IPEC Questionnaire on Nitrosamines

Questionnaire for Excipient Nitrosamines Risk Evaluation

Notice

This risk evaluation is based on IPEC Questionnaire version 2-October 2025

Introduction

Several authorities issued guidance and information on nitrosamine impurities within which are requests for the Market Authorization Holder (MAH) to conduct a risk evaluation with regards to nitrosamine formation in their drug products. Excipients can contribute to the formation or content of nitrosamines in drug products through precursor substances present in the excipient (e.g., nitrites, amines, or other nitrogen containing compounds). This questionnaire aims to provide information about excipients to assist the MAH in their evaluation of the risk of the presence or formation of nitrosamine impurities in the final drug product. It is **NOT** a requirement of the excipient manufacturer to conduct a nitrosamine risk assessment. Indeed, this is not possible without specific knowledge of the actual drug product formulation and properties of the drug substance (active pharmaceutical ingredient [API]) and other drug components.

This questionnaire reflects the guidance, for example, from the EMA assessment report "Nitrosamine impurities in human medicinal products"[1], the related EMA guidance[2] including the "Questions and answers for marketing authorization holders"[3], the US FDA Guidance for Industry "Control of Nitrosamine Impurities in Human Drugs"[4], the US FDA Guidance for Industry "Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs)"[5] and how they may be adapted for pharmaceutical excipients.

The information generated should also assist medicinal product manufacturers to address similar requests from other regulatory authorities, based on IPEC's current understanding of global activities on this subject.

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[1] European Medicines Agency (EMA): Assessment report, procedure under Article 5(3) of Regulation EC (No) 726/2004, Nitrosamine impurities in human medicinal products: https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-assessment-report_en.pdf

[2] European Medicines Agency (EMA): Nitrosamine impurities, Guidance for marketing authorization holders: https://www.ema.europa.eu/en/human-regulatory/post-authorisation/referral-procedures/nitrosamine-impurities#guidance-for-marketing-authorisation-holders-section.

[3] European Medicines Agency (EMA): Questions and answers for marketing authorization holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human products: https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-questions-answers-marketing-authorisation-holders/applicants-chmp-opinion-article-53-regulation-ec-no-726/2004-referral-nitrosamine-impurities-human-medicinal-products_en.pdf

[4] U.S. Food & Drug Administration, Control of Nitrosamine Impurities in Human Drugs. Revision 2, September 2024. https://www.fda.gov/media/141720/download

[5] U.S. Food & Drug Administration, "Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities." August 2023. https://www.fda.gov/media/170794/download

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The questionnaire includes a matrix to consider the structure and the origin of the excipient as a first risk indication. In addition, excipient suppliers are encouraged to share their conclusion.

The use of a standard format will facilitate data collection from excipient suppliers and thus enable a more efficient process of conducting the required risk assessments by medicinal product manufacturers / Marketing Authorisation Holders.

Excipient suppliers can use this form as a reference to better provide MAHs and the medicinal product manufacturers information related to nitrosamine risk assessment to the best of their knowledge, considering available supplier information and likely chemical production processes where information from the supplier is not available.

This information for nitrosamine risk evaluation is prepared for:

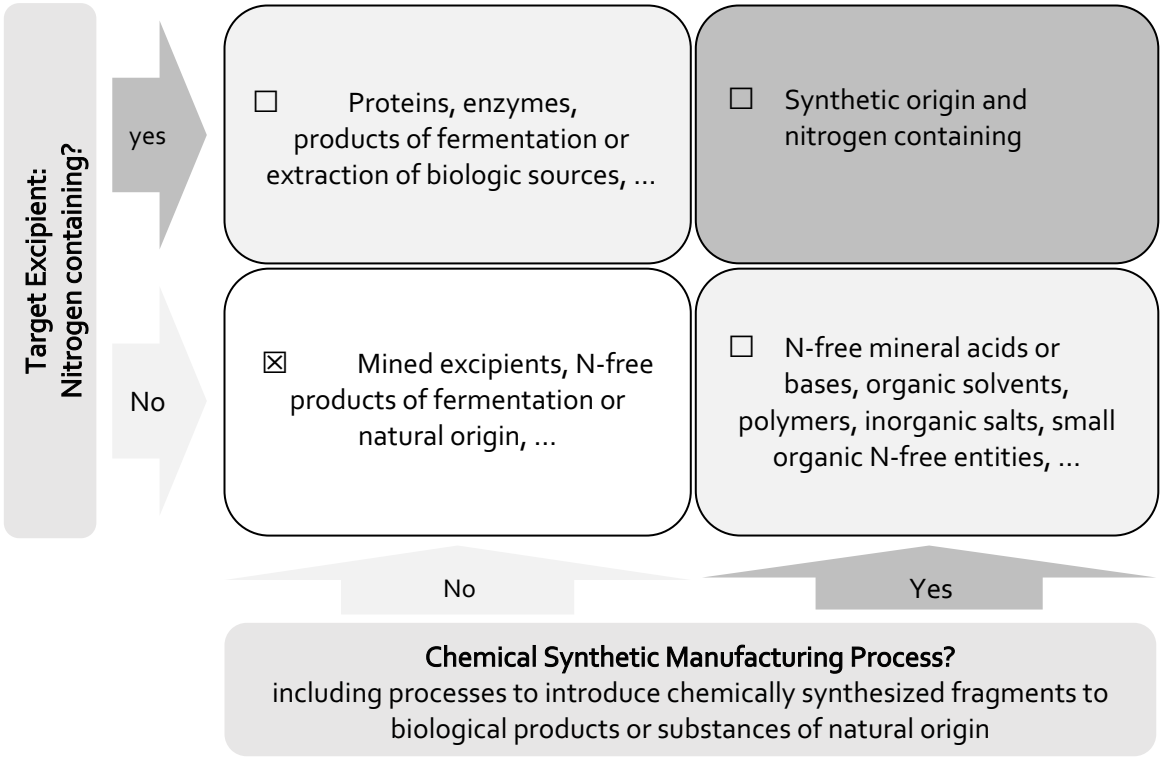
Supplier product number and name:	Pharmatose®, Respitose®, Lactochem®, Lactopress®, Lactohale®, SuperTab® (EU/NL), Wynhale®, HMS, Lactose DT
Supplier:	DFE Pharma GmbH & Co. KG

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1) Please tick the applicable category based on structure and origin of the excipient in support to evaluate the risk of formation of nitrosamines[6].



2) Is sodium nitrite ($NaNO_2$) or any other nitrite or nitrosating agent[7]:			Not available/ applicable or unknown
- used in any steps in the manufacturing process[8] as reagents/catalyst?	YES ☐	NO ☒	
- known to be used in the preparation of raw materials or intermediates used in the manufacturing process?	YES ☐	NO ☒	☐
- known to be used in the preparation of	YES☐	NO☒	☐

[6] Nitrogen-free materials are considered to be of lower inherent risk for nitrosamine contamination as they are typically manufactured and do not contain without nitrosatable structures. Nitrosamines have been observed in medicinal products with N-containing APIs of chemical synthetic origin. EMA concludes that there is a very low risk of nitrosamines being present as impurities in biological medicinal products, although it can't be completely ruled out.[1]

[7] see Guidance 1 in Annex

[8] in this document, "manufacturing process" refers to the manufacturing steps that are outlined in the flow chart of the manufacturing procedure for the mentioned product.

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reagents/catalysts/processing aids used in the manufacturing process? - known or likely to be generated during the manufacturing process? - deliberately added to the process, including components of cell culture media or for fermentation?	 YES☐ YES ☐	 NO☒ NO ☒	 ☐ ☐
3) Have you analysed the excipient for*: - Nitrites? - Nitrosamines? *Default testing is **NOT** mandatory but may be performed if considered relevant for a specific excipient. Test methods: - Nitrites: ISO 14673-2:2004 | IDF 189-2:2004. - Nitrosamines (NDMA; NMEA; NDEA; NDIBA; NDPA; NDBA; NPIP; NPYR; and NMOR): GC/TEA. ..... Note: Presently, nitrite testing of excipients is not harmonized and results may vary depending on the method used by different manufacturers of the same excipient. Users are encouraged to test themselves when comparing suppliers.	 YES ☒ YES ☒	 NO ☐ NO ☐	Test result *< 0.03 ppm (Reporting limit)* *< 2 ppb (LOD)*
4) Is water used in the manufacturing process? If "Yes": i. Is the water used prepared by distillation, by ion exchange or by reverse osmosis? ii. If 'no' and potable water is used, where possible, please report the maximum level of nitrite: - Maximum levels in accordance with Directive (EU) 2020/2184: 0.5 ppm - Maximum levels in accordance with the current Drinking-Water Standards for New Zealand Regulations 2022: 3 ppm (Note: Nitrite is a controlled impurity in potable water with a WHO guideline limit of 3 mg/L and a European limit of 0.5 mg/L.) **Note:** In the production of DFE Pharma lactose products both: potable water (compliant with EU and local legislation) and RO	YES☒ YES ☒ YES ☒	NO☐ NO ☐ NO ☐	 Information not available ☐

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water (derived from potable water) can be used. This information is included in the specific product PFS. Potable water used, complies with the current Directive (EU)2020/2184 on drinking water (EU sites) or with the current Drinking-Water Standards for New Zealand (New Zealand site).			

5) Are there any hydroxylamines, hydrazines, hydrazides or hydrazones present in the manufacturing process? If yes, please provide any relevant information about the chemical name / structure: Not applicable	YES ☐	NO ☒	Information not available ☐
6) Vulnerable amines: Are there any secondary and/or tertiary amines[9] present as[8]: - Raw material[10]? - Intermediate? - Reagent? - Processing aid? - Catalyst? - Solvent? - Final excipient? - Known impurity of the final excipient? If yes, are those amines present in the - Same - Previous - Subsequent step as any nitrosating agent mentioned in question 2? Please provide any relevant information about the chemical name / structure of amine(s): Lactose is manufactured from whey, which contains whey protein as a natural constituent. Proteins are polyamides composed of amino acids as monomers, a few of which contain different amine structures. Due to two crystallisation steps and refining, lactose is effectively purified from protein residues and	YES ☐ YES ☐ YES ☐ YES ☐ YES ☐ YES ☐ YES ☐ YES ☐ YES ☐ YES ☐ YES☐	NO ☒ NO ☒ NO ☒ NO ☒ NO ☒ NO ☒ NO ☒ NO ☒ see comment) NO ☐ NO ☐ NO ☐	Information not available ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ Not applicable ☒ ☒ ☒

[9] see Guidance 3 in Annex
[10] IPEC General Glossary of Terms and Acronyms: https://www.ipec-europe.org/guidelines.html

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free amino acids. As an additional note: Proline is the only amino acid with a secondary amine structure. In proteins, proline is bound within the peptide chain; however, even if present as free proline and reaction with nitrite would occur, its nitrosated form (N-nitrosoproline) is not classified as carcinogenic by the International Agency for Research on Cancer (IARC).			
7) Is there any amide, primary amine or quaternary ammonium salt present as: - Raw material? - Intermediate? - Reagent ? - Processing aid? - Catalyst? - Solvent? - Final excipient? - Known impurity of the final excipient? Information about the chemical name / structure:	YES ☐ YES ☐ YES ☐ YES ☐ YES ☐ YES ☐ YES ☐ YES ☐	NO ☒ NO ☒ NO ☒ NO ☒ NO ☒ NO ☒ NO ☒ NO ☒ see comment 6)	Information not available ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐
8) Recycled/recovered Solvents[11]: - Are recycled / recovered nitrogen containing solvents used in the manufacturing process?	YES ☐	NO ☒	Information not available ☐
9) Equipment: - Is the excipient produced in multipurpose equipment? - In case of multipurpose equipment, is the equipment used for manufacturing of any material involving nitrites, nitrosating agents or material with identified risk of formation of nitrosamines? - Are chloramines used as part of cleaning procedures used for manufacturing equipment?	YES☐ YES ☐ YES ☐	NO☒ NO ☐ NO ☒	Not applicable ☒
10) Additional comments, if any, not covered in the questionnaire **Conclusion:** ***The risk of presence of nitrosamine in the product is low and nitrite precursors are present only at trace levels (typically < 0.03 ppm).***			

[11] see Guidance 2 in Annex

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Annex[12]:

Guidance 1 (Sources of nitrosating agents)

Nitrosating agents, and their precursors, to be considered include:

- Nitrites (e.g., sodium nitrite, $NaNO_2$) and nitrosyl halides (e.g. ClNO, BrNO), dinitrogen trioxide (N_2O_3), dinitrogen tetroxide (N_2O_4) and organic nitrites (e.g. t-BuONO).
    - It should be noted that nitrite itself is not a nitrosating agent, but it can lead to nitrosating agents (e.g., HNO2, NO, ClNO, BrNO, N2O3, etc..) under certain conditions (e.g. aqueous acidic).
- Nitrosating agents used in the manufacturing process should be considered.
- Impurities acting as nitrosating agents (e.g. from the input materials or water) should be considered if these input materials or water are used in proximity of a vulnerable amine.[13, 14] It should be noted that the risk from water is very low as described below.

Additional considerations for potential nitrosation risks:

- Certain input solid materials used during synthesis (e.g. NaCl, NaOH, K_2CO_3 and charcoal) can contain low levels (ppm) of nitrosating agents). Trace analytical methods for nitrite analysis have been reported[15] and can be used to establish nitrite levels in input materials. It should be noted that the grade of the materials may lead to different nitrite contents. Liquid reagents, organic solvents and aqueous solutions at low pH are generally considered to not contain nitrite.
- Analysis has shown that nitrite levels in process water are typically very low (less than 3 ppb for potable water and less than 0.1 ppb for purified water)[16] therefore, an understanding of the nitrite content of the water used has the potential to mitigate water as a risk factor.
- Side reaction in nitration reactions. Nitric acid typically contains nitrogen dioxide and therefore dinitrogen tetroxide as an impurity, additional nitrous acid may also be produced, leading to nitrosation, if any reducing agents are present.[13, 17]

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[12] This information is partly transferred from the EFPIA decision tree for drug substances, 3rd revision, published June 2024, efpia-nitrosamines-risk-management-workflows-jun-24-udpate.pdf. Described in: Dirat, O. et al. Drug Substance and Drug Product Workflows for Quality Risk Management for the Presence of Nitrosamines in Medicines. Organic Process Research & Development 2025, 29, 1538-1553. https://doi.org/10.1021/acs.oprd.5c00097

[13] Horne, S. et al. Regulatory Experiences with Root Causes and Risk Factors for Nitrosamine Impurities in Pharmaceuticals J. Pharm. Sci 2023, 112, 1166-1182. https://doi.org/10.1016/j.xphs.2022.12.022

[14] Bream, R. et al. Formation of N-Nitrosamine Drug Substance Related Impurities in Medicines: A Regulatory Perspective on Risk Factors and Mitigation Strategies Org. Process Res. Dev. 2023, 27, 1736-1750. https://doi.org/10.1021/acs.oprd.3c00153

[15] Boetzel, R et al. A Nitrite Excipient Database: A useful Tool to Support N-Nitrosamine Risk Assessments for Drug Products,J. Pharm. Sci. 2022, 112, 1615-1624. https://doi.org/10.1016/j.xphs.2022.04.016

[16] Suresh Kumar, A. B., et al. "Nitrite in Pharmaceutical Manufacturing Water: Development of an Ultra-Sensitive Analytical Method, Typical Data, and Discussion of Potential Nitrosamine Formation in Drug Substance and Drug Product from Water." Organic Process Research & Development 28.7 (2024): 2614-2622. https://pubs.acs.org/doi/10.1021/acs.oprd.4c00037

[17] López-Rodríguez, R et al. Pathways for N-Nitroso Compound Formation: Secondary Amines and Beyond. Org. Process Res. Dev. 2020, 24, 1558-1585. https://doi.org/10.1021/acs.oprd.0c00323

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- Nitroalkanes, halogenated nitro alkanes, Fremy's salt, nitroso sulfonamides and
- nitroaromatics can all under some circumstances give rise to nitrosating agents. [17]
- Hydroxylamines, hydrazines, [18] hydrazides and hydrazones can under oxidative conditions (air, hypochlorite, oxygen, ozone and peroxides) give rise to nitrosating agents.[17, 19, 20]
- Chloramines are known to generate nitrosamines under certain conditions and so should also be considered.[17,19] Ozone and other strong oxidants may lead to the formation of nitrosamines.[17, 19, 20]
- NOx present in air could lead to the formation of nitrosamines and/or introduce nitrosating agents in materials. Processing operations under inert atmosphere do not present this potential risk. Certain operations performed under air should be assessed (e.g., certain drying and milling operations). In this context, it has been observed that mechanical stress can favor the formation of nitrosamines.[21]
- A review of nitrosating agents has been provided by Bream et al.[14]

Guidance 2 (Potential indirect risks)

Consider all potential sources (nitrosamines, nitrosating agents and vulnerable amines) in input materials.

The potential presence of nitrosamines in input materials should be considered, especially if secondary amines are used.[22]

Use of recovered materials (solvents, reagents, catalysts) and associated controls should be assessed. Third party recycling of solvents from a different process should be a particular focus. Recovered materials risks are significantly lower if the recycling is dedicated to the same manufacturing process and/or when performed for early steps. When recycling materials, the following considerations can be useful:

1. Is the pre-recovered stream likely to contain any vulnerable amines, nitrosating agents or nitrosamines.
2. Is the recovery process likely to introduce and / or purge any of the above.
3. Is the recovered material reasonably expected to contain any new or increased levels of vulnerable amines, nitrosating agents or nitrosamines. If so, what is the impact (consider fate and purge).

Carry-over from other processes using shared equipment should be considered. Steps performed under GMP (using solvents/reagents with appropriate controls, and controls on their recovery and reuse, as well as use of appropriate cleaning protocols) are considered to be a lower carry-over risk.

[18] Lunn, G. et al. Aerial oxidation of hydrazines to nitrosamines. Environ. Mol. Mutagen. 1991, 17, 0893–6692. https://doi.org/10.1002/em.2850170109

[19] Nawrocki, J et al. Nitrosamines and Water, J. Hazard. Mater. 2011, 189, 1-18. https://doi.org/10.1016/j.jpba.2022.114872

[20] Jires et al. N-Nitrosation in the absence of nitrosating agents in pharmaceuticals? J. Pharm. Biomed. Anal. 2022, 218, 114872. https://doi.org/10.1016/j.jpba.2022.114872

[21] Basoccu, F et al. Mechanochemistry for healthcare: revealing the nitroso derivatives genesis in the solid state. ChemSusChem, 2023, e202301034. https://doi.org/10.1002/cssc.202301034

[22] Spiegelhalder et al. Contamination of Amines with N-Nitrosamines. Angew. Chem., Int. Ed. Engl. 1978, 17, 367– 368, https://doi.org/10.1002/anie.197803672

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Guidance 3 (Sources of secondary and tertiary amines)

A "vulnerable" amine is an amine that is capable of reacting with a nitrosating agent to form a stable nitrosamine.

Only secondary and tertiary amines (and salts thereof) are able to form nitrosamines, as primary amines will react with nitrosating agents to produce unstable diazonium species, and tetra substituted quaternary ammonium salts, being coordinatively saturated (and positively charged) cannot directly undergo nitrosation. Note that some quaternary ammonium salts, principally those containing methyl or benzyl substituents, are known to de-alkylate under certain conditions, generating the corresponding tertiary amines which can go on to be nitrosated.[23] Secondary amines are of most concern as they can react with nitrosating agents significantly faster than most tertiary amines. Besides nitrite concentration and pH, the secondary amine pK_a impacts the nitrosation rate, with low pK_a amines generally being more readily nitrosated even at low nitrite concentrations.

Simple tertiary alkylamines react approximately 1000 slower than the corresponding secondary amines while tertiary amines that contain stereo-electronic features (e.g. gramine) or tertiary alkyl aniline derivatives can in some instances form nitrosamines through a multitude of mechanistic pathways. [24, 25, 26]

Therefore, all secondary and tertiary aliphatic and aromatic amines (amine functionality not being part of the aromatic ring system) should be considered including those:

- present as part of the starting materials, intermediates or excipient structure,
- introduced as reagents, catalysts, solvents,
- present as impurities in the input materials or generated in the process (e.g. by hydrolysis of tertiary amides).

Specifically, amines may be introduced as impurities or degradants of:

- Common amide-containing solvents such as *N,N*-dimethylformamide (DMF), *N,N*-dimethyacetamide (DMAC) and *N*-methylpyrrolidinone (NMP). These solvents can contain secondary amine impurities or generate secondary amines via hydrolysis under various reaction conditions.

[23] a) W. A. Mitch et al. Quaternary Amines as Nitrosamine Precursors: A Role for Consumer Products? Environ. Sci. Technol. 2010, 44, 1224–1231. https://doi.org/10.1021/es902840h b) T.-L. Ho Dealkylation of Quaternary Ammonium Salts with 1,4-Diazabicyclo[2.2.2]octane. Synthesis 1972, 702, https://doi.org/10.1055/s-1972-21977 eand related references.
[24] Curran, T. A et al. Consideration of the Extent That Tertiary Amines Can Form N-Nitroso Dialkylamines in Pharmaceutical Products, Org. Process Res. Dev.2023, 27, 1714–1718, https://doi.org/10.1021/acs.oprd.3c00073
[25] Ashworth, I. W. et al. Formation of Dialkyl-N-nitrosamines in Aqueous Solution: An Experimental Validation of a Conservative Predictive Model and a Comparison of the Rates of Dialkyl and Trialkylamine Nitrosation Org. Process Res. Dev. 2023 27, 1759–1766, https://doi.org/10.1021/acs.oprd.2c00366
[26] S. Diab, et al. Formation of N-Nitrosamines by Reaction of Secondary Dialkylamines with Trace Levels of Nitrite in Aqueous Solution: An Automated Experimental and Kinetic Modeling Study Using Di-n-butylamine, Org. Process Res. Dev. 2024 28, 293-304, https://doi.org/10.1021/acs.oprd.3c00404

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- Common tertiary amine bases such as triethylamine, diisopropylethylamine and *N*-methylmorpholine.
- Quaternary ammonium salts such as tetrabutylammonium bromide (TBAB)
- Primary amines such as monoethylamine
- Starting materials, intermediates or the excipient itself

Other amine-containing functional groups can also indirectly lead to the formation of nitrosamines under certain conditions, such as 1,1-dialkyl hydrazines which have been reported to oxidize to form nitrosamines.[27]

This evaluation should include the use of all chemicals within a process, including those used during the work-up and isolation as well as during reactive chemistry.

Any secondary and/or tertiary amines which might be reasonably expected to reside in the excipient should be flagged with approximate levels for inclusion within the assessment of the drug product.

[27] G. Lunn et al. Aerial oxidation of hydrazines to nitrosamines Environ. Mol. Mutagen. 1991, 17, 0893-6692. https://doi.org/10.1002/em.2850170109

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