BIOSPECTRA
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BioSpectra Questionnaire for Nitrosamines Risk Evaluation

Introduction

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 active.

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] and how they may be adapted for pharmaceutical excipients. This questionnaire additionally applies to Active Pharmaceutical Ingredients, Process Chemicals, and their raw materials.

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.

The use of a standard format will facilitate data collection from suppliers and thus enable a more efficient process of conducting the required risk assessments by drug product manufacturers / Marketing Authorisation Holders. With this form, suppliers can provide information for nitrosamine risk evaluation to the best of their knowledge, considering available supplier information and likely chemical production processes where information from the supplier is not available.

This BioSpectra Questionnaire for Nitrosamines Risk Evaluation was derived from the current IPEC Federation Questionnaire for Excipient Nitrosamines Risk Evaluation Version 1 – February 2023, 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

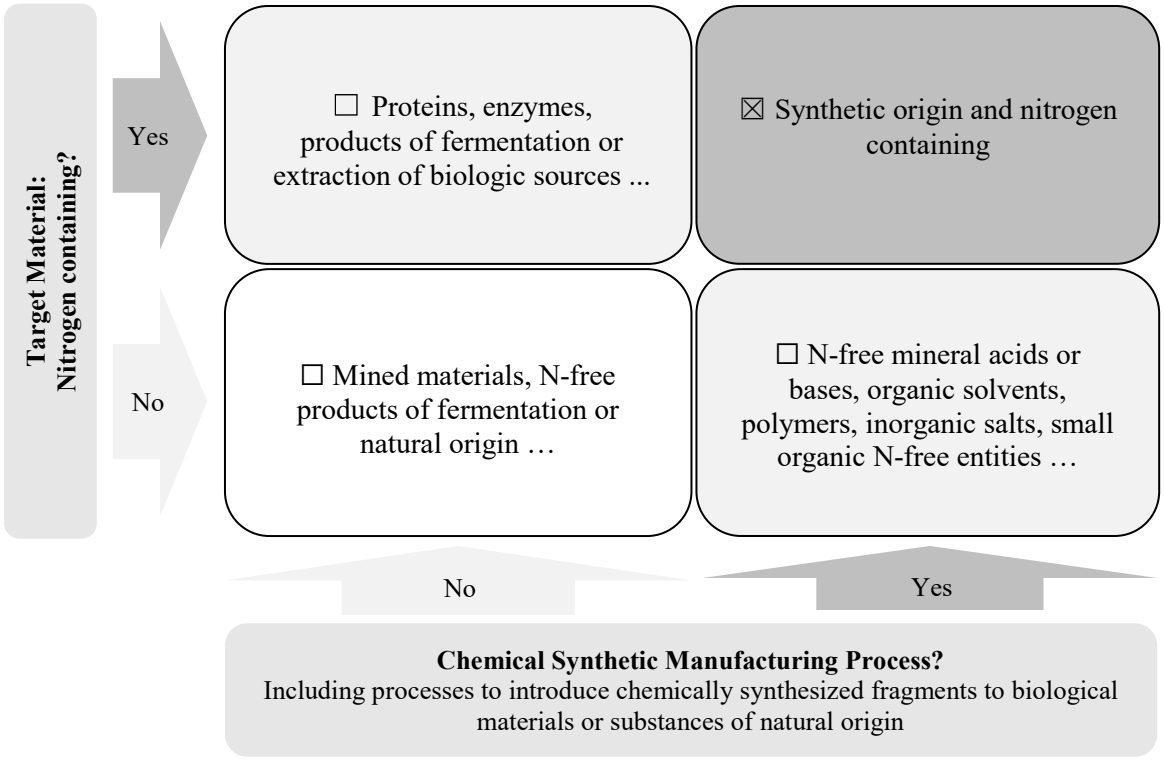
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This information for nitrosamine risk evaluation is applicable to the following:

Material name	Bis-Tris, Bio Excipient Grade
Product number(s)	BTRI-3250
Company Name:	BioSpectra Inc.
Completed by / Date	Cassie Baun 4/3/24
Job title	Senior Compliance Specialist
Signature	

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1) Please pick the applicable category based on structure and origin of the material in support to evaluate the risk of formation of nitrosamines[5].



2) Is sodium nitrite ($NaNO_2$) or any other nitrite or nitrosating agent[6]:			Not available/ applicable or unknown
- used in any steps in the manufacturing process[7] 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 components of cell culture media or for fermentation?	**YES** ☐	**NO** ☒	☐

[5] 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]

[6] see Guidance 1 in Annex.

[7] 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.

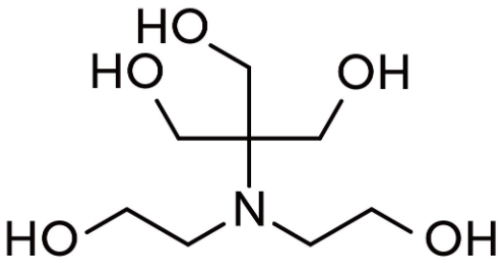
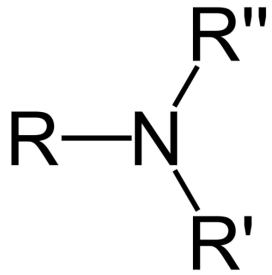
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3) Have you analysed the material and are results available for*: - Nitrites? - Nitrates? - Nitrosamines? *Default testing is **NOT** mandatory but may be performed if considered relevant for a specific material. If yes, please provide test results for the tested analyte and a general indication of the applied test method and indicate if testing was performed in-house or contracted out. Test results: 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[8]? 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.)[9]	**YES** ☒ **YES** ☒ _____ ppm _____ ppm	**NO** ☐ **NO** ☐ Not Available ☐ ☐	Not Applicable ☐ ☒ ☒

[8] 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

[9] 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

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5) Are there any secondary and/or tertiary amines[10] present in the manufacturing process as[7]:			Not available/ applicable or unknown
- Raw material[11]?	**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** ☐	☐
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): Bis-Tris chemical structure contains a tertiary amine. Solvent refers to approved Bis-Tris recycled mother liquor utilized in the BioSpectra Bis-Tris manufacturing process. Bis-Tris Chemical Structure:  Tertiary Amine Chemical Structure:  ……			

[10] see Guidance 2 in Annex
[11] IPEC General Glossary of Terms and Acronyms: https://www.ipec-europe.org/guidelines.html

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6) Is there any amide, primary amine[12] or ammonium salt used or present in the manufacturing process as: - Raw material - Intermediate - Reagent / Base - Processing aid - Catalyst - Solvent - Washing Fluid - Process Chemical, Excipient, or Active Pharmaceutical Ingredient, etc. supplied to customer? Please provide information about the chemical name and structure: Not Applicable ……	 **YES** ☐ **YES** ☐ **YES** ☐ **YES** ☐ **YES** ☐ **YES** ☐ **YES** ☐ **YES** ☐	 **NO** ☒ **NO** ☒ **NO** ☒ **NO** ☒ **NO** ☒ **NO** ☒ **NO** ☒ **NO** ☒	Not available/ applicable or unknown ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐
7) Recycled/recovered Solvents[13]: - Are recycled / recovered nitrogen containing solvents used in the manufacturing process? Please provide information about the usage, and any controls in place: Bis-Tris Mother liquor is analysed for Absorbance and must be approved for use in the BioSpectra Bis-Tris manufacturing process. ……	**YES** ☒	**NO** ☐	Not available/ applicable or unknown ☐
8) 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 ☐ ☐ ☐

[12] 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

[13] see Guidance 3 in Annex.

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9) Additional comments, if any, not covered in the questionnaire

If "information is not available" has been ticked to any option in question 2), please include any additional comments here.

Not Applicable

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Annex[14]:

Guidance 1 (Sources of nitrosating agents)

Nitrosating agents to be considered include nitrites (e.g., sodium nitrite, $NaNO_2$) and nitrous acid (HNO_2), nitric oxide (NO), nitrosyl halides (e.g., ClNO, BrNO), dinitrogen trioxide (N_2O_3), dinitrogen tetroxide (N_2O_4) and organic nitrites (e.g., t-BuONO). Nitrosating agents purposely used in the manufacturing process and/or introduced as impurities (e.g. from the input materials or water) should be considered.

Other potential nitrosation risks:

- Side reaction in nitration reactions. Nitric acid typically contains nitric oxide as an impurity, additional nitrous acid may also be produced, leading to nitrosation, if any reducing agents are present.
- Hydroxylamine under oxidative conditions.
- Chloramines are known to generate N-nitrosamines under certain conditions and so should also be considered.[15]
- Ozone may lead to the formation of N-nitrosamines by initial oxidation of amines to nitrite.[12]
- Use of azide salts and azide compounds is commonly followed by quenching with nitrous acid or nitrites and may lead to nitrite residues. [13]
- Nitric acid and nitrates under reducing conditions may result in by-products with nitrosating activity.[13]

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

Guidance 2 (Sources of secondary and tertiary amines)[16]

Secondary amines are of greatest concern, however tertiary amines can also undergo nitrosation via more complex pathways. All secondary and tertiary aliphatic and aromatic amines should therefore be considered including those present as part of the starting material, intermediate or final structure as well as those introduced as reagents, catalysts, solvents or as impurities.

Tertiary amine bases (i.e., triethylamine, diisopropylethylamine and N-methyl morpholine) may contain secondary amines as impurities.

Secondary Amines may also be introduced as impurities or degradants:

- Of common amide containing solvents such as N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMAC) and N-methyl pyrrolidinone (NMP)
- Of quaternary ammonium salts such as tetrabutylammonium bromide (TBAB)
- Of primary amines such as methylamine
- Of starting materials, intermediates, or the product itself

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

[14] This information is partly transferred from the EFPIA decision tree for drug substances, published 1 Nov 2019.
[15] Nawrocki, J et al. Nitrosamines and Water, J. Hazard. Mater. 2011, 189, 1-18.
[16] SCCS (Scientific Committee on Consumer Safety), Opinion on Nitrosamines and Secondary Amines in Cosmetic Products, 27 March 2012.

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Guidance 3 (Potential contamination risks)

Consider all potential sources of contamination (*N*-nitrosamines, nitrosating agents, vulnerable amines, etc.) in input materials.

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

Cross contamination 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) are a lower cross contamination risk.

Guidance 4 (Determining an acceptable level)

Interim acceptable daily intakes for chronic exposure to several common N-nitrosamines have been defined. See literature reference[17] for EMA interim acceptable daily intake for chronic exposure to common N-nitrosamines.

Processes to determine acceptable intakes for all other N-nitrosamines should be in alignment with the EFPIA paper.[18]

These levels should be adjusted for less than lifetime exposures as described in ICH M7.[19]

Calculate acceptable limits in ppm relative to the substance using the maximum daily dose. Higher limits may be justified for ICH S9 indications. [20]

Guidance 5 (Conducting purge assessments)[21]

Where a nitrosating agent and amine have the potential to be concurrently present an assessment of the process conditions should be conducted to determine if a N-nitrosamine could potentially be formed and what the maximum realistic level could be. Nitrosation occurs more rapidly under acidic conditions (apart from organic nitrites) and may also be catalysed by certain anions and aldehydes (notably thiocyanate and formaldehyde).[10, 22]

During purge calculations consider the likely physicochemical characteristics of the N-nitrosamine which may be formed. For instance, NDMA has a BP of 153°C and will partition in both aqueous and organic layers. It is highly soluble in water and organic solvents. Other, higher molecular weight, N-nitrosamines will behave differently.

N-nitrosamines are relatively stable compounds though the following conditions are known to result in de-nitrosation:

- Strongly acidic condition with a nucleophile trap (e.g. HCl with MeOH)
- Metal reducing conditions (e.g. Zn AcOH; Ni/Al KOH)
- Pd/C Hydrogenation
- Grignards
- Strong oxidants (H_2O_2; $KMNO_4$)

[17] EMA, Temporary interim limits for NMBA, DIPNA and EIPNA impurities in sartan blood pressure medicines, 20 August 20, 2019.
[18] EFPIA position with respect to safety related aspects of EMA and Health Canada requests for N-nitrosamine evaluations, 2019.
[19] ICH M7, Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, 31 March 2017.
[20] ICH S9, Nonclinical Evaluation for Anticancer Pharmaceuticals, 29 October 2009.
[21] Barber, C et al. A consortium-driven framework to guide the implementation of ICH M7 Option 4 control strategies. Regul. Toxicol. Pharmacol. 2017, 90, 22-28.
[22] Williams, D. L. H. Nitrosation reactions and the chemistry of nitric oxide. 2004, Amsterdam, Elsevier.

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