April – June 2001:

Pre-experiments
Two animals per sex were treated i.p. with the test substance at single doses of 2000, 1000, 750, and 500 mg/kg bw in 1% CMC in a volume of 10 ml/kg bw. Animals were examined for acute toxic symptoms at intervals of around 1 h, 2-4 h, 6h, 24 h, 30 h, and 48 h after administration of Kojic acid. In the highest treatment group all animals died within the first hour after application of the test substance. Toxic effects described for the other dose groups were reduced spontaneous activity, abdominal position, eyelid closure and apathy. 750 mg/kg bw was chosen as highest dose for the main experiment.

Main experiment
The potential of Kojic acid to induce micronuclei in polychromatic erythrocytes in the bone marrow of NMRI mice was investigated. The test substance was administered intraperitoneally as a single dose of 187.5, 375 or 750 mg/kg bw. Twenty-four and 48 hours after administration bone marrow cells of five female and five male mice were collected for micronuclei analysis. At least 2000 polychromatic erythrocytes (PCEs) were scored per animal. Cytotoxic effects were described as ratio between PCEs and normochromatic erythrocytes (NCEs). Vehicle and cyclophosphamide (40 mg/kg bw) were used as negative or positive control.

Results
After treatment with Kojic acid the number of NCEs was not substantially increased as compared to mean NCE values of the vehicle control indicating that Kojic acid had no cytotoxic properties in the bone marrow. However, following exposure clinical signs like reduced spontaneous activity, eyelid closure, apathy and abdominal position were observed indicating to systemic availability of Kojic acid in exposed animals. In comparison to the corresponding vehicle control there was no biologically relevant or statistically significant enhancement in the number of cells with micronuclei at any preparation interval after administration of the test substance and with any dose level tested.

Conclusion
Under the experimental conditions used Kojic acid did not induce micronuclei in bone marrow cells of treated mice and, consequently, Kojic acid is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 88

Study 2

Guideline: /
Species: ddY mice
Group sizes: 6 males/group
Test substance: Kojic acid
Batch: /
Purity: /
Dose levels: 125, 250, 500, 1000 mg/kg bw, twice 24 h apart ("2-repeated dose study")
125, 250, 500 mg/kg bw, five times at 24 h intervals ("5-repeated dose study")
Route: i.p.
Vehicle: 0.9% physiological saline
Sacrifice times: 6 h after the final dose
GLP: /
Date: May 1980

Pre-experiments:
Two males were treated i.p. with the test substance at single doses of 125, 250, 500, 1000, 2000 and 4000 mg/kg bw in 0.9% physiological saline in a volume of 10 ml/kg bw. Animals

were examined for mortality for 30 hours. 1000 and 500 mg/kg bw were chosen as the highest doses for the 2-repeated and the 5-repeated dose study, respectively.

Main experiment:
The test substance was administered intraperitoneally twice at a 24 hours interval at doses of 125, 250, 500 or 1000 mg/kg bw/day or five times at 24 hours intervals at doses of 125, 250, or 500 mg/kg bw/day. Test substance was dissolved in 0.9% physiological saline and applied at volumes of 10 ml/kg bw. Six hours after the final dose bone marrow cells were collected for micronuclei analysis. 1000 polychromatic erythrocytes (PCEs) were scored per animal. Vehicle and mitomycin C (2 and 0.8 mg/kg bw for 2 and 5 times application, respectively) were used as negative or positive control.

Results
A single dose of 1000 mg/kg bw was reported to be lethal for 5 of 6 animals. Both in the 2-repeated and in the 5-repeated dose study a biological relevant increase in the number of micronuclei was not observed.

Conclusion
Under the experimental conditions used Kojic acid is not mutagenic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 24

Comment
The test is performed before the implementations of OECD guidelines. Purity of Kojic acid and the batch number are not mentioned. The test has only confirmatory value.

Bone marrow micronucleus test in rats and mice

Guideline:	/
Species:	ddY mice
	Fischer 344 rats
Group sizes:	4 males/treatment group, 3 males/positive control
Test substance:	Kojic acid
Batch:	5312 (food additive)
Content:	100.6%
Dose levels:	0, 500, 1000 mg/kg bw
Route:	Gastric intubation
Vehicle:	0.5 % sodium carboxymethylcellulose
Sacrifice times:	4 days after treatment
GLP:	/
Date:	Before December 2005

Four male mice and rats were used for each treatment group and three animals for positive controls. Doses administered are based on the approximate maximum tolerated dose for each species determined by acute toxicity experiments where oral gavage of 2000 mg/kg bw resulted in death of 4/4 mice and 4/4 rats within 3 hours. 24 hours after oral administration, partial hepatectomy was performed on 9 week old animals by removing three major lobes of the liver. After four days, livers were removed. Isolated hepatocytes were scored for the number of micronucleated hepatocytes (MNHEPs) among 1000 hepatocytes. Additionally, for 3 week old mice assays were performed by the same procedure but without partial hepatectomy, livers were removed at 72, 96, and 120 hours.

Results
Four days after partial hepatectomy mean values of MNHPCs in mice increased dose dependently and with 1000 mg/kg bw the value was significantly increased compared to the

control. In rats, however, no increase was observed. Furthermore, Kojic acid was found to have no micronucleus inducing ability in infant mice without hepatectomy.

Ref.: AR4

Comment
Data are from a paper from the open literature. This is no standard test. The relevance of these positive results in hepatectomised mice is unclear. This publication is not taken up in the safety review.

Unscheduled DNA Synthesis test in rats

Guideline:	OECD draft guideline 486, 1991
Species:	Wistar HanIbm rats
Group sizes:	4 males/group
Test substance:	Acide Kojique (Kojic acid)
Batch:	6K32
Purity:	considered 100%
Dose levels:	0, 150, 1500 (2 hours exposure only) mg/kg bw
Route:	Oral gavage, once
Vehicle:	Deionised water
Sacrifice times:	2 and 16 h after treatment
GLP:	in compliance
Date:	July – November 1997

Kojic acid was assessed in the in vivo UDS assay for its potential to induce DNA repair in the hepatocytes of rats. Four animals per group received single doses of the test substance orally administered in a volume of 10 ml/kg bw. The highest dose used was chosen on the basis of a pre-experiment for toxicity and was estimated to be close to the maximum tolerated dose. Deionised water and 2-acetylaminofluorene were used as negative and positive controls. Hepatocytes were isolated 2 and 16 hours after treatment. For each dose level, including controls, hepatocytes from three treated animals were assessed. At least three cultures were established of each animal. Slides from 2 cultures/animal were prepared and the unscheduled synthesis of DNA was evaluated by the incorporation of tritiated methyl thymidine (5 µCi/ml, specific activity 20 Ci/mmol) in 50 cells/slide.

Results
Viability of hepatocytes from treated groups was not substantially affected. Enhanced mean nuclear and cytoplasmic grain counts, as well as slight shifts of the percentage distribution of the nuclear grain counts to higher values at the 2 and 16 hours treatment interval after administration of 1500 mg/kg bw were observed. However, net grain values of all treated groups were consistently negative and comparable to the current vehicle control. In vivo treatment with the positive control revealed distinct increases in the number of nuclear and net grain counts.

Conclusion
Under the experimental conditions used Kojic acid did not induce DNA-damage leading to unscheduled DNA synthesis in the hepatocytes of the treated rats and, consequently, is not genotoxic in rats in the in vivo UDS test.

Ref.: 26

DNA adducts

Study 1

Guideline: /
Species: F344/DuCrj rats
Group sizes: 3 males/group
Test substance: Kojic acid
Batch: 44261
Purity: 100.3%
Dose levels: 0, 0.5 or 2% diet concentrations given for 7 or 28 days
Route: Oral via the diet
Vehicle: /
Sacrifice times: 1 day after the last day of treatment
GLP: /
Date: November 2004 - June 2005

Kojic acid was administered orally to male F344/DuCrj rats to investigate the formation of DNA adducts in the liver using the ^{32}P-postlabeling method. Rats received the test substance via the diet at concentrations of 0.5 or 2.0% for 7 or 28 days. 2-acetylaminofluorene (40 mg/10 ml/kg bw) was used as positive control and administered by gavage once 16 hours before specimen collection. All animals were observed for clinical signs daily, and weighed weekly. At the end of treatment animals were sacrificed. Organs were examined grossly and liver weights were determined. DNA adducts in removed livers were determined by ^{32}P-postlabeling. Chromatography was performed using three solvent systems for analysis of test substance to investigate the existence of unknown DNA adducts.

Results
There were no treatment-related clinical signs or abnormal findings in gross pathology reported for any treatment group. Body weights were significantly different from the control after day 7 of treatment with 2% Kojic acid. In this group slightly lower food consumption was noted. Liver weight, however, was not different from those of controls. In the 2.0% treatment group with 2 of the 3 solvent systems unclear patterns were observed in a part of the autoradiogram. These effects could not be reproduced in a second experiment and therefore it was concluded that they were not derived from DNA adducts. For controls and the 0.5% treatment group no spots of DNA adducts were detected while distinct spots were described for the positive control.

Conclusion
Under the conditions of this study Kojic acid had no potential to form DNA adducts in the liver of rats.

Ref.: 99

Comment
No data on body weights, food consumption or clinical signs are provided with the study.

Study 2

Guideline: /
Species: F344 rats
Group sizes: 20 males/group
Test substance: Kojic acid
Batch: /
Purity: /

Dose levels: 0 or 2% given for 1 (8-OHdG, only) or 2 weeks
Route: Oral via the diet
Vehicle: /
Sacrifice times: At the end of treatment
GLP: /
Date: Before November 2005

The formation of DNA adducts in the thyroids of male rats, subjected to dietary administration of 2% Kojic acid for two weeks, was assessed by ^{32}P-postlabeling analysis. 8-hydroxy-deoxyguanosine (8-OHdG) was measured in the thyroid of male rats exposed to 2% Kojic acid for one or two weeks with a high-performance liquid chromatography system coupled to an electrochemical detector. DNA was extracted from thyroids obtained from the control and the treated groups (20 thyroid lobes per animal from 10 animals per group were combined and two samples were achieved; 8-OHdG: at least 6 lobes from 3 rats were combined as one sample, 5 or 6 samples were analysed).

Results
No spots indicating formation of specific DNA adducts were detected in the thyroids of rats given diet containing 2% Kojic acid for two weeks. Values for the amounts of 8-OHdG tended to be reduced at 1 week after administration of diet containing 2% Kojic acid, and were significantly decreased after 2 weeks as compared to the controls.

Conclusion
Under the conditions of this study Kojic acid had no potential to form DNA adducts including 8-OHdG in the thyroid of rats.

Ref.: AR6

Comment
Data are from a paper from the open literature.

Induction of lacZ mutations

Guideline: /
Species: Muta ^{TM}Mice (CD_2-lacZ80/HazfBRstrain)
Group sizes: 10 males/group
Test substance: Kojic acid in corn oil
Batch: 121K2600
Purity: 99.9%
Dose levels: 800, 1600 mg/kg bw
Route: Oral gavage
Vehicle: Corn oil
Sacrifice times: At the end of treatment
GLP: in compliance
Date: March – September 2002

The ability of Kojic acid to induce gene mutations in the lacZ transgene in liver tissues from Muta T^{M}Mice was evaluated.

Dose range-finding study
Kojic acid was tested at levels of 2000, 1700 and 1400 mg/kg bw and day. Mortality was seen at 2000 mg/kg bw/day in both sexes. Clinical signs of toxicity at all dose levels were flattened posture, ataxia, hypoactivity, recumbency, few faeces and laboured breathing. Body weight loss occurred at all dose levels.

Main study

Animals were dosed orally by gavage for 28 consecutive days and sacrificed 35 days after the initial administration. Doses were on the basis of the results of the dose range-finding study. Only male animals were treated in the main study because no sex differences were observed in the pre-study. Corn oil was used as negative control. Thyroid gland and liver were removed from all surviving animals. Due to technical problems with DNA recovery from thyroid tissue DNA extractions have only been performed from liver samples of treated and control animals. Aliquots of isolated DNA were introduced into competent *E. coli* C lac^- $galE^-$ Kanr ($galE^-$ Ampr) bacteria using a proprietary bacteriophage lambda vector (packaging). Plating was performed for total titre and in the presence of phenylgalactose (P-gal, 0.3% w/v) for $lacZ^-$ mutants. Plates were scored for clear plaques and mutation frequencies were calculated. If possible data were generated for at least 200,000 plaque forming units per liver sample for all animals. Concurrent packaging of positive control DNA confirmed the correct functioning of the packaging reactions on each occasion that mutation frequency data were generated for use in the final analysis.

Results
No mortality occurred in animals dosed with Kojic acid at 800 mg/kg bw/day whereas one animal was found dead on day 6 in the 1600 mg/kg bw/day group. Various signs of clinical toxicity were observed indicating sufficient systemic bioavailability of Kojic acid. At necropsy no gross lesions were noted. Weight losses during the study were reported for animals in the treated groups and in the control group, however, the majority of the animals were gaining weight by the end of the study. No increases in group mean mutant frequencies were observed from DNA extracted from liver samples when compared to those from vehicle treated animals.

Conclusion
Treatment with Kojic acid for 28 days did not result in an increased mutant frequency in the liver of Muta T^MMice and, consequently, Kojic acid is not mutagenic in this in vivo gene mutation test.

Ref.: 94

Comment
Test was performed without positive control. The actual mutant frequency ("summary of results, individual animal and group mean mutation frequency, MF)" is reported as Appendix 11 of the unsigned "Interim Summary Report" issued January 2003. The signed "Final Report" was issued October 2002.

Summary of *in vivo* Mutagenicity / Genotoxicity

Species	Dose	Tissue	Result
Comet assay			
Wistar rats	1000, 2000 mg/kg bw	Liver, stomach and colon	negative
Dominant lethal test			
BDF1 mice	350, 700 mg/kg bw	-	negative
Micronucleus test			
NMRI mice	187.5, 375, 750 mg/kg bw	Bone marrow	negative
ddY mice	125, 250, 500, 1000 mg/kg bw	Bone marrow	Negative [1]
ddY mice	500, 1000 mg/kg bw	liver	Positive after hepatectomy [2]
Fisher rats	500, 1000 mg/kg bw	liver	negative
Unscheduled DNA synthesis			
Wistar HanIbm rats	150, 1500 mg/kg bw	liver	negative
DNA adducts			
F344/DuGrj rats	0.5 or 2% in diet	liver	negative
F344 rats	0.02, 0.2 or 2% in diet	thyroid	negative

Summary of *in vivo* Mutagenicity / Genotoxicity

Species	Dose	Tissue	Result
Induction of LacZ mutations			
Muta ™mice	800, 1600 mg/kg bw	liver	negative

[1] Test has only a confirmatory value
[2] The relevance of this test is unclear

3.3.7. Carcinogenicity

Rats, initiation and promotion assay, liver

Guideline:	/
Species/strain:	F344 rats
Group size:	Experiment 1: 10 males/group Experiment 2: 20 males/group Experiment 3: 25 males/group
Test substance:	Kojic Acid
Batch:	/
Purity:	97.7 to > 99.5%
Dose levels:	0, 0.125, 0.5, 2.0% in diet (all experiments) Average daily intake calculated: 0, 65.6, 261.4, 1013.2 mg/kg bw/day
Exposure period:	20 weeks
GLP:	/

Effects of Kojic acid on the induction of hepatic pre-neoplastic lesions in *N*-bis(2-hydroxypropyl)nitrosamine-initiated (experiment 1) and non-initiated (experiment 2) models, and its promoting influence in a medium-term liver bioassay (experiment 3) were investigated at dietary doses of 0.125, 0.5 or 2% in male F344 rats. In experiment 1, rats received a single sc injection of 2000 mg/kg DHPN for initiation. Starting 1 week after this treatment, rats were fed diet containing 0, 0.125, 0.5, 2.0% Kojic acid for 20 weeks. In experiment 2 rats were fed Kojic acid without initiation. For experiments 1 and 2 body weights were recorded weekly. At the end of experiments rats were sacrificed. Livers were removed and prepared for histopathology. Immunohistochemistry for gluthatione S-transferase P form (GST-P) from 5 or more nucleated hepatocytes in cross section and proliferating cell nuclear antigen (PCNA) from 5000 hepatocellular nuclei of surrounding parenchyma in each animal was conducted. In experiment 3 rats received a single ip injection of 200 mg/kg DEN. Starting 2 weeks thereafter, the animals were then fed Kojic acid containing diet for 6 weeks and subjected to two-thirds partial hepatectomy at week 3. At the end of week 8 animals were sacrificed and subjected to further investigation as in experiments 1 and 2.

Results
In experiment 1, two animals in the highest dose group died because of marked thyroid enlargement. Surviving rats in this group showed a decrease in terminal body weights and an increase in relative liver weights compared to the control. Numbers and areas of GST-P-positive foci increased dose-related. In the 2% Kojic acid group significant increases in numbers (22.3 ± 13.0 vs 8.5 ± 3.4 in the control) and areas (0.37 ± 0.29 vs 0.05 ± 0.03 in the control) of GST-P-positive foci and toxic changes such as vacuolation of hepatocytes and microgranulomas were reported. Single cell necrosis and proliferation of small bile ducts were noted. The development of GST-P-positive foci was pronounced in the animals with hepatocellular toxic changes. Immunohistochemistry for PCNA revealed no apparent overall differences between control and treated groups. In the 2% Kojic acid group PCNA positively varied greatly among animals due to low values in the animals showing steeply depressed

body weights, but PCNA-positive hepatocytes appeared to be localized abundantly in degenerating lesions.

In experiment 2, effects observed were similar to those from experiment 1, but dose-related increases in absolute and relative liver weights without any decrease in terminal body weight was found in the 0.5 and 2% Kojic acid groups. Numbers (0.65 ± 0.57 vs 0.17 ± 0.28 in the control) and areas (0.005 ± 0.005 vs 0.0007 ± 0.0012 in the control) of GST-P-positive foci and hepatocellular proliferating cell nuclear antigen (PCNA) expression (3.8 ± 2.3 vs 2.6 ± 0.7 in the control) were significantly increased by the 2% Kojic acid treatment. The PCNA-positive hepatocytes were abundantly localized around the vacuolated and granulomatous legions.

In experiment 3, dietary administration of Kojic acid led to a significant decrease in body weight gain and an increase in relative liver weight in a dose-related manner. Significant increases in numbers (16.9 ± 3.2 vs 8.4 ± 2.7 in the control) and areas (1.62 ± 0.39 vs 0.77 ± 0.34 in the control) of GST-P-positive foci were observed with 2% Kojic acid.

The authors concluded a tumour-promoting and possible hepatocarcinogenic activity of Kojic acid in the diet at 2% probably due to enhanced replication of hepatocytes related to toxic changes.

Ref.: AR9

Rats, initiation assay, liver

Guideline:	/
Species/strain:	F344 rats
Group size:	15 males/group for tumour initiation test 9 males/group for tumour promoter test
Test substance:	Kojic Acid
Batch:	2Y181
Purity:	100.3%
Dose levels:	0, 0.5, 1 or 2% in diet for tumour initiation test 0 or 2% in diet for tumour promoter test
Exposure period:	4 weeks
GLP:	in compliance

Tumour initiation test
Animals received 0 (group 1), 0.5% (group 2), 1% (group 3) or 2% (group 4) of the test substance or 2-acteaminofluorene as positive control (2-AAF, 0.01% in group 5 and 0.001% in group 6) with the diet for 4 weeks. After the exposure period all animals received the basal diet for 1 week and subsequently a diet containing 0.5% phenobarbital sodium salt (SBP) as promoter for 6 weeks. Six weeks after the beginning of the experiment two-thirds partial hepatectomy was performed in all animals.

Tumour promoter test
Animals received 0 (group 7), or 2% (group 8) of the test substance or 2-AAF as positive control (0.01% in group 9 and 0.001% in group 10) with the diet for 4 weeks. After the exposure period all animals received the basal diet for 7 weeks. Six weeks after the beginning of the experiment two-thirds partial hepatectomy was performed in all animals.

Investigations
Animals were checked twice a day for behaviour, signs of toxicity and mortality. Body weights were measured at the commencement of the experiment and weekly thereafter. Daily food consumption values per rat as well as intake of test substance, 2-AAF and SPB were calculated. At the end of the experiment all surviving animals were killed. Organs were examined macroscopically. Liver weights were recorded. Sections from three liver lobes were stained immunohistochemically for GST-P.

Results
No alterations in survival rate and general conditions related to the test substance were found. Death of 2 rats in group 6, and 1 in group 8 and 9, respectively were related to partial hepatectomy performed before death. Significant decrease of body weight during initiation period was observed with 2.0% Kojic acid (groups 4 and 8). However, return to control levels was apparent during the subsequent SPB or basal diet period. A tendency for decrease in the food consumption values during SPB or basal diet period was observed in line with the increase of body weight change. No differences related to the Kojic acid treatment were found in final body and liver weights, independent of promoter treatment. On liver immunohistochemistry the numbers of GST-P positive foci developing in treated groups with subsequent promoter treatment (groups 2-4) were similar to the control group values and effects of Kojic acid treatment were not observed. There was no development of GST-P positive foci in the Kojic acid group without promoter treatment (group 8). In the groups included as positive controls the development of GST-P positive foci was statistically significant increased with 0.01% 2-AAF only (groups 5 and 9). It was concluded that Kojic acid does not possess initiation potential for the rat liver.

Ref.: 96

Rats, initiation assay, liver

Guideline:	/
Species/strain:	F344 rats
Group size:	Experiment 1: 5 males/group, five weeks old Experiment 2: 8-12 males/group, seven weeks old
Test substance:	Kojic Acid
Batch:	/
Purity:	/
Dose levels:	Experiment 1: 0, 2.0% in diet Experiment 2: 0, 1000, 2000 mg/kg bw in 0.5% carboxymethylcellulose
Exposure period:	Experiment 1: 3, 7, 28 days Experiment 2: single dose
GLP:	/

Experiment 1
Five rats per group were fed a diet containing 0, or 2% Kojic acid for 3, 7, or 28 days. All animals were injected with bromodeoxyuridine (BrdU: 100 mg/kg bw) ip once a day for the final 2 days of the exposure and once on the day of termination 2 hours before sacrifice. At autopsy livers were removed and weighed, slices were prepared for BrdU immunostaining. Labelling indices (LIs) were calculated as percentages of cells positive for BrdU incorporation divided by the total number of cells counted. Additionally, 8-oxodeoxyguanosine (8-OxodG) was measured in nuclear DNA to examine the formation of oxidative DNA adduct and cell proliferating activities of hepatocytes in the liver. Levels were assessed by HPLC-ECD detection.

Results
Body weight gain of the 2% Kojic acid group was significantly decreased on day 28 compared to the control group. Absolute liver weight values were significantly increased on day 7 but decreased on day 28 in rats who received 2% Kojic acid, while relative liver weights were significantly increased at all time points investigated. Only the LI values of hepatocytes of rats exposed to Kojic acid in the diet for 3 and 7 days were significantly increased as compared with the relevant control values. All 8-OxoG levels in the liver DNA of treated rats were slightly higher than the relevant control values, but without statistical significance.

Experiment 2
Male F344 rats were subjected to two-thirds partial hepatectomy on day 0. 12 hours after the completion of surgery animals were treated once orally with the vehicle (0.5% carboxymethylcellulose, group 1, 8 rats), 1000 (group 2, 12 rats) or 2000 mg/kg bw (group 3, 10 rats), respectively, at volumes of 10 ml/kg bw. Subsequently, animals were fed basal diet for 2 weeks and diet containing 0.015% of N-2-acetaminofluorene (2-AAF) for another 2 weeks. Three weeks after Kojic acid administration rats received a single 0.8 ml/kg bw dose of CCl_4. At the end of week five survivors were sacrificed and slices of all liver lobes were stained immunohistochemical for gluthathione S-transferase placental form (GST-P) as marker of preneoplastic foci. The mean area and number of GST-P positive foci per unit area of all liver sections was calculated.

Results
One rat in the control group died during the experiment. On immunohistochemical examination, slight increases were observed in the mean area and the numbers of GST-P positive foci, however, differences were not significant.

Ref.: AR10

Rats, promotion assay, thyroid

Guideline:	/
Species/strain:	F344 rats
Group size:	20 males in group 1, 25 males in groups 2-4
Test substance:	Kojic Acid
Batch:	/
Purity:	/
Dose levels:	0, 2 or 4% Kojic acid
Exposure period:	12 weeks
GLP:	/

Time course changes in thyroid proliferative lesions as well as related hormone levels in the blood of male F344 rats given N-bis(2-hydroxypropyl)nitrosamine (DHPN: 2800 mg/kg body weight, single s.c. injection, diluted in 0.85% NaCl, given to rats in groups 2 to 4) as an initiation treatment followed by pulverized basal diet containing 0% (Group 2), 2% (Group 3) or 4% (Group 4) Kojic acid were examined at weeks 1, 2, 4, 8 and 12. Blood was collected for hormone analysis from 4 to 5 rats and animals were subjected to autopsy and histopathological investigation subsequently. As an untreated control group (Group 1), rats were given basal diet for 13 weeks and examined in the same manner.

Results
One rat died in the DHPN + 4% Kojic acid group at week 8 due to tracheal blockage caused by an extremely hypertrophied thyroid. Body weight gain was reduced significantly from week 1 in DHPN + 4% Kojic acid group and from week 2 in the DHPN + 2% Kojic acid group. While relative liver weights in both Kojic acid treated groups were significantly greater than those in the DHPN-alone group at each time point, absolute liver weights in the DHPN + 4% Kojic acid group were significantly decreased from week 2. The absolute liver weights in the DHPN + 2% Kojic acid group were significantly increased only at week 2 and decreased at week 8. Relative pituitary weights in the DHPN + 2% and the DHPN + 4% Kojic acid groups were significantly increased from weeks 4 and 8, respectively. Absolute and or relative thyroid weights were significantly increased in a treatment period-dependent manner in both DHPN + Kojic acid groups from week 2 to week 12, while relative Serum T_3/T_4 levels in the DHPN + 2% Kojic acid and DHPN + 4% Kojic acid groups were significantly reduced as compared with the DHPN-alone group at each time point. Serum TSH levels in both DHPN + Kojic acid groups were significantly increased at each time point in a treatment period-dependent manner from weeks 1 to 12, and extent of elevation was more remarkable in the DHPN + 4% Kojic acid group. At week 2, there were no statistically

significant intergroup differences in liver T_4-UDP-GT activities on a milligram microsomal protein basis, however, values were slightly higher in the Kojic acid treated groups. Histopathologically, no thyroid proliferative lesions were observed in the untreated control group or the DHPN-alone group. However, diffuse follicular cell hypertrophy and decreases colloid in the thyroid were apparent in all rats of the DHPN + Kojic acid groups at each time point. In addition, focal follicular cell hyperplasias and adenomas of the thyroid were observed at high incidence in the DHPN + 2% Kojic acid group from week 4 and in the DHPN + 4% Kojic acid group from week 8. Multiplicities of focal follicular cell hyperplasias and adenomas of the thyroid in the DHPN + 2% Kojic acid group were significantly greater than those in the DHPN + 4% Kojic acid group at week 8. In the pituitary, an increase in the number of TSH producing cells with expanded cytoplasm was apparent from weeks 4 to 12 in both DHPN + Kojic acid groups. It was concluded that thyroid proliferative lesions were induced by Kojic acid administration due to continuous serum TSH stimulation through the negative feedback mechanism of the pituitary-thyroid axis, resulting from depression of serum T_3 and T_4.

Ref.: 58

Rats, promotion assay, thyroid

Guideline:	/
Species/strain:	F344 rats
Group size:	Experiment 1: 8 males/group 1, 10 males/groups 2-3 Experiment 2: 10 males/group
Test substance:	Kojic Acid
Batch:	/
Purity:	/
Dose levels:	0 or 2% Kojic acid
Exposure period:	Experiment 1: 12 weeks Experiment 2: 20 weeks
GLP:	/

In the order to examine whether Kojic acid exerts a promoting effect on thyroid carcinogenesis, male F344 rats were initiated with *N*-bis(2-hydroxypropyl)nitrosamine (BHP; 2800 mg/kg bw, single s.c. injection) and, starting 1 week later, received pulverized basal diet containing 0 or 2% Kojic acid for 12 weeks. Untreated control rats were given basal diet for 13 weeks. As an additional experiment, two groups without BHP initiation received basal diet or diet containing 2% Kojic acid for 20 weeks. Half of the rats in each group of experiment 1 were killed at week 4 and the remainder after 12 weeks exposure. Half of the rats per group of experiment 2 were killed at weeks 4 and 20. Prior to sacrifice body weights were recorded and blood samples were taken for hormone analysis.

Results
Body weights were decreased in Kojic acid treated animals of experiment 1 at both time points. Absolute and relative thyroid weights were increased in all groups dose- and time-dependently up to 25 fold compared to controls after Kojic acid treatment. Relative liver weights were also increased at each time point in both experiments in rats treated with Kojic acid. Additionally absolute liver weights were significantly increased in experiment 2 after 20 weeks of Kojic acid exposure. Serum T_3 and T_4 levels were significantly decreased (half to one-third of values of the BHP alone group) and serum TSH was markedly increased (13-19 times higher than the values of the BHP-alone group) in the BHP + Kojic acid group at weeks 4 and 12. Similar changes in serum thyroid-related hormones were observed in the group with 2% Kojic acid alone at week 4, but not at week 20. Focal thyroid follicular hyperplasias and adenomas were observed in 4/5 and 3/5 rats in the BHP + Kojic acid group at week 4, respectively. At weeks 12, these lesions were observed in all rats in the BHP + Kojic acid group. Animals of the Kojic acid alone group showed marked diffuse hypertrophy of follicular epithelial cells at weeks 4 and 20. No changes in thyroid-related

hormone levels or thyroid histopathological lesions were observed in either the BHP alone or the untreated control groups. Measurement of liver T_4-uridine diphosphate glucuronosyltransferase (UDP-GT) activity at week 4 revealed no significant intergroup differences. It was concluded that thyroid proliferative lesions were induced by Kojic acid administration 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.

Ref.: 57

Rat, promotion assay, thyroid

Guideline:	/
Species/strain:	F344 rats
Group size:	Experiment 1: 15 males in groups 1, 5, 6, 7 and 10 males in groups 2, 3, 4 Experiment 2: 5 males in groups 1 and 2 10 males in group 3
Test substance:	Kojic Acid
Batch:	/
Purity:	/
Dose levels:	Experiment 1: 0, 0.002, 0.008, 0.03, 0.125, 0.5, 2.0% in diet calculated as 0, 0.1, 4.2, 15.5, 65.6, 261.4 or 1013.2 mg/kg bw/day Experiment 2: 0, 0.5, 2.0% in diet
Exposure period:	20 weeks
GLP:	/

In experiment 1 male F344 rats were initiated with N-bis(2-hydroxypropyl)nitrosamine (DHPN, 2000 mg/kg bw as single sc. injection). One week after initiation animals received basal diet containing 0, 0.002, 0.008, 0.03, 0.125, 0.5 or 2.0% Kojic acid (groups 1 to 7) for 20 weeks. Five rats each in groups 1, 5, 6, and 7 were sacrificed at week 12, and 10 animals each in all groups at week 20. In experiment 2 three groups without DHPN initiation received 0, 0.5 or 2% Kojic acid for 20 weeks. During the experiments, body weight and food consumption in all groups were measured every week. At the end of the experiments blood was collected for hormone analysis. At autopsy thyroids and pituitary weights were recorded. Histopathologically, thyroid proliferative lesions were classified into focal follicular cell hyperplasias, adenomas and carcinomas.

Results
Overall 4 animals diet due to tracheal blockage caused by extremely hyperthrophied thyroids (one in week 12 and two in week 20 in experiment 1 group 7, and one in experiment 2, group 3 in week 20, respectively). There was no significant difference in body weight gain between treated and control groups at weeks 12 and 20, while food consumption in the DHPN + 2% Kojic acid group was slightly but not significantly lower than in the DHNP-alone group. Relative thyroid weights were significantly increased at weeks 12 and 20 in a dose-dependent manner in the DHPN-initiated groups given 0.5% Kojic acid or more. Relative pituitary weights tended to be increased in the DHPN + 2% Kojic acid group. Also in experiment 2 relative thyroid weights were significantly increased in the group given 2% Kojic acid alone, compared to those in the control group. Serum T_4 level were significantly decreased in the DHNP-initiated groups given 0.125% Kojic acid or more at week 12. No significant changes in serum T_3 levels were observed in the groups treated with

DHPN and Kojic acid and a significant increase was evident in the 2% Kojic acid alone group at week 20. Some rats in the highest dose groups (group 7 in experiment 1 and group 3 in experiment 2) showed pronounced elevation of serum TSH at each time investigated. Histopathologically, the incidences of focal thyroid follicular cell hyperplasias in the DHPN-initiated groups treated with 0.125, 0.5 and 2% Kojic acid at week 20 were 5/10, 10/10 and 8/8 rats, respectively. At week 20 adenomas were observed in 7/10 rats in the DHPN + 0.5% Kojic acid group and in 8/8 rats in the DHPN + 2.0% Kojic acid group, while carcinomas were developed in 6/8 rats in the DHNP + 2.0% Kojic acid group. In groups without DHPN initiation, only focal follicular cell hyperplasia was observed in 1/9 rates in the highest dose group.

It was concluded that the NOAEL for thyroid tumour-promoting effect for Kojic acid is 0.03% or 15.5 mg/kg bw/day under experimental conditions chosen and that the substance possesses weak tumourigenic activity in rats due to continuous serum TSH stimulation by a non-genotoxic mechanism.

Ref.: 86

Rats, initiation assay, thyroid,

Guideline: /
Species: F344 rats
Group sizes: 20 males/group
Test substance: Kojic acid
Batch: /
Purity: /
Dose levels: 0, 0.02, 0.2 or 2% in the diet, given for 8 weeks
GLP: /

A two-stage rat thyroid tumorigenesis model was used to investigate possible tumour initiation activity, male F344 rats were given diet containing 0 (group 1), 0.02 (group 2), 0.2 (group 3) or 2% Kojic acid (group 4) for 8 weeks followed by administration of 0.1% sulfadimethoxine (SDM), a thyroid promoter in the drinking water for 23 weeks with a subsequent 13-week recovery period. Rats given N-bis(2-hydroxypropyl)nitrosamine (DHPN, 700 mg/kg bw, 4 times by sc injection, group 5) during the initiation period followed by administration of 0.1% SDM and rats given diet containing 2% Kojic acid for the initial 8 weeks (group 6) or for the entire 31 weeks (group 7) of the experiment or basal diet alone (group 8) were used as controls. During the experiments, body weights in all groups were measured weekly. At the end of the 31-week administration period animals were subjected to blood sampling for hormone analysis. At the end of the experiment animals were sacrificed and necropsied. Thyroids were weighed and fixed. Thyroids of 1 to 9 animals were used for pathological investigations.

Results
Five rats in group 5, 3 in group 7 and one in groups 4 and 6 died of tracheal obstruction due to extremely hypertrophied thyroids during the administration or recovery periods. Tracheal obstruction also deteriorated the general condition of animals. Rats in groups 5 and 7 showed significant inhibition of body weight at the end of administration which persisted until the end of the recovery phase in group 7 (2% Kojic acid for 31 weeks). Absolute and relative thyroid weights of all treated groups (1-7) were significantly higher than those of the untreated control group at the end of administration period. Values decreased at the end of recovery period except for group 5; however, they did not reach the level of untreated animals at the end of administration period. At the end of administration period serum T_3 levels in groups 1, 4, and 5 as well as T_4 levels in all treatment groups except for group 6 were significantly decreased as compared with the untreated control group values at the end of administration period. For group 6 T_3 and T_4 levels were significantly increased compared to untreated controls. TSH levels increased in all treated groups except for group 6. Increases were dose-dependent and dependend on treatment duration in those groups

who received Kojic acid. At the end of recovery period except for group 5 T_3 and T_4 levels still were slightly higher, TSH levels had approximately returned to the normal range in the treatment groups except for group 5. No histopathological lesions were observed in group 8 (untreated control). Carcinomas and adenomas were reported for all animals of group 5 (positive control). No carcinomas and adenomas were observed in the groups treated with Kojic acid except for one adenoma in group 7 (2% Kojic acid for 31 weeks). Number of animals with focal follicular cell hyperplasia was significant higher in groups 4 (4/10), 5 (9/9), and 7 (6/9) at the end of the administration period and in groups 5 (6/6) and 7 (5/8) at the end of the recovery period. Values for mean of total areas of thyroid proliferative lesions per animal as well as values for mean percentages of PCNA positive cells to appr. 150 – 700 follicular cells counted per proliferative lesion were significantly increased in groups 5 (positive control) and 7 (2% Kojic acid for 31 weeks).

It was concluded that Kojic acid has no tumour initiation activity in the thyroid and that earlier observed thyroid tumourigenic activity is attributable to a non-genotoxic mechanism.
Ref.: AR6

Mice, thyroid and liver

Guideline:	/
Species/strain:	(C57BL/6NxC3H/N)F_1 mice
Group size:	65 males or 8 females/group
Test substance:	Kojic Acid
Batch:	/
Purity:	/
Dose levels:	0, 1.5 or 3% in diet
Exposure period:	20 months
GLP:	/

Mice were given 0, 1.5 or 3% Kojic acid containing basal diet *ad libitum* from the age of 6 weeks. Doses were chosen on the basis of preliminary sub chronic test results in the range of 0 to 5%. Animals were observed daily and moribund animals were killed and autopsied along with those animals which died spontaneously. Body weights were noted every month. At 6 and 12 months after the commencement, subgroups of five animals were sacrificed and examined. At month 19 Kojic acid diet was switched to normal diet in 10-14 animals of each treated group. At the end of the 20 month experimental period (week 88), all surviving animals were sacrificed. Organ weights were recorded and histopathological examinations were performed. Additionally free T_3 and TSH were determined at months 6, 12 and 20.

Results
Survival rates at 18 month were 67%, 56%, and 76% in the controls, the 1.5% and the 3% Kojic acid diet groups for males, respectively, and 91%, 87%, and 91% in the controls, the 1.5% and the 3% Kojic acid diet groups for females. Body weights were significantly reduced in all treated groups. Diet consumption, however, did not decrease in mice receiving Kojic acid with the diet. Thyroid weights were increased significantly in all treated animals of both sexes, however, effects were more pronounced in males. Except for the thyroid there were no significant differences among groups in the major organ weights of values for haematological and serum biochemical parameters. Incidences of tumours in the thyroid increased from 2% in the control to 65% and 87% in the treated groups for males and to 8% and 80% in the treated groups for females. Tumours were classified as hyperplasia and follicular adenomas. In all male groups the incidences of hepatomas were high but without any significant intergroup variation. In females incidence in the high dose group was significantly elevated compared to controls. In treated male mice incidences of thyroid adenomas significantly decreased when diet was switched to normal 30 days before termination. Serum free T_3 levels decreased significantly in females of both treatment groups and in males of the high dose group, while TSH levels increased only in females of

the 1.5% treatment group after 6 months and in males of the 3% treatment group after 20 month.

It was concluded that Kojic acid induces thyroid adenomas in male and female B6C3F_1 mice, presumably by a mechanism involving decrease in serum free T_3 levels and increased TSH.
Ref.: 29

Mice, thyroid and liver

Guideline:	/
Species/strain:	p53(+/-) CBA mice P53(+/+) wild-type mice
Group size:	7 - 13 males/group
Test substance:	Kojic Acid
Batch:	/
Purity:	/
Dose levels:	0, 1.5 or 3% in diet
Exposure period:	26 weeks
GLP:	/

Heterozygous p53 –deficient CBA mice in which exon 2 of the lateral p53 allele was inactivated and wild-type littermates were fed basal diet containing 1.5 or 3% Kojic acid or basal diet only (controls). Animals were observed daily for their general condition and weighed once weekly. At the end of the treatment period, surviving animals were sacrificed after blood sampling for hormone analysis. Animals were autopsied. Organ weights were recorded. Organs with macroscopic lesions were sectioned for histopathological examination. Tissue sections were additionally immunohistochemically stained for proliferating cell nuclear antigen (PCNA). 5000 hepatocellular nuclei in normal background parenchyma in each animal were counted for determination of PCNA.

Results
One p53(+/+) male receiving 3% Kojic acid was found dead at week 13. The 3% Kojic acid groups of both p53(+/-) and p53(+/+) mice showed reduction in body weight gain as compared with the control groups, terminal body weights being 8 and 15% lower, respectively with statistical significance. Absolute thyroid weights were significantly increased in a dose related fashion by 209 and 444% in the 1.5 and 3% Kojic acid groups, respectively, in p53(+/-) mice, and by 140 and 374% in p53(+/+) mice. Absolute and relative liver weights in the treated groups showed higher values in both p53(+/-) and p53(+/+) mice than in the respective control groups, but the difference was not significant except for the relative weight in the 3% p53(+/+) mice. Serum T_3 levels were not altered by Kojic acid treatment, but serum T_4 levels declined dose dependently by 35 and 58% in the 1.5 and 3% Kojic acid groups of p53(+/-) mice, respectively, and by 50 and 65% in p53(+/+) mice with statistical significance in all treated groups. Serum TSH level was significantly elevated in the 1.5% group of p53(+/-) mice only. Histopathological examination revealed changes attributable to the Kojic acid treatment in the thyroid and liver. In the thyroid, diffuse hypertrophy and hyperplasia of the follicular epithelial cells accompanied by increase in cytoplasmic colloid-like droplets were observed in all treated p53(+/-) and p53(+/+) mice. There were no benign or malignant neoplasms of the thyroid in any groups. In the liver, hepatocellular adenomas as well as altered hepatocellular foci of eosinophilic cell-, clear cell-, and/or mixed cell-types were observed in the 1.5 and 3% Kojic acid groups of both p53(+/-) and p53(+/+) mice. The incidences of hepatic tumours were significantly increased in both 1.5% and 3% groups of p53(+/-) mice, while those of p53(+/+) mice were significantly increased only in the 3% group. When compared for percent incidences of hepatic proliferative changes, the p53(+/-) mice showed greater prevalence than wild-type mice, and the difference was significant for adenomas in the 1.5% group and altered foci in the 3% group. As nonproliferative lesions in the liver, focal

hepatocellular necrosis and inflammatory cell infiltration appeared to be enhanced in the 1.5 and 3% groups of p53(+/-) and p53(+/+) mice. The animals with necrotic changes in the liver showed elevated PCNA expression in hepatocytes of background parenchyma, and the average of PCNA in the 3% Kojic acid group of p53(+/-) mice was significantly higher than that in the control group. In p53(+/+) mice, effects of the compound were masked by the strong increase in PCNA-positive nuclei in animals showing hepatic necrosis. There were no remarkable findings that could be attributed to the Kojic acid treatment in any of the other tissues and organs examined.

Ref.: AR7

Mice, initiation assay, liver

Guideline:	/
Species/strain:	ICR mice
Group size:	3, 6, 7 or 9 males in groups 1 to 4, respectively
Test substance:	Kojic Acid
Batch:	/
Purity:	100.6%
Dose levels:	0 or 3.0% in diet
Exposure period:	Initiation: 4 weeks Promotion with phenobarbital: 14 weeks
GLP:	/

In order to evaluate the tumour-initiating activity of Kojic acid in mouse liver, an in vivo initiation assay in liver was performed using partially hepatectomized mice. Male ICR mice were fed on a basal diet (BD) containing 0 or 3% Kojic acid for 4 weeks, followed by distilled water (DW) containing 0 or 500 ppm phenobarbital (PB) for 14 weeks. Two weeks after the treatment with PB, two-thirds partial hepatectomy was performed in all mice. At the end of experimental period animals were sacrificed and liver slices were performed in order to evaluate γ-glutamyltransferase positive foci as a marker of preneoplastic foci in the liver as well as proliferating cell nuclear antigen.

Results
No treatment related death was reported and differences in food consumption and body weight changes were reported to be not remarkable. In microscopic examinations, no proliferative lesion was observed in any of the groups. There were slight but not significant differences in the mean numbers of γ-glutamyltransferase-positive cells between the Kojic acid + DW (1.52 ± 1.52) and the Kojic acid + PB (3.89 ± 1.04) groups. Mean values are 1.04 ± 1.04 in the BD + DW group and 3.33 ± 1.65 in the Kojic acid + DW group. In the immunohistochemical analyses of the proliferating activity of hepatocytes, significant increases in the labelling index of proliferating cell nuclear antigen (PCNA) were observed in the BD + PB (6.32 ± 3.32) and Kojic acid + PB (9.57 ± 3.29) groups as compared to the BD + DW group (1.28 ± 1.93). In the kojic acid + DW group the mean value for PCNA was 6.81 ± 5.99. Because no significant difference in the positivity of PCNA was observed between the BD + PB and the Kojic acid + PB groups it was concluded that Kojic acid has no tumour-initiating activity in the liver of mice.

Ref.: AR8

Comment
As PCNA was enhanced for the Kojic acid + DW group compared to the BD + DW group, an effect of Kojic acid on proliferation of liver cells in mice cannot be excluded.

Mice, tumour incidence

Guideline: /

Species/strain: $B_6/C_3/F_1$ mice
Group size: 50/group
Test substance: Kojic Acid
Batch number: /
Purity: /
Dose levels: 0, 0.16, 0.4, 1%
Exposure period: 78 weeks
GLP: /

Male and female $B_6/C_3/F_1$ mice were administered Kojic acid at daily doses of 0, 0.16, 0.4, and 1% for 78 weeks. Doses were fixed according to results of a 7 week preliminary study with doses up to 5%. Observation for clinical signs and death occurred daily. Body weight and food intake were checked once a week during the initial 13 experimental weeks and thereafter once in 4 weeks. At the end of the experiment animals were sacrificed and necropsied. Organ weights were recorded and histopathological investigations were performed.

Results
Although a few intercurrent deaths occurred in both the male and female experimental groups, there was no significant difference between each of the Kojic acid groups and the respective control group; the female and male cumulative survival rates ranging from 92 to 100% in the experimental groups. Gross external examination resulted in a few cases of inflammatory changes of external genitals. Swelling of preputial glands, Harderian gland enlargement, and palpable masses in the femoral subcutis in the in the male experimental groups including the control, but neither gross clinical signs nor external abnormalities attributable to Kojic acid administration were recognized. Body weights were decreased in males from week 3 and in females from week 11 in the highest dose group and additionally significantly reduced in males of the 0.16% group. Food consumption was increased at several time points in all treated groups compared to controls. Significant decreases in absolute organ weights were reported for lungs (males at 1%), adrenals (males at 0.4 %) and salivary glands (males at 0.16%). Increases in absolute organ weights were noted for prostate (0.4%), adrenals (females at 0.16%), and thyroids (dose-depending, for males at 0.4 and 1% and for males at all doses). Relative organ weights differed for kidneys (males at 0.4 and 1%, females at 1%), liver (females at 1%), and heart (males at 0.4% and females at 1%), adrenals (females at 0.16 and 1%), thyroids (males at 0.4 and 1%, females at all doses), pituitary and brain (females at 1%). At necropsy and histopathological examination, tumour changes were observed mainly in the forms of hepatic adenomas and hemangiomas, pulmonary adenomas, malignant lymphomas, leukaemia, or pituitary adenoma as well as non-tumourigenic changes consisting mainly of nodular hyperplasia in the liver, adrenal subcapsular spindle cell hyperplasia, uterus cystic endometrial hyperplasia. However, tumour incidences in Kojic acid treated groups did not significantly differ from controls.

It was concluded that Kojic acid is not tumourigenic to $B_6/C_3/F_1$ mice under conditions tested.

Ref.: 28

Mice, promotion and initiation assay, skin

Guideline: /
Species/strain: CD-1(ICR) mice
Group size: 10 to 15 females/group
Test substance: Kojic Acid in cream formulation
Batch: 2Y051
Purity: 100.3%
Dose levels: 0, 0.3 or 3%

	Dermal exposure
Exposure period:	20 weeks
GLP:	in compliance

In order to examine the initiation and promotion potential of Kojic acid using a mouse medium-term skin carcinogenesis bioassay animals were grouped as follows:

Group	Initiation	Promotion	No. of animals
1	DMBA	vehicle	15
2	DMBA	0.3% Kojic acid	15
3	DMBA	3% Kojic acid	15
4	DMBA	TPA	15
5	Acetone	0.3% Kojic acid	10
6	Acetone	3% Kojic acid	10
7	vehicle	TPA	15
8	3% Kojic acid	TPA	15

Dose levels for positive controls were 100 µg/mouse in 0.1 ml acetone for 9,10-dimethyl-1,2-benzanthracene (DMBA), and 100 µg/mouse in 0.2 ml acetone for phorbol-12-myristate 13-acetate (TPA). Stability, homogeneity and content of Kojic acid in a cream formulation was assessed and found to be in acceptable range of ± 10%. Three days before commencement of the treatment the back skin of the animals was shaved. Shaving was repeated at least every three weeks. Control or test substances were applied to a skin area of about 4 cm^2 to the back skin. For initiation mice in groups 1-6 were treated once at the commencement of the experiment with the initiator (groups 1-4) or with acetone (groups 5, 6). Mice in groups 7 and 8 were applied 50 mg of the test substance daily for one week. For promotion one week after the commencement of the experiment mice in groups 1-3, 5 and 6 received 50 mg of the test substance 5 times weekly for 19 weeks. Groups 4, 7 and 8 were treated with TPA twice weekly for 19 weeks one (group 4) or two (groups 7, 8) weeks after commencement of the experiment. All animals were checked for behaviour, signs of toxicity, and mortality once a day and for skin nodules weekly. Body weights were recorded weekly. After termination of the promoter treatment all surviving animals were killed and examined macroscopically. Histological examination of the back skin was performed. Liver weights were recorded additionally.

Results
Two animals (one in group 7 and one in group 8) died accidentally after treatment. No further mortality was observed. Body weight from week 3 until the end of the experiment as well as absolute and relative liver weights were significantly increased in group 4 (positive control). In groups 2 and 6 body weight significantly decreased in week 2 or in weeks 3 and 4, respectively. One squamous cell papilloma developed in the skin of one mouse of the group receiving 3% Kojic acid after DMBA initiation (group 3). In the positive control (group 4) nodules in the skin were observed from week 7 on; at week 11 all animals had developed at least one nodule, histopathological investigation revealed squamous cell hyperplasia in 14, squamous cell papilloma in 15 and squamous cell carcinoma in one animal.

It was concluded that Kojic acid does not possess initiation and promotion potential for skin carcinogenesis.

Ref.: 97

Comment on carcinogenicity

From the studies available it is concluded that kojic acid is a non-genotoxic carcinogen in rodents. For the thyroid tumour induction a tumour promoting effect based on hormonal disruption is obvious. The goitrogenic (thyrotropic) effect is linked to inhibition of iodine uptake, a subsequent decrease in serum T_3/T_4 levels followed by a compensatory increase in TSH release with the consequence of thyroid cell proliferation. In contrast to rodents an

increase in TSH in humans does not pose a significant concern regarding potential thyroid carcinogenesis in humans. There is convincing evidence that humans are considerably less sensitive than rodents with regard to perturbation of thyroid hormone homeostasis but it is not clear if these species differences are of qualitative or quantitative nature. Despite these comments, a threshold on the basis of hormonal disruption can be assumed.

Ref.: A4, A5

Summary: Carcinogenicity

	Species	Sex	Dose levels	Exposure period	Organ	Initiation	Promotion	Result
1	F344 rats	m	0.125, 0.5, 2.0 % in diet	20 weeks	liver	DHPN: 2000 mg/kg bw	Kojic acid: 20 weeks	GST-P-positive foci
					liver	-	-	GST-P-positive foci
					liver, hepatctotomised	DEN: 200 mg/kg	Kojic acid: 6 weeks	GST-P-positive foci
2	F344 rats	m	0.5, 1 or 2 % in diet	4 weeks	liver, hepatctotomised	Kojic acid	phenobarbital: 6 weeks	negative
3	F344 rats	m	2 % in diet	3, 7 or 28 days	liver	-	-	effect on proliferation possible
			1000, 2000 mg/kg bw	single application	liver, hepatctotomised	Kojic acid	2-AAF: 2 weeks	effect on proliferation possible
4	F344 rats	m	2 or 4 % in diet	12 weeks	thyroid	DHPN: 2800 mg/kg bw	Kojic acid: 12 weeks	thyroid proliferative lesions
5	F344 rats	m	2 % in diet	12 weeks	thyroid	BHP: 2800 mg/kg bw	Kojic acid: 12 weeks	thyroid proliferative lesions, adenomas
				20 weeks	thyroid	-	-	hypertrophy
6	F344 rats	m	0.002 to 2.0 % in diet	20 weeks	thyroid	DHPN: 2000 mg/kg	Kojic acid: 20 weeks	adenomas, carcinomas, NOAEL: 15.5 mg/kg bw
7	F344 rats	m	0.02 to 2% in the diet	8 weeks	thyroid	Kojic acid	0.1% (SDM), 23 weeks	hyperplasia, proliferative lesions
8	F_1 mice	f/m	1.5 or 3 % in diet	20 months	thyroid	-	-	hyperplasia and follicular adenomas (f/m)
					liver	-	-	incidences of hepatomas (f)
9	p53(+/-) CBA mice	m	1.5 or 3 % in diet	26 weeks	thyroid	-	-	hypertrophy and hyperplasia
					liver	-	-	adenomas
10	ICR mice	m	3 % in diet	4 weeks	liver, hepatctotomised	Kojic acid	phenobarbital: 14 weeks	effect on proliferation possible
11	$B_6/C_3/F_1$ mice	f/m	0.16, 0.4, 1 %	78 weeks	all	-	-	negative
12	CD-1 mice	f	0.3 or 3 % in cream formulation	20 weeks	skin	DMBA: 100 µg/mouse	Kojic acid: 19 weeks	negative
						Kojic acid	TPA: 100 µg/mouse , 19 weeks	negative

3.3.8. Reproductive toxicity

3.3.8.1. One generation reproduction toxicity

Study 1

Guideline:	/
Species/strain:	CRL:COBS CD (SD) BR rats
Group size:	20/group
Test substance:	Kojic acid in 1% methylcellulose
Batch:	8312
Purity:	/
Dose levels:	0, 25, 150 or 900 mg/kg bw/day by oral gavage
Treatment period:	males: 9 weeks prior to mating and during mating females: 2 weeks prior to mating, day 1 - 7 of gestation
GLP:	/

Kojic acid was applied in dose levels of 25, 150 or 900 mg/kg bw/day by oral gavage. The control group received the vehicle alone. Dosing of males commenced when they were approximately 7 weeks of age and continued daily throughout the 9-week pre-mating period and the mating period. Sexually mature females were administered the test substance daily through the 2-weeks pre-mating period and up to day 7 of gestation. Animals were observed daily for clinical signs. Body weight, food and water consumption were recorded. On day 20 of pregnancy animals were killed. Females were investigated macroscopically. Litter data as number of corpora lutea, number and distribution of live young as well as foetal deaths, litter weight and foetal abnormalities were recorded. Half the pubs in each litter were investigated for visceral abnormalities. The remainder were prepared for skeletal examination. Testes of males which had failed to inseminate females were weighed and preserved for histological examination.

Results
At 900 mg/kg bw/day treatment was associated with increased activity, slight aggressiveness and increased, brown stained salivation, lethargy, prone posture, lacrimation, dyspnoea, unsteadiness on feet or catalepsy. Males appeared more affected than females. Brown staining of fur was described as well as dark coloration of urine samples from males during week 5. At 150 mg/kg bw/day slightly increased activity and salivation were observed. Mortality of one female at 25 mg/kg bw/day occurred unrelated to treatment. Body weight gain was retarded for both sexes at 900 mg/kg bw/day. In this treatment group food consumption for males during week 9 was significantly lower than among controls. The number of animals successfully mating and median pre-coital time were comparable for all groups. However, alternative comparison showed that at 900 mg/kg bw/day a significantly lower proportion of pregnancies was induced during the first 4 days of mating compared with the controls. A single total litter loss occurred in the highest dose group. The affected animal showed a single early resorption. No conclusive association with treatment was indicated and intergroup comparisons were restricted to dams with viable offspring. Values for corpora lutea, implantations and pre-implantation loss were comparable in controls and the 25 and 150 mg/kg bw/day groups. In the highest dose group decrease in number of corpora lutea per dam combined with an increase in pre-implantation loss revealed a significantly lower number of implantations per litter. Post-implantation loss was comparable for all groups. Mean foetal weights were decreased only in the 900 mg/kg bw/day group, but differences were not significant. No major malformations were observed in any group and incidences of minor visceral and skeletal anomalies were essentially comparable for all groups. Intergroup differences in the incidence of skeletal variants were not statistically significant.

Ref.: 40

Comment

Sialodacryoadenitis infection was observed among males and females in all groups starting during their acclimatisation periods. A NOAEL of 150 mg/kg bw/day for parental- and embryotoxicity can be derived from this study.

Study 2

Guideline:	/
Species/strain:	Sprague Dawley rats
Group size:	7 males for control, 8 males for treatment groups
Test substance:	Kojic acid
Batch:	/
Purity:	/
Dose levels:	50 µg/day in propylene glycol
Treatment period:	21 days
GLP:	/

Male rats (150 – 200 g bw) of proven fertility were orally administered a suspension of Kojic acid in propylene glycol at a dose of 50 µg/rat/day for 21 days. The control group received propylene glycol alone. Fertility performance of the individual rat was studied from day 16 to day 21 of treatment. Each male was caged separately with two females of proven fertility. Presence of sperms in the vaginal smear indicated that the females had mated to the particular male and day of mating was taken to be day 1 of pregnancy. Laparotomy was performed on day 8 of pregnancy, to examine and record corpora lutea and implantation sites. Litter content was examined and litter size recorded at term. Teratogenic effects and deaths as well as postnatal behaviour of the dams were recorded. Male rats were sacrificed on day 22 and tissues were collected and weighed. Sections of testis were performed for histological examination. Fructose content in coagulating gland and acid phosphatase activity in ventral prostate were estimated. Spermatozoa collected from caput, corpus, cauda epididymis and vas were examined microscopically and their number, morphology and mortality were recorded.

Results
Kojic acid significantly reduced body weight in males and females as well as weights of testis and epididymis in males. Fructose content of coagulating gland and acid phosphatase activity in ventral prostate were not affected by Kojic acid. There were no effects of Kojic acid on spermatogenesis or sperm parameter. 6/7 (control group) or 6/8 (Kojic acid treated group) males succeeded in mating and altogether 8 females were mated in both groups, respectively. Implantation and litter sizes were reduced in the treated group. Loss of viability among the litter on second or third day post-delivery and cannibalistic behaviour of dams were also noted.

Ref.: 78

Study 3

Guideline:	/
Species/strain:	ddy-SLC mice
Group size:	35/group
Test substance:	Kojic acid in 1% methylcellulose
Batch:	8823
Purity:	/
Dose levels:	0, 25, 150 or 900 mg/kg bw/day by gavage (1ml/kg bw)
Treatment period:	days 6 – 15 of gestation
GLP:	/

Kojic acid was orally administered to ddy-SLC strain mice by gavage for 10 days from days 6 to 15 of gestation and effects on pregnancy, development of foetuses and live born offspring were studied. Caesarean section was performed on 2/3 of the pregnant mice of each group on day 18 of gestation to observe toxicity and teratogenicity in the foetuses, and the other 1/3 of mice were allowed to deliver naturally to observe the second generation mice. Four male and female newborns per litter were chosen on day 4 after birth and 2 male and female offspring per litter were chosen at weaning to observe their growth and reproduction ability. The other weanlings were used for skeletal examination.

Results
During gestation period, only animals of the 900 mg/kg bw/day group exhibited calmness and ataxia and in some cases coma and dyspnea. No relevant changes were observed in body weight, food consumption, water intake, course of gestation findings in delivery and lactation in the groups treated with Kojic acid. Body weight changes of pregnant dams in the 25 mg/kg bw/day group significantly surpassed those of the controls during the treatment period and body weight gain during gestation was also increased significantly. With regard to organ weights a decrease for heart weight was observed in dams of the 900 mg/kg bw/day group. No relevant effects of treatment were observed in numbers of corpus luteum verum, implantations, living foetuses, resorbed and dead embryos, or in the survival rate of foetuses, weight of placenta and sex ratio in foetuses of the treated groups, however, in the 900 mg/kg bw/day group a significant decrease in body weights of male foetuses was observed. In the high dose group the incidence of minor changes and anomalies in the viscera was increased. Hypoplasia of lung and heart was observed in 0 (control), 5.1% (25 mg/kg bw/day), 4.8% (150 mg/kg bw/day) and 7.6% (900 mg/kg bw/day) of foetuses. A significant retardation of ossification was also observed in the highest dose group and a significant, dose-depending decrease in number of foetuses with ossified calcaneus in the 150 and 900 mg/kg bw/day groups, while animals with retarded ossification of occipital bone and number of cervical ribs were declined in the lowest dose group. For weanlings no skeletal differences were observed in treated groups. Body weight for F_1 offspring was increased at birth in the 25 mg/kg bw/day group. Three week old F_1 mice revealed significantly increased kidney weights in both sexes at 900 mg/kg bw/day. Motor reflex function, muscular strength, equilibrium response on an inclined plane and learning ability test by water T-maze were not affected by Kojic acid in reared offspring, while for motor function test by using a rota-rod and emotional test in open-field changes in animals of the treated groups compared to control were noted, however these changes were not dose-depending. In F_1 dams heart weight was reduced significantly on day 18 of pregnancy in the highest dose group and in 13-week old males adrenal prostata gland weights were decreased in the 900 and the 25 mg/kg bw/day group, respectively. No effect considered to be due to treatment was observed in the reared offspring concerning time point of descending of the testes and opening of the vagina. No effects were noted on the sexual cycle, copulation rate, conception rate and foetal finding (F2) in the reproduction ability test of F_1 (each one male and female from a dam) carried out in the 11^{th} week after delivery.

Conclusion
A NOAEL for maternal toxicity and for embryotoxicity of 150 mg/kg bw/day can be derived from this study.

Ref.: A1

Study 4

Guideline:	/
Species/strain:	ddy-SLC mice
Group size:	35/group
Test substance:	Kojic acid in 1% methylcellulose
Batch:	1527R

Purity: /
Dose levels: 0, 30, 160 or 800 mg/kg bw/day by gavage (1ml/100 g bw)
Treatment period: days 15 of gestation - 21 post partum
GLP: /

Kojic acid was orally administered at doses of 0, 30, 160 or 800 mg/kg to SLC: ddY strain mice once daily from day 15 of pregnancy to day 21 postpartum to assess the effect of treatment on dams and F_1 offspring. Spontaneous parturition was allowed for all the dams and the second generation was subjected to postnatal observations. The number of the offspring was adjusted to four males and four females per litter on day 4 post partum. At weaning, 2 males and 2 females per litter were selected for analysis of their growth and reproductive ability. The other remaining weanlings were subjected to skeletal examination.

Results
Dams (F0) that had been treated with 800 mg/kg/day showed signs of calmness and ventral posture. Convulsions were also observed in some of these animals. A significant decrease in food consumption and water intake was observed at the terminal stage of gestation. In addition, a significant decrease in body weight was observed at this stage, and a significant reduction in body weight gain during the lactation period was observed in this dosage group as well. The length of gestation was also significantly prolonged, however, no anomalies were observed in the lactation behaviour of this group. No significant adverse effects were noted in the dams in the 30 mg/kg/day treatment group. Significant decreases in the absolute and relative organ weights were observed for the kidney at 160 mg/kg bw/day, thymus at 800 mg/kg bw/day and liver at 160 and 800 mg/kg bw/day. In the highest dose group absolute spleen weight was reduced additionally.

At birth the number of live female newborns and total number of live newborns from dams in the 800 mg/kg/day treatment group were significantly lower than the control values. For one dam all offspring were stillborn on day 21 of pregnancy. No further significant differences from control values were noted in the numbers of implantation sites, total newborns, perinatal mortality, live male newborns, sex ratio or body weight of life newborns at any dosage. A significant inhibition of body weight gain was observed in female weanlings of the dams given 800 mg/kg/day. In three week old F_1 offspring relative organ weights were decreased for liver (160 and 800 mg/kg bw/day groups), brain, kidney and adrenals (160 mg/kg bw/day group), and testis (30 mg/kg bw/day group). Skeletal observation of weanlings (F_1) revealed no effect of treatment on the rate of ossification or on the incidence of variations or malformations. No anomalies were observed in the reflex function test, auditory examination, muscular strength test, equilibrium response test, motor function test using a rota-rod, open-field emotional test, or the water T-maze learning ability test in the offspring (F_1), however some significant changes were noted for females of the highest dose group in the open-field behaviour test and the water T-maze learning ability test. No effect on developmental stages as assessed by the age (days) of ear detachment, emergence of abdominal hair or opening of eyelids was observed in any of the dosage groups. Vaginal opening was delayed at 30 and 160 mg/kg bw/day, incisor eruption was retarded significantly and dose-dependent at 160 and 800 mg/kg bw/day. No differences were observed in the age (days) at descent of testes and the oestrous cycle. Offspring copulation index and fertility index of the offspring in the reproduction study performed at week 11 after birth (1 male and 1 female F_1 mouse per litter) were unaffected in any of the treatment groups. Changes were a smaller number of live male foetuses at 30 mg/kg bw/day and a significant and dose-dependent higher placental weight at 160 and 800 mg/kg bw/day. At 800 mg/kg bw/day F_1 dams showed significantly decreased body weights, thymus and liver weights. No changes were observed for F2 foetuses.

Ref.: 41

Comment
A NOAEL of 30 mg/kg bw/day for maternal toxicity and for embryotoxicity can be derived from this study.

3.3.8.2. Embryotoxicity/Teratogenicity

Study 1

Guideline:	/
Species/strain:	New Zealand White rabbit
Group size:	13 females/group
Test substance:	Kojic acid in 1% methylcellulose)
Batch:	8320 (preliminary study: 8208)
Purity:	/
Dose levels:	0, 20, 100 or 500 mg/kg bw/day by gavage at a volume of 2 ml/kg bw
Treatment period:	days 6 – 18 post-coitum
GLP:	/

Dosages of 0, 20, 100 or 500 mg/kg bw/day were administered to female rabbits by intragastric intubation during days 6 to 18 of gestation. Animals were killed on day 29 of pregnancy, litter values were determined and foetuses were examined for abnormalities. Dose levels based on a pilot and preliminary toxicity study in which treatment at 750 mg/kg bw/day was associated with initial bodyweight loss and marked post-dosing reactions like tachypnoea, mydriasis, lethargy and catalepsy.

Results
At 500 mg/kg bw/day post-dosing reactions similar to those recorded in the preliminary study at 750 mg/kg bw/day were mainly evident after day 12 of gestation (7th dose), catalepsy was not observed. At 20 and 100 mg/kg bw/day sporadic post-dosing reactions were observed only in occasional animals. One animal at 100 and two animals at 500 mg/kg bw/day were killed following evidence of enteric disorder. This was not considered to be related to treatment. At 500 mg/kg bw/day bodyweight gain was marginally lower than that of controls throughout. In the other dose groups weight gains were not conclusively different from those of controls. The numbers of pregnant animals per group and preimplantation losses were essentially comparable for all groups. Single total litter losses occurring among controls, at 20 and at 100 mg/kg bw/day were considered to be unrelated to treatment and intergroup comparisons were restricted to dams with viable young. There were no treatment related intergroup differences in litter size, post-implantation loss, litter and mean foetal weights reported. Major malformations observed included one heart defect in the 20 mg/kg bw/day group and three effects from two litters in the 100 mg/kg bw/day group. Effects were considered to be unrelated to treatment as in the highest dose group no major malformations were observed. Minor anomalies were significantly increased in the highest dose group, however, effects were considered not to be related to Kojic acid.

Ref.: 38

Comment
The SCCP considers the minor anomalies observed in the highest dose group of relevance with respect of Kojic acid treatment. A NOAEL of 100 mg/kg bw/day can be derived for maternal toxicity and for embryotoxicity from this study.

Study 2

Guideline:	ICH, 1993
Species/strain:	Wistar rats
Group size:	6 females/group
Test substance:	Kojic acid (53758) in 0.5% methylcellulose
Batch:	8A44
Content:	98 – 102%

Dose levels: 0, 100, 300, 1000 mg/kg bw/day by gavage
Treatment period: days 6 – 17 post-coitum
GLP: in compliance

Three groups of mated female Wistar rats (221 – 283 g) received the test substance by oral gavage at 100, 300 or 1000 mg/kg bw daily from day 6 to 17 post-coitum. In addition a group of six mated females was given the vehicle alone and acted as control group. Clinical signs and mortality were checked daily. Food consumption and body weight were recorded at designated intervals during pregnancy. On day 20 of pregnancy, females were sacrificed. Foetuses were removed by hysterectomy and females were examined macroscopically. Litter parameters as number of corpora lutea, implantation sites, early and late resorptions, or dead and live foetuses were recorded. Foetuses were weighed, sexed and submitted to an external examination.

Results
There were no death in any group and no clinical signs were observed in any female. No abortions or total resorptions occurred. Body weight in treated females was reduced at 300 and 1000 mg/kg bw/day. Food consumption was reduced in these dose groups at the end of the treatment period, however, changes were not related to the test substance by the authors. No relevant macroscopic findings were recorded at necropsy of the females from any group. The number of corpora lutea and implantation sites were similar in the 0, 100, and 1000 mg/kg bw/day groups. In the 300 mg/kg bw/day group the number of implantation sites was lower than that of the controls (8.8 per female versus 12.2) resulting in a significantly higher pre-implantation loss (24.3 versus 0%). This finding was not considered to be related to the administration of the test substance as effect was not dose-depending. The number of foetuses per female was reduced at 300 and 1000 mg/kg bw/day compared to the control group but values were not significant (8.5 and 10.8 versus 12.2, respectively). No post-implantation loss occurred in any group. The test substance did not influence body weight or sex ration of foetuses. No malformations or anomalies were observed. It was concluded that the NOAEL for maternal toxicity, embryo- and foetotoxicity is 100 mg/kg bw/day under the experimental conditions chosen.

Ref.: 20

Comment
Only six females per group were investigated.

Study 3

Guideline: /
Species/strain: Sprague Dawley rats
Group size: 7 females/group
Test substance: Kojic acid
Batch: /
Purity: /
Dose levels: 50 µg/day in 0.1 ml propylene glycol
Treatment period: day 1 - 5 of gestation
GLP: /

The test substance was given orally to mated females in 0.1 ml propylene glycol daily at a concentration of 50 µg/rat for days 1 to 5 of pregnancy. Animals of the control group received the vehicle alone. On day 8 of pregnancy females were laparotomized to examine corpora lutea and implantation sites. Litter size was recorded at term. Newborns were checked for teratogenic effects and behaviour of dams was observed after delivery.

Results

One female of the treated group died before delivery, 2 animals showed nasal and mouth infections. Significant loss in litter size was observed in females treated with Kojic acid. Furthermore reduction in implantation sites as well as loss of viability among the litter 2 to 3 days after littering was reported. No teratogenic effects could be observed but mortality of litter was increased significantly. Cannibalistic behaviour was reported from day 2 after delivery on for females treated with Kojic acid. It was concluded that Kojic acid possesses anti-implantation, abortifacient and embryotoxic effects.

Ref.: 35

Comment
This study is merely briefly described and is of limited value.

3.3.9. Toxicokinetics

Guideline:	/
Species:	JCL-Wistar rats
Group sizes:	3 males/group 2 pregnant females/group transfer to breast milk: 3 females
Test substance:	^{14}C-Kojic acid
Batch:	/
Purity:	99.9%
Dose levels:	10 µCi/100 g bw (100 mg/kg bw) for all groups and administration routes (oral, sc, dermal, single application) 10 µCi/100 g bw (100 mg/kg bw/day) for 7 days subcutaneously
GLP:	/

^{14}C-Kojic acid was biosynthesized by adding ^{14}C-U-glucose into a cultured broth of *Aspergillus* SP-No.6-MA-181 strain. Kojic acid was extracted with hot acetic ethyl ester after 3 days, re-crystallized and dried subsequently. Purity of radioactivity was confirmed by spotting ^{14}C-Kojic acid on a thin layer plate, with 99.9% purity obtained. Treatment of animals was performed by oral (gavage), sc or dermal application of the administration solution of 10 µCi/0.1 ml at doses of 10 µCi/100 g bw (100 mg ^{14}C-Kojic acid/kg bw). For dermal application a vinyl chloride sheet (30 x 30 x 2 mm) was adhered to the dorso-lumbar area one day before administration. Test substance was applied to the sheet and dried. A plastic cover was adhered to the sheet. Blood concentrations were determined as specific activity (dpm/ml) 0.5, 1, 3, 6, 24 and 48 hours after oral, sc or dermal administration, respectively. Urine and faeces were sampled in a metabolic cage. From male rats bile was collected for the duration of 0 – 10 min, 30 min – 1 h, 1 – 3 h, 3 – 6 h, 6 – 24 h. Additionally, an operation as a test for enterohepatic circulation was performed were bile of one treated rat flow into the duodenum of a second rat. Collection of bile was performed from the second rat. Transport of test substance to breast milk was examined by investigation of the stomach content of 3 day old lactating rats from females treated subcutaneously. Organs and tissues of two animals/group were examined for radioactivity 0.5, 1, 3, 6, 24 and 48 hours after administration. For investigation of sex differences distribution of radioactivity to tissues, organs and foetuses in females (2/group) orally treated on day 11 or 20 of pregnancy with the test substance was examined for radioactivity 10, and 30 minutes as well as 3 hours after administration. Macro-autoradiography was performed additionally. Thin layer chromatography was performed of urine obtained within 24 hours as well as liver and foetus samples obtained 3 hours after dermal (5.08 µCi/3.3 mg Kojic acid/330 mg applied cream/100 g body weight) or sc. (low dose: 5.18 µCi/3.3 mg Kojic acid/0.1 ml/100 g body weight; high dose: 51.8 µCi/3.3 mg Kojic acid/0.1 ml/100 g body weight) administration from females on day 17 and 18 of pregnancy. Additionally bile samples were collected from treated non-pregnant females.

Results

Single administration in males
Oral exposure
Test substance was transported to the intestine within 3 hours and to the cecum within 6 hours after administration. Distribution of radioactivity in tissues and organs was very rapid and maximum values were reached within 30 minutes after administration. Very high radioactivity was measure in liver, kidney and pancreas, high radioactivity in lungs, heart and spleen. Maximum values for radioactivity in blood samples were measured 0.5 and 1 hours after application ($4.58 \times 10^4 \pm 1.05 \times 10^4$ dpm/ml and $5.56 \times 10^4 \pm 1.01 \times 10^4$ dpm/ml corresponding to 20.63% and 25.05% of radioactivity, respectively, AUC_{0-6} = 71.8 µg eq/ml x h). Radioactivity decreased to background level within 24 hours. Test substance measured in bile samples amounted to approximately 0.5 µCi/10µCi within 24 hours. No radioactivity was measured in the samples from the test for enterohepatic circulation. The total amount excreted with the urine within 48 hours was about 70% of the administered dose. Main urinary excretion occurred in the first 6 hours after application. Faecal excretion was negligible, 0.82% of the administered dose was excreted with faeces within 48 hours. Main excretion occurred 3 to 24 hours after application.

Subcutaneous exposure
Distribution of radioactivity in tissues and organs was slightly slower than after oral administration. High radioactivity was measured in the kidney and the liver after 0.5 and 1 hour and between 0.5 and 6 hours in the digestive tract. Maximum values for radioactivity in blood samples were measured 0.5 and 1 hours after application ($2.95 \times 10^4 \pm 5.58 \times 10^3$ dpm/ml and $4.81 \times 10^4 \pm 6.11 \times 10^2$ dpm/ml corresponding to 13.29% and 21.67% of radioactivity, respectively, AUC_{0-6} = 50.2 µg eq/ml x h). Radioactivity decreased to background level within 48 hours. Test substance measured in bile samples amounted to approximately 0.76 µCi/10µCi within 24 hours and was slightly higher than for the oral and dermal exposure routes. No radioactivity was measured in the samples from test for enterohepatic circulation. The total amount excreted with the urine within 48 hours was about 50% of the administered dose. Main urinary excretion occurred in the first 6 hours after application. Faecal excretion was negligible, 2.62% of the administered dose was excreted with faeces within 48 hours. Main excretion occurred 3 to 24 hours after application.
A recovery test of $^{14}CO_2$ in expired air after single subcutaneous administration was conducted in males. The total recovery rate was 1.4% of the administered dose within 5 hours.

Dermal exposure
Distribution of radioactivity in tissues and organs was slower and concentrations were lower after dermal application. No remarkable high radioactivity was detected in the liver. Highest values observed 30 minutes after application were seen in the pancreas. In the kidney radioactivity was highest 1 to 6 hours after application. For the same time period radioactivity increased in the digestive tract. Maximum values for radioactivity in blood samples were measured 0.5 hours after application ($11.1 \times 10^4 \pm 1.0. \times 10^4$ dpm/ml corresponding to 5% of radioactivity AUC_{0-6} = 18.3 µg eq/ml x h). Radioactivity decreased to background level within 24 hours. Test substance measured in bile samples amounted to approximately 0.5 µCi/10µCi within 24 hours. No radioactivity was measured in the samples from test for enterohepatic circulation. The total amount excreted with the urine within 48 hours was about 56% of the administered dose. Main urinary excretion occurred in the first 24 hours after application. Faecal excretion was negligible. 1.58% of the administered dose was excreted with faeces within 48 hours. Main excretion occurred 3 to 24 hours after application.

Single administration in females
Pregnant females, subcutaneous exposure
Radioactivity was distributed rapidly in tissues and organs and was measured 10 minutes after application. There were no differences in tissues and organ contents in both groups investigated (day 11 and day 20 of pregnancy). Very high values were observed in the

kidney (10 and 30 minutes after application) and high values in liver, pancreas, spleen and salivary gland 10 and 30 minutes after application as well as in lungs (30 minutes after application) and kidney (3 hours after application). Radioactivity measured in the foetus was very high 30 minutes after application. Values decreased in the 11th day of pregnancy group but persisted to be very high in the 20th day of pregnancy group. High values were also measured in both treatment groups in the uterus, the ovary and the placenta 30 minutes after application. In the 20th day of pregnancy group also high values were measured in the amniotic fluid.

Nursing dams, subcutaneous exposure
Radioactivity in the stomach wall and stomach content of lactating rats was measured 0.5, 1, and 3 hours after application of the test substance. Less than 1% of the applied dose was found after 3 hours of application.

Repeated dose in males
Subcutaneous exposure
Radioactivity in blood and urine samples increased until the 4th administration and showed a tendency to reach equilibrium which was almost 3 times higher than values 24 hours after the first application of the test substance. Distribution of test substance was measured 10 minutes, 1, 6, 24, and 48 hours after the last administration. Radioactivity was partly several times higher in all organs and tissues after repeated dose administration than in the comparable group after single administration especially in the intestinal tract in the 1 hour group, in the pancreas and in adipose tissue.

Isolation of 14C-labeled metabolites
In the urine and in the liver Kojic acid was detected. Metabolites in all organs or tissues detected were glucuronate (6.4 – 39.6% of total radioactivity) and mainly sulphate (35.6 – 93.7% of total radioactivity) conjugates of Kojic acid.

Ref.: 42, AR11

Comment
The ratio for oral / dermal AUC values is 4.

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

Study 1

Guideline:	/
Species/strain:	Hartley/Dunkin guinea pigs
Group size:	10 males
Test substance:	Kojic acid
Batch:	/
Purity:	/
Concentration:	Induction: 5% test substance (w/v) in absolute alcohol, 0.2 ml Challenge: 1% test substance (w/v) in absolute alcohol, 0.2 ml
GLP:	/

Test material was applied to the shaven dorsal nick region of ten guinea-pigs, daily for five consecutive days. A similar mid-dorsal site was treated daily with absolute alcohol (0.2 ml). After each induction guinea-pigs were irradiated with UV-light (light source comprising five 18" long Blacklite tubes – 15 watt/tube -) located twelve inches away from the skin for 15 minutes. Animals were observed for the presence of erythema. After a rest period of 10 days a challenge application was made to the pre-treated site in the dorsal nick region and absolute alcohol to the middorsal site. The sites were then exposed to UV-irradiation for 15 min and assessed for the presence of erythema after 0, 24, 48 and 72 hours.

Results
No dermal reactions were observed at the control sites during induction period, while slight erythema were recorded for eight animals at the third, fourth, and fifth induction exposure in the treated group. Following challenge no dermal reactions were observed.

Conclusion
Under the test conditions reported Kojic acid was not photosensitising. The SCCP considers Kojic acid to be slightly photoirritant.

Ref.: 15

Study 2

Guideline:	/
Species/strain:	albino guinea-pigs, Hartley/Dunkin
Group size:	10 males
Test substance:	Kojic acid
Batch:	/
Purity:	/
Concentration:	5% test substance (w/v) in absolute alcohol, 0.5 ml
GLP:	/

Test substance was applied to a patch of Whatman paper. Two patches were placed on the abraded skin of animals. One site (site 1) was covered by aluminium foil to protect the skin from UV-light. Guinea-pigs then were irradiated with UV-light (light source comprising five 18" long Blacklite tubes – 15 watt/tube -) located six inches away from the skin for 30 minutes. Procedure was repeated daily for five consecutive days. Both application sites were assessed for the presence of oedema and erythema prior to re-exposure on each day.

Results
On site 1 (treated and occluded) no dermal reactions were observed before or after UV-exposure in any of the animals tested. On site 2 (treated and unoccluded) slight erythema were seen in 3/10 animals on isolated occasions. One animal developed a slight erythema which persisted over two days.

Conclusion
Under the test conditions reported Kojic acid was reported to be not or slightly photoirritant.

Ref. 14

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

Photomutagenicity

Guidelines:	OECD 471, 1997
Species/strain:	*E. coli* WP2 (Trp^+)
Replicates:	Triplicates, two independent tests
Test substance:	Kojic acid
Solvent:	DMSO
Batch:	8 A 44
Purity:	100%
Concentrations:	0, 33, 100, 333, 1000, 2500, 5000 µg/plate, with and without metabolic activation
Treatment:	plate incorporation test
GLP:	in compliance
Date:	May – August 1998

The assay was performed with *E. coli* WP2 (Trp^+) in two independent experiments. Each concentration was tested in triplicate. Kojic acid was dissolved in DMSO. No precipitation occurred up to the highest dose investigated. Test concentrations of Kojic acid were based on the results of a pre-experiment for toxicity with the strain WP2. Toxicity was evaluated on the basis of reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn. Irradiation was performed using a metal halogenide light source which emits a spectrum simulating sunlight. The doses were determined using a UV-meter with two individual detectors, one for UV-A and one for UV-B light. The optimal UV dose was established in a separate pre-experiment. Bacteria were exposed to different doses of UV light using different exposure times. Following irradiation bacteria are plated on selective medium. After incubation revertant colonies are counted to measure both, photomutagenicity and phototoxicity of the irradiation. An UV dose was chosen that increased the number of revertant colonies to approximately twice the number of revertants without irradiation (10 seconds of irradiation, approximately 10 mJ/cm^2 UVA and approx. 0.5 mJ/cm^2 UVB). 8-Methoxypsoralen served as positive and cultures treated with solvents as negative controls.

Results
After treatment and irradiation a significant increase in revertant colony numbers was observed at 2500 µg/plate in experiment 1 and at 2500 and 5000 µg/plate in experiment 2. However, irradiation did not further increase the number of revertant colonies above the level of the corresponding treated but not irradiated controls.

Conclusion
Within the scope of this assay and under the conditions used in this study irradiation with artificial sunlight has no relevant influence on the mutagenic potential of Kojic acid in this photomutagenicity assay with *E. coli* WP2

Ref.: 27

3.3.11. Human data

See 3.3.3

3.3.12. Special investigations

The overall biological effects of Kojic acid in the gene expression profiling of human skin A375 malignant melanoma cells were examined. Cells were either cultured alone or in the presence of Kojic acid at concentrations of 0.32, 1.6, 8, 40, 200 or 1000 µg/ml for 72 hours. MTT was used to assess viability of cells following treatment. Total RNA was quantified in cells exposed to 8 µg/ml Kojic acid for 24 hours. RNA was amplified and gene expression analysis was performed on microarrays.

Results
Cell growth was inhibited dose-dependently by Kojic acid by 40% (highest concentration) or 20% (0.32 – 40 µg/ml). A total of 361 differentially expressed genes were distinctively changed with 136 up-regulated and 225 down-regulated genes. Seven of the down-regulated genes were identified as tumour suppressor genes in melanoma cancer cells.

Ref.: AR5

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

For calculation of MoS, an NOAEL of 6 mg/kg bw/day is used, based on histopathological findings and altered iodine uptake after oral administration in rats (4-week treatment, ref. 41).

(Skin care formulation, 1% Kojic acid)

Face

Maximum absorption through the skin	**A (µg/cm²) µg/cm²**	**=**	**7.28**
Skin Area surface	**SAS (cm²)**	**=**	**565 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**4.11 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60 mg/kg**	**=**	**0.069**
No observed adverse effect level (mg/kg) (28-days, rat, oral)	**NOAEL**	**=**	**6 mg/kg**
Margin of Safety	**NOAEL / SED**	**=**	**88**

Hands

Maximum absorption through the skin	**A (µg/cm²) µg/cm²**	**=**	**7.28**
Skin Area surface	**SAS (cm²)**	**=**	**860 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**6.28 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60 mg/kg**	**=**	**0.104**
No observed adverse effect level (mg/kg) (28-days, rat, oral)	**NOAEL**	**=**	**6 mg/kg**
Margin of Safety	**NOAEL / SED**	**=**	**58**

Face and hands

Maximum absorption through the skin	**A (µg/cm²) µg/cm²**	**=**	**7.28**
Skin Area surface	**SAS (cm²)**	**=**	**1425 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**10.37 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60 mg/kg**	**=**	**0.173**
No observed adverse effect level (mg/kg) (28-days, rat, oral)	**NOAEL**	**=**	**6 mg/kg**
Margin of Safety	**NOAEL / SED**	**=**	**35**

It has to be considered, that consumers who want to bleach their skin not only apply the products on hands and face only, but also on at least parts of the arms as well as on neck and décolleté.

Kojic acid is a fungal metabolite commonly produced by many species of *Aspergillus*, *Acetobacter* and *Penicillium*. *Aspergillus flavus* is used in the production of a number of foods, including soy bean paste (miso), shoyu (soy sauce) or sake which are produced throughout the world. An additional exposure via food can be assumed at least for consumers who feed on Asian food regularly.

3.3.**14. Discussion**

Physico-chemical specification
According to the submission, Kojic acid is used as a skin whitening agent at a concentration of 1% in leave-on creams, which are generally applied to the face. But it can also be used on the hands and forearms. However, the SCCP is aware of products on the market containing Kojic acid at concentrations higher than 1%.

No data was provided on the stability of Kojic acid in the test solutions and in the marketed products. The test batches were not identified in many cases and purity of the test substance was often not reported.

General toxicity
Acute toxicity of Kojic acid is low. Mean LD_{50} values for oral administration are 1800 or > 2000 mg/kg bw for rats and 5100 mg/kg bw for mice, 2600 or 2700 mg/kg bw after subcutaneous application in rats or mice, respectively and > 2000 mg/kg bw for rats after dermal exposure. For intraperitoneal administration the mean LD_{50} is 2400 mg/kg bw for rats and 2600 mg/kg bw for mice. After repeated doses of Kojic acid in rodents the main target organs affected are the thyroid and the pituitary gland as well as the liver. An increase in thyroid weight was shown at doses above 0.125% Kojic acid in the diet given to male rats for 28 consecutive days. Reduced serum level of T_3 and T_4 were reported in several studies. TSH levels increased in a dose- and time-dependent manner after Kojic acid administration. In male rats the uptakes of ^{125}I, as well as the numbers of colloid in thyroid follicles and follicular cell hypertrophy were significantly changed at 0.003% and a NOAEL of approximately 6 mg/kg bw/day can be derived with respect to these effects. These findings were affirmed by additional results from subchronic and chronic toxicity studies. With respect to hyperplasia and thyroid adenomas a NOAEL of 15.5 mg/kg bw/day was derived for male rats from a 20 week-feeding study. Increased thyroid weights as well as diffuse hypertrophy and hyperplasia of the follicular epithelial cells were also reported for female and male mice, administered to 1.5% Kojic acid orally. Effects were more pronounced in males. It is obvious that thyroid proliferative lesions were induced by Kojic acid administration due to continuous serum TSH stimulation through the negative feedback mechanism of the pituitary-thyroid axis, resulting from depression of serum T_3 and T_4 levels.

After dermal exposure for 28 consecutive days a NOAEL of 100 mg/kg bw/day could be derived for rats. A study on dermal toxicity in rabbits can not be evaluated due to bacterial infection of the testing animals. After application of 0.3% Kojic acid to the skin for 19 weeks body weight was significantly reduced in female mice.

Irritation/Sensitisation
Kojic acid was not irritant to rabbit skin or mucous membranes but slightly photoirritant. In the Guinea pig and in humans, Kojic acid was found to be a sensitizer. The substance was not photosensitising.

Dermal absorption
From a 1% formulation an average amount of 3.63 µg/cm^2 became physiologically available through human skin in-vitro. The maximum value was 7.28 µg/cm^2. According to the SCCP Notes of Guidance the maximum value is used for MOS calculation as only 8 samples were investigated in this study. Percutaneous absorption was also studied in an open, uncontrolled, single application study, when 500 mg of a cream formulation containing 1% Kojic acid was applied to cheeks of women. Kojic acid was detected in plasma of all patients, however, the applied amount was very low (approximately 0.1 mg/kg bw/day) and the application area small.

Mutagenicity/Genotoxicity
Overall, the genotoxicity program on Kojic acid investigated the three endpoints of genotoxicity: gene mutations, structural chromosome aberrations and aneuploidy. Kojic acid was mutagenic in most of the bacterial gene mutation assays. However, these findings could not be confirmed in two in vitro gene mutation tests in mammalian cells: Kojic acid was not mutagenic in a mutation test in hamster V79 cells (performed without S9-mix only) and in a mouse lymphoma assay at the *hprt* locus. Kojic acid was clastogenic in two in vitro chromosome aberration tests and was positive in a sister chromatid exchange test. The relevance of clastogenic effects after Kojic acid treatment in an in vitro micronucleus test in Hep 2G cells at high concentrations only is unclear. In a test on phototoxicity with *E. coli* Kojic acid induced mutagenic effects but irradiation with artificial sunlight had no relevant influence on the mutagenic potential of the test substance indicating that Kojic acid is probably not photomutagenic.

The positive findings from the in vitro tests could not be confirmed with in vivo tests. Kojic acid treatment did not result in DNA adducts in liver and thyroid cells, indicating that it probably does not bind to (liver and thyroid) DNA. An in vivo unscheduled DNA synthesis (UDS) test was negative indicating that treatment with Kojic acid did not lead to DNA damage that is repaired by excision repair. Kojic acid was not clastogenic in a comet assay in the liver, stomach and colon and in an in vivo bone marrow micronucleus test after single and multiple doses. Finally Kojic acid was not mutagenic in an in vivo gene mutation assay with transgenic mice. The negative results from the dominant-lethal test indicate that Kojic acid probably is not a germ cell mutagen.
The only positive in vivo results were found in an in vivo micronucleus test in hepatocytes after partial hepatectomy. However, the relevance of these positive results is very limited. Based on all results, it can be concluded that Kojic acid can be considered to have no genotoxic potential *in vivo* and additional tests are unnecessary.

Carcinogenicity
In the rat liver the marker for preneoplastic foci was slightly increased in one study after 20 weeks of exposure to Kojic acid with the diet up to 2% as well as in a tumour promotion assay. Effects on proliferation were considered possible in a second feeding study up to 28 days and up to 2% Kojic acid while a further 28-day feeding study with a maximum of 2% Kojic acid showed no initiating potential for liver tumours. All investigations were performed with male rats only. In mice hepatocellular adenomas, altered hepatocellular foci as well as nonproliferative lesions like focal hepatocellular necrosis and inflammatory cell infiltration were observed after 1.5% Kojic acid administered orally for 26 weeks. Proliferation of liver cells in mice was enhanced, when 3% Kojic acid were administered for 4 weeks. Investigations were performed in male mice except for one 20 month feeding study, were incidences of hepatomas were reported for female mice. Liver weights were increased in male and female mice and also in the F_1 offspring of Kojic acid treated dams.

Thyroid proliferative lesions, hyperplasia and adenomas were reported when male rats were investigated in feeding studies up to 4 % Kojic acid for 12 to 20 weeks. In one promotion assay carcinomas were observed. A NOAEL of 15 mg/kg bw/day was derived from this study. In mice hypertrophy in males as well as hyperplasia and adenomas in females and

males were reported in two feeding studies up to 3 % Kojic acid for 26 weeks or 20 months, respectively. According to a negative study on DNA-adducts in the thyroid it can be concluded that thyroid tumorigenic activity may be attributable to a non-genotoxic mechanism.

In a carcinogenicity test with mice Kojic acid was not carcinogenic when fed on a diet containing up to 1% for 78 weeks.

When female mice were dermally exposed to 0.3 – 3.0% Kojic acid for 19 weeks, no initiation and promotion potential for skin carcinogenesis was observed.

From the studies available it is concluded that kojic acid is a non-genotoxic carcinogen in rodents. For the thyroid tumour induction a tumour promoting effect based on hormonal disruption is obvious. The goitrogenic (thyrotropic) effect is linked to inhibition of iodine uptake, a subsequent decrease in serum T_3/T_4 levels followed by a compensatory increase in TSH release with the consequence of thyroid cell proliferation. In contrast to rodents an increase in TSH in humans does not pose a significant concern regarding potential thyroid carcinogenesis in humans. There is convincing evidence that humans are considerably less sensitive than rodents with regard to perturbation of thyroid hormone homeostasis but it is not clear if these species differences are of qualitative or quantitative nature. Despite these comments, a threshold on the basis of hormonal disruption can be assumed.

Reproductive toxicity
Kojic acid showed no effects on fertility of rats and mice in various one-generation studies. The test substance did not induce malformations. Effects observed were changes in litter parameter and organ weights in the offspring. NOAEL values for maternal toxicity as well as for embryotoxicity are in the range of 100 to 150 mg/kg bw/day for rats, at 100 mg/kg bw/day for rabbits and at 30 mg/kg bw/day for mice. Cannibalistic behaviour during lactation period was reported in two studies for rats who received 50 µg Kojic acid daily for 21 consecutive days before mating (males) or from day 1 to day 5 of gestation. This effect, however, was not reported by other authors and its relevance is unclear.

Kinetics and safety assessment
Kojic acid is rapidly absorbed and distributed to all organs after oral, dermal or subcutaneous administration. After dermal application, maximum values in blood samples were measured after 0.5 hours. The ratio for oral / dermal AUC values is 4. The test substance was excreted mainly with the urine. Excretion was minor via bile and negligible via respiratory air and faeces. Kojic acid did not undergo enterohepatic circulation. Very high concentrations reached the foetus 30 minutes after single subcutaneous application in pregnant females and persisted in later stages of development. Transfer to mother milk was low. After repeated subcutaneous exposure concentrations in blood and urine samples increased and showed a tendency to reach equilibrium which was almost 3 times higher than values 24 hours after the first application of the test substance. Concentrations in organs and tissues were partly several times higher after repeated dose administration than after single administration. Main metabolites were sulphate conjugates of Kojic acid and the glucuronide. Data on kinetics after single application of Kojic acid are summarised in the following table:

Species	Dose (mg/kg bw)	Route	C_{max}	AUC_{0-6} (µg eq/ml)	AUC_{0-24}	Ref.
Rat	100	oral	25.07 ± 4.56 µg eq/ml		101.54 ± 19.35 µg eq/ml	32
Rat		oral	25.05%	71.8		42/AR11
Rat		sc	21.67%	50.2		42/AR11
Rat		dermal	5%	18.3		42/AR11
Human	Appr. 0.1	dermal	1.54 ng/ml		19.4 ng/ml	100

It was discussed in the review of safety aspects submitted to the SCCP that percutaneous absorption in the rat is higher than in humans and that occlusion additionally enhances penetration of Kojic acid. The relative systemic exposure in rats after topical application under occlusion was approximately 20% of the respective exposure following oral administration. After oral exposure to 100 mg/kg bw AUC values in the rat were approximately 5000 times higher than for humans exposed dermally to a dose which was 1000 times lower. These data support the conclusion, that skin penetration in rats and humans is considerable. Additionally, it has to be considered, that data on kinetics of Kojic acid in the rat were obtained with doses of 100 mg/kg bw. The NOAEL dose, however, is lower and could be derived at 6 mg/kg bw/day from the studies provided. For these reasons a safety approach based on kinetic data can not be used.

In rats repeated exposure resulted in higher blood levels of Kojic acid than after single administration. In humans repeated use of bleaching products may also result in higher systemic exposure than determined after single administration.

For bleaching products a mean amount of 1 g formulation containing 1% Kojic acid applied twice daily to the face only can be assumed, corresponding to a daily application of 20 mg or 0.33 mg/kg bw Kojic acid on the face. Studies with Japanese women were conducted by single application of 500 mg of a 1% Kojic acid formulation resulting in a dose of 5 mg or approximately 0.1 mg/kg bw (50 kg bw estimated for Japanese women). It has to be taken into account, that consumers may be exposed to higher doses, especially, when they apply Kojic acid containing bleaching products also to other parts of the skin, e. g. hands and arms, neck and décolleté.

For the safety assessment a skin penetration rate of 7.28 µg/cm^2 and a NOAEL of 6 mg/kg bw, based on histopathological findings and altered iodine uptake after oral administration in a 28 day study with rats, was used for MOS calculation.

4. CONCLUSION

Based on the information provided, margins of safety of respectively 35 (face and hands), 58 (hands) and 88 (face) have been calculated suggesting that the use of Kojic acid at a maximum concentration of 1.0% in skin care formulations poses a risk to the health of the consumer. In addition, other parts of the skin might be exposed to Kojic acid.

Kojic acid has the potential to induce skin sensitisation.

Relevant data on kinetics of Kojic acid after dermal application may be submitted to refine the MOS approach.

5. MINORITY OPINION

Not appliacble

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