Scientific Committee on Consumer Safety

SCCS

OPINION ON

Kojic Acid

The SCCS adopted this opinion at its 15th plenary meeting

of 26 – 27 June 2012

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Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat. They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.
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SCCS

The Committee shall provide opinions on questions concerning all types of 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.).

Scientific Committee members

Jürgen Angerer, Ulrike Bernauer, Claire Chambers, Qasim Chaudhry, Gisela Degen, Elsa Nielsen, Thomas Platzek, Suresh Chandra Rastogi, Vera Rogiers, Christophe Rousselle, Tore Sanner, Jan van Benthem, Jacqueline van Engelen, Maria Pilar Vinardell, Rosemary Waring, Ian R. White

Contact
European Commission
Health & Consumers
Directorate D: Health Systems and Products
Unit D3 - Risk Assessment
Office: B232 B-1049 Brussels
Sanco-SCCS-Secretariat@ec.europa.eu

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Prof. J. Angerer
Dr. U. Bernauer
Dr. C. Chambers
Prof. G. Degen
Dr. W. Lilienblum (associated scientific advisor)
Dr. S.C. Rastogi
Prof. V. Rogiers (rapporteur)
Prof. T. Sanner (chairman)
Dr. J. van Benthem
Dr. J. van Engelen
Prof. R. Waring
Dr. I.R. White

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1. BACKGROUND

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

Esters of Kojic Acid and other derivatives might also be used, e.g. Kojic dipalmitate, Kojic isopalmitate and chloro-Kojic Acid are mentioned in CosIng.

The first opinion (SCCP/1182/08) was adopted 30 September 2008 by the Scientific Committee on Consumer Products with the conclusion:
"Based on the information provided, margins of safety (MoS) of respectively 35 (face and hands), 58 (hands) and 88 (face) have been calculated suggesting that the use of Kojic Acid at a maximum concentration of 1.0% in skin care formulations poses a risk to the health of the consumer. In addition, other parts of the skin might be exposed to Kojic Acid. Kojic Acid has the potential to induce skin sensitisation.
Relevant data on kinetics of Kojic Acid after dermal application may be submitted to refine the MoS approach."

The current submission III consists of the industry response to the above mentioned opinion and a new dermal penetration study. Reference is also made to the recent Cosmetic Ingredient Review report (CIR).

2. TERMS OF REFERENCE

1. *Does the SCCS consider the use of Kojic Acid in a concentration of up to 1.0% in cosmetic products safe for the consumers given the provided data?*

2. *Does the SCCS foresee further scientific concerns to the safe use of Kojic Acid and/or its derivatives?*

3. OPINION

3.1. RELEVANT PARTS OF SCCP OPINION ON KOJIC ACID (SCCP/1182/08)

3.1.1 General toxicological profile of Kojic Acid

The toxicological profile of Kojic Acid was discussed in detail in SCCP/1182/08 (Ref. 1) and is summarized as follows:

Acute systemic toxicity and local effects

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

Repeated dose systemic toxicity

After repeated oral doses of Kojic Acid in rodents, the main target organs affected appear to be the thyroid and the pituitary gland as well as the liver. An increase in thyroid weight was shown at doses above 0.125% (95.3 mg/kg bw/day) Kojic Acid in the diet given to male rats for 28 consecutive days in a study specifically designed to study effects of Kojic Acid on the thyroid function. Reduced serum level of T_3 and T_4 were reported in several studies. TSH levels increased in a dose- and time-dependent manner after Kojic Acid administration. In male rats the uptakes of ^{125}I, as well as the numbers of colloid in thyroid follicles and follicular cell hypertrophy were significantly changed at 0.003% (23.8 mg/kg bw/day) and a NOAEL of approximately 6 mg/kg bw/day was derived with respect to these effects. These findings were affirmed by additional results from subchronic and chronic toxicity studies. With respect to hyperplasia and thyroid adenomas a NOAEL of 15.5 mg/kg bw/day was derived for male rats from a 20 week-feeding study. Increased thyroid weights as well as diffuse hypertrophy and hyperplasia of the follicular epithelial cells were also reported for female and male mice, administered 1.5% Kojic Acid orally. Effects were more pronounced in males. From the available studies, it is obvious that thyroid proliferative lesions were induced by Kojic Acid administration due to continuous serum TSH stimulation through the negative feedback mechanism of the pituitary-thyroid axis, resulting from depression of serum T_3 and T_4 levels.

In a chronic feeding study, Kojic Acid was administered to Sprague Dawley rats for 26 consecutive weeks at 125, 250, 500 and 1000 mg/kg bw/day. The substance induced some clinical signs (including exophthalmos), kidney and liver related effects. A reduction in body weight gain and increased absolute and relative weights of several organs, including the thyroid gland, were found. The majority of the effects were only observed at the highest dosage levels. No histopathological changes were noted. Based upon the results of this study, a NOEL of 125 mg/kg bw/day was determined. Specific thyroid markers, such as TSH, T_3 and T_4 levels, were not analysed in this study.

A 28 day dermal study in Wistar rats was carried out under GLP. Daily administered doses were 0, 100, 300 and 1000 mg/kg in a 0.5% aqueous methylcellulose solution. No topical effects could be attributed to treatment. Body weight and food consumption were measured once a week. They were similar in control and treated groups and food consumption was unaffected by treatment. Decreased lymphocyte and white blood cells

counts were observed in male and female rats given 300 or 1000 mg/kg/day at the end of the 4-week treatment period. These were partially reversed in males at the end of a treatment-free period of 2 weeks. Absolute and relative spleen weights were decreased in females at the highest dosage. No histopathological changes were associated with it. Based on the changes observed in lymphocytes and white blood cell counts a NOEL value of 100 mg/kg/day was established.

Mutagenicity/Genotoxicity

Overall, the genotoxicity program on Kojic Acid investigated the three endpoints of genotoxicity: gene mutations, structural chromosome aberrations and aneuploidy. Kojic Acid appeared to be mutagenic in the bacterial gene mutation assays, but these findings could neither be confirmed in the gene mutation test in hamster V79 cells (performed without S9-mix only) and in the mouse lymphoma assay at the *hprt* locus. Kojic Acid was clastogenic in two *in vitro* chromosome aberration tests and was positive in a sister chromatid exchange test. The relevance of clastogenic effects after Kojic Acid treatment in an *in vitro* micronucleus test in Hep 2G cells at high concentrations only is unclear. In a test on phototoxicity with *E. coli* Kojic Acid induced mutagenic effects but irradiation with artificial sunlight had no relevant influence on the mutagenic potential of the test substance indicating that Kojic Acid is not expected to be photomutagenic.
The positive findings from the *in vitro* tests could not be confirmed with *in vivo* tests. Kojic Acid treatment did not result in DNA adducts in liver and thyroid cells, indicating that it probably does not bind to (liver and thyroid) DNA. An *in vivo* unscheduled DNA synthesis (UDS) test was negative. 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, the SCCP concluded that Kojic Acid can be considered to have no genotoxic potential *in vivo* and that additional tests are unnecessary.

Carcinogenicity

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

Thyroid proliferative lesions, hyperplasia and adenomas were reported when male rats were investigated in feeding studies up to 4 % Kojic Acid for 12 to 20 weeks. In one promotion assay, carcinomas were observed. A NOAEL of 15 mg/kg bw/day was derived from this study. In mice, hypertrophy in males as well as hyperplasia and adenomas in females and males were reported in two feeding studies up to 3 % Kojic Acid for 26 weeks or 20 months, respectively. According to a negative study on DNA-adducts in the

thyroid it can be concluded that thyroid tumorigenic activity may be attributable to a non-genotoxic mechanism.

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

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

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

Reproductive toxicity

Kojic Acid showed no effects on fertility of rats and mice in various one-generation studies. The test substance did not induce malformations. Effects observed were changes in litter parameters 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.

Toxicokinetics

Toxicokinetic studies with the rat revealed that 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 was 4. The test substance was excreted mainly with the urine. Excretion was minor via bile and faeces and negligible via expired air. Kojic Acid did not undergo enterohepatic circulation. Very high concentrations reached the foetus 30 minutes after single subcutaneous application in pregnant females and persisted in later stages of development. Transfer to mother milk was low. After repeated subcutaneous exposure concentrations in blood and urine samples increased and showed a tendency to reach equilibrium which was almost 3 times higher than values 24 hours after the first application of the test substance. Concentrations in organs and tissues were partly several times higher after repeated dose administration than after single administration. Main metabolites were sulphate conjugates of Kojic Acid and the glucuronide.

It was discussed in the review of safety aspects submitted to the SCCP that percutaneous absorption in the rat is higher than in humans and that occlusion additionally enhances penetration of Kojic Acid. The relative systemic exposure in rats after topical application under occlusion was approximately 20% of the respective exposure following oral administration.

An *in vitro* dermal absorption study with a 1% Kojic Acid formulation showed an average amount of 3.63 µg/cm² to become systemic available. The maximum value was 7.28 µg/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.

Studies with Japanese women (n=6) were conducted by single application 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 C_{max} was 1.54 ± 0.38 ng/ml with a mean $AUC_{0->\infty}$ of 19.4 ng/ml), which was slightly above the limit of quantification (1 ng/ml). The dermal transfer of Kojic Acid to blood was considered to be low by the applicant, but the SCCP commented that the area for measuring dermal penetration into blood was rather small.

For bleaching products containing Kojic Acid a mean amount of 1 g formulation containing 1% Kojic Acid applied twice daily to the face only, can be assumed. This would correspond to a daily application of 20 mg or 0.33 mg/kg bw Kojic Acid on the face. It has to be taken into account, that consumers may be exposed to higher doses, especially, when they apply Kojic Acid-containing bleaching products also to other parts of the skin, e. g. hands and arms, neck and decollage.

In addition, as in rats repeated exposure resulted in higher blood levels of Kojic Acid than after single administration, the SCCP considered that in humans repeated use of bleaching products containing Kojic Acid may also result in higher systemic exposure than determined after single administration.

Overall, toxicokinetic data in the rat and in humans were available, but at different dose levels. Moreover, the rat data concerned a dose level of 100 mg/kg bw, whereas the NOAEL was lower (6 mg/kg bw/day). Therefore a safety approach based on kinetic data could not be used.

3.1.2 Safety evaluation (including calculation of the MoS)

In 2006, the SCCP calculated the following MoS values for Kojic Acid, taking into consideration that the compound is used as a skin whitening agent at a concentration of 1% in leave-on creams, which are generally applied to the faceand hands.

For calculation of MoS, an NOAEL of 6 mg/kg bw/day was used, based on thyroid effects after oral administration in rats (28 day treatment). For absorption through the skin, the maximum obtained value of 7.28 µg/cm² from the *in vitro* study with human skin was used.

The resulting MoS values were:

Face:	**MoS = 88**
Hands:	**MoS = 58**
Face and hands:	**MoS = 35**

3.1.3 Additional discussion points and SCCP conclusion

The SCCP pointed out that consumers who want to bleach their skin not only apply the products on hands and face, but also on parts of the arms as well as on neck and decollage.

The SCCP stated being aware of products on the market containing Kojic Acid at concentrations higher than 1%.

Kojic Acid is a fungal metabolite commonly produced by many species of *Aspergillus*, *Acetobacter* and *Penicillium*. *Aspergillus flavus* is used in the production of a number of foods, including soy bean paste (miso), shoyu (soy sauce) or sake which are produced

throughout the world. An additional exposure via food can be assumed at least for consumers who consume Asian food regularly.

The SCCP noted that no data was provided on the stability of Kojic Acid in the test solutions and in the marketed products. The test batches were not identified in many cases and purity of the test substance was often not reported.

Based on calculated margins of safety of 35 (face and hands), 58 (hands) and 88 (face), respectively, the SCCP concluded that the use of Kojic Acid at a maximum concentration of 1.0% in skin care formulations poses a risk to the health of the consumer. In addition, other parts of the skin might be exposed to Kojic Acid and the compound has the potential to induce skin sensitisation.

The SCCP stated that relevant data on kinetics of Kojic Acid after dermal application may be submitted to refine the MoS approach.

3.2 NEWLY INTRODUCED DATA

3.2.1 Arguments against SCCP/1182/08 conclusion and SCCS comments

Following the assessment by SCCP, the applicant submitted arguments contesting the SCCP conclusions as well as additional data.

Concerning the extent of kojic acid becoming systemically available, the applicant argued that on the basis of a well-conducted study in 6 female human subjects after application of 500 mg of a cream containing 1% Kojic Acid, the resulting mean maximal plasma levels were 1.54 ng/ml with a mean $AUC_{0-\infty}$ of 19.4 ng/ml (Ref. 2). Such plasma concentrations were considered as trace levels. Given the intrinsically low toxicity of Kojic Acid, the applicant considered the possibility of adverse effects at such low exposure levels to be negligible. Even if Kojic Acid were to be applied to the hands or other skin areas, doubling the measured plasma levels mentioned above would, according to the applicant, result in a minimal to negligible systemic availability.

The applicant further contested the view of SCCP that a single-dose human dose would underestimate the human risk after repeated exposure as the plasma levels were below the limit of quantification after 24 hours, meaning that accumulation was not expected to occur. In addition, the typically used *in vitro* dermal absorption study also implies a single dose application.

Concerning the calculation of the MoS, the applicant argued against the use of the NOAEL value of 6 mg/kg bw/day originating from a 28-day study in the rat and based on thyroid effects as such effects in the rat is known for its potential irrelevance to man. The main reason for this is that thyroxine is rapidly metabolized in rodents due to the absence of freely circulating thyroid-hormone binding globulin that is significantly present in humans. Furthermore, the human thyroid appears to be less sensitive to prolonged TSH stimulation than that of the rat. Therefore, it is also less sensitive to tumour formation.

The following new data was provided:

- An *in vitro* dermal absorption study as the applicant considered that the study assessed in SCCP/1182/06 concerned an exploratory study that did not follow current guidelines (only 8 samples were used). This study is described in section 3.2.2.
- A brief description of a repeated insult patch test (HRIPT) (Ref. 3), intended to show that the test substance is not sensitising.
- A student thesis, describing that Kojic Acid does not cause decreased epidermal melanocytes or melanin granules in black guinea pigs at 1%, in contrast to

hydroquinone (Ref. 4). The applicant mentions that this study shows that Kojic Acid has no skin-bleaching activity at a concentration of 1%.

SCCS comments

- For the human dermal absorption study with the 1% Kojic Acid cream, the SCCP already described several shortcomings. The composition of the cream formulation used in the study was lacking. Also individual data on medical and physical examinations were missing and the application area was considered rather small (2 cheeks) for measuring dermal penetration into blood.
- 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 is unsuitable to override the concerns in relation to sensitisation stated in opinion SCCP/1182/08. The SCCS considers HRIPT experiments as unethical.
- The black guinea pig study did not assess on any safety aspect related to Kojic Acid. A solution of Kojic Acid in DMSO:Ethanol (1:4) did not have a depigmenting effect at 1% concentration. The results were quite surprising, as the current safety assessment of Kojic Acid concerns the use as a skin whitening agent (by retarding melanin production) at 1%.
- The SCCS added to the kojic acid submission 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, induced thyroid follicular cell tumors and liver preneoplastic lesions were observed. From the study it could be concluded that the NOAEL is below 227 mg/kg bw/day in male rats (Ref. 5).

3.2.2 *In vitro* dermal absorption study

Guideline:	OECD TG 428: Percutaneous Absorption: *In vitro* Method (2004), SCCS/1358/10
Date of test:	17-29 September 2010
Test system:	Excised, dermatomed (400µm) human skin on a static diffusion cell
N° of samples:	12 samples (4 donors, 3 skin samples/donor) per tested concentration
Test substance:	Leave-on skin care formulation containing 1% of Kojic Acid (full composition stated in appendix 1 to this opinion)
Batch:	Radiolabelled Kojic Acid: TS00130/001
Purity:	99.2%
Applied amount:	2 mg formulation/cm², rinsed off after 24 hours
Receptor fluid:	Phosphate Buffered Saline (PBS)
Exposure period:	24 hours
GLP/QAU:	In compliance

The *in vitro* percutaneous absorption of [^{14}C]-Kojic Acid was determined in human dermatomed skin by using a leave-on skin care formulation containing 1% of Kojic Acid Prior to dosing, the membrane integrity was checked by measurement of electrical resistance. The doses were applied to the 12 intact skin membranes (from 4 human donors) at a rate of 2 mg/cm², corresponding to a nominal 20 µg of Kojic Acid /cm². The exposure period was 24 hours following which, the skin surface was washed with 2% sodium dodecyl sulphate (SDS) in water (2 x 762 µl), followed by rinsing with water (2 x 762 µl). The washing procedure was repeated until decontamination appeared complete, after which the skin surface was dried with sponges. Subsequently, the *stratum corneum* was removed by tape stripping removing a maximum of 20 strips from each skin

membrane. The flange skin was cut away from the dermis and the epidermis on the remaining skin disc was separated from the dermis using a heat separation technique.
The application rates and exposure conditions used in this study were designed to simulate predicted normal human exposure to the test material.
The penetration process was monitored using [^{14}C]-radiolabelled Kojic Acid, which was incorporated into the formulation prior to application. The receptor fluid was phosphate buffered saline. The 24 hour penetration profile was determined by collecting receptor fluid samples 0.5, 1, 2, 4, 8, 12, 16, 20 and 24 hours following application. The samples were analysed by liquid scintillation counting (LSC).

Results

None of the 12 dosed cells were rejected. Mean recovery of the applied test material was 95.4%, with individual cell values ranging from 87.5% to 99.9%.
After a short lag phase of 4 hours, the mean Kojic Acid penetration rate was 0.004 µg/cm²/h between 4-12 hours, after which penetration through human skin increased slightly to 0.009 µg/cm²/h between 12-24 hours. Between 0-24 hours, the penetration rate was, on average, 0.006 µg/cm²/h.
The amounts (mean ± SD) of Kojic Acid that penetrated through human skin at 4, 8 and 12 hours were 0.004 ± 0.005 µg/cm², 0.015 ± 0.023 µg/cm² and 0.036 ± 0.064 µg/cm², respectively. These respective amounts expressed as percentages of the applied dose were 0.018, 0.073 and 0.175%. 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 following table provides the amounts of Kojic Acid in µg/cm² as measured in the different compartments. Results are given as a percentage of the applied dose and in µg/cm^2.

Amount of Kojic Acid in:	**1% Kojic Acid formulation (n= 12)**	
	%	µg/cm²
Skin wash	89.2 ± 3.94	18.2 ± 0.801
Donor chamber	0.065 ± 0.065	0.013 ± 0.013
Flange	0.170 ± 0.161	0.035 ± 0.033
Stratum corneum	2.65 ± 1.91	0.539 ± 0.389
Epidermis	2.64 ± 1.62	0.538 ± 0.329
Dermis	0.344 ± 0.167	0.070 ± 0.034
Receptor fluid (24h)	0.698 ± 1.30	0.142 ± 0.265
Total non-absorbed	91.8 ± 3.86	18.7 ± 0.786
Total absorption	3.68 ± 2.40	0.749 ± 0.489
Recovery	95.48 ± 3.33	19.44 ± 0.68

Conclusion

Based upon the results of the above study, 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 extent of Kojic Acid penetration through human skin amounted to only 0.698% (0.142 ± 0.265 µg/cm²) of the applied dose, after 24 hours. 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).

Ref.: 6

SCCS comments

The formulation used (see Appendix 1 for full composition) raises some questions:

1) In the report a discrepancy with respect to the concentration used seems to be present (0.88% instead of 1.0%).

2) The formulation contains a film forming component (5% nylon), a high amount of silicones {dimethicone (25%) and phenyltrimethicone (4%)} and a high amount of hydrophilic polyols (29%). Silicones represent a complex family of different structure-related compounds but with very differing properties (Ref 7, 8 ,9). It is not clear how a combination of the ingredients in the formulation affects the dermal absorption of Kojic Acid and whether this formulation is representative for the formulations on the market (lotions, creams and soaps).

3) The formulation is prepared by mixing a water and an oil phase just before use. To obtain and maintain a stable and homogeneous emulsion, the presence of an appropriate emulsifying system is necessary, in particular since a high amount of hydrophilic polyols and hydrophobic silicones is present (Ref. 10). From the report, it is not clear which emulsifying system has been used. Some dimethicone derivatives (e.g. polyethers) might exhibit emulsifying properties (Ref. 7), but no explanation in this respect is provided. With regard to the homogeneity of the emulsion during the dermal absorption test, detailed information is present (letter of December 2011). Data on the stability of Kojic Acid, already requested in the previous opinion, is not provided.

4. DISCUSSION

For the application of Kojic Acid at 1% in skin whitening cosmetic cream formulations, the calculated MoS values in SCCP/1182/08 for use in products applied to face and/or hands were all below 100. Therefore the Committee concluded that the requested use of the compound could not be considered as safe.

The applicant provided arguments against this conclusion, consisting of the following main elements:

1) The **dermal absorption** value used in SCCP/1182/08 comes from an explanatory study, using too few skin samples. The mean dermal absorption value was 3.63 µg/cm^2 and the maximum value of 7.28 µg/cm², which was used for the calculation of the MoS by the SCCP. The applicant submitted a new *in vitro* dermal absorption study resulting in a much lower absorption rate (mean value of 0.75 µg/cm²).
2) The applicant considers the **observed thyroid effects** in the rats with Kojic Acid **irrelevant for man** and therefore suggests using a different NOAEL value. Nevertheless, in the proposed calculations, the applicant uses the thyroid-based 6 mg/kg bw/day NOAEL value of the 28-day oral study in the rat. With the dermal absorption value of 0.75 µg/cm² instead of 7.28 µg/cm², the MoS values calculated by the applicant are 850 (face), 558 (hands) and 337 (face and hands).

1) Dermal absorption data

The dermal absorption value of 0.75 µg/cm² originates from a study in which a formulation is used that may not be representative of the majority of Kojic Acid-containing formulations. As mentioned in chapter 3.2.2, it contains a high amount of silicones, polyols and nylon. In an explanatory letter (December 2011), the applicant confirmed that the formulation was a w/o emulsion. Both phases of the emulsion have to be mixed before application. It was further confirmed that most leave-on cream

formulations are mainly o/w emulsions. The applicant argued that given the fact that Kojic Acid is a hydrophilic molecule, a w/o emulsion provides better stability and protection against oxidation. It is further argued that this type of formulation promotes rather than inhibits the penetration of the substance.
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 3.63 µg/cm² with a SD of 2.38 µg/cm² (highest value was 7.28 µg/cm²). As in the latest version of 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).

2) The choice of the NO(A)EL

The SCCS acknowledges that some thyroid effects are known to be rodent-specific. However, to give exclusion that the effects observed in this particular case are irrelevant for men and that Kojic Acid is not a thyroid tumour inducer, the original raw data with respect to genotoxicity/mutagenicity from 2006 are re-examined according to the current standards. In addition, the RIVM report (601516009) (Ref. 11) on the overall assessment of the relevance of thyroid effects observed in rodents for humans is taken into consideration.
The re-evaluation of the genotoxicity/mutagenicity *in vitro* and *in vivo* data (Full report in **Appendix 2**) leads to the conclusion that the opinion on genotoxicity/mutagenicity drafted in 2008 is still valid. 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.
Since Kojic Acid is considered to be non-genotoxic for men, the thyroid tumours observed in rats are not relevant for human carcinogenicity risk. In contrast to the risk of carcinogenicity, the disturbance of the hypothalamus-pituitary-thyroid (HPT) axis itself is considered to be toxicologically relevant for humans since regulation of thyroid function is comparable in rats and humans, although the latter are less susceptible to such disturbances. Therefore, disturbance of the HPT-axis is considered to be a hazard indicator for humans and should be taken into account when setting a NOAEL value. Upon expert judgement a reduced interspecies assessment factor could be taken into account (Ref. 11).

This means that the conservative NOAEL of Kojic Acid derived from the 28-day oral study in rat still is used.

Therefore, in the MOS calculation, the conservative NOAEL value of 6 mg/kg/day as obtained in the 28-day, oral rat study, focussing on the effects on the HPT-axis, is taken into account. As this value is derived from a subacute study, a default assessment factor of 3 is applied to correct for duration extrapolation (to extrapolate from subacute to subchronic exposure).

As the newly introduced dermal absorption study is considered not to be representative for the products present on the market (as explained above), the conservative dermal absorption of 8.39 µg/cm^2 is taken into account.
This leads to the following MoS values:

Face

Maximum absorption through the skin	A (µg/cm²)	=	8.39 µg/cm²
Skin Area surface	SAS (cm²)	=	565 cm²
Dermal absorption per treatment	SAS x A x 0.001	=	4.11 mg
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	SAS x A x 0.001/60	=	0.079 mg/kg
No observed adverse effect level (28 days, rat, oral)	NOAEL	=	6 mg/kg bw/d
No observed adverse effect level extrapolated to subchronic study (6-month, rat, oral)	NOAEL/3	=	2 mg/kg bw/d
Margin of Safety	**NOAEL / SED**	**=**	**25.3**

Hands

Maximum absorption through the skin	A (µg/cm²)	=	8.39 µg/cm²
Skin Area surface	SAS (cm²)	=	860 cm²
Dermal absorption per treatment	SAS x A x 0.001	=	6.28 mg
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	SAS x A x 0.001/60	=	0.120 mg/kg
No observed adverse effect level (28 days, rat, oral)	NOAEL	=	6 mg/kg bw/d
No observed adverse effect level extrapolated to subchronic study	NOAEL/3	=	2 mg/kg bw/d
Margin of Safety	**NOAEL / SED**	**=**	**16.7**

Face and hands

Maximum absorption through the skin	A (µg/cm²)	=	8.39 µg/cm²
Skin Area surface	SAS (cm²)	=	1425 cm²
Dermal absorption per treatment	SAS x A x 0.001	=	10.37 mg
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	SAS x A x 0.001/60	=	0.199 mg/kg
No observed adverse effect level (28 days, rat, oral)	NOAEL	=	6 mg/kg bw/d
No observed adverse effect level extrapolated to subchronic study	NOAEL/3	=	2 mg/kg bw/d
Margin of Safety	**NOAEL / SED**	**=**	**10.1**

In the MoS calculation
(i) A conservative value for the dermal absorption is applied (mean plus 2 SD),
(ii) A conservative NOAEL, derived from the disturbance of the HPT-axis in rats, is used,
(iii) An extrapolation factor from subacute to subchronic exposure is used

It is generally known that humans are much less susceptible to HPT-axis disturbances than rats (ref. 11). For these reasons, the reduced interspecies assessment factor of 1 is taken into consideration.

Therefore, the SCCS is of the opinion that upon re-examination of the available data for Kojic Acid, its use as a whitening agent at a concentration of 1.0 % in leave-on creams, when applied to face and/or hands is safe for the consumer.

It should, however, be noticed that whenever human skin barrier is disturbed, as is for example the case after peelings, or application to larger skin surfaces are involved, the use of Kojic Acid is not considered to be safe.

As far as the derivatives of Kojic Acid are concerned, the SCCS did not receive any data, meaning that no conclusion can be drawn on the safety of the derivatives.

5. CONCLUSION

Re-examination of the available data for Kojic Acid, used as a skin whitening agent at 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 is safe for the consumers.

As far as the derivatives of Kojic Acid are concerned (e.g. esters of Kojic Acid, Kojic Acid dipalmitate, Kojic Acid isopalmitate and chloro-Kojic Acid), the SCCS did not receive any data, meaning that no conclusion can be drawn on the safety of the derivatives.

When human skin barrier is weakened, (e.g. after peelings) or Kojic Acid is applied on larger skin surfaces, the use of Kojic Acid is of concern.

6. MINORITY OPINION

None

7. REFERENCES

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Appendix 1: Skin care formulation used in the *in vitro* dermal absorption study (see 3.2.1)

The formulation used is prepared in two phases which are mixed by vortexing at the start of the study. They consist of:

- A **water** phase:

Test substance name:	Amount (g):
Water	32.645
Preservative	0.2
Glycerin	23
Propylene glycol	6
EDTA	0.05
Kojic Acid	0.88
Lactic acid	0.005
Total	62.78 g per 100 g of final formulation

- An **oil** phase:

Test substance name:	Amount (g):
Dimethicone	25
Phenyl trimethicone	4
Oil	3
Preservative	0.1
Nylon	5
Total	37.1 g per 100 g of final formulation

Appendix 2: Re-evaluation of genotoxicity/mutagenicity data (see 4. Discussion)

In vitro tests

The results obtained in *in vitro* tests seem to be contradictory. However, re-evaluation of the tests and separation of them into tests measuring direct endpoints and indicator tests provides more clarity.
The 3 genotoxic endpoints (gene mutations, structural chromosome aberrations and numerical chromosome aberrations) are covered in reliable tests.
Of the 6 gene mutation tests in bacteria only 2 are considered reliable and used in this opinion (refs 22 and 89). One Ames test (ref 22) was positive. The second was negative. However, not only 2 strains were used but also the concentrations tested were much lower which may explain the negative results in this test.
The positive results found in bacteria were not confirmed in mammalian cells. The most reliable gene mutation test in mammalian cells was negative. The top concentration used was the one required in the OECD guideline (10 mM or 5000 µg/ml whichever is the lowest; for Kojic acid 10 mM = 1421 µg/ml). Therefore the fact that the top concentration did not show the correct required level of cytotoxicity (10-20% survival according to the OECD guideline) is no longer relevant.
Chromosome aberrations were covered in 2 tests. A chromosome aberration test was negative at short harvest times but positive at longer ones. The authors of the test report indicate that the positive results may be due to cytotoxicity (in this case a reduction in number of cells not a reduction in mitotic index). The authors conclude: "*In the absence of metabolic activation and after 18 or 28 h exposure to cytotoxic concentrations (cell numbers reduced by approximately 50%) the test item produced a weak increase in chromosome aberrations which was slightly in excess of our historical control incidence. No similar increase was seen after 4 h of exposure or in all tests performed in the presence of metabolic activation. Although a weak effect of the test item may not be excluded the positive response may be related to cytotoxicity.*" When re-evaluating the report, it seems that the authors may be right. In a micronucleus test the results were reliable (and negative) in the part done with SVK14 cells; less convincing were the positive results with HepG2 cells. Positive results were only found at concentrations well above the required concentrations in OECD guidelines. Consequently, to date this test was considered negative.

Consequently, under *in vitro* conditions, the indications for a mutagenic potency of Kojic acid are limited and in fact restricted to one reliable positive Ames test and one positive CA test at longer harvest times but where the authors consider cytotoxicity as reason for the positive reaction.

test	ref	concentration	S9	result	remarks
Ames test	21	500-4000 µg/plate	+/-	weak mut	Poor description of test compound and results; limited value
Ames test	93	30--5000 µg/plate	+/-	mutagenic	Unsure whether the compound tested was kojic acid; limited value
Ames test	A3	100-6000 µg/plate	+/-	mutagenic	Poor description of test compound and results; limited value
Ames test	22	33-5000 µg/plate	+/-	mutagenic	Reliable test

test	ref	concentration	S9	result	remarks
Ames test	AR2??	33-5000 µg/plate	+	negative	Unable to find this test. Not in Circa????
Ames test	89	3-1000 µg/plate	-	negative	Reliable test. However, only 2 strains tested
Ames test	59	10-10000 µg/plate	+/-	mutagenic	Unreliable test; no value
GM E.coli K12	83	1-2 – 100 µl	+/-	negative	Unreliable test; no value
GM mammalian cells	23	30 – 10000	-	negative	But unpublished report. Only results section available. No indication on exposure. Only without S9 mix. Therefore less reliable
GM mammalian cells	91	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.
CA test	A3	3 – 6 µg/ml	+/-	mutagenic	Poor description of test compound and results; limited value.
CA test	90	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.
SCE test	A3	3 – 6 µg/ml	+/-	mutagenic	Poor description of test compound and results; limited value. Indicator test
MN-test		500 – 8000 µg/ml	+/-	negative	Reliable test
		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 concentrations

***In vivo* tests**

Under *in vivo* conditions none of the *in vitro* positive effects could be confirmed.
Firstly, the results of the DNA adduct tests demonstrated that Kojic acid does not generally bind to DNA.
The positive finding in the Ames tests is covered by 3 tests which measure or are considered to measure gene mutations *in vivo*. The UDS test, not being considered as an adequate *in vivo* test to confirm positive results in an *in vitro* gene mutation test, was well performed and negative. The Comet assay which is developed as a test measuring clastogenicity, but as recently was demonstrated, which is also rather sensitive in detecting compounds which induce gene mutations, was negative as well. Finally, the best test to confirm *in vitro* gene mutations, a gene mutation test using transgenic mice was negative too. However, unfortunately in this latter test a positive control was not included which may weaken the negative results.
The positive clastogenic finding in an *in vitro* chromosome aberration test was likewise well covered with negative *in vivo* tests. Firstly the Comet assay was negative and secondly a well performed *in vivo* micronucleus test was negative as well. Next to this a single positive result was found in an *in vivo* micronucleus test in which micronucleus induction was studied in hepatocytes of hepatectomised rats and mice. Clearly, this is not a standardized test and the value of the positive result in very limited.

test	species	dose	tissue	result	remarks
DNA adducts	F344/DuG rj rats	0.5 or 2% in diet	liver	negative	Indication for DNA binding, No OECD guideline. "Indicator test"
DNA adducts	F344 rats	0.02, 0.2 or 2% in diet	thyroid	negative	Indication for DNA binding, No OECD guideline. "Indicator test"
UDS test	Wistar HanIbm rats	150, 1500 mg/kg bw	liver	negative	Reliable test. Indicator test.
In vivo GM	Muta ™mice	800, 1600 mg/kg bw	liver	negative	Test performed without positive control. Therefore less reliable.
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
MN test	NMRI mice	187.5, 375, 750 mg/kg bw	Bone marrow	negative	Reliable test
MN test	ddY mice	125, 250, 500, 1000 mg/kg bw	Bone marrow	negative [1]	Poor description of test compound and results; limited value.

test	species	dose	tissue	result	remarks
MN test	ddY mice Fisher rats	500, 1000 mg/kg bw	liver after hepatectomy [2]	positive mice, negative rats	The relevance of a positive results after hepatectomy is unclear It certainly is a standard test. Limited value.
Dominant lethal test	BDF1 mice	350, 700 mg/kg bw	-	negative	Reliable test

Conclusion

Having re-evaluated several of the *(in vitro)* tests, the conclusion from the opinion as drafted in 2008 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 and additional tests are unnecessary.***