ADMIN

Nitrosation

EXPERT PANEL MEETING
December 4-5, 2023

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Jinqiu Zhu, PhD, DABT, ERT, DCST, CIR Toxicologist
Date: November 9, 2023
Subject: *N*-Nitrosation and the Safety Evaluation of Cosmetic Ingredients

Enclosed is revised version of the CIR Resource Document – *N*-Nitrosation and the Safety Evaluation of Cosmetic Ingredients (*resource_document_nitrosation_122023*), as well as the transcripts of the discussion at the previous Panel meeting (*transcripts_ document_nitrosation_122023*). The Panel last reviewed this document at the June 2023 meeting, reaching a consensus on the need for further insights and edits from an outside expert conversant with the toxicity of *N*-nitroso compounds and related *N*-nitrosation pathways. In August 2023, the CIR Science and Support Committee (SSC) also reviewed and provided feedback on this draft document (*CIRSSCcomment_resource_document_nitrosation_122023*). The CIR SSC recommended a restructuring of the document to include clearer, segmented sections with subheadings. Detailed comments were further made for each section of the document, concerning reference selection, data refinement, editorial modifications, structural reorganization, regulatory clarifications, and so forth. Rather than covering nitrosation in general, the CIR SSC emphasized this document should focus on potential nitrosation associated with secondary amines during the production or storage of cosmetics and personal care products.

Following the feedback received, the document has been substantially revised. Dr. Ronald C. Shank, who previously served as an esteemed member of the Expert Panel for Cosmetic Ingredient Safety and is a renowned expert on the topic of nitrosation and associated toxicities, dedicated his expertise to make the revisions (*Communication_Dr. Shank_122023*). In his revision, Dr. Shank removed much of the repetition on the role of precursors for nitrosation, added a brief discussion on the metabolic activation of *N*-nitrosamines, and moved the whole risk assessment discussion to the "Annex." Dr. Shank specifically pointed out a document issued in 2012 by the Scientific Committee on Consumer Safety (SCCS), namely "Opinion on Nitrosamines and Secondary Amines in Cosmetic Products" (ref. 24 in the draft resource document), which has most of the elements needed as a resource for the Panel.

The Panel is requested to review the revised draft resource document to determine its alignment with their expectations for this iteration and whether it accurately represents their current understanding of the risks involved with regard to *N*-nitroso compounds during the evaluation of cosmetic ingredient safety. The Panel should to decide if further revisions are needed.

Personal Care Products Council
Committed to Safety, Quality & Innovation

TO: Bart Heldreth Ph.D., Executive Director – Cosmetic Ingredient Review
Expert Panel for Cosmetic Ingredient Safety

FROM: CIR Science and Support Committee of the Personal Care Products Council

DATE: August 18, 2023

SUBJECT: Nitrosation Resource Document

The CIR Science and Support Committee (CIR SSC) appreciates the opportunity to comment on the June 2023 draft of the Nitrosation Resource document. As this is an important document, we will also want the opportunity to comment on all future drafts of this document.

The potential for nitrosation is an issue the cosmetics industry has been aware of and managing for many years. Secondary amines should be the focus for cosmetic products as nitrosamines formed from secondary amines are commonly stable compounds. Nitrosamines of secondary amines can be introduced to a finished product as a raw material impurity or formed during manufacturing or storage of the finish product under certain defined conditions. We understand that it is a small subset of ingredients and products, that are of special concern and not a wide ranging concern for cosmetic products.

Rather than trying to cover nitrosation in general, this document should focus on potential nitrosation in cosmetic and personal care products. To make the document more readable, we suggest that it be restructured into shorter sections as follow:

1. What is a nitrosamine
2. Toxicology of nitrosamines
3. Regulations – Cosmetics
4. Nitrosatable species (primary versus secondary, versus tertiary amines; amides, etc.)
5. Nitrosating agents – provide perspective on which are relevant in cosmetics (nitrite, but not nitrosyl halides)
6. Rates of reaction, e.g., pKa of amines
7. Nitrosation accelerators, e.g., aldehydes
8. Nitrosation inhibitors, e.g., antioxidants
9. Methods for measurement, e.g., LC-MS/MS or GC-MS/MS as the preferred techniques, reference ISO 15819 (NDELA)
10. Strategies to minimize nitrosamines in cosmetics (reference to Cosmetic Europe Guidance and ISO 14735)

The Cosmetics Europe 2009 technical guidance document should be used to help focus the CIR resource document.

The following are specific comments on the draft reviewed by the Expert Panel for Cosmetic Ingredient Safety during the June 12-13, 2023 meeting.

Background

The discussion of Nitrosation of amines should be expanded. Primary amines do not form stable nitrosamines. Most tertiary amines nitrosate very slowly and are not generally a concern for cosmetics. Secondary amines should be the focus area for cosmetic products because these are stable compounds. N-Nitrosamines of secondary amines can be introduced into a finished product as a raw material impurity or formed during manufacture or storage of the finished product.

Nitrosation of amides is very slow because the lone pair of electrons on the nitrogen are associated with the carbonyl group of the amide. Primary amides are not stable and will degrade. Secondary amides can nitrosate but require strong nitrosating agents and the reaction is slow. Overall, nitrosation of amides is not considered a problem for cosmetic products, although there is potential for amides to contain amine impurities.

As stated above, the focus of the document should be the formation of N-nitrosamines in cosmetics, which should be made clear from the beginning of the document. Historically, the focus has been on diethanolamine in cosmetics and the possible formation of N-nitrosodiethanolamine (NDELA). In 2011, the SCCS indicated that mainly two nitrosamines (NDELA and N-nitrosobis(2-hydroxypropyl)amine (NBHPA)) have been found in cosmetics. A short list of nitrosamines have ever been found in cosmetics (N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosomorpholine (NMOR), N-nitrosopyrrolidine (NPYR), NBHPA, NDELA, and N-nitrosopara-aminobenzoic acid (NPABA)).

Factors influencing the N-nitrosation of ingredients in cosmetic products

This section covers a lot of information, some of which is repeated from the background section. As discussed above, splitting the report into shorter sections would help to make the document more readable.

While the list of nitrosating agents presented in this section is correct, some are not relevant to cosmetic products.

It should be noted that 2-Bromo-2-Nitropropane-1,3-Diol (Bronopol) has two roles in nitrosation. The nitro group provides the nitrosating agent and the formaldehyde catalyzes the reaction.

This section should not mention the “European Cosmetics Directive” as this was replaced by the “Cosmetics Regulation” in 2009.

Information about controlling N-nitrosamines in pharmaceuticals should be moved to the regulatory section.

The document states that “N-Nitrosamines can be quantitatively detected down to 1 ppb.” This statement is too general because the exact limit of quantification (LOQ) will depend on the specific nitrosamine, analytical method used, and the sample matrix.

Current regulations on nitrosamine formation in cosmetic ingredients and cosmetic formulations

The first part of this section is a review of a publication and does not concern regulations. It should be moved to the background section.

We are especially concerned with the section below (in italics) and reference 32. The reported data are not consistent with the surveillance conducted by the German authorities (Annex III of SCCS/1486/12) or reference 34. Since not all cosmetics have nitrosamine impurities and those that have tested positive can have a wide range in values, it does not make sense to list the results from one study that evaluates data as far back as the 1970s and 1980s. Of importance, reference 34 used a validated analytical method and found 13 of 103 cosmetic products in Korea and 4 of 12 raw materials in which NDELA was detected. The range found was from trace (<20 µg/kg) to 214.2 µg/kg. It is also important to indicate that there are a wide range of consumer exposure characteristics that are important when discussing risk (i.e., rinse-off versus leave-on, applied to hair or applied to skin, etc.)

"*Contamination of cosmetic products with NDELA is attributed to the interaction of di- or triethanolamine (DEA and TEA, respectively), commonly used ingredients in cosmetics, with a nitrosating agent.*[32,34] *The remaining 1% of observed contamination comes from N-nitrosomorpholine (NMOR, ~ 0.99%) and N-nitrosodimethylamine (NDMA, ~ 0.01%). Hair care products (total N-nitrosamine: 1900 ± 1900 ng/g (average ± standard deviation), similarly hereinafter), shampoos (220 ± 50 ng/g), and lotions (100 ± 25 ng/g) all showed quantifiable N-nitrosamine concentrations, with NDELA being the major congener in all cases. Among identified exposure sources through literature review (n = 6), average total N-nitrosamine concentration in cosmetic products ranges from 400 to 49,000 ng/g."*

This section should include a table summarizing regulations, including the FDA drug regulations/guidelines.

The following sentence should be deleted as it does not add useful information and it is contradicted by the descriptions of the regulations that follow: *"While regulatory oversight could significantly reduce daily N-nitrosamine exposure, regulating N-nitrosamines in personal care products would be challenging due to the aforementioned attenuations of carcinogenicity or rates of nitrosation, and numerous existing laws and regulations governing the manufacturing and sale of cosmetics and personal care products."*

The SCCS section needs to be re-written for clarity. Begin with the regulations in the Annexes of Directive 76/768/EEC which include purity specification of 50 ppb N-nitrosamines for raw materials and all nitrosamines potentially formed. Secondary amine content in raw materials should be as low as achievable and not exceed 5% in raw materials. In finished cosmetic products, a maximum secondary amine content of 0.5% should be maintained.

The 160 ppb value is not an estimated safety level. It is the maximum level of NDELA in finished cosmetic products applied topically that would pose a minimal health risk to the consumer. This assessment was completed by Cosmetics Europe, not by SCCS. The details about the conservative deterministic aggregate exposure assumption should also be detailed in this document.

A T25 value is mentioned, but no number is provided. What is the number?

More details on how the risk assessment was done and all the assumptions used should be included in the text. As written, this does not read well, and is not sufficient. For risk assessments, descriptions of the point of departure, the threshold safety value, exposure assumptions, etc. all need to be described.

If kept in the document, the following, with the indicated revision, should be in the Background section: *"N-Nitrosation may occur in trace concentrations in diverse environments, including air, soil, water, stored or preserved foods, and the gastrointestinal tract of both animals and humans.[38] N-Nitrosamine impurities may show up in a variety of cosmetic ingredients. For instance, surfactants and emulsifiers, which are ammonia or amine salts, may serve as potential source of N-nitrosamines. Raw materials based on secondary amines, such as DEA, have the highest potential for N-nitrosamine formation. N-Nitrosamine levels in certain cosmetics may increase over the months following product opening.[39] Although ~~commonly present~~ trace nitrosamines could be present as contaminants in cosmetic products containing primary, secondary, and tertiary alkyl amines, N-nitrosamines are not listed on product labels since they are impurities or formed by reactions of chemical mixtures in products."*

Reference 16 is guideline for drug products only. The CIR document indicates that is also for food and cosmetics. This is not correct.

Reference 41 is for the drug, metformin, not for foods.

VSD is incorrectly defined as "Valuable Safety Dose". It should be "Virtually Safe Dose". It is customarily a risk level of 1 in 1,000,000. For Proposition 65, the state of California uses 1 in 100,000.

Please provide a reference for FDA guidance on minimizing the formation of nitrosamines during the manufacture of cosmetics. Reference 40 does not include FDA guidance on nitrosamines in cosmetics.

Has FDA issued guidance to manufacturers on preventing N-nitrosamine formation during the manufacture of cosmetics? If so, please provide the reference.

The following sentences should be deleted. Nitrosamines are not intentionally added and will not be included in product labels. *"The US Fair Packaging and Labeling Act (FPLA) requires cosmetic manufacturers to label their products with ingredient information and necessary warning statements to assess consumers in making informed decisions.[45] While the FPLA does not establish specific provisions for N-nitrosamines or N-nitrosation in cosmetics, manufacturers must comply with labeling requirements if these substances are present and could potentially pose health risks."*

Safety aspects considered by the Panel for mitigating N-nitrosation potential in cosmetic formulations

Please add "some" before products, as N-nitrosamines have not been found in all products.

It is not clear why indole rings are being used as an example. A better example may be secondary amines like diethanolamine since this is historically more relevant.

The Expert Panel only evaluates the safety of ingredients. It is not correct to state that they consider the "cosmetic package, or at the point of use".

Please change "would investigate" to "recommends the manufacturer investigate"

The example of DEA should focus on what was said in regard to the potential for NDELA formation, rather than other mechanisms of cancer that have been attributed to DEA.

In the following, replace “detected in cosmetics” with “detected in cosmetics that contain secondary amines and a nitrosation source”: *“Although the levels of N-nitrosamines detected in cosmetics are usually very low”*

In the last paragraph, it should be made clear that not all cosmetic products are impacted. It is just the small number that contain secondary amines and a nitrosation source.

Unless there is a specific reference that can be cited, the document should not state that FDA has guidance for industry to prevent the formation of N-nitrosamines in cosmetics.

Conclusion

In the first sentence, please qualify the N-nitrosamine exposure by saying “When exposure and duration of exposure are high enough, some N-nitrosamines pose health risk…”

RE: revised nitrosamine resource document

Bart Heldreth <heldrethb@cir-safety.org>

Tue 10/17/2023 2:37 PM

To:Ron Shank <rcshank@hs.uci.edu>
Cc:Jinqiu Zhu <ZhuJ@cir-safety.org>;Monice Fiume <fiumem@cir-safety.org>

1 attachments (217 KB)

resource_document_nitrosation_122023_DRAFT Shank 1 edited 1b.docx;

Thanks, Ron!

I can do the Chem structures. We will get to work on this and include your suggestions in our forward to the Panel.

Truly,
Bart

Dr. Bart Heldreth
Executive Director
Cosmetic Ingredient Review

From: Shank, Ronald <rcshank@hs.uci.edu>
Sent: Tuesday, October 17, 2023 2:32 PM
To: Bart Heldreth <heldrethb@cir-safety.org>
Subject: revised nitrosamine resource document

Dear Bart

I have edited rather extensively the draft resource document on N-nitrosamines and attach it as “resource document draft Shank 1b”. I have removed much of the repetition on the role of precursors for nitrosation, added a brief discussion on the metabolic activation of N-nitrosoamines, and moved the risk assessment discussion to the “Annex”. My chemical structure computer program is not supported by Windows 11 on my new computer, so I was unable to draw the metabolic pathway for N-nitrosodiethanolamine; so I cut and pasted the pathway from the publication by Li and Hecht; hopefully someone on your staff can draw the pathway if needed,

My understanding is that this is meant to be an internal document to be used as a reference for the Panel as they review ingredients where N-nitrosation may be an issue. There is already available a document that I feel the Panel can use without generating a new one; that document is the one issued in 2012 by The Scientific Committee on Consumer Safety “Opinion on Nitrosamines and Secondary Amines in Cosmetic Products”. That document has most of the elements needed as a resource for the Panel. That document and another on the metabolic activation of N-nitroso compounds should meet the needs of the Panel; (“Metabolic Activation and DNA Interactions of Carcinogenic *N*-Nitroamines to Which Humans are Commonly Exposed” by Yupeng Li and Stephen S. Hecht in the International Journal of Molecular Science in 2022 (May, Vol. 23(9). Steve Hecht would be a great resource for the Panel to consult on

this topic; he has an endowed chair in the Masonic Cancer Center in the University of Minnesota (hecht002@umu.edu).

I have tried to edit the draft you sent me should CIR decide to go ahead with your own resource document. You will see that I suggest the discussion of safety evaluations be moved to the Annex section. The reason for this is that the Panel has been using quite appropriately the phrase ‘safe when formulated to minimize the formation of N-nitroso componds’. FDA tries to use risk assessment in the evaluation of carcinogens in drugs because of the benefit drugs have in treating medical ailments; the same risk-benefit argument isn’t applicable to cosmetics.

Please let me know if I need to provide further explanation for my revisions; I would like to help.

Ron

JUNE 2023 PANEL MEETING

Dr. Belsito Team - June 12, 2023

DR. BELSITO: Nitrosation. That document is terribly redundant and needs to be incredibly edited and it was beyond me to do it. And it's an important document. Comments?

DR. SNYDER: Yeah, I mean I have lots of editorial comments.

DR. BELSITO: I have lots of edits, but even beyond my edits it needs to be --

DR. SNYDER: And it's not very efficiently done because it's all with comments. I'd like to see a Word document, or suggests that maybe the CIR considers employing Ron Shank to review the document -- work with Jinqiu on the document before we see another iteration of it. This is his area of expertise. I think he would do a very good job on it.

DR. KLAASSEN: Oh, he'd do an excellent job. This is his baby.

DR. SNYDER: Yeah.

DR. BELSITO: And he's a good editor.

DR. SNYDER: The first question I had was who is the targeted audience for this document?

DR. BELSITO: It's a boilerplate, like our respiratory boilerplate.

DR. SNYDER: Because there's parts of it where you say manufacture should, blah, blah, blah, blah, blah. I thought, well, it's for us, it's not for manufacturers, right?

MS. FIUME: It's the background information. So, same as in the epi section where we refer them to the resource document.

DR. SNYDER: Okay.

MS. FIUME: So, when you're talking about nitrosation, if you enter the discussion that would be built in and we could refer them to the resource document. It's for background information as to why nitrosation is an issue.

DR. SNYDER: Okay.

DR. RETTIE: Yeah, I'd agree with the redundancy. I think that's probably pretty easily attacked, that particular part. And I'd like to see a few more figures in there and I'd like to see a scheme for nitrosamide formation and how you get to the DNA alkylating species for the breakdown. If only to add that --

DR. BELSITO: So, diagrams of what we're looking at?

DR. RETTIE: Diagrams, schemes, reaction pathways. Not too many.

DR. KLAASSEN: No. I had the same point. It could be made much clearer that way.

DR. RETTIE: Yeah.

DR. BELSITO: Okay. So, it needs to be finessed. We need some diagrams. And our recommendation is that perhaps we enlist Dr. Shank to do one last hurrah for CIR?

MS. FIUME: I will pass that along.

DR. BELSITO: Okay.

MS. FIUME: Does that mean we can get a Taho and meet with him there?

DR. BELSITO: Yes. I think we need to change the bilaws that say we always have to meet in D.C. Particularly since we have three panel members from the --

DR. SNYDER: West coast.

MS. KOWCZ: West coast.

DR. BELSITO: -- from the west coast, yeah.

DR. RETTIE: So, I live in a lovely, secluded island. You're all welcome to come and I'll host you.

DR. SNYDER: I live in a really secluded island.

DR. BELSITO: Scottland's not an island.

DR. RETTIE: Adrian's wall, we consider that to be.

DR. BELSITO: Okay. I think we're done.

Dr. Cohen Team – June 12, 2023

DR. COHEN: So, we have a Nitrosation Resource Document. It was a hard read. This was a tough read for me. But it was very well -- it was -- I like the depth in which we went into it. I think there was some redundancy in it. But I like that we got a chance to

review it. And I think it sort of speaks for itself. And maybe we could have some more edits in here. There was a comment in Wave 3 that this should be open to the public for comment. Does the group think it's ready for public review yet?

DR. BERGFELD: I'm going to say no. But, you know, we had a conversation, some of us, earlier about the fact that we would like that Ron Shank have a look at this. This was his area of interest.

DR. COHEN: Yes, as a consult.

DR. BERGFELD: As a consult. We'd like to sort of involve some of our ex CIR Panel members if they're able to do some of the work that they were doing, because we counted on them so much. So, if that would be something you'd consider, letting him go through it and then coming back and seeing what the editorials are.

DR. HELDRETH: Yeah. That was actually part of the reason why we wanted to bring this forward. You know, nitrosation was kind of Ron's baby during his tenure. Now we have some new experts with nitrosation.

I know that Dr. Tilton has worked on looking at nitrosation when it comes to vaping products and other tobacco-related products like that. We thought it was worthwhile to bring nitrosation to the forefront in the Panel's mind, considering there may be different viewpoints on the science of it.

DR. BERGFELD: So, we could consider this a working document for now?

DR. HELDRETH: For sure.

DR. BERGFELD: With all the experts putting their pen to it?

DR. HELDRETH: Yes.

DR. BERGFELD: Good.

DR. HELDRETH: Any edits or changes anybody has, make sure you include them in your Panel returns and Jinqiu can make those edits before we send them off to Ron.

DR. COHEN: I had a lot of comments on it.

DR. HELDRETH: Great. Appreciate that.

DR. COHEN: Yeah. I think we wait for another round of a review on this before it goes out for public comment. Does that not work for everyone? It's a very detailed report. One quick thing. As I was reviewing this -- I mean, this would be sort of an interesting paper as well. What happened with the hair dye epidemiology document? We discussed publishing that. And are we any closer to doing that?

DR. HELDRETH: It's currently available on our website for anybody to access. But we could certainly publish it in whatever journal.

DR. COHEN: Yeah, I don't think this would go in the International Journal of Toxicology. We should try to find a really high impact epidemiology journal, medical epidemiology journal. We don't have a contract that everything that comes out of here goes to International Journal of Toxicology, right?

DR. HELDRETH: No, just what we choose to send them. No.

DR. BERGFELD: Well, there was some comment too about the CIR having a better profile amongst other experts that utilize the materials. So, we might take a look at that and maybe this document, maybe even the inhalation document as well as sort of a summary of the CIR's activities over the years. We've done two such articles in the past. Maybe an updated one.

DR. HELDRETH: Sure.

DR. BERGFELD: New panel members, introduce them as well.

DR. COHEN: I think the hair dye epidemiology was amazing work. So much work went into that. It's really an amazing document.

DR. BERGFELD: And overtime.

DR. COHEN: And I think the chances of someone finding that document is really low. It's really low, on the CIR website. I think if it was published, it would get a lot of hits. That would have a tremendously high impact and be referenced constantly.

Full Panel – June 13, 2023

DR. COHEN: We spent some time on this. It's a very important report to have on Nitrosation. It's still in the draft format, so there are some redundancies in here. And I don't think it's ready for public comment yet. And we had recommended that perhaps we had an outside resource review it, and help edit it. And we had nominated Ron Shank if he was interested in doing it, since he's a nitrosation expert. And we could pull him back in for some work on this. And then we could re-review it here and then put it out for public comment.

DR. BELSITO: We had the exact same conclusion.

DR. BERGFELD: I think that we also called on David to help us with that as well. Thank you. All right, any other comments about the resource document?

DR. COHEN: You just volunteered me.

DR. BERGFELD: It's in draft form. It's a moving document. It's going to be changed slightly.

DR. COHEN: One quick thing.

DR. BERGFELD: Yeah.

DR. COHEN: Because Don brought it up before with the hair dye epidemiology, which was an amazing report. And I think we had agreed that we might want to publish that independently in an epidemiology journal. Our team wanted to persuade the staff to move that along for us to consider editing it and submitting it to a high-impact epi journal, not the International Journal of Toxicology.

DR. BERGFELD: Dave?

DR. ROSS: I agree with that comment about that document, but I'd also just like to, in the Nitrosation document, going back to volunteer Dr. Rettie and Tilton, without their consent, to comment on that document also.

DR. TILTON: The non-clinical people.

DR. COHEN: Second that motion.

DR. BERGFELD: I would like to also say that in the discussion in the team meeting with Dr. Cohen, we talked about the publication timeline and when these things would be published. And we're talking about prioritizing some of these, and moving them ahead a little bit faster and taking a look at how our publications are moving through the system, and maybe renegotiating with a publisher about getting the backlog caught up.

Going on to Dr. Belsito, you're going to talk about the amended 2024 Priorities List.

SEPTEMBER 2023 PANEL MEETING

Dr. Cohen Team - September 11, 2023

DR. COHEN: All right. So, I think we're moving onto the Nitrosation Impurities. This is an Intake Limits for Nitrosation Drug Substance-Related Impurities Guidance.

So, just last month the FDA, CDER, released recommended acceptable intake limits for nitrosamine drug substance related impurities, and it was to give guidance for manufacturers and applicants for drugs -- over the counter and prescription -- with a framework for predicting mutagenic and carcinogenic potential, the NDSRIs. And just provides a framework for acceptable intake.

The CIR is working to bring the nitrosamine resource document to the Panel table at the end of the year, and Ron Shank is working with the team. And the question is this nitrosation document, what impact did it have on any of you in helping craft this resource document from our end?

In a different but very similar vein, PCPC had recommendations -- I think it was in Wave 2 -- about how the resource document should look. And they broke it down to about ten subtypes that I think really -- it didn't simplify it, it just organized it in a way that I thought was reasonable and that maybe we'd be able to understand better. And I liked their recommendations very much and I thought we should adopt them but, David, I'm sure you have a lot to say on this.

DR. BERGFELD: Are we ready to adopt before Ron Shank looks at it?

DR. COHEN: No.

DR. ROSS: No.

DR. COHEN: No, no, no, these are just --

DR. BERGFELD: Okay.

DR. COHEN: -- the questions. I just wanted to give my opinion on what I thought -- what the PCPC had put forth, I thought it was very reasonable.

DR. ROSS: Yeah. I think we can move forward to consider these, but I would like your deliberations with Ron. I'd like those completed first. I mean, I look at some of these categories and the recommended Ais, and clearly there's a recognition that all nitrosamines are not created equal. They have different carcinogenic potencies which, I think, we're all aware of. So, it's nice to see that delineated here.

The other caveat here I have for you is that the FDA has also communicated to industry acceptable intake levels for specific substances. That's in addition to this. And we have to make sure that we're adhering to those guidelines also. So that's point one.

Point two, I haven't test driven this on some nitrosamines but if I draw a structure, where does it fall out based on their guidelines of how many carbons and how many bonds? So, I need to do that. But I'm assuming you're going to do that, Jinqiu, yeah?

DR. ZHU: Yes, I will.

DR. ROSS: Yeah. So, I think a summary would be, yeah, we should work towards incorporating this with those caveats, but obviously we can't do it tomorrow. We have to wait until we hear the results of those deliberations.

DR. COHEN: Can you (inaudible)?

DR. ROSS: You know, it was just in the document that FDA provides the industry, acceptable intake levels for specific nitrosamines. Which I don't know if they're going to be different to what you would derive from these guidelines, but they've already been communicated to industry. And we should also make sure that we're adhering to those guidelines as well as what's in this new document.

DR. SLAGA: Right.

DR. COHEN: Yeah, Tom. Tom?

DR. SLAGA: Yeah. I agree very much with everything that's been stated, with we have to take what FDA says and look at it in detail in which we have. I'm very much interested to hear and see what Ron Shank submits. How he's going to twist this around. We get that document in December?

DR. BERGFELD: December.

DR. COHEN: Presumably.

DR. SLAGA: Yeah. I think, at that point, we can do some comparisons and come up with a few things.

DR. COHEN: Okay.

DR. HELDRETH: That was our intention, was to provide this document so that the Panel could give us a little bit of feedback. And then Jinqiu is going to prepare his best draft and forward that onto Ron. And then once we get Ron's comments back, Jinqiu would revise that draft. And that -- along with a copy of Ron's comments -- is what would come to the panel in December.

DR. COHEN: Okay. Susan?

DR. TILTON: I agree that the approach that was put forward for categorizing nitrosamine impurities by potency should be considered and incorporated. I mean, it's similar to what we've been talking about with some of these other assays when we're thinking about doing things in a quantitative manner. Organizing by potency certainly helps with that.

So, it would be important to make sure that this is incorporated in a way that's really relevant for cosmetics. And I think the recommendations that were made for the nitrosation document in general, we will have to see what comes out of the discussion but I think the recommendations were primarily to try to organize it in a way that reduce a lot of the redundancies going on.

So, any way in which it can be organized in a manner to make it more straightforward is going to be helpful.

DR. COHEN: Okay. Any other comments? I think I've transcripted what you all said and I'll bring that across tomorrow.

DR. BERGFELD: Could I clarify? This is going to be a resource document then? CIR resource document on -- the title will be Nitrosamine in Cosmetics?

DR. HELDRETH: Yes. That's the plan essentially.

DR. COHEN: I think that was my understanding of it, yes.

DR. BERGFELD: I think we ought to say that up front because this is a drug. FDA, I guess what they call it.

DR. COHEN: Okay. I'll make some clarity on that.

DR. BERGFELD: Guidance, yeah.

DR. BJERKE: The FDA did make a distinction that it's both oral and topical.

DR. COHEN: Topical, yeah. And prescription and over the counter so. All right, any other comments? Did you get enough information from us there or did we just walk around in circles?

DR. ZHU: I think I'm fine.

DR. COHEN: You're okay?

Dr. Belsito Team - September 11, 2023

DR. SNYDER: Nitrosation, also a Wave 2 to that. Ron Shank has gratefully agreed to work on this. But, just to give some further guidance, any comments?

DR. KLAASSEN: Well, I just thought that that FDA document was interesting, and you might want to consider what's included in that.

DR. SNYDER: My first question is why did they limit it to OTCs and drugs? Why not have it be comprehensive?

DR. BELSITO: Well, because that's the separate little niche that looked at nitrosamines, where the drugs came up.

DR. EISENMANN: It's much more complex than what's in for cosmetics, though. It's much more limited.

DR. BELSITO: Yeah. It's much more complex for cosmetics because we have to look at product type, exposure, absorption. Whereas, drugs, they're basically looking at just PO.

DR. EISENMANN: I think it's different issues. So I'm not sure there should be an emphasis on -- not that it shouldn't be mentioned, but it shouldn't necessarily be an emphasis on that document because it is for drugs.

DR. KLAASSEN: No.

DR. EISENMANN: And it's more about when you're making an active -- how to prevent that.

DR. KLAASSEN: Right.

DR. EISENMANN: There's different issues for cosmetics, as it sometimes happens within the product rather than as you're making an ingredient.

DR. KLAASSEN: Right. That's a concept that --

DR. BELSITO: Right. It's a formulation issue.

DR. EISENMANN: Right, right.

DR. BELSITO: Right. Whereas in drugs it's not.

DR. EISENMANN: Right.

DR. BELSITO: And also in drugs, FDA is purely looked at PO. So in skin you have the issues of product type, which is going to influence it, the exposures.

DR. SNYDER: So what I was confused by -- because, if you go to Page 29 of the document, this hierarchical thing about potency categories, it says these NDSRIs, Nitrosamine Drug Substance-Related Impurities. That's what we're dealing with, right, impurities?

DR. BELSITO: Yeah. But, in our case, they can be formed in formulation. Right? Much of the nitrosamine stuff we do is, you know, shouldn't be formulated with N-nitroso, comma, shouldn't be formulated with da, da, da, da. And most of it has to do with DEA.

DR. KLAASSEN: It's a problem in the synthesis of drugs.

DR. BELSITO: Okay.

DR. KLAASSEN: In the synthesis, sometimes you get these byproducts where we are more concerned about forming them.

DR. SNYDER: Okay. But the same concerns as the intake or the exposure would be --.

DR. KLAASSEN: Similar. There are absolute similarities.

DR. SNYDER: Right. Yeah.

DR. KLAASSEN: But it's not identical.

DR. BELSITO: No one's arguing about the systemic toxicity. But the approach that the FDA is taking is looking at the impurities in a specific agent, and we're more concerned about the formation of these in a formulation.

DR. SNYDER: Okay.

DR. BELSITO: Right?

DR. SNYDER: Yeah.

DR. KLAASSEN: They had the concept in there that I like, that a nitrosamine isn't a nitrosamine. Some of them are really reactive --

DR. BELSITO: Right.

DR. KLAASSEN: -- others, yeah, just a little bit. I like that in there. But we're basically, in this Committee, trying to stop the formation of any nitrosamine. We're not just trying to stop the formation of the real active ones. But, anyhow, it was good science. I enjoyed reading that.

DR. RETTIE: Yeah. I thought it was worth reading about the alpha carbon primacy.

DR. SNYDER: Right.

DR. RETTIE: And, in thinking about that, we wonder about the toxicity of these things in cosmetics.

DR. SNYDER: I like their language; the appropriate control strategy to keep below the acceptable intake. We're trying to always look for that kind of language that is consistent across the scientific world.

DR. BELSITO: How many French fries can you eat?

DR. SNYDER: Yeah, I know. Not many anymore.

DR. RETTIE: There's a very interesting review (inaudible). It's written by (inaudible). That is almost at odds with -- because it tries to address the acceptable intake piece for the drugs and drug molecules that are being produced. And it's not clear that the acceptable intake levels for his examples parallel exactly or even closely the information in these documents here. And I didn't really know what to make of that. He's not answering my emails.

DR. BELSITO: Okay. But, after reading this, any advice to Jinqiu and Ron as they put together our nitrosation document?

DR. SNYDER: Quite high confidence that we will get a very nice document.

Full Panel – September 12, 2023

DR. BERGFELD: All right. I think we should move on. I think we've discussed this and we'll see it again. So we're going on to the FDA Nitrosation Impurities Guidance. Dr. Belsito?

DR. BELSITO: Yeah. So this document, first of all, we had looked at it before we made a number of edits and then finally ended up suggesting that Jinqiu work with Ron Shank to come up with a publishable document. The CIR did bring us one question and that would be, what influence should the recommended acceptable intake levels for nitrosamine drug substance related impurities guidance from industry have on this document? And we thought that -- I mean, it can be included in the document, but in terms of cosmetics, we really need to look at product type exposure, potential absorption. So it's really -- cosmetics are almost more complicated than a po drug, where you're looking simply at known systemic toxicity of a given concentration of nitrosamine.

So, I think it's something you want to add to the report, but it doesn't solve the issue for nitrosamines. Particularly, since in cosmetics nitrosamines are largely going to be generated in product by combination of ingredients and not be a part of the final active drug that is the case in terms of the drugs FDA has looked at,

DR. BERGFELD: I interpreted this FDA document of guidance as something we would look at and reference, but we'd make up our own guidance.

DR. BELSITO: Yes, exactly.

DR. COHEN: Right, but we probably --

DR. BERGFELD: Well, cosmetics are much different, yeah.

DR. COHEN: But as far as intakes are concerned, we probably want to work within those parameters, right, to make sure we're not exceeding that.

DR. BERGFELD: Exactly.

DR. COHEN: If that's something we can understand and calculate at the time.

DR. KLAASSEN: I think what's important here is that we make sure that Ron Shank knows about this document. And he knows the area and can take the appropriate things out of here. I think that's all that's necessary. Again, you probably don't find this on PubMed. Just make sure that he knows about this document, it's current, and he can do what he wants with it.

DR. BELSITO: And Allan made a comment about the various potencies of the different nitrosamine classes. Allan, if you want to say something about that.

DR. RETTIE: Yeah, I had a hard time following the document in terms of acceptable intake for oral drugs, and looking at the compounds that they were specifying numbers for, and trying to relate that to this nitrosamine document. There didn't seem to be -- I mean, there was some parity when you compared, but it wasn't clear to me that it was very, very good.

And so, I just felt it was a bit difficult to know how to apply that to cosmetics. Certainly oral drugs are very different from what we're talking about here. So, I thought it might just have limited utility.

DR. BERGFELD: Me too.

DR. RETTIE: Good to note, as Dr. Bergfeld said, and to know about but any extrapolations across, I thought, would be a bit difficult.

DR. BERGFELD: Anyone else wish to comment?

DR. COHEN: PCPC had some recommendations on how the document ought to lay out, and I thought they were pretty good. It was a nice organization. It doesn't have to be exactly like that, but --

DR. BERGFELD: I think that Ron should be appraised of all that.

DR. COHEN: Yeah.

DR. BERGFELD: David, anything?

DR. ROSS: I was just going to comment, I agree. It can be used as a guide, but it's not going to solve all our problems. All nitrosamines are not created equal, this just underlines that.

DR. BERGFELD: All right. Let's move on, then, to the FDA Cosmetic Registration Draft Guidance. Dr. Cohen.

EXPERT PANEL FOR COSMETIC INGREDIENT SAFETY

Resource Document

N-Nitrosation and the Safety Evaluation of Cosmetic Ingredients

Meeting Review - 12/2023 - DRAFT

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.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. - Ron Shank, Ph.D., former member of the Panel, provided expert opinion herein - The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This resource document was prepared by Jinqiu Zhu, Ph.D., D.A.B.T., E.R.T., D.C.S.T., CIR Toxicologist.

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BACKGROUND

What is a *N*-nitrosamine and its presence in cosmetics

Amines, particularly secondary amines, have the potential to undergo *N*-nitrosation reactions in the presence of nitrosating agents such as nitrite and nitrous acid, resulting in the formation of *N*-nitrosamines. Nitrosamines belong to the class of *N*-nitroso compounds, depicted in Figure 1, where the R_1 and R_2 groups attached to the amine nitrogen can range from a hydrogen atom (in each case) to complex chemical substituents (although, typically alkyl groups).

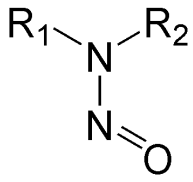

Figure 1. Generic *N*-nitrosamine structure

N-Nitrosation may occur in diverse environments, including air, soil, water, stored or preserved foods, and the gastrointestinal tract of both animals and humans.[1] *N*-nitrosamines can contaminate cosmetic products due to impurities in raw materials, manufacturing processes, and as a result of reactions during storage and packaging, where initial ingredients may interact with nitrosating agents.

Amides can be *N*-nitrosated to form *N*-nitrosoamides (Figure 2), but there are few reports that such compounds have been detected in cosmetics. Most *N*-nitrosamines and *N*-nitrosamides so far tested have proven to be mutagenic and carcinogenic. Some *N*-nitrosamines have shown to be carcinogenic in more than 40 animal species.[2]

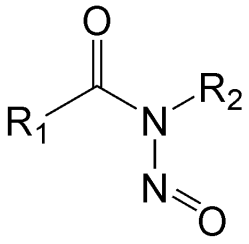

Figure 2. Generic *N*-nitrosamide structure

Some *N*-nitrosamines can be present in a variety of cosmetics ingredients. For example, some surfactants and emulsifiers, including ammonia or amine salts, can potentially be sources of *N*-nitrosamines. Raw materials containing secondary amines, such as diethanolamine (DEA), have the highest potential for *N*-nitrosamine formation. *N*-Nitrosamine levels in certain cosmetics may increase over the months following product opening.[3]

The issue of *N*-nitrosation potential has long been recognized and managed by the cosmetics industry. Minimizing the likelihood of *N*-nitrosamine formation in cosmetics is important. However, exposure to *N*-nitrosamines is widespread through daily consumption of food and drinking water, and considerable efforts have been undertaken to establish internationally-recognized acceptable daily intake limits for *N*-nitrosamines.[4-6] Moreover, certain substances with nitrosatable structures may be susceptible to *N*-

nitrosation, but only under non-cosmetic use conditions; these would not be physiologically relevant or applicable to cosmetic formulations.[7] Therefore, it is crucial to elucidate the considerations guiding the Panel's approach to addressing concerns related to the occurrence of *N*-nitrosamines as contaminants in cosmetic products.

The *N*-nitrosamines most often observed in cosmetic products are *N*-nitrosodiethanolamine (NDELA) and *N*-nitrosobis(2-hydroxypropyl)amine (NBHPA).[8] Other nitrosamines are rarely found in cosmetics and/or raw materials.[4,8] A concise list of nitrosamines, beyond NDELA and NBHPA, have been identified in cosmetics, including *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosomorpholine (NMOR), *N*-nitrosopyrrolidine (NPYR), and *N*-nitroso-p-aminobenzoic acid (NPABA).[9]

TOXICOLOGY OF *N*-NITROSAMINES

Acute exposure to most *N*-nitrosamines induces cellular necrosis in the tissues in which they are metabolized by cytochrome-P450 enzymes, especially liver, kidney, and lung. *N*-nitrosamides do not require metabolic activation to induce cellular necrosis.

Most *N*-nitrosamines possess potent genotoxic properties in *in vitro* bacterial reverse mutation tests and have been shown to be carcinogenic in laboratory animals; prolonged or excessive exposure to *N*-nitrosamines may increase the risk of certain types of cancer in humans.[2,10-12] Studies have demonstrated the ability of such chemicals to permeate the epidermis, as well as the respiratory and gastrointestinal tracts, leading to systemic absorption.[13-16] To manifest mutagenic and carcinogenic activity, *N*-nitrosamines need to undergo metabolic activation to a reactive carbocation or diazonium compound which will bind covalently to DNA.[17,18] This metabolic transformation leads to irreversible chromosomal and genetic alterations.

The metabolism of NDELA, one of the most often detected in cosmetics (hair dye formulations) is given below (Figure 3) as typical for *N*-nitrosamines.[18] The metabolism of NDMA, which has been studied to a greater extent, is also shown (Figure 4). NDMA undergoes α-methyl hydroxylation which decomposes to α-hydroxy*N*-dimethylamine, yielding methyl diazohydroxide and formaldehyde; methyl diazohydroxide forms the strong electrophile methyldiazonium ion which covalently binds to DNA, RNA, and protein. Replication of the adducted DNA leads to cellular transformation and carcinogenesis.

Several hundred *N*-nitroso compounds have been tested for carcinogenic potential and most have shown to be carcinogenic in a variety of tissues.[2] Within the library of *N*-

nitroso compounds there is at least one that induces cancer of any major organ, liver, kidney, lung, skin, brain, pancreas, urinary bladder, colon, etc. *N*-nitrosoethylurea (NEU) has been shown to induce cancer transplacentally.[19,20]

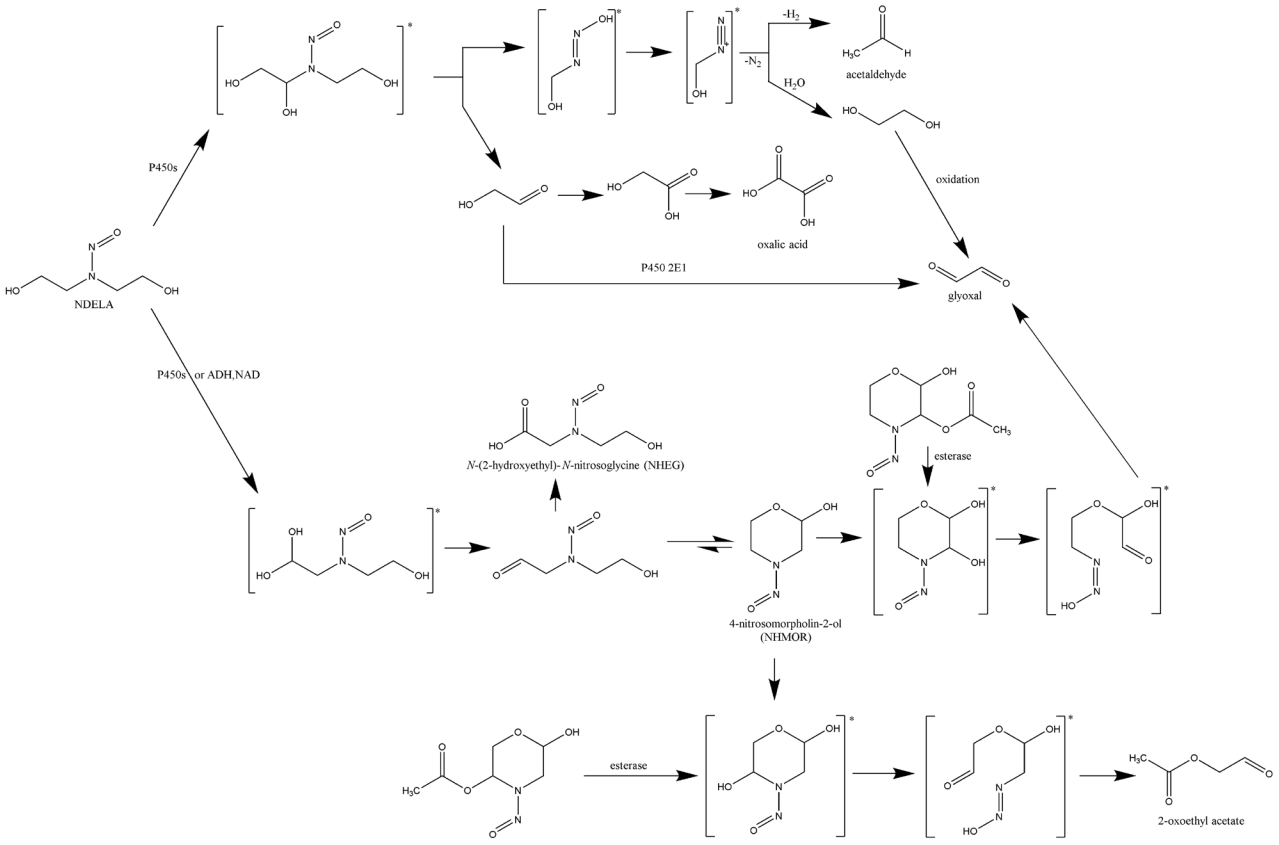

Figure 3. Mechanisms of NDELA metabolic activation.[18, CIR Staff]

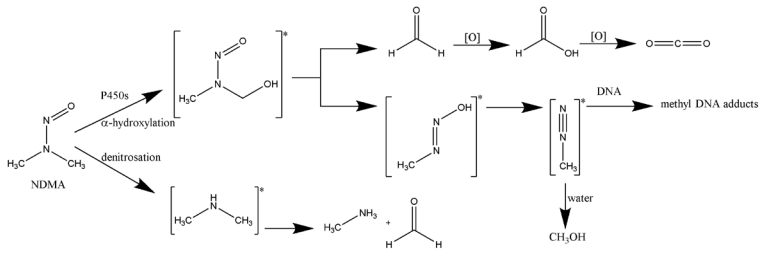

Figure 4. Mechanisms of NDMA metabolism.[18, CIR Staff]

REGULATION OF *N*-NITROSAMINE FORMATION IN COSMETIC INGREDIENTS AND COSMETIC FORMULATIONS

In a notice published in the United States Federal Register of April 10, 1979 (44 FR 21365), the FDA expressed concern about *N*-nitrosamine contamination in cosmetics, stating that cosmetics containing *N*-nitrosamines may be deemed adulterated and subject to enforcement action.[21] Marketing adulterated cosmetics is illegal regardless of whether safety issues arise from ingredients or contaminants. Companies and individuals who market cosmetics have a legal obligation to ensure product safety.[22] The FDA is responsible for monitoring cosmetics to detect potential safety issues, including potential *N*-nitrosamine contaminants. FDA inspectors carry out examinations to verify that manufacturers adhere to the suggested procedures to prevent the formation of *N*-nitrosamines during the manufacturing process of cosmetics.[22] For example, the FDA recommends that cosmetics manufacturers voluntarily remove ingredients that can combine to form *N*-nitrosamines, such as NDELA, and conduct testing to identify the causes of NDELA contamination in cosmetics. Table 1 provides a brief summary of major regulations and guidelines pertaining to *N*-nitrosamines in medicines, food, and cosmetic products within the US and European Union (EU).

In the EU, the presence of *N*-nitrosamines was prohibited in cosmetic products under the Cosmetics Regulation (European Commission [EC]) No 1223/2009 of the European Parliament and of the Council of the European Union in Annex II. Secondary alkyl- and alkanol-amines and their salts were also banned to reduce contamination from carcinogenic *N*-nitrosamines that are formed after the reaction with nitrosating agents. Moreover, Annex II specifies that *N*-nitrosamines should not form part of the composition of cosmetic products above trace levels that are technically unavoidable in Good Manufacturing Practices (GMPs). Regulations outlined in the Annexes of EU Cosmetic Regulations 76/768/EEC set a specified purity level for *N*-nitrosamines in raw materials at 50 ppb, which includes all nitrosamines potentially formed. The content of secondary amines in raw materials should be kept as minimal as possible and should not surpass 5%. In finished cosmetic products, the content of secondary amines should be restrained to a maximum of 0.5%.

For certain substances regulated under Annex III, minimum raw material purity should be higher than 99%, and limits have been set for maximum secondary amine content in the raw materials (e.g., 5% and 0.5% for fatty acid dialkanolamides and monoalkylamines, respectively) and finished product (e.g., 0.5% for monoalkylamines, monoalkanolamines and their salts), as well as for maximum *N*-nitrosamine impurities (e.g., 50 µg/kg for fatty acid dialkylamides and dialkanolamides). Such a limit does not

apply to finished products, and relevant ingredients should not be used with nitrosating systems and should be kept in nitrite-free environments. Nevertheless, the Cosmetic Regulation (EC) specifies in Article 17 that "The non-intended presence of a small quantity of a prohibited substance, stemming from impurities of natural or synthetic ingredients, the manufacturing process, storage, migration from packaging, which is technically unavoidable in good manufacturing practice, shall be permitted provided that such presence is in conformity with Article 3." At present a maximum permitted concentration for *N*-nitrosamines, such as NDELA, in cosmetics products has not been established in the EU.[23] While *N*-nitrosamines may be present as traces that are technically unavoidable in GMPs, the finished product should not cause harm to human health when applied under normal or reasonably foreseeable conditions of use.[9]

NITROSATABLE SPECIES IN COSMETIC INGREDIENTS AND COSMETIC FORMULATIONS

The formation of *N*-nitrosamines in cosmetic products may result through the confluence of three factors: i) the presence of precursor ingredients having structures that are capable of undergoing nitrosation (i.e., nitrosatable structures), ii) the availability of a nitrosating agent, and iii) the existence of suitable reaction conditions. The occurrence of *N*-nitrosation is conditional, depending on both the nitrosating agent and the substrate, and can occur under acidic, neutral, or alkaline conditions.[24,25]

While *N*-nitrosamines can be formed from primary, secondary, and tertiary amines, *N*-nitrosamine formation is most favored with the secondary amines, under manufacturing, storage, or use conditions. For amines with different degrees of substitution (i.e., not secondary), the likelihood of *N*-nitrosation is much less. The attention in cosmetic products should be primarily on secondary amines, as the resultant *N*-nitrosamines are the most stable and likely to result in consumer exposure.

Primary amines do not yield stable *N*-nitrosamines. These can easily interact with nitrosating agents, leading to the formation of unstable *N*-nitroso compounds but swiftly decompose, resulting in diazonium salts rather than producing *N*-nitrosamines.[24,26] Some tertiary amines may demonstrate reactivity towards *N*-nitrosation.[24,27] However, the majority of these are not notably reactive towards *N*-nitrosation under standard conditions.[28] The nitrosation of the majority of tertiary amines proceeds at a notably slow rate and generally does not pose a concern for cosmetics.

Certain cosmetic constituents that are considered relevant precursors (e.g., secondary amine traces or "nitrosatable precursors") for the formation of *N*-nitroso compounds, may be generated during the formulation of cosmetic products or through

the decomposition of raw materials.[9] For example, DEA and diisopropanolamine may be present as impurities and decomposition products of raw materials such as monoalkanolamines, trialkanolamines, and fatty acid mono- and dialkanolamides. Dimethylamine or morpholine may be present as decomposition products of certain preservatives. Consequently, the occurrence of *N*-nitrosamine contaminants in finished cosmetic products can be attributed to the utilization of specific cosmetic ingredients, which are subject to specific restrictions on use (e.g., maximum secondary amine content in the raw material or in the finished product as well as minimum raw material purity has been established in Annex III of EU Cosmetic Regulation (2009) for monoalkanolamines, monoalkanolamines and their salts).[24] The most probable origins of nitrosatable agents are dialkanolamines, which are utilized in the synthesis of dialkanolamides, with diethanolamide being predominantly used in cosmetic products.

It has been recognized that specific structural elements present in secondary amines can inhibit cytochrome P450-dependent metabolic activation of *N*-nitrosamine and thus attenuate mutagenicity/ carcinogenicity (e.g., ethyl-*t*-butyl nitrosamine), or even result in generation of non-carcinogenic *N*-nitrosamines.[13,24] Other structures that can mitigate formation of carcinogenic *N*-nitrosamines may also exist. For instance, strongly basic or acidic centers present in a given *N*-nitroso compound may significantly reduce mutagenicity/carcinogenicity (e.g., *N*-nitroso-*N*'-methylpiperazine, or most *N*-nitroso amino acids and their esters).[29]

N-Nitrosamides also pose genotoxic and carcinogenic risks. However, nitrosation of amides proceeds at a markedly slow rate due to the lone pair of electrons on the nitrogen being associated with the amide's carbonyl group. *N*-nitrosated primary amides exhibit instability and are prone to degradation. While secondary amides possess the capability to undergo nitrosation, these necessitate potent nitrosating agents, and the reaction the reaction progresses slowly; such potent nitrosating agents are not present in cosmetic formulations. In general, the nitrosation of amides is not considered a major issue for cosmetic products. However, there remains a real potential for the presence of nitrosatable amine impurities within amides.[24]

REACTION RATES OF AMINES

The kinetics of amine nitrosation primarily hinge on the acid-base equilibria of the amine and nitrous acid, with dinitrogen trioxide (N_2O_3) acting as the nitrosating agent, reacting with the free electron pair of the unprotonated amines to form *N*-nitroso compounds, and displacing NO_2. This process is influenced by pH levels (the nitrous acid/nitrite equilibrium has a pKa of 3.4), with optimal rates occurring at pH 3 - 4,

manifesting a bell-shaped curve to greater acidity or alkalinity.[30,31] For example, morpholine, an amine of less basicity, exhibits a reaction rate that is at least 316 times more rapid at pH 3.4 under comparable conditions. Additionally, it is recognized that the intrinsic rate constants in the reaction of the free amine with N_2O_3 are notably similar across various amines.[31,32]

The availability of pKa values for amines enables the prediction of *N*-nitrosation rates under specified conditions.[33] Substituting one alkyl group of a secondary amine with an aromatic group such as a benzene ring diminishes the amine's basicity by around 5 pKa units. However, the presence of substituents on the benzene ring may substantially influence the *N*-nitrosation rate. This is attributed to their ability to modify both the basicity of the amine and the intrinsic rate constant for *N*-nitrosation.[34]

NITROSATION ACCELERATORS

The formation of *N*-nitrosamines from secondary amines can be catalyzed by aldehydes, especially formaldehyde, through the intermediacy of imines, and these transformations are observed to occur readily at pH values ranging between 5 and 10.[24] Similar reactions can also occur with ingredients in organic solvents like dichloromethane, which serves as a source of formaldehyde. Furthermore, substances that release formaldehyde, including formaldehyde donors like hydroxymethylurea and hydroxymethylsarcosine, can also act as catalysts in nitrosation, possibly catalyzing their own nitrosation to produce *N*-nitroso compounds.

Of note, certain preservatives that are allowed in cosmetics, such as 2-bromo-2-nitropropane-1,3-diol (bronopol) and 5-bromo-5-nitro-1,3-dioxan (bronidox), have been identified as potent nitrosating agents. Bronopol can decompose to act as formaldehyde, which further catalyzes the production of nitrosamines from secondary amines. Whenever these preservatives are formulated with secondary amines, substantial *N*-nitrosamine formation can be anticipated. Formulation of such preservatives with *N*-nitrosatable ingredients in cosmetics must be avoided. Cosmetic products may also contain unavoidable traces of acetaldehyde, primarily derived from botanical ingredients or ethanol, which may catalyze *N*-nitrosation.[24,35] Other moieties, such as halide ions, thiosulfates, thiols, and certain phenolic compounds, may also act as effective catalysts for *N*-nitrosation reactions.[34] Consequently, such accelerators should not be used in formulations containing secondary amies.

NITROSATION INHIBITORS

There are a wide range of substances that can compete with amines for the nitrosating agents, thereby inhibiting *N*-nitrosamine formation under specific conditions, including water or oil soluble inhibitors, as listed in a technical guidance document published by Cosmetics Europe.[9] Antioxidants and chelating agents, such as ascorbic acid, gallic acid, and sodium citrate, are among the potential inhibitors that can react preferentially with nitrite, nitrogen oxides, or the iminium ions produced during aldehyde-catalyzed reactions. Achieving effective inhibition necessitates employing a combination of inhibitors that can both prevent iminium ion formation and scavenge nitrite. Appropriate traps for iminium ions include citrate, adipate, and tartrate anions. To be used together with these iminium ion traps, ascorbic acid and its salts, erythorbic acid and its salts, sodium ascorbyl phosphate, or magnesium ascorbyl phosphate are suitable nitrite scavengers. From the perspective of formulation stability, sodium ascorbyl phosphate and magnesium ascorbyl phosphate are the preferred options for nitrite scavenging.[9]

Cosmetic formulations may require both hydrophobic and hydrophilic nitrosation inhibitors to be effective in both phases. In the practical application of inhibitors, it's important to note that none of the agents can destroy N-nitrosamines already existing in raw materials; inhibitors should ideally be introduced to the formulation before incorporating any organonitrogen ingredients; additionally, there exists a limitation to the extent of inhibition achievable in real systems, and there are specific restrictions on which of the potential inhibitors can be integrated into cosmetics. In every instance, the formulation, production, and subsequent storage should be conducted at the lowest feasible temperature.

In addition, according to the EU Cosmetics Regulation (2009), the utilization of any of these inhibitors is deemed as the introduction of a cosmetic ingredient, thus necessitating compliance with the requirements and regulations regarding product safety and product information.

MEASUREMENT OF NITROSAMINES IN COSMETIC PRODUCTS AND RAW MATERIALS

The apparent total nitrosamine content (ATNC) method acts as an effective screening tool for evaluating the presence of the volatile and nonvolatile *N*-nitrosamines in cosmetic products and raw materials, and can be applied to a majority of cosmetic samples.[9] Given that this method is not prone to false negatives, if samples are determined to be below the acceptable limits, additional testing may not be necessary; however, due to the potential for interference from non *N*-nitroso compounds, any

positive result exceeding acceptable limits necessitates confirmation through a more specific technique whenever feasible. If the composition of the cosmetic matrix implies that NDELA or other specific nitrosamines might be contributing to a positive result in the ATNC, additional analysis may be needed by employing chromatographic techniques, such as gas chromatography (GC) coupled to thermal energy analyzer, single or tandem mass spectrometry (MS or MS/MS); or high performance liquid chromatography (HPLC) with thermal energy analyzer, ultraviolet light (UV) detector, or MS and MS/MS detection.[9,36] The GC-thermal energy analyzer method is highly specific but is time-intensive and might induce *in situ* formation of nitrosamines. The HPLC post-column derivatization and HPLC/MS/MS methods are both standardized by ISO (e.g., ISO 10130 and ISO 15819) and are preferred in regulatory context. New analytical methods that may simultaneous determine a couple of prohibited *N*-nitrosamines are also under development; e.g, a novel method based on vortex-assisted dispersive liquid–liquid microextraction (VA-DLLME) following by GC-MS analysis is proposed.[36] The proposed method ought to possess robust analytical characteristics and, being both simplistic and cost-effective procedure, should be suitable for quality control of cosmetics to ensure the safety of users and compliance with the regulations governing cosmetic products.

POSSIBLE STRATEGIES FOR MINIMIZING NITROSAMINE FORMATION IN COSMETICS

To mitigate the formation of nitrosamines, it is critical to employ a strategic approach incorporating several minimization strategies.[9] These include the reduction or elimination of incidental nitrite sources and secondary amino sources, alongside the implementation of suitable inhibitors.

In adherence to GMPs, the presence of adventitious nitrite can be mitigated by employing purified water during manufacturing, utilizing containers made of nitrite-free steel or plastic is crucial for storing raw materials and products, limiting exposure to air containing nitrogen oxides throughout the production process, and isolating the manufacturing process from hydrocarbon fuel equipment and open flames. Furthermore, removing unnecessary nitrates/nitrites from raw materials and reducing the use of materials manufactured in environments rich in nitrogen oxides are crucial steps in minimizing the levels of adventitious nitrite.

Traces of secondary amines in cosmetic products can originate from substances such as diethanolamine, diisopropanolamine, dimethylamine and morpholine. These can exist as impurities and decomposition products of raw materials like monoalkanolamines, trialkanolamines, and fatty acid mono- amine oxides, and

dialkanolamides, as well as certain preservatives. Therefore, monoalkanolamines, monoalkylamines, trialkanolamines, trialkylamines, and their salts, along with fatty acid dialkylamides and dialkanolamides, are subject to restrictions in Annex III of EU Cosmetic Regulation (2009).[37] The restrictions relate to their minimum purity, maximum secondary amine content, maximum *N*-nitrosamine content, requirements for storage in nitrite-free containers, defined usage levels, and mandatory avoidance of nitrosating systems.

Alongside the selection of suitable raw materials, it is important to incorporate inhibitors of nitrosamine formation. Notably, there is no one-size-fits-all solution, so suitable inhibition strategies need to be evaluated for each product type, with considerations given to the type of emulsifiers used and the specific requirements based on the emulsion and amine types. Potential inhibitors may involve antioxidants and some substances which can either preferentially interact with nitrite and nitrogen oxides - acting as nitrite scavengers - or with iminium ions that are generated during the formaldehyde-catalyzed pathway to the formation of *N*-nitrosamines, where low levels of formaldehyde may be present, utilizing specific inhibitors of iminium ions is recommended.

The generation of *N*-nitrosamines in products can be effectively controlled through appropriate formulation strategies, specifically by avoiding the use of amines or amino derivatives in combination with a nitrosating agent.[9,38] Furthermore, it is necessary to conduct tests under normal conditions of use to verify the absence of *N*-nitrosamine formation. To this end, it is crucial to consider the kinetics of the *N*-nitrosation reaction, as its reaction rates can vary significantly, differing by orders of magnitude, and are highly influenced by the basicity of the amine. In general, the reaction leading to the formation of *N*-nitrosamines can involve a variety of nitrosating species, and is typically pH dependent. Given that the formation of *N*-nitrosamines typically occurs under acidic conditions, it is possible to implement alternative strategies during cosmetic manufacturing for mitigating their production.[24] Under neutral or basic conditions, the kinetics of these reactions are significantly reduced, which is crucial for the safety evaluation of *N*-nitrosatable substances under cosmetic use conditions.[39] For example, although the nitrogen atom of pyridine is known to be susceptible to *N*-nitrosation, this reaction occurs only under non-physiologic conditions or under strictly anhydrous conditions that are not applicable to hair dye product formulations, or any aqueous cosmetic formulations.[7]

SAFETY ASPECTS CONSIDERED BY THE PANEL FOR MITIGATING *N*-NITROSATION POTENTIAL IN COSMETIC FORMULATIONS

Although the levels of *N*-nitrosamines detected in cosmetics that contain secondary amines and a nitrosation source are usually very low, precautions should be taken in situations where contact with nitrosating agents during production, formulation, storage, and usage cannot be reliably eliminated. The nature of the ingredients and their propensity for *N*-nitrosation should all be duly considered.

The Panel notes small amounts of secondary amines could be present as impurities in cosmetic ingredients or formulations; for example, ethanolamine typically contains a small amount of DEA as an impurity.[40] Accordingly, the Panel expressed concerns regarding the levels of incidental free secondary amines that could potentially facilitate the generation of *N*-nitrosamines. In this regard, it is expected that cosmetic manufactures undertake a thorough evaluation of the potential risks associated with *N*-nitrosamine impurities. A range of factors, such as chemical structure, raw materials, and synthesis pathway, may render an ingredient susceptible to *N*-nitrosamine formation. Collaborative research between the cosmetic industry and the FDA is essential to comprehensively understand the mechanisms underlying *N*-nitrosamine formation, to establish robust analytical techniques for their detection, as well as to devise strategies for preventing their occurrence in cosmetic products. The Panel recognizes that some concerns regarding the formation of *N*-nitroso compounds in cosmetic products may be based on experimental conditions that are not representative of plausible use conditions. For instance, lecithin has been reported to be metabolized to choline by bacterial phospholipases in a model system, which can subsequently release dealkylated dimethylamine, an *N*-nitrosatable compound in the presence of nitrates.[41] However, the Panel has determined that such experimental conditions do not reflect the use of this ingredient in cosmetic products. It is noteworthy to highlight that nitrosatable compounds may not undergo *N*-nitrosation when applied during normal or reasonably foreseeable conditions of product use; consequently, the exclusion of nitrosating agents from formulations is not deemed necessary under such circumstance. In this context, the Panel may assess the ingredients and their associated contaminations on a case-by-case basis, aiming to ensure a high degree of consumer protection and minimize the risk of exposure.

Although *N*-nitrosamines are not deliberately added to consumer products, they have been detected as contaminants in various products, including cosmetics, food, beer, tobacco, and rubber products. Since *N*-nitrosamines may be present in trace amounts as unintended consequences, the implementation of sensitive analytical methods is crucial to detect and monitor their levels. The Panel observes that

nitrosamine contamination issues do not impact all cosmetic products. Only a minority, those containing secondary amines and a source of nitrosation, are affected. Cosmetic manufacturers are required to conduct quality control analyses for raw materials and products whose constituents may inadvertently lead to *N*-nitrosamine formation. Avoidance of impurities and incompatibilities between ingredients is essential to prevent potential *N*-nitrosation reactions. The Panel recommends that cosmetic ingredient manufacturers adhere to the guidance issued by relevant regulatory agencies, for industry, to identify and prevent objectionable levels of *N*-nitrosamine impurities in cosmetic products, or situations wherein such *N*-nitrosamine may be formed.

CONCLUSION

N-Nitrosamines are not cosmetic ingredients. When exposure and duration of exposure are high enough, some *N*-nitrosamines may pose considerable health risks in cosmetic formulations owing to incidental formation resulting from amine ingredients therein. These amines have the potential to interact with nitrosating agents through diverse routes, such as manufacturing or storage contamination, or reaction with nitrosating agents during product use. Thus, the Panel is concerned with the presence, in cosmetic formulations, of either a) ingredients or impurities which may act as substrates capable of being *N*-nitrosated, or b) ingredients, impurities, or in-use materials which may act as nitrosating agents. Additionally, while the formation of *N*-nitrosamides is unlikely in cosmetics, contamination of amide ingredients with *N*-nitrosatable amines is entirely possible. To address these concerns, the Panel directs formulators to be aware of situations wherein *N*-nitrosamines may be formed, and consider strategies that prevent the formation of *N*-nitrosamines. For example, in cases where a secondary amine is used as an ingredient, or the available evidence suggests the possible presence of secondary amine contamination, the Panel cautions formulators to adopt strategies that avoid exposure to *N*-nitrosating agents (e.g., do not formulate with nitrosating ingredients; consider possible contamination by nitrosating agents from ingredient impurities, from packaging, or from final product use).[40,42]

Table 1. Regulations and guidelines on *N*-nitrosamines in medicines, food, and cosmetic products in the US and EU.

Agency	Title	Regulation/Recommend/Guidance	Year	Ref.
US FDA	Control of Nitrosamine Impurities in Human Drugs (Revision 1): Guidance for Industry	FDA advises manufacturers consider the potential causes of nitrosamine formation and to assess the risk of nitrosamine contamination or formation in the active pharmaceutical ingredients (APIs) and drug products. Manufacturers should prioritize the evaluation of APIs and drug products based on elements like maximum daily dose, treatment duration, therapeutic indication, etc. Manufacturers are advised to refer to the ICH Guidance for Industry *Q9 Quality Risk Management* (2006) for specifics regarding the identification, analysis, and management of quality risks. Manufacturers of APIs and drug products should implement suitable measures to avoid unacceptable levels of nitrosamine impurities.	2021	4
US FDA	Recommended Acceptable Intake Limits for Nitrosamine Drug Substance Related Impurities (NDSRIs): Guidance for Industry	In this guidance, FDA provides updated information on acceptable intake (AI) limits and recommended testing methods for NDSRIs. AI limits recommended for the five predicted carcinogenic potency categories range from 26.5 to 1500 ng/day, e.g., NDEA (Potency Category 1) with an AI limit of 26.5 ng/day; NDMA (Potency Category 2) with an AI limit of 100 ng/day. An approach has been proposed to categorize the carcinogenic potency of nitrosamine impurities in drug products and active pharmaceutical ingredients.	2023	43
US FDA	CPG Sec 510.600 Dimethylnitrosamine in Malt Beverages	A practical level of safety has been determined to be 5 ppb Dimethylnitrosamine (DMNA). Malt beverages containing more than 5 ppb DMNA may be considered to be adulterated under section 402 of the Act.	2005	44

US FDA	CPG Sec 578.500 Dimethylnitrosamine in Barley Malt	The action level of 10 ppb DMNA in barley malt applies to all barley malt produced after October 1, 1980. (Nitrosamines at levels greater than an established action level are considered avoidable contamination under section 406 of the Federal Food, Drug, and Cosmetic Act.)	2005	45
US FDA	CPG Sec 500.450 Volatile N-Nitrosamines in Rubber Baby Bottle Nipples	An action level of 10 ppb for individual nitrosamines applies to both consumer and hospital rubber baby bottle nipples initially introduced or initially delivered for introduction into interstate commerce on or after January 1, 1985.	2005	46
European Commission	ANNEX II- List of substances prohibited in cosmetic products	Cosmetic products containing *N*-nitrosamines including NDELA are banned.	2009	37
European Commission	ANNEX III- List of substances which cosmetic products must not contain except subject to the restrictions laid down	The threshold for N-nitrosamines in raw materials is set to 50 ppb, including all potentially formed nitrosamines. Secondary amine content in raw materials should be as low as achievable and not exceed 5% in raw materials. In finished cosmetic products, a maximum secondary amine content of 0.5% should be maintained.	2009	37
Cosmetics Europe	Technical Guidance Document on Minimizing and Determining Nitrosamines in Cosmetics	The guidance document offers general recommendations on strategies to reduce the potential formation of nitrosamines in cosmetic products. Analytical methods and each's relevance and limitations are outlined. It suggests an analytical approach for examining nitrosamines in both cosmetic products and raw materials.	2009	9

ANNEX I

SAFETY ASESSMENT OF *N*-NITROSAMINE IN COSMETICS

Primary sources of human exposure to *N*-nitrosamines recognized as consumption of tobacco and alcohol, in addition to food intake.[47] Importantly, cosmetics are not a primary source of *N*-nitrosamines and not every cosmetic product contains nitrosamine impurities; those products that have been tested positive reveal a wide range of concentration values. For instance, a study has demonstrated that out of 103 cosmetic products in Korea, 13 were found to have NDELA, and it was detected in 4 out of 12 raw materials. The range detected spanned from trace levels (< 20 µg/kg) to 214.2 µg/kg, utilizing a ultra-high performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) method.[48] It is also important to indicate that there exists a broad spectrum of consumer exposure characteristics that are vital to consider in risk characterization, including considerations like rinse-off versus leave-on products, and whether they are applied to hair or skin, among other factors..

California Proposition 65, officially known as the Safe Drinking Water and Toxic Enforcement Act of 1986, has defined a no-significant-risk-level (NSRL) for NDELA at 0.3 µg/d. The NSRL is designated by California's Office of Environmental Health Hazard Assessment (OEHHA) and represents the daily intake level associated with a 10^{-5} lifetime carcinogenic risk (LCR).[49] In addition, Cosmetic Europe derives a maximum level of NDELA at 160 ppb in finished cosmetic products that poses a minimal health risk to the consumer, which corresponds to an exposure dose of 24 ng/kg bw/d back-calculated from the equations below.[8,23]

LCR (10^{-5}) = Exposure dose / (HT25/0.25), where

HT25 = Human Dose Descriptor

HT25 (mg/kg bw/d) = $T25/(bw_{human}/bw_{animal})^{0.25}$

The default human bw is 60 kg

T25 = Animal Dose Descriptor (defined as the chronic dosage that can induce tumor development at a specific tissue site in 25% of the tested animals after correction for spontaneous incidence and within the standard lifetime of the species; dose descriptor T25 is used for setting specific concentration limits for non-threshold carcinogens).

The mean T25 of 2.09 mg/kg bw/d for NDELA was derived from six rat studies, which corresponds to HT25 at 0.60 mg/kg bw/d.[8]

A maximum level of NDELA (i.e., 160 ppb) in finished cosmetic products that may pose a minimal health risk to the consumer can be derived in accordance with the following equation:

$$SED = \frac{A \times C\ (\%) \times DAp\ (\%)}{\text{Body weight (60 kg)}}$$

Wherein,

SED (g/kg bw/day) = systemic exposure dose. The value of 24 ng/kg bw/d can be used as a toxicological reference value (TRV) in humans, since this daily exposure to NDELA is derived based on the daily intake level posing a 10^{-5} lifetime carcinogenic risk.

A (g/day) = estimated daily aggregate exposure to cosmetic products of 15.1 g/day, which is the sum of the calculated daily exposures for all cosmetic products through the dermal route and takes into consideration the corresponding retention factors by SCCS.[50]

DAp (%) = for conservative estimating, a maximum dermal absorption of 60% for NDELA is used

C (%) = the concentration of NDELA in the finished cosmetic product

For genotoxic and carcinogenic compounds, a margin-of-exposure (MoE) $\geqslant$ 10,000 is considered to be protective.[8,51] A contamination of 50 µg NDELA/kg, as currently regulated in the EU Cosmetics Regulation (2009) for raw materials, is associated with a margin-of-exposure (MoE) > 10,000 in all four of the cosmetic product types under investigation (i.e., mascara, shower gel, handwash soap, and body lotion), when using the BMDL10 (a benchmark dose associated with 10% extra tumor risk adjusted for background) at 0.73 mg/kg bw/d as point-of-departure (PoD).[8] In another risk assessment conducted with 195 cosmetic samples, that cover a wide range of categories including baby products, body lotion, cleansing foam, eye cream, face cream, hair conditioner, hair styling product, hand cream, shampoo, shower gel, sun cream, and skin toner, concentrations of NDELA, NDEA, triethanolamine (TEA), and DEA in products were determined by liquid chromatography–tandem mass spectrometry (LC–MS/MS).[52] Exposure to maximum levels of NDELA and NDEA detected in cosmetics resulted in MoE > 10,000; in addition, the margin-of-safety (MoS) calculation of TEA and DEA detected concentrations in finished products was > 100,000.

ANNEX II

Current boilerplates applied in CIR reports for addressing concerns related to *N*-nitrosamine formation are presented below.

N-NITROSAMINE FORMATION CAVEAT

BACKGROUND

Nitrosamines are compounds containing the R_1R_2N-N=O functional group. Nitrosation is the process of converting organic compounds (e.g., alkyl and aryl amines and amides) into nitroso derivatives (e.g., nitrosamines and nitrosamides) by reaction with nitrosating agents. These agents include nitrous acid (HNO_2), oxides of nitrogen (e.g., nitrites, nitrates, and dinitrogen trioxide), and other compounds capable of generating a nitrosonium ion, NO^+.

Of concern in cosmetics is the conversion of secondary amines (R_1-NH-R_2) into *N*-nitrosamines that may be carcinogenic. Of the approximately 300 *N*-nitroso compounds that have been tested, 85% of the 209 nitrosamines and 92% of the 86 nitrosamides have been shown to produce cancer in laboratory animals (Shank and Magee, 1981; NRC, 1981). Nitrosation can occur under physiologic conditions. Depending on the nitrosating agent and the substrate, nitrosation can occur under acidic, neutral, or alkaline conditions. However, nitrosation occurs most commonly under acidic conditions. Atmospheric or physiological NO_2 may also participate in the nitrosation of amines in aqueous solution (Challis et al., 1982).

Another concern is when nitrosamines may be present in a cosmetic as an impurity of an ingredient. This concern became apparent during the safety assessment of morpholine (08/1989) wherein the Expert Panel for Cosmetic Ingredient Safety determined that, under conditions of cosmetic use, it is highly unlikely that morpholine is totally free of carcinogenic *N*-nitrosamines. Nitrosation of morpholine to form *N*-nitrosomorpholine occurs readily. Accordingly, concern was raised about the contamination of morpholine with *N*-nitrosomorphine.

Even though amines (exist as impurities or decomposition products of raw materials) may not be mutagenic or carcinogenic alone, in the presence of a nitrosating agent they may exhibit mutagenic and carcinogenic potential, due to the reactions recited above. While many secondary amines are readily nitrosated to form isolatable *N*-nitrosamines and *N*-nitrosamides, primary alkyl and aryl amines ultimately yield diazonium salts, instead of nitrosamines. Tertiary alkyl amines also do not tend to react with nitrosating agents to form nitrosamines. While tertiary aryl amines do undergo nitrosation, the reaction occurs on the aromatic ring (i.e., not on the amine; *C*-nitrosation), and does not result in the formation of nitrosamines.

In general, the nitrosation of amides is not considered a major issue for cosmetic products. However, there remains a real potential for the presence of nitrosatable amine impurities within amides.

Consequently, the Panel generally cautions that cosmetic products containing secondary amines or amides should be free of nitrosating agents. Manufacturers can accomplish this by formulating these ingredients in a way that avoids the formation of nitrosamines, and by eliminating the presence of impurities that contain nitrosating agents.

DISCUSSION

[Ingredient(s)] should not be used in cosmetic products in which *N*-nitroso compounds can be formed. **[*Discuss rationale*.]**

CONCLUSION (include this statement as part of the Conclusion)

- The Expert Panel cautions that products containing these ingredients should be formulated to avoid the formation of *N*-nitrosamines.

for hairdyes: unless the Panel instructs otherwise, the issue of nitrosamine formation, and the caveat, are addressed in the Discussion section (the caveat is not included in the Conclusion)

REFERENCES

1. US Environmental Protection Agency (EPA). *Ambient Water Quality Criteria for Nitrosamines.* 1980. EPA 440/5-80-064.
2. Shank RC, Magee PN. Toxicity and carcinogenicity of N-nitroso compounds. In: *Mycotoxins and N-Nitroso Compounds: Environmental Risks.* Boca Raton, FL: CRC Press, Inc; 1981:185–217.
3. Lavilla I, Cabaleiro N, Bendicho C. Main Chemical Contaminants in Cosmetics: Regulatory Aspects and Analytical Methods (Chapter 14). In: *Analysis of Cosmetic Products (Second Edition).* Elsevier Science; 2018:331-383.
4. US Food and Drug Administration (FDA). Control of Nitrosamine Impurities in Human Drugs - Guidance for Industry, Revision 1. In:2021.
5. Chain EPoCitF, Schrenk D, Bignami M, et al. Risk assessment of N-nitrosamines in food. *EFSA J.* 2023;21(3):e07884.
6. US Food and Drug Administration (FDA). Information about Nitrosamine Impurities in Medications. https://www.fda.gov/drugs/drug-safety-and-availability/information-about-nitrosamine-impurities-medications. Accessed 03/10/2023.
7. Burnett CL, Bergfeld WF, Belsito DV, et al. Safety Assessment of 2-Amino-3-Hydroxypyridine as Used in Cosmetics. *Int J Toxicol.* 2020;39(2_suppl):91S-97S.
8. Scientific Committee on Consumer Safety (SCCS). *Opinion on NDELA in Cosmetic Products and Nitrosamines in Balloons.* 2012. SCCS/1486/12.

9. Colipa (The European Cosmetics Association). Technical Guidance Document on Minimising and Determining Nitrosamines in Cosmetics. https://cosmeticseurope.eu/download/TjBjaHR5ekxhQ0Vxbkc1eEtKU2NTdz09. Published 2009. Accessed 02/05/2023.
10. International Agency for Research on Cancer (IARC). Agents Classified by the IARC Monographs, Volumes 1–133. https://monographs.iarc.who.int/agents-classified-by-the-iarc/. Updated 03/24/2023. Accessed 04/05/2023.
11. The U.S. Department of Health and Human Services (HHS). Report on Carcinogens, Fifteenth Edition. In:2021.
12. Shank RC. Toxicology of N-nitroso compounds. *Toxicol Appl Pharmacol.* 1975;31(3):361-368.
13. Low H. Nitroso compounds: safety and public health. *Arch Environ Health.* 1974;29(5):256-260.
14. Bronaugh RL, Congdon ER, Scheuplein RJ. The effect of cosmetic vehicles on the penetration of N-nitrosodiethanolamine through excised human skin. *J Invest Dermatol.* 1981;76(2):94-96.
15. Lijinsky W, Losikoff AM, Sansone EB. Penetration of rat skin by N-nitrosodiethanolamine and N-nitrosomorpholine. *J Natl Cancer Inst.* 1981;66(1):125-127.
16. US Food and Drug Administration (FDA). Cosmetic Product Manufacturers: Guide to inspections of cosmetic product manufacturers. In:1995.
17. López-Rodríguez R, McManus JA, Murphy NS, Ott MA, Burns MJ. Pathways for N-Nitroso Compound Formation: Secondary Amines and Beyond. *Org Process Res Dev.* 2020;24(9):1558-1585.
18. Li Y, Hecht SS. Metabolic Activation and DNA Interactions of Carcinogenic N-Nitrosamines to Which Humans Are Commonly Exposed. *Int J Mol Sci.* 2022;23(9).
19. Diwan BA, Rehm S, Rice JM. Age- and dose-dependent transplacental carcinogenesis by N-nitrosoethylurea in Syrian golden hamsters. *J Cancer Res Clin Oncol.* 1996;122(11):643-652.
20. Donovan PJ. Cell sensitivity to transplacental carcinogenesis by N-ethyl-N-nitrosourea is greatest in early post-implantation development. *Mutat Res.* 1999;427(1):59-63.
21. Congressional Research Service. FDA Regulation of Cosmetics and Personal Care Products. In:2012.
22. US Food and Drug Administration (FDA). Cosmetics Safety Q&A: Contaminants. Do cosmetics contain harmful contaminants, such as lead? What does FDA do to guard against them? Web site. https://www.fda.gov/cosmetics/resources-consumers-cosmetics/cosmetics-safety-qa-contaminants. Updated 02/25/2022. Accessed 02/18/2023.
23. Colipa (The European Cosmetics Association). Determination of a safe level of NDELA in finished cosmetic products - Cosmetics Europe Industry Guidelines. https://www.ctpa.org.uk/file.php?fileid=3483. Accessed 02/08/2023.
24. Scientific Committee on Consumer Safety (SCCS). *Opinion on Nitrosamines and Secondary Amines in Cosmetic Products.* 2012. SCCS/1458/11.
25. Rostkowska K, Zwierz K, Różański A, Moniuszko-Jakoniuk J, Roszczenko A. Formation and Metabolism of N-Nitrosamines. *Pol J Environ Stud.* 1998;7(6):321–325.
26. Wichitnithad W, Nantaphol S, Noppakhunsomboon K, Rojsitthisak P. An update on the current status and prospects of nitrosation pathways and possible root causes of nitrosamine formation in various pharmaceuticals. *Saudi Pharm J.* 2023;31(2):295-311.
27. Loeppky RN, Shevlin G, Yu L. Rapid nitrosamine formation from tertiary nitrogen compounds: An overview. In Significance of N-Nitrosation of Drugs. *Drug Dev Eval.* 1990;16:253-266.
28. Loeppky RN. Nitrosamine and N-Nitroso Compound Chemistry and Biochemistry (Chapter 1). In: *Nitrosamines and Related N-Nitroso Compounds.* Vol ACS Symposium Series Vol. 553. American Chemical Society; 1994:1-18.
29. Preussmann R. Carcinogenicity and Structure-Activity relationships of N-Nitroso compounds. In: *The Significance of N-Nitrosation of Drugs.* New York: Gustav Fischer; 1990:3-17.
30. Mirvish SS. Kinetics of dimethylamine nitrosation in relation to nitrosamine carcinogenesis. *J Natl Cancer Inst.* 1970;44(3):633-639.
31. Mirvish SS. Formation of N-nitroso compounds: chemistry, kinetics, and in vivo occurrence. *Toxicol Appl Pharmacol.* 1975;31(3):325-351.
32. Williams DLH. *Nitrosation Reactions and the Chemistry of Nitric Oxide.* Amsterdam: Elsevier; 2004.

33. Eisenbrand G. Endogenous Nitrosation -Findings and Problems. In: Nicolai Hv, ed. *The Significance of N-Nitrosation of Drugs.* New York: Gustav Fischer; 1990.
34. Williams DLH. *Nitrosation reactions in the chemistry of nitric oxide.* Amsterdam, Oxford: Elsevier; 2004.
35. Scientific Committee on Consumer Safety (SCCS). *Opinion on Acetaldehyde.* 2012. SCCS/1468/12.
36. Schettino L, Benede JL, Chisvert A. Determination of nine prohibited N-nitrosamines in cosmetic products by vortex-assisted dispersive liquid-liquid microextraction prior to gas chromatography-mass spectrometry. *RSC Adv.* 2023;13(5):2963-2971.
37. EUR-Lex. Access to European Union. https://eur-lex.europa.eu/homepage.htmlEUR-Lex. Updated 07/2023. Accessed 09/20/2023.
38. Challis BC, Trew DF, Guthrie WG, Roper DV. Reduction of nitrosamines in cosmetic products. *Int J Cosmet Sci.* 1995;17(3):119-131.
39. Ziebarth D, Scheunig G. Effects of some inhibitors on the nitrosation of drugs in human gastric juice. *IARC Sci Publ (1971).* 1976(14):279-290.
40. Fiume MM, Heldreth BA, Bergfeld WF, et al. Safety Assessment of Ethanolamine and Ethanolamine Salts as Used in Cosmetics. *Int J Toxicol.* 2015;34(2 Suppl):84S-98S.
41. Johnson W, Jr., Bergfeld WF, Belsito DV, et al. Safety Assessment of Lecithin and Other Phosphoglycerides as Used in Cosmetics. *Int J Toxicol.* 2020;39(2_suppl):5S-25S.
42. Fiume MM, Heldreth B, Bergfeld WF, et al. Safety Assessment of Diethanolamine and Its Salts as Used in Cosmetics. *Int J Toxicol.* 2017;36(5_suppl2):89S-110S.
43. US Food and Drug Administration (FDA). Recommended Acceptable Intake Limits for Nitrosamine Drug SubstanceRelated Impurities (NDSRIs) Guidance for Industry. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/recommended-acceptable-intake-limits-nitrosamine-drug-substance-related-impurities. Published 2023. Updated 08/04/2023. Accessed 08/24/2023.
44. US Food and Drug Administration (FDA). Complance Policy Guide (CPG) Sec 510.600 Dimethylnitrosamine in Malt Beverages. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cpg-sec-510600-dimethylnitrosamine-malt-beverages. Published 2005. Updated 08/24/2018. Accessed 08/24/2023.
45. US Food and Drug Administration (FDA). Compliance Policy Guide (CPG) Sec 578.500 Dimethylnitrosamine (DMNA) in Barley Malt. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cpg-sec-578500-dimethylnitrosamine-dmna-barley-malt. Published 2005. Updated 02/26/2015. Accessed.
46. US Food and Drug Administration (FDA). Compliance Policy Guide (CPG) Sec 500.450 Volatile N-Nitrosamines in Rubber Baby Bottle Nipples. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cpg-sec-500450-volatile-n-nitrosamines-rubber-baby-bottle-nipples. Published 2005. Updated 08/24/2018. Accessed 08/24/2023.
47. Gushgari AJ, Halden RU. Critical review of major sources of human exposure to N-nitrosamines. *Chemosphere.* 2018;210:1124-1136.
48. Joo KM, Shin MS, Jung JH, et al. Determination of N-nitrosodiethanolamine, NDELA in cosmetic ingredients and products by mixed mode solid phase extraction and UPLC-tandem mass spectrometry with porous graphitic carbon column through systemic sample pre-cleanup procedure. *Talanta.* 2015;137:109-119.
49. California's Office of Environmental Health Hazard Assessment (OEHHA). Office of Environmental Health Hazard Assessment Proposition 65. No Significant Risk Levels (NSRLs) for Carcinogens and Maximum Allowable Dose Levels (MADLs) for Chemicals Causing Reproductive Toxicity. In:2013.
50. Scientific Committee on Consumer Safety (SCCS). *Notes of guidance for the testing of cosmetic ingredients and their safety evaluation - 9th revision.* 2016. SCCS/1564/15.
51. European Food Safety Authority (EFSA). Margin of Safety. https://www.efsa.europa.eu/en/topics/topic/margin-exposure#published-on-this-topic. Updated 04/27/2023. Accessed 05/18/2023.
52. Lim DS, Roh TH, Kim MK, et al. Risk assessment of N-nitrosodiethylamine (NDEA) and N-nitrosodiethanolamine (NDELA) in cosmetics. *J Toxicol Environ Health A.* 2018;81(12):465-480.