

ADMIN

Nitrosation Resource Document

EXPERT PANEL MEETING

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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: May 19, 2023
Subject: *N*-Nitrosation and the Safety Evaluation of Cosmetic Ingredients

Enclosed is a draft resource document to provide clarity on the Panel's standpoint concerning the risks associated with *N*-nitrosation reaction that may potentially occur during the production and formulation stages of cosmetic ingredients, and to delineate the approach for effectively assessing and managing the potential risks of nitrosamines during the safety evaluation process. This iteration of the document, named *ResourceDocument_Nitrosation_062023* in the pdf, is to be reviewed herein for the first time by the Panel.

The document examines the possible sources of nitrosamine impurities in cosmetic products, provides a concise overview of the existing regulatory framework governing the formation of nitrosamines in cosmetic ingredients and formulations, as well as conducts an in-depth analysis of the diverse factors that influence nitrosamine formation. Additionally, the document offers an extensive exposition of the Panel's considerations during the evaluation and determination process of potential risks linked to *N*-nitrosation in cosmetic formulations and applications.

The Panel is requested to review the draft resource document and assess the extent to which it concurs with their current perspective on the risks associated with *N*-nitroso compound formation during their review process of cosmetic ingredient safety. The Panel should determine how, and to what extent, the document should be revised.

EXPERT PANEL FOR COSMETIC INGREDIENT SAFETY

Resource Document

N-Nitrosation and the Safety Evaluation of Cosmetic Ingredients

06/2023 - 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. 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.

BACKGROUND

Cosmetic formulations containing amines, particularly secondary amines, and nitrogenous derivatives such as certain amides, 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 R1 and R2 groups attached to the amine nitrogen can range from a hydrogen atom (in each case) to complex chemical substituents (although, typically alkyl groups). Some *N*-nitrosamines possess strong genotoxic properties 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.¹⁻³ Studies have demonstrated the ability of such chemicals to permeate the epidermis, as well as the respiratory and gastrointestinal tracts, leading to systemic absorption.⁴⁻⁷ In order for *N*-nitrosamines to demonstrate mutagenic and carcinogenic activity, the metabolic activation is necessary due to their stability under physiological pH conditions.⁸ The activation process involves cytochrome P450-dependent enzymes, which effect either a single α -hydroxylation, or processive α -hydroxylations to form an *N*-nitrosamide (chemical structure in which a nitroso group is bonded to the nitrogen atom of an amide, Figure 2). As direct-acting *N*-nitroso compounds, *N*-nitrosamides are unstable at physiological pH and will undergo nonenzymatic decomposition.^{9,10} Ultimately, highly reactive carbocations or diazonium compounds are generated which are capable of alkylating various cellular macromolecules, including DNA, RNA, and chromosomes.

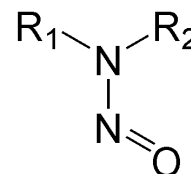


Figure 1. Generic *N*-nitrosamine structure

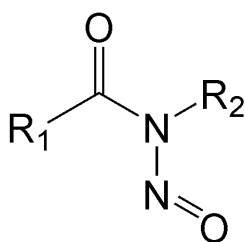


Figure 2. Generic *N*-nitrosamide structure

N-Nitrosamines can contaminate cosmetic products due to impurities in raw materials, manufacturing processes, as well as storage and packaging, where precursor ingredients come into contact with nitrosating agents. Nitrosation reactions may occur under physiological conditions.¹¹ The presence of atmospheric nitrogen dioxide (NO₂) may also contribute to the nitrosation process of amines in aqueous solution.¹² While detected in a wide array of materials (even naturally occurring in some ripe fruit), *N*-nitrosamines are not listed on product labels due to their classification as impurities. In 1979, the US Food and Drug Administration (FDA) expressed concerns about the presence of *N*-nitrosamine-contaminated cosmetics and stated that such products could be considered adulterated and subject to enforcement actions.^{7,13} Minimizing the likelihood of *N*-nitrosamine formation in cosmetics is important. On the other hand, 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.¹⁴⁻¹⁶ 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.¹⁷ Therefore, it is necessary to clarify the considerations of the Panel's approach to address issues related to generation of *N*-nitroso compounds that have carcinogenic potential. The current document aims to examine the potential source and root causes of *N*-nitrosamine impurities in cosmetic products, provide a concise overview of the existing regulatory framework governing *N*-nitrosamine formation in cosmetic ingredients and formulations, analyze the diverse factors that influence the formation of *N*-nitrosamines, and further

elucidate how the Panel assesses and determines the potential risks linked to *N*-nitrosation in cosmetic formulations, as applied during a comprehensive safety evaluation of cosmetic ingredients.

FACTORS INFLUENCING THE *N*-NITROSATION OF INGREDIENTS IN COSMETIC PRODUCTS

Formation of *N*-nitrosamines in cosmetic products may result through the confluence of three factors: i) the presence of precursor ingredients with nitrosatable structures, ii) a nitrosating agent, and iii) appropriate reaction conditions. Depending on the nitrosating agent and the substrate, *N*-nitrosation can occur under acidic, neutral, or alkaline conditions.^{11,18} *N*-Nitrosamines can be formed from primary, secondary, and tertiary amines. The secondary amines, in general, are the most reactive compounds toward nitrosating agents, under manufacturing, storage, or use conditions, that favor *N*-nitrosamine formation.

In addition to secondary amines, secondary amides represent an important group of NH-containing compounds that can form stable *N*-nitroso compounds. Similarly, related compounds such as heteroamides, carbamates, and ureas, which have electron-withdrawing groups attached to the amino moiety, are commonly subjected to *N*-nitrosation by electrophilic reagents like nitrogen oxides, nitrosyl halides, and nitrosonium salts. Solvent studies have revealed that *N*-nitrosamide formation was the highest when the reaction was carried out under neat conditions.⁸

The basicity (i.e., alkalinity) of amines, which is determined by the structure, plays a crucial role in regulating the rate of *N*-nitrosation. The availability of pKa/pKb values for amines enables the prediction of *N*-nitrosation rates under specified conditions.¹⁹ Moreover, the substitution of an alkyl group of a secondary amine with an aromatic ring exhibits a more pronounced impact on the *N*-nitrosation rate, as substituents in the aromatic ring can affect both the amine basicity and the intrinsic rate constant for *N*-nitrosation.²⁰

For amines with different degrees of substitution (i.e., not secondary), the likelihood of *N*-nitrosation is less. Primary amines may readily react with nitrosating agents to generate unstable *N*-nitroso products that rapidly decompose and ultimately yield diazonium salts, instead of *N*-nitrosamines.^{18,21} The transformation from tertiary amines requires the cleavage of the carbon-nitrogen bond of one of the alkyl groups attached to the nitrogen atom (i.e., converting the tertiary amine to a secondary amine). While some tertiary amines may exhibit reactivity towards *N*-nitrosation,^{18,22} most are not particularly reactive towards *N*-nitrosation under standard conditions.²³

N-nitrosamines can be effectively controlled through appropriate formulation strategies, specifically by avoiding the use of amines or amino derivatives in combination with a nitrosating agent.^{24,25} 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.¹⁸ 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.²⁶ 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.¹⁷

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.²⁴ For example, diethanolamine (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 Europe for monoalkanolamines, monoalkanolamines and their salts).¹⁸

Various factors can either significantly promote or impede *N*-nitrosation reactions. The formation of *N*-nitrosamines from secondary amines can be catalyzed by aldehydes, especially formaldehyde, through the intermediacy of imines.¹⁸ In the presence of catalysts, *N*-nitrosation proceeds readily at pH values ranging from 5 to 10. Notably, permitted preservatives 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. Whenever these preservatives are formulated with secondary amines structures, substantial *N*-nitrosamine formation can be anticipated. Formulation of these 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.^{18,27} Other moieties, such as halide ions, thiosulfates, thiols, and certain phenolic compounds, may also act as effective catalysts for *N*-nitrosation reactions.²⁰

There are substances that can compete with amines for the nitrosating agents, thereby inhibiting *N*-nitrosamine formation.²⁴ 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. Such *N*-nitrosamine formation inhibitive strategies are formulation-specific and the efficacy of a potential inhibitor must be established for each individual application, taking into account the aspect of stability within specific formulations. Cosmetic formulations may require both hydrophobic and hydrophilic nitrosation inhibitors to be effective in both phases. However, it is important to note that none of these reagents can eliminate *N*-nitrosamines that are already present in raw materials. In addition, according to the European Cosmetics Directive, 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.

N-nitrosating agents, such as sodium nitrite (NaNO₂), nitrous acid (HNO₂), nitric oxide (NO), and nitrosyl halides (e.g., ClNO), can be inadvertently transferred into relevant cosmetic products during manufacturing, highlighting the need for appropriate monitoring and mitigation measures. For instance, potential contamination may arise from residual nitrites in water. The World Health Organization (WHO) has assigned a limit of 3 mg/l (or 3 ppm) for nitrite in potable water, which may be linked to the formation

of *N*-nitrosamines.²⁸ In addition, the catalytic enhancement effect of the cationic surfactant decyltrimethylammonium bromide, as well as other micelle-forming agents, on the *N*-nitrosation of amines by nitrite has been documented.¹⁸ This observation implies that such impurities present in these surfactants may contribute to an enhanced *N*-nitrosation risk.²⁹ Nitrosating agents can also originate from nitrates present as potential impurities in raw materials or from gaseous nitrogen oxide. Nitrous esters are particularly potent nitrosating agents. The formation of *N*-nitrosamines has been observed to be directly proportional to the levels of both amines and nitrites.¹⁴ Consequently, any rise in nitrite content will lead to a substantial increase in *N*-nitrosamine content.³⁰ As impurities present in a cosmetic product are substances/particles not intentionally added as part of the formulation, it is important to use raw materials that meet purity specifications and to avoid any contact with adventitious nitrosating agents such as nitrite-treated raw material containers, atmospheric NO_x sources at production, packaging, and storage units.²⁴ The control limits of *N*-nitrosamine impurities in pharmaceuticals have been proposed by global regulatory authorities,²¹ which might provide insight for addressing safety concerns about exposure to *N*-nitrosamine contaminations during cosmetic usage; e.g., if multiple *N*-nitrosamines are present in pharmaceuticals, the sum of *N*-nitrosamines must be lower than 26.5 ng/day. *N*-Nitrosamines can be quantitatively detected down to 1 ppb.²⁸

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 nitrosamines.^{4,18} 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).³¹

CURRENT REGULATION ON NITROSAMINE FORMATION IN COSMETIC INGREDIENTS AND COSMETIC FORMULATIONS

Major sources of human exposure to total *N*-nitrosamines have been investigated in a critical review encompassing a literature set of 122 studies, with refined quantifications of exposure estimations by diet and lifestyle.³² These estimates suggest that individuals who regularly consume beer and smoke tobacco are expected to experience the highest daily exposure from tobacco use (88%), food intake (8%), beer consumption (4%), and potable water consumption (< 1%). By comparison, individuals who abstain from alcohol and tobacco consumption would have a 12-fold lower daily *N*-nitrosamine exposure, primarily through food ingestion (94%) and potable water consumption (6%). Although personal care products have also been considered as potential contributors to daily *N*-nitrosamine exposure, currently available data are insufficient to calculate the exposure risk from this source. *N*-Nitrosamines may be found in personal care products primarily due to the presence of *N*-nitrosodiethanolamine (NDELA), which accounts for 99% of all observed *N*-nitrosamines in these products.^{32,33} 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.

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 and or rates of nitrosation, and numerous existing laws and regulations governing the manufacturing and sale of cosmetics and personal care products.³² California Proposition 65, officially known as the Safe Drinking Water and Toxic Enforcement Act of 1986, has established a no-significant-risk-level (NSRL) for NDELA at 0.3 $\mu\text{g}/\text{d}$ (NSRL is defined by California's Office of Environmental Health Hazard Assessment (OEHHA) as the daily intake level posing a 10^{-5} lifetime risk of cancer).³⁵ In a risk assessment conducted by Scientific Committee on Consumer Safety (SCCS), the estimated safety level of NDELA in finished cosmetic products has been calculated to be 160 ppb, based on a toxicological reference value (TRV) of 24 ng/kg bw/d (an exposure dose considered to pose a minimal health risk to the consumer), as well as an adjusted T25 value (T25 value is 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 life time of the species).^{33,36} A contamination of 50 μg NDELA/kg, as currently regulated in the Cosmetic Directive 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).³⁶ 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, *N*-nitrosodiethylamine (NDEA), TEA, and DEA in products were determined by liquid chromatography–tandem mass spectrometry (LC–MS/MS).³⁷ 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 $> 100,000$.

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 cosmetics ingredients. For instance, surfactants and emulsifiers, which are ammonia or amine salts, may serve as potential sources 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 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. In a notice published in the 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.¹³ 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.⁴⁰ The FDA is responsible for monitoring cosmetics to detect potential safety issues, including potential *N*-nitrosamine contaminants.

The US Federal Food, Drug, and Cosmetic Act (FD&C Act) provides a regulatory framework to ensure the safety of consumers from potential harm caused by *N*-nitrosamine exposure in cosmetics, food, and drugs.¹⁶ The FDA has determined when exposure to *N*-nitrosamines at low levels do not represent a public health risk (e.g., acceptable intake limit level established in foods) when consumed as part of a regular diet over a lifetime (i.e., 70 yr).⁴¹ However, the recent identification of *N*-nitrosamine impurities in some medications has raised concerns about long-term exposure to these compounds and their potential carcinogenic effects. In recent times, regulatory agencies are actively pursuing to impose limits for *N*-nitrosamine impurities in medicinal products, which are significantly below the Valuable Safety Dose (VSD, i.e., a generic allowable lifetime exposure of 1.5 µg/d based on a carcinogenic risk of 1 in 100,000).^{28,42} Mutagenic impurities may also be classified into different categories in order of decreasing regulatory concern. While the FDA recognizes the cancer risk associated with prolonged exposure to *N*-nitrosamines, it does not anticipate an increased risk of cancer with short-term exposure, even at levels above the acceptable limits.⁴¹ The FDA has investigated the presence of *N*-nitrosamines in pharmaceutical products and has proposed control limits for *N*-nitrosamine impurities in these products.²¹ A mitigation strategy outlined in FDA's guidance involves the implementation of a supplier qualification program that systematically evaluates the presence of nitrite impurities within inactive ingredients (excipients) suppliers, aiming to minimize the likelihood of *N*-nitrosamine formation in the final pharmaceutical formulation.⁴³

The FDA has issued guidance to manufacturers on minimizing the formation of nitrosamines during the manufacturing process of cosmetics. FDA inspectors conduct checks to ensure that manufacturers are following the recommended procedures.⁴⁰ 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. The acceptable limits of *N*-nitrosamines in pharmaceuticals have been established based on their toxicity levels. In some cases, the FDA has permitted manufacturers to distribute batches of drugs that exceed the acceptable *N*-nitrosamine intake levels to alleviate drug shortages. The FDA evaluates each product and contamination on a case-by-case basis to balance the risks of exposure with the benefits of continued use of the drug.⁴⁴ Both the FDA and Cosmetics Europe, have issued guidance to manufacturers on preventing *N*-nitrosamine formation during the manufacturing process.^{7,24} However, the FDA's guidance documents do not establish legally enforceable responsibilities. The US Fair Packaging and Labeling Act (FPLA) requires cosmetic manufacturers to label their products with ingredient information and necessary warning statements to assist 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.

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 in Annex II. Secondary alkyl- and alkanolamines and their salts were also banned to reduce contamination from carcinogenic *N*-nitrosamines that are formed after the reaction with nitrosating agents. Additionally, 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). 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 EC Cosmetic Regulation 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.³³

In regard to the practically inevitable presence of *N*-nitrosamines, an International Organization for Standardization (ISO) Technical Report has been published, titled “ISO/TR 14735: Cosmetics – Analytical methods – Nitrosamines: Technical guidance document for minimizing and determining *N*-nitrosamines in cosmetics.” This document offers guidance on strategies for reducing the occurrence of *N*-nitrosamine formation in cosmetics, as well as proposes reliable analytical approaches for detecting and quantifying these trace compounds in raw material and finished products.⁴⁶ 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.²⁴

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

N-Nitrosation reactions in formulations containing *N*-nitrosatable ingredients, regardless of *N*-nitroso derivative content (e.g., nitrosamines/nitrosamides), have been reported in products. The Panel is aware of unintended *N*-nitrosamine impurities in raw materials and finished products, and evaluates the carcinogenic potential of *N*-nitrosamines in relation to their parent chemical groups. As part of this evaluation, information concerning the possibility of ingredients to undergo *N*-nitrosation is identified (e.g., substrate structures that modify reactivity towards nitrosating agents), such as the presence of the indole ring (indoles have been demonstrated to be readily nitrosated to form *N*-nitrosamines).^{47,48} Additionally, the propensity to give rise to *N*-nitrosamines in a raw material, in a cosmetic package, or at the point of use, is considered.

In cosmetics, only a limited number of ingredients which can result in *N*-nitrosamines have been identified.¹⁸ The Panel suggests that evaluations of these compounds should be conducted on a case-by-case basis. Specifically, the Panel would investigate the potential *N*-nitrosamines that could be formed in cosmetics and assess their carcinogenic potency. For instance, although a dermal carcinogenicity study revealed positive findings for DEA, the Panel considered the hepatocarcinogenicity that was reported in mice to have little relevance to the safety of DEA in personal care products. Furthermore, the renal lesions observed in mice could be attributed to DEA-induced choline deficiency, which is a mechanism of little relevance in humans. Even if DEA-induced choline deficiency was not the cause of the renal lesions, it was concluded there is still no carcinogenic risk to humans since DEA does not appear to penetrate human skin to any significant extent at concentrations relevant to human exposures from the use of personal care products.^{49,50}

Although the levels of *N*-nitrosamines detected in cosmetics 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. However, it is also worth noting that certain low levels of *N*-nitrosamines may not pose a significant health risk to consumers under realistic in-use exposure scenarios.

The Panel notes small amounts of secondary amines that could be present as impurities in cosmetic ingredients or formulations (e.g., ethanolamine typically contains a small amount of DEA as an impurity).⁵⁰ Accordingly, they 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 manufacturers 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.⁵¹ 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 will 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. 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 the FDA, 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

Some *N*-nitrosamines pose considerable health risks owing to the prevalence of amines in cosmetic formulations, which 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.

N-Nitrosamines are not cosmetic ingredients. Thus, the Panel is concerned with the presence, in cosmetic formulations, of either a) ingredients or impurities which may act as substrates capable being *N*-nitrosated, or b) ingredients, impurities, or in-use materials exposures which may act as nitrosating agents (or the presence of both a and b). To address this concern, 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 of the formulation to *N*-nitrosating agents (e.g., do not formulate with nitrosating ingredients; consider contamination by nitrosating agents from ingredient impurities (GMPs), from packaging, or from final product use).

ANNEX

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. Nitrosamides are compounds containing the $R_1C(O)R_2N-N=O$ 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 and amides 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 and amides 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.

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)

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