Scientific Committee on Consumer Safety

SCCS

OPINION

on Kojic acid

- CORRIGENDUM -

The SCCS adopted this document

at its plenary meeting on 15–16 March 2022 and
the CORRIGENDUM by written procedure on 10 June 2022

ACKNOWLEDGMENTS

Members of the Working Group are acknowledged for their valuable contribution to this Opinion. The members of the Working Group are:

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This Opinion has been subject to a commenting period of eight weeks after its initial publication (from 5 November to 14 January 2022). Comments received during this period were considered by the SCCS. For this Opinion, main changes of the content occurred in sections 3.2.3, 3.2.4, 3.4, and in the respective discussion sections as well as in conclusion section 4 under response to question 2.

The Corrigendum includes changes in the following sections: abstract, 3.2.4 calculation of SED/LED, 3.4 safety evaluation, 3.5 discussion and relevant conclusions 1 and 2.

All Declarations of Working Group members are available on the following webpage:
Register of Commission expert groups and other similar entities (europa.eu)

1. ABSTRACT

The SCCS concludes the following:

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of Kojic acid, does the SCCS consider Kojic acid safe when used in cosmetic products up to a maximum concentration of 1 %?*

   On the basis of the safety assessment, and considering the concerns related to potential endocrine disrupting properties of Kojic acid, the SCCS is of the opinion that Kojic acid is safe when used as a skin lightening agent in cosmetic products at concentrations of up to 1%.

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of Kojic acid in cosmetic products?*

   /

3. *Does the SCCS have any further scientific concerns with regard to the use of Kojic acid in cosmetic products?*

   As Kojic acid is sometimes added to peeling agents, a weakened skin barrier may be of additional concern because of greater dermal absorption.

   Only the topical use of Kojic acid in cosmetics has been considered in this Opinion. Other uses (*e.g.* food) of natural or synthetic sources have not been considered.

   As far as the derivatives of Kojic acid are concerned, *e.g.* esters of Kojic acid such as Kojic acid dipalmitate and Kojic acid isopalmitate, and derivatives such as chloro-Kojic acid, these have not been included in this Opinion as no data has been submitted.

Keywords: SCCS, revision, scientific opinion, Kojic acid, CAS No 501-30-4, EC No 207-922-4, Regulation 1223/2009

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), scientific opinion on Kojic acid, preliminary version of 26-27 October 2021, final version of 15-16 March 2022, Corrigendum of 10 June 2022, SCCS/1637/21

About the Scientific Committees

Two 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.
These Committees are: the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) and they are made up of scientists appointed in their personal capacity.

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

SCCS

The Committee shall provide Opinions on questions concerning health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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2. MANDATE FROM THE EUROPEAN COMMISSION

1. Background on substances with endocrine disrupting properties

On 7 November 2018, the Commission adopted the review[1] of Regulation (EC) No 1223/2009 on cosmetic products ('Cosmetics Regulation') regarding substances with endocrine disrupting (ED) properties. The review concluded that the Cosmetics Regulation provides the adequate tools to regulate the use of cosmetic substances that present a potential risk for human health, including when displaying ED properties.
The Cosmetics Regulation does not have specific provisions on EDs. However, it provides a regulatory framework with a view to ensuring a high level of protection of human health. Environmental concerns that substances used in cosmetic products may raise are considered through the application of Regulation (EC) No 1907/2006 ('REACH Regulation').
In the review, the Commission commits to establishing a priority list of potential EDs not already covered by bans or restrictions in the Cosmetics Regulation for their subsequent safety assessment. A priority list of 28 potential EDs in cosmetics was consolidated in early 2019 based on input provided through a stakeholder consultation. The Commission carried out a public call for data[2] in 2019 on 14[3] of the 28 substances (to be treated with higher priority-Group A substances) in preparation of the safety assessment of these substances. Kojic acid (CAS No 501-30-4, EC No 207-922-4) is one of the above-mentioned 14 substances for which the call for data took place.

2. Background on Kojic acid

Kojic acid is a secondary metabolite commonly produced by many species of filamentous fungi including Aspergillus and Penicillium. Due to its inhibitory effect on tyrosinase activity and melanogenesis, Kojic acid has been widely used as a skin lightening/whitening or depigmenting agent in cosmetic products. In addition, the ingredient Kojic acid (CAS No 501-30-4, EC No 207-922-4) with the chemical name '5-Hydroxy-2-(hydroxymethyl)-4H-pyrane-4-one', is also included in the European database for information on cosmetic substances and ingredients (CosIng) with the reported functions of 'bleaching' and 'antioxidant'.
Kojic acid has been subject to safety evaluations by the SCCP in 2008[4] and 2012[5]. In particular, the SCCP Opinion from 2012 (SCCP/1481/12) concluded that '*…a concentration of 1.0% in leave-on creams, which are generally applied to the face and/or hands leads to the conclusion that it [Kojic acid] is safe for the consumers'*. Currently, Kojic acid is not regulated under the Cosmetic Regulation (EC) No. 1223/2009.

Kojic acid has been reported to interfere with either iodine organification or iodine uptake by the thyroid, resulting in altered thyroid functions, hence it was included in the priority list for safety assessment. During the call for data, stakeholders submitted scientific evidence to demonstrate the safety of Kojic acid in cosmetic products. The Commission requests the SCCS to carry out a safety assessment on Kojic acid in view of the information provided.

[1] https://ec.europa.eu/transparency/regdoc/rep/1/2018/EN/COM-2018-739-F1-EN-MAIN-PART-1.PDF
[2] https://ec.europa.eu/growth/content/call-data-ingredients-potential-endocrine-disrupting-properties-used-cosmetic_products_en
[3] Benzophenone-3, kojic acid, 4-methylbenzylidene camphor, propylparaben, triclosan, Homosalate, octocrylene, triclocarban, butylated hydroxytoluene (BHT), benzophenone, homosalate, benzyl salicylate, genistein and daidzein
[4] https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_148.pdf
[5] https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_098.pdf

Terms of reference

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of Kojic acid, does the SCCS consider Kojic acid safe when used in cosmetic products up to a maximum concentration of 1%?*

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of Kojic acid in cosmetic products?*

3. *Does the SCCS have any further scientific concerns with regard to the use of Kojic acid in cosmetic products?*

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Primary name and/or INCI name

Kojic acid (INCI)

(SCCP/1182/08)

3.1.2 Chemical names

IUPAC name: 5-hydroxy-2-(hydroxymethyl)pyran-4-one

Other names and synonyms:
4H-Pyran-4-one, 5-hydroxy-2-(hydroxymethyl)-
5-Hydroxy-2-hydroxymethyl-4-pyrone
2-Hydroxymethyl-5-hydroxy-4-pyrone
5-Hydroxy-2-hydroxymethyl-γ-pyrone

(SCCP/1182/08; Irving, 2011; ECHA, 2021)

3.1.3 Trade names and abbreviations

Kojic acid (KA)
Rita KA
Tonelite Kojic acid

(CIR, 2010)

AEC KA
KASL

(Saeedi *et al*., 2019)

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

(SCCP/1182/08)

Trade name Mixtures:
Botacenta SLC 175
Melarrest A
Melarrest L

(CIR, 2010)

Dermawhite HS
Phytoclar
Rice extract "COS"
Vegewhite

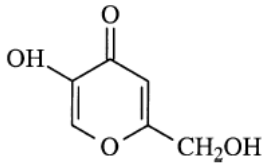
(SCCP/1182/08; NCBI, 2021)

3.1.4 CAS / EC number

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

(SCCP/1182/08)

3.1.5 Structural formula



(SCCP/1182/08)

3.1.6 Empirical formula

Empirical formula: $C_6 H_6 O_4$

(SCCP/1182/08)

3.1.7 Physical form

White to light yellow crystalline powder

(SCCP/1182/08)

Prismatic needles from acetone, ethanol and ether or methanol and ethyl acetate
(O'Neil, 2006; Lide, 2007; Lewis & Hawley, 2007)

3.1.8 Molecular weight

Molecular weight: 142.11

(NCBI, 2021)

3.1.9 Purity, composition and substance codes

Purity: > 97%

(Kynoch, 1977d from SCCP/1182/08)

98 – 102% (batch 8A44)

(Manciaux, 1998a; 1998b; 1998c; Richard; 1998 from SCCP/1182/08)

> 98%

(Jinnai *et al*., 2019)

3.1.10 Impurities / accompanying contaminants

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

(IARC, 2000 from SCCP/1182/08)

Sample P005464:
Arsenic: ≤ 2 ppm
Chloride: ≤ 50 ppm
Heavy metals: ≤ 10 ppm
Sulfate: ≤ 120 ppm
Aflatoxins < 1.08 ppb

(SGS laboratories, 2003 from SCCP/1182/08)

3.1.11 Solubility

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

(IARC, 2000 from SCCP/1182/08)

Mole-fraction solubility x 10^{-3} at 298.15 K: 10.96 (methanol), 7.385 (ethanol), 5.244 (n-propanol), 2.010 (ethyl acetate), 33,10 (2-methoxyethanol), 23.32 (2-ethoxyethanol), 10.32 (1,4-dioxane), 184.3 (DMA), 248,9 (DMSO), 16.94 (acetic acid), 201.2 (NMP), 134.7 (DMF), 4.549 (acetone), 5.527 (water)

(Sun *et al*., 2021)

Soluble in ethanol, ethyl ether, acetone, DMSO; slightly soluble in benzene

(Lide, 2007)

Soluble in water, acetone; slightly solluble in ether; insoluble in benzene

(Lewis, 2007)

Sparingly soluble in pyridine

(O'Neil, 2006)

In water soluble $9.35x10^{+5}$ mg/L at 25 °C (est)

(US EPA, 2008)

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

(SGS laboratories, 2003 from SCCP/1182/08)

3.1.12 Partition coefficient (Log Pow)

Log K_{ow} = -0.64 (experimental value)

(Kontoghiorghes, Jackson & Lunec, 1986)

3.1.13 Additional physical and chemical specifications

Appearance: odourless, slightly bitter taste
Melting point: 152-154°C (CIR, 2010)
pH: 4.7 (1 w/v% in water)
p*Ka* value: 7.66 at 25°C (Serjeant & Dempsey, 1979)
UV absorption: λmax 270 nm (solvent: water)
Characterization by UV spectrum; IR spectrum; NMR spectrum; Mass spectrum; HPLC chromatogram

(SGS laboratories, 2003 from SCCP/1182/08)

Density: 1.542 g/cm^3

(Sun *et al*., 2021)

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.

(Burdock *et al*., 2001 from SCCP/1182/08)

3.1.14 Homogeneity and Stability

No data submitted.

SCCS comments on physical specifications
- A full report of the chemical characterisation of Kojic acid in terms of purity and identity in representative batches should be provided and the validity of the analytical methodologies used must be shown.
- Hazardous impurities like heavy metals and aflatoxins may be present and should be kept at trace levels under continuous monitoring.
- No data were provided on the stability of Kojic acid in the test solutions and in the marketed product.
- For several tests, the purity of the test substance was not reported.

3.2 EXPOSURE ASSESSMENT & TOXICOKINETICS

3.2.1 Function and uses

From SCCP/1182/08
Kojic acid is used as a skin lightening agent in cosmetic products in use concentrations of 1%. It is used in leave-on creams, which are generally applied to the face, but it can also be used in hand creams. The SCCS furthermore commented that products are on the market containing Kojic acid at concentrations higher than 1%.

(SCCP/1182/08)

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%.

(Burdock *et al.*, 2001; Palmer, 1979 ; Cabanes, 1994 from SCCP/1182/08)

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

(Morton *et al*., 1945 from SCCP/1182/08)

SCCS comment
In the SCCS Notes of Guidance (SCCS/1628/21), the application frequency for face cream is 2.14 times/day and 2 times/day for hand cream. Commercially available Kojic acid - containing creams provide user application instructions varying between once to twice per day. For previously assessed skin lightening ingredients (alpha and beta arbutins) a frequency of application of 2 was used, which is also the frequency applied here. The potentially exposed surface consists of face, hands and neck (as for alpha arbutin) and represents 1745 cm².

3.2.2 Dermal / percutaneous absorption

From SCCP/1182/08
An *in vitro* dermal absorption study with a 1% Kojic acid formulation showed an average amount of 3.58 ± 2.38 µg $_{eq}$/cm^2. The maximum value was 7.28 µg eq/cm^2. According to the SCCP Notes of Guidance (6th Revision applicable in 2008) the maximum value was used for MoS calculation, as only 8 samples were investigated in this study and the composition of formulation given in the dossier was not legible.

(Leclerc, 2002)

A human dermal study with Japanese women (n=6) was furthermore submitted. The study applied a single dose of 500 mg of a 1% Kojic acid formulation on the left and right cheeks resulting in a dose of 5 mg or approximately 0.1 mg/kg bw (50 kg bw estimated for Japanese women). Kojic acid was detected in plasma samples of all subjects, but not at all time points. The mean Cmax was 1.54 ± 0.38 ng/ml with a mean AUC0->∝ of 19.4 ng/ml), which was slightly above the limit of quantification (1 ng/ml). The potential dermal transfer of Kojic acid to blood appeared to be very low and no adverse events were observed, which led the authors to conclude that there is no problem regarding the safety of Kojic acid. However, several shortcomings were noted by the SCCP. Specifically, the composition of the cream formulation used in this study was not given, individual data on medical and physical examinations are missing and the application area is rather small for measuring dermal penetration into blood.

(Fukase, 2005)

It was discussed in the review of safety aspects submitted to the SCCP (SCCP/1182/08) 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.

From SCCP/1481/12
An *in vitro* dermal absorption study (OECD TG 428 compliant, GLP) was submitted by the applicant. The study was performed on excised, dermatomed (400 µm) human skin on a static diffusion cell. A total of 12 samples (4 donors, 3 skin samples/donor) received a leave-on skin care formulation containing 1% of radiolabelled Kojic acid (99.2% pure). A dose of 2 mg formulation/cm^2 was applied and rinsed-off after 24 hours with 2% sodium dodecyl sulphate (SDS) in water (2 x 762 µl), followed by rinsing with water (2 x 762 µl). Receptor fluid samples were collected at 0.5, 1, 2, 4, 8, 12, 16, 20 and 24 hours following application. The mean recovery of the applied test material was 95.4%, with individual cell values ranging from 87.5% to 99.9%. The mean amount penetrated over the entire 24 hour exposure period was 0.142 ± 0.265 µg/cm², corresponding to 0.698% of the applied dose. The mean total systemically available dose of Kojic acid (remaining epidermis plus dermis and receptor fluid) was 3.68 % of the applied dose (corresponding to 0.749 µg/cm²). Based upon the results obtained, the performing laboratory concludes that the Kojic acid component of a 1% leave-on skin care formulation penetrated through human dermatomed skin at a very slow rate.
The SCCP noted several shortcomings regarding the formulation used, and concluded that the formulation used may not be representative of the majority of Kojic acid-containing formulations on the market as it contains a high amount of silicones, polyols and nylon. Further arguments by the applicant about the appropriateness of the chosen formulation and for the discrepancy between the results of the two studies were found insufficient.

(Davies, 2011)

The discussion section of the 2012 Opinion (SCCP/1481/12) concluded:
'The SCCS is of the opinion that the applicant does not provide evidence about the appropriateness of the chosen formulation and that no reasonable explanation is given for the discrepancy between the results of the two studies. For these reasons the dermal absorption study results cannot be used for the calculation of the overall MoS of Kojic acid in cosmetic products. Consequently, the dermal absorption values present in the previous opinion SCCP/1182/08 are kept, providing an average amount of 3.58 µg/cm² with a SD of 2.38 µg/cm² (highest value was 7.28 µg/cm²). As in the Notes of Guidance (7th revision, SCCS/1416/11) it is stated that, when a dermal study has some shortcomings, the mean value plus 2SD should be used, the value taken into account for the calculation of the MoS becomes therefore 8.39 µg/cm² (mean dermal absorption is 3.63 and SD 2.38 µg/cm²) instead of the highest value, used in the earlier opinion (SCCP/1182/08).'

SCCS comment
After consultation of the original study report by Leclerc (2002), an error in the mean dermal absorption value was noted. The correct mean dermal absorption value is 3.58 ±2.38 µg/cm² instead of 3.63±2.38 µg/cm². Following the most recent SCCS Notes of Guidance (SCCS/1628/21), in case of significant deviations and/or very high variability, the mean + 2SD (8.34 µg/cm²) may be used.

3.2.3 Other studies on toxicokinetics

Single administration

From SCCP/1182/08
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 *via* 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. Data on kinetics after single administration of Kojic acid are summarised in the following Table 1:

Table 1: Overview of *in vivo* toxicokinetics data of Kojic acid after single administration

Species	Dose (mg/ kg bw)	Route	C_{max}	AUC_{0-6} (µg eq/ml x h)	AUC_{0-24}	Ref.
Rat, male	100	Oral	25.07 ± 4.56 µg eq/ml		101.45 ± 19.35 µg eq/ml	(Higa *et al.*, 2000)
Rat, male		Oral	20.63% (after 0.5 hours) and 25.05% (after 1 hour)	71.8		(Suzuki *et al.*, 1978; Sansho Seiyaku Co., Ltd., 2001)
Rat, male		Subcutaneous	13.29% (after 0.5h) and 21.67% (after 1h)	50.2		(Suzuki *et al.*, 1978; Sansho Seiyaku Co., Ltd., 2001)
Rat, male		Dermal	5% (maximum after 0.5h)	18.3		(Suzuki *et al.*, 1978; Sansho Seiyaku Co., Ltd., 2001)
Human	Appr. 0.1	Dermal	1.54 ng/ml		19.4 ng/ml	(Fukase, 2005)

Repeated administration
From SCCP/1182/08
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 in all organs or tissues detected were sulphate conjugates of Kojic acid (35.6 – 93.7% of total radioactivity) and glucuronides (6.4 – 39.6% of total radioactivity).
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.
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.

SCCS comment
No new data was submitted or identified from the open literature.

SCCS overall comment on toxicokinetics
SCCS considers that Kojic acid is well absorbed after oral exposure, and therefore will not correct for oral bioavailability.

Additional information submitted during commenting period
Valuable additional information on the clinical study carried out by Fukase (2005) was submitted to the SCCS in December 2021 whereby the composition of the cream formulation containing 1% Kojic acid and a clear indication of the application area was given. SCCS notes that a limitation of this *in vivo* percutaneous absorption study (n=6) is that no radiolabelled compound was used, which would have allowed to determine pharmacokinetic parameters for Kojic acid (dermal bioavailability, distribution volume, ...). Also, urinary samples could have allowed to determine the elimination rate.

3.2.4 Calculation of SED/LED

Dermal exposure:

The human percutaneous absorption study of Fukase (2005) showed that after a single application of 500 mg cream containing 1% Kojic acid on the entire face focusing on the cheeks, the AUC (0-24h) was close to 20 ng/ml.h, leading to a total amount of Kojic acid measured in plasma over 24h period of 0.04 mg. SCCS proposes to use this amount measured in plasma to calculate the SED as, in contrast to the *in vitro* dermal absorption study, the elimination of Kojic acid within 24h is taken into consideration via this approach. The mean Cmax was found to be 1.54 ng/mL.h, but cannot be considered for SED calculation.

Further, a number of important points need to be taken into consideration:
- The AUCs were calculated over a period of 24h and are not infinite. This could lead to an underestimation of the real value since for 2 patients Kojic acid was still measured in the blood after 24h. Data in rodents showed that excretion of Kojic acid after dermal or subcutaneous administration is up to 24h. It has been reported that 50% and 56% of the subcutaneous and dermal administered radioactivity, respectively, were excreted in the urine within 48h. Excretion in the feces seemed to be very low.
- The reported AUC values in the study are within a factor 3 (min = 10, max = 31.4 ng/ml.h)

Taking into account both the short observation time (0-24h) and the variation observed in the AUC values, the SCCS decided to take the 95 percentile of the AUC as value, calculated as follows:
AUC = AUC mean + 1.65x standard error = 19.4 + 1.65x7.9 = 32.4 ng/ml.h.

Considering a plasma volume of 0.06L/kg bw (or 60 ml/kg bw), the amount of Kojic acid measured in plasma over 24h based on AUC was calculated as follows:
32.4 ng/ml x 60 ml/kg bw x 48 kg bw (mean bw measured in the study) = 0.093 mg/day.

- The SCCS noted, however, that according to the SCCS Notes of Guidance (SCCS/1628/21), the daily exposure to face cream is equal to 1.54 g/day (565cm^2 surface area). When considering also application of the face cream to the neck area (320 cm^2), the estimated daily exposure for face + neck becomes: 1.54 g/day x ((565 + 320)/565) = 2.41

g/day. If the Kojic acid content of the cream is 1%, the daily exposure to Kojic acid is 24.1 mg/day for application to the face + neck, which corresponds to 4.82 times more Kojic acid than in the *in vivo* percutaneous absorption study (application of 5 mg of Kojic acid). Assuming a linearity between dose and AUC, the total amount of Kojic acid measured in plasma in 24h can be estimated as: 0.093 mg/day x 4.82 = 0.448 mg/day and the SED becomes: 0.448 mg/day/60 kg bw= 0.0075 mg/kg bw/day.

In case of hand cream, analogous calculations can be done whereby an estimated daily amount applied of 2.16 g/day (SCCS/1628/21) results in 21.6 mg/day of Kojic acid and thus 4.32 times more than in the *in vivo* percutaneous absorption study or 0.093 mg/day x 4.32 = 0.402 mg/day. This leads to a SED of: 0.402 mg/day/60 kg bw= 0.0067 mg/kg bw/day.

For aggregate exposure (face + neck + hands), the total daily exposure is 4.57 g/day, resulting in 45.7 mg/day of Kojic acid being 9.14 times more than in the *in vivo* percutaneous absorption study or 0.093 mg/day x 9.14 = 0.850 mg/day. This leads to a SED of: 0.850 mg/day/60 kg bw= 0.0142 mg/kg bw/day.

Area of application	Surface area (cm^2)	Estimated daily amount applied cream (g/day)	Calculated daily amount applied Kojic acid (mg/day)	SED* (mg/kg bw/day)
Face+neck	565 + 320 = 885	2.41	0.448	0.0075
Hands	860	2.16	0.402	0.0067
Aggregate (face+neck+hands)	1745	4.57	0.850	0.0142

*SED was calculated based on the 95th percentile of AUC values (0-24h) obtained in a clinical study (Fukase 2005) using a cream containing 1% of Kojic acid.

SCCS comment
Bleaching products may not only be applied to face, neck and hands but also to other parts of the skin, *e.g.* arms and décolleté. This may result in even higher exposure levels of consumers to Kojic acid.

3.3 TOXICOLOGICAL EVALUATION

3.3.1 Irritation and corrosivity

3.3.1.1 Skin irritation

From SCCP/1182/08
A single dose of 0.5 g Kojic acid (Batch No 8224) in 0.5 ml purified water was not irritant to intact and abrased albino rabbit skin (n=6) when applied for 24 hours under occlusive conditions.

(Kynoch & Ligett, 1978)

SCCS comment
According to the value for solubility in water as submitted to the SCCP, namely 43.85g/L, the applied dose of Kojic acid would not be appropriately dissolved into the solvent.

3.3.1.2 Mucous membrane irritation / eye irritation

From SCCP/1182/08
A single dose of 0.05 mL of 3% aqueous solution of Kojic acid was applied to the eye of rabbits (mean bw of 2.8 kg) and scored without washing. Kojic acid caused no eye disturbances in the preliminary test, but mild transient hyperemia was observed in the second experiment in 2 out of 4 animals. The overall eye irritability was reported to be very weak. In a supplementary test no specific response was observed for up to 72 hours.

(Shino, 1978)

SCCS comment
No new data was submitted or identified from the open literature.

SCCS overall comment on skin and mucous membrane irritation
The SCCS agrees with the former Opinion that Kojic acid is not irritant to rabbit skin or mucous membranes.

3.3.2 Skin sensitisation

From SCCP/1182/08 and SCCP/1481/12

In a guinea pig study with 10 animals using the Magnusson Kligman method, Kojic acid was considered to be non-sensitising.

(Kynoch & Elliot, 1978 from SCCP/1182/08)

In humans, patch testing with 220 female patients including 5 Kojic acid sensitive patients, resulted in facial dermatitis in the Kojic acid sensitive patients, 1-12 months after starting application of cosmetic products with Kojic acid.

(Nakagawa, Kawai & Kawai, 1995 from SCCP/1182/08)

Additionaly, a case report was described of a 30 year old woman in whom an eczematous eruption appeared after use of a cream formulation containing 3% Kojic acid. The individual also showed strong positive reactions in a confirmatory patch test with Kojic acid at 1 and 5%. Taking all the information together, the SCCP concluded that Kojic acid was found to be a sensitiser in guinea pigs and humans

(Serra-Baldrich, Tribó & Camarasa, 1998 from SCCP/1182/08)

The 2008 SCCP Opinion (SCCP/1182/08) described a GLP compliant guinea pig Buehler test where 2 out of 20 animals showed a positive reaction (erythema grade 1 or 2) after challenge, indicating a sensitising potential of Kojic acid.

(Manciaux, 1998b from SCCP/1182/08)

A brief description of a repeated insult patch test (HRIPT) was provided, from which the applicant intended to show the test substance is not sensitising. The HRIPT was poorly documented and the test substance was not defined (there was a handwritten annotation '*Product contains 1% Kojic acid*') and the exact dosage levels were lacking. This study was found unsuitable to override the concerns in relation to sensitisation stated in opinion SCCP/1182/08. Furthermore, the SCCS considers HRIPT experiments as unethical.

(Eisenberg & Frank, 2006 from SCCP/1481/12)

New human data from open literature
A case-report describes a positive patch-test reaction to 0.5% (++), 1% (+++), 3% (++++) in a 40 year-old woman who developed an acute dermatitis on the face and neck area after applying for three days a commercial product containing 0.5% Kojic acid.

(Mata *et al.*, 2005)

One case-report, concerning a 54-year-old Spanish female, described the development of allergic contact dermatitis and hyperpigmented lesions after chronic use of a depigmenting cream on the patient's arms. Patch testing was positive to the commercial product, and to Kojic acid at 1% in an aqueous solution. The same Kojic acid solution tested negative in 20 control subjects.

(García-Gavín *et al*., 2010)

Another case-report of a 54 year-old woman with a skin reaction to a facial cream describes a strong positive (++) patch-test response to Kojic acid at 1% after 96 hours. All other ingredients of the cream were negative.

(Tejera-Vaquerizo & García-Gavín, 2019)

SCCS overall comment on skin sensitisation
The skin sensitising potential of Kojic acid was tested in two guinea pig tests using two different testing protocols, of which one was negative. In the other test, a Buehler test, 2 of the 20 guinea pigs had mild erythema. According to OECD TG406, at least 15% of the animals has to be positive to consider a test chemical as a skin sensitiser. Hence, under the conditions of these tests, Kojic acid was not considered to be a skin sensitiser in guinea pigs. Considering all information in humans, the SCCS is of the opinion that the occurrence of allergic contact dermatitis from Kojic acid is very low.

3.3.3 Acute toxicity

3.3.3.1 Acute oral toxicity

From SCCP/1182/08
Three studies for acute oral toxicity were submitted to the SCCP for evaluation in 2008.

CFLP mice (21-27g) were treated by oral intubation with a single dose of Kojic acid (40% w/v) in 0.5% methylcellulose at 1000, 4000 and 16 000 mg/kg bw (range finding screen) or 4000, 6400, 10000 and 16000 mg/kg bw (main experiment) over an observation period of 14 days. A control group, receiving the vehicle alone (40 ml/kg), was included. The range finding test indicated and LD50 in the range of 4000-16000 mg/kg bw. From the main experiment an LD50 of 5100 mg/kg bw was derived (95% confidence limit: 3900-6700 mg/kg bw).

(Kynoch, 1977c)

Groups of CFY rats (102-123g) were treated by oral intubation with a single dose of Kojic acid (40% w/v) in 1% methylcellulose. The doses were 1000, 4000 and 16 000 mg/kg bw for the range finding screen (2 of each sex per group) and 1000, 1600, 2500 and 4000

mg/kg bw for the main experiment (5 of each sex per group). A control group was included, receiving the vehicle alone (10 ml/kg). After an observation period of 14 days an LD50 of 1000 to 4000 mg/kg bw was determined based on the range finding screen. 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 that died after treatment. The LD50 and its 95% confidence limits were calculated to be 1800 (1500 – 2000) mg/kg bw.

(Kynoch, 1977d)

An acute oral study performed according to OECD 401 (1987) in Wistar rats (5 males and 5 females per group) was submitted. 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. 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 LD50 of the test substance administered to rats by the oral route was > 2000 mg/kg bw.

(Manciaux, 1998a)

3.3.3.2 Acute dermal toxicity

From SCCP/1182/08

One acute dermal toxicity study was available (OECD compliant, GLP). 5 Male rats (body weight 274 ± 16 g) and 5 female rats (body weight 205 ± 9 g) were treated with single doses of 2000 mg/kg bw Kojic acid (53758) in its original form. 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. 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 LD50 was higher than 2000 mg/kg bw.

(Manciaux, 1998c)

3.3.3.3 Acute inhalation toxicity

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3.3.3.4 Acute intra-peritoneal toxicity

From SCCP/1182/08

CFLP mice (20-28g) 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 study consisted of a range finding screen with doses of 1000, 4000 and 16 000 mg/kg bw (2 animals of each sex per group), and a main experiment with doses of 1600, 2500, 4000, 6400, and 10000 mg/kg bw (5 animals of each sex per group). 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 of the range finding test indicated that the LD50 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 LD50 and its 95% confidence limits were calculated to be 2600 (2200 – 3000) mg/kg bw.

(Kynoch, 1977)

Groups of CFY 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 1000, 4000 and 16 000 mg/kg bw (range finding screen; 2 males and 2 females per group) or 1000, 1600, 2500 and 4000 mg/kg bw (main experiment; 5 males and 5 females per group). 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 the observation period. Results of the range finding test indicated that the LD50 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 that died after treatment. The LD50 and its 95% confidence limits were calculated to be 2400 (2000 – 3000) mg/kg bw.

(Kynoch, 1977)

3.3.3.5 Acute subcutaneous toxicity

From SCCP/1182/08

CFLP 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 1000, 4000 and 16 000 mg/kg bw (range finding screen) or 1600, 2500, 4000, 6400, 10000 and 16000 mg/kg bw (main experiment). 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 of the range finding test indicated that the LD50 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 LD50 and its 95% confidence limits were calculated to be 2700 (1900 – 3900) mg/kg bw.

(Kynoch, 1977)

Groups of CFY 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 1000, 4000 and 16 000 mg/kg bw (range finding screen) or 1000, 1600, 2500, 4000, 6400 and 10000 mg/kg bw (main experiment). 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 of the range finding test indicated that the LD50 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 LD50 and its 95% confidence limits were calculated to be 2600 (2000 – 3200) mg/kg bw.

(Kynoch, 1977)

3.3.3.6 Acute intravenous toxicity

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SCCS comment
No new data was submitted or identified from the open literature.

SCCS overall comment on acute toxicity
The SCCS agrees with the former Opinion that 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.

3.3.4 Repeated dose toxicity

3.3.4.1 Repeated Dose (28 days) oral / dermal / inhalation toxicity

From SCCP/1182/08
A 28-day study (dermal application) was described using New Zealand White strain rabbits (2.0 to 2.5 kg bw; 5 males and 5 females/group). The animals received 0, 0.65, 6.5 or 65% corresponding to 0, 13, 130, 1300 mg/kg bw/day of Kojic acid (Batch numbers 780213, 8224, 8313) in 1% aqueous methylcellulose. 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. Animals were observed daily for local effects, clinical signs and mortality, whilst body weight and food consumption were recorded weekly. Blood samples for haematological and biochemical parameters were taken prior to treatment and in the control, for the highest dose group this was done prior to termination. Animals were killed after the end of treatment period for autopsy and histopathology. 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 corpuscular 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.

(Kynoch *et al*., 1979)

SCCS comment
Effects on skin and eyes can not be evaluated due to the bacteriological infection of the 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 therefore obtained.

Another 28-day dermal study (OECD 410; GLP) administered Kojic acid to Wistar Hannover rats. The animals were allocated to three treated and one control group of 16 males and 16 females (control and high dose-level groups) or 10 males and 10 females (low and intermediate dose-level groups). 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. The animals received the Kojic acid in 0.5% aqueous methylcellulose solution (w/w) daily by cutaneous route, for four weeks, at the dose levels of 100, 300 and 1000 mg/kg/day. Control animals received the vehicle alone. 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, and weekly recordings of food consumption and body weight were made. 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 of two weeks. Blood levels for T_3, T_4 and TSH were not measured. After termination, representative organs were weighed and the animals were submitted to a detailed macroscopic post-mortem examination.
No death occurred during the study and no relevant clinical signs were observed. Furthermore, no treatment-related topical effects were observed and the overall body weight gains, final body weights and food consumption were similar in control and treated groups. 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 recovery period for animals 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. 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.

(Roger, 1999)

A 28-day study was described consisting of 3 separate experiments with Kojic acid in the diet of male F344 rats.

In the first experiment, groups of nine animals received 0 (control), 0.008; 0.03, 0.125, 0.5 or 2.0% Kojic acid containing diet for 28 days (calculated as 0, 5.85, 23.8, 95.3, 393.6, 1387.3 mg/kg bw/day). 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. In the groups with a diet, containing ≥ 0.125% of Kojic acid, thyroid weight increased 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 T3, T4 and TSH level were also only affected in the 2.0% group.

For the second experiment, 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. 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 T3, and T4 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.

In a third experiment, 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. 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. T3 and T4 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.

(Fujimoto *et al.,* 1999)

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

Over the course of four weeks, male F344 rats (8 animals/group) received a basal diet containing Kojic acid at 0, 0.008, 0.03, 0.125, 0.5, 2.0% (calculated as 0, 5.85, 23.8, 95.3, 393.6, 1387.3 mg/kg bw/day). At 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. 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 T3 and T4 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.

(Tamura *et al*., 1999)b

SCCS comment
Based on the histopathological findings and altered ^{125}I uptake, a NOAEL of 6 mg/kg bw/day can be derived.

Male F344 rats (10 animals/group) received Kojic acid in 0.5% carboxymethylcellulose at doses of 0, 4, 15, 62.5, 250, 1000 mg/kg bw/day in 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/100g, corresponding to 100 mg/kg bw/day). Blood samples were collected 10, 30 minutes and 1, 3, 6, and 24 hours after administration. The results showed that at 1000 mg/kg bw/day, a decrease in motility, inhibition of body weight gain and food consumption were observed. 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 from 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_{0-24h} was calculated to be 101.54 ± 19.35 µg eq/ml.

(Higa *et al*., 2000)

SCCS 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 $AUC_{0-24\ h}$ was calculated to be 101.54 ± 19.35 µg eq/ml.

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

From SCCP/1182/08
13 weeks of consecutive administration of Kojic acid in 1% aqueous solution of carboxymethylcellulose was performed in male SD rats (20 animals/group) by oral gavage at dose levels of 0, 250, 500, 1000, 2000, 3000 mg/kg bw/day, followed by 4 weeks treatment-free. After dose setting and 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 and observed daily, and food and water intakes were measured weekly. Deceased animals were autopsied at time of death.
In the highest dose group, all animals died within the first three 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. Autopsies 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 in body weight gain occurred in the groups receiving 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.

(Kariya *et al*., 1979)

SCCS comment
Pulmonary lesions were noted in all groups 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.

3.3.4.3 Chronic (6 months) toxicity

Male rats (SLC-SD; 10 animals/group at 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 dosing groups of 250, 500, 1000 mg/kg bw/day were followed by a 5-week recovery period. Treated animals were observed for abnormalities daily, whilst body weight, feed consumption and water intake were determined twice a week until 13 weeks after the initial administration followed by 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. Two animals in the highest dose groups died because of injuries due to administration, but these were not related to the substance. 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.

(Chronic toxicity test and recovery, 1980)

From SCCP/1481/12
The SCCS added to the Kojic acid opinion of 2012 a published article of a 55-week chronic toxicity study of dietary administered Kojic acid of 0% (control), 0.05 % (227mg/kg bw/day) and 2% (968mg/kg bw/day) to male F344 rats. At the highest dosage, Kojic acid induced thyroid follicular cell tumors and liver preneoplastic lesions could be observed. From this study it could be concluded that the NOAEL is below 227 mg/kg bw/day in male rats.

(Ota *et al.*, 2009)

SCCS comment
No new data was submitted or identified from the open literature. Studies on repeated dose, *i.e.* subchronic and chronic oral toxicity, were performed with male rats only.

SCCS overall comment on repeated dose toxicity
The SCCS agrees with the former Opinion that the most conservative NOAEL that could be identified from repeated dose studies with Kojic acid is based on the reduced uptake of ^{125}I and the changed numbers of colloid in thyroid follicles and of follicular cell hypertrophy at a dose level of 23.8 mg/kg bw/day in rats (28-day, oral), leading to a NOAEL of 6 mg/kg bw/day.

3.3.5 Reproductive toxicity

3.3.5.1 Fertility and reproduction toxicity

From SCCP/1182/08
Kojic acid in 1% methylcellulose was administered in dose levels of 25, 150 or 900 mg/kg bw/day by oral gavage in CRL:COBS CD (SD) BR rats (20 per group). The control group received the vehicle alone. At 900 mg/kg bw/day treatment was associated with behaviour changes. 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 and 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, however, 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, a 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. The SCCP commented that 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 could be derived from this study.

(Palmer, 1979a)

Kojic acid was administered by oral gavage in 1% methylcellulose to ddy-SLC strain SPF mice from days 6 to 15 of gestation and effects on pregnancy, development of foetuses and live born offspring were studied. The administered dose levels were 0, 25, 150 or 900 mg/kg bw/day (1ml/kg bw). 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. In the 900 mg/kg bw/day group a decrease for heart weight was observed in dams as well as a significant decrease in body weights of male. 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 foetuses and incidence increased with dose. 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 F1 offspring was increased at birth in the 25 mg/kg bw/day group. Three week old F1 mice revealed significantly increased kidney weights in both sexes at 900 mg/kg bw/day. In F1 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. A NOAEL for maternal toxicity and for embryotoxicity of 150 mg/kg bw/day can be derived from this study.

(Anon, 1980)

Kojic acid was orally administered in 1% methylcellulose at doses of 0, 30, 160 or 800 mg/kg to ddY-SLC mice (35 per group) once daily from day 15 of pregnancy to day 21 postpartum to assess the effect of treatment on dams and F1 offspring. Spontaneous parturition was allowed for all the dams and the second generation was subjected to postnatal observations. 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 F1 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 (F1) 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 (F1), 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. Vaginal opening was delayed at 30 and 160 mg/kg bw/day, incisor eruption was retarded significantly and dose-dependently at 160 and 800 mg/kg bw/day. 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 F1 dams showed significantly decreased body weights, thymus and liver weights. No changes were observed for F2

foetuses. The SCCP commented that a NOAEL of 30 mg/kg bw/day for maternal toxicity and for embryotoxicity can be derived from this study.

(Mineshita, 1983)

Male Sprague Dawley 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 (7 males) received propylene glycol alone. Fertility performance of the individual rat was studied from day 16 to day 21 of treatment. Each male (8 per group) was caged separately with two females of proven fertility. 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 observed.

(Choudhary, 1994)

3.3.5.2 Developmental toxicity

From SCCP/1182/08

Pregnant New Zealand white rabbits (13 females/group) were examined for abnormalities after exposure to doses of Kojic acid in 1% methylcellulose at 0, 20, 100 or 500 mg/kg bw/day at day 6 to 18 of gestation. The animals were terminated on day 29 of pregnancy. Post-dosing effects like tachypnoea, mydriasis and lethargy were observed at 500 mg/kg bw/d after day 12 of gestation (7^{th} dose). At 20 and 100 mg/kg bw/d sporadic post-dosing reactions were observed in few animals. Three animals (1 at 100 mg/kg bw/d; 2 at 500 mg/kg bw/d) were terminated following enteric disorder that was not considered treatment related. At 500 mg/kg bw/d bodyweight gain was found slightly lower compared to control groups. Bodyweight was not significantly different from controls in the other dose groups. The number of pregnant animals per group and preimplantation losses were comparable amongst all groups. Single total litter losses occurring amongst the control group, at 20 and at 100 mg/kg bw/d were considered to be unrelated to treatment and intergroup comparisons were restricted to dams with viable youngs. 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/d group and three effects from two litters in the 100 mg/kg bw/d group. Effects were considered to be unrelated to treatment as the highest dose group did not show major malformations. Minor anomalities were significantly increased in the highest dose group, however not considered treatment related by the authors. The SCCP considered 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 was derived for maternal toxicity and for embryotoxicity.

(Palmer, 1979b)

In a study in Sprague Dawley rats (7 females/group) Kojic acid at 50 µg/day in 0.1 ml propylene glycol was given orally from day 1 to 5 of pregnancy. Animals of the control group received the vehicle alone. 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 by the authors that Kojic acid possesses anti-implantation, abortifacient and

embryotoxic effects. The SCCP considered the study of limited value because of its limited description.

(Choudhary *et al.*, 1992)

In another teratogenicity study, Kojic acid (53758) in 0.5% methylcellulose was administered to mated female Wistar rats (221-283 g) daily by oral gavage at 0, 100, 300, 1000 mg/kg bw/d from day 6 to 17 post-coitum. The study was performed according to the ICH guideline "S5 Detection of Toxicity to Reproduction for Medicinal Products" (1993). Termination of the animals was performed on day 20, foetuses were removed by hysterectomy and females examined macroscopically. No deaths occurred in any group and no clinical signs were observed in the female rats. Furthermore, 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 considered related to the test substance by the authors. No relevant macroscopic findings were recorded at necropsy of the females from any group. The numbers 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 test substance related, since the effect was not dose-dependent. 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 furthermore 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. The SCCP commented that only six females per group were investigated.

(Richard, 1998)

SCCS comment
No new data was submitted or identified from the open literature.

SCCS overall comment on reproductive toxicity
The SCCS agrees with the former Opinion that 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 of mothers was reported after delivery in two studies where rats received 50 µg Kojic acid daily for 21 consecutive days before mating (males) or from day 1 to day 5 of gestation (females). This effect, however, was not reported by other authors and its relevance is unclear.

3.3.6 Mutagenicity / genotoxicity

3.3.6.1 Mutagenicity / genotoxicity *in vitro*

A summary of available *in vitro* data on mutagenicity / genotoxicity of Kojic acid is included in Annex 1, Table 2.

3.3.6.2 Mutagenicity / genotoxicity *in vivo*

A summary of available *in vivo* data on mutagenicity / genotoxicity of Kojic acid is included in Annex 2, Table 3.

SCCS overall comment on mutagenicity / genotoxicity
Since 2012, only one additional paper has been found on mutagenicity testing of Kojic acid. In this paper (Ogiwara *et al*., 2015) micronucleus and comet assays were performed in male rats administered orally with 250, 500 and 1000 mg/kg/day for 14 days, and at 125, 250 and 500 mg/kg/day for 28 days. As a result, no increased frequencies of micronuclei or DNA damage in comet assay were observed in bone marrow, peripheral blood leucocytes or liver. After re-evaluation of the available literature data, the conclusion from the SCCS/1481/12 opinion is still valid. It therefore can be concluded that:

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.***

3.3.7 Carcinogenicity

A summary of newly identified data on carcinogenicity of Kojic acid since the opinion SCCS/1481/12 is included in Annex 3, Table 4.

A summary of views presented by different scientist groups/committees on relevance of rodent thyroid tumor data after exposure to Kojic acid for humans can be found in Annex 4, Table 5.

SCCS overall comment on carcinogenicity
Since 2012, additional relevant studies have been identified on carcinogenicity testing of Kojic acid. In the study by Higa *et al*. (2007) in medium-term carcinogenesis test in rats, 2.0% Kojic acid was orally given to F344/DuCrj rats for 4 weeks of the initiation period, followed by the combination of partial hepatectomy and treatment with a hepatocarcinogenesis promoter, phenobarbital. Although the numbers of GSTP-positive foci of two cells or more and 0.1 mm or more in diameter increased slightly, it is suggested that the observed slight increase was the effect of promotion activity of Kojic acid rather than the initiation activity. In support, no clear adducts derived from Kojic acid were detected in the livers of the Kojic acid exposed animals.

The results of Higa *et al*. (2007) indicate that Kojic acid did not have initiation nor promotion activity of skin carcinogenesis. In the skin carcinogenesis bioassay for initiation-promotion potential, 3.0% Kojic acid cream formulation was applied to the back of the mice for 1 week (once a day, total 7 times) and for 19 weeks (5 times a week, total 95 times) during the initiation and the promotion stages, respectively. No skin nodules were observed in any animal skins formed due to Kojic acid treatment given in either stage.

The results of the study by Chusiri *et al.* (2011) indicated that Kojic acid administered in the diet (up to 2%) did not have initiation effects on rat hepatocarcinogenesis, but did promote hepatocarcinogenesis. Thus, the results suggest that Kojic acid is a non-genotoxic hepatocarcinogen in rats.

After re-evaluation of the available literature data and opinions from different scientist groups/committees on relevance of rodent thyroid tumor data, the SCCS concludes that:

Thyroid tumorigenesis by Kojic acid

1. The positive findings from the *in vitro* genotoxicity tests were not confirmed *in vivo,* thus Kojic acid has been considered to have no genotoxic potential *in vivo*. It was shown not to form DNA-adducts.
2. Kojic acid induces thyroid follicular-cell tumours in rodents by interfering with thyroid hormone homeostasis (most probably by hampering iodine uptake, less likely by UDP-glucuronylation of T4). Experimental data underline the importance of TSH signaling in the development of thyroid malignancies in animals after exposure to Kojic acid. There is some evidence that humans are less sensitive than rodents with regard to perturbation of thyroid hormone homeostasis.
3. Although it can be assumed that Kojic acid could interfere with thyroid hormone homeostasis in humans, there are currently no compound-specific quantitative data available to substantiate this assertion.
4. The margin of safety can be applied for Kojic acid and can be based on thyroid-pituitary disruptive effects themselves (with consequently observed changed colloid content or development of follicular cell hypertrophy/hyperplasia), in lieu of tumor effects.

Liver tumorigenesis by Kojic acid

1. Some chemicals that cause thyroid tumours in rats or mice and have no detectable genotoxic activity often also produce hepatocellular tumours, particularly in mice. A correlation has been established between potency for hepatic microsomal enzyme induction and capacity for tumour promotion in rat liver by enzyme inducers of the phenobarbital type (CYP2B1, CYP2B2) (McClain & Rice, 1999).
2. The data on liver microsomal enzyme induction by Kojic acid are rather scarce. It was proposed that Kojic acid treatment at high doses in the promotion stage can induce overexpression of P450, such as CYP2B1 (Chusiri *et al*., 2011). This may contribute to an increase of 8-OHdG formation through ROS, which then promotes an increase of cell proliferation and finally increased induction of GST-P positive foci (a biomarker of early stages of liver carcinogenesis).
3. Kojic acid has no initiation activity on rat hepatocarcinogenesis, while high doses may exert promotion activity, showing the existence of a possible threshold for rat.
4. The human relevance of the observations on rat hepatocarcinogenesis is not clear, but rather implausible under the normal cosmetic use of Kojic acid. Hence, the effect was not considered by the SCCS while calculating MoS.

3.3.8 Photo-induced toxicity

3.3.8.1 Phototoxicity / photo-irritation and photosensitisation

From SCCP/1182/08

In a phototoxicity text in 10 male guinea pigs the induction was performed with 5% Kojic acid in 0.2 ml absolute alcohol during five consecutive days to the shaven dorsal neck region. After each induction guinea-pigs were irradiated with UV-light (wavelength 300-420nm) located twelve inches away from the skin for 15 minutes. Challenge was performed on the same area with 1% Kojic acid in 0.2 ml absolute alcohol after a 10-day resting

period, followed by 15 min of UV-irradiation and assessed for the presence of erythema after 0, 24, 48 and 72 hours. 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. The SCCP commented that Kojic acid was not photosensitising, yet slightly photoirritant.

(Elliot & Seaber, 1978)

A phototoxicity test in 10 male albino guinea pigs was performed using two patches of Whatman paper with Kojic acid (5% test substance (w/v) in 0.5 ml absolute alcohol) placed on the abraded skin of the animals. One of the application sites was protected from UV-light using aluminium foil. Next, guinea pigs were irradiated with UV-light (wavelength 300-420nm) six inches away from the skin for 30 minutes. The procedure was repeated daily for five consecutive days. No dermal reactions were observed after treatment without irradiation (occluded patch) in all animals. The treated and unoccluded site showed slight erythema in 3/10 animals on isolated occasions. One animal developed a slight erythema which persisted over two days. The authors concluded that under the test conditions, Kojic acid was reported to be not or slightly photoirritative.

(Elliot & Seaber, 1978)

SCCS comment
No new data was submitted or identified from the open literature.

SCCS overall comment on photo-irritation and photosensitisation
The SCCS agrees with the former Opinion that Kojic acid is slightly photoirritative. The substance is not photosensitising.

3.3.8.2 Photomutagenicity / photoclastogenicity

From SCCP/1182/08
A photomutagenicity test (OECD 471, GLP compliant) in E. coli WP2 (Trp+) was reported with Kojic acid (8A44; 100% purity) in DMSO at 0, 33, 100, 333, 1000, 2500, 5000 µg/plate (+/- S9). The test was performed in triplicate, for two independent tests. Irradiation was performed using a metal halogenide light source which emits a spectrum simulating sunlight. A pre-experiment determined an optimal irradiation dose to be 10 seconds at 10 mJ/cm^2 UVA and 0.5 mJ/cm^2, leading to the number of revertant colonies to be approximately twice the number of spontaneous revertants without irradiation in the WP2 strain. After incubation, revertant colonies are counted to measure both photomutagenicity and phototoxicity of the irradiation. 8-Methoxypsoralen served as positive control and cultures treated with solvents as negative controls. 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. Within the scope of this assay and under the conditions used in this study, irradiation with artificial sunlight was concluded to have no relevant influence on the mutagenic potential of Kojic acid.

(Wollny, 1998)

New data identified from the open literature
One study investigated photomutagenicity/photogenotoxicity in three test systems with a bacterial gene mutation assay, chromosomal aberrations test in CHL cells and micronuclei in skin of HR-1 male mice. The plate method was applied in the absence or presence of UV

irradiation (sunlight simulator SOL500; transmitting 50% of light at 335 nm). A slight increase of revertant colonies was observed in *S. typhimurium* TA102 strain and *E. coli* WP2/pKM101 in the UV irradiation groups as compared with the groups without UV irradiation but not in strain TA98. No statistically significant increase of CHL cells with structural aberration of chromosome or polyploid cells was observed at any dose level without UV irradiation. With UV irradiation, cells with structural aberration of chromosomes showed a statistically significant increase (frequency of occurrence: 40.0%) at high dose (1.4 mg/mL) and a statistically significant increase of polyploid cells (frequency of occurrence: 3.8%) was observed at medium dose (0.70 mg/mL). In mice, the frequency of MN in Kojic acid groups did not increase significantly in epidermal cells with/without light irradiation condition at any dose levels compared with the negative control.

(Higa *et al*., 2007)

SCCS comment
Kojic acid induced a weak photo-mutagenic effect in photo-reverse mutation assay with S typhimurium TA102 and *E. coli* WP2/pKM101, however, in TA102 the fold increase was less than 3. In the lack of historical negative control values the results can be treated as equivocal. There is some evidence that Kojic acic can induce chromosome aberration at high dose with light irradiation in the photo-chromosome aberration assay in cultured CHL cells although not without light irradiation. No photoclastogenic effect was observed in mice skin exposed to Kojic accid with or without irradiation.

SCCS overall comment on photomutagenicity/photoclastogenicity
UV irradiation with artificial sunlight has no relevant influence on the mutagenic effect in combination with Kojic acid in bacterial gene photo-mutation assays. There is some evidence that Kojic acic can induce chromosomal aberrations at high dose with light irradiation in the photo-chromosome aberration assay in cultured CHL cells although not without light irradiation. However, no signifficant increase in MN frequency was found in epidermal cells of mice in Kojic acid groups with/without light irradiation at any dose levels compared with the negative control.

3.3.9 Human data

Human data has been identified and discussed under section 3.3.2. Skin sensitisation.

3.3.10 Special investigations

3.3.10.1 Assessment of endocrine disrupting potential

The applicant argued that the toxicological effects of Kojic acid on the thyroid gland, derived from the observation that hepatic function is elevated and thyroid hormones are lost in response to hepatic injury, are not occurring in humans. Because the effects are judged to be irrelevant to humans, it was concluded by the applicant that there is no issue with respect to the endocrine-disrupting potential of Kojic acid.

Furthermore, argumentation was provided with respect to the general mechanisms of thyroid tumorigenesis, enzyme induction in liver microsomes, and Kojic acid specific mechanisms of thyroid tumorigenesis (Annex 5).

A break-down of all the available data (summerised in Annex 6, Table 6) according to the OECD conceptual framework for testing and assessment of endocrine disruptors (EDs) can be done as follows (OECD, 2018):

3.3.10.1.1 Non-test information, *in silico*, read across, *in chemico*

No data available for Kojic acid.

3.3.10.1.2 *In vitro* assays

No data available for Kojic acid.

3.3.10.1.3 *In vivo* assays that provide data about selected endocrine mechanism(s) / pathway(s)

No data available for Kojic acid.

3.3.10.1.4 *In vivo* adverse effects on endocrine relevant endpoints

None of the available studies were performed in compliance with recognised standards or guidelines (*e.g.* OECD). Nevertheless, thyroid and liver related effects could be identified in rodents with a clear trend towards a decrease in serum T3/T4 levels followed by a compensatory increase in TSH release with the consequence of thyroid cell proliferation (Annex 6, Table6). Histopathological examination of the thyroid of rodents exposed to Kojic acid generally show significantly increased weight. The available data indicates that the mechanism by which Kojic acid interferes with the thyroid hormone homeostasis in rodents acts independently from T4-uridinediphosphate glucuronosyltransferase (T4-UDP-GT) (Mitsumori *et al.*, 1999; Tamura *et al.*, 1999a) and likely disturbs the synthesis of thyroid hormones by suppressing the iodine uptake as well as the organification thereof (Tamura *et al*., 1999b; Higa *et al*., 2002). Other mechanisms (*e.g.* inhibition of TPO, SULT expression, hormone release etc.) underlying the changes in thyroid hormone levels after Kojic acid administration have not been studied (Bartsch *et al*., 2018).

SCCS comment
Even though none of the available studies were performed in compliance with recognised standards or guidelines (*e.g.* OECD), the available data provides an indication of HPT-axis disturbances and resulting serum thyroid hormone changes in rodents. However, uncertainty about the mechanisms underlying these changes remains. Few alternative non-animal assays are available to study the molecular targets of the thyroid. Currently, no OECD *in vitro* regulatory guidelines to test chemical interactions with molecular initiating events (MIEs) in the thyroid axis are established.

Recently the US EPA established an Adverse Outcome Pathway (AOP) network that links the accepted chemical targets of thyroid activity to down-stream adverse out-comes (Noyes *et al*., 2019). This work mapped out the differences and similarities in thyroid toxicity pathways between species, sometimes leading to different adverse outcomes, and thereby aids cross-species extrapolation in safety assessment. Based on this analysis, the US EPA concluded that for decision-making purposes, serum thyroid hormone changes provide a clear indication of altered thyroid homeostasis with the potential to adversely affect development. This could be especially relevant when the thyroid feedback systems are yet

to be fully developed and thyroid hormone reserves, critical to neurological development, are low (reviewed by de Escobar *et al.* 2004; Skeaff, 2011; Williams, 2008; and Zoeller & Rovet, 2004). The SCCS agrees with this conclusion.

As far as thyroid tumour induction is concerned, a tumour-promoting effect based on hormonal disruption has been observed. The US EPA study (Noyes *et al*., 2019) came to the following conclusion: *'Elevated TSH in rodents leads to thyroid hypertrophy and potential thyroid cancer, an adverse outcome that has limited relevance to human thyroid cancer due to species differences in sensitivity' (*Capen and Martin 1989; EC 2017; Hurley 1998; McClain *et al*.,1988). The same conclusion was also made previously by several expert groups (Capen *et al*., 1999; EU Commission group of Specialised Experts, 1999).

Taken together, the SCCS is of the opinion that changes in serum thyroid hormone levels observed in animal studies are evidence of endocrine effects and cannot be disregarded as such. Furthermore, safety assessment and/or regulatory decisions based on the endocrine effects in animals are protective for humans against down-stream (non-cancer) effects, such as developmental neurotoxicity.

SCCS overall conclusion on ED properties
Based on histopathological findings and altered iodine uptake in rats, a NOAEL of 6 mg/kg bw/day remains appropriate for risk assessment of Kojic acid.

Based on the findings of several research groups, it is apparent that differences are being measured between rodents and humans with respect to sensitivity to thyroid toxicity. What this means in quantitative terms is not very clear and therefore the interpretation of the results remains rather speculative. According to the SCCS Notes of Guidance (SCCS/1628/21), in case of different susceptibility to HPT-axis disturbances in rats and humans, a change of the inter-species toxicodynamic default factor may be necessary. In the case of Kojic acid, the SCCS decided not to reduce this factor because there is currently no compound-specific quantitative data available for humans that allows for this reduction.
Recently, it could be demonstrated for pharmaceutical compounds with thyroid disturbing effects that organ-on-chip devices, applied in a human/rodent comparative study involving liver and thyroid follicle cells, provide the possibility to derive mechanistic data which allow interpretation of the observations made. The SCCS is of the opinion that this new NAM methodology opens possibilities for retrieving essential mechanistic information for compounds like Kojic acid that could provoke species-depending HTP-axis disturbances.

3.3.10.2 Toxicogenomics

From SCCP/1182/08
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. 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 downregulated genes were identified as tumour suppressor genes in melanoma cancer cells.

(Cheng *et al.*, 2006)

3.3.10.3 Immunomodulatory potential of Kojic acid

New data identified from open literature
The influence of Kojic acid on functional properties related to macrophage activation was studied in a further *in vitro* study using 50 µg/ml Kojic acid. One hour of incubation of macrophages with Kojic acid showed enhanced cell spreading and an increase in cell surface exposure, associated with a rearrangement of microtubules, actin filaments and intermediate filaments. A further increase in phagocytic activity towards yeast was detected, when compared to untreated cells. ROS (reactive oxygen species) production was heightened in the presence of Kojic acid, but not the NO (nitric oxide) production. Cell viability of macrophages was furthermore not affected following Kojic acid treatment. The authors concluded that Kojic acid was shown to modulate macrophage activation through several mechanisms.

(Rodrigues *et al.*, 2011)

The ability of Kojic acid to influence innate immune responses was studied *in vitro* using human peripheral blood monocytes. Kojic acid (50 µg/mL) was added to a culture of purified monocytes isolated from human blood. After 48 hours of exposure, cultures were analyzed by light microscopy, scanning electron microscopy, transmission electron microscopy and flow cytometry. Treatment with the test substance induced morphological alterations in monocytes, such as increased cell size, as well as numerous cellular projections. Increased labeling of cell surface EMR1-F4/80 was detected using the flow cytometer but labeling of CD11b and CD14 was decreased. Kojic acid exposure was found to increase IL-6 cytokine production but did not cause cytotoxic effects in monocytes. The authors concluded that Kojic acid promotes the differentiation of monocytes into macrophages thus has the ability to act as an immunomodulatory agent.

(Da Costa *et al.*, 2018)

SCCS comment
Studies on the immunomodulatory potential of Kojic acid are of limited importance to the overall risk assessment of the substance.

3.4 SAFETY EVALUATION (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

The calculation of the systemic exposure dose (SED) was carried out using data from a clinical percutaneous absorption study, as described in section 3.2.4. As point of departure for risk assessment, a NOAEL of 6 mg/kg bw/day, based on a 28-day oral repeated dose rat study is used (see section 3.3.4.1). Since the point of departure is based on a subacute 28-day study, an additional assessment factor of 3 was added to the risk assessment to extrapolate to a subchronic 90-day study, in accordance with the SCCS Notes of Guidance (SCCS/1628/21). Furthermore, as Kojic acid is well absorbed after oral exposure, no correction for oral bioavailability is used, resulting in an adjusted NOAEL of 2 mg/kg bw/day. Following MoS calculations for separate product types and aggregated exposure can be calculated:

Area of application	SED (mg/kg bw/day)	Adjusted NOAEL (mg/kg bw/day)	MoS
Face+neck	0.0075	2	**267**
Hands	0.0067	2	**199**
Aggregate (face+neck+hands)	0.0142	2	**141**

Although it can be assumed that Kojic acid after exposure at a sufficient dose for a sufficient time can potentially also interfere with thyroid hormone homeostasis in humans, there are currently no compound-specific quantitative data available to substantiate this assertion. Therefore a change of the interspecies toxicodynamic default factor of 2.5 has not been applied in the case of Kojic acid.

3.5 DISCUSSION

Physicochemical properties

A full report of the chemical characterisation of Kojic acid in terms of purity and identity in representative batches should be provided and the validity of the analytical methodologies used must be shown. Hazardous impurities like heavy metals and aflatoxins may be present and should be kept at trace levels under continuous monitoring. No reports on the stability of the test substance in test solutions and products in the marketplace were submitted. In many cases, the purity of the test substance was not reported.

Exposure assessment & Toxicokinetics

To estimate the SED, SCCS used the 95th percentile of AUC values (0-24h) obtained in a clinical study (Fukase 2005) after single application of a cream containing 1% Kojic acid. SCCS used the amount measured in plasma to calculate the SED as, in contrast to the *in vitro* dermal absorption study, the elimination of Kojic acid within 24h is taken into consideration using this approach.

Based on the available information, the SCCS considers that Kojic acid is well absorbed after oral exposure, and therefore will not correct the oral POD used for the MoS calculation to take into account oral bioavailability.

The resulting SED values are 0.0075mg/kg bw/day (face & neck), 0.0067 mg/kg bw/day (hands) and 0.0142 mg/kg bw/d (aggregate exposure).

Toxicological Evaluation

Irritation and corrosivity

Kojic acid was not an irritant to rabbit skin or mucous membranes.

Skin sensitisation

Kojic acid was not considered to be a skin sensitiser in guinea pigs. In humans, the occurrence of allergic contact dermatitis from Kojic acid is very low.

Acute toxicity

No new data were submitted or identified. Acute toxicity of Kojic acid is low. Mean LD50 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 LD50 is 2400 mg/kg bw for rats and 2600 mg/kg bw for mice.

Repeated dose toxicity

No new repeated dose data were submitted or identified. The most conservative NOAEL that can be identified from repeated dose studies with Kojic acid is based on the reduced uptake of ^{125}I, as well as the changed amount of colloid in thyroid follicles and follicular cell hypertrophy at a dose level of 23.8 mg/kg bw/day in rats (28-day, oral), leading to a NOAEL of 6 mg/kg bw/day.

Reproductive toxicity

No new data were submitted or identified for 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.

Mutagenicity / genotoxicity/photomutagenicity/photoclastogenicity

The available data has been re-evaluated together with new data identified from the open literature. The positive findings from the *in vitro* tests with Kojic acid could not be confirmed with *in vivo* tests. 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.
Kojic acid with UV irradiation induced a weak photo-mutagenic effect in photo-reverse mutation assay in *E. coli* WP2/pKM101 and S. typhimurion TA102 but not in TA98. In the lack of historical negative control values the SCCS considers these result as equivocal. There is some evidence that Kojic acic can induce chromosomal aberrations at high dose with light irradiation in a photo-chromosome aberration assay in cultured CHL cells, although not without light irradiation. However, no signifficant increase in MN frequency was found in mouse epidermis after exposure to Kojic acid with or without light irradiation at any dose levels compared with the negative control.

Carcinogenicity

The positive findings from the *in vitro* genotoxicity tests were not confirmed *in vivo,* thus Kojic acid has been considered to have no genotoxic potential *in vivo*. Kojic acid induces thyroid follicular-cell tumours in rodents by interfering with thyroid hormone homeostasis (most probably by hampering iodine uptake, less likely by UDP-glucuronylation of T4). Experimental data underline the importance of TSH signaling in the development of thyroid malignancies in animals after exposure to Kojic acid. There is some evidence that humans are less sensitive than rodents with regard to perturbation of thyroid hormone homeostasis.
Although it can be assumed that Kojic acid could interfere with thyroid hormone homeostasis in humans, there are currently no compound-specific quantitative data available to substantiate this assertion.
A correlation has been established between potency for hepatic microsomal enzyme induction and capacity for tumour promotion in rat liver by enzyme inducers of the phenobarbital type (CYP2B1, CYP2B2). The data on liver microsomal enzyme induction by Kojic acid are rather scarce. It was proposed that Kojic acid treatment at high doses in the promotion stage can induce overexpression of P450, such as CYP2B1. This may contribute to an increase of 8-OHdG formation through ROS, which then promotes an increase of cell

proliferation and finally increased induction of GST-P positive foci (a biomarker of early stages of liver carcinogenesis).
Kojic acid has no initiation activity on rat hepatocarcinogenesis, while high doses may exert promotion activity, showing the existence of a possible threshold for rat. The human relevance of the observations on rat hepatocarcinogenesis is not clear, but rather implausible under the normal cosmetic use of Kojic acid.

Photo-induced toxicity

Kojic acid was slightly photoirritant. The substance was not photosensitising.

Human data

Considering all information in humans, the SCCS is of the opinion that the occurrence of allergic contact dermatitis from Kojic acid is very low.

Special investigation: assessment of endocrine disrupting potential (including human data)

Re-analysis of available repeated dose studies confirmed the conclusion of previous SCCS Opinions that Kojic acid exposure in rats is associated with a decrease in serum T3/T4 levels followed by a compensatory increase in TSH release with the consequence of thyroid cell proliferation. Increased TSH levels in rodents provide indication of a chemical with the ability to perturb the HPT-axis in different species, including humans.
Whilst for some chemicals it is possible to judge thyrotoxic effects in rodents as irrelevant to humans based on comprehensive mechanistic data, such data is limited for Kojic acid. Novel tools are currently being developed to help evaluate how xenobiotics may interfere with thyroid hormone homeostasis. A multi-organ-chip model, based on 3D-Co-culture of human liver cells and thyroid follicles, shows promising results as both direct effects on the thyroid gland as well as indirect effects mediated by the liver, can be explored (Kühnlenz *et al*., 2019). Comparison of such multi-organ-chip model, based on human cells, with an equivalent model, based on rodent cells, has the potential to elucidate the relevance of the different findings between humans and rodents and could contribute substantially to our mechanistic knowledge of this complex interaction in the future (Boehm *et al*., 2019).

4. CONCLUSION

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of Kojic acid, does the SCCS consider Kojic acid safe when used in cosmetic products up to a maximum concentration of 1 %?*

   On the basis of the safety assessment, and considering the concerns related to potential endocrine disrupting properties of Kojic acid, the SCCS is of the opinion that Kojic acid is safe when used as a skin lightening agent in cosmetic products at concentrations of up to 1%.

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of Kojic acid in cosmetic products?*

   /

3. *Does the SCCS have any further scientific concerns with regard to the use of Kojic acid in cosmetic products?*

   As Kojic acid is sometimes added to peeling agents, a weakened skin barrier may be of additional concern because of greater dermal absorption.

   Only the topical use of Kojic acid in cosmetics has been considered in this Opinion. Other uses (*e.g.* food) of natural or synthetic sources have not been considered.

   As far as the derivatives of Kojic acid are concerned, *e.g.* esters of Kojic acid such as Kojic acid dipalmitate and Kojic acid isopalmitate, and derivatives such as chloro-Kojic acid, these have not been included in this Opinion as no data has been submitted.

5. MINORITY OPINION

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6. REFERENCES

Anon (1980). Teratogenicity Study of Kojic Acid. Effect of Kojic Acid on Reproduction of Mice. Study by administration during the fetal organogenic period. Environmental Health Bioresearch Center Co., Ltd., unpublished report.

Axelrod, A.A. & Leblond, C.P. (1955): Induction of thyroid tumors in rats by low iodine diet. *Cancer* 8: 339-367.

Bartsch, R. *et al*. (2018). Human relevance of follicular thyroid tumors in rodents caused by non-genotoxic substances. *Regulatory Toxicology and Pharmacology*, 98, 199-208.

Bjeldanes, L. F., & Chew, H. (1979). Mutagenicity of 1, 2-dicarbonyl compounds: maltol, kojic acid, diacetyl and related substances. *Mutation Research/Genetic Toxicology*, 67(4), 367-371.

Boehm, *et al*. (2019, September). Development of a rodent liver-thyroid 2-Organ-Chip for 23 thyroid toxicity testing. [EUROTOX, Poster presentation].

Borzelleca, J. F. *et al.* (1987). Lifetime toxicity/carcinogenicity study of FD & C Red No. 3 (erythrosine) in rats. *Food and Chemical Toxicology*, 25(10), 723-733.

Brendler-Schwaab, S. & Krämer-Bautz, B. (2004). Kojic Acid: Comet test *in vivo* in male rat liver, stomach and colon. Bayer Healthcare AG, Toxicology Department, Report No AT01715, December 17, unpublished report.

Burdock, G.A. *et al.* (2001). Evaluation of Health Aspects of Kojic Acid in Food. *Regulatory Toxicology and Pharmacology*, 33, 80-101.

Burnett C.L., *et al*. (CIR) (2010). Final report of the safety assessment of kojic acid as used in cosmetics, *International Journal of Toxicology*, 29 (6_suppl) 244S–273S.

Cabanes J *et al*. (1994). Kojic Acid, a Cosmetic Skin Whitening Agent is a Slow-binding Inhibitor of Catecholase Activity of Tyrosinase. *Journal of Pharmacy and Pharmacology*, 46; 982-985.

Capen, C. C., & Martin, S. L. (1989). The effects of xenobiotics on the structure and function of thyroid follicular and C-cells. *Toxicologic Pathology*, 17(2), 266-293.

Capen, C. C. *et al.* (1999). Species differences in throid, kidney and urinary bladder carcinogenesis: proceedings of a workshop; November; Lyon, France. Lyon, France: International Agency of Cancer Research (IARC). IARC scientific publications, (147).

Capen, C. C. (1996): Hormonal imbalances and mechanisms of chemical injury of thyroid gland. In Monographs on Pathology of Laboratory Animals: Endocrine System. 2nd ed. (TC Jones, CC Capen and U. Mohr, Eds) pp. 217 238 Springer Verlag. Berlin.

Cheng, S.-L. *et al.* (2006). Toxicogenomics of Kojic Acid on Gene Expression Profiling of A375 Human Malignant Melanoma cells. *Bioliological and Pharmaceutical Bulletin*, 29(4) 655-669.

Choudhary, D.N. *et al.* (1992). Effect of Some Mycotoxins on Reproduction in Pregnant Albino Rats. *Journal of Food Science and Technology*, 29,4, 264-265.

Choudhary, D.N. (1994). Sahay GR and Singh JN. Antifertility and cannibalistic properties of some mycotoxins in albino rats. *Journal of Food Science and Technology*, 31, No. 6, 497-49.

Chronic Toxicity Test and Recovery on Kojic Acid in Male Rats. Kyudo Company Ltd., Dept of Toxicology, April 1980, unpublished report.

Chusiri, Y. *et al.* (2011). Modulation of Cytochrome P450 Expression by Kojic Acid in Rats. *Thai Journal of Toxicology*, 26(2), 93-93.

Curran, P.G. & DeGroot, .L.J. (1991). The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. *Endocrine Reviews,* 12, 135-150.

Da Costa, J. P. *et al.* (2018). Biological effects of kojic acid on human monocytes *in vitro*. *Biomedicine & Pharmacotherapy*, 101, 100-106.

Davies, D.J. (2011) Kojic Acid - *In vitro* Percutaneous Absorption of [14C]-Kojic Acid in a Leave-on Skin Care Formulation through Human Dermatomed Skin. Study Number QD0849/003. Report JV2136-REG, issued 24/01/2011.

de Escobar, G. M. *et al.* (2004). Maternal thyroid hormones early in pregnancy and fetal brain development. *Best Practice & Research Clinical Endocrinology & Metabolism*, 18(2), 225-248.

EC. (2017). Supporting the Organisation of a Workshop on Thyroid Disruption—Final Report. European Commission, Framework Contract ENV. A.3/FRA/2014/0029 on implementation of the Community strategy on Endocrine Disrupters. https://publications.europa.eu/en/publication-detail/-/publication/472d2c88-a8b1-11e7-837e-01aa75ed71a1/language-en [accessed 6July2021].

ECHA (28/05/2021) Substance infocard 5-hydroxy-2-hydroxymethyl-4-pyrone. ECHA. https://echa.europa.eu/substance-information/-/substanceinfo/100.007.203

Eisenberg, R. & Frank, J. (2006). Repeated Insult Patch Test with Sample 1:DWHC I (Cream). Study number C06-0308.01.

Elliot, P.H. & Seaber, J.A. (1978). Screening test for Delayed Contact Photohypersensitivity with Kojic Acid in the Albino Guinea Pig. Huntingdon Research Centre, Report No 9931/D16/78, 8.12.1978, unpublished report.

Elliot, P.H. & Seaber, J.A. (1978). Effect of Ultraviolet Light on Skin Treated with Kojic Acid in the Albino Guinea Pig. Huntingdon Research Centre, Report No 9933/D28/78, 11.12.1978, unpublished report.

EU Commission Group of Specialiced Experts in the field of Carcinogenicity, Mutagenicity and Reprotoxicity (1999). Non-genotoxic thyroid carcinogens in rodent bioassay. ECB1/49/99. Ispra.

EU Commission Group of Specialised Experts in the fields of Carcinogenicity, Mutagenicity and Reprotoxicity (1999) Non-genotoxic thyroid carcinogens in the rodent bioassay, ECBI/49/99 Add.1 Rev.2.

Feltes, M. (1997). Acide kojique. Test du micronoyeau *in vitro* sur lignees cellulaires SVK14 et HepG2. L'OREAL Life Sciences Research, Genetic Toxicity Dept., Aulnay, France, August 28, unpublished report.

Fujimoto, N. *et al*. (1999). Changes in thyroid function during development of thyroid hyperplasia induced by kojic acid in F344 rats. *Carcinogenesis* 20, 8, 1567-1571.

Fujimoto, N. *et al*. (1998). Induction of thyroid tumors in (C57BL/6N x C3H/N)F1 mice by oral administration of kojic acid. *Food and Chemical Toxicology*, 36, 697 703.

Fukase, H. (2005). Percutaneous absorption study of Kojic Acid in humans. CPC Clinic, Medical facility, Kagoshima, Japan, April 28, unpublished report.

Furth, J. (1954): Morphologic changes of associated with thyrotropin secretary tumors. *American Journal of Pathology,* 30, 421-463.

García-Gavín, J. *et al*. (2010). Pigmented contact dermatitis due to kojic acid. A paradoxical side effect of a skin lightener. *Contact Dermatitis*, 62(1), 63-64.

Gopinath, C. *et al*. (1987). The endocrine glands. In Atlas of Experimental Toxicological Pathology (C. Gopinath, DE. Prentice, and DJ. Lewis, Eds.), pp. 104 121. MTP Press Limited. Lancaster.

Higa, Y. *et al*. (2007). Kojic acid-absence of tumor-initiating activity in rat liver, and of carcinogenic and photo-genotoxic potential in mouse skin. *The Journal of Toxicological Sciences*, 32(2), 143-159.

Higa, Y. *et al*. (2000). Studies on thyroid function in rats subjected to repeated oral administration with kojic acid. *The Journal of Toxicological Sciences*, 25(3), 167-175.

Higa, Y. *et al*. (2002). Effects of kojic acid on thyroidal functions in rats by single-dose administration and in cultured rat thyroid cells (FRTL-5 cells). *The Journal of Toxicological Sciences*, 27(5), 423-431.

Hill, R. N. *et al*. (1989). Thyroid follicular cell carcinogenesis. *Toxicological Sciences*, 12(4), 629-697.

Hill, R. N. *et al*. (1998). Risk assessment of thyroid follicular cell tumors. Environmental Health Perspectives, 106(8), 447-457.

Honarvar, N. (2001) .Micronucleus assay in bone marrow cells of the mouse with kojic acid. RCC-CCR project number 696303, unpublished report.

Hood, A., & Klaassen, C. D. (2000). Differential effects of microsomal enzyme inducers on *in vitro* thyroxine (T4) and triiodothyronine (T3) glucuronidation. *Toxicological Sciences*, 55(1), 78-84.

Hurley, P. M. (1998). Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. *Environmental Health Perspectives*, 106(8), 437-445.

IARC Monograph on the Evaluation of Carcinogenic Risks to Humans. Some Thyrotropic Chemicals.Vol. 79, 10-17 October 2000.

IARC Scientific Publications No. 147. (1999). Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis. Ed. C.C. Capen, E. Dybing, J.M. Rice and J.D. Wilbourn. IARC, Lyon.

Irving, H. M. N. H. (2011). Guide to Trivial Names, Trade Names and Synonyms for Substances Used in Analytical Nomenclature.

Ishikawa, S. *et al*. (2006). Characterization of genotoxicity of kojic acid by mutagenicity in Salmonella and micronucleus induction in rodent liver. *Genes and Environment*, 28(1), 31-37.

Iwahara, S. & Sakamoto, K. (1980). Mutation Induction Test of Kojic Acid. Hatano Research Institute, Food and Drug Safety Centre, Study No. 54-079, 54-119, May, unpublished report.

Iwahara, S. (1981) Mutagenicity Study on Kojic Acid. Mutation Induction Test in Chinese Hamster V79 Cells. Food and Drug Safety Centre, Hatano Research Institute, November, unpublished report.

Iwahara, S., (1981). Dominant lethal test of Kojic acid in mice Hatano Research Institute, Food and Drug Safety Center, November, unpublished report.

Jinnai, H. *et al*. (2019). Discussion on the Endocrine Disrupting Potential of Kojic Acid. Sansho Pharmaceutical Co., Ltd. Unpublished report.

Kariya, K *et al*. (1979) Subacute toxicity test of Kojic Acid: 4- and 13-week consecutive oral administration. Department of Pharmacology Kobegakuin University / Pathology Dept Osaka University Medical College, May, unpublished report.

Kennedy, T. H. & Purves, H. D. (1941). Studies on experimental goitre. I: The effect of Brassica seed diets on rats. *British Journal of Experimental Pathology*, 22(5), 241.

Kim, S. B. *et al*. (1987). Desmutagenic effect of α-dicarbonyl and α-hydroxycarbonyl compounds against mutagenic heterocyclic amines. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 177(1), 9-15.

Kontoghiorghes, G. J. *et al*. (1986). *In vitro* screening of iron chelators using models of free radical damage. Free radical research communications, 2(1-2), 115-124.

Kühnlenz, *et al*. (2019, September). Establishment of a Multi-Organ-Chip based identification platform for endocrine disruptors. [Poster presentation].

Kynoch, S.R. *et al*. (1979). The Effect of Repeated Application of Kojic Acid to the Skin of Rabbits for Thirty Days. Huntingdon Research Centre, Report No 7995/SS018 -28.3.79, unpublished report.

Kynoch, S.R. & Ligett M.P. (1978). Irritant Effect of Kojic Acid on Rabbit Skin. Huntingdon Research Centre, Report No. 9556/14D/78, 7.9.1978, unpublished report.

Kynoch, S.R. (1977a). Acute intraperitoneal toxicity to Mice of Kojic Acid, Huntingdon Research Centre, Report No. D6/77/8088, unpublished report.

Kynoch, S.R. (1977b). Acute intraperitoneal toxicity to rats of Kojic Acid. Huntingdon Research Centre, Report No. D3/77/8085, unpublished report.

Kynoch, S.R. (1977c). Acute Oral Toxicity of Kojic Acid to Mice. Huntingdon Research Centre. Rep. No. 8086/D4/77, unpublished report.

Kynoch, S.R. (1977d). Acute Oral Toxicity to Rats of Kojic Acid. Huntingdon Research Centre, Report No. 8084/D1/77, unpublished report.

Kynoch, S.R. (1977e). Acute Subcutaneous Toxicity of Mice to Kojic Acid. Huntingdon Research Centre, Report No. D5/77/8087, unpublished report.

Kynoch, S.R. (1977f). Acute Subcutaneous Toxicity of Rats to Kojic Acid. Huntingdon Research Centre, Report No. D2/77/8083, unpublished report

Leclerc, C. (2002). *In vitro* percutaneous absorption of [14C] Kojic acid in human dermatomed skin; L'Oréal, May, unpublished report.

Lewis Sr, R. J. & Hawley, G. G. (2007). Hawley's condensed chemical dictionary, John Wiley & Sons, Inc. New York, NY, p732.

Lide, D. R. (2007). Handbook of Chemistry and Physics, volume 88th edition. CRC Press, 22(24), 154.

Lloyd, M. (2002). Kojic Acid: Mutation at the hprt locus of L5178Y Mouse Lymphoma Cells Using the Microtitre Fluctuation technique. COVANCE Laboratories Ltd., Harrogate, UK, rep. No. 413/45-D6173, May 16, unpublished report.

Watanabe, M. K. *et al*. (2015). Evaluation of the repeated-dose liver, bone marrow and peripheral blood micronucleus and comet assays using kojic acid. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 780, 111-116.

Manciaux, X. (1998a) - 53758 (KA): Acute Oral Toxicity in Rats. Centre International de Toxicologie (CIT) - Study n° 16692 TAR, unpublished report.

Manciaux, X. (1998b) - 53758: (Kojic Acid) Skin Sensitization Test in guinea pigs (Buehler test) Centre International de Toxicologie (CIT) - Study No 16696 TSG, unpublished report.

Manciaux, X. (1998c) - 53758: (Kojic Acid) Acute Dermal Toxicity in Rats. Centre International de Toxicologie (CIT) - Study No. 16693 TAR, unpublished report.

Marzin, D. (1997) Mutagenicity test on Bacteria (Salmonella typhimurium his-) Using B. N. Ames Technique with 53758. Report No IPL-R970118/53758/L'OREAL, Institut Pasteur de Lille, 31 January, unpublished report.

Mata, T.L. *et al*. (2005). Allergic contact dermatitis due to kojic acid. *Dermatitis*, 16(2), 89; quiz 55-6.

McClain, R. M. *et al*. (1988). Studies on the mode of action for thyroid gland tumor promotion in rats by phenobarbital. *Toxicology and Applied Pharmacology*, 94(2), 254-265.

McClain, R.M. & Rice, J.M. (1999) A mechanistic relationship between thyroid follicular cell tumours and hepatocellular neoplasms in rodents. In: Capen, C.C., Dybing, E., Rice, J.M. & Wilbourn, J.D., eds, Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis (IARC Scientific Publications No. 147), Lyon, IARCPress, pp. 61–68.

Mineshita, T. (1983) Reproduction Study of Kojic Acid – A Study in Mice Treated During Perinatal and Lactation Periods. Environmental Health Bioresearch Center Co., Report No 0461, October 1983, unpublished report.

Mitsumori, K. *et al*. (2000). Rapid induction of uterine tumors with p53 point mutations in heterozygous p53 deficient CBA mice given a single intraperitoneal administration of N ethyl N nitrosourea. *Carcinogenesis* 21, 1039-1042.

Mitsumori, K. *et al*. (1999). Promoting effect of kojic acid due to serum TSH elevation resulting from reduced serum thyroid hormone levels on development of thyroid

proliferative lesions in rats initiated with N-bis(2-hydroxypropyl)nitrosamine. *Carcinogenesis* 20, 1, 173-176.

Morris, H.P. (1955). The experimental development and metabolism of thyroid gland tumors. *Advances in Cancer Research*, 3, 51 115.

Morton, H. E. *et al*. (1945). Toxicity and antibiotic activity of kojic acid produced by Aspergillus luteo-virescens. *Journal of Bacteriology*, 50(5), 579-584.

Nakagawa, M. *et al*. (1995). Contact allergy to kojic acid in skin care products. *Contact Dermatitis*, 32(1), 9-13.

Nakano, M. (2005). DNA adduct formation study of Kojic acid in the liver of rats. Sumika Technoservice Corporation, Osaka, Japan, Study No STS0439, 14 June, unpublished report.

Napalkov, N.P. (1976): Tumors of thyroid gland, in Turusov VS (ed): Pathology of Tumors in Laboratory Animals : Part 2. Vol 1. Tumors of the Rat. IARC Scientific Publication No.6. Lyons, France: IARC, pp. 239-272.

National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 3840, Kojic acid. Retrieved September 14, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/Kojic-acid

Nohynek, G. J. (2006) Kojic acid, Review of Safety Aspects L'Oréal, submission September 2006.

Noyes, P. D. *et al*. (2019). Evaluating chemicals for thyroid disruption: opportunities and challenges with *in vitro* testing and adverse outcome pathway approaches. *Environmental Health Perspectives*, 127(9), 095001.

OECD (2018), Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption, OECD Series on Testing and Assessment, No. 150, OECD Publishing, Paris, https://doi.org/10.1787/9789264304741-en.

Ohshima, M. & Ward, J.M. (1984): Promotion of N-methyl-N-nitrosourea-induced thyroid tumors by iodine deficiency in F344n/NCr rats. *Journal of the National Cancer Institute,*73, 289-296.

Ohshima, M. & Ward, J.M. (1986): Dietary iodine deficiency as a tumor promoter and carcinogen in male F344/NCr rats. *Cancer Research,* 46, 877-883.

Omura, H. & Nonaka, M. (1980). A Study on Mutagenic Activity of Kojic Acid. Kyushu University, Department of Food Chemistry, May, unpublished report.

O'Neil, M. J. (2006). The Merck Index, an Encyclopedia of Chemicals, Drugs and Biologicals. Whitehouse Station: Merck Research Laboratories, Division of Merck and Co.

Onodera, H. *et al.*, (2001). Susceptibility of liver proliferative lesions in heterozygous p53 deficient CBA mice treated with Phenobarbital after initiation of dimethylnitrosamine. *Journal of Toxicologic Pathology* 14, 273 278.

Ota, Y. *et al*. (2009). A 55-week chronic toxicity study of dietary administered kojic acid (KA) in male F344 rats. *The Journal of Toxicolocical Sciences,* 43, 305-313.

Palmer, A. (1979a). Effect of Kojic Acid on Fertility and Early Pregnancy of the Rat. Huntingdon Research Centre, Rep. No. SSO/21/79429, June, unpublished report.

Palmer, A. (1979b). Effect of Kojic Acid on Pregnancy of the New Zealand White Rabbit. Huntingdon Research Centre, Rep. No. SSO/22/79320, April, unpublished report.

Paynter, O. E. *et al*. (1988). Goitrogens and thyroid follicular cell neoplasia: evidence for a threshold process. *Regulatory Toxicology and Pharmacology*, 8(1), 102-119.

Reiss, J. (1986). Detection of genotoxic properties of mycotoxins with the SOS chromotest. *Naturwissenschaften*, 73(11), 677-678.

Richard, J. (1998) - 53758: (Kojic Acid) Preliminary Study for Effects on Embryofetal Development by Oral Administration (gavage) in rats. Centre International de Toxicologie (CIT) - Study No. 16699 RSR, unpublished report.

Ripamonti, E. *et al*. (2018). Endocrine disruption by mixtures in topical consumer products. *Cosmetics*, 5(4), 61.

RIVM report 601516009/2002 Part II, Editors R.Luttik and S.M.G .J.Pelgrom; subpart 2: Follicular Thyroid Tumours in Rodents (M.T.M. van Raaij) with Appendix, pp 27-
42.

Rodrigues, A. P. D., *et al*. (2011). Kojic acid, a secondary metabolite from Aspergillus sp., acts as an inducer of macrophage activation. *Cell Biology International*, 35(4), 335-343.

Roger, R. (1999). 53758: (Kojic Acid) - Four-week study by cutaneous route in rats followed a 2-week treatment free period. Centre International de Toxicologie (CIT) - Study No 17002 TSR, unpublished report.

Saeedi, M. *et al*. (2019). Kojic acid applications in cosmetic and pharmaceutical preparations. *Biomedicine & Pharmacotherapy*, 110, 582-593.

Sansho Seiyaku Co., Ltd. (2001). Absorption, distribution, metabolism, and excretion (ADME) of kojic acid in the revision 2001. 52 pages, unpublished report.

SCCP (Scientific Committee on Consumer Products), Opinion on Kojic acid, 30 September 2008, SCCP/1182/08.

SCCS (Scientific Committee on Consumer Safety), Opinion on Kojic acid, 26-27 June 2012, SCCP/1481/12.

SCCS (Scientific Committee on Consumer Safety), SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation 11th revision, 30-31 March 2021, SCCS/1628/21.

Schulz, M. (2002). *In vitro* chromosome aberration test in Chinese hamster V79 cells with kojic acid. RCC-CCR project number 696302, unpublished report.

SGS Serjeant, E. P. & Dempsey, B. (1979). Ionisation constants of organic acids in aqueous solution. Oxford: Pergamon Press. IUPAC chemical data series, no. 23.

Serra-Baldrich, E. *et al*. (1998). Allergic contact dermatitis from kojic acid. *Contact Dermatitis*, 39(2), 86-87.

SGS laboratories Mycotoxin analysis, 23 May 2003; SGS Multilab, 76178 Rouen, France, Assay Report No D200305/0276.

Shibuya, T. *et al*. (1982). Mutagenicity and dominant lethal test of kojic acid: Ames test, forward mutation test in cultured Chinese hamster cells and dominant lethal test in mice. *The Journal of Toxicological Sciences*, 7(4), 255-262.

Shimo, T. *et al*. (1994). Time course observation of thyroid proliferative lesions and serum TSH levels in rats treated with thiourea after DHPN initiation. *Cancer letters*, 85(2), 141-149.

Shino, T. (1978) Irritation test of Kojic Acid Aqueous Solutions Against the Eye Mucosa of Rabbits. Department of Dermatology, Kyushi University - 14.11.1978, unpublished report.

Skeaff, S. A. (2011). Iodine deficiency in pregnancy: the effect on neurodevelopment in the child. *Nutrients*, 3(2), 265-273.

Sun, R. *et al*. (2021). Kojic acid in fourteen mono-solvents: Solubility data, Hansen solubility parameter and thermodynamic properties. *The Journal of Chemical Thermodynamics,* 152, 106280.

Suzuki, M. *et al*. (1978) Study on the absorption, distribution, metabolism and excretion of 14C kojic acid in rats. Laboratory for Biological Science, Co. Ltd., unpublished report.

Swarm, R. L. *et al*. (1973). Observations on the thyroid gland in rats following the administration of sulfamethoxazole and trimethoprim. *Toxicology and Applied Pharmacology*, 24(3), 351-363.

Takizawa, T. *et al*. (2001). Modifying effects of flumequine on dimethylnitrosamine-induced hepatocarcinogenesis in heterozygous p53 deficient CBA mice. *Journal of Toxicologic Pathology*, 14(2), 135-135.

Takizawa, T. *et al*. (2003). Hepatocellular tumor induction in heterozygous p53-deficient CBA mice by a 26-week dietary administration of kojic acid. *Toxicological Sciences*, 73(2), 287-293.

Takizawa, T. *et al*. (2004). Enhancement of hepatocarcinogenesis by kojic acid in rat two-stage models after initiation with N-bis (2-hydroxypropyl) nitrosamine or N-diethylnitrosamine. *Toxicological Sciences*, 81(1), 43-49.

Tamura, T. *et al*. (1999)a. Inhibition of thyroid iodine uptake and organification in rats treated with kojic acid. *Toxicological Sciences*, 47(2), 170-175.

Tamura, T. *et al*. (1999)b. Time course observation of thyroid proliferative lesions and serum levels of related hormones in rats treated with kojic acid after DHPN initiation. *The Journal of Toxicological Sciences*, 24(3), 145-155.

Tamura, T. *et al*. (2001). Dose-threshold for thyroid tumor-promoting effects of orally administered kojic acid in rats after initiation with N-bis (2-hydroxypropyl) nitrosamine. *The Journal of Toxicological Sciences*, 26(2), 85-94.

Tamura, T. *et al*. (2006). Absence of *in vivo* genotoxic potential and tumor initiation activity of kojic acid in the rat thyroid. *Toxicology*, 222(3), 213-224.

Tejera-Vaquerizo, A., & García-Gavín, J. (2019). Allergic Contact Dermatitis to Kojic Acid. *Actas dermo-sifiliograficas*, 110(3), 243-244.

Tennant, R. W. *et al*. (1995). Identifying chemical carcinogens and assessing potential risk in short-term bioassays using transgenic mouse models. *Environmental Health Perspectives*, 103(10), 942-950.

Tennant, R. W. *et al*. (1996). Evaluation of transgenic mouse bioassays for identifying carcinogens and noncarcinogens. *Mutation Research/Reviews in Genetic Toxicology*, 365(1-3), 119-127.

Vegarra, M.M. (2002). Kojic acid: induction of lacZ mutations in tissues of treated Muta™Mouse. Covance Laboratories, Vienna, USA, No. 23674-0-305, 3 October, 2002, unpublished report.

Volkner, W. (1997) *In vivo / in vitro* unscheduled DNA synthesis in rat hepatocytes with Kojic Acid. Research and Consulting Company (RCC), Study No. 593300, unpublished report.

Wei, C. I. *et al*. (1991). Mutagenicity studies of kojic acid. *Toxicology letters*, 59(1-3), 213-220.

Williams, G. R. (2008). Neurodevelopmental and neurophysiological actions of thyroid hormone. *Journal of Neuroendocrinology*, 20(6), 784-794.

Wollny, H.E (1998). Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay with Kojic Acid. Research and Consulting Company (RCC) - Study No. 612701, unpublished report.

Wollny, H.E. (1998). Escherichia Coli Photomutagenicity Assay with Kojic Acid. Research and Consulting Company (RCC) - Study No 612702, unpublished report

Wollny, H.E. (2001). Salmonella typhimurium Reverse Mutation Assay with Kojic Acid. RCCCCR Project Number 696301, November 22, unpublished report.

Wynford-Thomas, D. *et al*. (1982). Dissociation of growth and function in the rat thyroid during prolonged goitrogen administration. *European Journal of Endocrinology*, 101(2), 210-216.

Zoeller, R. T., & Rovet, J. (2004). Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. *Journal of Neuroendocrinology*, 16(10), 809-818.

7. GLOSSARY OF TERMS

See SCCS/1628/21, 11th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 181.

8. LIST OF ABBREVIATIONS

See SCCS/1628/21, 11th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 181.

ANNEX 1, Table 2: Overview of available *in vitro* genotoxicity/mutagenicity data of Kojic acid

Test	Test system	Concentration	S9	Result	Remark	Reference
Ames test	Salmonella typhimurium TA 1535, TA 100, TA 1537 and TA98	500-4000 µg/plate	+/-	Weak mutagenic activity	Poor description of test compound and results; limited value	(Iwahara & Sakamoto, 1980)
Ames test	Salmonella typhimurium TA 1535, TA 100, TA 1537, TA 98 and TA102	30-5000 µg/plate	+/-	Mutagenic	Unsure whether the compound tested was Kojic acid; limited value	(Marzin, 1997)
Ames test	Salmonella typhimurium TA98 and TA100	100-6000 µg/plate	+/-	Mutagenic	Poor description of test compound and results; limited value	(Wei *et al*.,1991)
Ames test	Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537 and Escherichia coli WP2	33-5000 µg/plate	+/-	Mutagenic	Reliable test	(Wollny, 1998)
Ames test	Salmonella typhimurium TA98 and TA100	3-1000 µg/plate (-S9), 33-5000 µg/plate (+S9)	-/+	Negative (reduced nr of revertants)	Reliable test. However, only 2 strains tested	(Wollny, 2001)
Ames test	Salmonella typhimurium TA98 and TA100	10-10,000 µg/plate	+/-	Mutagenic	Unreliable test; no value	(Bjeldanes & Chew, 1979)
GM E. Coli K12	E. Coli K12	1-2 – 100 µl	+/-	Negative	Unreliable test; no value	(Reiss, 1986)
Ames test	Salmonella typhimurium TA98 and TA100	-	-	-	Description test is very poor. Not relevant	(Kim *et al*., 1987)
GM mammalian cells	Chinese hamster V79 cells	30 – 10000	-	Negative	Unpublished report. Only results section available. No indication on exposure. Only without S9 mix. Therefore less	(Iwahara, 1981)

					reliable	
GM mammalian cells (HPRT)	L5178Y Mouse Lymphoma cells	300 – 1421µg/ml	+/-	Negative	Looks OK. Although cytotoxicity did not reach the required level (10-20% survival at the top dose), the test compound was tested up to the required top concentration.	(Lloyd, 2002)
Chromosomal aberration	CHO-KI cells	3-6 ug/ml	+/-	Mutagenic	Poor description of test compound and results; limited value	(Wei *et al*., 1991)
	Chinese Hamster V79 Cells	250 - 1420 µg/ml	+/-	Negative/ mutagenic	Reliable test. Positive at longer harvest times and without S9 only. Authors consider cytotoxicity as reason but use worst case approach *i.e.* weak mutagenic.	(Schulz, 2002)
SCE	CHO-KI cells	3-6 ug/ml	+/-	Mutagenic	Poor description of test compound and results; limited value	(Wei *et al*., 1991)
MN-test	SVK14 cells	500 – 8000 µg/ml	+/-	Negative	Reliable test	(Feltes, 1997)
	HEPG2	1000 - 8000 µg/ml	-	Uncertain	Positive at concentration which are above the required level (in OECD guidelines) of the top concentration and (thus) at cytotoxic	(Feltes, 1997)

					concentrations	
Photomutagenicity	E. coli WP2 (Trp+)	33-5000 µg/plate	+/-	Negative	Irradiation with artificial sunlight had no relevant influence on the mutagenic potential of Kojic acid	(Wollny, 1998)
Photogenotoxicity	Salmonella typhimurium, TA102, TA98 E. coli WP2 (pKM 101) Plate method GLP study, with and without UV	78.1-5000 µg/ml	-	Slightly positive for photo-mutagenicity in E. coli WP2/pKM101 and S. typhimurium TA102 but negative in TA98	Irradiation with UV light had slight mutagenic influence on Kojic acid No GLP study, no historical controls	(Higa *et al*., 2007)
Photo-genotoxicity Chromosomal aberrations,	CHL cells, with and without UV	0.088-1.4 mg/ml	-	Negative for genotoxicity, positive for photo-genotoxicity in the highest concentration	In the presence of irradiation with UV light Kojic acid induced chromosomal aberrations	(Higa *et al.*, 2007)

ANNEX 2, Table 3: Overview of available *in vivo* (photo)genotoxicity data of Kojic acid

Test	Species	Dose	Tissue	Result	Remark	Reference
DNA adducts	F344/DuG rj rats	0.5 or 2% in diet	Liver	Negative	Indication for DNA binding, No OECD guideline. "Indicator test"	(Nakano, 2005)
DNA adducts	F344 rats	0.02, 0.2 or 2% in diet	Thyroid	Negative	Indication for DNA binding, No OECD guideline. Indicator test.	(Tamura *et al.*, 2006)
USD test	Wistar HanIbm rats	150, 1500 mg/kg bw	Liver	Negative	Reliable test. Indicator test.	(Volkner, 1997)
In vivo GM	Muta ™mice (D2-lacZ80/HazfBR strain)	800, 1600 mg/kg bw	Liver	Negative	Test performed without positive control. Therefore less reliable.	(Vegarra, 2002)
Comet assay	Wistar rats	1000, 2000 mg/kg bw	Liver, stomach and colon	Negative	No OECD guideline. Indicator test. Still a reliable test measuring both clastogenicity and mutagenicity	(Brendler-Schwaab & Krämer-Bautz, 2004)
MN test	NMRI mice	187.5, 375, 750 mg/kg bw	Bone marrow	Negative	Reliable test	(Honarvar, 2001)
MN test	ddY mice	125, 250, 500, 1000 mg/kg bw	Bone marrow	Negative	Poor description of test compound and results;	(Omura & Nonaka, 1980)

					limited value.	
MN test	ddY mice Fisher rats	500, 1000 mg/kg bw	Liver after hepatectomy	Positive mice, negative rats	The relevance of a positive results after hepatectomy is unclear It certainly is a standard test. Limited value.	(Ishikawa *et al.*, 2006)
Dominant lethal test	BDF1 mice	350, 700 mg/kg bw	-	Negative	Reliable test	(Shibuya *et al.,* 1981)
Photo-genotoxicity, Micronucleus assay,	male HR-1 mice, with and without UV	1 and 3%	Skin	Negative	Kojic acid did not induce micronuclei with or without UV	(Higa *et al.,* 2007)
8-oxodG, 32-P postlabelling, non-GLP	Male Rat F344/DuCrj (SPF), oral exposure	0, 0.5, 2.5%	Liver	Negative	Kojic acid did not induce DNA adducts (8-oxodG)	Higa *et al.*, 2007)
8-oxodG, by HPLC-ECD	Male F344 rats	diet containing 0–2% Kojic acid (together with exposure to 2-AAF and partial hepatectomy)	Liver	Negative	No significant induction of 8 oxodG	(Chusiri *et al.,* 2011)
Micronucleus assay	Oral administration, six-week-old male rats	250, 500 and 1000 mg/kg/day for 14 days, and at 125, 250 and 500 mg/kg/day for 28 days.	Liver, bone marrow and peripheral blood leucocytes	Negative	No induction of micronuclei in any tested tissue	(Ogiwara *et al.,* 2015)
Comet assay, OECD guideline	Oral, six-week-old male rats	250, 500 and 1000 mg/kg/day for 14 days, comet assay 21h after last administration	Liver, and peripheral blood leucocytes	Negative	No induction of strand breaks	(Ogiwara *et al.,* 2015)

ANNEX 3, Table 4: Overview of newly identified carcinogenicity data of Kojic acid

Carcinogenicity						
Mouse medium term skin carcinogenicity test for initiation and promotion, based on GST-P positive foci	Male rats F344	0.5-2% of Kojic acid for 4 weeks Animals killed after 11 weeks	Liver	Equivocal for initiation, negative for promotion	Highest dose positive for initiation	(Higa *et al*., 2007)
Mouse medium term skin carcinogenicity test for initiation and promotion, based on GST-P positive foci	Female mice, CD-1 (ICR)	For initiation: 0,3, 3% of Kojic acid in cream once a day for 7 days, For promotion: 50 mg of Kojic acid 5 times per week for 20 weeks	Skin	Negative	No initiation or promotion activities on skin	(Higa *et al.,* 2007)
carcinogenicity test for initiation and promotion, based on GST-P positive foci	Male F344 rats	administered in diet containing 0–2% Kojic acid		Negative for initiation, positive for promotion in higher concentration	Higher concentrations of Kojic acid promote hepatocarcinogenesis in rats. Indication that Kojic acid is a non-genotoxic hepatocarcinogen	(Chusiri *et al*., 2011)

ANNEX 4, Table 5: Summary of views presented by different scientist groups/committees on relevance of rodent thyroid tumor data after exposure to kojic acid for humans

	Tumor data from rodents relevant for humans	Tumor data from rodents NOT relevant to humans
1998 US EPA (Hill *et al*., 1998)	Accordingly, we cannot qualitatively reject the animal model; it seems reasonable that it may serve as an indicator of a potential human thyroid cancer hazard. However, to the extent that humans are susceptible to the tumor inducing effects of thyroid-pituitary disruption and given that definitive human data are not available, humans appear to be quantitatively less sensitive than rodents to developing cancer from perturbations in thyroid-pituitarystatus. Recognizing these things and based upon thyroid carcinogenesis mode of action considerations, the EPA adopted the following three science policy positions: 1. It is presumed that chemicals that produce rodent thyroid tumors may pose a carcinogenic hazard for the human thyroid. 2. In the absence of chemical-specific data, humans and rodents are presumed to be equally sensitive to thyroid cancer due to thyroid-pituitary disruption. This is a conservative position when thyroid-pituitary disruption is the sole mode of action in rats because rodents appear to be more sensitive to this carcinogenic mode of action than humans. When the thyroid carcinogen is a mutagenic chemical, the possibility that children may be more sensitive than adults needs to be evaluated on a case-by-case basis. 3. Adverse rodent noncancer thyroid effects (e.g., thyroid gland enlargements) following short- and long-term reductions in thyroid hormone levels are presumed to pose human noncancer health hazards. 4. A nonlinear dose-response relationship (margin of exposure) should be used when thyroid-pituitary disruption is judged to be the sole mode of action of the observed	

	thyroid and related pituitary tumors (Table 2, Example 3). Thyroid-pituitary perturbation is not likely to have carcinogenic potential in short-term or highly infrequent exposure conditions. The margin of exposure procedure generally should be based on thyroid-pituitary disruptiveeffects themselves, in lieu of tumor effects, when data permit. Such analyses will aid in the development of combined noncancer and cancer assessments of toxicity. Results of the margin of exposure procedure will be presented in a way that supports risk management decisions for exposure scenarios of differing types (e.g., infrequent exposure, short durations).	
1999 IARC (Capen *et al*., 1999)	**Species differences in thyroid carcinogenesis** The weight of the evidence suggests that rodents are more sensitive than human subjects to thyroid tumour induction due to hormonal imbalances that cause elevated TSH levels. – Agents that lead to the development of thyroid neoplasia through an adaptive physiological mechanism belong to a different category from those that lead to neoplasia through genotoxic mechanisms or through mechanisms involving pathological responses with necrosis and repair. – Agents that cause thyroid follicular-cell neoplasia in rodents solely through hormonal imbalance can be identified on the basis of the following criteria: • No genotoxic activity (agent and/or metabolite) was found in an overall evaluation of the results of tests *in vivo* and *in vitro*. • Hormone imbalance was demonstrated under the conditions of the assay for carcinogenicity. • The mechanism whereby the agent leads to hormone imbalance has been defined. – When tumours are observed both in the thyroid and at	

	other sites, they should be evaluated separately on the basis of the modes of action of the agent. – Agents that induce thyroid follicular-cell tumours in rodents by interfering with thyroid hormone homeostasis can, with some exceptions, notably the sulfonamides, also interfere with thyroid hormone homeostasis in humans if given at a sufficient dose for a sufficient time. These agents can be assumed not to be carcinogenic in humans at concentrations that do not lead to alterations in thyroid hormone homeostasis.	
2001 IARC monograph 79 (Capen *et al*., 1999)	Taken form Capen *et al*. 1999 (IARC monograph) as above plus the text below: **Specific chapter on Kojic acid** **Mechanistic considerations** Kojic acid is a directly acting genotoxin. It is also a potent goitrogen in rodents, causing decreased serum thyroid hormone concentrations, increased thyroid-stimulating hormone concentrations, increased thyroid gland weights and diffuse follicular cell hypertrophy and/or hyperplasia. Kojic acid inhibits iodine uptake by the thyroid and inhibits iodine organification at high doses. The antithyroid effects of kojic acid are therefore the probable mechanism by which it produces thyroid gland tumours; however, a role of genotoxicity cannot be excluded in the light of the positive findings. **Evaluation** There is inadequate evidence in humans for the carcinogenicity of kojic acid. There is limited evidence in experimental animals for the carcinogenicity of kojic acid.	

2002 RIVM (RIVM report, 601516009/2002)		If the substance is non-genotoxic and it has been demonstrated (based on the information indicated under point 2) that the substances induces a prolonged disturbance in the HPT-axis, the thyroid tumours observed in rats are not considered to be relevant for human carcinogenicity risks. This implies that these tumours are not sufficient evidence for considering the substance as potential carcinogenic for humans and hence classification is not indicated. Disturbance of the HPT-axis is considered to be a hazard indicator for humans and should be taken into account when setting NOAELs and health based limit values. If disturbance in the HPT-axis is the major/critical toxicological endpoint in rats, the interspecies assessment factor to be used for establishing a toxicological limit value may be reduced on a case-by-case basis, because of the fact that humans are substantially less susceptible to disturbances in the HPT-axis than rats. The Specialised Experts agreed that there is convincing scientific evidence that humans are considerably less sensitive than rodents (especially rats) regarding: (i) perturbation of thyroid hormone homeostasis induced by non-genotoxic xenobiotics (ii) development of epithelial thyroid tumours after long-term exposure to such agents. Non-genotoxic carcinogenic substances producing thyroid tumours in rodents with low or medium potency by a clearly established perturbation of the thyroid hormone axis, in general, do not need to be classified. Other rodent thyroid carcinogens merit classification in either category 2 or 3.

2017 Brunel University London and DTU National Food Institute Denmark (EU, 2017)	These observations underline the importance of TSH signalling in the development of thyroid malignancies. Without TSH signalling, malignancies do not develop. Several studies analysed in two large meta-analyses (McLeod *et al*. 2012, Zheng *et al*. 2016) have confirmed that higher serum TSH is associated with an increased risk of follicular and papillary thyroid cancer in humans. **5.5.8 Conclusion** It would appear that traditional toxicological studies of thyroid carcinogenesis with their focus on analysing TH levels are likely missing key events leading to thyroid cancer in the rat. These events seem to revolve around the activation of de-differentiating and proliferative pathways in the thyroid, accessible only through functional and transcriptomics analyses not normally conducted in classical toxicological studies. Further studies of this kind are needed to substantiate the relevance of de-differentiating signalling pathways for the induction of follicular thyroid tumours in the rat. Until such evidence emerges there appears to be little reason to deviate from the USEPA and IARC guidance regarding the identification of thyroid carcinogens.	
2018 Karlsruhe Institute of Technology (KIT) (Bartsch *et al*., 2018)		In conclusion: rats develop thyroid tumors resulting from constant stimulation of the thyroid gland and the continuous increase of TSH levels. In humans, as indicated by unchanged T3, T4 and TSH levels no disturbance of the thyroid homeostasis even after long-term high doses of drugs that enhance elimination of thyroid hormones is observed. Consequently, non-genotoxic substances that only cause thyroid adenomas/carcinomas in rats, which can be attributed to a disturbance in thyroid function such as the induction of phase II enzymes e.g. UGTs, are considered of no relevance to humans and do not warrant classification as carcinogenic. This also applies to tumors induced by

		substances that impair thyroid hormone synthesis or release such as impaired iodine uptake, inhibition of iodine peroxidase, of thyreoglobulin synthesis, of deiodinases or of hormone release from the thyroid follicles when there is evidence for increased thyroid stimulation by increased TSH levels. Mice are less sensitive to disturbances of thyroid hormone homeostasis. However, in case thyroid tumors are also associated with increased TSH levels the conclusion applies to this species as well.
2019 US EPA (Noyes *et al*., 2019)		Elevated TSH in rodents leads to thyroid hypertrophy and potential thyroid cancer, an adverse outcome that has limited relevance to human thyroid cancer due to species differences in sensitivity although this too is an area of renewed interest (EU 2017).

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ANNEX 5: Applicants' argumentation with respect to the endocrine disrupting potential of Kojic acid

Mechanisms of Thyroid Tumorigenesis

Numerous studies have reported that chronic treatment of rodents with Goitrogenic compounds such as Thiouracil and its derivatives results in the development of follicular cell adenomas. Thiouracil and its derivatives showed this effect in rats (Napalkov, 1976) and mice (Morris, 1955). This phenomenon also has been observed in rats that consumed brassica seeds (Kennedy & Purves, 1941), erythrosine (FD&C Red No. 3) (Capen & Martin, 1989; Borzelleca, 1987), sulfonamides (Swarm *et al.*, 1973), and many other compounds (Hill *et al.*, 1989; Paynter *et al.*, 1988). The pathogenetic mechanism of this phenomenon has been understood for some time and are widely accepted by the scientific community. These goitrogenic agents either directly interfere with thyroid hormone synthesis or secretion in the thyroid hormone catabolism and sub sequent excretion into the bile, or disrupt the peripheral conversion of thyroxine (T4) to triiodothyronine (T3). The ensuing decrease in circulating thyroid hormone levels resulting in a compensatory increased secretion of pituitary thyroid stimulating hormone (TSH). The receptor mediated TSH stimulation of the thyroid gland leads to proliferative changes of follicular cells that include hypertrophy, hyperplasia, and ultimately, neoplasia in rodents.

Hepatic Microsomal Enzyme Induction

Hepatic Microsomal Enzymes play an important role in thyroid hormone economy because glucuronidation is the rate limiting step in t he biliary excretion of T4 and sulfation primarily by phenol sulfotransferase for the excretion of T3. Long-term exposure of rats to a wide variety of different chemicals may induce these enzyme pathways and result in chronic stimulation of the thyroid b y disrupting the hypothalamic pituitary thyroid axis (Curran and DeGroot, 1991). The resulting chronic stimulation of the thyroid by increased circulating levels of TSH often results in a greater risk of developing tumors derived from follicular cells in 2 year or lifetime chronic toxicity/carcinogenicity studies with these compounds in rats. Recent studies have suggested that glucuronidation and enhanced biliary excretion of T3 may be the reason why serum TSH is increased in short term (7 days) studies with some microsomal enzyme inducing chemicals (*e.g.* phenobarbital, pregnenolone-16 α-carbonitrile) but is less affected with others (3 methylcholanthrene, PCB) (Hood and Klaassen, 2000). However, microsomal enzyme inducers are more effective in reducing serum T4 than serum T3 (Hood and Klaassen, 2000). Outer-ring deiodinase (ORD) activity, an enzyme involved in the peripheral conversion of T4 (major secretory product of the thyroid) to T3, was reduced (not increased as would be expected if this was the mechanism) following the administration of four well characterized enzyme inducers in rats. Type I ORD was measured in thyroid, kidney, and liver whereas type II ORD was quantified in brown adipose tissue, pituitary gland, and brain. Excessive secretion of TSH alone (*i.e.*, in the absence of any chemical exposure) also has been reported to produce a high incidence of thyroid tumors in rodents (Ohshima and Ward, 1984, 1986). This has been observed in rats fed an iodine deficient diet (Axelrod and Leblond, 1955) and in mice that received TSH secreting pituitary tumor transplants (Furth, 1954). The pathogenetic mechanism of thyroid follicular cell tumor development in rodents involves a sustained excessive stimulation of the thyroid gland by TSH. In addition, iodine deficiency is a potent promoter of the development of thyroid tumors in rodents induced by intravenous injection of N methyl N nitrosourea (Ohshima and Ward, 1984). The subsequent parts of thyroid section showed specific mechanisms by which xenobiotic chemicals disrupt thyroid hormone synthesis and secretion, induced hepatic microsomal enzymes that enhanced thyroid hormone catabolism or inhibited enzymes involved in monodeiodination in peripheral tissues that result in perturbations of thyroid hormone economy which in rodents predisposes to the development of follicular cell tumors in chronic studies.

Mechanisms of Thyroid Tumorigenesis treated with Kojic acid

Tumorigenic activity of Kojic acid in the thyroids of B6C3F1 mice was earlier demonstrated after dietary treatment for 20 months (Fujimoto *et al.*, 1998), while no thyroid tumors were found in p53 (+/-) or p53 (+/+) CBA mice, despite the high susceptibility of heterozygous p53-inactivated mice to genotoxic carcinogens (Mitsumori *et al.*, 2000; Tennant *et al.*, 1995, 1996). This might be due to variation in the strain of mice used and duration of the administration period. However, the Kojic acid treated CBA mice showed diffuse hypertrophy and hyperplasia of thyroid follicular cells, which were typical histopathological features associated with goitrogenic substances in rodents (Capen, 1996; Gopinath *et al.*, 1987), as reported in B6C3F1 mice. Kojic acid might thus exert tumorigenic potential in the thyroids of p53 (+/-) and p53 (+/+) CBA mice with more prolonged exposure. In this experiment, serum T4 levels in mice receiving Kojic acid were reduced in a dose-related manner, but dose-proportional effects on T3 or TSH was also reported in F344 rats receiving Kojic acid at dietary concentrations up to 2% for 20 weeks (Tamura *et al.*, 2001) and B6C3F1 mice given 1.5 or 3% Kojic acid for 20 months (Fujimoto *et al.*, 1998). Moreover, in the previous study in rats, administration of sulfadimethoxine or thiourea, a goitrogenic anti thyroidal compound, was associated with a reduction of thyroid hormones and elevation of TSH after a one week treatment but no apparent alteration of T3, T4, and/or TSH levels on prolonged administration to rats of phenobarbital, propylthiouracil or pregnenolone-16 α-carbonitrile. These findings suggested that hormonal desensitization may be induced by prolonged anti-thyroidal treatments (Shimo *et al.,* 1994; Wynford Thomas *et al.*, 1982). Kojic acid was reported to interfere with thyroid iodine uptake and its organification (Fujimoto *et al*., 1999; Tamura *et al*., 1999a) but not elicited any changes in the activity of hepatic uridine diphosphate glucuronosyl transferase or histopathological hypertrophy or swelling of hepatocytes in F344 rats (Mitsumori *et al*., 1999). There was also no hepatocellular hypertrophy in this study, although the Kojic acid-treated animals showed somewhat elevated liver weights. Considering the results, Kojic acid might exert goitrogenic action *via* hormonal mechanisms in p53 (+/-) and p53(+/+) mice of CBA-background, as observed in B6C3F1 mice and F344 rats. The fact that Kojic acid failed to form DNA adducts in the thyroid glands of rats by dietary feeding at 2% supports the form DNA adducts in the thyroid glands of rats by dietary feeding at 2% supports the inference.

Hepatocellular adenomas as well as altered hepatocellular foci were observed in Kojic acid-treated groups not only p53(+/-) mice but also in their wild-type littermates. Since no spontaneous hepatocellular proliferative lesions were observed in control animals in line with the previous 26-week studies (Mitsumori *et al.*, 2000; Onodera *et al.*, 2001; Takizawa *et al.*, 2001), these proliferative lesions in the liver could be attributed to the treatment with Kojic acid. The fact that focal hepatocellular necrosis and inflammatory cell treatment with Kojic acid. The fact that focal hepatocellular necrosis and inflammatory cell infiltration were enhanced in the 1.5 and 3% Kojic acid groups suggests a hepatotoxic potential of Kojic acid. The elevated proliferation induced for hepatocytes were roughly parallel the occurrence of necrotic lesions both in occurrence of necrotic lesions both in p53(+/-) and p53(+/+) mice, and might make large variability in the p53(+/+) mice. Although the effects of Kojic acid were masked by relatively variable control level of the 0% Kojic acid group in p53(+/+) mice, the elevated proliferation index in the 3% group of p53(+/-) mice might also be indicative of the hepatic regeneration. In the 20-month carcinogenicity study conducted by Fujimoto *et al.*, (1998), a slight (10%) but statistically statistically significant increase in the incidence of hepatocellular carcinomas was observed only in female B6C3F1 mice receiving 3% Kojic acid in the diet, but no hepatic disorders were observed on histopathological examination as well as serum biochemistry. The findings suggest a high susceptibility of CBA-background mice particularly p53(+/-) mice to hepatotoxicity of Kojic acid, and thus associated secondary cell proliferation might influence the induction of hepatocellular tumors. In addition, significant tumorigenic dose was lowered and the prevalence of hepatic proliferative lesions was higher in the p53(+/-) mice as compared to their wild-type counterparts. In particular, incidences of hepatic tumors at a dose of 1.5%

and altered foci at a dose of 3% Kojic acid in p53(+/-) mice were significantly higher than in p53(+/+) mice. Since p53(+/-) mice are sensitive to genotoxic carcinogens (Mitsumori *et al.*, 2000; Tennant *et al.*, 1995, 1996), the possibility that Kojic acid exerts carcinogenic action through, 1995, 1996), the possibility that Kojic acid exerts carcinogenic action through genotoxicity could not could not be ruled out from this experiment.

1 **ANNEX 6, Table 6: Overview of toxicological studies with Kojic acid studying endocrine-related endpoints**

Study	Test compound	Guideline	Dose	Duration	Test system/Species	Target	NOAEL (mg/kg bw/d)	Ref
Rats, oral, diet ^{125} I uptake and hormone determination	Kojic acid	/	Experiment 1: 0, 0.008, 0.03, 0.125, 0.5, or 2% in diet	4 weeks	F344 rats, 9 males / group (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 T3, T4 and TSH level were also only affected in the 2.0% group.	23.8 mg/kg bw/day (thyroid weight) 5.85 mg/kg bw/day (iodine uptake)	(Fujimoto *et al.*, 1999)
Rats, oral, diet ^{125} I uptake, hormonal and histological examinations	Kojic acid	/	Experiment 2: 0 or 2% in diet	4 weeks	F344 rats; 8 males or 8 females/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 T3, and T4 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.		
Rats, oral, diet ^{125} I uptake and the other half for hormonal and histological examinations	Kojic acid	/	Experiment 3: 0 or 2% in diet	4 weeks	F344 rats; 8 males /group (Experiment 3)	Organic 125I formation returned to normal after 6 hours, 125I uptake per unit thyroid weight rose to 70% of the control level within 24 hours. T3 and T4 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.		
Rats, oral, diet hormone analysis, Histopathological examination of thyroid and pituitary tissues	Kojic acid	/	0, 0.008, 0.03, 0.125, 0.5, 2.0% Kojic acid in diet Average daily intake calculated: 0, 5.85, 23.8, 95.3, 393.6, 1387.3 mg/kg bw/day	4 weeks	F344 rats; 8 animals/group	Body weight differences insignificant. 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 T3 and T4 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	NOAEL of 6 mg/kg bw/day (histopathological findings and altered ^{125}I uptake)	(Tamura *et al.*, 1999b)

						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.		
Rat, oral, gavage Clinical and histopathological examination. Recording of iodine uptake and iodination. Hormone analysis in blood	Kojic acid in 0.5% carboxymethyl cellulose	/	0, 4, 15, 62.5, 250, 1000 mg/kg bw/day or 0, 0.008, 0.03, 0.125, 0.5, 2.0% Kojic acid in diet dosing volumes of 5 ml/kg bw, single oral administration of ^{14}C-Kojic acid (10 µCi/100 g or to 100 mg/kg bw/day)	28 days	F344/ DuCrj rats 10 males/group	decrease in motility, inhibition of body weight gain, and a decrease in food consumptionat 1000 mg/kg bw. 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 T4 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 T3 level in week 1 at 250 mg/kg bw/day. Absorption of Kojic acid was rapid. Tmax of blood concentrations of radioactivity was 1.0 ± 0.0 hours with Cmax of 25.07 ± 4.56 µg eq/ml. T1/2 was 4.8 ± 0.3 hours. Elimination was nearly complete within 24 hours. AUC0-24	NOAEL of 62.5 mg/kg bw/day (decreased T3 level) Cmax was 25.07 ± 4.56 µg eq/ml and AUC0-24 h was calculated to be 101.54 ± 19.35 µg eq/ml.	(Higa *et al*., 2000)

						h was calculated to be 101.54 ± 19.35 µg eq/ml.		
Chronic (6m) study, oral gavage observation for abnormalities macroscopic and microscopic of organs, analysis of urine, blood biochemistry	Kojic acid	/	0, 125, 250, 500, 1000 mg/kg bw/day in 1% aqueous solution of carboxymethylcellulose (0.5 ml/100 g bw) by gavage	SLC-SD rats 10-20 males/group	26w + 5w recovery	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	no effect level of 125 mg/kg bw/day	(Chronic toxicity test and recovery, 1980)

						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. **SCCS comment** Only the weight of the thyroid was determined, whilst no measurements of thyroid function were provided.		
DNA adducts in thyroid, oral dietary study	Kojic acid	/	0 or 2% given for 1 (8-OHdG, only) or 2 weeks	F344 rats 20 males/group	1 or 2 weeks	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		(Tamura *et al.*, 2006)

						significantly decreased after 2 weeks as compared to the controls.		
Rats, initiation and promotion assay, liver hepatic pre-neoplastic lesions in N-bis(2- hydroxypropyl)nitrosamine(DHPN)-initiated (experiment 1) and non-initiated (experiment 2) models, and its promoting influence in a medium-term liver bioassay (experiment 3)	Kojic acid (purity 97.7 to > 99.5%)	/	Experiment 1: initiation with s.c. 2000 mg/kg of DHPN and fed 0, 0.125, 0.5 or 2% Kojic acid in diet or 65.6, 261.4, and 1013.2 mg/kg/day Experiment 2: No initiation with 0, 0.5 or 2% Kojic acid in diet or Experiment 3: a single i.p. of 200 mg/kg DEN. Fed diet containing 0%, 0.125%, 0.5%, or 2% Kojic acid for 6weeks, and subjected to two-thirds partial hepatectomy at week 3	F344 rats Experiment 1: 10 males/group Experiment 2: 20 males/group Experiment 3: 25 males/group	20 weeks	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-Ppositive 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 hepatocellular proliferating cell nuclear antigen (PCNA) revealed no apparent overall differences between control and treated groups. 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		(Takizawa *et al.*, 2004)

						0.17± 0.28 in the control) and areas (0.005 ± 0.005 vs 0.0007 ± 0.0012 in the control) of GSTP-positive foci and PCNA expression (3.8 ±2.3 vs 2.6 ± 0.7 in the control) were significantly increased by the 2% Kojic acid treatment. 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.		
Rats, promotion assay, thyroid Time course changes in thyroid proliferative lesions as well as related hormone	Kojic acid	/	0, 2 or 4% Kojic acid	F344 rats 20 males in group 1, 25 males in groups 2-4	12 weeks	One rat died in the DHPN + 4% Kojic acid group at week 8 due to tracheal blockage caused by an extremely hypertrophied thyroid. While relative liver weights in both Kojic acid treated groups were significantlygreater 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		(Tamura *et al.*, 1999)

levels						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 T3/T4 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 pointin 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 T4-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 at all concentrations tested, due to continuous serum TSH stimulation through the negative feedback mechanism of the pituitary-thyroid axis, resulting from depression of serum T3 and T4.		
promoting effects on thyroid carcinogenesis	Kojic acid	/	0 (group 1, 2) or 2% Kojic acid (group 3) Groups 2 and 3 received 2800 mg/kg N-bis(2-hydroxypropyl)nitrosamine (BHP)	F344 rats - Experiment 1: 8 males/group 1, 10 males/groups 2-3 Experiment 2: 10 males/group	Experiment 1: 12 weeks Experiment 2: 20 weeks	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 T3 and T4 levels were significantly decreased (half to one-third of values of the BHP alone group) and serum TSH was markedly increased		(Mitsumori *et al.*, 1999)

						(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 T4-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 T3 and T4 caused by a mechanism independent of T4-UDP-GT activity.		
Rat, promotion	Kojic acid	/	Experiment 1: 0, 0.002,	F344 rats - Experiment	20 weeks	Overall 4 animals died due to tracheal blockage caused by extremely hyper-throphied thyroids	NOAEL of 0.03% or 15.5	(Tamura *et al.*,

assay, thyroid			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	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		(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). 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 T4 level were significantly decreased in the DHNP-initiated groups given 0.125% Kojic acid or more at week 12. No significant changes in serum T3 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	mg/kg bw/day (thyroid tumour-promoting effect)	2001)

						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.		
Rats, initiation assay, thyroid, (two-stage rat thyroid tumorigenesis model)	Kojic acid	/	0 (Group 1), 0.02 (Group 2), 0.2 (Group 3) or 2% (Group 4) in the diet for 8 weeks. Group 5 received 4 DHPN at 700 m/kg bw s.c. with 2 weeks intervals, group 6 2% Kojic acid, group 7 2% Kojic acid for 31 weeks.	F344 rats Group sizes: 20 males/group	8 weeks BD or Kojic acid treatment, followed by 23 weeks 0.1% SDM in drinking water and 8 weeks recovery	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. 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. At the end of administration period serum T3 levels in groups 1, 4, and 5 as well as T4 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 T3 and T4 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 T3 and T4 levels still were slightly higher, TSH levels had approximately returned to the normal range in the treatment		(Tamura *et al.*, 2006)

						groups except for group 5. 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.		
Mice thyroid and liver	Kojic acid ad libitum basal diet	/	0, 1.5 or 3% in diet	(C57BL/6N xC3H/N)F1 mice - 65 males or 8 females/group	20 months	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.		(Fujimoto *et al.*, 1998)

						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 T3 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 B6C3F1 mice, presumably by a mechanism involving decrease in serum free T3 levels and increased TSH.		
Mice thyroid and liver	Kojic acid in basal diet	/	0, 1.5 or 3% in diet	p53(+/-) CBA mice P53(+/+) wild-type mice Group size:	26 weeks	One p53(+/+) male receiving 3% Kojic acid was found dead at week 13. 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		(Takizawa *et al.*, 2003)

				7 - 13 males/group		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 T3 levels were not altered by Kojic acid treatment, but serum T4 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.		
In vivo single dose administration	Kojic acid in 0.5% carboxymethylcellulose		1000 mg/kg	Male F344/DuCrj rats	Up to 72 hours after administration	The ^{125}I uptake activity into the thyroid (% of dose) increased time-dependently in the control group and remained constant from 6		(Higa *et al*., 2002)

in rats	(Wako Pure chemical Industries Ltd., Inc., Osaka, Japan)					hours to 24 hours after administration. In the animals treated with Kojic acid, the ^{125}I uptake activity was significantly lower than in the control group. The ^{125}I uptake activity at 24 hours after injection of Na ^{125}I, following Kojic acid administration 48 hours before, recovered to an extent comparable with that of the control at 24 hours after Kojic acid administration. The ^{125}I organification activity in the control group was approximately 90% from 30 minutes to 24 hours after administration. The activity in the animals treated with Kojic acid was significantly lower than control from 30 minutes to 6 hours after administration. In the animals where Na ^{125}I was given i.p. 24 or 48 hours after Kojic acid administration, the organification activity recovered to an extent comparable with that of the control. Serum T3 showed to increase from 30 to 6 hours after Kojic acid administration, whilst serum T4 decreased 2 to 48 hours after administration. Serum TSH level did not fluctuate significantly in association with Kojic acid administration. The authors concluded that since Kojic acid is absorbed, metabolised and excreted rapidly, the function of iodine organification in rats revers as rapidly as 24 hours after Kojic acid administration. Therefore, the		

						thyroid hyperthrophy observed in rats is considered to be seen only when Kojic acid is given at a massive dose or for along period of time. **SCCS comment** Newly identified study from open literature.		
In vivo short-term administration in rats	Kojic acid (purity ≥ 98%) in 0.5% carboxymethylcellulose	/	Group 1 (control): 5 ml/kg bw of 0.5% carboxymethylcellulose Groups 2 to 4: Kojic acid at 0.6, 3.0 or 1,875 mg/kg bw	Male F344 rats (4 per group)	14 days	No significant differences were noted between low and medium dose groups and the vehicle control. Significant weight loss and decreased food intake was observed at the highest dosage of kojic acid. A further significant increase was noted in relative liver and thyroid weights as compared with the control groups. An increase in T3 levels were found in rats fed with low and medium doses of kojic acid, while a decrease in T4 level was found in rats treated with the high dose of kojic acid. TSH in the blood could not be detected in this study. In addition, rats treated with the high dosages of Kojic acid had significantly decreased serum ALP levels. A significant decrease in CYP2B1 protein expression was observed in the livers of Kojic acid treated rats at the low dose, whilst the medium and high doses significantly increased CYP2B1 expression. In addition, treatment with Kojic acid decreased CYP2E1 expression at all doses and expression of CYP2C11		(Chusiri *et al*., 2011)

						was significantly decreased at high dosages of kojic acid. **SCCS comment** Newly added study from open literature.		

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