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BIOSPECTRA QUESTIONNAIRE FOR NITROSAMINES RISK EVALUATION

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1. INTRODUCTION:

1.1. Several authorities issued guidance and information on nitrosamine impurities within which are requests for Marketing Authorization Holders (MAHs) to conduct a risk evaluation with regards to nitrosamine formation in their drug products. Excipients, Active Pharmaceutical Ingredients, Process Chemicals, and their raw materials 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 these substances, and their raw materials, to assist the MAH in their evaluation of the risk of the presence of nitrosamine impurities in the final drug product. It is not the requirement of the Active Pharmaceutical Ingredient, Excipient, Process Chemical, and their raw material manufacturers to conduct a nitrosamine risk assessment, indeed this is not possible without specific knowledge of the actual and specific drug product formulation and properties of the drug substance (active pharmaceutical ingredient [API]) and other drug components. This questionnaire reflects the guidance 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. This questionnaire additionally applies to Active Pharmaceutical Ingredients, Process Chemicals, and their raw materials.

1.2. The information generated should also assist companies to address similar requests from other regulatory authorities, based on our current understanding of global activities on this subject. The questionnaire includes a matrix to consider the structure and the origin of the material as a first risk indication. In addition, suppliers are encouraged to share their conclusion.

1.3. This BioSpectra Questionnaire for Nitrosamines Risk Evaluation was derived from the current IPEC Federation Questionnaire for Excipient Nitrosamines Risk Evaluation Version 2 – October 2025, and other relevant sources. Reference https://www.ipec-europe.org/articles/questionnaire-for-excipient-nitrosamines-risk-evaluation.html.

[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. https://www.fda.gov/media/141720/download

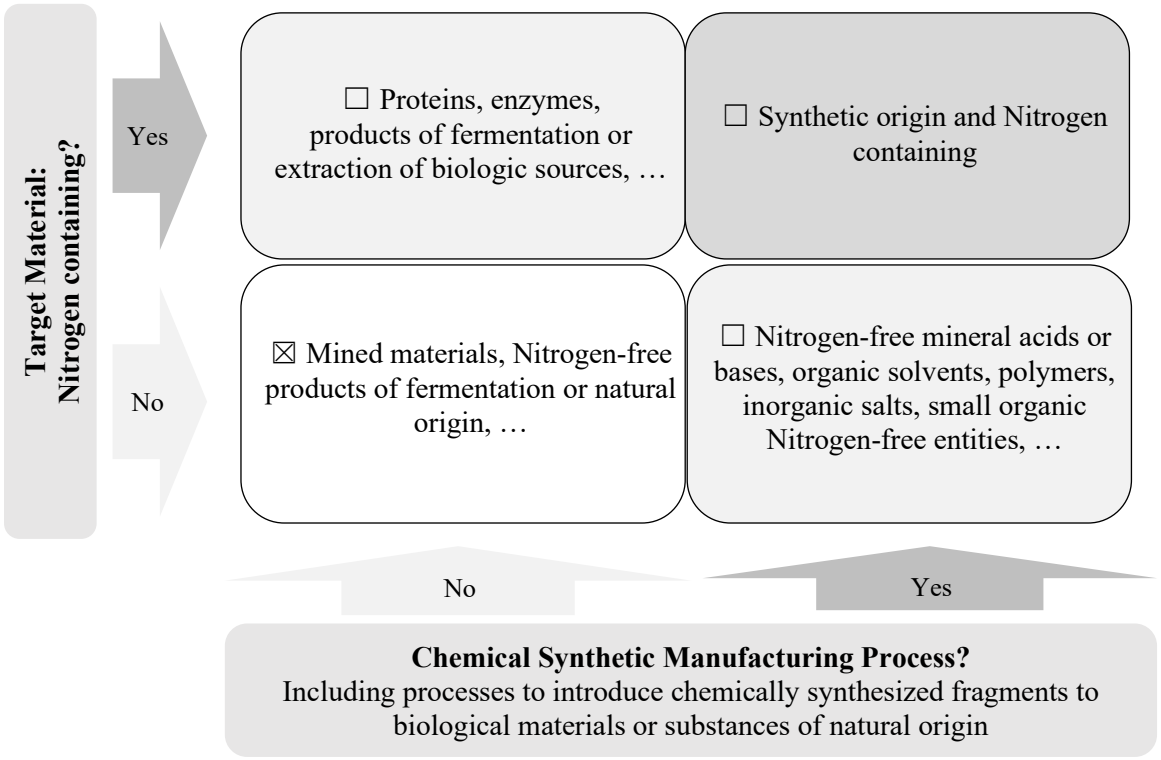
[5]U.S. Food & Drug Administration, "Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities." August 2023. https://www.fda.gov/media/170794/download

2. ASSESSMENT INFORMATION:

Material name	Sodium Decanoate, Bio Excipient Grade
Product number(s)	NDEC-3201
Company Name:	BioSpectra, Inc.
Completed by Name	Cassie Baun
Date	6/23/26
Job title	Senior Compliance Specialist
Signature	

3. NITROSAMINE RISK ASSESSMENT:

1) Please pick the applicable category based on structure and origin of the material 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 reagents/catalysts/processing aids used in the manufacturing process?	**YES** ☐	**NO** ☒	☐
- known or likely to be generated during the manufacturing process?	**YES** ☐	**NO** ☒	☐
- deliberately added to the process, including but not limited to components of cell culture media or for fermentation?	**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.
[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.

3) Have you analysed the material and are results available? Note: Default testing is **NOT** mandatory but may be performed if considered relevant for a specific material.: - Nitrites? - Nitrates? - Nitrosamines? If yes, please provide test results and expected levels for the tested analyte, and a general indication of the applied test method, the method Limit of Quantification (LoQ) or Limit of Detection (LoD) and indicate if testing was performed in-house or contracted out. Test results and Details: Not Applicable Note: Presently, nitrite testing is not harmonized, and results may vary depending on the method used by different manufacturers of the same material. Users are encouraged to test themselves when comparing suppliers.	**YES** ☐ **YES** ☐ **YES** ☐	**NO** ☒ **NO** ☒ **NO** ☒	Test result, if available- report below or provide separately
4) Is water used in the manufacturing process[9]? 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: - Nitrites - Nitrates (Note: Purified water according Ph. Eur. Complies with a Nitrates level of maximum 0.2ppm) (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.)[10]	**YES** ☒ **YES** ☒ _____ ppm _____ ppm	**NO** ☐ **NO** ☐ Not Available ☐ ☐	Not Applicable ☐ ☒ ☒
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 ☐

[9]EMA Guideline on the quality of water for pharmaceutical use: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-quality-water-pharmaceutical-use_en.pdf
[10]Ian W. Ashworth, Olivier Dirat, Andrew Teasdale, and Matthew Whiting. Potential for the Formation of N-Nitrosamines During the Manufacture of Active Pharmaceutical Ingredients: An Assessment of the Risk Posed by Trace Nitrite in Water, Org. Process Res. Dev. 2020, 24 (9), 1629-1646: https://www.sciencedirect.com/org/science/article/abs/pii/S1083616021021551

6) Vulnerable Amines: Are there any secondary and/or tertiary amines[11] present in the manufacturing process as[8]:			Not available/ applicable or unknown
- Raw material[12]?	**YES** ☐	**NO** ☒	☐
- Intermediate?	**YES** ☐	**NO** ☒	☐
- Reagent?	**YES** ☐	**NO** ☒	☐
- Processing aids?	**YES** ☐	**NO** ☒	☐
- Catalyst?	**YES** ☐	**NO** ☒	☐
- Solvent?	**YES** ☐	**NO** ☒	☐
- Process Chemical, Excipient, or Active Pharmaceutical Ingredient, etc. supplied to customer?	**YES** ☐	**NO** ☒	☐
- Known impurity of the material supplied to customer?	**YES** ☐	**NO** ☒	☐
If yes, are those amines present in the			Not available / applicable or unknown
- Same	**YES** ☐	**NO** ☐	☒
- Previous	**YES** ☐	**NO** ☐	☒
- Subsequent	**YES** ☐	**NO** ☐	☒
step as any nitrosating agent mentioned in question 2?			
Please provide any relevant information about the chemical name and structure of the amine(s): Not Applicable			
7) Is there any amide, primary amine[13] or ammonium salt used or present in the manufacturing process as:			Not available/ applicable or unknown
- Raw material	**YES** ☐	**NO** ☒	☐
- Intermediate	**YES** ☐	**NO** ☒	☐
- Reagent / Base	**YES** ☐	**NO** ☒	☐
- Processing aid	**YES** ☐	**NO** ☒	☐
- Catalyst	**YES** ☐	**NO** ☒	☐
- Solvent	**YES** ☐	**NO** ☒	☐
- Washing Fluid	**YES** ☐	**NO** ☒	☐
- Process Chemical, Excipient, or Active Pharmaceutical Ingredient, etc. supplied to customer?	**YES** ☐	**NO** ☒	☐
- Known impurity of the material supplied to customer?	**YES** ☐	**NO** ☒	☐
Please provide information about the chemical name and structure: Not Applicable			

[11]see Guidance 2 in Annex
[12]IPEC General Glossary of Terms and Acronyms: https://www.ipec-europe.org/guidelines.html
[13]Primary amines may reduce the risk of nitrosamines formation. See KK Nanda et al., J Pharm Sci 2021 (12), 3773; DOI: https://doi.org/10.1016/j.xphs.2021.08.010; M Homsak, Processes 2022 (10), 2428; https://doi.org/10.3390/pr10112428

8) Recycled/recovered Solvents[14]: - Are recycled / recovered nitrogen containing solvents used in the manufacturing process? Please provide information about the usage, and any controls in place: Not Applicable	**YES** ☐	**NO** ☒	Not available/ applicable or unknown ☐
9) Equipment: - Is the product manufactured 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 available/ applicable or unknown ☐ ☐ ☐
10) Additional comments, if any, not covered in the questionnaire *If "information is not available" has been ticked to any option, please include any additional comments here.* Not Applicable			

[14]see Guidance 3 in Annex.

4. ANNEX[15]:

4.1. *Guidance 1 (Sources of nitrosating agents)*

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

4.1.1.1. Nitrites (e.g., sodium nitrite, $NaNO_2$) nitrosyl halides (e.g., ClNO, BrNO), dinitrogen trioxide (N_2O_3), dinitrogen tetroxide (N_2O_4), and organic nitrites (e.g., t-BuONO).

4.1.1.1.1. It should be noted that nitrite itself is not a nitrosating agent, but it can lead to nitrosating agents (e.g., HNO_2, NO, ClNO, BrNO, N_2O_3, etc..) under certain conditions (e.g. aqueous acidic).

4.1.1.2. Nitrosating agents purposely used in the manufacturing process and/or introduced as impurities (e.g. from the input materials or water) should be considered.

4.1.1.3. 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.[16, 17] It should be noted that the risk from water is very low as described below.

4.1.2. Other potential nitrosation risks:

4.1.2.1. Certain input solid materials used during synthesis (e.g. NaCl, NaOH, K2CO3 and charcoal) can contain low levels (ppm) of nitrosating agents. Trace analytical methods for nitrite analysis have been reported[18] 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.

4.1.2.2. 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)[19] therefore, an understanding of the nitrite content of the water used has the potential to mitigate water as a risk factor.

4.1.2.3. 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. [16, 20]

4.1.2.4. Nitroalkanes, halogenated nitro alkanes, Fremy's salt, nitroso sulfonamides and nitroaromatics can all under some circumstances give rise to nitrosating agents.[20]

4.1.2.5. Hydroxylamines, hydrazines[21,] hydrazides and hydrazones can under oxidative conditions (air, hypochlorite, oxygen, ozone, and peroxides) give rise to nitrosating agents. [20, 22, 23]

4.1.2.6. Chloramines are known to generate N-nitrosamines under certain conditions and so should also be considered. [20, 22]

4.1.2.7. Ozone and other strong oxidants may lead to the formation of N-nitrosamines by initial oxidation of amines to nitrite. [20, 22, 23]

4.1.2.8. 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.[22]

4.1.2.9. A review of nitrosating agents has been provided by Bream et al. [17]

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[15]This information is partly transferred from the EFPIA decision tree for drug substances, 3rd revision, published June 2024.

[16]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

[17]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

[18]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

[19]Pfizer internal data shared on 25th Oct 2021 at Global Workshop on Nitrosamine Impurities: Global Workshop on Nitrosamine Impurities (cvent.com)

[20]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

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

[22]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

4.2. *Guidance 2 (Sources of secondary and tertiary amines)*

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

4.2.2. 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.

4.2.3. 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]

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

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

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

- 4.2.5.1. 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.
- 4.2.5.2. Common tertiary amine bases such as triethylamine, diisopropylethylamine and N-methylmorpholine.
- 4.2.5.3. Quaternary ammonium salts such as tetrabutylammonium bromide (TBAB).
- 4.2.5.4. Primary amines such as monoethylamine.
- 4.2.5.5. Starting materials, intermediates, or the excipient itself.

4.2.6. 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]

4.2.7. 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.

4.2.8. 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.

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[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 DOI: 10.1055/s-1972-21977 and 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

[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

4.3. ***Guidance 3 (Potential indirect contamination risks)***

4.3.1. Consider all potential sources of contamination (*N*-nitrosamines, nitrosating agents, vulnerable amines, etc.) in input materials. The potential presence of nitrosamines in input materials should be considered, especially if secondary amines are used.[28]

4.3.2. Use of recovered materials (solvents, reagents, catalysts) is of particular concern if appropriate controls are not put in place. 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:

4.3.2.1. Is the pre-recovered stream likely to contain any vulnerable amines, nitrosating agents or nitrosamines.

4.3.2.2. Is the recovery process likely to introduce and / or purge any of the above.

4.3.2.3. 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).

4.3.3. 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.

4.3.4. The materials DMF, ortho-xylene and tributyltin chloride were highlighted by the EMA as materials at risk of cross contamination by N-nitrosamines. Sodium azide was highlighted by Health Canada for risk of cross contamination with nitrite.

[28]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