Amended Safety Assessment of Kojic Acid as Used in Cosmetics

Status:	Draft Tentative Amended Report for Panel Review
Release Date:	August 15, 2025
Panel Meeting Date:	September 8-9, 2025

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Samuel M. Cohen, M.D., Ph.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume, M.B.A. This safety assessment was prepared by Christina Burnett, M.S., Senior Scientific Analyst/Writer, CIR.

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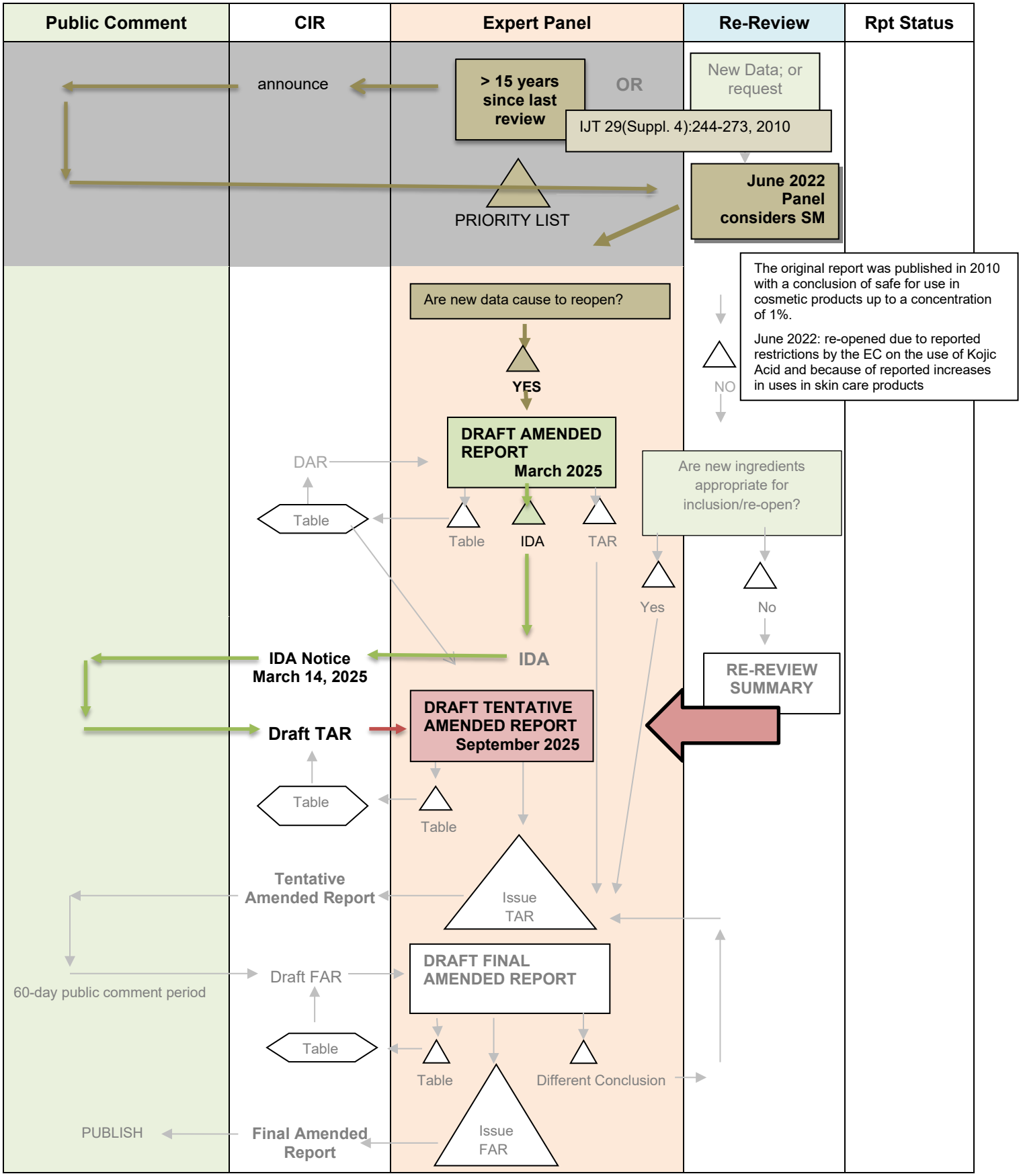
RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Kojic Acid

MEETING September 2025



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, M.S., Senior Scientific Analyst/Writer, CIR
Date: August 15, 2025
Subject: Amended Safety Assessment of Kojic Acid as Used in Cosmetics

Enclosed is the Draft Tentative Amended Report on the Safety Assessment of Kojic Acid as Used in Cosmetics. (It is identified as *report_KojicAcid_092025* in the pdf document). At the June 2025 meeting, the Panel determined that the data were insufficient to support safety of this cosmetic ingredient, and an Insufficient Data Announcement (IDA) was issued identifying the additional data needs:

- A margin of exposure (MOE) calculation for whole body exposure
- An explanation as to why the European Union (EU) restricted use of Kojic Acid to the face and hands only

Since the IDA, CIR has received two data submissions, one which came through the Council (*data1_KojicAcid_092025*) and the other that was directly received from the submitter (*data2_KojicAcid_092025*), containing MOE calculations for whole body exposure to Kojic Acid and the rationale for the EU restrictions. The MOE values for whole body application were less than 100, indicating a potential for health risks when used at high levels of exposure. The concern of potential risk to human health when Kojic Acid is used at high levels is what prompted the EU to restrict the use of Kojic Acid to the face and hands only.

Comments received from the Council on the Draft Amended Report that was reviewed by the Panel in June have been addressed (*PCPCcomments_KojicAcid_092025* and *response-PCPCcomments_KojicAcid_092025*). Additional supporting documents for this report package include a flow chart (*flow_KojicAcid_092025*), report history (*history_ KojicAcid_092025*), a search strategy (*search_KojicAcid_092025*), a data profile (*dataprofile_KojicAcid_092025*), transcripts from most recent meetings that Kojic Acid has been discussed (*transcripts_KojicAcid_092025*), the minutes from all the meetings at which Kojic Acid were discussed during the original reviews (*originalminutes_KojicAcid_092025*), and the original report (*originalreport2010_KojicAcid_092025*).

A draft Abstract and Discussion have been included in this report version. The Panel should carefully consider and discuss the data (or lack thereof), and issue a Tentative Amended Report with a safe, safe with qualifications, insufficient data, unsafe, or split conclusion, and identify any additional items for inclusion in the Discussion.

For your recollection:

Data need	Received?	Notes
MOE calculation for whole body exposure	Yes	MOE value below 100
Explanation as to why the EU restricted use of Kojic Aci to the face and hands only	Yes	MOE values indicated a potential risk to human health when used high levels of exposure occur

Kojic Acid History

2010 – The CIR Final Report on the Safety Assessment of Kojic Acid was published in the *International Journal of Toxicology*. The Panel concluded that Kojic Acid is safe as a cosmetic ingredient up to a concentration of 1%.

June 2022 – The Panel determined that this safety assessment should be re-opened for re-evaluation due to reported restrictions by the European Commission on the use of Kojic Acid and because of reported increases in uses in skin care products.

March 2025 – The Panel determined that the data were insufficient to support safety of this cosmetic ingredient and issued an IDA. The additional data needs were:

- An MOE calculation for whole body exposure
- An explanation as to why the EU restricted use of Kojic Acid to the face and hands only

Kojic Acid Data Profile* – September 2025 – Christina Burnett																														
	Use				Toxico-kinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	New Rpt	Old Rpt	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Kojic Acid CAS No. 501-30-4	X	O	X	X	XO	XO	O	O	O		XO	XO			O	XO	XO	O	O		O	O		O	O	O		O	O	XO

* "X" indicates that new data were available in this category for the ingredient; "O" indicates that data from the original assessment were available

Kojic Acid

Ingredient	CAS #	PubMed	FDA	CompTox	ChemPort	NIOSH	NTIS	NTP	FEMA	EU	ECHA	SIDS	SCCS	AICIS	FAO	WHO	Web
Kojic Acid	501-30-4	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Search Strategy

PubMed

Search performed from 2005 to present
(kojic acid) OR (501-30-4[EC/RN Number]) – 1173 hits, 29 relevant

LINKS

Search Engines

- Pubmed - http://www.ncbi.nlm.nih.gov/pubmed
  - appropriate qualifiers are used as necessary
  - search results are reviewed to identify relevant documents
- CompTox: https://comptox.epa.gov/dashboard/chemical/pubmed-abstract-sifter/DTXSID3039242; https://www.epa.gov/comptox-tools/downloadable-computational-toxicology-data#LM
- eChemPortal: https://www.echemportal.org/echemportal/
- DeepDyve: https://www.deepdyve.com/
- Connected Papers - https://www.connectedpapers.com/

Pertinent Websites

- wINCI - https://incipedia.personalcarecouncil.org/winci/ingredient-custom-search/
- FDA Cosmetics page - https://www.fda.gov/cosmetics
- eCFR (Code of Federal Regulations) - https://www.ecfr.gov/
- FDA search databases: https://www.fda.gov/industry/fda-basics-industry/search-databases
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras
- SCOGS database: https://www.fda.gov/food/generally-recognized-safe-gras/gras-substances-scogs-database
- Inventory of Food Contact Substances Listed in 21 CFR: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=IndirectAdditives
- Drug Approvals and Database: https://www.fda.gov/drugs/development-approval-process-drugs/drug-approvals-and-databases
- FDA Orange Book: https://www.fda.gov/drugs/drug-approvals-and-databases/approved-drug-products-therapeutic-equivalence-evaluations-orange-book
- OTC Monographs - https://dps.fda.gov/omuf
- Inactive Ingredients Approved For Drugs: https://www.accessdata.fda.gov/scripts/cder/iig/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- EUR-Lex - https://eur-lex.europa.eu/homepage.html
- Scientific Committees (SCCS, etc) opinions: https://health.ec.europa.eu/scientific-committees_en https://health.ec.europa.eu/scientific-committees/scientific-committee-consumer-safety-sccs_en
- ECHA (European Chemicals Agency – REACH dossiers) – https://echa.europa.eu/
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- EFSA (European Food Safety Authority) - https://www.efsa.europa.eu/en
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/

- International Programme on Chemical Safety http://www.inchem.org/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) IRIS library - https://apps.who.int/iris/
- a general Google and Google Scholar search should be performed for additional background information, to identify references that are available, and for other general information - www.google.com https://scholar.google.com/

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: March 4, 2025

SUBJECT: Draft Amended Report: Amended Safety Assessment of Kojic Acid as Used in Cosmetics (draft prepared for the March 13-14, 2025 meeting)

The Personal Care Products Council respectfully submits the following comments on the draft amended report, Amended Safety Assessment of Kojic Acid as Used in Cosmetics.

Key Issues

In several places in the CIR report, e.g., Introduction, Cosmetic Use, Summary, Discussion, the CIR report on Kojic Acid should clearly state that in the United States products that whiten the skin are considered drugs, while in many jurisdictions, such as Europe, products that whiten the skin are considered cosmetics.

Cosmetic Use – Although the description of MoCRA in the Cosmetic Use section is better, it is still not very clear. The report currently states: "As a result of the Modernization of Cosmetics Regulation Act (MoCRA) of 2022, the VCRP was terminated in 2023, and as of 2024, manufacturers and processors have been mandated to register and list their products (and ingredients therein) with the FDA (i.e., RLD). An exception is made for small businesses, which are exempt from MoCRA reporting for most cosmetic product categories. However, to utilize the exemption, the small business must not sell eye area products, injected products, internal use products, or products that alter appearance for more than 24 h."

We suggest the following changes (shown in red): "As a result of the Modernization of Cosmetics Regulation Act (MoCRA) of 2022, the VCRP was ~~terminated~~ discontinued in 2023, and as of 2024, manufacturers and processors ~~have been mandated~~ are required to register facilities and list their products (and ingredients therein) with the FDA (i.e., RLD). An exception is made for small businesses (average gross annual sales in the US of cosmetic products for the previous 3-year period is less than $1,000,000, adjusted for inflation), which are exempt from MoCRA reporting for most cosmetic product categories. ~~However, to utilize the exemption, the small business must not sell~~ Eye area products, injected products, internal use products, or products that alter appearance for more than 24 h, and the facilities that manufacture these

products are not included in this exemption."

Additional Considerations

Method of Manufacture – Please correct "galatosone" to "galactosone"

Cosmetic Use – For the RLD information, it would be helpful to state the main types of products reported to contain Kojic Acid.

Dermal Penetration, old report summary – It would be helpful if some details of the rat study (especially the dermal portion) were included in this report. Although the SED calculations from the Nohynek et al. (2004) study were presented in the Dermal Penetration section in the original report, they should be presented in the Risk Assessment section of the re-review so that they can be compared to the SCCS SED calculations. The primary reference for these SED calculations should be stated so it is clear that the values were not calculated by CIR or the SCCS.

Short-Term – Please revise: "Treatment sites were covered for 6 h each day with gauze before being removed with warm water." (replace "removed" with "washed" – or say that Kojic Acid was removed)

Please correct: "The rabbits were killed after the end of treatment prior and underwent necropsy and histopathology." (delete "prior", or indicate what happened "prior")

At what doses of Kojic Acid were the erythematous papules and abscesses observed in rabbits?

Short-Term; Summary – Please correct "A/G ration" ("ration" should be "ratio")

Developmental and Reproductive Toxicity, old report summary – A description of a developmental and reproductive toxicity study is incomplete unless it states when during gestation the animals were treated. If they were guideline studies, stating the guideline, e.g., OECD number, would be a sufficient description.

Genotoxicity, old report summary – Are the units "ml/ml" for the study with light irradiation in Chinese hamster lung cells, correct?

Carcinogenicity, old report summary – Please revise: "Kojic Acid is a group 3 carcinogen" to "Kojic Acid is in IARC group 3". As stated in the CIR report, group 3 is for substances that are not classifiable, there are not enough data to consider them to be carcinogens.

Kojic Acid – September 2025 – Christina Burnett	
Comment Submitter: Alexandra Kowcz, Personal Care Products Council **Date of Submission: March 4, 2025**	
Comment	**Response/Action**
Key Issue: In several places in the CIR report, e.g., Introduction, Cosmetic Use, Summary, Discussion, the CIR report on Kojic Acid should clearly state that in the United States products that whiten the skin are considered drugs, while in many jurisdictions, such as Europe, products that whiten the skin are considered cosmetics.	Added in Cosmetic Use section where EU regulations are discussed. The Panel may want to discuss if it is necessary to add in other sections of the report.
Key Issue, Cosmetic Use – Although the description of MoCRA in the Cosmetic Use section is better, it is still not very clear. The report currently states: "As a result of the Modernization of Cosmetics Regulation Act (MoCRA) of 2022, the VCRP was terminated in 2023, and as of 2024, manufacturers and processors have been mandated to register and list their products (and ingredients therein) with the FDA (i.e., RLD). An exception is made for small businesses, which are exempt from MoCRA reporting for most cosmetic product categories. However, to utilize the exemption, the small business must not sell eye area products, injected products, internal use products, or products that alter appearance for more than 24 h." We suggest the following changes (shown in red): "As a result of the Modernization of Cosmetics Regulation Act (MoCRA) of 2022, the VCRP was terminated discontinued in 2023, and as of 2024, manufacturers and processors have been mandated are required to register facilities and list their products (and ingredients therein) with the FDA (i.e., RLD). An exception is made for small businesses (average gross annual sales in the US of cosmetic products for the previous 3-year period is less than $1,000,000, adjusted for inflation), which are exempt from MoCRA reporting for most cosmetic product categories. However, to utilize the exemption, the small business must not sell Eye area products, injected products, internal use products, or products that alter appearance for more than 24 h, and the facilities that manufacture these products are not included in this exemption."	Boilerplate language for this paragraph has been updated.
Method of Manufacture – Please correct "galatosone" to "galactosone"	Correction made.
Cosmetic Use – For the RLD information, it would be helpful to state the main types of products reported to contain Kojic Acid.	Added product categories.
Dermal Penetration, old report summary – It would be helpful if some details of the rat study (especially the dermal portion) were included in this report. Although the SED calculations from the Nohynek et al. (2004) study were presented in the Dermal Penetration section in the original report, they should be presented in the Risk Assessment section of the re-review so that they can be compared to the SCCS SED calculations. The primary reference for these SED calculations should be stated so it is clear that the values were not calculated by CIR or the SCCS.	Detail added.
Short-Term – Please revise: "Treatment sites were covered for 6 h each day with gauze before being removed with warm water." (replace "removed" with "washed" – or say that Kojic Acid was removed) Please correct: "The rabbits were killed after the end of treatment prior and underwent necropsy and histopathology." (delete "prior", or indicate what happened "prior")	Revised to "washed". Deleted "prior".

At what doses of Kojic Acid were the erythematous papules and abscesses observed in rabbits?	No further details provided.
Short-Term; Summary – Please correct "A/G ration" ("ration" should be "ratio")	Corrections made.
Developmental and Reproductive Toxicity, old report summary – A description of a developmental and reproductive toxicity study is incomplete unless it states when during gestation the animals were treated. If they were guideline studies, stating the guideline, e.g., OECD number, would be a sufficient description.	Old summary data expanded with more detail.
Genotoxicity, old report summary – Are the units "ml/ml" for the study with light irradiation in Chinese hamster lung cells, correct?	Corrected to "mg/ml".
Carcinogenicity, old report summary – Please revise: "Kojic Acid is a group 3 carcinogen" to "Kojic Acid is in IARC group 3". As stated in the CIR report, group 3 is for substances that are not classifiable, there are not enough data to consider them to be carcinogens.	Reworded.

JUNE 2022 PANEL MEETING – STRATEGY MEMO

Belsito Team – June 16, 2022

Dr. Belsito - OK. Okey doke. So we have, I think 4 minutes, but let's try and knock off we want to try and knock off the Kojic Acid. That's *(inaudible) use area Monice?

Monice Fiume (CIR) - Uh, sure.

Dr. Belsito - And that's in wave.

Monice Fiume (CIR) - I believe they should be in the original submission. A strategy memos.

Dr. Belsito - In the admin memo is that it?

Monice Fiume (CIR) - Let me see how it was put into the book. It's should say strategy memo. On the flash, it starts with SM and then Kojic Acid, SM aluminum prostaglandins and use table, yeah.

Dr. Belsito – Yeah. That's. Umm. Yeah. OK. So first of all, let me talk about aluminum. Since I'm the person who chooses the allergen of the year, we do not have to reopen it. The issue is with vaccines, not with cosmetic use.

Monice Fiume (CIR) - OK.

Dr. Belsito - And it's not even that common with vaccines. So definitely a no to reopening aluminum. And kojic acid. Umm. So this is basically new data that the SCCS has acted upon, and namely data from the US EPA noise 2019. It came to the conclusion that elevated TSH and rodents leads to thyroid hypertrophy and potential thyroid cancer and adverse outcome that is limited relevance to human thyroid cancer due to species differences in sensitivity. And the same conclusion was also made previously by several other expert groups, but through some reason, SCCS. US has decided that. They are. Not to reduce the interspecies factor. And has come up with some different conclusions than what we had. Nonetheless, I mean, there's clearly new data. The 2019 EPA study since we last looked at kojic acid. So do we need to reopen this?

Dr. Rettie - There was some mention of possible endocrine disruption as well. Did that come from the Europe study European group?

Dr. Belsito - You know, that came the noise group *(inaudible). That's the thyroid stuff that I just.

Dr. Rettie - Uh, so those were related in, in rats. The yeah. OK, go.

Dr. Belsito - Yeah.

Dr. Klaassen - This there's basically in Europe, anything that's considered an endocrine disruptor is kind of like a fire bomb. And so there's an endocrine disruptor they get overly excited, let's say about its relevance to humans. This this thyroid problem is not considered a problem in the United States and for the FDA or the EPA. So I have no problem with the thyroid aspect and think it's just an overreaction of the European to endocrine disruptors in general.

Dr. Rettie - OK. Thanks.

Dr. Belsito - For Curt, you don't think we need to go back and revisit it?

Dr. Klaassen - I don't think so personally, no.

Dr. Liebler - Isn't the question isn't the question whether to do it on our 15 year clock, which would bring it up in 2025, or rereview it now?

Dr. Belsito - Correct.

Dr. Klaassen - Right.

Dr. Liebler - And so you know, I it sounds like we wait until it's due rather than rush to rereview it now.

Dr. Klaassen - I agree.

Dr. Belsito - Then what are your thoughts, given the EPA data that's new to us?

Dr. Liebler - I mean I guess I agree with Curt, but I'm willing to be talked out of it if Don, if you feel that the that the new data justifies speeding up?

Dr. Belsito - Well, I mean, it's now, you know, 2022, June of 2022, 2025 is going to be here before we know it. You know even if we agreed to reopen it, it would just Monice how would that work? It would go onto the priority list for 2023 and it probably would be what December of 2023 before we start looking at it?

Monice Fiume (CIR) - It may be earlier than that, it doesn't necessarily have to go onto the priority list because the panel can reopen anything at any given time. If you want it added to the 2023 priority list, we could do it that way, but it would just be issued really as a report that is being reopened for cause.

Dr. Belsito – Paul?

Dr. Snyder - Yeah, I was. I was a little bit like Dan. I was kind of on the fence on this. I think that the issues for me, that change in that the kojic acid not safe when used it at up to 1% for skin lightening due to endocrine disrupting properties, I was I had a question. What drove that conclusion? And so if we reopened, we could kind of better understand that conclusion. The other issues I had two other issues. Were there any data in the SEC SCCP report that weren't in our report? And so that would be, were there any significant new data that we really need?

Dr. Belsito - Just that EPA study.

Dr. Snyder - That we really need to have a look at and then any differences in the formulations of kojic acid used as a skin lightening agent versus cosmetic product. Even though the skin lightening is the drug effect. But if there was a difference in formulation that might help us better understand that because I think we always kind of deal with that and discuss that. So I could go either way, I mean I.

Dr. Belsito - I think we'll find that the cosmetic uses of this have skyrocketed since we last looked at it. It is a hot ingredient in the quote UN quote, Cosmeceuticals I know that that is not an appropriately accepted term, but that's what dermatologists referred to as Cosmetic products that are marketed with very heavy marketing claims to improve. Whatever that keeps them out of the OTC category. But the consumer doesn't understand the difference so.

Dr. Snyder - On that basis, I think we, I think we should reopen then I would sway that would sway me to the if it, if it's skyrocketed and this this change in this levels and things I would think it would be a good due diligence on our part to just make sure that we're good with our conclusion.

Dr. Belsito - Yeah, I mean, I said we should open it to rereview. It's going to come up in another couple years anyway, so let's do it now.

Dr. Snyder - Yeah, I'm agree with that.

Dr. Klaassen - I have no problem with that.

Dr. Liebler - Same here.

Dr. Belsito - OK, Monice so this one we will reopen.

Monice Fiume (CIR) - OK and just to give insight according to the VCRP, the number of uses for kojic acid is currently 87.

Dr. Belsito - That's voluntary. And what was it when we last looked at it?

Monice Fiume (CIR) - Let me see.16.

Dr. Belsito - You know.

Monice Fiume (CIR) - So yeah, so it's gone up.

Dr. Belsito - And there are in a lot of the companies that are using it, are probably leading are almost certainly not members of PCPC and are probably not reporting. Because it's used in a lot of these little boutique bronze.

Cohen Team – June 16, 2022

Transcript of discussion missing.

Full Panel – June 17, 2022

Dr. Belsito - OK so panel safety assessment on this was issued in 2010. We concluded two endpoints of concern, dermal sensitization and skin lightening would not be seen at use concentrations below 1 cent and so we went with the safe for use in cosmetic products at that level. I will remind you it is effective in lightening and skin lightening would be considered a drug or facts. They get to a number of studies on endocrine disruption, particularly thyroid, and recognize that the rodent thyroid and is sensitive to chemical substances and physiologic perturbations in ways different than in humans. So we thought that that was not an issue here. We're now reviewing it because it's been more than 15 years. Yeah. Well, we'll soon be 15 years, I should say in 2025. It's not yet at 15 years. And the question was, should we accelerate review? And I thought we should because the use of this product is increasing rapidly, you know, from the VCRP data Monice, I think it went from what, 11 or 12 up to 84. I think that that's probably gross underrepresentation. Kojic acid has become a huge issue and or huge product in in this in "cosmeceuticals". I know they don't exist, but it, you know dermatologist are using it like crazy. And the companies that I see

with patients bringing kojic acid products are relatively small, probably don't participate in PCPC and are probably not reporting their uses to the VCRP. So I think we need to relook at this because it's a hot consumer item.

Dr. Bergfeld - Any other comments?

Dr. Cohen - Yeah. So, Don, we, we had a virtually parallel discussions. I think most of the discussion of kojic acid and the dermatology lexicon relates to a drug effect, a lightening effect. and I didn't think that the three year typical window was that far out that we needed to reaccelerate it and we have in there 1% and I understand the new the European regulation is .7%. I listen. I'm not digging in on this. It's just that we thought that there wasn't enough there to accelerate it. And this also coupled with the fact that our publication expectation on this is 2 to three years anyway. So that was our comment on it.

Dr. Belsito - We discussed that also, but it's only going to be a matter of a couple years before it's going to come up so. Or I'll let other Members in my group and other people comment. I just think that yeah, the Europeans have changed their opinion. And the use is certainly more than 84 products. And when you look at the marketing, you know it's marketed skin brightening. You know, not lightening. You know you so they avoid any drug claims, you know.

Dr. Cohen - So. So Don, I think your points and your teams points are well taken instead of maybe having your team persuade our team, maybe I'll just ask our team if there's any objections to the acceleration cause it, we went back and forth on this. So Tom, Ron, Dave, any issues if we affirm the Belsito's team of reaccelerated more for public awareness issues.

Dr. Slaga - Uh, Tom here. No matter of fact, I was my original.

Dr. Cohen - It was.

Dr. Ross - Fine with me there, Ross.

Dr. Bergfeld – Ron?

Dr. Shank - I don't see it as so important, but it's strictly up to the dermatologist.

Dr. Cohen - OK. So Don, we can affirm your motion to accelerate the review.

Dr. Belsito - Thank you.

Dr. Bergfeld - I think that we've actually had a vote to accelerate by all the comments made and the individuals commenting, so we will accelerate this ingredient. Thank you. So we're moving on to the next ingredient.

MARCH 2025 PANEL MEETING – DRAFT AMENDED REPORT

Belsito Team – March 13, 2025

DR. SNYDER: The next one is Kojic Acid. Bring it up here, Kojic Acid. This is an amended safety assessment. The original review was published in 2010. It was safe as used up to 1 percent. It's 15 years since that was looked at, so it was reopened.

In June of 2022, we reopened due to the EU restrictions that we learned due to increased uses in skin care products. In 2024 RLD data, there were 1,114 formulations, mostly skin care preps, up to 1 percent in leave-ons. So, the report's on Page 60, did anybody have any comments?

MS. BURNETT: I did want to note that, at the time, in June 2022, when the Panel looked at this and decided to reopen it, the EU had one conclusion. And then they changed it.

DR. SNYDER: Okay.

MS. BURNETT: And the current opinion is in the report. But, at that time, they had declared it insufficient data. And then, the data was produced.

DR. SNYDER: Went as safe?

MS. BURNETT: Yeah.

DR. SNYDER: Okay.

DR. BELSITO: But they approved it only for hands and face, which I don't understand. Right?

MS. BURNETT: Correct. Yeah. that's what it says.

MS. FIUME: But is it only hand and face?

DR. BELSITO: On PDF Page 63, for this ingredient, the regulation states that Kojic Acid may only be used in face and hand products at a maximum concentration of 1 percent.

MS. BURNETT: Okay.

MS. FIUME: Okay. sorry. I was looking at the SCCS. Yes, the EU said 1 percent, face and hand. Yes.

DR. BELSITO: And I didn't understand that. Do we have any idea why they limited it to hands and face? I mean, they did their MOE calculation -- sorry, Curt, for using that word -- based on the amount that would be applied to the hands and face. But I just don't understand that restriction.

MS. BURNETT: I mean, I don't know for sure what their logic was, but maybe they were thinking that if you are wanting to lighten your skin, those are the places that you would most likely apply it. Don't know if anyone would actually take a bath in it.

DR. BELSITO: But I mean, as a cosmetic ingredient that we're looking at, skin lightening would not be an effect that we would look at.

MS. BURNETT: Correct.

DR. SNYDER: No. That's a drug effect. Yeah.

DR. BELSITO: Right. I mean, again, the EU, I'm trying to think are -- sunscreens are cosmetics in Europe, they're over the counter here. Maybe skin lightening creams are also cosmetics in Europe, and that's what they were looking at. But we have uses other than hands and face in our report. Right?

DR. SNYDER: Yes.

MS. BURNETT: Correct.

DR. SNYDER: Well, maybe we can get clarification on that by the time we come around in the next version of this. You think we can dig that out?

MS. BURNETT: Maybe.

DR. SNYDER: Try.

MS. BURNETT: I mean, sometimes you can find deliberations for the SCCP, sometimes you can't. But it's just not in the opinion. I don't know.

MS. FIUME: But it's the EU, they have different wordings.

MS. BURNETT: Right.

MS. FIUME: So we would have to figure out which --

MS. BURNETT: For sure. That's hard to find, their deliberations, if it's the EU.

DR. SNYDER: Okay. But we have no data to suggest that we need to limit it to the hands and face. Right, Don?

DR. BELSITO: Yeah. I mean, that's my point. We have, you know, RLD data that suggests that it's used in products other than the hands and face. You know, we have a lot of other data that I think we could basically say -- what I had here was heavy metal boilerplate. Thyroid effects were not relevant. And other genotox data was Okay. the DART data was clean. Sensitization data was clean. Skin lightening, not to be expected with a cosmetic product.

And what we would need to do -- Curt, again, my apologies -- margin of exposure to determine use beyond hand and face if we were concerned about it.

DR. KLAASSEN: I'd like to add to that, that with Kojic Acid, they actually did the study for absorption in humans.

DR. BELSITO: My conclusion there was probably safe as used but need further data supporting on face and hand use in Discussion, perhaps a margin of exposure assessment, and would like clarification why the EU limited to hands and face.

DR. RETTIE: So, from a radiolabeled study in dermatomed skin, they had 17 percent. Is that the number you're looking at?

DR. KLAASSEN: Yeah.

DR. SNYDER: Okay. So we'll try to get that clarification. Other than that, we're moving it forward, safe as used.

DR. BELSITO: I mean, I think we need to do a few calculations.

DR. SNYDER: Okay.

DR. BELSITO: But I didn't see any data that suggested that there were issues. Did you?

DR. SNYDER: No, I did not. I agree. Okay. you got that, Christina? Okay. Thank you.

Cohen Team – March 13, 2025

DR. DAVID COHEN: Okay. Kojic Acid.

The original review of Kojic Acid was performed in 2010 with the conclusion that Kojic Acid is safe for use in cosmetic products at a concentration up to 1 percent. It has been 15 years since this has been reviewed. Excuse me one second. In June of 2022, the Panel determined that the safety assessment should be reopened because of restrictions by the European Commission and use of Kojic Acid reportedly being used in skin care preparations.

There's some original report data that is put in here because it was sort of critical to finishing this report. In 2024, RLD data reported it in over 1,100 formulations. The VCRP had it in 123. Most were leave-on products. And a concentration of use survey conducted by the Council, in 2024, indicated its use at 1 percent in leave-on products, down from 2 percent in 2008.

The EU has this as Annex III and has restricted its use to 1 percent for face and hand products. And it could be used as a skin lightening agent in cosmetic products up to 1 percent.

Okay. I can open it up. I thought some of the material from the HRIPT from the old one ought to be pulled forward. Otherwise, it looks like we don't have anything in the new report on it. I thought it was pretty good information.

DR. BERGFELD: What did you think about the boilerplate in the previous discussion? Would that be added, or we'd update that?

DR. DAVID COHEN: What part?

DR. BERGFELD: The boilerplate for inhalation. Are we going to update that one?

DR. DAVID COHEN: It needs to be updated to the new boilerplate.

DR. BERGFELD: Yeah. And then the question I had was, are you going to restrict it to 1 percent or less because of depigmentation?

DR. ROSS: Present practices of use.

DR. DAVID COHEN: I'll come back around to it. What's that?

DR. ROSS: Present practices of use because --

DR. BERGFELD: Yeah. It's 1 percent.

DR. ROSS: Everything is aligning at 1 percent. You know, the Annex III, the previous, and our current concentrations.

DR. BERGFELD: As a dermatologist, pretty common ingredient for us to bleach the skin?

DR. ROSS: Yeah, right.

DR. DAVID COHEN: Or to even the tone of the skin.

DR. BERGFELD: Even the tone?

DR. DAVID COHEN: It's really for that, right? It's for tone evening rather than just skin bleaching, right?

MS. BURNETT: I do want to note that in the United States, skin lightening is not considered a cosmetic effect.

DR. DAVID COHEN: No, it's a drug effect.

MS. BURNETT: Right. So I do need wording for Discussion on how to address that. If you are satisfied with how it was addressed in the original Discussion, I can just pull it forward. But if you want it re-emphasized or written differently, please let me know.

DR. DAVID COHEN: Let me go through -- let me run the Panel first on this for other issues. David?

DR. ROSS: I didn't have too much on this absorption. It looked okay. Tox, no concerns and acute subchronic and chronic. DART, I didn't have anything. I'll let Sam comment on genotox and carcinogenesis.

Thyroid was cropping up there, and there's quite a few questionable points on the thyroid cancers in animals relative to humans. There's some nice reviews out there which highlight that.

This was a competitive inhibitor of tyrosinase, but it had no effects on melanocytes, which was interesting. The irritation looked okay, 1 in 3 percent. It was mild in rabbits. Sensitization, up to 30 percent, wasn't a sensitizer. Humans, HRIPTs, as you commented, David, 1 percent and 2 percent were okay.

The dermal depigmentation, they used that black guinea pig model, which was extensively commented on in the previous reviews of this material at CIR. And it was found to be acceptable in that model at 1 and 4 percent in the 2010 report. And it was very little effect of Kojic Acid relative to phenylhydroquinone, which was the positive control.

Christina pulled the original Japanese paper for me in 2002, and I looked at that, and that looked fine. Phototox was good. Ocular, 3 percent, it's not an irritant. And there are some MOEs out there, and you can expand that discussion or leave it as is. It looks okay.

So if you agree with the black guinea pig model for depigmentation and the MOE estimate, you're probably safe as used at 1 percent. I mean, that's where I was.

DR. DAVID COHEN: Okay. Susan?

DR. TILTON: Yeah, I agree. That was a good summary. No additional concerns about the toxicity data.

I guess there was a question posed to us about how some of the data was presented previously in tables within the report for the genotoxicity and the carcinogenicity studies, and maybe I'll let Sam comment on that.

I mean, from my perspective, it can just be included in the report. I mean, I haven't seen a report come through where the data has been presented that way. So that doesn't seem consistent with how we would typically present the data. And it's discussed within the report. So that was my only comment.

DR. DAVID COHEN: So you were safe as used at 1 percent?

DR. TILTON: Oh yeah, safe as used at 1 percent.

DR. BERGFELD: Are you going to put 1 percent into the Conclusion or just safe as used?

DR. DAVID COHEN: I might want to put it in the Conclusion. It's a cosmeceutical product, right? So you have in there that the 4 percent and the 1 percent, it doesn't go to depigmentation. But, I mean, it may have effect on dyschromias in a positive way, but that is a drug effect.

MS. BURNETT: So you're essentially reaffirming the original conclusion?

DR. DAVID COHEN: Yeah. Yeah. I am. What do you got, Sam?

DR. SAMUEL COHEN: Yeah, I would certainly reaffirm that as well. But on the genotox studies, there were some older Ames studies, chromat, and SCE studies. Again, the qualifier for the SCE you can -- but the Ames tests and the chromat were in vitro, and there were concerns about cytotoxicity. So they probably were not valid. And there are some recent in vitro tests that have all been negative consistently.

On top of that, in vivo, there's a negative micronucleus assay, a negative comet assay, and a negative DNA adduct study. There's also negative UDS, but you have to put the qualifier for that. So I think the in vivo data clearly here is overriding the in vitro.

And then with regard to carcinogenicity, the thyroid and liver tumors, these are most likely what are called CAR activator related. And those are not relevant to human cancer risk. And the adrenal tumors in rats are also not relevant to human risk. There's a nice review by Greim, G-R-E-I-M, et al. in Critical Reviews in Toxicology, maybe 10, 15 years ago. If you can't find it, let me know. I can send it to you.

So I have no concern in the fact that even IRAC classified as a 3, they don't have any concern, which is very unusual for IR. And then on top of it, is that the cytotoxicity data is all in vitro, and it's in millimolar concentrations, which is, in orders of magnitude higher than anything you're gonna attain in human exposure.

DR. ROSS: Yeah, I pulled the Greim papers myself.

DR. SAMUEL COHEN: Yeah, he did a nice job on that.

DR. ROSS: Yeah.

DR. DAVID COHEN: Sounds like quite a paper.

DR. SAMUEL COHEN: Actually, I refer to it a lot anytime I see adrenal tumors in rats. I just say it's not relevant to humans, see Greim et al.

DR. DAVID COHEN: So if we go as safe as used, and we have this Discussion issue, this is still going to come back as a draft tentative, isn't it? We have some time to think about the Discussion and the skin lightening?

DR. BERGFELD: Is this a draft item?

DR. DAVID COHEN: Right, it's not going to skip over tentative, will it?

MS. BURNETT: No, no.

DR. HELDRETH: So it's a Draft Amended Report right now. If you're giving a conclusion now, it'll go out as a Tentative Amended Report. And then it'll come back to you at a future meeting as a Draft Final Report.

MS. BURNETT: A discussion will be formulated for the public comment coming out of this meeting. If you want me to essentially update the previous discussion, I can do that.

DR. DAVID COHEN: That's why I want to think --

MS. BURNETT: If there's a point that you want me to take out, I obviously would update the inhalation boilerplate and anything else that's a boilerplate that hasn't been updated since 2010.

DR. DAVID COHEN: Okay.

DR. BERGFELD: Well, it sounds to me that the third paragraph in that previous Discussion that deals with the thyroid, we have to also add the adrenal gland comments.

DR. DAVID COHEN: I'm just going back to that for a second.

DR. HELDRETH: Yeah, and if you have any updates to the Discussion like that, please put them in your Panel returns to help Christina get it right.

DR. DAVID COHEN: I agree with you, Wilma, yeah, to the adrenal glands can go in there. Okay.

DR. BERGFELD: Have we decided on the conclusion containing the 1 percent restricted concentration, or are we just putting that in the Discussion?

DR. ROSS: I guess it depends whether you got evidence that it's not safe above 1 percent.

DR. BERGFELD: Yeah. But you could put that in the Discussion. You could say that in a sentence after you'd report all the other stuff you have in there. Where did that go?

DR. DAVID COHEN: Now, maybe we just go safe as used as described in the report because what about 1.2 percent? We don't have data to see that it's not safe.

DR. ROSS: Yeah, we don't. So, I agree with you, Dave.

DR. DAVID COHEN: Yeah, just safe as used. At first, I wanted to go with it because of the way it's used, but I don't want to apply that arbitrarily.

DR. BERGFELD: It's right there in the first paragraph, though. It says, "Therefore, the expert Panel finds that Kojic Acid should only be used up to a concentration of 1 percent in cosmetic products."

DR. DAVID COHEN: That's not --

DR. BERGFELD: That's the first paragraph of the previous Discussion.

DR. DAVID COHEN: In the Discussion?

DR. BERGFELD: Yeah. Yeah.

DR. DAVID COHEN: Yeah, yeah, in the Discussion. Okay. I thought you were mentioning a conclusion.

DR. BERGFELD: Well, I did ask that.

DR. DAVID COHEN: No, you did. You did.

DR. BERGFELD: It could go in the Discussion.

DR. DAVID COHEN: And I think we answered it.

DR. BERGFELD: Yes.

Full Panel – March 14, 2025

DR. SNYDER: So Kojic Acid, the original review was published in 2010, safe as used up to one percent. It's been 15 years so it was reopened in June of 2022, due to the EU restrictions and due to increased uses in skin care products. We received new RLD data that suggest that there's over 1,100 formulations, mostly skin care preps, up to 1 percent in leave-ons, and in products other than hands and face.

The Belsito Team motions to move this forward to an amendment safety assessment, safe as used. With one caveat, pending -- we understand the wide restrictions in the EU were to the hands and face -- if we can get that information. Widely restricted to hands and face.

DR. DAVID COHEN: So, what's the motion specifically?

DR. BERGFELD: Safe as used.

DR. SNYDER: Safe as used.

DR. DAVID COHEN: Safe.

DR. BELSITO: But we would like to look at margins of exposures for other uses on other body parts. Our discussion ended up being that we thought, perhaps, and we don't know, just like sunscreens are cosmetics in Europe and OTC here, skin

lightening creams may be cosmetics in Europe. And they were looking at it specifically in terms of use on face and hand as a skin lightener.

But, the data is, I mean, it's really clean. The thyroid effects are not relevant. The genotox data is okay. The DART data is clean. The sensitization data is clean. You know, you basically have a heavy metal boilerplate skin lightening effects. And we've dealt with those before by saying cosmetics should not have skin lightening effects. So, the restrictions to hands and face do not make sense since we're told they're used in other body parts, right?

DR. DAVID COHEN: No, no. I have no issue with the motion. The issue is, if we're moving it forward but you still want to see this. And what if you don't get to see this? What happens here?

DR. SNYDER: We'll deal with it then, because we're going to see the report again, right, before it goes final?

DR. DAVID COHEN: Comes as a final.

DR. BELSITO: Yes.

DR. SNYDER: So that's okay, yes.

DR. DAVID COHEN: Right, and I'm just fast-forwarding.

DR. SNYDER: We're fairly confident that it's going to be for the reasons Don stated. So we don't think there's any reason to hold it up. Right, Don?

DR. BELSITO: Right.

DR. DAVID COHEN: Reasonable expectations we'll get it?

DR. BELSITO: I think we can easily do the calculations; we have the data.

DR. BERGFELD: Dr. Ross?

DR. ROSS: Yes, I think this one, you know, Don, you could do those calculations. You know, in our team --

DR. BELSITO: I couldn't, you could.

DR. ROSS: Well, in our team, we discussed those MOEs that we have. And SCCS basically used a very conservative NOAEL, most conservative one they could find. And they say that in their discussion. But, I didn't think it was an overwhelming margin of exposure even with what they were using. So, I mean, we can do more MOE calculations to add to that.

One other thing we can do is expand the MOE Discussion. There is some literature out there that we didn't quote, which has an MOE in it. And we also have NOAEL values in the dossier, in sub-chronic and chronic studies, which you could incorporate and expand that MOE discussion if you wish. So you could do that. That would be my comment.

You know, if you're expanding their calculations to use on other areas of the body, then you may drive that MOE down when you do accumulative. You know what I mean, if you add in all of the exposure areas? So I just want to make that caution. If you do that then you may not get these same answers that you're looking at right now.

Jinqiu also looked at this. I don't know if he has any comments, but he can add to it if he wishes. But, we can certainly expand the MOE discussion if you like, Don.

DR. BELSITO: I mean, I think we can look at it. If you want to go out as insufficient pending an MOE, I'm okay with that too. There aren't that many uses beyond the hands and the face, but I certainly agree with your point. The more you add cumulative exposure, the lower the margin of exposure is going to become. We'll see.

DR. DAVID COHEN: Well, so I guess that's the question at hand. We're clearing this, but you still have questions. And, are those questions not important enough that if they don't happen, or they don't come, you're still going to pass it through.

DR. BELSITO: I don't know. Paul, you're leading, I mean, what do you want to do here?

DR. SNYDER: Yes, I think it's a good discussion. I'll rescind my motion. And I'll move a motion that we will issue an Insufficient Data Announcement. And the data needs will be the calculation of the margin of safety based upon all of the uses.

DR. DAVID COHEN: Second.

DR. BERGFELD: Second.

DR. DAVID COHEN: Other comments, the previous discussion discusses 2 percent as an appropriate concentration. So, we have editorial changes for the Discussion that will be in the returns, getting it back to 1 percent.

DR. BELSITO: Right.

DR. DAVID COHEN: New inhalation boilerplate, adrenal tumor should go in the Discussion as well.

DR. SNYDER: Yes, we had similar editorial comments, so we accept those.

DR. BERGFELD: Curt, did you have a comment?

DR. KLAASSEN: Yes. In this margin of exposure they have 267, 199, and 141. Am I supposed to interpret that as the 267 is for face, 199 is for neck, and 141 is for hands?

DR. DAVID COHEN: Is that all of them?

MS. BURNETT: The 267 is the face and neck. The 199 is hands. And then the 141 is an aggregate of all those.

DR. ROSS: That's how I had it defined.

DR. KLAASSEN: Okay. All right.

DR. ROSS: So the aggregate obviously got lower, right. So, we start adding more things into that, if we use that very conservative NOAEL, then that's going to push that close to the line. But, you know, if we expand that discussion and look at other NOAELs we could use that are in the dossier, you could, I think, make a more balance decision then.

DR. BERGFELD: So at the moment we're going out as insufficient for the MOE re-review I'm going to say. Okay, let's call the question. All those in favor of this motion please indicate by raising your hand. Don, is your hand up, yes, unanimous, thank you. Go ahead, Bart, you want to say something?

DR. HELDRETH: Before we move on, I just wanted to narrow down what CIR is actually tasked with at this point. So, of course we're now insufficient for those MOE, and possibly find out why the SCCS decided on the amount they did. If we get to a point where that information is not available, because not everything is available publicly about what goes into an annex, or how that was translated in the European Union, if that information is not found, do you want Jinqiu to start building these draft MOEs for these other exposures and circulate them to Dave Ross and Curt Klaassen to look at in advance of the meeting?

DR. BERGFELD: Absolutely.

DR. SNYDER: I think that's a great idea to have it in advance of the meeting so that we come prepared, so we're not doing that on the fly at the meeting. Thank you. It's a good idea.

SEPTEMBER 2009 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT

Belsito Team – September 24, 2009

DR. KLAASSEN: I like this new style. I don't know about other people, but this is the one that has (off mike) new way or a different way of covering them.

DR. BELSITO: Yes.

DR. LIEBLER: Yeah, I like it too.

DR. BELSITO: Yeah, very nice. Okay. So before we start looking at the documents, we were handed three new piece of data here.

MS. BURNETT: Yeah, I have a few more back at the office, but the Japanese studies on the carcinogenesis are – well, they're saying the Japanese lab just keeps playing around with it, so I didn't (off mike) all out to figure their (off mike).

DR. BELSITO: Okay. So –

MS. BURNETT: Dr. Snyder provided the one (off mike) study.

DR. BELSITO: Okay. So Paul provided that, so you can summarize these studies for us.

DR. SNYDER: Well, because they gave it a NOAEL in the male rat of .5 percent or 227 milligrams per kilogram per day (off mike) it looks like (off mike) NOAEL for the male rat.

DR. BELSITO: So this was a 55-week feeding study in male rats. And so it was an NOAEL or –

DR. SNYDER: NOAEL

DR. BELSITO: Okay, of 227 milligrams per kilogram per day for renal toxicity or carcinoma.

DR. SNYDER: Well, it was (off mike) for the thyroid and the –

SPEAKER: Yeah, liver.

DR. SNYDER: Thyroid and liver agent.

DR. BELSITO: Okay. And then there is the Takazawa study, "Enhancement of Hepatocarcinogenicity by Kojic Acid," 2-stage model after initiation with nitrosamines. So the authors conclude that at least with nitrosamines at 2 percent, kojic acid is tumor-promoting and possibly hepatocarcinogenic –

DR. SNYDER: And that's 1,000 milligrams per kilogram per day.

DR. LIEBLER: And what they're really looking at is not tumorous, but these GSTP-positive foci, which is, you know, several arrows upstream of any possible tumor. And, you know, the magnitude of the effects that they're seeing is quite modest at a high dose of kojic acid, so their conclusion is that possible hepatocarcinogenic activity, but the carcinogenic potential is likely to be weak.

DR. BELSITO: Okay. SO that obviously would need to be added and go into discussions as we move forward.

And then the last is just an abstract from 2004.

DR. SNYDER: One question. Is (off mike) – if you go to page – of the Takazawa paper, he has this reference (off mike).

MS. BURNETT: I have that back in the office, too.

DR. SNYDER: No, but, I mean, is it in the report? Is it referenced in the report? Because it's harder to find the references because they're not alphabetical.

MS. BURNETT: Yeah, I know. I don't think that one's in, but I have it sitting on my desk to go in.

DR. SNYDER: Because they actually give us a .03 to .06 milligrams per kilogram per day may be absorbed through the skin.

MS. BURNETT: Yeah, I have that one back at the office.

DR. KLAASSEN: That would be perfect for this review.

DR. BELSITO: Okay. And then we have an abstract. There's no heading. Is that what you're referring to?

MS. BURNETT: Oh, (off mike) up here.

DR. SNYDER: Oh, yeah, it is. Yeah.

DR. BELSITO: Okay.

DR. SNYDER: Yeah, I didn't catch that when (off mike).

SPEAKER: That was the one we were just referring to. How did that come out?

DR. BELSITO: So comparing the values with the NOEL and oral subchronic animal studies of 250 milligrams per kilogram per day, they calculate a margin of safety as 4,200 to 8,900-fold.

DR. LIEBLER: So there should be a full paper on that, right?

DR. BELSITO: Yes.

MS. FIUME: Yes.

DR. BELSITO: Okay.

DR. LIEBLER: 2004.

MS. BURNETT: I (off mike) and I didn't – you know, like you guys wanted it (off mike). I gave you the hard ones.

DR. LIEBLER: (off mike)

DR. BELSITO: Yes, yeah. It has absorption and alkaline solutions at 309 to 312, and then there was some questionable slight phototoxicity to it without any photosensitization. And the use concentrations are up to 4 percent is the highest they have.

MS. BURNETT: I did get an update from the council on the additional ingredients and they did not have any reported concentrations other than –

DR. BELSITO: They did not have any?

MS. BURNETT: For the kojic isopalmitate and chlorokojic acid. There are no reported concentrations of use for them.

DR. BELSITO: So in 2009, all of these values disappear, is that what you're saying?

MS. BURNETT: No, no, no. Kojic acid is used.

DR. BELSITO: Right.

MS. BURNETT: The proposed additional ingredients, the chlorokojic acid, the –

DR. BELSITO: Kojic isopalmitate.

MS. BURNETT: Yeah.

DR. BELSITO: And kojic dipalmitate.

MS. BURNETT: Have no concentrations of use.

DR. BELSITO: Right, okay.

MS. BURNETT: So they hadn't run a survey on those when I wrote the SLR.

DR. BELSITO: Right. But we've dealt with that before. Just to make a note. So it was 5 percent kojic acid and alcohol that had some very questionable phototoxicity. But it'd be nice to know what the pH of that was because it seems that the absorption is really very pH-dependent, so I think there could be some issues with that in terms of phototoxicity. It certainly needs to be defined a little bit better.

On page 23 of the document, on the reproductive toxicity, the last paragraph actually the last three lines, it says: Body weight gain in the 25 milligram per kilogram maternal mice was slightly, but significantly, greater than the control values. Is that slightly – is that "significant" of "slightly, but not significant?" I would just check that because normally when you're writing something and you say it was "slightly greater, but not significant" rather than "slightly, but significant."

MS. BURNETT: For some reason that sticks out in my mind that that was written as it's reported.

DR. BELSITO: Okay.

MS. BURNETT: But I will check.

DR. BELSITO: Just making sure. And then page 24, the second through fourth lines:

The authors concluded that kojic acid causes an anti-implantation effect and abortifacient effect and litter death in albino rats, which is mainly due to the compound's toxicity.

Do you mean compound's "maternal toxicity?" I'm assuming "maternal" should go in there.

MS. BURNETT: Probably

DR. BELSITO: Okay. And on page 27, I guess I was just interested in Curt and Paul and Dan's comments on the genotox.

DR. SNYDER: Genotoxins.

DR. BELSITO: Yeah. The in vivos, though, were all negative, which I think is important to note, but the in vitro was like everywhere. So I was just curious what you guys thought about that.

DR. KLAASSEN: Well, it's all followed up with carcinogenicity test, so.

DR. LIEBLER: Right. I mean, the in vitro stuff usually was with a lot of kojic acid. And, you know, I think Christina's summary basically says that this is a mixed bag of systems and results.

DR. BELSITO: Okay. So you're between the negative in vivos and the carcinogenicity.

DR. LIEBLER: Data.

DR. BELSITO: We're not going to worry about this mixed bag of in vitro stuff.

DR. LIEBLER: Right.

DR. BELSITO: Okay. Page 36, under the photogenotoxicity, the next to the last line of the first paragraph, it says: The UVA doses were 10 millijoules per centimeter-squared of UVA. UVA's usually measured in joules, so I think it was probably 10 joules per centimeter-squared. UVB is millijoules.

MS. BURNETT: I'll check that.

DR. BELSITO: Yeah, check it. And 10 joules per centimeter-squared is a standard UVA dose for phototoxicity.

So I guess we answered all my questions that I had about the carcinogenicity data from 38 on.

On page 42, bottom paragraph, fourth line, is that the right chemical, acteaminofluorene?

DR. LIEBLER: Probably acetyl.

SPEAKER: 42, bottom.

MS. BURNETT: I will go back and check that.

SPEAKER: (off mike)

SPEAKER: Yeah. Where are you? What line?

SPEAKER: Seventh line up from the bottom.

SPEAKER: Yeah.

DR. LIEBLER: Oh, yes, acetyl. I'm sure that's acetylaminofluorene. That's AAF. Yeah, that's a standard abbreviation for it. It's a hepatocarcinogen.

DR. KLAASSEN: Classic (off mike).

DR. LIEBLER: Yeah, right. That's just a typo.

DR. BELSITO: So it's acetylaminofluorene?

DR. LIEBLER: Right.

DR. BELSITO: Okay.

Just to point out to everyone involved that the sensitization and irritation data that we have on these are really limited and there was no human data. That I find hard to believe.

MS. BURNETT: And that's how many?

DR. BELSITO: And the Japanese are throwing this into everything.

MS. BURNETT: Right. For whatever reason. And they allude to some irritation, but I was not able to find anything.

DR. BELSITO: Okay. Because I think that, at least from my standpoint, at the end of the day, however we work out this issue with the carcinogenicity, and it seems like we've worked through that based upon margins of safety and yadda, yadda, yadda, I think that there's going to be insufficient data for phototoxicity. I think there's going to be insufficient data for sensitization and irritation. And more importantly, I don't have a dose response for a skin lightening effect that is marketed quite clearly as a cosmeceutical for lightening skin. So I think that, from my standpoint, those would be real insufficiencies.

And then the only other comment that I had was on page 3 and on page 51 about EU.

DR. SNYDER: Well, SCCP, you can limit it at the end of (off mike).

DR. BELSITO: Yeah. It says: The European Commission determined the use of kojic acid, a maximum concentration of 1 percent in skin care formulations. It poses a risk to human health due to potential systemic effects and skin sensitization.

So they don't limit it. They say it poses a risk. And then on page 51, it says: The Europeans determined the use of kojic acid at a maximum concentration of 1 percent in skin care products pose a risk to health due to potential systemic effects and skin sensitization.

MS. BURNETT: That's the same.

DR. BELSITO: No, but it doesn't make sense, "at al maximum concentration of 1 percent?" Which means that – I don't understand how that works. A maximum concentration of 1 percent, it poses a risk. So does that mean that above 1 percent it doesn't, below 1 percent it doesn't? I mean, the terminology, "a maximum concentration" is what throws me off there. So is the data suggesting that below 1 percent there's no risk? Do you see what I'm saying? I don't understand the meaning of a sentence that says at a maximum concentration of this, it poses no risk. Normally you would say at concentrations less than or equal to 1 percent there is not risk or at concentrations greater than or equal to 1 percent there is a risk.

DR. SNYDER: And what are they referring to as systemic effects?

DR. BELSITO: Right. So I guess I would like to see that study. It's reference 15.

MS. BURNETT: I think I provided it with the online data.

SPEAKER: Can you speak up a little bit?

MS. BURNETT: I said I think I provided with the online data, but I can snag it and bring it tomorrow.

DR. SNYDER: I guess that's one issue we hadn't really thought about. If everything's online and we discuss it here, (off mike) computer (off mike).

DR. BELSITO: Yeah, it'd be nice, but then you're going to need to have –

DR. LIEBLER: We're going to get issued laptops?

SPEAKER: Only some (off mike).

DR. BELSITO: Yeah.

DR. LIEBLER: I've got mine. You want to look something up?

DR. BELSITO: No, but we need to have Internet access, too.

DR. LIEBLER: Yeah, I got that. I charged it to your room.

DR. BELSITO: Just –

DR. LIEBLER: Yeah, I can try and grab – I can just grab a paper. I mean, it'll take me a few minutes to get – but.

DR. BELSITO: So –

DR. LIEBLER: It might be a good idea, though. I mean, Don's got a good point, just in case somebody doesn't bring – somebody on staff for each team has a laptop and can go and grab stuff as needed because, I mean, you can do it in a couple of minutes.

DR. SEIDMAN: It'd be nice to have a screen, too.

DR: LIEBLER: Yeah, we could do that.

DR. SEIDMAN: LED.

DR. LIEBLER: Or at least be able to grab it and read it.

DR. BELSITO: Yeah.

SPEAKER: It's 2009.

DR. BRESLAWEC: No, I completely agree. It think that's a great idea. I mean, if we're going to be sending you stuff that's not hard copy, the least we can do is provide access.

DR. SNYDER: I mean, that is (off mike) discussions.

DR. BRESLAWEC: Absolutely.

DR. SNYDER: (off mike) we take a look at, you know, this table or look at this data or, you know.

DR. BRESLAWEC: Yeah. No argument, it's a great suggestion.

DR. KLAASSEN: I'm actually thinking (off mike) at this point not be able to have time, but go completely electronic.

DR. LIEBLER: Yeah, I think so (off mike).

DR. KLAASSEN: And actually everything, you know. I'm the most unlikely person to get this because this is the first time that I've come to this meeting that I have a cell telephone.

DR. LIEBLER: We just say cell phone.

DR. KLAASSEN: Oh, cell phone. See, I didn't know how to say the right word.

DR. LIEBLER: But yours doesn't have a (off mike) on the side, does it?

DR. KLAASSEN: I'm getting to the point of looking here and looking there. It'd be easier just to have it all in one place, you know, for each thing that you're doing.

MS. BURNETT: Kojic acid was the first report where I think all of the unpublished data came in on a CD-ROM. I didn't have any hard copies.

DR. LIEBLER: Yeah. See, what would be really convenient is if each of us could have a login on your server and then we had all of the documents either listed as PDFs of other downloadable common formats.

MS. BURNETT: Yeah.

DR. LIEBLER: You simply access those and download them. None of them would be too big.

DR. BRESLAWEC: One of our concerns was that we didn't know how you all would react to it at all, so we didn't want to completely switch over. And my sense is that the reaction is positive to going to pretty much all data online or as much of it as we can. And now that we have that feedback, we can (off mike) that.

DR. BELSITO: Yeah, I think that, you know, instead of – I mean, I would like still the written document that's going to be published, but I think all of the supporting document – the only comment I would make is because this was a new thing and because I've been in Europe for two weeks, where sometimes they charge you 30 euros for a day's access of Internet, you know, when I came across documents where the old reports weren't attached, I really didn't want to spend 30 euros to get an old report. So if I had known that ahead of time, if there had been a sheet before I left that said the following information is available online, then I could have gone online, snagged it, and just saved it on my laptop.

DR. SEIDMAN: Just for a point of clarification, you'd probably still want the books online as well so you could download these in additional to having (off mike).

DR. BELSITO: Yeah, if we get to the point where there are this many books, and I wouldn't have carried them with me. This was a small number; it was easy enough to carry.

DR. SEIDMAN: In Word (off mike).

DR. BRESLAWEC: Please mention this at the main session tomorrow.

DR. BELSITO: Yeah. But I think Word would be nice because I don't – I try to, you know – I mean, I don't know how to make corrections in PDF.

DR. SEIDMAN: You need a special program. You need Adobe Professional or Adobe Standard.

DR. BELSITO: Right.

DR. SEIDMAN: You can't use Adobe Reader. That's why we had trouble.

DR. LIEBLER: Yeah. Now, so, when I started two meetings ago, they made it possible for me to download the – they sent me the books as PDFs.

DR. BELSITO: Right.

DR. LIEBLER: And I was able to mark those up as PDFs and just give them back my marked up files on a USB key.

This time I didn't get those for some reason. And it's no big deal, I used the books and a pen. But that's actually a pretty convenient way to do it. And again, if you had those accessible on the server you could just download the PDFs. And getting Adobe Professional on your computer is no big deal.. It just has a – in this case, the only thing you have that you don't have in Adobe Reader is you have a toolbar that has, you know, text highlight, insert note here, stuff like that, that's –

DR. SEIDMAN: It's a standalone program they have to buy unlike Adobe Reader, which is free, you know.

DR. LIEBLER: Right, but, you know, that's not a real barrier.

DR. SEIDMAN: Yeah.

DR. BELSITO: Particularly if CIR gives it to us.

SPEAKER: They would (off mike).

DR. SEIDMAN: Us? What are you talking about?

DR. LIEBLER: It's cheap.

DR. KLAASSEN: Well, the relative cost of this, it's – I mean, it's not what it was 10 years ago.

Dr. LIEBLER: But that way you could literally have everything that you needed for the meeting on your laptop.

DR. BELSITO: Yeah.

DR. BRESLAWEC: Well, we'd like to work for that. It makes it easier for us as well.

DR. BELSITO: Yeah. No, definitely. And I think that, you know, oftentimes, you know, it is a voluminous waster of paper because, you know, you send us 80 pages of, you know, a Hilltop research report and we're interested in only 3 or 4 pages.

DR. SNYDER: Conclusion.

DR. BELSITO: Well, no, conclusion. I mean, sometimes you're interested in the individual data, scanning data to see about outliers, but, by and large, there are only three pages you're concentrating on. Okay.

So let's see, are there any other comments on this document?

DR. LIEBLER: I have a few.

DR. BRESLAWEC: Did that report clarify the 1 percent maximum (off mike)?

DR. BELSITO: Yeah, it was based upon calculation of margin of safety, not – and – do you have it?

SPEAKER: I gave it back.

DR. BELSITO: Did Dan get a chance to see it?

DR. LIEBLER: Yeah.

DR. BELSITO: Okay.

DR. LIEBLER: I'll look at it.

DR. BELSITO: Go ahead, Dan.

DR. LIEBLER: So I have a few things ranging from the chemically trivial to the more significant, I guess. On page 1, under Physical and Chemical Properties, according to the review article by Velik, "the phenolic hydroxyl group," it's actually not "phenolic," it's just "enolic," so you can ditch the P-H on that word.

DR. BELSITO: Which line is that, Dan?

DR. LIEBLER: The middle line under Physical and Chemical Properties, "the phenolic hydroxyl group." It's not really "phenolic" because there's an oxygen in the ring so it can't be phenolic. It's enolic hydroxyl group.

MS. BURNETT: Okay.

DR. LIEBLER: And then next page, under Analytical Methods, "Kojic acid can be detected with chromatographic or electrophreic," it probably should be "electrophorectic," R-E-T-I-C, "techniques that take advantage of UV light absorption maxima." You could probably just say "absorption" because the actual maxima for kojic acid is shown in Table 1 are different. They're not 254 and 280.

MS. BURNETT: Right.

DR. LIEBLER: And when I saw 254 and 280, that struck me as too much of a coincidence because that's normally the wave lengths of filters that you commonly have put in UV detectors.

MS. BURNETT: Okay.

DR. LIEBLER: And so something that absorbs like kojic acid at 268 in an acidic or neural solution it has, you know, a wide enough band that you pick up some absorbance at 254. So it's a relatively little detail, but you can detect it at 254, but the maxima is higher.

MS. BURNETT: Okay. Do you have notes?

DR. LIEBLER: I did. I made notes for you. And then on page 9, there's a section about aflatoxin and kojic acid, and as far as I can tell it's really just about whether or not aflatoxin and kojic acid are produced together in these various microorganisms. And unless there's aflatoxin contaminating the kojic acid that's used in cosmetic ingredients, I think this information's not really relevant to our evaluation.

DR. BELSITO: So you would recommend deleting that?

DR. LIEBLER: Deleting that section unless there's some issue of kojic acid and aflatoxins co-purifying, which I find hard to believe.

And then on page 48 and 49, there's a section about effects on thyroid and T3 and T4 levels. And so presumably, the mechanism of action of kojic acid in skin lightening is by inhibiting peroxidases – or –

SPEAKER: Tyrosinases.

DR. LIEBLER: Right, tyrosinase, which is a peroxidase. And this kind of compound is capable of inhibiting a lot of different peroxidases because it simply is easily oxidized as an alternate substrate. And so thyroid peroxidase inhibition may be the mechanism of action by which kojic acid exerts these effects because that's how T3 and T4 get made, the iodines get put on the ring by thyroid peroxidase. So what I would suggest is could you look and see if there's any possible literature on thyroid peroxidase inhibition by kojic acid?

MS. BURNETT: Thyroid?

DR. LIEBLER: Thyroid peroxidase.

MS. BURNETT: Peroxidase.

DR. LIEBLER: That's the enzyme that adds the iodine atoms to the phenyl rings, to the tyrosine rings, in T3 and T4, (off mike) those.

MS. BURNETT: Okay.

DR. LIEBLER: There might be relevant literature on that.

And I think that was it for me.

DR. BELSITO: Well, Curt?

DR. KLAASSEN: I guess I was a little surprised that there wasn't more data on the metabolism of kojic acid.

There's only, you know, one short paragraph on here, and you know, it just says that it's a glucuronate and a sulfate. And I just wonder if you could double-check to see if there's a little bit more data up there. It seems like with all of this carcinogenicity work that they would have done more plain biotransformation of a chemical.

DR. BELSITO: Okay. Other comments? Okay. So, where we are with this, let me try summarizing, is that we're fairly comfortable with the carcinogenicity issues based upon exposure. And that would include the use concentration up to 4 percent, which is the highest concentration we're given, is that correct? So it'd be insufficiencies boil down to phototoxicity in a basic solution or we're told that it absorbs in the UVB range; sensitization and irritation at concentration of use; and a dose response for skin lightening effects.

DR. BRESLAWEC: Dr. Belsito? Are you looking for dose responses for the effect or for toxicity?

DR. BELSITO: Well, we're looking at – I mean, we don't necessarily need a dose response, I guess, if they're going to use it at 4 percent. We need information that 4 percent does not result in skin lightening. Is that a better way of saying it?

DR. BRESLAWEC: Thank you.

DR. BELSITO: So absence of skin lightening at whatever concentration they maximally want to use this in a cosmetic.

Now as I recall, we had sort of pushed this up in priority because of FDA concerns about this skin lightening use. And so I guess I'd like to hear from the FDA folks if they have any other concerns that we're not addressing at this point.

Anything else on kojic acid? Okay.

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DR. MARKS: We're going to look at kojic acid now, and this is the first time we've seen this safety assessment so we need to look at data needs, we look to look at the ingredients. Two out of the four proposed ingredients are not used. I'll open the discussion. Ron, Ron, and Tom, in terms of data needs. Is there sufficient data to move on with this report?

DR. SHANK: I don't think so. I think we need skin depigmentation data at the use concentration of 4 percent. That's the only insufficient data need that I came up with.

DR. BERGFELD: Ron, I will tell you that it is used as a depigmenter especially with Asians.

DR. SHANK: But not as a cosmetic. Right?

DR. BERGFELD: It's in cosmetics. It's not a prescription. On the West Coast they just love it.

DR. KATZ: I can't comment about it because I actually didn't understand something I read in the book either about reference to it being or not being looked at by drugs, and my question was whether or not it was ever considered for monograph, and if there's any reason that it should have been or just the reference that it's not used in OTC drugs is just a reference to state of fact that it's not used in OTC drugs.

DR. BERGFELD: I know it's compounded. I have friends who are using it and compounding it in Japan and brining it over.

MS. WEINTRAUB: I have a question about the process. Kojic acid has not been approved by the FDA for such use. Then what is our role here?

DR. MARKS: Our role is to determine whether it's safe to be used in cosmetics, and of course Ron raises the issue of if it has, and there is good evidence, hypopigmentation of one of its effects. We need to know what level as a cosmetic can it be used without worrying about hypopigmentation if I interpreted what you said, and I had the same concerns, Ron. I have no idea.

MS. WEINTRAUB: Even though FDA only approves the use as an antioxidant?

DR. KATZ: That addresses the question that I had raised. I'm not aware that it ever came in as an ingredient either through the monograph or through an NDA process to be approved as a drug. I may not have made it very clear. The wording was strange to me that if it's used as a cosmetic ingredient, then FDA would not have approved it. FDA would only have approved it if it came in for a drug approval. I agree with what Jim is saying, that the question really is is this something that should be a cosmetic or should it be something that should go into the FDA to be looked at as a drug which is a whole different issue than what's really being asked for here.

DR. BERGFELD: I think it's concentration related like so many things. The hydroquinone on page 50 that's talking about clinical testing and therapeutic use was at 2 percent glycolic acid and 2 percent kojic acid. It's my understanding that 1 and 2 percent hydroquinone was on the market as just an over-the-counter product. I understand also that it had been relooked at and possibly FDA had pulled it off the market, just the 2 percent hydroquinone. There was a bleach on the market for cosmetic use.

DR. KATZ: There is a bleach on the market for cosmetic use, but it can't really be called a bleach. As a drug you're correct in that it is a topic of a tentative final monograph and they were trying to finalize the monograph I think about 2 years ago. There was a call for data and FDA was waiting to receive data to substantiate the safety of its use in over-the-counter drugs. As far as I know, that monograph for hydroquinone for a drug has not yet been finalized. I know that they've been assessing information that they've received, but I'm not aware that they finalized that monograph.

DR. BERGFELD: I know that the American Academy of Dermatology did response in letter form though.

DR. MARKS: In terms of?

DR. BERGFELD: A report on hydroquinone.

DR. MARKS: Supporting hydroquinone.

DR. SEIDMAN: As FDA regulated kojic acid, the submission would come in with proposed doses for a specific intended use. I'm a little confused about where the distinction is then between cosmetics and a drug because if I've understood you correctly, you're saying it's a matter of dose.

DR. KATZ: No, it's concentration and it's also use. What distinguishes between a cosmetic and a drug usually is whether or not the ingredient itself and how it's being prescribed is something that one can use safely without intervention for a drug in general, but there are certain other distinctions and whether it affects structure function claim itself is a structure function claim os some other mediating of disease, prevention, et cetera, which would make something a drug rather than a cosmetic. So it really depends on how something is being used and the concentration in which it's being used that might make part of the distinction.

DR MARKS: I think for us the issue is not whether it's being used as skin lightening, that's toxicologic end point we're worried about, we're looking at this as a cosmetic which presumably is used as an antioxidant and/or a skin-conditioning emollient. So it would seem like something if it's being used in Asia as a treatment at 1 percent concentration, there's got to be something less than that that does not have the potential of hypopigmentation but we just don't know that so that that would be a data need.

DR. SEIDMAN: Use concentrations go up to 4 percent in facial creams.

DR. MARKS: Yes, so that would be concerning to me. The other thing continuing on skin impact is that it's a sensitizer, that's on page 30, although in guinea pig testing ti was safe up to 30 percent, but then, Wilma, going back to the study you referred to on page 51, it says at 1 percent preparation, a side effect of this treatment is contact allergy and I don't see any RIPTs to establish a safe level. I would also be interested potentially, although I think we already hazard alerts occurred on what a local lymph node assay would show, but to me it's really what is the RIPT, what is the safe level that this can be used in a cosmetic which isn't a sensitizer. I'm not sure, and Tom mentioned it and I'll refer to that also, on page 3 the SCCP determined a maximum concentration of 1 percent and I'm not sure how they arrived at that. Again I would think it would be less than that if you have 1 percent preparations used for the treatment of melasma in Asia, and contact allergy is a significant side effect.

DR. BERGFELD: Is it significant? On page 50 of women who have an irritant response, and then on page 51 you have a citation of a single report, a 1 percent preparation used two a day for 2 months and the side effect of this treatment is contact allergy but we don't have any numbers there.

DR. MARKS: I guess the problem I had on page 50, without a study it was a mixture of glycolic acid and hydroquinone and glycolic acid is a known irritant, and they talk about what could be an irritation reaction there. They really to my mind don't define whether it's allergic contact or just irritation. That study didn't have as much meaning as the following paragraph on the next page at the top where they talk about this preparation and contact allergy as a side effect. That's not quantified and I'd like to know how often that occurs. As I said, I'd like to see what an RIPT looks like so that we could get a sense of what is a safe level.

DR. SLAGA: There is no data to support that.

DR. MARKS: Right.

DR. SLAGA: It's a statement.

DR. MARKS: Absolutely, but it's an alert in my mind.

MS. BURNETT: That study was an efficacy study, so I just culled out what was most important from that efficacy study to report it in the report.

DR. MARKS: For me, not only would I want to see what is the safe level in terms of the side effect of hypopigmentation, but I'd also want to see what is a safe level for sensitization.

The other things, impurities, Ron, Tom, Ron? I'm going to do that many different ways to change it up. Rather than Ron, Ron and Tom, I may do Ron, Tom, Ron. At any rate, impurities are okay in that impurities are okay in that part? And then the other thing is the other geno and carcinogenicity. That's all fine?

DR. SLAGA: Yes, even on the skin it's a much higher dose and it has no effect. As a skin carcinogen, there are some internal tumor-promoting types of things, but I don't have any concern with that.

DR. MARKS: Impurities?

DR. SLAGA: No.

DR. HILL: No.

DR. MARKS: Do we need that?

DR. SLAGA: No, it's fairly pure from my understanding.

DR. MARKS: Let's go to the four ingredients. We had these data needs certainly for kojic acid. I think we would move forward at this point with insufficient.

DR. SLAGA: Yes.

DR. MARKS: As I said earlier, the chlorokojic acid and the kojic isopalmitate are not used. The kojic dipalmitate does have uses. Forget the number. Is there any problem with extending it from kojic acid to those other three ingredients?

DR. SHANK: I would not add the chlorokojic acid. That's not a simple ester. The dipalmitate is a simple ester. That's okay. But chlorokojic acid could be something entirely different so I would not include that.

DR. HILL: I didn't see it in here: Did you run across information as to how that ester is handled biologically? Certainly that will be when dermally administered a very different beast than kojic acid.

MS. BURNETT: I have found no information on that. It was essentially nonexistent.

DR. HILL: I know, because I would expect to see very different behavior, maybe no difference in the end results, but very different behavior in terms of dermal penetration, how deep in the skin it got, what exited the skin, how fast, what were the species? Do we end up with a monoester leaving and entering the circulation or just kojic acid? Because if the only thing that escapes the skin is kojic acid, then we know what the data suggests. If on the other hand the ester or a monoester escapes into the general circulation, and there are two possible monoesters, but I think the one that's listed here would be the most stable and most likely to come out without hydrolysis and what happens to that, and without that data I don't see what's there for kojic acid supports that diester at all in my mind.

MS. BURNETT: Unfortunately what I could find is in there. There are hardly any data. Also to clarify, I didn't include it in the transmittal memo, but the industry provided me a memo that said that there was no concentration of use for the additional ingredients and that what we have is what's provided.

DR. SHANK: I don't see the advantage of adding another cluster to a report that's going to go insufficient data, so I would leave it just kojic acid.

DR. ANSELL: Yes, and we've just seen this report. The only cosmetic application for this is as an antioxidant and we really question the response we got as it relates to 4 percent, whether that is truly a cosmetic application and should be included within this report. The effective concentrations at the tenth percent are much more likely to be the cosmetic application. So we would propose on tabling or giving us an opportunity to contact FDA and see if there's an opportunity to identify who actually made this report and whether it's truly a cosmetic application because of the concerns that we started the discussions with.

DR. MARKS: I'm going to go back and then, Jay, I'll address your comments because whether it's at 4 percent, 2 percent, or .05 percent, we don't know what the safe level for hypopigmentation -- sensitization at this point is. So I think I would probably say we still should not table at this point or hold, but to just put it out as insufficient, and really what we need are those end points of toxicologic effect. Ron and Ron, you would limit this report to kojic acid alone and not extend?

DR. SHANK: Correct.

DR. MARKS: Because you had mentioned the chloro not being a simple ester, Ron, and then, Ron, you were concerned about the metabolism of dipalmitate also. Correct?

DR. HILL: I think the systemic exposure difference between that and kojic acid itself with that diester. It's a long-chain diester compared with kojic acid itself, so I'm not sure all of the dermal toxicity studies that are done with kojic acid are relevant to the diester in this case. Without knowing something about how fast that ester is hydrolyzed and do we get kojic acid rapidly before it ever penetrates much into the skin, I guess then that would be no problem from where I sit.

DR. BERGFELD: I'm sorry, what did you really say?

DR. HILL: What did I really say? That at least transdermal behavior and if even the hemiester, in other words, the half-ester survives intact to penetrate into the lower levels of skin, then we start to worry about the tumor-promoting activity of skin-type cancers, or if in fact pervasive esterases rapidly hydrolyze that diester before it ever gets anywhere that it can do any harm.

DR. BERGFELD: So your real recommendation would be if you're putting out a want list, that if you had the skin absorption studies for these esters, they might be included?

DR. HILL: Absorption and biological fade, in other words, how rapidly the ester is hydrolyzed. And if there is information as to biological activity, then maybe that half-ester that we're not including could be included because that would the logical metabolite I believe.

MS. BURNETT: Since it's not being used, I'm not sure we're going to get data on that. I don't know if industry would want to pursue that.

DR. ANSELL: If the report is limited to kojic acid, then it takes all the ester off the table, so all the questions become moot.

DR. HILL: If the curiosity would be satisfied or if we learned something about the bio-handling, put in the chemical abstracts number into the chemical abstracts and see what pops up would be very easy. Nothing popped up?

MS. BURNETT: No.

DR. HILL: Not even patents or you didn't open it to that?

MS. BURNETT: No.

DR. MARKS: So we'll recommend tomorrow an insufficient data conclusion, we're concerned about a limit for hypopigmentation as a side effect of the cosmetic, with sensitization we need RIPT, and we will limit this report to just kojic acid. Are there any other comments?

DR. ANDERSEN: What did you think of the summaries?

DR. MARKS: Thank you, Alan. They got into the meat of this. I actually liked the summaries personally, but I think there was some disagreement in my team, maybe referred to "Cliff's Notes" or even less than that. The other thing is if the summaries now rather than in the final article. So I actually liked the summaries, but I'll let my other panel members speak for themselves. Tom?

DR. SLAGA: I have no problem with the summary. We're supposed to read it anyway, but at the same time it directs your thoughts going down to reading it in detail. So I have no problem. Ron?

DR. SHANK: I don't see that they're necessary.

DR. HILL: If they're just duplicating what's in the ultimate summary at the end of the document, they probably aren't necessary. I liked the concept. I had specific criticisms of some of them, but I just made notes. And also perhaps with increased use of elegant tables, those summaries would become somewhat less necessary, but I don't know that's true.

DR. MARKS: Alan, what's your sense in terms of the editors of the journal? Is this something that they are really pushing for? Again if that's the case and we certainly want to put this besides on the Website in the public arena in the form of a journal publication, then if this is what they're aiming for then it seems to me that we should try and accommodate that.

DR. ANDERSEN: We've tried the experiment at one level, but let me direct your attention to pages 27 to 36. That's nine pages of genotox data. The peer-review feedback that we're getting from the tox journal is what's the take-home message? We did it as a summary for the whole genotox section. We actually talked about maybe a better way of doing it might have been to summarize the bacterial assays in that section and then summarize the mammalian assays. We're not wedded to a specific approach, but what we're getting is that pushback from the journal that says you've got to slog through all of this stuff and we're certainly getting the suggestion of condense it, you're too wordy, but even once you condense it, what's the take-home message to this section? We're particularly guilty in the absorption, distribution, metabolism and excretion section in which when we present a dozen studies none of which were done relating to the cosmetic use of the product, it's all data kluged together for some other reason, that it reads like it's kluged together and the read is left with, huh? One study says this, one study says this. You don't make any attempt at all to unify this, and we've talked about it extensively and have simply had to say guilty as charged. It's how we've been doing it, we want to change so that the material is conveyed more consistently in a way that can be compared, and one of the things is as you go through a section of material, summarize it so that there's a take-home message.

DR. MARKS: I guess one of the things in terms of the repetition of the summary on page 51, and this is a marked difference from the way the reports have been done in the past, is do you eliminate the summary section of the paper since that's occurring piece meal throughout the paper?

DR. BERGFELD: No.

DR. MARKS: Then you have the discussion which puts it in the context of say a broader vantage point.

DR. ANDERSEN: What we were thinking is as we go through and in whatever fashion we end up putting these internal summaries, that we effectively have written the summary, we just pull them out and repeat them in the summary section. You could ask isn't that just duplicating? But we also have been criticized by the journal for having summaries that repeat a lot of the data. We've also been guilty of that. It's a function of who's written the report. But we tend to put a lot of stuff in summaries and we're being told to back it down, summary not regurgitation, and this was also a way of helping us be more concise. Those are the pressures we're trying to respond to and the fact that it's being pointed out that we tend to be a little bit wordy seems to be a valid criticism. It's a long report. Make these shorter. You don't have to read so much.

DR. SLAGA: If you take the genotoxicity as the example that you just went over, it's positive in bacterial systems and it's positive in some of the mammalian but not. To me in the discussion is where you should state what you think that really means and then in light of the skin carcinogenesis studies, it obviously does not have an effect on the skin so that you're really not weighing that genotoxicity as very positive because in the past we would say if it's positive in both we want to see skin carcinogenesis. We have that, so therefore we say it's not important in this case.

DR. ANSELL: In our review of these, we didn't find this particular one objectionable, but we do have a concern that the summaries read awfully like conclusions and we don't want to start drawing conclusions from any single set of data for exactly the reasons that Tom just said. So if we go down this road, I think we need to be careful that we're summarizing data.

DR. SLAGA: We're not drawing conclusions. That's only a summary.

DR. ANSELL: Right, but we saw earlier a summary which drew conclusions as I mentioned earlier, and so when we start talking about that it is mutagenic, that has to be taken within the context of the entire package which is what you do in the overall summary, so that we raised that as a concern.

DR. ANDERSEN: So that if you're going to do summaries, constrain them to just capturing what's in that section and not interpreting it.

DR. ANSELL: Yes. In fact, we thought the tables were perhaps a good idea, although the panel has their own opinion on that apparently. We had some technical comments as to what the tables looked like, but we did not object to condensing it through the use of tables.

DR. SEIDMAN: I think give the Panel's role, you're right that we can't draw conclusions on the data per se, and Alan may weigh in on that. Still I think it's CIR's job to point out where there are inconsistencies in the data or where we have concerns about the data because something wasn't reported, so we can weigh in about the quality of the data, and I think that's valuable. And we can weigh in on the weight of evidence. If 90 percent of the studies point to genotoxicity, we can say that, and we don't say though that it's genotoxic, we're just weighing in on what the data report. Do you agree with that distinction or is that too fuzzy?

DR. ANSELL: No, I think that's exactly what we're talking about, but that weigh in, and indeed we fully supported bringing on people with just the expertise to do that, should not be done piecemeal, it has to be done in the discussion as opposed to trying to draw a conclusion on each little dataset.

DR. SEIDMAN: I don't feel one way or the other about summary statements to be quite honest. I prefer tables and then to make summary statements at the end, but still I'd like to know if you're objecting to the summary statements that discuss

what's missing from the data or inconsistencies in the data? Is that something you would personally care for or you'd prefer not having that and just going to the summary section for that kind of information?

DR. ANSELL: I'll defer to the Panel on that.

DR. BERGFELD: I would like to go back in history a little bit. What I see is just an upgrade for user-friendliness and transmitting good information. The Panel has put together a document that nobody has. They've consolidated a lot of information and the readers and public who's reading this is quite diverse including the dermatologists. So I like what's being said here. I like the fact that we're having some summary statements and some of the more laborious types of recording, and whether you would want to go to tables and keep some of these longer pieces and tables I have no objection to. I do have an objection though to when it comes to altering the summaries. I think the summaries are excellent as are the extracts. It's the front and the back of an article, the focus issue, it anchors the article. I think that we put the discussion in there to discuss just what we're talking about, the lack of and why we went this way because we used such and such and that discussion was a real addition to our documents when we put it in a number of years ago. So I think this is looking very good and I think the upgrades and the suggestions are very good, and I think that over time that it will even be a slicker document.

DR. MARKS: Does that answer your question, Alan?

DR. ANDERSEN: I think so.

MS. WEINTRAUB: I think they are very useful. I think though some of the summarize don't quite summarize all of the information in that section. Some of the summaries really were almost verbatim from one study one group of studies, but then as you read on in that section, there were other studies as well that the summary did not mention at all. So I think we still have to be careful that the summary actually summarizes the entire section.

DR. ANDERSEN: To get it right.

MS. WEINTRAUB: Yes, but I think in terms of, I don't know how many consumers actually read these, but I think it definitely helps to really focus reading and knowing what type of information is supposed to be discussed and should be discussed in the section so that I think it's an overall asset.

DR. ANDERSEN: We've been on a lot of summer booklists.

MS. WEINTRAUB: "New York Times" bestsellers.

DR. MARKS: I think the conclusion is that we by and large like the idea of having each section summarized since we're being pushed that way by the journal, that we'll maintain the summary section at the back which is as Alan pointed out will probably be repeated from those individual sections, and that the discussion is to put this all in perspective when there are differences in results in various section of the paper and they need to be put in perspective.

DR. HILL: One of my specific criticisms that I mentioned was on the issue that she discussed.

DR. MARKS: Again just to repeat, we will recommend tomorrow an insufficient conclusion, that we need a safe level for sensitization, and we're going to limit this report to only kojic acid.

DR. ANDERSEN: The phraseology was interesting, safe level of sensitization I understand. Safe level for pigmentation, you want the level at which it doesn't pigment, absence of pigmentation?

DR. MARKS: That's correct. Are there any other comments? The next group of ingredients are the cyclomethicones.

DR. HILL: I'm sorry, one question I had while I was glancing through you were asking is maybe the aflatoxin information was there for our consumption, but that falls into the category of information that I'm not sure needs to stay in the final. I know about aflatoxins, but I didn't see the relevance to a cosmetic in human use.

MS. BURNETT: It can be deleted and probably will be.

DR. ANDERSEN: Good pick-up.

Full Panel – September 25, 2009

DR. BERGFELD: -- I think it's time to move on to the last item in this particular grouping, and that's the kojic acid group, Dr. Belsito presenting.

DR. BELSITO: This is "Kojic Acid and Related Ingredients," and again this is a new cosmetic ingredient for use, and my team looked at the document, and we thought that while there is a good amount of data, the data was still not sufficient to look at safety and that what we needed was information on sensitization and irritation and concentration of use. The phototoxic potential of this chemical in basic solution if it was to used in a basic solution because it does absorb in the high UVB range and also since it's been touted as a cosmeceutical for skin lightening, some notion of what percentage this may or may not cause skin lightening would be needed. And additionally there was a request from Dan, and he might want to expand upon

this, if we could get some information about inhibition of thyroid peroxidase by kojic acid, because we know it does seem to be a tyrosine inhibitor, that would be helpful as well.

DR. BERGFELD: Dan.

DR. LIEBLER: My comment was based on the fact that the chemical nature of this compound makes it a pretty good inhibitor of many peroxidases, and because there were several pages of material on effects on thryox – thyroid hormone, this process is also governed by a peroxidase, and so I thought that that would be valuable to include in terms of understand mechanism.

DR. BERGFELD: Anyone over here? Ron?

DR. MARKS: Yeah, we concur with that. The only other thing we would suggest is limiting the report to just kojic acid. The other ingredients which were listed – those other three – were either – the biologic fate of these ester is really not known, nor are they simple ester, so we would limit only the kojic acid, and we would move to issue an insufficient report with those needs.

DR. BERGFELD: I guess your option is either to table it and have it go out as an announcement or go out insufficient, is that correct?

DR. ANDERSEN: Oh, I think the appropriate step is an insufficient data announcement. It signals that the Panel believes there are specific additional data that it would like to look at, and that's the position that you should stake out at this point. You have the option of reviewing the data that you receive and making an alternate approach once you have the data, so it's just –

DR. BERGFELD: Don, your opinion on this?

DR. BELSITO: Oh, yeah. I mean, I think we should –

DR. BERGFELD: You didn't make a motion.

DR. BELSITO: No, we should proceed. I made a motion for insufficient. I think it's been seconded.

DR. BERGFELD: Okay, I didn't hear that.

DR. BELSITO: Yeah, that was my motion. Insufficient.

DR. BERGFELD: Okay.

DR. BELSITO: I gave a list.

DR. BERGFELD: Okay.

DR. BELSITO: And Jim's only comment was to delete the other ingredients other than kojic acid.

DR. BERGFELD: Okay. Any other comments?

DR. ANDERSEN: I have a question in terms of the deletion. I understood the discussions that related to the chlorokojic acid. I'm a little concerned about the reluctance on the esters. They seem to be of the same class that the Panel has argued that simple water hydrolysis is very effective in breaking these particular kinds of structures, and even if that doesn't work, esterases are plentiful in the skin. So it is a little concerning to me why we can't expand to include the monoester and the diester. So, I wouldn't mind some further discussion of that.

DR. MARKS: Ron Hill, would you mind?

DR. HILL: There's no answer – three's no information whatsoever, at least in what I had access to, as to in fact does that very lipophilic ester, which is quite chemically different because of that modification from kojic acid, pass through the skin and what escapes the other side of the skin in normal use. And so while I would agree that probably the phenolic ester would be cleaved fairly rapidly, I'm not sure that's true within the layers of skin within the environment of skin, and the monoester cleavage – I mean, the hydroxy methyl ester cleavage might not go so quickly, and if that escapes into the circulation or, for that matter, some information – well, that could have effects, but I think of greater concern is does the diester or either of the monoesters – it would presumably be the hydroxy methyl ester – have biological effects within the skin, in particular promotion or – well, promotion of any carcinogenesis within dermal layers, because such lipophilic compounds can, in fact, have effects – biological effects – on the kinds of targets that may promote tumorigenesis, and so there's no data whatsoever on those esters in terms of biology if, in fact, in the layers of skin they're there and doing something.

DR. BERGFELD: Dan.

DR. LIEBLER: So, I have no objection to considering these esters. I'd like to just make one correction as a follow-up to Alan's comment and a clarification on Ron's – is that even though – first of all, these compounds, even though in principle and solution under the right conditions, they can hydrolyze. For these esters, those conditions will probably not be achieve in skin under conditions where you don't have a lot of skin damage. It would have to be too acidic or too basic. There is a lot of

esterase activity in skin but not enough to make much of a dent in the mole fraction of material applied. If you – we actually studied tocopheryl acetate, a vitamin E acetate ester, in the '90s in my lab quite a bit, and other tocopheryl family members and applied material at least in rodent skin had a very small mole fraction of ester mediated hydrolysis. So, even though there are already enzymes there, you're talking about putting such a whopping amount of material on the skin that the fractional hydrolysis is relatively small. So, most of the material would probably be there in the esterified form, and then whatever biologic activities it has it's going to have there.

DR. BERGFELD: Thank you.

DR. ANDERSEN: That's – that helps the thinking process. Thank you.

DR. BERGFELD: So, the motion is to vote on an insufficient data announcement – or report.

DR. ANDERSEN: Well, I think – did Don accept the amendment to the motion to constrain to kojic acid?

DR. BELSITO: Yeah, is that your recommendation, Dan?

DR. LIEBLER: We could constrain it.

DR. BELSITO: Fine.

DR. BERGFELD: So, call for the motion. All those in favor of an insufficient data report. Thank you. Unanimous.

DR. ANDERSEN: It will make it easier for us, because there's just no information on all the other stuff. Nada, nothing, it's –

DR. BERGFELD: Would you be considering just mentioning that in the report or not – or just avoiding it?

DR. ANDERSEN: No.

DR. BERGFELD: Okay.

DR. ANDERSEN: We're focused now and that'll make it easier.

DECEMBER 2009 MEETING – SECOND REVIEW/DRAFT TENTATIVE REPORT

Belsito Team – December 7, 2009

DR. BELSITO: Thanks. Okay. Okay, kojic acid. So we can get back at them with this one, huh? Where is this? Pinky? Yeah. Pink Book II. Okay, we got a phototox and we got a primary skin irritation in rabbits on kojic acid. Where are my cheat notes here?

MS. BURNETT: Carol also has an HRIPT study and she will hopefully have information on the concentration that was used in the study so we provide this tomorrow.

DR. BELSITO: Okay. So, back in September, we looked at this and we went out insufficient, asked for dermal sensitization and irritation at current use concentrations, phototoxicity, and so we got phototoxicity here. It was done on male, albino guinea pig skin and it was done on 30 male albinos, experimental. And the treated group got 1 or -- 10 animals got 1 percent kojic acid, 10 got 3 percent. In the control, positive control was 10 percent anthracene ointment, and the anthracene mice got the expected phototoxicity, the petrolatum didn't, and then the kojic acid. There was nothing at 1or 3 percent in a total of 20 guinea pigs, so that's what we have for phototoxicity.

For irritation on rabbit skin, again, 1 and 3 percent and the number of rabbits -- 12 male rabbits, and there's nothing at 3 percent. And the concentration of use for kojic acid in cosmetics is --

DR. BERGFELD: 4 percent.

DR. BELSITO: Okay, so 3 percent is close to 4 percent, but is not 4 percent. So, we don't have dermal sensitization, we have irritation in a small number of rabbits. And is the sensitization going to be in rabbits also or is it going to be a human repeat insult patch like we said?

DR. ANSELL: Supposedly human.

DR. BELSITO: Okay, and in the end, do we know, is it a good end?

MS. BURNETT: I haven't seen the study yet.

DR. ANSELL: Yeah, and we're still chasing after the concentration it was run at, which is why we haven't provided it as yet.

DR. BELSITO: Okay, so that's all we're going to get on this, is we're going to get an HRIPT. That's the only thing that's outstanding?

DR. BERGFELD: How about the hypopigmentation?

DR. BELSITO: Well, that's what I'm going to get to because then there's still several other points.

DR. BERGFELD: Well, except that they are -- IP could give that to you maybe.

DR. BELSITO: It wouldn't be long enough.

DR. ANSELL: We do believe we have -- let me invite Tom up to talk a little bit about the hypopigmentation.

DR. RE: Tom Re from L'Oreal. We have a supplier who has expressed interest in providing a study to delineate a concentration that doesn't cause skin bleaching, but they wanted feedback from the panelists to what type of data that the panel feels would suffice. Would an individual study on tyrosinase inhibition be sufficient or do you want an in vivo study either in animals or humans?

DR. BELSITO: Well, animals, I think, are always difficult to do for hypopigmentation, but, I mean, I think the -- probably the least expensive study that would address the point would be looking at effects on tyrosinase, you know, the enzyme, supposedly mediator, that's responsible for the lightening effect. So if they could do that, I mean --

DR. BERGFELD: Well, the problem with that is, at least clinically, it's known to lighten the skin color and even f you had inhibition of tyrosinase in that other model, what if it didn't lighten it to make it white? How are you going to make that differential? Because some of the reasons it's used is the antioxidant property of it, which includes the lightening property.

So, I'm not sure that that's applicable if you're looking at humans. Maybe it should be an animal model.

DR. ANSELL: Well, it might help if I understood exactly what the concern is. What is the pathology associated with the hypopigmentation? Why --

DR. BERGFELD: Whether it goes to complete cessation of tyrosinase.

DR. BELSITO: Well, even if it doesn't, Wilma, cosmetics should not have a biological effect, and if it effects the pigmentation of the skin, then it's a drug. So, it doesn't matter whether it causes complete de-pigmentation or just some slight lightening. I mean, I guess the point is, is that the cosmetic industry has gotten themselves into their own dilemma here by promoting this as skin lightening in their marketing terms and so now the question is, does it, in fact, lighten skin? And if it does, at what concentration does that occur?

DR. ANSELL: If anyone is, in fact, claiming that it lightens the skin, then they are selling an illegal, unapproved drug. It doesn't make it an unsafe cosmetic, it makes it an illegal drug.

DR. BELSITO: Well, I understand that, but then there must be a reason why they -- you know, I mean, it's all the cosmeceutical marketing, that gray zone where they sort of promise things, but not quite promise it, so.

DR. ANSELL: Well, we've crossed from kind of a safety discussion into a regulatory discussion. In fact, such claims would not necessarily be illegal outside the U.S.

DR. BELSITO: Well, I agree. That's not the discussion I want to get into. The point is, is that for whatever reason, there must be some thought that this would help with blemish problems. It's certainly thought to be true among the Japanese, who are particularly fond of this cream to treat their melasma, so the issue is at what level would this occur? And so -- but what we're now being asked to do is to help industry design a study that would give us, or at least suggest a way, of getting to that problem. Would a study where -- I mean, I guess the question is, if it does "have a lightening effect on skin," what is the mechanism? Is the mechanism through an inhibition of tyrosinase or is there another mechanism? Does it affect melanosome transfer from melanocytes to keratinocytes? I mean, I'm assuming that it's through an effect on tyrosinase. If that's true then doing a study to look at effects on tyrosinase levels in skin would probably be the cheapest way to answer that question.

DR. BERGFELD: Could I give a piece of information? One of the Aveeno products that contains soy extract has done tyrosinase inhibitory studies and it's sold as an over-the-counter facial cream for women for radiance, sold for radiance in photo damage, but it definitely does have a tyrosinase inhibitor in it and it definitely -- in both animal and in the cell models and that's a cosmetic.

DR. BELSITO: Yeah, but I mean if you look at page 7, okay, kojic acid inhibits the activity of the enzyme tyrosinase in in vitro assays, so I'm assuming -- and if you assume that that's how it would have a skin lightening effect, then I would be comfortable with a study showing me a dose, you know, in vitro, human skin, going across the stratum corneum where there's -- if it could be done. And I don't know, I mean, I'm not an expert on whether the activity of tyrosinase on skin that's taken from an abdominoplasty or mammoplasty or whatever, how long that stays intact. But if you can show me a level that doesn't get across the stratum corneum and doesn't affect tyrosinase, I'd be comfortable with that in terms of a level for skin lightening effect, but I don't know if the other panel members would. But that still doesn't answer the question that Dan had before about thyroid peroxidase.

DR. LIEBLER: Yeah, I'm going to pull that one. Based on my reading of this and thinking about it some more, I'm not really concerned about thyroid peroxidase anymore, because I don't think you would achieve levels of compound needed to produce the thyroid effects that were observed.

DR. BELSITO: Right, but I think that that would -- and, again, a study on human skin to me would be much more beneficial. You can't do a skin lightening study on humans. I mean, that's not going to get through any ethics board, so it would have to be on animals.

DR. BERGFELD: Page 7 has some of that.

DR. LIEBLER: I think technically it may be quite difficult to do a study even in skin, human skin, in vitro to look at the doses that would inhibit tyrosinase because I think it would be a very difficult thing to measure. I'm not sure how specific the enzyme would be for tyrosine or any other substrate and how sample preparation, you know, homogenization, and so forth would affect the enzymes. So, I'm having a hard time envisioning how you could do a very good study to look at the ability of topically applied kojic acid to inhibit tyrosinase in situ and then somehow measure that accurately. It's one thing to get purified tyrosinase enzymes and do those types of studies that were mentioned here in the document that we have already.

DR. BRONAUGH: I think that -- some of these companies that make cultured skin preparations, make them with melanocytes, and you could probably use a cultured skin preparation to determine what dose in that preparation would inhibit tyrosinase. But if you're interested in the barrier properties of the skin, these cultured skin preparations don't have very good barrier properties.

DR. BERGFELD: Isn't it true that -- it states on page 2 that kojic acid is used as an antioxidant cosmetic line. Now, I don't know how you expand antioxidant to tyrosinase inhibitor, but I suspect it could.

DR. LIEBLER: I don't think they're necessarily related.

DR. BERGFELD: Are they not related?

DR. LIEBLER: No. Antioxidant most commonly means that it's something that chemically acts with oxidants, vitamin E being an excellent example of that. Sometimes things are called antioxidants when they change the levels of antioxidant enzymes by influencing gene expression. Technically that's not an antioxidant, but in common parlance that's come to be referred to as antioxidant as well. I don't think we have any evidence that kojic acid does that. If it does either of those it probably does the former, which is that direct reaction with oxidants.

DR. BELSITO: But to follow up on what Bob said, so if we -- if industry got an epidermal model with melanocytes, could calculate a dose at which there was no effect on tyrosinase, we do have good amount of percutaneous absorption material, you could then do a risk calculation based upon that, you know, concentration in cosmetics -- how much gets across the stratum corneum, what would have -- what has an effect on melanocytes -- and come up with some numbers at least. Because, I mean, if you're using that model, it's going to be a worst-case scenario because 100 percent of the kojic acid is going to be acting on those melanocytes.

DR. ANDERSEN: Just a question in terms of translating the data we already have potentially and to answering the question. We've got information in the report that there's an absence of inhibitory effects on melanin production at levels 10 micromolar.

It also says at 100 micromolar, but we've got other data that says there's tyrosinase inhibition at 100 micromolar. So if we target 10 micromolar and work backwards, is there a snowball's chance that you can reach those levels given what's in cosmetics and assume whatever dermal penetration you want ? I mean, it's -- might this simply be amenable to a calculation approach? Is it --

DR. BELSITO: Well, I mean, certainly you're right. I mean, looking at, again, page 7, you have 10 micromolar with cultured "melan-a" cells showing no inhibitory effect on melanin production and then tyrosinase was -- do we know that 10 micromolar is negative in that assay? We know that 100 micromolar is positive, but we don't know that 10 is negative.

DR. LIEBLER: Christina, is that reference 8 there in that -- is that the same publication for the lack of effect that 10 and 100 micromolar in the cultured melan-a cells? Do you remember? On page 7, the second paragraph that begins, "Kojic acid was a reference sample," and then it ends with "in cultured melan-a cells." Does that all refer to the same publication? Reference 8?

MS. BURNETT: Yeah.

DR. LIEBLER: Okay. Because that could be key -- a really key reference in making the argument that Alan just laid out for us.

DR. KLAASSEN: How are you going to figure out the concentration of the kojic acid around those cells? Even if you know 50 percent is absorbed or 10 percent is absorbed or 2 percent is absorbed, how do we get that -- change that percent into the micromolar?

DR. BELSITO: And then there are also some additional studies, Christina, that I guess you didn't pick up, references 33 through 36. I wonder if those would help us in retrospect aswe're going this way?

MS. BURNETT: I'm not positive because in all these studies the main chemical that was being researched was not kojic acid. They were using kojic acid as a positive control. So, I can go back and look, but from what I remember it was just, here's your concentration --

DR. BELSITO: Right, yeah, so one concentration of kojic acid and one that was positive, not negative.

MS. BURNETT: Right.

DR. BELSITO: Well, one thing, I think, is clear from the data that we're seeing on this tyrosinase inhibition is that it appears that at levels where you do observe some degree of tyrosinase inhibition there is no effect in melanin production because the first paragraph kojic acid was a potent inhibitor of tyrosinase, but they weren't seeing changes in the melanocytes. And then as we saw from that second and third paragraph, 100 micromolar did not affect melanin production, but did have some effects on tyrosinase activities. So if you could get to some way of showing us no effect on tyrosinase activity, I think based upon the data we could be fairly certain there wouldn't be any effect on pigment.

DR. BERGFELD: And so you would be left with this antioxidant description as its activity.

DR. BELSITO: Well, I mean, we're not going with a skin lightening activity for a cosmetic product.

DR. BERGFELD: No, no, but you would have dealt with that.

DR. BELSITO: Yeah, I mean --

DR. BERGFELD: And we know from the other antioxidants that we dealt with, namely the fruit acids, but it was pH-dependent and this has some pH dependency with UV and it was whether there was a salt of it or not. They acted differently. When there was a salt of the fruit acids, it was an emollient. When it was a pH which was rather acidic, it was an exfoliant, and it had other activity, obviously related to absorption.

Can you speak to why it's in these products?

DR. LIEBLER: So, when kojic acid is used as described in these cell studies, these are obviously short- term studies, kojic acid did not reduce pigmentation in mammalian cells, for example, in paragraph one. I think that's a different scenario than repeated use of a product over time that could affect the production of melanin. We don't know if it's going to have an effect on the production of melanin. That's the issue that I think that we're concerned about. And so I think these -- at least these cell model studies don't really address that issue satisfactorily.

DR. BELSITO: Okay, I mean, point well taken. In the acute setting you may not see some changes in melanin whereas in the chronic you might. But, I mean, again, I think it goes back to, you know, industry is coming up to the plate and asking us, you know, how can we answer that question. So, would we be comfortable on a study that looks at tyrosinase inhibition rather than a long-term study where they're painting an animal? Obviously it would have to be, you know, probably one of the --

DR. BERGFELD: Pigmented.

DR. BELSITO: -- pigmented, darker strained guinea pigs or whatever, and rely on that data. And that's what they're asking. So would we be comfortable on, you know, effects on tyrosinase and margin of safety on percutaneous absorption. Or do we want them to do a pigmented guinea pig paint-on study for 180 or whatever number of days?

DR. LIEBLER: I think if they brought us an in vitro tyrosinase inhibition study we'd be right where we are now, having to try and infer from that result what the impact -- what the relevance is to the likely affect on pigmentation in human skin.

DR. BELSITO: Would be over time.

DR. LIEBLER: Right, over time, right. So, I think that kind of study, even though it would be relatively easy to do, really wouldn't help us very much. I think the type of study that would give us an answer that would be more helpful would be the one you just described where you have a pigmented animal model, you apply it over time, and you look at the dose response for any pigmentation affect.

DR. BELSITO: So, Alan, parahydroxyanisole, is that the one we banned because of an effect on pigment?

DR. BERGFELD: It was the first one.

DR. BELSITO: And how long was that study done and on what strain, do we know?

DR. ANDERSEN: My guess is there's not enough information. There's not enough information in the compendium necessarily to answer that. Let's see, "Because of the de-pigmenting action of parahydroxyanisole in black guinea pigs at reported concentrations approaching those used in cosmetics, it is concluded that parahydroxyanisole is unsafe for use as a cosmetic ingredient." So, I'm assuming that there is a study in that safety assessment of parahydroxyanisole using black guinea pigs and skin lightening. I mean, we can find the -- it doesn't say when that was.

DR. BERGFELD: It used to just say on the top. The old document had it on the top.

DR. ANDERSEN: Yeah, but it doesn't now.

DR. BERGFELD: It needs to go back.

DR. ANDERSEN: Volume 4, number 5, Wilber.

DR. BELSITO: Okay. So then, Dan, would you be satisfied if industry looked up that study that was done on para-hydroxyanisole and reduplicated it with kojic acid and came up with a concentration where there was no de-pigmenting effect?

DR. LIEBLER: Yes.

DR. BRESLAWEC: I'd just like to add that that study was done many, many years ago, 20+. Perhaps the study model has changed.

DR. BELSITO: I don't think so.

DR. BERGFELD: You don't think so?

SPEAKER: Take a black guinea pig and paint them with a chemical and look at their pigment.

DR. RE: I actually just got this request from the supplier last week, so we really have -- we at L'Oreal have not even looked at the literature yet to see if there are more updated models or not. But I think, you know, I think that we can definitely by next meeting come back to you with a -- either we're not going to go or some indication of what the model should be.

DR. BELSITO: So, yeah, what you can take back to your supplier will come, I guess, tomorrow, but I guess from our team standpoint, measuring tyrosinase activity isn't going to help us.

So, we are -- where are we? It's pink. So we're going to get the HRIPT and find out if the dermal sensitization is sufficient. If that's sufficient from an HRIPT, then the irritation should be sufficient. But what we have for irritation right now is not sufficient to me. Twelve rabbits does not a safe compound make.

Phototoxicity seems okay and it's going to hinge on the skin lightening effects, but we have not a promise, but -- I mean, this is pink. So are we going formal and sufficient or are we going to table it until we hear back as to whether industry is going to do this study?

DR. BERGFELD: Table. My opinion.

DR. BELSITO: Madam Chairman says table. Dan? Paul? Kurt?

DR. LIEBLER: I would not argue with Madam Chairman.

DR. BELSITO: Okay, so we will --

SPEAKER: Never, ever?

DR. LIEBLER: Not right now.

DR. BELSITO: We will tentatively table this to see what industry's response will be to our skin lightening request.

DR. ANDERSEN: The converse view is you signaled back in September that these data were needed. You didn't receive them. Tough noogies.

DR. ANSELL: If I'm not mistaken, Alan, and you, of course, are the expert on your own procedures. The obligation is to come forward with an offer, not to come forward with the actual data.

DR. ANDERSEN: An offer is one of the things on the table, but this is a discussion of maybe there's an offer. So, it's --

DR. BERGFELD: For public relation's sake --

DR. ANDERSEN: There's nothing fundamentally wrong with tabling it, it's just if we're going to be hardnosed about continuing to move things along, at some point there's got to be an example. I guess we did it earlier today in which you were forceful in saying insufficient data. This one needs that test of, you know, you'd really much rather have the data than not have it. So, I'll shut up.

DR. BELSITO: Yeah, I mean, I think that we're -- we are prepared, you know. I mean, there's enough time between now and the April meeting for industry to decide whether they're going to do something. You can bring this back at the April meeting and if industry is going to do it, then we'll table it again for the data. If industry isn't going to do it, then we've got an insufficient report and we know where we're going with it.

DR. BERGFELD: Did I hear you say that you also needed the census HRIPT data?

DR. BELSITO: Well, I'm told that Carol is going to give us that tomorrow.

DR. BERGFELD: Okay.

DR. BELSITO: So, we'll see.

MS. BURNETT: Perhaps, she's waiting on --

DR. BELSITO: Perhaps.

MS. BURNETT: -- waiting on concentration data.

DR. BELSITO: Perhaps, so that could be another data request.

DR. EISENMANN: I have the study. I just need to know what concentration (inaudible).

DR. BELSITO: Okay, and the study was an HRIPT?

DR. EISENMANN: Yes.

DR. BELSITO: And what was the N?

DR. EISENMANN: I think in the 50s. I'd have to look back at that.

DR. BELSITO: Because that would make a difference, too, because if it's an N of 12 --

DR. EISENMANN: I'll have that number for you tomorrow.

DR. BELSITO: Thank you.

DR. BERGFELD: I'm sure it isn't.

DR. BELSITO: Okay, so we'll also look at the dermal sensitization issue tomorrow when we hear at what concentration it was tested.

MS. BURNETT: Before we move on, we had a few other items that the council commented on and I just want to have you guys address it. Dr. Mark's team addressed it too, so I just want to make sure that everybody's all on the same page.

The first item under the reproductive section --

DR. BELSITO: Page?

MS. BURNETT: Pages 23, 24. It'd be Choudhary studies 60 and 61. They would like your comments on the relevance of the studies and if there was any need for discussion.

DR. BELSITO: I didn't make any comments on it.

MS. BURNETT: Okay.

DR. BELSITO: Paul, did you?

DR. SNYDER: I mean, it's not a primary kojic acid study, so that's probably going to be the issue, there wasn't a (inaudible). There's nothing that comes out in the repro studies.

SPEAKER: Microphone.

DR. SNYDER: There's nothing that really comes out in the repro studies to -- I mean, it could -- it can stay or it can go, either way. I'm okay with it either way. It doesn't have any substantive new information or detractive information in my opinion.

MS. BURNETT: That's what I needed to know. Okay. And then the next discussion point, they would like further discussion on what you think on thyroid tumors in rodents and how they are relevant to humans.

DR. BELSITO: Page?

MS. BURNETT: They would be pages 45, and then I guess some research.

DR. BELSITO: Yes, thyroid gland hyperplasia, follicular adenomas were significantly increased.

MS. BURNETT: And I had added a citation by Hill, et al., at the end of that section on page 51. The Council would like to either have that stricken or the information from the SCCP opinion added so that it's balanced.

DR. KLAASSEN: Yes, I did put a question mark by that on page 51 when I read it. I – you know, this is -- I'm familiar with this article and I'm familiar with these thyroid tumors and, in general, we don't think that they probably are relevant for humans. So what we're seeing here in these relevance -- and, you know, what this sentence says here that Hill makes is basically kind of a government statement that -- where is it here? Okay, so, the EPA follows the position that chemically induced rodent thyroid tumors are presumed to be relevant to humans and that when interspecies information is lacking, the default is to assume co able carcinogenetic sensitivity in rodents and humans.

However, while it might say that there are many chemicals that produce these thyroid tumors in rodents and the way the thyroid hormone is handled by rodents is very different than in humans. Number one, the rodents don't have the thyroid-binding globulin and plasma and the half-life of thyroid hormone itself is much more rapid than in rodents than in humans, and there are other effects. And we have many drugs on the market that cause these thyroid tumors which appear to be identical to this and don't appear to be relevant. So, I'm not overly concerned. Plus we need to recall, you know, the dose difference between what this chemical can do when given at high doses, that the doses and the concentrations that are being absorbed across the skin are much less than will produce these effects.

I would suggest, quite frankly, that this paragraph that Hill wrote is -- really be removed because that's not really the general status of this area, but for people that work in this area.

DR. SNYDER: That's my interpretation of this data that was presented with, also, rodents are exquisitely sensitive to minor perturbations in thyroid hormone levels and circulation. We have data to suggest that that does occur in animals treated with kojic acid and so the expected response would be an increased proliferation of the thyroid and they all have increased organ weights consistent with that. So that mechanism appears to be the mechanism. We get no evidence. We've actually got studies that were looked at, genotoxicity and carcinogenic specific effects, initiation, promotion, in the thyroid, which were all negative. So, that doesn't appear to be the mechanism. So I think exactly what Dr. Klaassen is saying is correct, that this is something that's unique to the rodent, and we'll capture it or we should capture that in the discussion.

DR. BELSITO: Plus we've already captured that. I mean, this is this gentleman's opinion of doing a review of the literature. This is not primary research and we've already captured -- that was the point before -- that there are thyroid issues in animals treated with very high doses that if we feel we need to discuss it in the discussion, we can discuss it. We don't need this individual from the EPA giving us his opinion.

DR. LIEBLER: Maybe I could make a suggestion. The -- instead of this paragraph citing the Hill paper, as Curt's points out, perhaps there is a review, a perspective in the literature on rodent thyroid carcinogenesis not necessarily by kojic acid, but by other compounds, that might be used to illustrate Curt's point. Because I think Curt's point should be in this report in place of this Hill thing that makes the point about the sort of unique characteristics or nonhuman- like characteristics of thyroid carcinogenesis by other chemical substances. So, even if it doesn't necessarily cite kojic acid, and it might not have come up in your literature search, Christina, it might be the right thing to have there.

DR. BELSITO: But not there. This is under tumor initiation. It would be the right thing to have in the discussion as to why we felt that the thyroid effects were not relevant to humans.

DR. LIEBLER: Then that's fine. Yeah. Right, it should be in the document.

DR. SNYDER: The article, Charles Capen, "Toxicologic Pathology," 1996.

SPEAKER: For example.

DR. BELSITO: What were the page numbers?

SPEAKER: It's right here.

DR. KLAASSEN: You can also look in Casarett and Doull's Toxicology textbook.

DR. LIEBLER: Edited by whom?

DR. KLAASSEN: And if you want to look for the chemical that's been examined the most, it's phenobarbital. I have also written a dozen manuscripts on this area.

DR. BELSITO: Okay, any other points from the council? That's it?

MS. BURNETT: I think I have them covered.

DR. BELSITO: Okay, so industry is going to let us know what they're going to do about the skin lightening effect, Carol's going to let us know tomorrow what she's found out about sensitization that might be added in, we're keeping the data on the thyroid tumors in the document. In the discussion we're going to talk about why they're not relevant to humans referencing whichever reference we please, and we go from there. And industry, if we don't – our team would like to see this come back on the April meeting, and if we don't have a commitment from industry to do the studies, then it will go out as insufficient.

Marks Team – December 7, 2009

DR. MARKS: Okay. So at the September meeting this year, the panel issued an insufficient data announcement with a number of data needs: Sensitization and irritation data, phototoxicity, the dose response and skin lightening, and the role of kojic acid in inhibition of thyroid peroxidase. And we received some new data just a couple of hours ago.

MS. BURNETT: I looked at the new data and it doesn't fill the needs.

DR. MARKS: Not at all?

MS. BURNETT: It's just skin irritation and phototoxicity, which we already had some information on. And it doesn't really show anything different.

DR. SHANK: Right.

DR. BAILEY: I think it addressed some of the data needs, but not all.

DR. MARKS: Right. It would appear that both the skin irritation that's a slight mild irritant, and then I didn't have a lot of time to look at the phototoxicity, but, again, it would appear it would be not phototoxic, although there's some technical issues

(inaudible) in the study about whether there is some irritation. So I get some conflicting interpretation of this from my mind. How would you address those, Carol?

DR. EISENMANN: There's an HIRPT I have.

DR. MARKS: Oh, there is?

DR. EISENMANN: But I don't know the concentration yet, so I'm waiting for that. As soon as I get the concentration in the HIRPT -- I mean, it's negative, but I don't know the concentration of the material tested of kojic acid yet. So it's possible when I go back to my office this evening that it'll be there and I'll bring it tomorrow. I'm expecting that.

DR. MARKS: So it sounds like we may have the sensitization and irritation because presumably if it's negative it's not a significant irritant either if you have an HIRPT. So that would be helpful.

Did you -- did anybody else get to look through these? The phototoxicity, again, I was a little conflicted, but I think it's okay.

DR. SLAGA: It's least in cream was the way we wanted it.

DR. MARKS: Yes. Yeah, it's in cream. The concentration they used was 3 percent. And I think in use it may be up to 4 percent, but I think that's close enough, particularly if you have a concentration of HIRPT that is negative. That would be very helpful. So I think that may -- those first two data needs are going to be addressed.

I didn't see anything on skin lightening.

DR. SLAGA: No.

DR. SHANK: No.

DR. MARKS: And then the issue of the thyroid peroxidase, again --

DR. SHANK: Do we really need that?

DR. MARKS: Well, that's the next thing. We oftentimes, when we initially see an ingredient we kind of put out a lot of things we want and need. And then as we have more time to think about it and look at the data again we refine that. So do we really need the thyroid peroxidase data?

DR. SLAGA: I don't think we need it. I'm trying to recall how we originally stated that just to see if there was more data on that particular point. It really has not any relevance on the skin.

DR. SHANK: I agree.

DR. SLAGA: As long as it gets absorbed.

DR. SHANK: I agree.

SPEAKER: So we can remove that.

DR. BAILEY: So you would take that off of the list of data. What we may be down to then is skin lightening effect data --

DR. MARKS: Yes.

DR. BAILEY: -- to show limit on skin lightening.

DR. MARKS: Right.

DR. BAILEY: But on that, I mean, that's -- I mean, I understand the question, but I'm not sure how pathologically significant it would be. I mean, you get into the issue of skin lighteners and hydroquinone and the drug versus cosmetic effect and so forth. And certainly, you know, kojic acid can exert a skin lightening effect. But I'm just not sure from a cosmetic perspective a nondrug use as an antioxidant, how significant in a safety health question that would be in making a determination about safety for cosmetic uses.

I mean, for example, with hydroquinone, I think one of the conclusions was that it's safe for hair dye use, but not as a -- I don't know if it's stated OTC or non-drug use or whatever. We've made these distinctions in the past, so I'm wondering if we can't be in the same situation here. If we can come up with a reasonable basis for talking about skin lightening effects.

DR. MARKS: Well, it's used for eye makeup, so it's going to be applied on eyelids. And then it's also used in facial creams.

DR. BAILEY: I understand. And certainly the safety assessment should take that into account. But the skin lightening per se, is that an endpoint of concern?

DR. MARKS: Yeah. I think if you have skin of color it could be. And my understanding is it's being used in the Japanese market, too, as skin lightening. Is that correct?

DR. BAILEY: I think (inaudible).

DR. MARKS: So I think, you know, in the Japanese market it may be fine if you get a little lightening, but I'm not sure that I would feel that it would be good and safe in a much more heterogeneous population.

DR. BAILEY: But that's a good question that you could capture in your safety assessment. It's -- I mean, I think what I'm arguing for here is to table this while Carol gets some more data, we present that, and then maybe we can go back and talk about thresholds for skin lightening effects and provide something at the next meeting on that. Is that a reasonable thing, Tom?

DR. RE: Yeah, and if (inaudible) --

REPORTER: Actually, can you move closer?

DR. RE: I'm sorry. We have at least one supplier that has expressed interest in following up on the skin lightening, but they would like some direction as to what the panel would like to see in the way of data.

DR. MARKS: Basically, we want to see a threshold that under this concentration we're not concerned about skin lightening. Then that way we can say if it's -- I think the highest concentration was 4 percent. So if you used a product that contained 4 percent or less of kojic acid you would not be worried that there would be an effect on the pigmentation of the skin.

DR. RE: So you're talking about a clinical study that essentially does response?

Would an in vitro study on tyrosinase inhibition -- would that suffice?

DR. SLAGA: That would be more sensitive. It would probably come up that it would have effect on that at a lower concentration than in vivo.

DR. MARKS: I don't know. Do we have it comparable with hydroquinone where you take the test tube and compare it to the clinical?

DR. SLAGA: Yeah, I don't think --

DR. MARKS: So, I'm not sure you can translate an in vitro to an in vivo. That would be my only concern. There are pigmented animals that have been used in the past to look at skin lightening with hydroquinones.

DR. BAILEY: But if they could come up with a reasonable protocol for this comparison --

DR. MARKS: Right.

DR. SLAGA: And it's above 4 percent, which (inaudible).

DR. BAILEY: -- I assume that would be acceptable.

DR. RE: Okay.

DR. MARKS: Yeah. I think we need to have a -- you know, it'd be nice to say at whatever percentage down -- if it's above 4, then there's no safety issues at all from that point of view. Because that sounds like that now is the only concern we have.

There was some new carcinogenicity data, which we'll talk about --

DR. SLAGA: It's all very weak or it's actually tumor promotion at a weak stage. It normally occurs at a higher level, even at a weak promoting thing, so.

DR. MARKS: So you're not concerned about that. So we still have -- then the only thing, Tom, is the skin lightening.

DR. RE: And a pigmented animal model would be acceptable to the panel?

DR. SLAGA: Well, Ron brought up a good point. It's thinner than human skin, so it could come up where you actually -- I don't know how to do it with that. But in the past we would accept --

DR. RE: We'd be erring on the side of safety, though.

DR. SHANK: Yeah. It would be a much more sensitive model if it's negative in a pigmented rodent.

SPEAKER: What about hamsters?

DR. MARKS: As long as we have -- how do I want to say, you'd want a good control in that.

DR. RE: Sure.

DR. MARKS: To know that we really -- because, again, back in the hydroquinone and the de-pigmenting phenolics there were animal models used, but I don't know how widespread and how well accepted that was in terms of using that as a screen for human. I can't remember whether it was a mouse or whatever, rat.

DR. BRESLAWEC: I'm just wondering about the likelihood of actually getting this data – is that a real possibility -- from the industry?

DR. RE: As I said, we had a supplier who expressed interest. We need now to go back to them and see if their interest is real.

DR. BRESLAWEC: Thank you.

DR. MARKS: Okay. So -- go ahead, Ron.

DR. HILL: This was actually probably just something to write into the report. But back to the peroxidase activity I wondered, on page 45 is where the new section is added in there, and they see decreased serum T3 levels. They say likely mechanism with decreased serum T3 levels and increased thyroid stimulating hormone. I wondered in the drug uses of this compound -- I mean, surely somebody has looked at in the actual drug use where there's a significant effect on these levels. And that information ought to be out there and could at least be incorporated in the report.

DR. BRESLAWEC: Do we have any approved drug use of this?

DR. HILL: Isn't it used as a drug? Overseas?

DR. BAILEY: I want to say it was -- there was an RX use. And that data, I would guess, is probably not available. I don't know. Typically in these situations, somebody owns the data that's been provided to the FDA. And then if --

DR. HILL: If it's provided to the FDA, it's public record.

DR. KATZ: That depends.

DR. HILL: No?

DR. KATZ: Not necessarily.

DR. HILL: Okay.

DR. KATZ: If the drug itself has not been approved, then it would not be available for public record.

DR. HILL: Okay.

DR. KATZ: And some of the information may be proprietary.

DR. HILL: Okay.

DR. KATZ: So that it would not be available either even if the drug had been approved. You can request it under FOIA, but you may not get everything that you'd want because of what -- depending upon the nature of the data that's there.

DR. HILL: Well, could we make that request?

MS. BURNETT: I've already submitted a FOIA request.

DR. HILL: You did?

MS. BURNETT: And didn't receive anything.

DR. HILL: Didn't receive even a no?

MS. BURNETT: Well, not in the specific terms. I just asked for whatever available tox data they have and they didn't provide – they didn't have anything. I got a no. They provided me with the VCRP data and that was it.

SPEAKER: Thanks (inaudible).

DR. HILL: Somebody, somewhere, maybe not in the U.S., knows whether that use as a skin lightener at these higher levels would affect T3 levels and TSH levels. It might be in Japan or it might be in Europe, but somebody, somewhere, knows that answer. I'd be shocked if it isn't out there in the literature somewhere in case reports.

DR. BRESLAWEC: I want to point out that I'm pretty certain that kojic acid is not approved by the U.S. Food and Drug Administration for anything.

DR. HILL: Yes.

DR. BRESLAWEC: And as a dossier, if you will, that's under review, it's extraordinarily unlikely that we would get any information from FDA. The fact that Christina searched the literature and was not able to find anything suggests there's nothing in the published literature. And if somebody would like to submit it from their unpublished literature, we'll take it, but we have no way of acquiring that submission.

DR. HILL: So you did a fairly recent search for things like case reports on the literature and hadn't seen anything?

DR. BRESLAWEC: Absolutely.

MS. BURNETT: I always check right before the panel meeting.

DR. HILL: Okay. And you didn't see anything with T3 levels?

MS. BURNETT: Nothing.

SPEAKER: Interesting.

DR. MARKS: In getting back, even without that, Ron and Tom, you're not concerned about this eliminating that as the thyroid peroxidase again as a data need?

DR. SHANK: Correct.

DR. HILL: When did that go into the list of data needs? Because that was certainly --

DR. MARKS: At our last meeting.

MS. BURNETT: It was a request from Belsito's Team.

DR. HILL: Well, I guess if they have further concern we'll find out about that tomorrow, right?

DR. EISENMANN: I have a comment. The thyroid tumor issue, the citing of the EPA document. And I just wanted to make sure you know that the SCCP reviewed the data and concluded that thyroid tumors were not relevant to humans.

DR. HILL: Right.

DR. EISENMANN: So I don't know how you want to present that. If you want to stick with how it is. I suggest put both and say there's differing opinions or just your opinion. Get rid of the EPA reference and just put your opinion in the discussion. Either way is appropriate. But how it's stated right now in the summary of the section, it says that "is to assume comparable carcinogen sensitivity in rodents and humans." For this tumor I don't think that's appropriate anymore.

DR. HILL: The reason I wondered whether there was something out there on possible effects on the T3 levels or TSH levels, because they might be a relatively sensitive indicator of systemic exposure. And I wasn't very confident with the way that they estimated systemic exposure. And although I didn't have a chance to look up the paper, there was one place where they were talking about serum levels. And I wasn't clear whether they were measuring the parent compound only or they were including in that measurement the conjugates: The glucuronides and sulphatessulfates. And so I wasn't 100 percent confident in their – what I had in here in terms of the index of systemic exposure. And that goes to concentration of use. Because if we go up to 4 percent and there is significant systemic exposure, I was looking for some indication that that was still unimportant.

DR. MARKS: Tom? Ron?

DR. BAILEY: We do have the European assessment. I presumed that that was part of their deliberation on it. So it's not like this is, I think, a totally unknown issue. And so while it would be, you know, nice to have a study in front of us that answers that totally, I'm not sure I'm hearing that it's really needed.

DR. HILL: I agree. I just thought that if there happened to fortuitously be information that would give us some sense of that, I mean, no effect is no effect and it really doesn't tell you much other than --

DR. BAILEY: I mean, for hydroquinone, I think, because it's so studied there's probably information out there. But the kojic acid, I'm not familiar with a similar massive body of data because of the drug uses.

DR. MARKS: Christina, the reference to the SCCP on page 2, they say it's a maximum concentration of 1 percent in skin care formulations. And they did a margin of safety calculation. Do you know what that margin of safety was? What were they concerned about?

DR. EISENMANN: Thyroid effects.

DR. MARKS: Oh, the thyroid.

DR. EISENMANN: But noncarcinogenic thyroid effects.

DR. MARKS: Okay. Okay.

DR. SLAGA: Just, there's an editorial on the carcinogenicity and such. And that doesn't read through some statements about control versus treatment that are not correct (inaudible).

DR. MARKS: And then, Tom, I assume you'll also in the discussion, the second -- it's actually not a paragraph, it's sort of the second paragraph on page 55, these data -- "There were data that suggested carcinogenicity weak and tumor promotion activity."

DR. SHANK: Yeah.

DR. MARKS: Obviously you're going to leave that stand alone.

DR. SHANK: No.

DR. MARKS: Either it's going to be eliminated or you're going to have a follow-up sentence putting it in perspective.

DR. SHANK: Well, I had another phrase on the absorption, the slow rate of absorption across the skin.

DR. MARKS: Okay. So, we'll capture those, Christina.

DR. HILL: And that is where I say I was a little less than confident and comfortable that we had the solid answer on that. I had loose ends in that calculation that maybe just require looking at the original reference soon, like, later today.

DR. MARKS: Okay. Let's -- so, my sense is that the team would like to table this. That suggestion was made, I think, by John, which I will latch onto, that we table this with the idea that industry is going to provide us dose response studies for skin lightening effects of kojic acid. And that that's really the only safety concern we have now since it sounds like we have an HIRPT, which is going to be safe. And rather than issue, I think, an insufficient tentative report, we could table it awaiting the results of that industry study. Does that sound okay? Tom? Ron?

DR. SHANK: Yes.

DR. HILL: Yes.

MS. BURNETT: Can I just go back and clarify on the Hill reference? Did you want that removed or did you want me to add in the SCCP?

DR. SHANK: Could you say that again?

MS. BURNETT: The Hill reference, the EPA reference on the thyroid tumors. Did you want me to remove that or did you want me to add in the information of the SCCP regarding their margin of safety calculations?

DR. SHANK: I think add.

DR. EISENMANN: It's not the margin of safety calculation.

MS. BURNETT: Well --

DR. EISENMANN: It's the conclusion about thyroid tumors.

MS. BURNETT: Right.

DR. SHANK: I would add it.

MS. BURNETT: You want to add it?

DR. EISENMANN: And leave in the Hill?

DR. SHANK: Yeah.

DR. EISENMANN: Okay. You have both.

MS. BURNETT: And one more thing that I have that was a council comment, they would like you to discuss the -- I have two references in the reproduction section by Choudhary. They would like you to discuss them. It is on page 23, references 60 and 61. And just to let you know, it is the motility versus mortality. It's written as it's written in the --

DR. MARKS: So it's the last paragraph on 23?

MS. BURNETT: It's essentially the whole page.

DR. SLAGA: It's almost the whole page.

SPEAKER: Sixty and what? Sixty-one?

MS. BURNETT: And 61. It's essentially the whole page going on to the top of the next.

DR. SHANK: Oral administration.

DR. EISENMANN: They see effects of much lower doses. Or if they're really effects. I mean, it's much lower doses than -- other reports are -- NOEL are much higher. And I just thought that might be worth mentioning in the discussion.

DR. SHANK: You know, when the animals die and have infections and things like that, it sounds like the laboratory is not the best.

MS. BURNETT: I have the original studies with me if you need a closer look. It was pretty much -- it was two pages.

DR. SHANK: Two pages?

MS. BURNETT: They're not very well described (inaudible).

DR. SHANK: Uh-huh.

DR. MARKS: So, Carol, your question here was whether or not these two studies should be addressed in the discussion?

DR. EISENMANN: Addressed or delete them because they're so out of line of the other studies. Or, I mean, I don't care what -- I mean, I just --

DR. MARKS: I'll tell you what. That's going to be an editorial thing.

DR. EISENMANN: Okay.

DR. MARKS: I think. So why don't we let Dr. Shank read through those, think about it a little bit?

DR. EISENMANN: That's fine.

DR. MARKS: And then he can get back to Christina to decide whether or not -- how to handle those two studies. Does that sound good?

DR. SHANK: Yeah. They even allowed cannibalism.

SPEAKER: Huh?

DR. SHANK: They even allowed cannibalism. So you eat your data.

DR. MARKS: So I think the issue will be do we comment or do we delete? What's the precedent on that, Ron?

DR. EISENMANN: Or, I mean, you can ignore them if you want. Continue to ignore them completely if you want to, but I just thought --

DR. MARKS: Okay. Well, thank -- Carol, thank you for bringing that up.

SPEAKER: Mold does produce kojic acid. Yeah.

SPEAKER: So would did you call (inaudible)?

SPEAKER: Yes.

DR. MARKS: So, do you want to comment now or do you want to wait, Ron?

DR. SHANK: Yeah. I don't like --

DR. MARKS: Throwing away stuff.

DR. SHANK: -- throwing it away.

DR. MARKS: Yes. Exactly.

DR. SHANK: Because that makes us look biased.

DR. MARKS: Yes.

DR. SHANK: So I think we need to explain why we're not giving heavy weight to the data and that requires careful wording. So I need to think.

DR. MARKS: So, Ron, we'll have you give that to Christina then.

MS. BURNETT: Thank you.

MS. WEINTRAUB: Can I just bring up one point?

DR. MARKS: Sure.

MS. WEINTRAUB: And I'm sorry if you discussed this previously, but --

DR. MARKS: That's okay.

MS. WEINTRAUB: -- in reading the minutes from last time, which I think are very useful to have in front of the report, I saw that Dr. Katz and I had a discussion about the role of FDA and their position on kojic acid in this context. And when I read that I remember feeling the same sense of uncertainty that I felt then. And I just wanted to make sure that I completely understood the dynamic in terms of FDA approving this for use for -- is it for drug purposes at this point, kojic acid?

DR. KATZ: Kojic acid is not approved by the FDA.

MS. WEINTRAUB: For any --

DR. KATZ: For any use.

SPEAKER: For any drug use.

DR. KATZ: Drug use. And for cosmetics, we don't approve anything.

MS. WEINTRAUB: Right. Right.

DR. KATZ: So that at this point in time it's not approved by the FDA.

MS. WEINTRAUB: But nothing is really.

DR. KATZ: That's right. Well, in terms of a drug.

MS. WEINTRAUB: Right.

DR. KATZ: And for cosmetics, that's correct. We don't approve ingredients that go into cosmetics.

MS. WEINTRAUB: Right. So --

DR. KATZ: Particular cosmetics.

MS. WEINTRAUB: So does the FDA have a position in terms of drug use on kojic acid? Or no position on that either?

DR. KATZ: Well, I'm not sure what you mean by a position. At this point in time since it's not approved, I'm not sure that you have a position one way or the other. I don't even know, in all honesty, whether or not kojic acid has been submitted as an ingredient for a drug that has not been approved. And that I can't answer because the drug side is totally separate from us. And the only way -- and that was part of the earlier discussion to know if the FDA has even addressed the issue -- is to make an FOIA request, which it appears was done. And the FDA has not presented or answered the request. Or if they did answer the request, they said that there was no information. So that from the drug side, that's about as much as you're going to get at this point in time.

MS. WEINTRAUB: So basically there's nothing to glean -- nothing to glean at all from FDA's sort of non- position on this?

DR. KATZ: Well, I wouldn't say the FDA has a non- position.

MS. WEINTRAUB: Or just they have not -- I mean, I understand that there is no higher people in cosmetics.

DR. KATZ: That's right.

MS. WEINTRAUB: Obviously, which is why we exist. And then in terms of drugs, we don't know what that process is. So there's just nothing that we can really glean from that.

DR. KATZ: Well, we do know what the process is from drugs.

MS. WEINTRAUB: Yes.

DR. KATZ: But we just don't have any information by which to -- the reason why I'm changing the wording a little bit is because the way that it's phrased is not really kind of accurate because the FDA does review applications that they get submitted, both in terms of investigational new drugs and NDAs, which were the new drug applications.

MS. WEINTRAUB: I just meant we don't know what the specific sort of place or where kojic acid is in that system, in that known process at this point.

DR. KATZ: At this point in time there are no known -- there are no approvals of kojic acid in an FDA drug. And that's what you do know.

MS. WEINTRAUB: Okay.

DR. BAILEY: If I could elaborate just a little bit, and that is there is an OTC drug monograph for skin bleaching products. Okay? And it's clearly classified as a drug effect. But the only ingredient that's in there is hydroquinone. And that's not a final monograph either. I think there's still deliberations that are going on. So kojic acid is not within the structure of an existing OTC drug monograph.

And what Linda is saying is that there are also -- we don't know there are no NDAs or other type of application that would indicate that the agency is looking at this from a prescription drug or some other way to get it into the monograph.

So, we know hydroquinone is a drug. We know skin bleaching is a drug. What we don't know is if anybody has applied for kojic acid unless an FOIA is submitted, which CIR has done.

DR. MARKS: Okay. Any other comments? Otherwise, we'll wrap up the discussion on kojic acid. And our team tomorrow will propose or move that this ingredient be tabled for an industry study in the future which will define the skin lightening effects of kojic acid.

DR. HILL: I have a report generation question. And since Halyna is here, maybe you could comment on this.

There were several places in the -- for the September meeting where I'd flagged that we had concentrations in the report in milligram per mil. And I had made the comment that I'd like to see them converted to micromolar concentrations. And I got the sense from the comments that maybe if that's not the way they calculated it in the original report we shouldn't put them in this review. But yet, milligram per mil -- I mean, I can do the calculation. I wondered if we could at least -- if we have a policy that we can't put them in the line of text, that we could at least footnote. Because if you know the molecular weight and

you know the milligram per mil, you can get that concentration. It's about a 10-second calculation. And it would really help in the reviews to have that information sometimes. Because when you have a concentration that inhibits an enzyme, for example, like tyrosinase or the peroxidase, you see milligram per mil, you see serum concentration, milligram per mil, and you don't really have a sense for how that relates to what the concentrations are that are necessary to inhibit those enzymes. And again, I, as a reviewer, can do that calculation if I happen to have a calculator with me.

DR. BRESLAWEC: Just to make it clear, you're talking about per surface area concentration (inaudible).

DR. HILL: No, no, no. I'm talking about actually solution concentrations, like the TC-50, 10.8 plus -- here's one example, and there are several like it -- 10.8, so it's plus or minus 4 milligram per mil. All right. What I really want to know is what's the micromolar concentration?

DR. BRESLAWEC: Yeah. We've not routinely done that calculation.

DR. HILL: Could we, I guess, is what I'm asking.

DR. BRESLAWEC: We'll certainly consider the request. Yes.

DR. HILL: It's pure substance and, you know, I mean -- and you know the molecular weight, you can do that.

DR. BRESLAWEC: We'll certainly --

DR. HILL: And if you don't want it in the footnote as a matter -- or in the text as a matter of policy, footnoting it would be awfully -- I mean, like I said, I can do those calculations.

DR. BRESLAWEC: Right. I think we're more trying to emphasize changing the values of what we get in terms of skin exposure from, you know, milliliter administered to surface area. And that's what we seem to be focusing more on in terms of the conversion.

DR. HILL: Well, I made that comment, too.

DR. BRESLAWEC: Yes. We will take your --

DR. HILL: I repeatedly made that comment, too. But I --

DR. BRESLAWEC: We'll take your suggestion under consideration.

DR. HILL: And same with serum concentrations when it's possible because, again, if you have some idea about how that relates to what concentrations -- and, of course, serum concentration is not the same as tissue, but sometimes it's pretty close. You at least have some idea we're in the same order of magnitude or, gosh, this is -- 4 is a magnitude above and we really don't care about that. We got this concentration. It's 10 nanomolar, but it takes 10 micromolar to inhibit an enzyme. Then kind of who cares? But if it's somewhere in the same magnitude, then you can immediately see that and immediately know whether there's a potential effect.

And I'm sorry. I guess this is -- I'm sitting on a plane, for example, and I have this -- I'm looking at this number and I don't have the calculator handy. Then it would be really awfully nice.

DR. BRESLAWEC: Okay. Actually, it may be something that you'd want to bring up in General Session and see how the others would react. That would be a good idea.

DR. HILL: Or we can just ask Alan how he feels about it. And he's not the only decider, but --

DR. BRESLAWEC: Yeah. Yeah. That's fine.

DR. BAILEY: Would that be a general request throughout the document or just for serum and those kinds of levels? Because it does add an extra burden to the staff, so if there's a particular that is -- and I fully appreciate you saying.

DR. HILL: It doesn't add a burden because we're talking about a 10-second calculation. And once you know the molecular weight, you can do that repeatedly for any time --

DR. BAILEY: Once you get the molecular weight --

DR. HILL: Well, okay, but we got that almost always in the documents. I mean, I'm being lazy, I guess, in the sense that I can do that calculation --

DR. BAILEY: Well, you guys are the reviewers, so, you know, they need to facilitate their review.

DR. HILL: That's all I'm asking. And it would be helpful.

DR. BAILEY: Yeah. Okay.

DR. MARKS: Okay. Any other comments?

DR. SHANK: I'd like to go back to page and these two studies about Choudhary on reproductive toxicity. Christina's handled it very well, very objective, and I think that's just fine.

The first part, you see these effects were seen and the authors said this is mainly due to maternal toxicity. Leave it at that.

The next one you say the authors concluded that the implantation and the cannibalistic effects in females decrease viability. I think that's fine just to leave it that way. And don't say anything in the discussion because anybody who knows about this kind of toxicity testing will say this is not the way you do it. And we don't need to say that the studies we felt were flawed.

Thank you, Christina.

DR. MARKS: Okay.

DR. SHANK: In the -- one other thing, a contradiction. Let me find it.

On page 53 at the bottom in the summary, the last paragraph says that kojic acid is rapidly absorbed and distributed to all organs and oral subcutaneous and dermal treatments. I don't think it was rapid and dermal.

And then on the next page we say absorption through kojic acid in human dermatomed skin was low. So, if you go back to the studies on kojic acid absorption in rats it says it was rapid and oral, not as rapid subcutaneous, and less in skin. So I don't think it's a good idea to include at the bottom of page 3 the rapid absorption in oral subcutaneous and dermal.

MS. BURNETT: Okay.

DR. HILL: And I'm not a dermatologist, so I'll just make that -- but when you say -- when you make a statement like approximately 17 percent of the applied dose being absorbed, does that always apply a 24-hour absorption study? Because otherwise, I mean --

DR. SHANK: Not always.

DR. HILL: Because 17 percent is absorbed in 10 minutes is a very different result than 17 percent is absorbed in 24 hours. And in terms of systemic exposure, the rate is really what matters much more than the amount.

I think I even made the comment here on one of the places that there were two statements where it had amounts where we really want it for rates. I just wrote it said rate and then it put 17 percent, and I wrote not a rate. And it was something else on the same line. Not a rate. Because, you know, even basic pharmacokinetics, you know how much that matters.

DR. MARKS: Okay.

DR. SHANK: One last thing, since this is used in sprays, different kinds of sprays, we should add the boilerplate to the discussion on aerosols. Particle size of aerosols. That's all.

DR. MARKS: There's a number of editorial things. Christina, I won't go over all those tomorrow, particularly since we're tabling it. But --

Okay. Does the team need some sort of idea -- since we're tabling it, are we tabling it for a year? Do you want a suggested timeline? Certainly, we should have some feedback.

DR. SLAGA: Well, it would be nice to have the feedback by next meeting if there is data coming.

DR. SHANK: By April we should have some idea as to whether industry is considering supplying data or not.

DR. RE: I will definitely let you know by next meeting whether or not the data is forthcoming.

DR. SHANK: Still interested. Okay.

DR. RE: And if it is, it's not going to be a year. It'll be, you know, a short timeframe.

DR. SHANK: Okay. Thank you.

DR. MARKS: Okay. That was easy. Let's go on to the next one.

Full Panel – December 8, 2009

DR. BERGFELD: Then moving on to the next ingredient, Dr. Marks, kojic acid group.

DR. MARKS: At the September 2009 CIR meeting, the panel issued an insufficient data announcement for data needs. First was dermal sensitization and irritation in current use concentrations. And we did receive data about irritation, sensitization, including this morning HRIPT, which would support that kojic acid is safe with relevance to these toxic effects.

We received phototoxic data and that would be okay. We did not receive anything about dose response for skin lightening effects and our team didn't feel we needed the thyroid peroxidase data or the role of kojic acid in this, but we had asked again the input from the Belsito Team about that need.

So, what we, in our discussions, felt the way to proceed is to table this ingredient. We got assurances from industry that we would have feedback by April concerning the skin lightening effects of kojic acid and that we would probably have scientific data to establish a threshold for skin lightening.

DR. BERGFELD: And that's a motion to table? Is there a second?

DR. BELSITO: Well, can we have a little discussion because there is --

DR. BERGFELD: Well, you can keep talking until we have another motion.

DR. BELSITO: There is an issue here that the sensitization data we got this morning was only to 1 percent, and this is used in face creams, lotions, up to 4 percent. So, we had, yesterday, agreed to table it awaiting industry's response whether they would do the skin lightening study à la para-hydroxyanisole, which went as a --

DR. BERGFELD: Unsafe.

DR. BELSITO: Unsafe, based on skin lightening. Thank you, Wilma. But now we have a second data need, which is going to be sensitization and concentration of use, so that's insufficient as well. So, I'm not so certain that we should be tabling it now. I just throw that out because we don't have sufficient sensitization data.

DR. BERGFELD: Discussion? Dr. Marks? Dr. Snyder?

DR. SNYDER: It's the same conundrum we had before where I think we do have insufficient data needs and then we also have this other issue that we're going to get some data that will help us explain another effect. And so we're justcaught between the two again.

I'm a little bit concerned about tabling all these reports because I think we need to keep moving them forward because, otherwise, we're defeating the purpose of expanding reports and moving them along. And I think many (inaudible) on the --

DR. MARKS: Well, Alan, would you like to proceed as insufficient? I'd like your -- because we certainly could do that.

DR. HILL: Could I say something before we comment on that? I'm a little confused about the skin lightening deal now because -- from our discussion yesterday, because, I mean, I did some reading overnight. I was looking for something else, but, I mean, the paper here is very clear that at 1 percent it's used for skin lightening in Asia. So, I mean, it would seem to be a moot point whether it has skin lightening effect because they're pretty clear that's what they're using it for at the 1 percent level.

And so, the questions that were sort of informally discussed in the interim, since yesterday is, do we care? Is that a drug use?

DR. BELSITO: But that's a claim that is used. I don't, you know --

DR. HILL: Or is it like an herbal where there is no claim, but everybody knows that's what it's used for, it's just not claimed so on the label.

DR. BELSITO: We don't have any data to indicate that 1 percent will, in fact, cause skin lightening, whether they claim -- and it could be additional ingredients that are put in with kojic acid like hydroquinone that caused the skin lightening. So, we don't really know what those are coming from, so we need the data.

DR. HILL: Or the mechanism.

DR. BELSITO: Right.

DR. BERGFELD: Alan, can you respond to table versus insufficient data announcement?

DR. ANDERSEN: I -- my preference would be to move forward with issuing a tentative report with an insufficient data conclusion. You have already issued an insufficient data announcement. And on your hallmark question, you received no data, and that's the question of skin lightening. You should proceed on that basis.

You also received data that did not fully address the sensitization question, so that remains an ongoing data need. So my preference would be to take the next step. It's not a final decision. The industry still has the opportunity to do two things, one is either provide additional data or to raise their hands and say we will undertake a study to develop the needed data.

At that point, you can indeed table it since you know there's something going on. But if the response is, well, we're not going to undertake a study and we're not going to provide you the data, at least then your positioned to make a final decision and say that's it.

So, I'd rather you issue a tentative report with an insufficient data conclusion.

DR. BERGFELD: John Bailey, can you respond?

DR. BAILEY: Yeah, first, I would say on the sensitization, I would ask the panel, the data supports that it's not sensitized at 1 percent. And so the panel's conclusion could be 1 percent is safe even though there are higher levels reported in the use. So, I would -- I think the notion that it's insufficient needs to be very, you know, very closely linked to the higher levels above 1 percent.

I would -- you know, we have made a commitment to address the skin lightening effects. I have questions about whether -- I mean, we really have to be careful to separate the drug uses from what we're dealing with right now, and that is the safety of an ingredient for cosmetic use separate from the claims. You know, the claims are FDA's domain and they would need to address that, so we don't regulate or address claims in this deliberation.

So, you know, it comes down to, is a level of skin lightening safe or unsafe as determined by whatever data you have before you? And so, you know, in other words, is there a pathology associated with skin lightening effects? I can't answer that. That's your decision, but we really need to be careful to keep those two issues separate.

DR. BERGFELD: So, what I hear you saying is that this should be tabled. You have sensitization at 1 percent and you have a promise to do the tyrosinase inhibitory testing from L'Oreal.

Linda Katz? Dr. Katz? Any comment about the FDA and it's dealing with this particular ingredient? Nothing.

DR. KATZ: As far as I'm aware, that this is not a drug there, so that I can't really speak to the drug issue anyway. That would be CDER's jurisdiction, but I'm not aware of it being approved as a drug. And as far as a cosmetic, again, there's no premarket approval for cosmetic use, so that would be the FDA's current position.

DR. BERGFELD: Thank you.

DR. LIEBLER: Wilma, perhaps this panel could look at the skin lightening, actually, as an adverse effect of the cosmetic product as opposed to a drug effect because we're not about drugs. But so perhaps the consideration of this as an adverse effect might guide our thinking a little bit better than thinking about it as a drug.

DR. BERGFELD: That's a nice approach. Thank you.

DR. ANDERSEN: Well, and that parallels how the panel dealt with para-hydroxyanisole.

DR. BERGFELD: So, Dr. Marks, how would you like to proceed?

DR. MARKS: Well, my sense is, the motion that I made that it be tabled would tend to support that, with the option in the future of having a conclusion at 1 percent limit as safe, since we have an RIPT to indicate that, but we still have data needs as far as the skin lightening adverse effect. And we can await industry's response that by April, we'll know whether a study has been devised and proceeding. So, that's sort of different. I'd have to withdraw the table motion if we wanted to move onto -- continue insufficient.

DR. BERGFELD: Your table motion was not seconded, though. We're now reconsidering tabling. Are you seconding or are you commenting?

DR. BELSITO: Commenting.

DR. BERGFELD: Thank you.

DR. BELSITO: Again, we don't -- I mean, what we were told yesterday is that our request for the studies to look at skin lightening will be brought back to industry. There was no definite commitment on the part of industry to do it at this point. We don't know. And I think with this additional data need I would prefer that we go ahead as insufficient, and if industry comes up in April and says, yes, we're going to do this study, we can agree to table it. If they come up and say, no, we're not going to do it, well, we're done with the report. We've already issued our insufficient, we can move on, we don't need to look at it again.

So, that's the reason why now that there's this other data need that we clearly have that I would like to go insufficient.

DR. BERGFELD: John Bailey?

DR. BAILEY: As long as we understand that, again, the -- if data or a strong commitment to provide data is provided, that the panel will take that and do deliberation and, you know, either continue the insufficient data status or move it back to tabled or whatever is appropriate, I just don't want a hammer to come down in 60 days and say that no more deliberation. Let's put that on the record.

DR. BERGFELD: Well, the consideration right now is either to table or to go out as a tentative final insufficient, not to retain the status quo of insufficient data announcD

R. BAILEY: I think there is a high interest in the agreed and I would not call it a promise, but I think that there's sufficient interest that we can -- you know, we will have a commitment to provide the information. I don't want to say something I can't necessarily follow through on.

DR. BERGFELD: So, there is a motion to table. Is there a second? Is there another motion?

DR. BELSITO: The motion would be insufficient for sensitization, irritation at concentration of use, and for a dose at which there is no skin lightening effect.

And in terms of your prior question about thyroid peroxidase, we deleted that from our list of requests.

DR. BERGFELD: So, there's a motion to go forward -- yes, John?

DR. BAILEY: Could we word that a little differently? I would say not to provide information on a no-dose effect, but to study the skin lightening effect, present information on skin lightening effects of kojic acid.

DR. BERGFELD: Is that good?

DR. BELSITO: Yeah, I mean, actually what we want is pick a concentration, the highest one industry wants to use in a cosmetic product, and show us that it doesn't cause skin lightening. We don't need a dose response effect to see at what point you get skin lightening. We just want proof that there's no skin lightening at a concentration that industry wants to use this chemical.

DR. BERGFELD: So, that's a motion to go forward with the tentative final insufficient. Is there a second?

DR. SLAGA: Second.

DR. BERGFELD: A second. Any further discussion? Seeing none, call the question -- yes?

MS. BURNETT: On the discussion yesterday of the thyroid tumor effects, the teams had different positions and I just want to have that clarified for my own purposes. Dr. Marks' team would like to keep the Hill document -- the EPA, FDA information, and also incorporate the SCCP's position. Dr. Belsito's Team said to remove that citation from Hill and to use different sources. So, I just want to make sure I go the right way.

DR. BERGFELD: I think that we can handle this after the motion as a discussant point if you don't mind. So, I'm going to call for the question, all those in favor of the motion to move forward as an insufficient data -- tentative report insufficient.

All right, so it's unanimous now. Christina has asked how to deal with the thyroid abnormalities. Two opposing thoughts from each team. So, Paul, you're making a motion or a movement to speak?

DR. SNYDER: I think that either -- I think there's a common ground between the two. I think we need to see the next iteration of how you draft the wording. I think that we're not concerned about the thyroid lesions, and I provided you with a reference yesterday on a mechanistic publication on that in rodents. And so I think that once both teams see that and see the language that you would craft from that to deal with that, and why we're not concerned with that, I think we can then make a determination as to what language you want to keep and what language you want to remove.

Curt, I guess, might speak to it, but he was a little bit more adamant about removing the Hill language. I was more in favor of removing the Hill language and adding some additional language that would justify our reasoning behind why we're not concerned.

DR. BERGFELD: Ron Shank?

DR. SHANK: Well, I think this is what Christina is asking for, how does she write it up in the next iteration? Does she leave in the Hill and add more or does she take the Hill material out? And I'm reluctant to just delete the data because we think it doesn't fit.

DR. BELSITO: It's not data, it's a review.

DR. SHANK: Review.

DR. BERGFELD: Curt?

DR. KLAASSEN: Yeah, I mean, I think, as long as it's all put in perspective, you know, I have no real problem of lumping it in. The way it is, it's not balanced. It is his opinion. The overall opinion of people that are doing research in the area is quite contrary to his opinion, but -- so, I think -- I have no trouble of leaving, you know, his reference in there, but it needs to be clear that it's his opinion that's been published. There's really no data behind his opinion, and so it just needs to be word smithed and put all in perspective and I think it will be okay.

DR. BERGFELD: We can work with it.

DR. KLAASSEN: And if not, we'll modify it the next time around.

DR. BERGFELD: Curt, would you agree to work with Christina on that --

DR. KLAASSEN: Sure.

DR. BERGFELD: -- prior to the meeting? And is there a second reference that would balance his opinion?

DR. KLAASSEN: Yes, we gave her --

DR. BERGFELD: Do we have that? Good.

DR. KLAASSEN: -- that yesterday.

DR. BERGFELD: Okay. All right, is there anything else that we should discuss around this? Nothing. Okay, we'll move on to the next ingredient. Thank you for bringing that up, by the way.

MARCH 2010 MEETING – THIRD REVIEW/DRAFT FINAL REPORT

Belsito Team – April 5, 2010

DR. BELSITO: So kojic acid. This is the Blue. Okay, so we got a repeat insult patch test on a product containing 2 percent kojic acid. And 230 subjects, 218 completed the study. There were six less to follow up, five voluntary withdrawals, and one critical deviation in that the subject was over 70 years of age. And there was anyway no evidence of sensitization in this study on 2 percent kojic acid. Otherwise --

DR. EISENMANN: Did you see the study on --

DR. BELSITO: Pardon?

DR. EISENMANN: The guinea pig. The black guinea pig study.

DR. BELSITO: Right. Yeah. So in December we went with an insufficient -- we asked for dermal sensitization, irritation, and concentration of use repeat insult patch testing. So we have that now at 2 percent. We didn't see any irritation or significant sensitization. We asked for a dose response for skin lightening sufficient to demonstrate a threshold. And in the second wave of material posted there was a guinea pig study on lightening. Right? A black guinea pig study. And then there was some irritation (inaudible) rabbit studies and a further toxicity study at.6 percent. Is that all correct? And then in the guinea pig -- black guinea pig study there was no lightening at 1 or 4 percent. And I guess, and I really don't know, is whether -- I mean, is that black guinea pig -- is that the way to look at this or not? I mean, was there a causative control? And the answer was I didn't see one.

DR. EISENMANN: Well, the other different type of compounds. I mean, they did see --

DR. SNYDER: Phenyl hydroquinone.

DR. EISENMANN: Right. They did see lightening with a different type of compound.

MS. BURNETT: Yeah. It wasn't the primary ingredient or chemical that was being reviewed. It was reviewed in addition to hydrophenol and arbutin, and a few others.

DR. LIEBLER: I wasn't sure how to interpret this study because of two things. One is I don't know how relevant this model is compared to human skin lightening.

DR. BELSITO: Right.

DR. LIEBLER: And second, I don't think that the information presented about the kojic acid really allows us to establish a threshold.

DR. BELSITO: Threshold.

DR. LIEBLER: Essentially reports are negative data for kojic acid. I think two concentrations used. But I think taking a threshold you've got to have a positive effect and a negative effect of two different doses at minimum.

DR. BELSITO: Well, unless it's used below 4 percent. Or 2 percent.

DR. EISENMANN: Well, they'd be satisfied with the 1 percent on this.

DR. BELSITO: Right. And the information that we have in terms of concentration of use is the highest is at 2 percent in a face and neck cream. So, while we didn't get a dose response, we got -- I mean, if you accept the black guinea pig model, we have data suggesting that neither 2 nor 4 percent induced any kind of pigmentary change. So at that point do we really need a dose response? We have a level two times higher than what's reported to be used in cosmetics, not altering pigmentation. We have a sensitization and irritation study at 2 percent that's negative, and those were the data points. I mean, essentially that's what we were asking for.

I guess the issue I have, again, is whether you know, I'm not enough of a pigment biologist and of dermatology to know whether that black guinea pig model is the right way of looking for chemical depigmentation. In other words, when people study hydroquinone, what models did they use to look at that? Because we are faced with a lot of data. You know, on pages 44 and 45, they talk to us about the effectiveness, for instance, of a gel containing 2 percent kojic acid and I think, granted, it also continued hydroquinone. But then another study, Garcia and Fulton, compared the effectiveness of kojic acid and hydroquinone. There was at least one study. I think that was a combination where kojic acid is used to treat melasma.

DR. SNYDER: I thought (inaudible) at the last paragraph before it goes to summary.

SPEAKER: Pregano, Pregnano.

DR. BELSITO: Yeah. Yeah, the treatment is widely used in Asia. I mean, and it is. I mean, did we get any information from -- I mean, how does Japan regulate this? Because they promote it very heavily as a -- to improve --

DR. EISENMANN: It's my understanding it's 1 percent in quasi-drugs.

DR. BELSITO: In quasi-drugs. And how does Japan define a quasi-drug? Do they need to look at safety and efficacy? Do they just need to look at safety? I mean, what kind of data do they require?

DR. BAILEY: The quasi-drugs are sort of a funny category because it includes hair dyes. In the quasi-drug category. So I think they look at safety and also effectiveness, but not effectiveness necessarily in a medicinal way. More, does it perform? So I think it's a little different than, you know, like we have with drugs and OTC drugs and cosmetics, where in Europe where -- when there are so many OTC drugs regulated as cosmetics, you know.

DR. BELSITO: I guess --

DR. BAILEY: It's an odd -- it's a little bit of an odd (inaudible).

DR. BELSITO: You know, we clearly have the information we asked for. And at face value the information we asked for is negative. However, before going out with a final safe as used up to 2 percent, I would like to at least get some comments of an independent pigment biologist as to the appropriateness of this guinea pig study. I mean, I'm just not comfortable saying that that is an adequate study, particularly given all the other claims that may just be snake oil claims on kojic acid 1 percent and higher improving depigmentation.

So, and I guess that would be my comment. I would like to table it for a little bit more thought and maybe if, you know, ask the other team or anyone who can identify a pigment biologist. Wilma may know some people or I could, you know, do some research, you know, as to whom might be the appropriate person to throw this report at and just get a comment back from them. That's my thought. But I think in terms of sensitization and irritation your HRIPT took care of that, so I think that data need goes away. And temporarily the data need for a dose response for a skin lightening effect go away since we have 2 and 4 percent. The question is, is that an adequate model?

DR. KLAASSEN: Yeah, I'm not an expert in that area either. However, it seems like it probably is because, you know, working through the tyrosines, you know, I would guess it's probably a bit of a model of anything that we do in toxicology, but that's only from having read this, not from being an expert.

DR. BAILEY: We do have as a control hydroquinone, right?

DR. EISENMANN: Well, not in this paper (inaudible) that they actually saw --

DR. BAILEY: It's the quinone structure that's --

DR. EISENMANN: They work by different mechanisms.

DR. BAILEY: -- (inaudible) those two. If you look at the structure of kojic acid I think you're also going to see probably something quinone-like in that, too. I mean, it's a -- I mean, you could envision that this would be a quinone.

DR. LIEBLER: It's an enolate.

DR. BAILEY: Yeah.

DR. LIEBLER: It, you know, it can inhibit tyrosinase. It can inhibit peroxidase chemistry as I believe that's elsewhere in the background. Is that right? Is that this compound or is it something else I'm thinking of?

DR. SNYDER: I think you're right.

DR. LIEBLER: It wasn't thyroid. Was it this one (inaudible)?

SPEAKERS: Yeah.

DR. SNYDER: Okay. So we've been down that road, yeah.

SPEAKER: Yep, yep.

DR. SNYDER: So it is able to inhibit peroxidase chemistry.

DR. LIEBLER: Okay.

DR. SNYDER: Okay. And part of the mechanism for inhibiting pigment -- my understanding is it could inhibit peroxidase chemistry involved in making dopa polymers, which are the melanins. And it also could affect the packaging of the pigment because once this pigment is made it's packaged and extruded into outside the cells. Outside the melanocytes. Right? And that's the way the pigment is packaged in large part it accounts for differences in skin (inaudible) at least. So I just don't know how much of that part of the biology in the guinea pig model translates to humans.

SPEAKER: Right.

DR. SNYDER: And so I don't know if the effect that was observed in the black guinea pig model is because of peroxidase inhibition or tyrosinase inhabitation to be more specific. Or if it has to do with the way the melanin is packaged and distributed in the skin. And that's where somebody who works -- who is familiar with this model might be able to provide some clarification.

DR. BAILEY: I mean, I think we do have what appears to be -- and I agree with the model part. I'm not knowledgeable in that area either, but we do have, you know, an apparent lack of effect versus a control. And I think as Carol said that, you know, I think the industry would be comfortable going down to 1 percent rather than to the 2 if that provides a greater margin of, you know, safety or effect. So I would take those into account, too. I don't know who's, you know, we'd have to go look and see who's the expert in this area.

DR. LIEBLER: It's possible we locate an expert and they come back and tell us, well, the effect of these compounds could be at both steps. And it's not possible for us to say that this model is not a reflection of human pigment biology or does not adequately reflect human pigment biology. And then where are we?

DR. BAILEY: Well, that's the other thing. I'm not sure what models, you know, what the array of models --

DR. BELSITO: Well, then I think we're back where we are, you know, and that is insufficient and we need the right model. So I think that's what we need to know. And in terms of reducing it to 1 percent and then we have no rationale for doing that, I mean, basically, you know, we're told it's used up to 2 percent. We have HRIPT at 2 percent that's negative. And then the second concern we had was pigmentary change and we have a study -- we either accept the model and say 1 percent and 4 percent are negative and, therefore, 2 percent is fine. Or we don't accept the model and say, you know, we need additional data in this model. And that's what I'm not comfortable going there. Certainly, hydroquinone, I mean, you saw some depigmenting effect. The drugs were applied or the chemicals were applied six days a week for five weeks. Maybe that's sufficient for hydroquinone and not for kojic acid. I just don't, you know, it's not my area of expertise. So since it's been a hang up since there are all these claims out there, I would just feel more comfortable having someone whose area of expertise it was saying, yeah, this is a good study and you can feel comfortable going ahead with it.

DR. BAILEY: So you're recommending to table it?

DR. BELSITO: I would like to table it and just have someone ask -- see if we can identify an independent individual to review this study and give us a one page response back as to adequacy.

DR. LIEBLER: I think that's a good idea.

DR. EISENMANN: Any suggestions on who?

DR. BELSITO: I don't know yet. I mean, I could make some suggestions, you know, but let's see if Wilma or Jim or someone else knows of someone in particular because I don't have any personal friends that I could suggest or call. I would make the suggestion by going back to the literature and see who's doing work in dermatology on pigmentary changes and that I know is a reputable person.

DR. BAILEY: I'm not sure what models have been used by the FDA either or were presented to FDA in the study about the quinones.

DR. BELSITO: Yeah. I mean, that would be interesting to know, too. If a black guinea pig was used for those then --

DR. BAILEY: And we can explore that.

DR. BELSITO: Yeah. If you could do that, that would be great.

DR. BAILEY: Okay.

DR. BELSITO: So we're going to table this and at least look at models for depigmentation and perhaps get an expert who can review that black guinea pig study and get back to us. But at this point we're not asking for any other additional data.

DR. BAILEY: Yeah, Tom Re of L'Oreal has been instrumental in looking at this. I don't know where Tom is right now. He's (inaudible), but perhaps he could (inaudible).

DR. BELSITO: (inaudible) tomorrow.

DR. BAILEY: So we can see if he has any thoughts also.

DR. BELSITO: Sure.

DR. SNYDER: Lunch?

DR. BELSITO: It's lunch. It's 5 after 12:00.

DR. SNYDER: We have some more stuff to discuss on this.

DR. BELSITO: Okay. So we're going to table and we'll come back from lunch and we'll do comments. Okay.

(LUNCH BREAK)

DR. BELSITO: Start with those.

DR. SNYDER: So, first of all, on page 7 of the kojic acid report, Blue No. 1. In many of these reports, the writers are using this terminology as it -- is it here in this report -- to a significant acute oral toxicant in mice or rats, and so are you okay with that terminology? Okay.

DR. LIEBLER: I flagged the same thing.

DR. SNYDER: Okay. So, I mean, because to me, one -- even at 1 gram per kilogram, that's not considered insignificant, so it's --

DR. LIEBLER: It just sounds -- it says, kojic acid is not a significant acute toxicant, blah, blah, blah, in all dose groups, clinical signs included -- and then there's a list of toxicity. So, I think it might be better to say it's not a potent toxicant.

DR. SNYDER: Well, that's why I'm going to current -- because what is the current thought in tox in regards to saying something is a mild, minimal, or moderate, or what is -- could we give them some guidance on that?

DR. KLAASSEN: That's used less and less. Yeah, I would, I think potent would be a -- it may be a better word to put in there.

DR. SNYDER: Okay, so if maybe you could relay this to the rest of the writers too because it's kind of a common --

MS. BURNETT: Okay.

DR. KLAASSEN: That's kind of old terminology.

DR. BELSITO: Well, instead of trying to classify it into potent, mild, moderate, minimal, if there aren't accepted guidelines can't we just say, kojic acid had LD-50 values greater than 1 gram per kilograms? State what we have, not interpret it?

DR. SNYDER: That's kind of what I wrote, I said, what it is, not what it isn't.

DR. BELSITO: Right.

DR. SNYDER: So, kind of in scientific writing you're better off to say what it is, not what it isn't.

DR. BELSITO: The acute oral LD-50 values of kojic acid --

DR. SNYDER: -- are in the 1 gram per kilogram range.

MS. BURNETT: Okay, can do that.

DR. SNYDER: And then on page --

DR. BELSITO: Or are greater than 1 gram per kilogram.

DR. SNYDER: One gram or greater.

MS. BURNETT: Okay.

DR. SNYDER: On page 13, the top paragraph there seems like it got lost, it's out of place, it says genotox but yet it gives LD-50s and it's under short-term dermal, so I don't know what happened there. It's a stand-alone paragraph that appears -- I don't know where that comes -- it's out of place.

DR. BELSITO: So, it should be in (inaudible)?

DR. SNYDER: Well, it's a genotox.

MS. BURNETT: I think --

DR. BAILEY: First two lines.

DR. SNYDER: Yeah.

MS. BURNETT: Yeah, I see that. My guess is --

DR. BELSITO: For five days. I mean it's acute oral.

MS. BURNETT: But I think it's because they did give LD-50 values, I probably put it both in the genotox section and here.

DR. SNYDER: Uh-huh. But it's under short-term dermal. That's the issue.

MS. BURNETT: I'll check to see where it's supposed to go. It might have gotten moved during editing.

DR. BELSITO: So, would five days be considered a short term oral or an acute oral?

DR. SNYDER: It's probably an acute oral, is what it is, because they've got LD-50 so that's probably where it goes, but does it have a genotoxic component?

MS. BURNETT: Let me see --

DR. BELSITO: Oh, not it says (inaudible) genotox, so --

MS. BURNETT: Let me check to see -- 52, 53 -- yeah, it's cited again under bacterial (inaudible)?

DR. BELSITO: It can't be bacterial, it was in vivo.

MS. BURNETT: It was probably -- there was a cerebral study that they did multiple things and --

DR. SNYDER: But it's not in its place where it's currently at, so we just need --

MS. BURNETT: Okay, I'll look at it.

DR. SNYDER: If it's already in the acute oral just strike it here.

MS. BURNETT: Okay.

DR. SNYDER: And then on page --

MS. BURNETT: Yeah, it was a dominant lethal study. So, it's also --

DR. BELSITO: Okay, so it's in genotox and is it already under acute oral?

MS. BURNETT: No, so I'll move it --

DR. SNYDER: Appropriately.

MS. BURNETT: Yeah.

DR. SNYDER: And then on page 18, this goes to something I mentioned to Bart earlier, I don't know if you were here or not, but in these italicized sections where we're going for brevity and hitting the high points, at the top of page 18 the sentence, "It appeared that the exposure of male deformed mating resulted in adverse effects," But the reproductive studies were in multiple species so I think we really need to -- if it's -- we've got to talk about specifically which species we're talking about.

MS. BURNETT: Okay.

DR. SNYDER: And make sure that we -- on male what? Rather than just saying "in males," particularly if there's more than one species is used.

MS. BURNETT: Okay.

DR. SNYDER: Then on page 24, first complete sentence where it starts, "Erratic toxic effects were observed in the first experiment. Results for treatment with S9 only reported in both experiments toxic effects." So, can we -- was it decreased viability, cytotoxicity? If we can be more specific there because I don't know what erratic toxic effects means as opposed to just toxic effects. So, if it was cytotoxicity that contributed to the inconsistency of the results, we probably ought to -- and then a question in regards to, so the new journal format, they want you to put the references right after -- if you mention the author's name as opposed to at the end of the sentence? Just in a general -- is that the new format for the journal?

MS. BURNETT: I think we're still working that out. I'll ask Kevin. You would prefer it at the end of the sentence or after?

DR. SNYDER: Well, it's just, when you were like on page 30, if you go to page 30, right above carcinogenicity the second paragraph, under the Higa --

MS. BURNETT: Higa.

DR. SNYDER: -- et al., is 8686, so that's not right, but --

MS. BURNETT: Did it show up like that in yours?

DR. BELSITO: Mm-hmm. It's 8686. Higa, et al., 8686.

DR. SNYDER: So, when you mention the author's name you put the reference, superscript, right there, as opposed to all other instances you put the reference at the end of the article.

MS. BURNETT: Okay.

DR. SNYDER: So, I'm just asking --

MS. BURNETT: Consistency will --

DR. SNYDER: Well, I'm just asking. It seems kind of odd to do it that way, but --

DR. BELSITO: It probably is a carryover from putting the authors and then in parentheses putting the year of publication. It should go at the end of the statement.

MS. BURNETT: Right.

DR. BELSITO: And then on page 37, I think you did a really good job of capturing that information I sent you about the thyroid effects. And so I just think we need to -- we need to clean up that italicized part there because there was no progression of lesions, the effects were reversible -- so there's lots of -- there's some other data -- there's some other information that you can put in there, (inaudible) put it in here regarding --

MS. BURNETT: Okay.

DR. SNYDER: You captured it -- the discussion section was really good in capturing those -- page 43 was really good --

DR. BELSITO: And you editorialized it?

DR. SNYDER: Yeah, yeah.

DR. BELSITO: (inaudible)

DR. SNYDER: So just so (inaudible). That's why.

MS. BURNETT: Okay. I will find it. All right, thank you.

DR. KLAASSEN: So, this is the kind of style that everybody's going to?

DR. SNYDER: Yeah.

DR. KLAASSEN: I like it.

DR. BELSITO: I think it's so much easier not to review all the old data, just to see it summarized and referenced as (inaudible).

DR. SNYDER: (inaudible) you can go look it up.

DR. BELSITO: Right.

DR. EISENMANN: We'll have to delete Table 4?

MS. BURNETT: We will delete Table 4. It was -- mainly how this came about is that during the last iteration I received data -- information saying that the VCRP reporting went down 10 ingredients which seemed odd because we've been hearing that from the people talking that it's being used in more things, so we checked Environmental Working Group's website and, boom, they had a whole bunch of -- and they're listing it as skin lighteners.

So, we just -- to illustrate that the VCRP data may not be complete, we decided to include what the EWG included and then we also contacted Health Canada to see what -- you know, what's going on in Canada and that's where Table 5 came from. So, we were never planning on leaving the EWG table in. We'd still like to leave the little blurb in the text in there.

DR. BELSITO: What page does that blurb in the text occur?

MS. BURNETT: Two. Page 2. It's highlighted.

DR. BAILEY: On Table 5, this is from Health Canada and so for a concentration range shown, they reported that the number of products that claim to have kojic acid in them at those levels -- I find it a bit hard to --

DR. LIEBLER: Ten to 30 percent?

DR. BAILEY: Yeah. I just -- I just don't see that. I mean, I -- you know, it just doesn't make sense based on what we know about its use from -- you know, from 5 percent -- I mean, that's more than a lot, it's like --

DR. LIEBLER: Tenfold higher.

DR. BAILEY: Yeah, so I'm just -- I don't know. I'm not sure how much this helps.

DR. BELSITO: Well, but it's --

DR. BAILEY: No, I understand it's the information that's out there and it's certainly worth talking about -- being aware of.

DR. KLAASSEN: You are or are not going to put this in the file --

DR. BELSITO: The Health Canada?

DR. KLAASSEN: Yeah.

DR. BELSITO: We said we're going to keep it.

MS. BURNETT: It might not be a table. It might go to just prose.

DR. BELSITO: I don't know. I would keep it in the table.

MS. BURNETT: Okay.

DR. BELSITO: I mean, the fact remains that we're told it's up to 2 percent, in Canada they have 17 -- at least 17 products that are reported to be -- have higher uses, and so I would -- yeah, I mean, this is -- I mean, it's reliable government data. I'm not sure -- right? I mean, Health Canada is like the FDA, right?

DR. SNYDER: So, it's mandatory, right?

MS. BURNETT: They have mandatory reporting.

DR. BELSITO: So, I don't know if they go out and test the concentrations but this is adequate government data that's -- you know, for what's available, very close to our market. So, I mean, I think it would -- you know, if we -- if we decide to go ahead with a "safe as used" up to 2 percent, you know, then I think it would bear a mention in our discussion that at least data that we receive from Health Canada indicated uses above this level and the panel has found -- would find that those uses would be -- there would be insufficient data to support those uses.

DR. BAILEY: Well, what I'm wondering is, and I don't know, but I'm wondering if these numbers are concentrations of a preparation. In other words, 30 percent of a material that contains 5 percent, you know, kojic acid that's added. I mean, the same sort of issue we were running into earlier about what's being studied, so, I mean, I just -- yeah, and actually, I will inquire the Health Canada about this. But I just have a hard time believing 10 to 30 percent. I'm wondering if this isn't some kind of formulation that's then added to --

DR. KATZ: Do you know if it's in fact a cosmetic and not a drug? If this is truly cosmetic use or if it's drug use?

MS. BURNETT: We asked -- as cosmetic, and that's what their response was. Like --

DR. KATZ: Because you might need to double check because they have a -- their OTC system is not that dissimilar to our OTC system and these could be OTC drugs. So, depending upon how you actually phrased the question, you might have asked over-the-counter.

MS. BURNETT: I'm pretty sure I asked, as cosmetic ingredients. It's maybe how they -- what they consider to be a cosmetic versus a pharmaceutical.

DR. KATZ: That's right.

MS. BURNETT: And you don't know.

DR. KATZ: And some of their products that would be here OTC drugs are now -- they're developing a third tier on some ingredients and some products and that might fall into their third tier. So, you might need to double check just to see how they're truly regulated.

DR. BAILEY: I'll send an e-mail to Louisa to see what says.

DR. BELSITO: Do you have connections there, John?

DR. BAILEY: Mm-hmm.

DR. BELSITO: Yeah, so I mean, just clarify it for us. I mean, one way or the other I'd like to include the report and if, in fact, there are uses above 2 percent, then it doesn't change what our conclusions would be, it just might change a little bit the discussion in terms of what we're aware of being out there in the1 market outside of the U.S.

DR. BAILEY: Okay. I'll see what I can find out.

DR. SNYDER: That's it.

DR. BELSITO: Okay, so obviously in the summary we need to add that new data that came through in the HRIPT, the 3 percent guinea pig. And basically we're tabling this and we're asking for some outside expert opinion and it's worth getting the guinea pig study. Okay.

DR. KLAASSEN: And you know, it -- we might find out tomorrow that Slaga knows that this is an excellent method.

DR. BELSITO: Yeah, I mean --

DR. KLAASSEN: He is a skin person (inaudible).

DR. BELSITO: We'll change what we sent based upon what they (inaudible).

Marks Team – April 5, 2010

DR. MARKS: So, in December the Panel issued a tentative safety assessment for kojic acid with an insufficient data conclusion, and the data needs per the memo from Christina were the dermal sensitization and irritation at use concentrations and then also a dose response for skin- lightening effects since that's a prominent effect of kojic acid from the literature and also a prominent concern we had since it's obviously not a cosmetic effect that we're -- it would be a drug effect.

MS. BURNETT: Dr. Marks, to let the team know, this morning you received another piece of information. It should say "TKO Research 2007, Another HRIPT Study."

DR. MARKS: Right. This is the April 1st one where they repeat insult patch tests using 2 percent kojic acid. It revealed no sensitization. So, we had actually that piece this morning and a previous one, which was the HRIPT that Alan mentioned, which was given out on the second day of the last meeting in December, which 1 percent was okay from an irritation and sensitization standpoint, so I think that data need is met. Is that correct?

DR. SHANK: I would think so. In an RIPT test, if you have a lot of people in that test with pigmented skin, would that test detect a skin lightener, depigmenter?

DR. BERGFELD: A shade --

DR. SHANK: Would one notice -- when one 1 does one of those RIPT tests, if the agent – if the ingredient -- if a test chemical were a skin lightener, would that show up if some of the patients had pigmented skin?

DR. MARKS: I don't think there -- first of all, I don't think there's any standard assay for that to begin with. We have a second piece of information in the data using a black guinea pig, but again the question is: Is that assay really relevant or acceptable to determine the safety of kojic acid? So, I would say no. It's a short-term -- the HRIPT is a short-term testing. Sometimes a depigmentation of skin after application of a chemical is delayed, and so I wouldn't feel that just a routine HRIPT and, oh, by the way, was lightening noticed during that period, would be an adequate screen.

DR. BERGFELD: May I comment? I think that if the patient in this situation had a whitening effect where the skin actually turned white, like we see with the hydroquinones if we use them, or some of the other ones, then you would say that. But just slight lightening you probably would not detect that nor would you be stating it as a side remark. And as I recall, reading some of the pigmentation studies, this kojic acid is really a minimal whitener, and there's no melanocyte damage, oxidative damage, so I would sense that the patient or individual's complexion would look fresher, however that's interpreted, but that the patient does say "fresher," but they would not detect whitening -- white, white.

DR. MARKS: Are there any comments from our visitors? Because we are going to be issuing, I think, an insufficient data conclusion if we can't resolve the lightening issue.

DR. ANSELL: Well, I would like to point out that the data we provided demonstrates the material is not a skin whitener. Rather, it interferes with the darkening process. And that the study from Ishima goes to the mechanism of the prevention in terms of interfering with tyrosinase activity in a reversible way. So, it's not a skin lightener; it prevents UV-induced darkening.

DR. BERGFELD: That's the study that shows in vitro tyrosinase inhibition you're referring to, Jay.

DR. SLAGA: So, it doesn't take normal skin and make it light, hmm?

DR. ANSELL: Yeah, the study indicates that it induces a reversible decrease in tyrosinase activity and that this is reversible

and that the cells recover this activity and the ability to form melanin after treatment is stopped, so what it's doing is reducing UV-induced hyperpigmentation.

DR. SLAGA: But the question, if you take normal skin without UV exposure, does it make the skin get lighter?

DR. BERGFELD: Yeah, well, the study showed that it got a little lighter but not significantly, and the tyrosinase activity inhibition would be true whether it be sun exposure or non-sun exposure, because that's an active enzyme system. But if the question is, is it enough lightening to restrict it in any way and, yes, there are no studies looking at doses, you know?

DR. SLAGA: Where are we on the dose response, the Japanese study that's being translated?

MS. BURNETT: You received it in the second wave of information. This one.

DR. SHANK: In that paper, with the black guinea pig study, the authors referred to two other papers also in Japanese that kojic acid is a skin lightener, but those papers are in Japanese. Can we get those? There was also -- in December there was an offer for -- a statement said that perhaps there would be a skin-lightening study done. Where are we on that?

DR. ANDERSEN: I don't think we've heard anything further about additional studies. I guess the question, though, comes back to the point that Jay made. In looking at the Tayama thesis work that was published in '02, there's a clear differentiation between the effects of hydroquinone and kojic acid. There's a different mechanism involved. Kojic acid, no decreased epidermal melanocytes, no decreased melanin granules that you see with hydroquinone; and those effects, I think, are what have classically led the Panel to say we don't want that level of activity in cosmetics. Kojic acid is acting differently, so maybe the question of the search for a level that causes no skin lightening is not hugely relevant anymore. It's more for Jim and Wilma from the --

DR. BERGFELD: I'd like to add you can get lightening by taking the stratum corneum off, so there are other ingredients or chemicals that we've dealt with that do a little bit of stripping and hydrating. So, I'm not so sure it doesn't fall into that sort of low-level category rather than something that's cytotoxic, which is a more dynamic biological activity.

DR. SHANK: Well, other than the cytotoxicity issue, is there -- I don't know how you'd classify the issue. Some people may not want the skin-lightening effect. So, should it be labeled that --

DR. BERGFELD: May have skin lightening --

DR. SHANK: -- it could lighten the skin. It would not be a toxicity issue; it would be a --

DR. BERGFELD: Cosmetic.

DR. SHANK: -- cosmetic issue.

DR. BERGFELD: That's a good idea.

DR. HILL: So, does that constitute something like a slight of hand in the sense that we don't have the FDA person here to comment, but it can't be labeled as a skin lightener else it's a drug rather than a cosmetic, yes?

DR. MARKS: Correct.

DR. HILL: But if it were labeled "This preparation may lighten the skin as a side effect," that seems to be almost --

DR. ANDERSEN: Well, I think it's going to depend --

DR. HILL: -- a backdoor claim?

DR. ANDERSEN: -- on the nature of the claim. If the claim is couched in appearance terms, then the ingredient can be a cosmetic. If the product as I saw time after time after time at the Academy of Dermatology meeting, those folks using 4 percent or so of kojic acid in products that appeared to be over-the-counter drugs were making a clear skin- lightening claim, not an appearance of skin lightening, but they didn't seem to have any question that they were drugs. And we're not talking about those products here; we're talking about the 2 percent kojic acid that has a claim that can be considered a cosmetic claim.

DR. MARKS: I think it can be handled like any other side effect in terms of if you look at the Japanese experience. In the references, 1 percent is used in Asia, and lightening may be the intended use or it could be viewed as a side effect just as allergic contact dermatitis has occurred with that concentration in Japan, even though we have now HRIPT indicating that 2 percent is a safe level for kojic acid, that it could it be handled as a side effect, so to speak, but I wouldn't want to see it as a claim. I guess the other comment when I go on page 45 of the report -- the one reference, though -- the last paragraph right above the summary, I think that reference is a secondary reference that would have been nice again that, "A side effect of this treatment is contact dermatitis. This treatment is widely used in Asia." It's Draelos. I wonder whether or not there was some -- again, getting back to Ron's points on primary Japanese references, again, to attest to the safety. To me, I guess if you were going to -- we had a 2 percent level of safety with sensitization. It's used up to 2 percent in the reports we have yet. For skin-lightening effect, at least using the black guinea pig, we have 1 percent is the safe level, so we may end up having to put a 1 percent just based on skin lightening.

DR. HILL: My recollection was that's why the dose responses curve was solicited. It just seems to me nobody -- okay, contact dermatitis is a side effect, but nobody's going to use it to cause contact dermatitis, okay, but name somebody who goes and purposely finds something with kojic acid in it. They're probably seeking the skin anti-darkening activity. And so if you put that on there and you label it as a side effect, it's -- I don't see the distinction really. I mean, and a level, I don't care, but --

DR. MARKS: Tom, you had a comment?

DR. RE: Yeah. Just one. There's only one major supplier --

DR. ANDERSEN: We'll have to use the mike.

DR. RE: Just one point. There's only one major supplier of cosmetic grade kojic acid, and that supplier does not recommend it being used over 1 percent. Two percent has been used, and the RIPT 2 percent is evidence that it has been used, but they don't recommend it above 1 percent. In fact, they have practically said that 2 percent is really a maximum level.

DR. MARKS: And the reason, Tom?

DR. RE: The reason for 1 percent?

DR. MARKS: Yes.

DR. RE: It's based basically on the guinea pig study where levels above 1 percent caused depigmentation, where a level of 1 percent there was no cytotoxicity and there's no active activity against melanocytes.

DR. MARKS: And I could go back and look at this study again, but did they follow these guinea pigs out, a la Jay, what you were saying, this is a reversible hypopigmentation based on inhibition of tyrosinase? Did those guinea pigs actually repigment with the high doses?

DR. SHANK: They didn't look at that.

DR. ANSELL: Right, the study was the one that I'm looking at, looked at the reversibility of the enzyme activity.

DR. MARKS: Right.

DR. ANSELL: Not the skin color per se. But, again, this study was looking at the reduction of hypopigmentation, not skin whitening, and specifically solar and senile lentigo.

DR. MARKS: Yeah, I think I would feel -- it's interesting, Tom, I was thinking 1 percent would be a safe level based on the guinea pig data that I think I could feel comfortable with that. I wouldn't go up to 2 percent even though we have sensitization data that would recommend that, so I think in my mind it's either insufficient versus limit to 1 percent.

DR. ANSELL: Well, we could support 1 percent. I mean, that seems quite reasonable.

DR. MARKS: Two Rons and Tom.

DR. SHANK: And that 1 percent is based on this Japanese paper that was just translated, is that correct?

DR. MARKS: Yes. Is that going to be enough safety data from your point of view, Ron? And I think a la what Alan said earlier, it would appear that its proliferation in the cosmetic dermatology world, that if there were really significant and prolonged depigmentation, which would be what we would be most concerned about, that that, I would have thought, would have appeared by now and also from the Japanese literature. So, I think experience would also support setting a level of 1 percent.

DR. SLAGA: I think that's a good compromise.

DR. BERGFELD: I'd like to make a comment about the comment you just made about we hadn't heard of any sort of liginous skin being developed from kojic acid. However, it is true on the West Coast at least that kojic acid is on the increased use by the dermatologists for its bleaching effect and lightening effect. So, whether they're using 4 percent or 2 percent, I'm unclear, but certainly they're using it, and they're using it as an OTC or a cosmetic ingredient. It's not a drug.

DR. ANSELL: Well, if they're using it for that purpose, it is a drug, and so –

DR. BERGFELD: I know that, but they're obtaining it without a prescription basically.

DR. MARKS: Well, I guess when we decide on our final report if we do set a limit, then it'll be incumbent on the manufacturers to take that into consideration. So, I'd probably -- not probably -- I would recommend we come to a conclusion with a final report. Actually, I guess since we're setting a limit, and we haven't done that, it would be actually a tentative final report with a 1 percent limit.

DR. HILL: Just glancing back over some, I think the most pertinent question here that was asked is: Is the skin lightening or anti-darkening effect reversible? I mean, I would guess it should be fully reversible based on the mechanism and action.

DR. MARKS: Correct.

DR. HILL: Because I get some sense here, glancing back at a couple of things, that the manner of use -- in other words, how long it's used and how often -- might have a big impact here on the safety of it. In other words, maybe using 2 percent over a short period of time is perfectly safe and fine; using 1 percent over a long period time I guess is probably okay, but it looks like there may be some issues in terms of sensitization, or at least contact allergy, rather. So, I'm wondering if it is -- I know you're saying not recommended above 1 percent, but is that recommendation based on I'd rather not be sued so this is my recommendation and really it's perfectly fine to use 2 percent, and then are we keeping things off the market that don't need to be kept off the market that people could make good use of?

DR. MARKS: I think we have this scientific data that 1 percent is safe in the black guinea pig, and that's probably the best model we have. And so I think we can make the recommendation of 1 percent based on data-driven evidence.

DR. HILL: And there's still opportunity even once this goes up for comment if need be, right, if there are people who want --

DR. MARKS: Right.

DR. HILL: -- 2 percent, they can still --

DR. MARKS: Absolutely.

DR. HILL: -- take the opportunity.

DR. MARKS: That's why it would be a tentative final report, and it has a 60-day period.

DR. RE: The black guinea pig was the study that was requested by the Panel, and I think that's the same model that was used with hydroquinone and other whitening agents.

DR. MARKS: Correct.

DR. SHANK: Yeah, the black guinea pig is an old model, and when it was initially studied with the hydroquinones, that was the animal model they used. I've not seen any -- actually this issue hasn't come up in the dermatologic world until, really, recently with a kojic acid.

DR. MARKS: So, Ron, Ron, and Tom, does safe with a limit of percent appear reasonable? I'm still troubled about basing this on --

SPEAKER: Mic please.

DR. SHANK: I'm still troubled about basing this percent on this translated Japanese paper. First concern is the focus of the paper was not on kojic acid; it was on a different chemical, and the studies were done on that. The focus was on melanocyte toxicity and loss of melanosomes. The data that are presented in tabular form are for the fifth day of exposure; yet, it went on for five weeks and there are no quantitative data there, and I wonder why that's the case. Why pick five days for your data if --

being a researcher myself, I can imagine that that was probably the best data point in the five-week study. I don't know that, but it's strange that you would pick five days if you're doing a five-week study and you pick day five to do your quantitative study and you don't mention the quantization elsewhere. So, I'm not content with basing the 1 percent on that one study.

Also those authors state two references -- references 26 and 27 -- in their paper, Japanese papers, that kojic acid is a skin lightener. So, I think I would stay with an insufficient data report, meaning information on repeated exposure levels that do not produce skin lightening. That's not a toxicity issue; that's a cosmetic issue I think.

MS. BURNETT: I will try to pull those two papers. We're going to get those translated. It can take a while, and we might not have them in time for the next Panel meeting.

DR. SHANK: Do you want those references? It was in the supplemental material, the translation of the Japanese paper. I'm sorry to keep referring to it as a Japanese paper, but I don't have the first page of that, so I can't say the authors. But it was a thesis on a different chemical, and kojic acid was included as a comparator, and in the reference section -- references 26 and 27 -- 26 is Sugai and 27 is Mashima; and the author of the translated paper on page 17 of that paper states that kojic acid has shown skin- lightening activity in even skin.

DR. MARKS: Okay. Well, with that in mind then what we would be doing, the team – Ron and Tom -- we would be issuing -- we will move, since I am presenting this tomorrow -- will move that a final tentative safety assessment, because now the conclusion hasn't changed, a final safety assessment be issued for kojic acid with an insufficient data conclusion and that we need the dose response for skin-lightening effects the same as previously so that we can --

DR. ANDERSEN: In humans, is that --

DR. BERGFELD: I think that's a problem in humans, because if you get --

DR. ANDERSEN: Well, would the guinea pig model be sufficient? is the question. I don't want to have work done that you won't like when you see it.

DR. SHANK: I'm not familiar with the various animal test models for skin lightening, so I'm afraid I couldn't answer that question. I would assume there must be some animal model that could be used.

DR. BERGFELD: Well, there's also an in vitro model. We've seen that with soy, which also interferes with tyrosinase, which is in cosmetic products. I know that there's an in vitro model.

DR. SHANK: Do we know that the human skin- lightening effect is due to tyrosinase inhibition? I don't know that we know that.

DR. SLAGA: Yeah, we don't that. We only know what the --

DR. BERGFELD: From the Japanese paper.

DR. SLAGA: Yeah.

DR. BERGFELD: True. I'd like to respond to the reason I would not do human and what we have seen with hydroquinone as well as p-hydroxyanisole, which is one of the -- put on an unsafe basis because depigmenting in one area sometimes gives you an autoimmune response where you get depigmentation in other areas, and we since don't do that. Totally know the mechanism of this particular chemical. I would hesitate to put it on a human and have that phenomenon occur.

DR. MARKS: I would say, Ron, that a more robust black guinea pig study would probably meet our needs, so not only having the thought in this case the five-day data on tyrosinase split carrying it out longer seeing what effect it actually has besides on tyrosinase but the actual pigmentation of the skin, whether there's lightening. We expect there'd be no depigmentation but do a dose response on the black-skinned guinea pig would probably adequate wouldn't you think, Wilma?

DR. BERGFELD: Yes.

DR. MARKS: Yeah.

DR. SHANK: Don't you think there are epidemiological type data out there on humans? These two Japanese papers say that it's a skin lightening -- they have concentration of kojic acid in those database?

DR. MARKS: I would -- yeah, that's Tom shaking his head here. That would, in my mind, also answer the question if you know you have products that have widespread use and the adverse -- the reporting or the safety data that should support that in humans, say as some sort of repeated application over a chronic period of time with -- address this -- that would be fine, also.

DR. SHANK: I don't know if epidemiological data is out there or not, but I can ask Ms. McGuire, and I want an intermediary here. But as I understand it, the skin- lightening effects of the kojic acid were demonstrated in an occupational setting where the workers had their hands in vats of kojic acid for extended periods of time and their skin became lighter.

DR. MARKS: That's how a lot of these are discovered -- by happenstance -- aren't they? Then the question, obviously, is was it reversible when they changed jobs. And depending on the cultural circumstances, even skin lightening may not be acceptable.

DR. HILL: I would guess whether it's reversible or not should be known from that. It would be hard to imagine it wasn't.

DR. MARKS: Often times there are reports and then no follow-up.

DR. HILL: Well, it might not be reported in open literature, but an inquiry to whatever companies had this incident. I mean, they might be willing to part with the information as to whether it was reversible, because up until -- I mean, I got to thinking about the mechanism issue -- is that if it is tyrosinase, there may be information as to how the -- what the potency of inhibition -- in other words, the binding constant of kojic acid on the human versus the black guinea pig enzyme -- is, but you would also need that in conjunction with how well this kojic acid penetrates the guinea pig skin from their test formulation versus state of use in human cosmetic preparations for that to have any informational value.

DR. MARKS: It would be interesting if the manufacturer has any safety data -- what's the reason at 1 percent they had, you know, no reports of adverse effects but at 2 percent and 4 percent they had a large number. So, I think, Alan, I think, to answer your question, what is really needed to overcome this insufficient conclusion concerning the skin-lightening threshold of kojic acid would either be another study in the black guinea pig actually establishing a threshold over a chronic period of time looking at skin lightening per se, not just applied days with tyrosinase activity, versus having an epidemiologic study. Ron Shank suggested that it would attest to the safety of it at a particular concentration.

DR. ANDERSEN: All right, it's good to capture that. I'm concerned that while the data presentation in the black guinea pig study were incomplete -- and that's bothersome -- the conclusion seemed to be at either 1 or 2 percent, there was no effect that could be seen in black guinea pig skin. So, in that range for depigmentation, kojic acid didn't do much to the black guinea pig. Now, again, I'd love to see the complete data.

DR. SHANK: When you say "no effect," actually there was no effect on melanosomes or melanocytes, okay?

DR. ANDERSEN: Mm-hmm.

DR. SHANK: There was skin lightening at 4 percent in their report. But I think we have to be careful to use that paper carefully, that their main concern was the study on melanocyte toxicity.

DR. ANDERSEN: Yes. No question.

DR. ANSELL: While that's certainly true, they also point out that the color metric could well be responsible due to crystallization of the substances being tested, which is why they saw such a trivial increase. The vehicle group, a white substance thought to be crystals of the applied substance, was attached to the skin surface, and the effect of the attached substance seemed to be greater than the deep pigmentation action.

DR. MARKS: Okay. So, again, tomorrow, team, what we're going to -- what I will move is that we issue a final safety assessment with an insufficient data conclusion, and it's the skin-lightening threshold which we are concerned about. And we need more robust either black guinea studies versus an epidemiologic study to attest to the safety of this material at least the use concentration now, which is 2 percent. So even though it's recommended 1 percent, if the data supports 1 percent, that's what the final -- but we don't have that at this point. Does that sound okay, Ron, Ron, Tom?

DR. BERGFELD: Did you want to explore in vitro studies, if they're available?

DR. MARKS: Well, I think anything there, but ultimately it's not going to be what does it -- presumably it's tyrosinase activity; presumably it's reversible. But, really, we're concerned about the end result, the lightening of the skin.

DR. SLAGA: In vitro it's kind of very difficult to extrapolate the amount of the whole animal unless you do --

DR. BERGFELD: I know --

DR. SLAGA: -- so, like, to me it would be to -- tyrosinase inhibition in vivo would be the --

DR. BERGFELD: I was just thinking about mechanisms.

DR. MARKS: Comment? Ron, you're shaking your head. Do you think we could add another -- is there an in vitro or in vivo other than -- that could be done other than the two suggestions we had? This is, again, for Alan when we go to industry if they're going to address this. Is there some other way that they could address it and reassure us from a safety point?

DR. HILL: I would honestly be reassured if the incident he was talking about where the occupational exposure was found to be fully reversible and that could be documented. To me that would be more than sufficient. If it is simply an irreversible inhibition of tyrosinase, I just -- did I say irreversible? I meant reversible inhibition of tyrosinase. I can't easily imagine any long-term consequences, any irreversibility that would come from that without really thinking out of the box. I mean, it could, but I doubt it. And, yeah, I mean, if there is information out there in terms of the IC50 values on human versus the black guinea pig enzyme or -- because that -- I mean, but otherwise, without knowing for sure that that's a mechanism in humans -- I think Wilma said that -- I'm not sure how much that really tells you.

DR. ANDERSEN: Don't we -- we've got data on tyrosinase inhibition, page 6, in the current document. The kojic acid inhibits this enzyme is not an issue. There's ample data demonstrating that. They don't --

DR. HILL: But relative affinities on human versus black guinea pig enzyme -- I don't think that's in there.

DR. ANSELL: Yeah, we don't have the human enzymatic, but we do have a 21-day human study looking at the prevention of hypopigmentation again, and although they are silent on the method -- mention of skin lightening, they do talk about the effectiveness in terms of preventing pigmentation in application of kojic acid cream for two months in a total of 204 cases.

DR. HILL: Where are you looking? I'm sorry.

DR. ANSELL: One of the Japanese papers -- unfortunately, we're getting a little confused with our Japanese authors, but this is a paper by Mishima Ogimawa Shibata, "Inhibitory Action of Kojic Acid on Melanogenesis and the Therapeutic Effects of Various Human Hyperpigmentation Disorders."

DR. MARKS: Did we get that one? Yeah, I don't -- we did? Did we get that one? It sounds like we didn't see that, Jay. Or if we did, it may have been that it came through in the electrons which somehow we missed.

Okay. Well, I don't think we're -- right now I think we can move on, because we'll have the Belsito's team input. But ours is going to be -- I will move -- our team will move that the final safety assessment is insufficient data, and then we can -- and we're still concerned about the skin lightening effect of kojic acid. And we need a threshold that we can feel comfortable that, one, either it does not occur or, two, that it's reversible.

Okay? Any other comments? I'm not sure we can -- oh, Jay, yes.

DR. HILL: Can we get a copy of that to look at later in the morning? I mean, I'm just asking.

DR. MARKS: Did you have that, Christina, that paper?

MS. BURNETT: Is this what I had in the paper already or is this something new?

DR. ANSELL: I'm not sure exactly where it came. It was just in the package of paper that I got. I do have another comment when we get to --

DR. MARKS: Sure.

DR. ANSELL: We have a question concerning the inclusion of a new table, Table 4, where we started to include a table which includes commercial products, trade names. This doesn't seem to go to either application or concentration associated with the safety. We're curious as to what it actually contributes -- a simple iteration of trade names solicited from second-party sources, page 51 and 52.

DR. MARKS: Do you want to address that, Christina or Alan?

DR. ANDERSEN: Well, I think it's data that are out there as provided by the Environmental Working Group. Their methodology is to look at labels of cosmetics that are available online, and these are the products that they found with kojic acid listed in the list of ingredients. So, it's simply another way to capture information on what's out there. What we thought was particularly significant is that the list is considerably longer than the list of numbers reported to the Voluntary Cosmetic Reporting Program. Now, we've known for a long time that the VCRP is underreported. I don't know whether these -- I don't know that we're saying these data fix it; it's just another perspective on products in which kojic acid is used. It is what it is.

DR. MARKS: Yeah, actually that brings up -- because there's another report. I was going to ask the number of uses that the EWG -- the Environmental Working Group -- is a reference group in another table, which is a standard table. We'll get to that later on. How comfortable do we feel with the data that's reported by the Environmental Working Group in terms of its accuracy, I guess?

DR. ANDERSEN: Yeah, that's a very good question. I don't have any sense of accuracy of that as a database. I don't know what it represents in terms of the universe. Is it also underreported? Are there products listed there for which it is not true that kojic acid is an ingredient? I don't really have a sense of the accuracy of those data from that standpoint.

DR. ANSELL: Well, I think my concern goes to not accuracy, although our experience has been that simple accumulation of data off the internet is often not particularly accurate. Mine goes to relevance. Have we identified an end-use application or concentration that would inform a safety assessment as opposed to a simple iteration of trade names? I think if you've identified

an application which is appropriate or an application which has not been included, then it needs to be carried into Table 3, for example, or into the discussion. But a simple iteration from a second party as to what they found on the internet I do not think informs a safety assessment.

DR. MARKS: Actually, I'd agree with you, Jay. The only reason I might and maybe not have it as a table, but mention that this is, that if you notice in the product, in their name of the product -- and this could be captured in one sentence if it's important from the safety point of view -- is many of these reference an effect on skin pigmentation, lightening or brightening or those with -- that appears in the name of the product very frequently. So maybe in that case it might be worthwhile referencing as a main use, but I don't think the table really adds anything to the safety assessment.

DR. ANDERSEN: Okay. There's the two footnotes to the table that I would be concerned that you not ignore, and that is that one product included on the label that kojic acid was used at 4 percent and another one reported 2 percent. Now, if those -- well, just those data were available from the label.

DR. ANSELL: Well, more specifically, EWG reported. CIR staff has not confirmed.

DR. ANDERSEN: No, we have not checked that.

MS. BURNETT: Actually, I went to the website for that producer and it says 4 percent.

DR. ANSELL: Well, then their product would fall well outside of the CIR review, and that should be referenced to -- that's --

MS. BURNETT: Right.

DR. ANSELL: -- whatever process we have come up with in terms of identifying --

DR. ANDERSEN: And it may not even be a cosmetic product. It may be, you know, practically an OTC drug.

MS. BURNETT: EWG reported it at 4 percent. I went to their -- the producer's website and confirmed that it said 4 percent. We didn't want to further analyze the EWG note. We just took it for what it was and just put it in there.

DR. ANDERSEN: But the sentence that was discussed that characterizes that there are further data from EWG and does that in just a short sentence is in the body, so if you just wanted to eliminate the table, we have captured at least the idea.

DR. MARKS: Another idea I had is, looking at Table 3, you could have another footnote there. Not only is it in the body, but for somebody looking at uses and concentrations, one could also say, again as I'll repeat according to data from the Environmental Working Group, the concentration is listed up to 4 percent -- just so you capture that. To me, that's a stylistic. I'm not sure we need to use two pages and Table 4 to capture that information and safety assessment.

DR. HILL: Does the International Journal of Toxicology publish supplemental material online? Is it something that can be put in a -- I mean, journals that I use, they do a lot of supplemental data, published only online and not in the hard copy of the journal.

DR. ANDERSEN: Not that I'm aware of.

DR. HILL: Okay. But we could query Mary Beth as to whether that's doable? Could that be done, that explore the --

DR. ANDERSEN: Yes.

DR. HILL: Because this would be a great -- I mean, then you have a reference and it's up there, unless we have concerns about -- real, genuine concerns about the accuracy of the data, and, of course, if this exact information is already available in EWG materials, then that -- then there's a link and you're good. But here, even if that's the case, you still get a nice snapshot, and I think there's merit in having it available somewhere.

DR. ANDERSEN: Well, that was one of the reasons why we included it -- was to get exactly this discussion going. But I wouldn't want to limit the discussion, because I direct your attention to Table 5, which I'd also like the team's view on. Again, if you capture the snapshot from the FDA VCRP data, you find there are 16 reported uses, and if you look, as we did, and ask Health Canada to tell us what's reported to them, there are rather more products in use with kojic acid over a considerably larger concentration range. It's a contact that we made with Health Canada, and we found that they were not only willing but eager to share their data on reported uses for ingredients, so we captured it and it's here. What do you think?

DR. MARKS: My concern is where do you make the cutoff and now do you go to Mexico? Do you go to Brazil? Do you go to Europe since we're really doing a safety assessment on ingredients which are available in the United States? So, I guess I would say that, yes, that should be captured in the body, say, that it's used up the 30 percent range of kojic acid in three products in Canada per se. But I'm not sure I'd create another table. You know, we don't know -- where it seems to be most widely available and used is in Japan and Asia, so it would be interesting to see what it is there, but we don't have that data. I'd ask the rest of the team to comment. So, my immediate response would be I would include it in a table, but I would, again, have it in the text for those that are interested.

DR. SLAGA: I agree. I think Table 4 and Table 5, as long as it's stated succinctly in the text, is sufficient.

DR. BERGFELD: I have a comment. I think what is in here is interesting, and I concur that you could put it in the text, but I would suggest that you go down a little further than 30 percent, and I would suggest that you do anything above 2 percent, because there's notable products at 1 to 3 at 45. And I think when you just say 3 products at 30, that misrepresents that group.

DR. MARKS: I didn't mean to limit it to that. I was just picking the highest concentration. If Canada hasn't seen an epidemic of skin lightening of marked degree at 30 percent, that actually is reassuring. I wouldn't limit it just to the -- you know, I would probably say just take the row of numbers that are X-number of products with concentrations ranging from less than 0.1 to 30 percent. And if you want to say the most frequent area -- or concentrations 1 to 3 percent, fine, but that can be done in one sentence.

DR. BERGFELD: I think we also have to ask the FDA if they're looking a kojic acid at 4 percent as an OTC to get the status of that, you know?

DR. SLAGA: Yeah.

DR. BERGFELD: Or anything above 2.

DR. HAVERY: You might want to ask Dr. Katz tomorrow, since she used to work for the Center of Drugs, but you probably know that kojic acid is not monographed, which means that any use intended to lighten or brighten the skin would be an unapproved drug.

DR. ANDERSEN: Well stated.

DR. HAVERY: Don Havery, FDA. And to the inclusion of Health Canada, it becomes very, very complicated. We don't know within their regulatory scheme whether these materials are being sold as drugs, whether they are being sold under conditions which would be significantly different than a cosmetic in the U.S. Japan has a whole category of quasi-drug products. So, unless we wanted to do an analysis of the teasing out the cosmetic -- a U.S. cosmetic from a U.S. drug within the context of a foreign regulatory scheme, it might be difficult to inform us as to whether the application would meet a U.S. sense of cosmetic application being a product that could be sold without physician intervention or review of labeling or any of the other distinguishing elements between OTC drugs and cosmetics.

DR. MARKS: Any other discussion? This has been robust and enlightening.

DR. SLAGA: Yes.

DR. BERGFELD: I didn't hear James' conclusion.

DR. MARKS: My conclusion was to just eliminate, as Tom said, Tables 4 and 5 and include capture the essence in the text. Jay, is that -- what was your -- how would you handle it?

DR. ANSELL: Well, to the extent we've identified elements which inform the safety assessment through these other reviews, I think they should be included. I'm not sure that in this particular case we've identified applications or concentrations which would be beyond those discussions we've already had, but I think it would depend very much on the verbiage. We were much more concerned about just a table just iterating, you know, a Google search on the Internet and including trade names within a CIR report.

DR. MARKS: Okay. Do the team members have any other comments about this or may we move on? Ron, did you have one?

DR. HILL: I was just going to see if we could get reference 37 as well, which is the -- because that's mammalian tyrosinase activity, and there are some statements in the main document that I flagged last time that I remember now that we were discussing it and that it should have some -- and I don't know if has human in there, but it says "mammalian," so that I'm guessing there are multiple species of enzyme -- enzymes from multiple species in there. And also it appears not to be competitive inhibition, so perhaps there are some details in that paper as to what kind of inhibition we're looking at. It's reversible, but not competitive, so are we looking at allosteric or what?

MS. BURNETT: I believe I have that in my filing cabinet. I can have it here tomorrow.

DR. HILL: Yeah, tomorrow's fine.

DR. MARKS: Are the results of that going to change our conclusions?

DR. HILL: I doubt it, but I --

DR. MARKS: Okay.

DR. HILL: If something comes up in the discussion tomorrow, I would like to be slightly educated. I would have looked up that reference and seen it already, except I thought this was going to go a little bit differently today.

DR. MARKS: Okay.

DR. HILL: Actually, I think you landed where I thought we were going to land, but circuitously.

DR. BERGFELD: That's right. They're enormous. I think it's time to get on with it then, and so we're going to go to the first report, kojic acid, with Dr. Marks presenting one of the final documents.

DR. MARKS: In December of last year the expert panel issued a tentative safety announcement for kojic acid with insufficient data conclusion. There were two data needs, essentially dermal sensitization and irritation at the current use concentration. Secondly, an indication of what dose response the skin-lightening effect of kojic acid occurred. We have received new data. Concerning the dermal sensitization we have an HRIPT test which shows that it's safe at this concentration which is 2 percent, the use concentration. Then for our team the more difficult issue was the skin lightening and there is a I think pivotal guinea pig study from Japan in which the primary purpose of that paper was to look at hydroquinone and kojic acid in 4 and 1 percent were evaluated in this guinea pig model and 1 percent had no effect, 4 percent did have some lightening effect, but when you look at the paper the author felt that part of this was due to a white substance deposited on the skin's surface. So with that in mind, that within the range there did not appear to be any significant lightening of the use concentration we moved that the final safety assessment is that this kojic acid is safe.

DR. BERGFELD: You're presenting that as a motion?

DR. MARKS: Correct.

DR. BERGFELD: Is there discussion or second?

DR. BELSITO: Discussion. I guess you're saying safe at current concentration of use as reported at 2 percent.

DR. MARKS: Correct.

DR. BELSITO: But there is significant skin lightening, but they explain it at least partly with powder at 4 percent.

DR. MARKS: Correct.

DR. BELSITO: And we don't know what 2 percent would do.

DR. MARKS: Correct. It was minimal. When you look at the actual values it seemed like there were minimal changes between 1 and 4 percent in that study. Also when you look at the mechanism, this would appear to be reversible. It's inhibition of tyrosinase. So we're not concerned that there are irreversible pigmentary changes such as you see with hydroquinone. Therefore, we felt that we could move that it was safe in light of the well-known effect of skin lightening.

DR. BELSITO: The P value at 4 percent compared to control was 0.01, the L value was 35.68 for 1 percent and the vehicle is 30.3, and the para-hydroquinone was 37.2 at 1 percent. The issue that our team had yesterday were is this a value model and I guess that's the first question to throw out because I'm not a pigment biologist so we're interested in Tom's feeling about this model for depigmentation.

DR. SLAGA: This model is used for this and actually I think the beauty of this particular paper is that they compared a number of different compounds including hydroquinone and at different doses. If you rank all of them, KA comes way down on the list. They even use the term very weak at the higher dose, but it's shown as a plus or minus in the table.

DR. BELSITO: So that you feel the system is good, and then I guess the next question is even if it's reversible, it's a biologic effect and cosmetics should not a biological effect at 4 percent. So I guess I have difficulty unless there was a study at 2 percent. I'd be more comfortable saying safe up to 1 percent in a cosmetic product as we have negative data at 1 percent.

DR. MARKS: Obviously we're extrapolating when we feel that 2 percent is safe, but if your team feels that the limit should be 1 percent based on the lightening effect then our team I'm sure would be comfortable with that and it would be up to industry to show that it's safe at the present use concentration of 2 percent which we think it would be, but you're absolutely right that we're extrapolating data to come to that conclusion.

DR. BERGFELD: John?

DR. BAILEY: We're comfortable with 1 percent so that if that's your conclusion, I think that will work for us.

DR. MARKS: With that in mind I'll withdraw my motion and reframe it that we move that it's safe at 1 percent.

DR. BELSITO: Second.

DR. BERGFELD: Is there any further discussion? Seeing none, I call for a vote. Do you have discussion?

DR. BELSITO: Just some editorial, that we felt that Table 4 in the document should be deleted, that's the survey by the Environmental Working Group, but that Table 5 should be kept because it is a government authority data and then in the discussion we'd have to point out that the products with concentrations above 1 percent, it's not clear what those are, whether they are OTC drugs or not, but they would not be appropriate as a cosmetic product.

DR. BERGFELD: Dr. Katz, would you make a comment on the FDA and kojic acid? Is there any activity at the FDA?

DR. KATZ: Not that I'm aware of. I don't know if it's being looked at by OTC drugs or not.

DR. BERGFELD: Thank you. Is there any other discussion? Did you have discussion regarding the text?

DR. MARKS: No, we felt that Table 4 should also be deleted. However, we felt also that Table 5 could be deleted and captured in the text because we didn't know at what government agency would you stop, if Mexico had similar ones, Europe had similar ones, Japan, then do you stop at one or do you have multiple tables? That's a relatively minor point and I don't think it's going to create stress either way for us.

DR. BERGFELD: Is there any other discussion?

DR. BAILEY: We would agree that Tables 4 and 5 probably should be taken out of the final report that is published.

DR. BERGFELD: Dr. Andersen?

DR. ANDERSEN: I think the effort to query the Environmental Working Group database and the Canadian database was at least partly related to the decrease in use, that we thought we were seeing such a low frequency of use in the VCRP. We all understand that the VCRP will never and can never be a complete finding of everything that is in use. It's voluntary reporting to FDA. It's a marvelous barometer for a lot of things, but to suggest that it is an absolute piece of information, we don't want to be in that position and I think everyone understands that. So we look at what other data are available. EWG gave us some additional information, Canada gave us some additional information, but it also confuses things because it's not clear that all of the data from Canada are just cosmetics, there may be some cosmetic drugs in that group and that just confuses it. I think the discussion has been marvelous and it gives us guidance on what to do with those two pieces of data for this particular report. I think it's wonderful.

DR. BERGFELD: Dr. Belsito?

DR. BELSITO: I would agree. I think that since the FDA system is voluntary and it's not going to capture everything that it's certainly worth going out and looking at how Canada reports simply because they're immediately to our north and a lot of those products are probably on our market and it's certainly worth looking at what the Environmental Working Group is finding, but we have no idea whether those products are still in use, whether the concentration ranges that they might report are in fact valid. I don't think that that final information should be in our report. I think what it should do is spur industry to go back to its constituents and say VCRP has no report of a product like this or a concentration range like this, but it's on the Environmental Working Group website or it's on Health Canada or whatever, what can you tell us about this. I think it's helpful, but when it comes to the final report I think unsubstantiated data like that at least from nongovernment agencies, and if you have a problem with where do we cut off the government agencies, it should not be in our report.

DR. BERGFELD: Are you inferring that it shouldn’t appear anywhere, a text or table both?

DR. BELSITO: I guess it depends upon how things turn out. I gather, and I'm blanking on the name of the ingredient, in one ingredient it turned out that the Environmental Working Group reported a class of cosmetics where the ingredient was used whereas the VCRP it was not reported when it went back to industry we found a report, so in that case maybe it doesn't need to be in the report. On the other hand, if we find the Environmental Working Group or Health Canada say that this product or a specific ingredient is being used in a type of product for which we have no report, we may want to make note that there have been reports from EWG or Health Canada or wherever of this ingredient being used in this type of cosmetic product for which we don't have reporting to FDA or substantiation from the industry. Then if it might be an issue in safety, we might want to bring it into the discussion. I think it's going to be on an ad hoc basis.

DR. HILL: I wanted to make sure that the panel people did not feel discouraged if the tables are not there in the final report and then they won't do this again because I felt like it did supply useful fodder for discussion and I wouldn't want them to be thinking that that was wasted effort.

DR. BERGFELD: Is there any other discussion? Dr. Bailey?

DR. BAILEY: Just one additional comment. I think that this is a great illustration of the contributions that this process makes because what you've done is to define the cosmetic uses for kojic acid, in other words, the nonbleaching cosmetic uses. If you look especially at the EWG list, much of what's on there are lightening or brightening or bleaching products which may fall outside of your conclusions so that I think that the benefit that you're providing is sort of drawing that line in the sand as to what's cosmetic and what's not. I think that's very helpful.

DR. BERGFELD: Thank you. Is there any other discussion? Seeing none I'm going to call for the vote. All those in favor of going forward with this ingredient? Unanimous. Thank you. No dissenters.

Amended Safety Assessment of Kojic Acid as Used in Cosmetics

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The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Samuel M. Cohen, M.D., Ph.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume, M.B.A. This safety assessment was prepared by Christina Burnett, M.S., Senior Scientific Analyst/Writer, CIR.

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ABBREVIATIONS

2-AAF	2-acetylaminofluorene
8-OxodG	8-oxodeoxyguanosine
ALP	alkaline phosphatase
AUC	area under the curve
BHP	N-bis(2-hydroxypropyl)nitrosamine
BrdU	bromodeoxyuridine
CAR	constitutive androstane receptor
C_{max}	peak concentration
CHO	Chinese hamster ovary
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
DART	developmental and reproductive toxicity
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook*
DHPN	diisopropanolnitrosamine
DMBA	9,10-dimethyl-1,2-benzanthracene
EC	European Commission
ECHA	European Chemicals Agency
EPA	Environmental Protection Agency
EU	European Union
FDA	Food and Drug Administration
GEDIAC	Grupo Español de Investigación Dermatitis de Contacto y Alergia Cutánea
GLP	good laboratory practice
GOT	glutamic-oxaloacetic transaminase
GPT	glutamic-pyruvic transaminase
GST-P	glutathione S-transferase-placental form
HRIPT	human repeated insult patch test
IARC	International Agency for Research on Cancer
iDMM	iPS cell-derived melanocyte medium
IL	interleukin
iPS	induced pluripotent stem
LDH	lactate dehydrogenase
LI	labeling indices
MDA	malondialdehyde
MoCRA	Modernization of Cosmetics Regulation Act
MOE	margin of exposure
MOS	margin of safety
MTT	methyl thiazol tetrazolium
NOAEL	no-observable-adverse-effect level
NOEL	no-observable-effect level
NR	not reported
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
PCNA	proliferating cell nuclear antigen
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RLD	Registration and Listing Data
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SDM	sulfadimethoxine
SED	systemic exposure dose
SPB	phenobarbital sodium salt
T3	triiodothyronine
T4	thyroxine
TG	test guideline
TPA	phorbol-12-myristate-13 acetate
TSH	thyroid-stimulating hormone
UDP-GT	diphosphate glucuronosyltransferase
US	United States
VCRP	Voluntary Cosmetic Registration Program

DRAFT ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) reassessed the safety of Kojic Acid, which is reported to function as an antioxidant in cosmetic products. The Panel reviewed the available data to determine the safety of this ingredient. The Panel issued an amended report…[TBD].

INTRODUCTION

This assessment reviews the safety of Kojic Acid as used in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*), Kojic Acid functions as an antioxidant in cosmetic formulations.[1] While Kojic Acid is purported to have skin-lightening properties, whitening of the skin is considered to be a drug effect in the US.

The Panel first reviewed the safety of Kojic Acid in a report published in 2010, with the conclusion "Kojic Acid is safe for use in cosmetic products up to a concentration of 1%."[2] In June 2022, the Panel determined that this safety assessment should be re-opened for re-evaluation due to reported restrictions by the European Commission (EC) on the use of Kojic Acid.[3] Excerpts from the summaries of the 2010 report are disseminated throughout this document, as appropriate, and are identified by *italicized* text. (This information is not included in the Summary section.)

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last performed in July 2025. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Some of the new data included in this safety assessment were found in the opinions by the Scientific Committee on Consumer Products (SCCP)[4] and the Scientific Committee on Consumer Safety (SCCS)[3,5] Please note that these sources provide summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when the SCCP and SCCS are cited.

CHEMISTRY

Definition and Structure

According to the *Dictionary*, Kojic Acid (CAS No. 501-30-4) is the heterocyclic compound that conforms to the structure in Figure 1.[1]

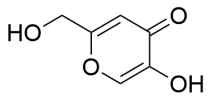

Figure 1. Kojic Acid

Chemical Properties

UV absorption appears to vary as a function of the pH.[2] *The enolic hydroxyl group at C5 gives Kojic Acid its weakly acidic property and allows it to form salts with a number of metals.*

Chemical properties for Kojic Acid are summarized in Table 1. Kojic Acid is a white to light yellow crystalline powder with a molecular weight of 142.11 g/mol.[2,3] The log K_{ow} is reported to be -0.64.[3]

Method of Manufacture

The method of manufacturing of Kojic Acid for cosmetic use is not clearly described in the published literature. However, Kojic Acid is a hydrophilic metabolite mainly derived from various species of *Aspergillus* and *Penicillium* by using glucose and yeast extract as carbon and nitrogen sources, respectively.[6] It also can be produced by using a variety of sugars (e.g., sucrose, lactose, galactose, arabinose, ribose, and starch) or using different organic wastes via microbial fermentation. Different fermentation procedures have been reported for large scale Kojic Acid production, including submerged batch fermentation.

Kojic Acid may also be synthesized by acetylation of tetraacetyl-galactosone hydrate by action of acetic anhydride and pyridine to yield diacetylkojic acid.[7] This is followed by deacetylation in the presence of ammonia in methanol or methoxide.

Impurities

The purity of Kojic Acid was reported to be greater than 97%.[3] Impurities may include heavy metals (10 mg/kg maximum) and arsenic (4 mg/kg). One sample was reported to contain $\leq$ 2 ppm arsenic, $\leq$ 50 ppm chloride, $\leq$ 10 ppm heavy metals, $\leq$ 120 ppm sulfate, and < 1.08 ppb aflatoxins.

Natural Occurrence

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

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of Kojic Acid in cosmetics. Data included herein were obtained from the FDA and in response to a survey of maximum use concentrations conducted by the Personal Care Products Council (Council), and it is these values that define the present practices of use and concentration. Frequencies of use obtained from the FDA include data from the Voluntary Cosmetic Registration Program (VCRP) database as well as Registration and Listing Data (RLD). As a result of the Modernization of Cosmetics Regulation Act (MoCRA) of 2022, the VCRP was discontinued in 2023 and, as of 2024, manufacturers and processors are required to register facilities and list their products (and ingredients therein) with the FDA (i.e., RLD). An exception is made for small businesses (average gross annual sales in the US of cosmetic products for the previous 3-year period is less than $1,000,000, adjusted for inflation), which are exempt from MoCRA reporting for most cosmetic product categories. Eye area products, injected products, internal use products, or products that alter appearance for more than 24 h, and the facilities that manufacture these products are not included in this exemption.[8] Please note, at this time, it is not appropriate to contrast data from the VCRP and RLD to determine a trend in frequency of use because there are numerous differences in the ways the data for the VCRP and the RLD were collected and processed, and because reporting frequency of use is now mandatory (as opposed to the past practice of voluntary reporting). Although the VCRP program is now defunct, trends in frequency of use from the RLD alone are not yet possible in that a baseline is currently not available.

According to RLD submitted to CIR in 2024, Kojic Acid is reported to be used in 1114 formulations, with the majority of the uses reported in personal cleanliness products (274 in bath soaps and body washes) and skin care preparations (374 in face and neck products; Table 2).[9] In 2023, VCRP data reported use in 123 formulations, most of which were leave-on products.[10] In the original 2010 report, Kojic Acid was reported in 16 formulations, most of which were leave-on products.[2] The results of the concentration of use survey conducted by the Council in 2024 indicate Kojic Acid is used at up to 1% in leave-on skin care preparations.[11] In 2008, the maximum concentration of use for Kojic Acid was reported to be 2% in leave-on skin preparations.[2]

RLD indicate that Kojic Acid is used in baby products (no concentrations reported) and according to RLD, the VCRP, and the results of the concentration of use survey, it is used in products that may come into contact with mucous membranes (0.001% in bath oils, tablets, and salts; 0.05% in bath soaps and body washes). Uses have also been reported in eye makeup preparations (no concentrations reported).

Some products containing Kojic Acid may be marketed for use with airbrush delivery systems. With the advent of MoCRA and the current product categories outlined by the FDA, it is now mandatory that cosmetic products used in airbrush delivery systems be reported as such for some, but not all, product categories in the RLD. In other words, a reliable source of frequency of use data regarding the use of cosmetic ingredients in conjunction with airbrush delivery systems is now available in some instances. None of the reported product categories for this ingredient as listed in the RLD include a designation using airbrush application, so it is possible that this ingredient is used with airbrush delivery systems, but not reported as such. Additionally, the Council currently surveys the cosmetic industry for maximum reported use concentrations of ingredients in products which may be used in conjunction with an airbrush delivery system; thus, this type of data may also be available when submitted. Please note that no concentration of use data were provided indicating airbrush application. Nevertheless, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety. Without information regarding the consumer habits and practices data or product particle size data (or other relevant particle data, e.g., diameter) related to this use technology, the data profile is incomplete, and the Panel is not able to determine safety for use in airbrush formulations. Accordingly, the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

In the European Union (EU), the SCCS concluded in 2022 that Kojic Acid is safe when used as a skin lightening agent in cosmetic products at concentrations of up to 1%.[3] (It should be noted that in Europe, products that whiten the skin are considered to be cosmetics; however, in the US, skin lightening is considered to be a drug effect.) The EC has categorized Kojic Acid in Annex III, the list of substances which cosmetic products must not contain except subject to the restrictions laid down.[12] For this ingredient, the regulation states that Kojic Acid may only be used in face and hand products at a maximum concentration of 1%. This restriction is based on the overall safety assessment performed by the SCCS, which

incorporated margin of exposure (MOE) evaluations that indicate higher exposure levels, such as those resulting from use on larger body areas, may pose a health risk.[13]

Non-Cosmetic

Non-cosmetic uses reported for Kojic Acid include therapeutic uses for melasma, antioxidant and preservative in foods, antibiotic, chemical intermediate, metal chelate, pesticide, and antimicrobial agents.[2]

Numerous studies have identified Kojic Acid as a topical treatment for hyperpigmentation.[6,14-16] Research has also been conducted on Kojic Acid for use as treatment for neurodegenerative diseases, including Alzheimer's disease,[17,18] and corneal injuries.[19] Kojic Acid is used in the production of a number of foods, including soybean paste (miso), shoyu (soy sauce), or sake.[5]

while Kojic Acid is purported to have skin lightening properties, it is currently not approved by the US FDA for such use in over-the counter pharmaceutical products. However, a study that quantified several known skin-lightening agents in commercial skin lightening products found Kojic Acid at concentrations as high as 3.9% (in a cream formulation).[20]

TOXICOKINETIC STUDIES

Dermal Penetration

Absorption of radiolabeled Kojic Acid at 1.045% in a formulation through human dermatomed skin resulted in 17% of the applied dose being absorbed.[2] *The in vivo percutaneous absorption of 1% Kojic Acid in a cream formulation (500 mg) was studied in 6 human volunteers. [This study was used in the 2022 SCCS opinion to calculate the margin of exposure (MOE).] The test material was applied to the entire surface of the facial skin (left and right cheeks). Kojic Acid was detected in the plasma of all the participants at one or more blood collection times (up to 24 h after application). All the concentrations in plasma were only slightly above the quantitation limit of 1 ng/ml. The mean peak concentration (C_{max}) was 1.54 ng/ml and the mean area under the curve ($AUC_{0-24\ h}$) was 19.4 h·ng/ml. There were no adverse effects observed in the participants. Based on pharmacokinetic studies in rat (described below under Absorption, Distribution, Metabolism, and Excretion) and in vitro percutaneous absorption values in human skin, the systemic exposure dose (SED) range was determined to be 0.03 to 0.06 mg/kg/d. The SED range was calculated using the application area of the hands and face (400 and 590 cm^2, respectively), a maximum application rate of 1.0 g of 1% Kojic Acid cream at 1 mg/cm^2 (total application of 10 mg Kojic Acid/d), and percutaneous absorption of 17% of the applied dose (3.6 µg/cm^2) in humans.*

In Vitro

The percutaneous absorption of ^{14}C-Kojic Acid was studied in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 428 with human dermatomed skin.[5] A leave-on skin care formulation containing 1% Kojic Acid was applied to 12 intact skin membranes on static diffusion cells at a rate of 2 mg/cm^2 (equivalent to 20 µg Kojic Acid/cm^2) for 24 h. The skin surface was then washed with 2% sodium dodecyl sulfate in water, followed by rinsing with water. The stratum corneum was then removed by tape-stripping. The 24-h penetration profile was determined by collecting receptor fluid (phosphate buffered saline) samples at 0.5, 1,2, 4, 8, 12, 16, 20 and 24 h following application. Mean recovery of the applied test material was 95.48 ± 3.33% (19.44 ± 0.68 µg/cm^2). The average penetration rate was 0.006 µg/cm^2/h. The mean amount of Kojic Acid that penetrated over the 24-h exposure period was 0.698% of the applied dose (0.142 ± 0.265 µg/cm^2). The mean total systemically available dose of Kojic Acid (remaining epidermis + dermis and receptor fluid) was 3.68% of the applied dose (0.749 µg/cm^2). The SCCS noted that there were several issues with the study, including a discrepancy in the reported concentration of Kojic Acid tested (0.88% instead of 1%) and the potential that the formulation may not be representative of the majority of the Kojic Acid-containing formulations on the market due to the high amount of silicones, polyols and nylon in the test material.[3,5]

Absorption, Distribution, Metabolism, and Excretion

In rats, Kojic Acid is rapidly absorbed and distributed to all organs in oral treatments.[2] *Kojic Acid is not as rapidly absorbed or distributed in subcutaneous treatments, is slowly absorbed in dermal treatments, and can be transferred at low levels to milk. Kojic Acid is mainly excreted in the urine; metabolites are sulfate and glucuronide conjugates of Kojic Acid.*

The toxicokinetics of ^{14}C-Kojic Acid was studied in male Wistar rats (number not reported) in a non-good laboratory practice (GLP) study.[21] The rats received a single oral (33 mg/kg in water/propylene glycol (2:1)), subcutaneous (same dose as oral), or dermal (1% in cream formulation at 33 mg/kg on 9 cm^2 area; 24-h application under occlusion) dose. With the oral and subcutaneous doses, Kojic Acid was rapidly absorbed and metabolized, with approximately 70% of the dose excreted in urine, 5% in feces, and 1.4% in expired radiolabeled carbon dioxide. The order of magnitude of plasma levels (C_{max} > 20 µg eq/ml following a single dose of 1.72 mg/kg) suggest a relatively high bioavailability after oral or subcutaneous administration. Appearance of radiolabel in expired air indicated that a minor portion of the administered Kojic Acid was metabolized completely. Topical administration resulted in lower plasma values, indicating a lower bioavailability, as compared with those following oral or subcutaneous administration. The calculated $AUC_{0-6\ h}$ values were 71.8, 63.9, and 13.3 µg ^{14}C eq · h/ml for oral, subcutaneous, and topical administration, respectively. It was determined that the bioavailability after topical application in rats under occluded conditions may be estimated to be in the order of 18% of a respective oral administration.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

In acute mouse studies with Kojic Acid, oral, subcutaneous, and intraperitoneal LD_{50} values were 5.1, 2.7, and 2.6 g/kg bw, respectively.[2] *In rats, the LD_{50} values were greater than 2 g/kg bw in oral and dermal studies, and 2.6 and 2.4 g/kg bw in subcutaneous and intraperitoneal studies, respectively.*

Short-Term Toxicity Studies

Dermal

A 4-wk dermal study in rats found that exposure at up to 1000 mg/kg/d Kojic Acid lowered lymphocyte counts at 300 and 1000 mg/kg/d and decreased absolute and relative spleen weights at 1000 mg/kg/d.[2] *The no-observable-effect level (NOEL) for this study was 100 mg/kg/d.*

The dermal toxicity of Kojic Acid was assessed in a 30-d study in New Zealand White rabbits.[3,4] Groups of 5 males and 5 females received 0, 13, 130, or 1300 mg/kg bw/d (0, 0.65, 6.5, or 65% w/v) Kojic Acid in 1% aqueous methylcellulose on abraded skin daily (2 ml/kg/d). Treatment sites were covered for 6 h each day with gauze before being washed with warm water. Animals were observed daily for clinical signs and mortality, and body weight and feed consumption were recorded weekly. Blood samples for hematological and biochemical parameters were taken prior to the start of treatment and in the control and in the highest dose group prior to study end. The rabbits were killed after the end of the treatment and underwent necropsy and histopathology.

All rabbits had slight dermal reactions, but effects were more persistent in the animals that were treated with Kojic Acid. Erythematous papules and abscesses were observed in several rabbits (no further details provided). Two animals had an infection of *Staphylococcus aureus*. One female of the 13 mg/kg bw/d group and 1 male of the 130 mg/kg bw/d group were found dead and 1 male of the control group was killed *in extremis*. Necropsy of these animals revealed lesions of the lung, liver, kidney, and brain that may have been contributing factors. In the 1300 mg/kg bw/d dose group, statistically significant changes were observed in the mean corpuscle hemoglobin concentration, the mean cell volume, and A/G ratio when compared to controls. In the 13 mg/kg bw/d dose group, the pituitary weight was significantly increased. Ophthalmoscopic examination revealed changes in the eyes in 1 control animal, 1 animal in the 13 mg/kg bw/d dose group, 3 animals in the 130 mg/kg bw/d dose group, and 3 animals in the 1300 mg/kg bw/d dose group. Plaques in the aorta were reported in 1 control male, 3 males and 1 female in the 13 mg/kg bw/d dose group, 1 male and 1 female in the 130 mg/kg bw/d dose group, and 4 males in the 1300 mg/kg bw/d dose group. Pale kidneys were reported in all treated groups. The SCCP noted that effects on skin and eyes could not be evaluated due to the bacterial infection in the animals. Because hematological and biochemical parameters after the treatment period were only studied in the 1300 mg/kg bw/d dose group, a conclusion on dose-dependency of statistically significant changes could not be made.[3,4]

Subchronic Toxicity Studies

Oral

In a 13-wk oral toxicity study, male rats received up to 3000 mg/kg/d Kojic Acid in 1.0% carboxymethycellulose.[2] *Rats that received 250 mg/kg or more of Kojic Acid had significant suppression of body weight gain when compared to the control group. The 1000 mg/kg dose group also had a slight decrease in erythrocyte counts and decreases of hematocrit value and hemoglobin concentration. Increases of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were observed in dose groups receiving 250, 500, and 1000 mg/kg. The 500 and 1000 mg/kg dose groups had increased alkaline phosphatase (ALP), and slight increases of total cholesterol, bilirubin, and calcium were observed in the 1000 mg/kg dose group. At necropsy, the absolute and relative weights of the adrenal glands were increased in the dose groups receiving 500 and 1000 mg/kg Kojic Acid. The NOEL was determined to be 125 mg/kg/d.*

Chronic Toxicity Studies

Oral

In a 26-wk study, groups of 10 male SLC-SD rats received 0, 125, 250, 500 or 1000 mg/kg bw/d Kojic Acid in 1% aqueous solution of carboxymethylcellulose (0.5 ml/100 g bw) via gavage.[3] A 5-wk recovery period occurred in the 250, 500, and 1000 mg/kg bw/d dose groups. The animals were observed for abnormalities daily, and body weight, feed consumption, and water intake were measured twice a week until 13 wk after the initial administration, then once a week thereafter. Urine was analyzed prior to necropsy at the end of the dosing period and at the end of the 5-wk recovery. Hematological and serobiochemical tests were performed before necropsy. Blood levels for triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) were not measured. Animals were killed and subjected to macroscopic and microscopic examinations.

In the groups receiving 250 mg/kg bw/d and more, excitation and subsequent sedation were observed for 2 and 3 h 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/d and above. A temporary decrease in feed consumption and increase in water intake were observed in the groups treated with 500 mg/kg and above. A decrease in urine volume was observed in the 2 highest dose groups, and at 1000 mg/kg bw/d a decrease of urinary pH was reported. Statistically significant hematological and biochemical differences reported included an increase in

creatinine in the 250 and 500 mg/kg bw/d groups; an increase in ALP values in the 500 and 1000 mg/kg bw/d groups; increases in GOT, GPT, bilirubin, and relative amounts of monocytes; and decreases in the number of erythrocytes, hematocrit and hemoglobin 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 250 mg/kg bw/d dose group and above. Decreases in absolute organ weights were reported for the heart (500 mg/kg bw/d and above dose groups) and for the spleen (500 mg/kg bw/d group only). Absolute organ weight increased in the adrenals in the 500 mg/kg bw/d and above dose groups. Thyroid weights were increased significantly at 500 and 1000 mg/kg bw/d. The NOEL for Kojic Acid was determined to be 125 mg/kg bw/d in this oral study.[3]

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

The no-observable-adverse-effect level (NOAEL) for maternal toxicity and embryotoxicity was 150 mg/kg/d in an oral two-generation developmental and reproductive toxicity (DART) study in female mice that received 0, 25, 150, or 900 mg/kg/d Kojic Acid in 1% methylcellulose on gestation days 6 - 15.[2] Fetuses of the 900 mg/kg dose group had a slight but significant decrease in the body weights (males), a slight but significant retardation of ossification (males and females), and a significant decrease in ossified calcaneus (males and females). In another two-generation oral study, female mice received 0, 30, 160, or 800 mg/kg on gestation day 15 to postpartum day 21. The dams in the 800 mg/kg/d does group, which had a significant decrease in feed consumption and water intake accompanied by a significant decrease in body weight at the end of gestation through the lactation period had a significantly decreased number of live female pups and total number of live pups at birth compared to controls, and a significant decrease in body weight gain was observed in female weanlings of this dose group. The F_1 mice had decreased relative organ weights and significant retardation of incisor eruption.

Female rats that received 50 µg/d Kojic Acid in propylene glycol on days 1 - 5 post-coitum had significant decreases in implantation sites, a significant increase in litter mortality, and a significant decrease in litter sizes. Cannibalistic behavior towards pups in treated dams was also observed. Significant decreases in the number of implantations occurred, but no decline in the number of corpora lutea were observed. No teratogenic effects were observed in the treated group. In an oral DART study in male and female rats that received 0, 25, 150, or 900 mg/kg/d Kojic Acid in methylcellulose prior to mating (9 wk prior in males and 2 wk prior in females) and through gestation day 7 in females, clinical signs of toxicity were observed in both sexes in the 900 mg/kg/d dose group. In the 900 mg/kg/d dose group, slightly delayed mating and lower values of mean litter size and number of implantations per litter were observed when compared to controls. No treatment-related effects were observed in any treatment group with regard to post-implantation loss, mean fetal weight, or embryonic or fetal development. In an oral developmental study in female rats that received 0, 100, 300, or 1000 mg/kg/d Kojic Acid in 0.5% methylcellulose on gestation days 6 - 17, no clinical signs of toxicity, abnormal macroscopic findings, abortions/resorptions, or death were observed in the dams at any dose level. There were no treatment-related effects on litter parameters, external malformations, or anomalies in fetuses in any dose group. In a fertility study of male rats that received 50 µg/d Kojic Acid in propylene glycol for up to 21 d (rats were mated on days 16 - 21), body weights were significantly decreased in treated males, and weights of the testis and epididymis were also significantly decreased when compared to controls. Implantations and litter sizes were significantly decreased in the treated group, and a loss of litter viability was observed on the second or third day after delivery. Cannibalism of the litter occurred in the dams mated with treated males 2 days after delivery that was not observed in the controls.

Female rabbits were given 0, 20, 100, or 500 mg/kg/d Kojic Acid in 1% methylcellulose via gavage on days 6 - 18 of gestation. No treatment-related effects on litter size, post-implantation loss, litter and mean fetal weights, or embryonic and fetal development were observed.

GENOTOXICITY STUDIES

Kojic Acid (tested at up to 10,000 µg/plate) was genotoxic in Salmonella typhimurium and Escherichia coli in several Ames studies performed with and without metabolic activation.[2] Genotoxicity was observed to Kojic Acid in Chinese hamster ovary (CHO) assays for sister chromatid exchanges and chromosomal aberrations (up to 6 mg/ml, with or without metabolic activation) assay, but genotoxicity was not observed in cell mutation assays in mouse lymphoma L5178 $TK^{+/-}$ cells (up to 1421 µg/ml, with or without metabolic activation) or in Chinese hamster V79 cells (up to 3000 µg/ml). Kojic Acid was a weak clastogen without metabolic activation in a chromosome aberration assay in Chinese hamster V79 cells when tested at up to1420 µg/ml, with and without metabolic activation, but the effects observed may have been related to cytotoxicity. In in vivo mammalian tests, Kojic Acid was not genotoxic in micronucleus tests in mice that received up to 4000 mg/kg bw intraperitoneally, a dominant lethal test in mice (up to 700 mg/kg orally), an unscheduled DNA synthesis test in rats (up to 1500 mg/kg orally), a comet assay in rats (up to 2000 mg/kg orally), or DNA adduct assays (up to 2% in diet). In an oral micronucleus study in both mice (3- and 9-wk old) and rats (9-wk old) with up to 1000 mg/kg Kojic Acid, mean values of micronucleated hepatocytes in 9-wk old mice were increased in a dose-dependent manner, with a significant increase over the negative control at 1000 mg/kg; however, this effect was not observed in 3-wk old mice or in the rats. Kojic Acid was a weak photo-mutagen in a photo-reverse mutation assay in S. typhimurium and E. coli when tested at up to 5000 µg/ml, and in a chromosomal aberration study with light irradiation in Chinese hamster lung cells when tested at up to 1.4 mg/ml.

Irradiation of Kojic Acid, both in a gene mutation assay with E. coli at up to 5000 µg/plate and in a micronucleus study in mice that were treated dermally with a cream containing up to 3% Kojic Acid, did not result in genotoxicity.

Additional in vitro and in vivo genotoxicity studies on Kojic Acid are summarized in Table 3. Negative results were observed in an SOS chromotest using *E. coli* K-12, with or without metabolic activation, at up to 2 mg Kojic Acid.[22] Genotoxicity was not observed in an in vitro micronucleus assay, with or without metabolic activation, in SVK14 human keratinocyte cells at 500-8000 µg/ml Kojic Acid.[3] Genotoxicity results were not clear in another in vitro micronucleus assay using HepG2 human liver cancer cells at 1000 - 8000 µg/ml Kojic Acid.[3] No induction of micronuclei or strand breaks were observed in an in vivo micronucleus assay and comet assay performed in male rats with up to 1000 mg/kg/d Kojic Acid.[23]

CARCINOGENICITY STUDIES

The International Agency for Research on Cancer (IARC) concluded that Kojic Acid is in group 3—not classifiable to human carcinogenicity.[2] Tumorigenic potential of Kojic Acid was observed in the liver, but not in the thyroid follicular epithelial cells in mice that were fed a diet containing up to 3% Kojic Acid for 26 wk; however, up to 1% Kojic Acid was not tumorigenic to mice in a 78-wk dietary study. In a 55-wk rat dietary study of 0.5 or 2% Kojic Acid, the no-observable-adverse-effect level (NOAEL) was determined to be below 0.5%. Changes in the liver, thyroid glands, and adrenal glands, including diffuse follicular cell hyperplasia in the thyroid glands in both treatment groups and adenomas and/or carcinomas in the 2% group, were observed.

Liver tumors observed in rodents often arise through a mode of action involving activation of the constitutive androstane receptor (CAR), leading to hepatocellular proliferation via enzyme induction.[24] However, this mechanism is not considered relevant to humans, as CAR activators do not induce the same proliferative response in human hepatocytes. Adrenal pheochromocytomas reported in rat studies are also generally considered not relevant to human cancer risk.[25] These tumors are frequently induced via secondary, non-genotoxic mechanisms such as sustained endocrine stimulation or mitochondrial dysfunction, which are not reflective of processes in humans.

Tumor Promotion and/or Tumor Initiation

Kojic Acid did not possess initiation or promotion potential for skin carcinogenesis in mice.[2] Several studies on mice and rat liver found Kojic Acid to have carcinogenesis-promoting potential but not an initiation potential. More robust summaries of these data may be found in Table 4.

Thyroid Effects

Studies on the effect of Kojic Acid on rodent thyroids found the chemical inhibits iodine uptake and organification in the thyroid, which causes a proliferative effect.[2] More robust summaries of these data may be found in Table 5.

In a 4-wk dietary study, groups of 8 male F344 rats received Kojic Acid at 0, 0.008, 0.03, 0.125, 0.5, 2.0% (0, 5.85, 23.8, 95.3, 393.6, 1387.3 mg/kg bw/d, respectively).[26] Blood samples were taken from 5 rats at the end of treatment period for hormone analysis and animals were necropsied. Histopathological examination of thyroid and pituitary tissues was performed. The remaining animals injected intraperitoneally with 0.4 ml of 0.1 M $Na^{125}I$ in saline 24 h prior to being killed for measurement of ^{125}I uptake and organification in the thyroid. There were no significant intergroup differences in the final body weights. Absolute and relative thyroid weights were increased in a dose-dependent manner in all treatment groups, with statistical significance at 0.5 and 2% Kojic Acid. Pituitary and liver relative weights also significantly increased compared to the control. ^{125}I uptake decreased in a dose-dependent manner, with statistical significance starting at 0.03% and higher. In addition, a significant reduction of organic formation of iodine and serum T3 and T4 levels were observed in the 2% Kojic Acid group along with a significant increase of TSH. Histopathologically, decreased colloid in the thyroid follicles and follicular cell hypertrophy in the thyroid were apparent at high incidences in the groups that received 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.

Kojic Acid has been considered to have no genotoxic potential in vivo; evidence indicates that humans are less sensitive than rodents with regard to perturbation of thyroid hormone homeostasis.[3] Rodent thyroid follicular cell tumors are often associated with sustained elevations in thyroid-stimulating hormone (TSH), leading to hypertrophy and tumor formation.[27] However, these effects are species-specific, as rodents exhibit a shorter half-life of thyroxine (T4) and heightened TSH responsiveness compared to humans. There are currently no compound-specific quantitative data available to substantiate the assertion that Kojic Acid could interfere with thyroid hormone homeostasis in humans. Reflecting these differences, both the IARC and the US EPA have emphasized that rodent thyroid tumorigenesis should be interpreted with caution when extrapolating to humans, as the underlying mechanisms are not predictive of human thyroid cancer risk. concluded that 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.[28]

The effects of Kojic Acid on thyroid function were studied in cultured rat FRTL-5 thyroid cells and by a single-dose oral administration in rats.[29] In the thyroid cells, Kojic Acid inhibited iodine organification dose-dependently, but did not inhibit iodine uptake. In rats receiving a single dose of 1000 mg/kg Kojic Acid, the ^{125}I uptake from blood into the thyroid

gland was significantly lower than that of the control group from 30 min to 24 h after administration. The ^{125}I organification activity of the Kojic Acid groups was significantly lower than controls from 30 min to 6 h after administration; however, this activity 24 or 48 h after administration recovered enough to be nearly comparable with the control group. These results suggest that the observed lower iodine uptake activity in the single-dose administration study in rats was due to the inhibition of iodine organification caused by Kojic Acid, which decreased iodine in the entire thyroid gland. Although serum T4 showed a tendency to decrease from 2 to 48 h after oral administration of Kojic Acid, serum TSH did not show any evident change associated with the administration of Kojic Acid in rats.

OTHER RELEVANT STUDIES

Cytotoxicity

The effect of Kojic Acid (4.22, 8.02, or 12.67 mM) on human liver HepG2 cells was assessed using the methyl thiazol tetrazolium (MTT) and crystal violet cell viability assays.[30] Kojic Acid significantly reduced cell viability in a dose-dependent manner following treatment up to 48 h, with an IC_{50} of 8.02 mM. A significant loss in cell viability was seen at 4.22 and 12.67 mM. After 24 h treatment, caspase - 3/7, -8, and -9 activities were all increased in HepG2 cells at 4.22 mM and 8.02 mM. The effect of Kojic Acid on the integrity of cell membrane was assessed by lactate dehydrogenase (LDH) assay, with LDH leakage observed at 12.67 mM.

The study further examined the impact of Kojic Acid on oxidative stress and antioxidant responses. Kojic Acid significantly increased the production of extracellular malondialdehyde (MDA), a by-product of lipid peroxidation, while significantly reducing protein carbonyl levels. However, 8-OHdG levels remain unchanged. The expression of antioxidant response proteins, Nrf2 and p-Nrf2, and catalase, a component in the detoxification of peroxides, were significantly downregulated. The gene expression of glutathione peroxidase was increased at 4.22 mM (1.51-fold) and 8.02 mM (1.07-fold), but unchanged at 12.67 mM. *NFκB* gene expression was decreased at 8.02 mM (0.24-fold), indicating a significant suppression of the NFκB inflammatory pathway. Mitogen-activated protein kinases including JNK 1/2 and p38 were also evaluated by Western blot: Kojic Acid significantly decreased JNK 1/2 expression but increased p38 expression at 4.22 and 8.02 mM. At the treated doses, no DNA or protein damage were detected. The researchers concluded that Kojic Acid exhibited minimal toxicity to HepG2 cells.[23]

Hepatic Effects

The effect of Kojic Acid on the expression of cytochrome P450 isozymes was studied in 4 groups of 4 male F344 rats.[31] The rats received 0.6, 3, or 1875 mg/kg bw Kojic Acid for 14 d. Kojic Acid at 1875 mg/kg bw significantly decreased body weight and serum T4 levels, but increased liver and thyroid gland weights. Protein expression of CYP2B1, CYP2E1, and CYP2C11 in liver tissues were analyzed by Western blotting. The pattern of CYP2B1 protein expression in the livers of Kojic Acid-treated rats showed that low-dose Kojic Acid significantly decreased the level of expression, but medium and high doses of Kojic Acid significantly increased CYP2B1 expression. Kojic Acid also decreased CYP2E1 expression in rat livers, and CYP2C11 was significantly decreased in livers of the rats that received the high dose of Kojic Acid.

Effects on Keratinocytes

Primary human melanocyte and keratinocyte co-culture systems were used to evaluate whether Kojic Acid induced changes in keratinocytes were associated with anti-melanogenic activities in melanocytes.[32] The cytokine secretion profiles in response to Kojic Acid (0.2 mM) were analyzed. Kojic Acid was found to have increased interleukin (IL)-6 and IL-8 production in melanocyte/keratinocyte co-cultures; however, only IL-6 directly suppressed melanogenesis while IL-8 did not. Kojic Acid did not increase IL-6 production in melanocyte monocultures; whereas in keratinocyte monoculture it significantly up-regulated IL-6 gene and protein expression. Anti-IL-6 antibody treatment antagonized the anti-melanogenic effect of Kojic Acid on the co-cultures. The authors concluded that anti-melanogenic activity of Kojic Acid on hyper-pigmented skin was associated with the Kojic Acid-induced IL-6 production in keratinocytes.

Tyrosinase Effects

Because of its well-documented ability to inhibit tyrosinase activity, Kojic Acid has been used in numerous studies as a positive control.[2]

The effects of Kojic Acid on melasma were studied using human induced pluripotent stem (iPS) cell-derived melanocytes.[33] The melanocytes were cultured in human iPS cell-derived melanocyte medium (iDMM). Kojic Acid (70 µg/ml) was directly added to iDMM. Microscopic analysis did not show any differences between human iPS cell-derived melanocytes treated with Kojic Acid when compared to untreated controls. The effects of Kojic Acid on tyrosinase activity were then investigated in human iPS cell-derived melanocytes. The addition of 70 µg/ml Kojic Acid for 2 wk had no effect on tyrosinase activity when compared to untreated controls.

Dermal Depigmentation

The depigmenting effects of Kojic Acid were studied in a black guinea pig study.[2] *Kojic Acid at 0.1 ml was applied at concentrations of 1 and 4% (w/v) in a 1:4 mixture of dimethyl sulfoxide and ethanol to the shaved dorsal area (4 x 4 cm^2 or 4 x 3 cm^2) of 4 JY-4 black guinea pigs. The vehicle alone was also tested. The test substance was applied once a day, 6 d/wk, for 5 successive weeks. After the application period had ended, the animals were killed and skin samples were prepared for*

examination. The depigmentation action was evaluated by macroscopic observation and spectrophotometric colorimetry. Optical and electron microscopy of epidermal melanocytes were also performed for morphological examination. The mechanism for which skin whitening occurs was also investigated by measuring oxygen consumption and the relation of free radicals to melanin synthesizing enzyme tyrosinase.

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

Toxicogenomics

In a gene expression study, human skin A375 malignant melanoma cells were cultured with 0. 0.32, 1.6, 8. 40, 200, or 1000 µg/ml Kojic Acid for 72 h.[34] Total RNA was quantified in cells exposed to 8 µg/ml Kojic Acid for 24 h. Cell growth was observed to be inhibited in a dose-dependent manner by Kojic Acid (20% for 0.32 - 40 µg/ml and ~ 40% for 1000 µg/ml). A total of 361 differently expressed genes were distinctively changed with 136 up-regulated and 225 down-regulated. Category classification of differentially expressed genes was conducted. Seven of the downregulated genes (APOBEC1, ARHGEF16, CD22, FGFR3, GALNT1, UNC5C and ZNF146) were identified as tumor suppressor genes in melanoma cancer cells.

Immunomodulatory Effects

The effects of Kojic Acid (50 µg/ml) on functional properties related to macrophage activation were assessed in an in vitro study.[35] Macrophages incubated for 1 h with Kojic Acid had enhanced cell spreading and an increase in cell surface exposure, associated with a rearrangement of microtubules, actin filaments, and intermediate filaments. Kojic Acid also potentiated phagocytosis by macrophages, as seen by the increase in phagocytic activity towards yeast, when compared to untreated cells. Reactive oxygen species production was increased in the presence of Kojic Acid, but not nitric oxide production. Cell viability of macrophages was furthermore not affected following Kojic Acid treatment.

The ability of Kojic Acid (50 µg/ml) to influence innate immune responses was studied in vitro using human peripheral blood monocytes.[36] Following a 48-h exposure, Kojic Acid 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 a 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. It was concluded that Kojic Acid promotes the differentiation of monocytes into macrophages, and thus has the ability to act as an immunomodulatory agent.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Kojic Acid at 1 and 3% was a mild dermal irritant in rabbits.[2] *In guinea pigs, 30% Kojic Acid was not a dermal sensitizer.*

In a human repeated insult patch test (HRIPT), subjects received 24-h occlusive induction applications of a cream product containing 1% Kojic Acid (amount sufficient to cover ¾ in^2 pad) on the same previously untreated site on the back 3 times/wk for 3 wk. Following a 2-wk non-treatment period, the challenge application was applied to a previously untreated site for 24 h, and the site was scored 24 and 72 h after patch removal. No responses were observed during either the induction or challenge tests. In another HRIPT study, the potential of a formula containing 2% Kojic Acid (0.2 mg on a 2 cm^2 pad) to induce sensitization was evaluated using 218 subjects. The test sites were semi-occluded. The patches were removed after 24 h, and the test sites were evaluated after 48 or 72 h. After a 2-wk non-treatment period, the subjects received challenge applications on previously untreated sites for 24 h, and the test sites were evaluated after 48 or 72 h. During the induction phase, 11 minimal or doubtful ("?") responses and 4 definite erythema ("+") responses were observed. Only one minimal or doubtful response was observed at 48 h, but was resolved at 72 h.

Phototoxicity

Slight skin reactions with 5 consecutive daily exposures of 15 or 30 min to UV light (5 Blacklite tubes of 15 W each; 350 nm) were observed in guinea pigs that received 5% Kojic Acid dermally.[2] *No phototoxicity was observed in guinea pigs that received 5 consecutive daily exposures of 38 min to UV light (10 Blacklite tubes; 320 nm) following topical treatments of 1 or 3% Kojic Acid.*

OCULAR IRRITATION STUDIES

Kojic Acid in a 3% aqueous solution was not an ocular irritant in rabbit eyes.[2]

CLINICAL STUDIES

Kojic Acid is reportedly used to treat melasma.[2] *In an efficacy test of formulations containing 2% Kojic Acid and glycolic acid (with or without hydroquinone) for the treatment of melasma in 39 patients for a month, burning and desquamation were reported in all patients. In another efficacy study in 40 Chinese women with a formulation containing 2% Kojic Acid, 10% glycolic acid, and 2% hydroquinone, patients experienced redness, itchiness, and exfoliation on the treatment sites, although these results were also observed on skin that was not treated with Kojic Acid. Another therapeutic study reported that the side effect of the treatment of melasma with 1% Kojic Acid was contact allergy.*

The efficacy of a topical therapy for the treatment of dyschromia was evaluated in a 12-wk clinical study in 55 healthy female patients with Fitzpatrick skin types I-IV.[15] At least 50% of the subjects had self-perceived sensitive skin. The topical therapy contained 1% Kojic Acid, 3% transexamic acid, 5% niacinamide, and 5% hydroxyethylpiperazineethane sulfonic acid. The test material was applied to clean facial skin twice daily. In addition to the efficacy parameters tested, the subjects were assessed for irritation. Minor transient effects such as erythema (2/55), itching (1/55), pruritis (1/55), redness (1/55) or stinging (1/55) were observed during the study. These effects quickly resolved after application of the test material.

Retrospective and Multicenter Studies

Of 220 female patients patch tested for suspected cosmetic-related contact dermatitis, 5 had reactions to Kojic Acid as well as products they owned that contained 1% Kojic Acid.[2] *The 5 patients had developed facial dermatitis within 1 to 12 mo of using Kojic Acid-containing cosmetic products. Reactions to 1 and 5% Kojic Acid in these patients were + and ++, respectively. The remaining 215 patients in the patch test group, including 3 that had previous exposures to Kojic Acid, did not have any reactions to Kojic Acid.*

Case Reports

A 30-yr-old woman was prescribed a cream containing 3% Kojic Acid, urea, hydroquinone, lactic acid, witch hazel, castor oil, citric acid, cellulose, and propylene glycol to treat hyperpigmentation.[2] *More than 4 mo later and a few weeks after a second medication was prescribed to use in conjunction with the cream, the patient presented with eczematous eruption on and around the hyperpigmentation. Patch tests with the Grupo Español de Investigación Dermatitis de Contacto y Alergia Cutánea (GEIDAC) series were negative, but a patch test of the cream was ++ after 4 d. Patching with 0.1, 0.5, 1, and 5% Kojic Acid resulted in positive results after 2 and 4 d, with a ++ reaction to 1 and 5% Kojic Acid. Patch tests of other components were negative. In another case report, a 54-yr-old woman with actinic lentigines on her arms and forearms developed pigmented contact dermatitis. The patient had been using a formulation similar to the one described above with 3% Kojic Acid. One year prior to presentation, the patient experienced progressive, asymptomatic erythematous and hyperpigmented area on her arms, but continued to use the formulation. A biopsy showed pigmentary incontinence, melanophagia, and moderate lymphohistiocytic infiltrate without a spongiotic epidermis. Patch tests with GEIDAC series, disperse dyes, and photopatch tests were negative. Patch tests with 1% Kojic Acid (aq.) and the compound "as is' were negative on day 2, but hyperpigmentation was present at both sites on day 4 and 7. These lesions persisted for 1 mo.*

A 40-yr-old woman developed acute dermatitis on the face and neck following application of a makeup product containing Kojic Acid on pigmented areas of her skin for 3 d.[37] The patient was patch tested with the European standard series, the makeup product, and individual components of the product, including 0.5, 1, and 3% Kojic Acid (pet.). Positive reactions were obtained for the makeup product (+++), Kojic Acid (0.5% ++, 1% +++, and 3% +++), lactic acid (++), and to polysorbate 80 (++) after 2 and 4 d. No reactions were observed to the other ingredients of the makeup product. Negative results were observed in 10 control patients tested. Further patch testing after 3 wk with lactic acid and polysorbate 80 were negative.

In another case report, a 54-yr-old woman presented with facial erythema associated with itching and a burning sensation.[38] The patient had been applying a serum to her face for approximately 1 mo prior. The patient was patch tested with the standard series recommended by GEIDAC and the serum. The 48- and 96-h readings were positive (+++) for the serum only. The patient was later tested with the individual ingredients of the serum, including 1% Kojic Acid in a water solution. No reactions were observed at 48 h, but a strong positive reaction (++) to Kojic Acid was observed at 96 h. The reaction persisted for 7 d. No reactions were observed to the other ingredients. No reactions were observed to 1% Kojic Acid solution in 12 control patients.

RISK ASSESSMENT

MOE is a quantitative factor calculated for cosmetic ingredients by dividing the NOAEL obtained for an ingredient in an animal experiment by the estimated SED for the ingredient in humans, generally according to US EPA and EC SCCS guidelines. The standard MOE value of 100 is derived from multiplying two factors: a 10-fold factor for extrapolating data from test animals to human beings (interspecies extrapolation) and an additional 10-fold for differences among the human population (intra-species extrapolation). An MOE value greater than 100 has traditionally been considered an indication of safety. The MOE is sometimes referred to as margin of safety (MOS), despite the parameters being definitionally different.

The values used to calculate the MOEs described here are summarized in Table 6. The SCCS calculated MOE values for Kojic Acid at 1% in a cream product for application scenarios on the face and neck, the hands, and an aggregate of these

applications (face + neck + hands).[3] The MOE values were 267, 199, and 141, respectively (the value for the hands appear to be a typographical error in the SCCS opinion and should be 299[39, CIR Staff]). These calculations are based on an adjusted NOAEL of 2 mg/kg bw/d. This value was derived from a 28-d oral repeated dose study in rats, which identified histological changes in the thyroid and altered iodine uptake at a NOAEL of 6 mg/kg bw/d.[26,40] To account for the subacute duration of the study, an assessment factor of 3 was applied to extrapolate to a subchronic (90-d) exposure scenario.[3] The SED for the application areas of face and neck, the hands, and the combination were 0.0075, 0.0067, and 0.0142 mg/kg bw/d, respectively. The SED was determined based on a human percutaneous absorption study involving a single application of 500 mg of a cream containing 1% Kojic Acid to the entire face. The internal exposure was derived by taking the 95th percentile of the $AUC_{0\text{-}24\ h}$ of 32.4 ng/ml·h, with a mean AUC of 19.4 ng/ml·h.[41] Assuming a plasma volume of 60 ml/kg bw and a mean body weight of 48 kg (as measured in the study), the total amount of Kojic Acid measured in plasma over 24 h after face exposure was calculated as:

32.4 ng/ml × 60 ml/kg bw × 48 kg = 0.093 mg/d.

The standard daily exposure to face cream is 1.54 g/d for a surface area of 565 cm².[42] Including the neck area (additional 320 cm²) increases the total surface area to 885 cm², resulting in an adjusted exposure of 2.41 g/d. At 1% Kojic Acid concentration, this corresponds to 24.1 mg/d, which is 4.82 times higher than the 5 mg used in the percutaneous absorption study. Assuming linearity between dose and systemic exposure, the internal exposure is scaled accordingly: 0.093 mg/d × 4.82 = 0.448 mg/d, resulting in an SED of 0.0075 mg/kg bw/d.

For hands exposure and combined exposures involving the face, neck, and body, similar calculations were performed based on standard daily application amounts and proportional adjustments. Details for these exposure scenarios are presented in Table 6. Notably, using the same NOAEL values and estimated daily amounts of applied cream for body and whole body (face + body) surface areas, the SEDs were determined to be to 0.0242 and 0.0558 mg/kg bw/d, respectively.[39,43] These correspond to MOEs for the body and whole body of 83 and 36, respectively, which are below the value of 100, indicating a potential health risk under high exposure conditions.

SUMMARY

Kojic Acid is reported to function as an antioxidant in cosmetics, according to the *Dictionary*. The Panel first reviewed the safety of Kojic Acid in a report published in 2010 with the conclusion "Kojic Acid is safe for use in cosmetic products up to a concentration of 1%."[2] In accordance with its Procedures, the Panel evaluates the conclusions of previously-issued reports approximately every 15 years, and it has been at least 15 years since this assessment has been issued. In June 2022, the Panel determined that this safety assessment should be re-opened for re-evaluation due to reported restrictions by the European Commission on the use of Kojic Acid.

According to RLD submitted in 2024, Kojic Acid is reported to be used in 1114 formulations. The 2023 VCRP data reported use in 123 formulations, most of which were leave-on products. In the 2010 original report, Kojic Acid was reported in 16 formulations, most of which were in leave-on products. The results of the concentration of use survey conducted by the Council in 2024 indicate Kojic Acid is used at up to 1% in leave-on skin care preparations. In 2008, the maximum concentration of use for Kojic Acid was reported to be 2% in leave-on skin preparations.

In the EU, the SCCS concluded in 2022 that Kojic Acid is safe when used as a skin lightening agent in cosmetic products at concentrations of up to 1%. (It should be noted that in Europe, products that whiten the skin are considered to be cosmetics; however, in the US, skin lightening is considered to be a drug effect.) The EC has categorized Kojic Acid in Annex III, the list of substances which cosmetic products must not contain except subject to the restrictions laid down. For this ingredient, the regulation states that Kojic Acid may only be used in face and hand products at a maximum concentration of 1%. Based on the SCCS opinion and MOE calculations, the EC determined that high exposure to Kojic Acid (when the concentration exceeds certain levels form applications beyond those to the face and hands), may increase health risk.

The percutaneous absorption of a leave-on skin care formulation containing radiolabeled ^{14}C-Kojic Acid at 1% was studied using human dermatomed skin. Mean recovery of the applied test material was 95.48 ± 3.33% (19.44 ± 0.68 µg/cm^2). The average penetration rate was 0.006 µg/cm^2/h. The mean amount of Kojic Acid that penetrated over the 24 h exposure period was 0.698% of the applied dose (0.142 ± 0.265 µg/cm^2). The mean total systemically available dose of Kojic Acid (remaining epidermis + dermis and receptor fluid) was 3.68% of the applied dose (0.749 µg/cm^2).

In a 30-d dermal toxicity study in rabbits, 0, 13, 130, or 1300 mg/kg bw/d (0, 0.65, 6.5, or 65% w/v) Kojic Acid in 1% aqueous methylcellulose was applied to abraded skin daily. Effects on the skin and eyes could not be accurately evaluated due to a bacterial infection in the animals. Necropsy of 3 animals that died during the course of the study (1 female in the 13 mg/kg group, 1 male in the 130 mg/kg group, and 1 male control) revealed lesions of the lung, liver, kidney, and brain that may have been contributing factors. In the 1300 mg/kg bw/d dose group, statistically significant changes were observed in the mean corpuscle hemoglobin concentration, the mean cell volume, and A/G ratio when compared to controls. In the 13 mg/kg bw/d dose group, the pituitary weight was significantly increased. A conclusion of dose-dependent statistical changes for hematology and biochemistry could not be made because parameters were only measured in the controls and the 1300 mg/kg dose groups. Plaques in the aorta were reported in 1 control male, 3 males and 1 female in the 13 mg/kg bw/d dose

group, 1 male and 1 female in the 130 mg/kg bw/d dose group, and 4 males in the 1300 mg/kg bw/d dose group. Pale kidneys were reported in all treated groups.

The NOEL for Kojic Acid in a 26-wk oral toxicity study in rats was 125 mg/kg bw/d. Male rats received 0, 125, 250, 500 or 1000 mg/kg bw/d Kojic Acid in 1% aqueous solution of carboxymethylcellulose and a 5 wk-recovery occurred in the 3 highest does groups. Statistically significant hematological and biochemical differences reported included an increase in creatinine in the 250 and 500 mg/kg bw/d groups; an increase in ALP values in the 500 and 1000 mg/kg bw/d groups; increases in GOT, GPT, bilirubin, and relative amount of monocyte; and decreases in number of erythrocytes, hematocrit and hemoglobin 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 250 mg/kg bw/d dose group and above. Decrease in absolute organ weights were reported for the heart (500 mg/kg bw/d and above dose groups) and for the spleen (500 mg/kg bw/d group only). Absolute organ weight increased in the adrenals in the 500 mg/kg bw/d and above dose groups. Thyroid weights were increased significantly at 500 and 1000 mg/kg bw/d.

Negative results were observed in an SOS chromotest using *E. coli* K-12, with and without metabolic activation, at up to 2 mg Kojic Acid. Genotoxicity was not observed in an in vitro micronucleus assay, with and without metabolic activation, in SVK14 human keratinocyte cells at 500-8000 µg/ml Kojic Acid. Genotoxicity results were not clear in another in vitro micronucleus assay using HepG2 human liver cancer cells at 1000-8000 µg/ml Kojic Acid. No induction of micronuclei or strand breaks were observed in an in vivo micronucleus assay and comet assay in performed in male rats with up to 1000 mg/kg/d Kojic Acid.

The effects of Kojic Acid on thyroid function in cultured rat thyroid cells found that Kojic Acid inhibited iodine organification dose-dependently, but did not inhibit iodine uptake. In rats receiving a single dose of 1000 mg/kg Kojic Acid, the ^{125}I uptake from blood into the thyroid gland was significantly lower than that of the control group from 30 min to 24 h after administration, and the ^{125}I organification activity of the Kojic Acid groups was significantly lower than controls from 30 min to 6 h after administration. This activity 24 or 48 h after administration recovered enough to be nearly comparable with the control group.

Kojic Acid (4.11, 8.02, or 12.67 mM) significantly reduced cell viability in human liver HepG2 cells in a dose-dependent manner following treatment up to 48 h, with an IC_{50} of 8.02 mM. Further examination of the impact of Kojic Acid on oxidative stress and antioxidant responses found that this ingredient exhibited minimal toxicity to HepG2 cells.

In rats, Kojic Acid at 1875 mg/kg bw significantly decreased body weight and serum T4 levels, but increased liver and thyroid gland weights. The pattern of CYP2B1 protein expression in the livers of Kojic Acid-treated rats showed that 0.6 mg/kg bw Kojic Acid significantly decreased the level of expression, but 3 and 1975 mg/kg bw of Kojic Acid significantly increased CYP2B1 expression. Kojic Acid also decreased CYP2E1 expression in rat livers, and CYP2C11 was significantly decreased in livers of the rats that received 1875 mg/kg bw Kojic Acid.

Kojic Acid (0.2 mM) was found to have increased IL-6 and IL-8 production in melanocyte/keratinocyte co-cultures; however, only IL-6 directly suppressed melanogenesis while IL-8 did not. Kojic Acid did not increase IL-6 production in melanocyte monocultures; whereas in keratinocyte monoculture it significantly up-regulated IL-6 gene and protein expression. Anti-IL-6 antibody treatment antagonized the anti-melanogenic effect of Kojic Acid on the co-cultures.

In a study using human iPS cell-derived melanocytes, microscopic analysis did not show any differences between human iPS cell-derived melanocytes treated with Kojic Acid when compared to untreated controls. The addition of 70 µg/ml Kojic Acid to the melanocytes for 2 wk had no effect on tyrosinase activity when compared to untreated controls.

In a gene expression study with human skin A375 malignant melanoma cells cultured with 0. 0.32, 1.6, 8. 40, 200, or 1000 µg/ml Kojic Acid, cell growth was observed to be inhibited in a dose-dependent manner. A total of 361 differently expressed genes were distinctively changed with 136 up-regulated and 225 down-regulated. Seven of the downregulated genes were identified as tumor suppressor genes in melanoma cancer cells.

Macrophages incubated for 1 h with Kojic Acid (50 µg/ml) had enhanced cell spreading and an increase in cell surface exposure, associated with a rearrangement of microtubules, actin filaments, and intermediate filaments. Kojic Acid also potentiated phagocytosis by macrophages, as seen by the increase in phagocytic activity towards yeast, when compared to untreated cells. Reactive oxygen species production was increased in the presence of Kojic Acid, but not nitric oxide production. Cell viability of macrophages was furthermore not affected following Kojic Acid treatment. Following a 48-h exposure, Kojic Acid (50 µg/ml) induced morphological alterations in human peripheral blood monocytes, such as increased cell size, as well as numerous cellular projections. Kojic Acid exposure was found to increase IL-6 cytokine production, but did not cause cytotoxic effects in monocytes.

In an efficacy study evaluating a topical therapy containing 1% Kojic Acid for the treatment of dyschromia, minor transient irritation effects were observed. Case studies in 2 women reported acute dermatitis to the face following application of skin products that contained Kojic Acid; patch testing yielded positive results to 0.5 to 3% Kojic Acid.

The SCCS calculated MOE values for Kojic Acid at 1% in a cream product for application scenarios on the face and neck, the hands, and an aggregate of these applications (face + neck + hands). The MOE values were 267, 199, and 141,

respectively (the value for the hands appears to be a typographical error in the SCCS opinion and should be 299). Further calculations using the same NOAEL values and using estimated daily amounts of applied cream for body and whole body (face + body) surface areas resulted in MOE values of 83 and 36, respectively, which are well below the value of 100, indicating a potential for health risks when used at high exposures.

DISCUSSION

[Note: This Discussion is in draft form, and changes will be made following the Panel meeting.]

In accordance with its Procedures, the Panel re-evaluates the conclusions of previously-issued reports approximately every 15 years. In 2010, the Panel published a final report on Kojic Acid and concluded that Kojic Acid is safe for use in cosmetic products up to a concentration of 1%. This report was reopened for re-evaluation due to reported restrictions by the EC on the use of Kojic Acid. This assessment is an amended report that reviews the safety of Kojic Acid as used in cosmetic formulations, in accordance with the product categories and concentrations of use identified in the Use section and Use table. The Panel concluded [to be determined].

The Panel noted that liver, thyroid, and adrenal tumors were observed in rodent studies. However, these effects are considered not relevant to human cancer risk due to well-characterized species differences in mode of action. Liver tumors are associated with CAR-mediated enzyme induction, which is a rodent-specific mechanism not operative in humans. Thyroid effects are linked to disruption of hormone homeostasis, to which rodents are particularly sensitive due to differences in thyroid hormone metabolism and regulation. Adrenal tumors observed in rats also involve mechanisms not predictive of human risk. Given these species-specific responses, the Panel stated that the observed tumorigenic effects do not indicate a significant carcinogenic risk to humans under cosmetic use conditions.

Data included in this report indicate that Kojic Acid may have a skin lightening effect. The Panel noted that skin lightening is considered a drug effect in the US and should not occur during the use of cosmetic products. Because of that caveat, the Panel's knowledge of the mechanism of action (i.e., inhibition of tyrosinase activity), and clinical experience, concern for this effect in cosmetics was mitigated. Nevertheless, cosmetic formulators should only use this ingredient in products in a manner that does not cause depigmentation.

The Panel expressed concern regarding heavy metals that may be present in this ingredient. They stressed that the cosmetics industry should continue to use the necessary procedures to minimize impurities in cosmetic formulations according to limits set by the US FDA and EPA.

The Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings) notes that airbrush technology presents a potential safety concern. Although frequency and/or concentration of use data are now available (and in some cases mandated) for ingredients marketed for use with airbrush delivery systems in certain product categories, no data are available for consumer habits and practices thereof, product particle size, or other relevant particle data (e.g., diameter). As a result of deficiencies in these critical data needs, the data profile is incomplete, and the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Accordingly, the Panel has concluded the data are insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

To be determined.

TABLES

Table 1. Chemical properties

Property	Value	Reference
Physical Form	Prismatic needles from acetone, ethanol + ether, or methanol + ethyl acetate White to light yellow crystalline powder	2 3
Molecular Weight (g/mol)	142.11	2
Density (g/ml)	1.542	3
Melting Point (ºC)	152-154	2
Water Solubility (g/l)	43.85 Soluble in water	3 2
Other Solubility	Soluble in ethanol, acetone; sparingly soluble in ether, ethyl acetate, chloroform, pyridine; insoluble in benzene	2
log K_{ow}	-0.64 -1.25	3 2
Disassociation constants (pKa @ 25 ºC) (pKa)	7.66 7.90, 8.03	3 2
UV Absorption (λ nm)	270 in water 215-216 and 268-269 in acidic or neutral solutions; 226-227 and 309-312 in alkaline solution 280 (pH of solution not reported)	3 2 2

Table 2. Frequency (RLD/VCRP) and concentration of use of Kojic Acid according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*	
	RLD (2024)[9]	**VCRP (2023)[10]**	**VCRP (2009)[2]**	**% (2024)[11]**	**% (2008)[2]**
Totals*	**1114**	**123**	**16**	**0.001-1**	**0.1-2**
summarized by likely duration and exposure**					
Duration of Use					
Leave-On	***	*87*	*12*	*0.005-1*	*0.1-2*
Rinse-Off	***	*33*	*4*	*0.05*	*NR*
Diluted for (Bath) Use	***	*3*	*NR*	*0.001*	*NR*
Exposure Type					
Eye Area	***	1	NR	NR	0.1-1
Incidental Ingestion	***	NR	NR	NR	NR
Incidental Inhalation-Spray	***	32[a]; 26[c]	4[a]	NR	NR
Incidental Inhalation-Powder	***	26[c]	2[b]	0.005-1[b]	1-2[b]
Dermal Contact	***	123	16	0.001-1	0.1-2
Deodorant (underarm)	***	2[a]	NR	NR	NR
Hair - Non-Coloring	***	NR	NR	NR	NR
Hair-Coloring	***	NR	NR	NR	NR
Nail	***	NR	NR	NR	NR
Mucous Membrane	***	26	2	0.001-0.05	NR
Baby Products	***	NR	NR	NR	NR
as reported by product category					
Baby Products	***5***				
Baby Shampoos	1	NR	NR	NR	NR
Baby Lotions/Oils/Powders/Creams	3	NR	NR	NR	NR
Baby Wipes	1	NA	NA	NR	NA
Bath Preparations	***69***				
Bath Oils, Tablets, and Salts	9	NR	NR	0.001	NR
Bubble Baths	2	NR	NR	NR	NR
Bath Capsules	1	NR	NR	NR	NR
Other Bath Preparations	61	3	NR	NR	NR
Eye Makeup Preparations (not children's)	***25***				
Eye Lotion	13	NR	NR	NR	0.1-1
Other Eye Makeup Preparations	12	1	NR	NR	NR
Hair Preparations (non-coloring)	***3***				
Tonics, Dressings, and Other Hair Grooming Aids	3	NR	NR	NR	NR
Makeup Preparations (not eye; not children's)	***7***				
Blushers and Rouges (all types)	1	NR	NR	NR	NR
Face Powders	6	NR	NR	NR	NR
Manicuring Preparations	***4***				
Other Manicuring Preparations	4	NR	NR	NR	NR
Personal Cleanliness	***303***				
Bath Soaps and Body Washes	274	22	1	0.05	NR
Deodorants (underarm)	1 (not aerosol)	2	NR	NR	NR
Douches	1	NR	NR	NR	NR

Table 2. Frequency (RLD/VCRP) and concentration of use of Kojic Acid according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*	
	RLD (2024)[9]	**VCRP (2023)[10]**	**VCRP (2009)[2]**	**% (2024)[11]**	**% (2008)[2]**
Disposable Wipes	12	NA	NA	NR	NA
Other Personal Cleanliness Products	9 (l.o.) 16 (r.o.)	1	1	NR	NR
Shaving Preparations	***2***				
Aftershave Lotions	1	NR	NR	NR	NR
Other Shaving Preparation Products	1	NR	NR	NR	NR
Skin Care Preparations	***742***				
Cleansing	172	10	2	NR	NR
Face and Neck (excluding shaving preparations)	312 (l.o) 62 (r.o.)	19	2	0.01-1 (l.o.; not spray)	2
Body and Hand (excluding shaving preparations)	44 (l.o.) 13 (r.o.)	7	NR	0.005-1 (l.o,; not spray)	1
Moisturizing	184	24	2	0.01	NR
Night	17	2	NR	NR	NR
Paste Masks (mud packs)	26	NR	NR	NR	NR
Skin Fresheners	49	6	2	NR	NR
Other Skin Care Preparations	55 (l.o.) 33 (r.o.)	26	6	NR	NR
Suntan Preparations	***3***				
Suntan Gels, Creams, and Liquids	3	NR	NR	NR	NR
Other Preparations (i.e., those preparations that do not fit another category)	***21***	NA	NA	0.66[d]	NA

NR – not reported; NA – not applicable (this category was not part of the VCRP)
l.o. – leave-on; r.o. – rinse-off
*The total FOU provided for RLD refers to the ingredient count supplied by FDA, and is not a summation of the number of uses per category because each product may be categorized under multiple product categories. For data supplied via the VCRP or by the Council survey, the sum of all exposure types may not equal the sum of total uses because each ingredient may be used in cosmetics with multiple exposure types.
**Likely duration and exposure are derived from VCRP and survey data based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)
*** In the RLD each ingredient may be reported under several product categories, making a summation of RLD misleading in comparison to VCRP data. Accordingly, RLD are presented below by product category (as supplied by FDA), but are not summarized by likely duration and exposure. [a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
[b] It is possible these products are powders, but it is not specified whether the reported uses are powders.
[c] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
[d] Facial toner used before applying facial peel

Table 3. Genotoxicity studies of Kojic Acid

Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
		IN VITRO			
1 - 2 mg	water	*E. coli* K-12	SOS chromotest; with and without metabolic activation	Negative	[22]
500 – 8000 µg/ml	Not reported	SVK14 human keratinocyte cells	Micronucleus assay, with and without metabolic activity; no further details provided	Not genotoxic, no further details provided	[3]
1000 – 8000 µg/ml	Not reported	HepG2 human liver cancer cells	Micronucleus assay, without metabolic activity; no further details provided	Results uncertain; positive at concentration that are above the OECD required level of the top concentration, which are cytotoxic concentrations; no further details provided	[3]
		IN VIVO			
250, 500, or 1000 mg/kg/d for 14 d, or, 125, 250, or 500 mg/kg/d for 28 d	0.5% sodium carboxymethylcellulose	Groups of 5 male Crl:CD (SD) rats	Mammalian micronucleus assay; oral administration via gavage for 14- or 28-d; cells from the liver, bone marrow, and peripheral blood were assessed for genotoxic effects	No induction of micronuclei; an increase of relative liver weight was observed in the 14-d 1000 mg/kg dose group, minimal hypertrophy of hepatocytes was also observed in this group	[23]
250, 500, or 1000 mg/kg/d for 14 d	0.5% sodium carboxymethylcellulose	Groups of 5 male Crl:CD (SD) rats	Comet assay in accordance with OECD TG 489; assay was performed in concert with the micronucleus assay described above; assay performed 21 h after last oral administration and cells from the liver and peripheral blood were assessed for genotoxic effects	No induction of strand breaks	[23]

Table 4. Tumor promotion and/or tumor initiation studies from the original safety assessment on Kojic Acid [2]

Concentration/Dose	*Vehicle*	*Test System*	*Procedure*	*Results*
			DERMAL	
up to 3%	*cream formulation*	*groups of 10 or 15 female CD-1 mice*	*The study utilized 9,10-dimethyl-1,2-benzanthracene (DMBA) as the tumor initiator and phorbol-12-myristate-13 acetate (TPA) as the promoter. Groups of mice were treated in the following manner: DMBA + vehicle, DMBA + 0.3% Kojic Acid, DMBA + 3% Kojic Acid, DMBA + TPA, acetone + 0.3% Kojic Acid, acetone + 3% Kojic Acid, vehicle + TPA, or 3% Kojic Acid + TPA. The control or test substances were applied to shaved backs (4 cm^2). The mice receiving DMBA or acetone were treated once at the beginning of the experiment while the mice treated with vehicle + TPA or 3% Kojic Acid + TPA received 50 mg of the test substances daily for 1 wk. A week after the study commenced, the treatment groups with DMBA or acetone received 50 mg of the test substances 5 x weekly for 19 wk. The remaining groups received TPA twice weekly for 19 wk at 1 or 2 wk after study commencement.*	*Kojic Acid in a cream formulation did not possess promotion potential for skin carcinogenesis. The positive controls (DMBA + TPA) yielded expected results that included skin nodules that were squamous cell hyperplasia, papilloma, or carcinoma, significantly increased body weight gain, and significantly increased absolute and relative liver weights. Body weight gain was significantly decreased in week 2 or weeks 3 and 4 in the DMBA + 0.3% Kojic Acid and acetone +3% Kojic Acid dose groups, respectively. Squamous cell papilloma was observed in 1 mouse from the DMBA + 3% Kojic Acid group.*
			ORAL	
0 or 3%	*diet*	*male ICR mice*	*A dietary study on the tumor-initiating potential of Kojic Acid The mice received the test material in the diet for 4 wk. The mice then received distilled water containing 0 or 500 ppm phenobarbital for 14 wk. A partial hepatectomy was performed on all mice 2 wk after the treatment with phenobarbital.*	*No proliferative lesions were observed in any dose groups during microscopic examinations. The authors concluded that Kojic Acid has no tumor-initiating activity in mouse liver; however, the SCCP concluded that the Kojic Acid effect on proliferation of liver cells cannot be excluded since Kojic Acid + distilled water PCNA values were increased compared to basal diet + distilled water.*
0, 0.125, 0.5, or 2%	*diet*	*male F344 rats (60 total)*	*20-wk dietary study; rats received a single subcutaneous injection of diisopropanolnitrosamine (DHPN) or the vehicle prior to receiving a diet containing test material*	*Kojic Acid was found to have carcinogenesis-promoting potential; rats that received 2% Kojic Acid with DHPN had a significantly increased ($p < 0.01$) relative liver weights. Histopathology revealed an increased incidence of microgranuloma and vacuolation of centrilobular hepatocytes. A significant increase ($p < 0.01$) in the number and area of glutathione S-transferase-placental form (GST-P) positive foci per unit area of liver in the DHPN and 2% Kojic Acid group was observed when compared to the DHPN only treated group. A similar increase was also observed in the 2% Kojic Acid group that did not receive DHPN. No treatment-related effects were observed in the other test groups that received Kojic Acid, with or without DHPN.*
0, 0.125, 0.5, or 2%	*diet*	*groups of 20 male F344 rats*	*20-wk dietary study of Kojic Acid were performed without DHPN initiation. The researchers also performed a medium-term liver bioassay to determine the promoting influence of Kojic Acid.*	*2% Kojic Acid was tumor-promoting and had weak hepatocarcinogenic potential. Dose-related increases in absolute and relative liver weights were observed in both treatment groups. Numbers and areas of GST-P positive foci were significantly increased ($p < 0.01$) in the 2 % Kojic Acid group when compared to the control group. Additionally, increased incidences of microgranuloma and vacuolation of hepatocytes were observed in the 2% Kojic Acid group. PCNA expression was significantly increased ($p < 0.05$) in the 2% Kojic Acid group, with proliferating cell nuclear antigen (PCNA)-positive hepatocytes mainly localized around the vacuolated and granulomatous regions. A dose-related decrease in body weight gains and an increase in relative liver weights were observed, with statistical significance ($p < 0.01$) observed in the 2% dose group. Significant increases ($p < 0.01$) in number and areas of GST-P positive foci were observed in the 2% dose group when compared to the control.*

Table 4. Tumor promotion and/or tumor initiation studies from the original safety assessment on Kojic Acid [2]

Concentration/Dose	***Vehicle***	***Test System***	***Procedure***	***Results***
0 or 2%	*diet*	*groups of 5 male F344 rats*	*A 2-part study investigated the initiation potential of Kojic Acid. In the first part of the study, the rats received Kojic Acid in the diet for 3, 7, or 28 d. All rats were injected with 100 mg/kg bw bromodeoxyuridine (BrdU) intraperitoneally once a day for the last 2 d of exposure and 2 h prior to termination. Labeling indices (LIs) were calculated as percentages of cells positive for BrdU incorporation divided by the total number of cells counted. In addition, 8-oxo¬deoxy¬guanosine (8-OxodG) was measured in nuclear DNA to examine the formation of oxidative DNA adduct.* *In the second part of this study, the rats were subjected to a two-third partial hepatectomy on day 0. At 12-h post-surgery, the rats were treated once orally with carboxymethylcellulose vehicle, or 1000 or 2000 mg/kg Kojic Acid. The rats were then fed basal diet for 2 wk and then diet containing 0.015% 2-acetylaminofluorene (2-AAF) for another 2 wk. At 3 wk post-Kojic Acid administration, rats received a single 0.8 ml/kg bw dose of carbon tetrachloride.*	*In the first part of the study on day 28, body weight gains in the 2% Kojic Acid group were significantly decreased compared to the control group. In the 2% Kojic Acid dose group, absolute liver weights were significantly increased on day 7 but decreased on day 28. Relative liver weights were significantly increased at all time points. The LI values of hepatocytes of the 2% dose group were significantly increased as compared to the controls on days 3 and 7. All 8-OxodG levels in the liver DNA in the 2% dose group were slightly higher than the control values but were not statistically significant.* *In the second part, slight decreases were observed in the mean area and numbers of GST-P positive foci, but these differences were not statistically significant. The researchers concluded that Kojic Acid has neither liver initiation activity nor the capability of 8-OxodG formation; however, the findings suggest that Kojic Acid has liver tumor-promoting effects.*
0, 0.5, 1 or 2%	*diet*	*groups of 15 male F344 rats*	*Liver carcinogenesis bioassay; rats received Kojic Acid or the positive control 2-AAF at concentrations of 0.001 or 0.01% for 4 wk. After the treatment period, all rats received basal diet for 1 wk, and then a diet containing 0.5% phenobarbital sodium salt (SPB) for 6 wk. In another portion of the study, groups of 9 rats received 0 or 2% Kojic Acid or 0.001 or 0.01% 2-AAF in feed for 4 wk, and then all rats received basal diet for 7 wk. At 6 wk after the beginning of the study, all animals from both portions of the study underwent a two-third partial hepatectomy.*	*No treatment-related effects or deaths were observed during the study. Rats that received 2% Kojic Acid in both portions of the study had significant decreases in body weights during initiation period of the study, but body weights returned to control levels during the SPB or basal diet treatments. Decreases in feed consumption during the initiation period occurred in the 1 and 2% Kojic Acid groups, but increases in feed consumption during the SPB or basal diet treatment were marked with increases in body weight change. No treatment-related differences were observed in final body or liver weights, with or without SPB. Numbers of GST-P-positive foci in Kojic Acid-treated groups were similar to the control values, with or without SPB. No other treatment-related effects were observed. The positive control groups yielded expected results. This study concluded that Kojic Acid did not possess initiation potential in the rat liver.*

Table 5. Thyroid effects studies from the original safety assessment of Kojic Acid [2]

Concentration/Dose	***Vehicle***	***Test System***	***Procedure***	***Results***
0, 1.5, or 3%	*diet*	*groups of 65 male and female B6C3F1 mice*	*20-mo study of the tumorigenicity of Kojic Acid*	*Thyroid weights were increased significantly in the Kojic Acid-treated groups of both genders, especially in the male groups; there were no significant differences in other major organ or tissue weights or hematological values or serum biochemical parameters in any of the treatment groups. Incidences of thyroid gland hyperplasia and follicular adenomas were significantly increased in all treatment groups. In mice that received normal feed 30 d prior to termination, incidences of thyroid gland adenomas were significantly decreased, although average thyroid weights were unchanged. The serum-free T3 levels in the 3.0% dose groups of both genders were significantly lower than the control at month 6, while the TSH levels were increased. The decreases in the free T3 levels continued at the later measurements, but changes in the TSH levels disappeared. It was concluded that chronic high doses of Kojic Acid induced thyroid adenomas in mice.*
0, 0.008, 0.03, 0.125, 0.5, or 2.% (doses equivalent to 0, 5.85, 23.8, 95.3, 393.6, and 1387.3 mg/kg bw/d)	*diet*	*groups of 8 male F344 rats*	*4 wk study to determine the mechanisms of serum thyroid hormone reduce and thyroid tumor-promotion effects of Kojic Acid exposure on rats*	*Absolute and relative thyroid gland weights were increased in all groups treated with Kojic Acid in a dose-dependent manner, with significant increases occurring at 0.5% or more. A statistically significant decrease in serum T3 and T4 levels was observed in the 2% group when compared with the control group. The serum TSH in the 2% group was significantly increased when compared to controls. There were no other significant differences in these parameters in the other dose groups. Thyroid 125I uptake was significantly decreased in a dose-dependent manner starting at 0.03% Kojic Acid. A significant reduction in organic formation of iodine was observed in the 2% group. Histopathologic examination revealed decreased colloid in the thyroid follicles and follicular cell hypertrophy in the thyroid in high incidences in groups that received 0.03% Kojic Acid or more. All rats in the 2% group had thyroid capsular fibrosis. In a quantitative morphometric analysis, the ratio of the area of follicular epithelial cells to the area of colloids was significantly increased in the 0.03% dose group and higher.*
0, 0.008, 0.03, 0.125, 0.5, or 2%	*diet*	*groups of 9 male F344 rats in the 1st experiment; groups of 8 male and 8 female F344 rats in the 2nd experiment; groups of 8 male F344 rats in the 3rd experiment*	*The mechanism of thyroid tumorigenesis from Kojic Acid was studied in a 3-part experiment. In the first experiment, rats received Kojic Acid in their diets for 4 wk. In the second experiment, male and female rats received diet containing 0 or 2% Kojic Acid; male groups were killed at weeks 1, 2, 3, or 4 and female groups were killed at weeks 2 and 4. For the final experiment in this study, groups of male rats received 0 and 2% Kojic Acid in diet for 4 wk. At the end of the treatment, Kojic Acid was replaced with basal diet for 0, 6, 12, 24, or 48 h.*	*In the first experiment, thyroid gland weights were increased in a dose-dependent manner in rats receiving 0.125% or more Kojic Acid, with the thyroid gland weights from the 2% dose group 9 times that of the controls. Uptake of ^{125}I into the thyroid gland was more sensitive to Kojic Acid treatment, with significant suppression at 0.03%. Organic ^{125}I formation was interrupted only in the 2% dose group. Serum T3, T4, and TSH levels were affected only in the 2% group.* *In the second experiment, thyroid gland weights of male rats increased linearly in the 4 wk of treatment with 2% Kojic Acid. A less prominent, but still significant, increase in thyroid gland weights was observed in females. The suppression of ^{125}I uptake was also time-dependent and in males, the decrease started at 1 wk after Kojic Acid treatment and reached about 2% of control values by wk 3, with organic ^{125}I formation significantly decreased by 50% compared to the controls. These effects were not as significant in females, with only 20% suppression of ^{125}I uptake at wk 4. Serum T3 and T4 levels were decreased to minimum levels after 2 wk of Kojic Acid treatment, but recovered thereafter although at lower than control values in both genders. Serum TSH started to increase at wk 1 and reached a maximum at weeks 2 and 3.* *In the third experiment, organic ^{125}I formation returned to normal limits after 6 h and ^{125}I uptake per unit of thyroid weight increased to 70% of the control values within 24 h. Serum T3 and T4 were 47% and 34% of the control values, respectively, after 4 wk of the Kojic Acid diet. The levels increased to normal limits within 48 h after returning to basal diet and high levels of TSH decreased to normal within 24 h. The histopathological investigation on thyroid glands in these 3 experiments found a diffuse type of hyperplasia. After 2 wk of returning to basal diet, normal thyroid follicular structure was apparent in enlarged thyroid glands.*

Table 5. Thyroid effects studies from the original safety assessment of Kojic Acid [2]

Concentration/Dose	***Vehicle***	***Test System***	***Procedure***	***Results***
0 or 2%	*diet*	*Groups of 10 male F344 rats*	*In a study to determine whether Kojic Acid causes a promoting effect on thyroid carcinogenesis, rats were initiated with N-bis(2-hydroxypropyl)-nitrosamine (BHP). One week later, the rats received basal diet containing Kojic Acid for 12 wk. A control group received no BHP initiation or Kojic Acid and were fed basal diet for 13 wk. Another 2 groups of rats not initiated with BHP received diet containing Kojic Acid for 20 wk.*	*Body weights were decreased in the rats that received Kojic Acid at both week 4 and 12. Rats exposed to Kojic Acid also had increased absolute and relative thyroid weights up to 25-fold greater than the control group, as well as increased relative liver weights at each time point. Absolute liver weights were significantly increased in rats exposed to Kojic Acid for 20 wk. Serum T3 and T4 levels were significantly decreased (approximately one half to one third the values of the BHP alone group) and serum TSH was significantly increased (13-19 times higher than the BHP alone group) in the BHP + Kojic Acid group at both time periods. Similar changes in other serum thyroid-related hormones were observed in the 2% Kojic Acid alone group at wk 4 but not at week 20. At wk 4, 4 of the 5 rats in the BHP + Kojic Acid group had focal thyroid follicular hyperplasias, while 3 of the 5 rats had focal thyroid follicular adenomas. These lesions were observed in all rats in the BHP + Kojic Acid group by wk 12. Rats that only received Kojic Acid had marked diffuse hypertrophy of follicular epithelial cells at wk 4 and 20. The BHP alone and the untreated control groups had no changes in thyroid-related hormone levels or histopathological lesions. There were no significant intergroup changes of the liver T4-uridine diphosphate glucuronosyltransferase (UDP-GT) activity*
0, 0.02, 0.2, or 2%	*diet*	*groups of 20 male F344 rats*	*The potential thyroid tumor initiation activity of Kojic Acid was evaluated in a 2-part study on rats. Rats received a diet containing 0, 0.02, 0.2, or 2% Kojic Acid for 8 wk that was followed by treatment with 0.1% sulfadimethoxine (SDM) in drinking water for 23 wk. A 13-wk recovery period followed the SDM treatment. Controls included a group that received 4 subcutaneous injections of BHP during the initiation period followed by an administration of 0.1% SDM, a group that received diet containing 2% Kojic Acid for the initial 8 wk alone, a group that received 2% Kojic Acid for the entire 31 wk, and a group that received only basal diet.*	*During the treatment and recovery periods, deaths from tracheal obstruction from extremely hypertrophied thyroids were observed in the BHP control group (5 in total), the 31-wk administration of Kojic Acid control group (3 in total), the 8-wk Kojic Acid control group (1 in total), and the 2% Kojic Acid + SDM treatment group (1 in total). Significant suppression of body weight gain was observed in the BHP and 31-wk Kojic Acid control groups during administration that continued until the end of the recovery period. All treated groups had significantly increased absolute and relative thyroid gland weights when compared to the basal diet control group at the end of the administration period. These values, however, were decreased at the end of the recovery period, except in the BHP control group. When compared to the untreated controls, serum T3 levels in the 0% Kojic Acid + SDM, 2% Kojic Acid + SDM, and BHP control group were significantly decreased at the end of the administration period, as were the serum T4 levels in all treatment groups except the 8-wk Kojic Acid control. The serum T3 and T4 levels in the 8-wk Kojic Acid control were significantly increased compared to the untreated controls. Dose-dependent significant increases in the serum TSH levels occurred in all treatment groups, except the 8-wk Kojic Acid control. These increases were also dependent on treatment duration in the groups that received Kojic Acid. Thyroid carcinomas and adenomas were observed in all rats of the BHP control group, while no histopathological lesions were observed in the untreated control group. One adenoma was observed in the 31-wk Kojic Acid control group, but no other carcinomas or adenomas were observed in the remaining treatment groups. At the end of administration, focal follicular cell hyperplasia was significantly higher in rats in the 2% Kojic Acid + SDM, BHP control, and 31wk Kojic Acid control groups. This effect was observed in the latter 2 groups until the end of the recovery period. The mean percentage of PCNA positive cells to follicular cells counted per proliferative lesion was significantly increased in the BHP control and the 31-wk Kojic Acid control group.*

Table 5. Thyroid effects studies from the original safety assessment of Kojic Acid [2]

Concentration/Dose	*Vehicle*	*Test System*	*Procedure*	*Results*
received 0, 4, 15, 62.5, 250, or 1000 mg/kg	*0.5% carboxy-methylcellulose*	*groups of 10 male F344/Du Crj rats*	*In a study on the effect of Kojic Acid on thyroid function, groups of male rats received Kojic Acid via gavage daily for 4 wk.*	*No abnormalities were observed in rats in the 0 to 250 mg/kg dose groups during treatment. Several rats in the 1000 mg/kg dose group had transient and slight decreases in motility 30 min to 1 h after dosing on days 18 to 28 of treatment. Body weights and feed consumption in the 1000 mg/kg dose group were significantly inhibited when compared to the control group. The absolute and relative weights of the thyroid glands were nearly comparable to the controls in the 4 to 250 mg/kg dose groups throughout the treatment period. Absolute and relative weights of the thyroid gland in the 1000 mg/kg dose groups were 1.2-fold and 1.3-fold greater than the control group, respectively. Serum T3 concentration in the 250 mg/kg dose group had a significant decrease only at week 1 when compared to the control group, but the other dose groups showed no significant differences compared to the control at weeks 2 to 4. The serum T4 concentration in the 1000 mg/kg dose group was significantly decreased at week 4, but no dosage of Kojic Acid affected the serum TSH concentration significantly. The 1000 mg/kg dose group had hypertrophy of epithelial cells in the thyroid gland at week 1 to 4; this was not observed in the 250 mg/kg dose group. Radioactive iodine uptake in the 4 to 250 mg/kg dose groups was comparable to the control group at week 1 to 4. In the 1000 mg/kg dose group, the iodine uptake was about 2-fold greater than the control group in week 1; the uptake in this group continued to be constant and high through wk 4. The TCA-precipitable radioactive iodine in the thyroid gland was also increased in the 1000 mg/kg dose group. The absorption of Kojic Acid was rapid as manifested by the Tmax of blood concentration of radioactivity, which was as short as 1.0 + 0.0 h and the t1/2 was 4.8 + 0.3 h. Blood concentrations of radioactivity had nearly disappeared by 24 h after treatment.*

Table 6. MOE calculations

Area of Application	Surface Area (cm^2)	Estimated Daily Amount of Applied Cream (g/d)	Calculated Daily Amount of Applied Kojic Acid (mg/kg bw/d)	SED (mg/kg bw/d)	Adjusted NOAEL (mg/kg bw/d)	MOE	Reference
Face + Neck	565+320=885	2.41	0.448	0.0075	2	267	[3,39,43]
Hands	860	2.16	0.402	0.0067	2	299*	[3,39,43]
Aggregate (Face + Neck + Hands)	1745	4.57	0.850	0.0142	2	141	[3,39,43]
Body	15,670	7.82	1.455	0.0242	2	83	[39]
Whole Body (Face + Body)	17,500	18.0	3.348	0.0558	2	36	[43]

*The SCCS reported a value of 199 in their opinion, which appears to be a typographical error.[39]

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

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Abstract

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

Keywords

cosmetics, kojic acid, safety

Introduction

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

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

Chemistry

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

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

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

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

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

Use

Cosmetic

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

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

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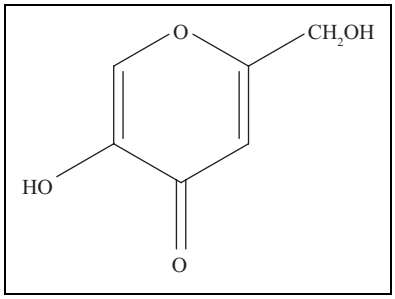

Figure 1. Kojic acid.

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

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

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

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

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

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

Table 2. Physical and Chemical Properties of Kojic Acid

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

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

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

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

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

Noncosmetic

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

Table 3. Cosmetic Product Uses and Concentrations for Kojic Acid

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

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

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

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

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

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

General Biology

Absorption, Distribution, Metabolism, Excretion

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

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

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

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

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

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

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

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

Dermal Penetration

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

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

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

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

Tyrosinase Inhibition

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

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

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

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

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

Animal Toxicity

Acute Oral Toxicity

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

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

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

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

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

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

Acute Subcutaneous Toxicity

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

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

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

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

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

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

Acute Intraperitoneal Toxicity

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

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

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

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

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

Acute Dermal Toxicity

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

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

Short-Term Oral Toxicity

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

Short-Term Dermal Toxicity

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

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

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

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

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

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

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

Chronic Toxicity

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

Ocular and Dermal Irritation

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

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

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

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

Dermal Sensitization

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

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

Dermal Depigmentation

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

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

Phototoxicity

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

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

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

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

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

Reproductive and Developmental Effects

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Genotoxicity

Bacterial Assays

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

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

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

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

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

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

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

Mammalian Cell Assays

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

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

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

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

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

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

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

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

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

In Vivo Mammalian Tests

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

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

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

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

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

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

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

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

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

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

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

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

Genotoxicity studies are summarized in Table 4.

Photogenotoxicity

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

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

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

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

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

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

Carcinogenicity

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

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

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

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

Table 4. Genotoxicity Studies for Kojic Acid

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

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

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

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

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

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

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

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

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

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

Carcinogenicity studies are summarized in Table 5.

Tumor Promotion

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

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

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

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

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

Tumor Initiation

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

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

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

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

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

Table 5. Carcinogenicity Studies for Kojic Acid

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

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

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

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

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

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

Dermal Tumor Promotion

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

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

Thyroid Effects

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Clinical Assessment of Safety

Case Studies

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

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

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

Clinical Testing and Therapeutic Use

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

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

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

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

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

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

Summary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Discussion

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

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

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

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

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

Conclusion

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

Author's Note

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

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

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Conflict of Interest

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

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 15, 2025

SUBJECT: Kojic Acid

Anonymous. 2025. Memo to Dr. Bart Heldreth in response to the insufficient data announcement for Kojic Acid on March 19, 2025 (with SCCS opinions attached).

CIR Executive Director,
Dr. Bart Heldreth

We are writing in response to the insufficient data announcement for Kojic Acid on March 19, 2025.

•A margin of exposure (MOE) calculation for whole body exposure
(The MOE is referred to as margin of safety (MoS) below)
In the SCCS OPINION CORRIGENDUM on Kojic Acid (SCCS/1637/21, 2022)[1)], the SCCS calculated MoS values for Kojic Acid at 1% in a cream product for application scenarios on the face and neck, the hands, and an aggregate of these applications (face + neck + hands). The MoS values were 267, 199※, and 141, respectively.
(※ We think there is a calculation mistake in the SCCS opinion. The MoS value for hands supposed to be 299)

MoS value for whole body exposure can be calculated analogously based on the same adjusted NOAEL of 2 mg/kg bw/day and the SED as described in SCCS OPINION[1)]. The SED was determined based on a human percutaneous absorption study involving a single application of 500 mg cream containing 1% Kojic Acid on the entire face. The internal exposure was derived by taking the 95th percentile of the AUC 0-24 h, and the amount of Kojic Acid in plasma based on AUC was estimated as 0.093 mg/day.
The daily exposure to body lotion is equal to 7.82 g/day according to the SCCS Notes of Guidance (SCCS/1647/22). If the Kojic Acid content of the cream is 1%, the daily exposure to Kojic Acid is 78.2 mg/day, which corresponds to 15.64 times more Kojic Acid than in the human percutaneous absorption study (application of 5 mg of Kojic Acid). Thus the total amount of Kojic Acid in plasma in 24h can be estimated as: 0.093 mg/day x 15.64 = 1.455 mg/day and the SED becomes: 1.455 mg/day/60 kg bw = 0.0242 mg/kg bw/day. MoS can be calculated as: 2 mg/kg bw/day/0.0242 mg/kg bw/day = 83.

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)**	**Adjusted NOAEL (mg/kg bw/day)**	**MoS**
Face+neck	565 + 320 = 885	2.41	0.448	0.0075	2	**267**
Hands	860	2.16	0.402	0.0067	2	**299**
Face+neck+hands	1745	4.57	0.850	0.0142	2	**141**
Body	15670	7.82	1.455	0.0242	2	**83**

Clearly, MoS values were greater than 100 for application scenarios on the face and neck, the hands, and an aggregate of these applications, but was less than 100 for whole body exposure.

As an additional explanation, in the 2022 SCCS OPINION[2)] before correction, the MoS values were calculated as 133, 154, and 71 for face and neck, the hands, and an aggregate of these applications (face + neck + hands) respectively, due to a SED calculation mistake (a frequency of application 2/day was taken into account which turned out to be not necessary). This miscalculation leads to a wrong conclusion that Kojic Acid is safe at a concentration up to 0.7%.

• An explanation as to why the European Union restricted use of Kojic Acid to the face and hands only

In the COMMISSION REGULATION (EU) 2024/996 of 3 April 2024, Kojic Acid was added in Annex III (Restricted Substances) and the reason was described as below:
(https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L_202400996)

The SCCS concluded in its opinion of 15-16 March 2022 that Kojic Acid is safe when used as a skin lightening agent in cosmetic products up to a maximum concentration of 1 %. Considering the SCCS opinion, it can be concluded that there is a potential risk to human health arising from the use of Kojic Acid in cosmetic products when its concentration exceeds certain levels. Therefore, Kojic Acid should be restricted to use as a skin lightening agent in face and hand products with a maximum concentration of 1 %.

Actually, in the former report of 2012 SCCS OPINION on Kojic Acid (SCCS/1481/12)[3)], the SCCS concluded that 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 is safe for the consumers. In addition, the SCCS mentioned that 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.
Following this 2012 SCCS opinion, measures have been taken in Germany and other EU Member States to destroy, withdraw products containing more than 1.0% Kojic Acid and products for body use containing Kojic Acid (<1.0%) from the market. Some of the examples are as below:
https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/292263?lang=en
https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/290649?lang=en

In addition, using the same calculation as that was in the 2022 SCCS OPINION CORRIGENDUM, MoS value for whole body exposure is 83 (less than 100), this also support that Kojic Acid is of concern when applied on larger skin surfaces than face and hands.

Sincerely yours,

Attached Reference:

1) SCCS/1637/21 (Final version & CORRIGENDUM) _2022.pdf

2) SCCS/1637/21 (Final version) _2022.pdf

3) SCCS/1481/12_2012.pdf

European Commission

Scientific Committee on Consumer Safety

SCCS

OPINION

on Kojic acid

- CORRIGENDUM -

Scientific Committees
on Consumer Safety
on Health, Environmental and Emerging Risks

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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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

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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

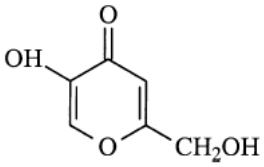
(SCCP/1182/08; NCBI, 2021)

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

/

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?*

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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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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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European Commission

Scientific Committee on Consumer Safety

SCCS

OPINION

on Kojic acid

Scientific Committees
on Consumer Safety
on Health, Environmental and Emerging Risks

The SCCS adopted this document
at its plenary meeting on 15–16 March 2022

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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.

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 not 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?*

   In the SCCS's opinion, the use of Kojic acid as a skin lightening agent in cosmetic products is safe for the consumer up to a maximum concentration of 0.7% Kojic acid in the final product.

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

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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

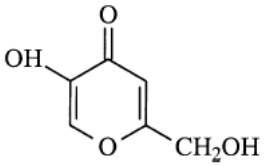
(SCCP/1182/08; NCBI, 2021)

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.

When a frequency of application of 2 times per day is assumed, the SED is calculated as follows:
SED = 0.093 mg x 2/60 kg=0.0031 mg/kg bw/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 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 x 4.82 = 0.448 mg. Taking into account a frequency of application of twice a day, the SED becomes: 0.448 mg/kg/d x 2/60 kg = 0.015 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 of Kojic acid and thus 4.32 times more than in the *in vivo* percutaneous absorption study or 0.093 mg x 4.32 = 0.402 mg. This leads to a SED of: 0.402 mg x 2/60 kg = 0.013 mg/kg bw/day.

For aggregate exposure (face + neck + hands), the total daily exposure is 4.57 g/day, resulting in 45.7 mg of Kojic acid being 9.14 times more than in the *in vivo* percutaneous absorption study or 0.093 mg x 9.14 = 0.850 mg. This leads to a SED of: 0.850 mg x 2/60 kg = 0.028 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	0.015
Hands	860	2.16	0.402	0.013
Aggregate (face+neck+hands)	1745	4.57	0.850	0.028

*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 and assuming a frequency of application of twice per day.

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:

Frequency of application	Area of application	SED (mg/kg bw/day)	Adjusted NOAEL (mg/kg bw/day)	MoS
Twice daily	Face+neck	0.015	2	**133**
	Hands	0.013	2	**154**
	Aggregate (face+neck+hands)	0.028	2	**71**

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.

In order to derive at a MoS of 100 for aggregated exposure for face and hand cream, the total SED should be maximally 0.02 mg/kg bw/day when applied twice per day. This can be achieved by reducing the Kojic acid concentration from 1% to 0.7%.

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.

Repeated administration may result in higher systemic exposure than just a single application.

An application frequency of 2 was assumed for Kojic acid containing products, in concordance with other previously evaluated skin bleaching substances (alpha and beta arbutins). Skin bleaching products are applied on face (including neck) and hands, for which also the aggregate exposure scenario is relevant, resulting in a maximum exposure area of 1745 cm^2. The SCCS is aware that skin bleaching products may be applied to other parts of the body. The resulting SED values are 0.015mg/kg bw/d (face & neck), 0.013 mg/kg bw/d (hands) and 0.028 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 not 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?*

   In the SCCS's opinion, the use of Kojic acid as a skin lightening agent in cosmetic products is safe for the consumer up to a maximum concentration of 0.7% Kojic acid in the final product.

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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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.

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-trophied 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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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

About the Scientific Committees

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

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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.***

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May 23, 2025

The Cosmetic Ingredient Review (CIR)

Subject: Submission to the CIR on Kojic Acid

Dear Sir or Madam:

This is Kazuhiro Iga of Sansho Pharmaceutical Co., Ltd. (former Sansho Seiyaku Co., Ltd.). We are the Japanese company who developed Kojic Acid in 1988 as an active ingredient for cosmetic products. We have been manufacturing and providing Kojic Acid since then.

According to the CIR Ingredient Status Report, Kojic Acid is stated as "Insufficient Data Announcement" and the followings are requested.

> The Expert Panel for Cosmetic Ingredient Safety requested the following additional information:
> 1. A margin of exposure (MOE) calculation for whole body exposure
> 2. An explanation as to why the European Union restricted use of Kojic Acid to the face and hands only

In order to defend Kojic Acid, we would like to provide additional information in response to the request.

Your attention to this matter would be greatly appreciated.

Yours faithfully,

Kazuhiro Iga
Director, Development Dept.
Email: iga@dermed.jp

Attachments:

1) SCCS_Opinion_on Kojic acid 2022
https://health.ec.europa.eu/document/download/04c4600e-fac7-43c7-bccc-18714258b644_en?filename=sccs_o_259.pdf
2) THE SCCS NOTES OF GUIDANCE 12TH REVISION
https://health.ec.europa.eu/document/download/32a999f7-d820-496a-b659-d8c296cc99c1_en?filename=sccs_o_273_final.pdf
3) Official Journal of the European Union COMMISSION REGULATION (EU) 2024/996 of 3 April 2024
https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L_202400996

1. A margin of exposure (MOE) calculation for whole body exposure

The SCCS (Scientific Committee on Consumer Safety) evaluated Kojic Acid as an endocrine disruptor and issued a final version of OPINON in June 2022, concluding that Kojic Acid is safe when used at concentrations of up to 1%. (Attachment 1)

In the OPINION, Kojic Acid was evaluated for the use on the face, neck and hands, which are the areas Kojic Acid-containing formulations are applied in Europe.

Since MOE for the whole body (face and body) exposure was not calculated in the OPINION, the amount of 18.0 g/day, which is stated as a standard exposure value for sunscreen lotion in the SCCS Notes of Guidance, 12th Edition (Attachment 2), is to be used in the MOE calculation.

The calculations were conducted as described in Section 3.2.4 p.17-18 of the OPINION. As for the systemic exposure dose (SED), it is as follows:

For aggregate exposure (face and body), the total daily exposure is 18.0 g/day, resulting in 180.0 mg/day of Kojic Acid being 36 times more than in the in vivo percutaneous absorption study or 0.093 mg/day x 36 = 3.348 mg/day. This leads to a SED of: 3.348 mg/day/60 kg bw= 0.0558 mg/kg bw/day.

Area of application	Surface area (cm2)	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
Whole body (face and body)	17500	18.0	3.348	0.0558

※ "Whole body" was added to the table in the OPINION on Kojic acid 2022 SCCS/1637/21 p.18

Based on the SED, MOE was calculated as described in Section 3.4 p. 39-40 of the OPINION.

Area of application	SED (mg/kg bw/day)	Adjusted NOAEL (mg/kg bw/day)	MOE
Face+neck	0.0075	2	267
Hands	0.0067	2	199
Aggregate (Face+neck+hands)	0.0142	2	141
Whole body (face and body)	0.0558	2	36

※ "Whole body" was added to the table in the OPINION on Kojic acid 2022 SCCS/1637/21 p.40

As a result, MOE for whole body exposure was below 100, which raises safety concerns.

2. An explanation as to why the European Union restricted use of Kojic Acid to the face and hands only

In COMMISSION REGULATION (EU) 2024/996 of 3 April 2024, it is stated about Kojic Acid as follows:

Considering the SCCS opinion, it can be concluded that there is a potential risk to human health arising from the use of Kojic Acid in cosmetic products when its concentration exceeds certain levels.

It is assumed that the European Commission (EC) determined that high exposure to Kojic Acid, causing MOE to fall below 100, may increase health risk. In consideration of the risk assessed by the SCCS, the EC presumably restricted the use of the product to the face and hands. (Attachment 3)