

BIO SPECTRA QUESTIONNAIRE FOR EXCIPIENT NITROSAMINES RISK EVALUATION

On September 19th, 2019, European Medicines Agency (EMA) published a new requirement that Marketing Authorization Holders (MAHs) for human medicines containing chemically synthesized active substances review their medicines for the possible presence of nitrosamines.

1. Information on nitrosamines for marketing authorization holders (EMA/189634/2019)¹
2. Questions and answers on “Information on nitrosamines for marketing authorization holders” (EMA/CHMP/428592/2019 Rev. 1)²

Other authorities started an equivalent approach, e.g. Health Canada³, TGA (Australia), Swissmedic⁴.

In September 2020, the US Food and Drug Administration (US FDA) published a guidance for the Control of Nitrosamine Impurities in Human Drugs⁵.

This questionnaire was developed with reference to EMA requirements on this topic. However, the information generated should also assist companies to address similar requests from other regulatory authorities, based on our current understanding of global activity on this subject.

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 drug product manufacturers / Marketing Authorisation Holders.

Information for nitrosamine risk evaluation is provided to the best of our 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:

Product Name:	Tris Hydrochloride, Bio Excipient Grade and Bio Pharma Grade
Product CAS Number:	1185-53-1
Supplier:	BioSpectra, Inc.
Supplier Contact Name: <i>(Please print full name)</i>	Cassie Baun
Supplier Signature:	<i>Cassie Baun</i>
Date Completed:	10/5/22

¹ Information on nitrosamines for marketing authorization holders. EMA/189634/2019: https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-information-nitrosamines-marketing-authorisation-holders_en.pdf

² Questions and answers on “Information on nitrosamines for marketing authorization holders”. EMA/CHMP/428592/2019 Rev. 1. https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-questions-answers-information-nitrosamines-marketing-authorisation_en.pdf

³ Health Canada: Information to Marketing Authorization Holders (MAHs) of Human Pharmaceutical Products Regarding Nitrosamine Impurities. October 2, 2019

⁴ <https://www.swissmedic.ch/swissmedic/en/home/news/mitteilungen/aufforderung-zlinhaberinnen-ham.html>

⁵ <https://www.fda.gov/media/141720/download>

<p>1) Is sodium nitrite (NaNO₂) or any other nitrite or nitrosating agent⁶:</p> <ul style="list-style-type: none"> - Used in any steps in the manufacturing process⁷ as reagents/catalyst? YES <input type="checkbox"/> NO <input checked="" type="checkbox"/> - Known to be used in the preparation of raw materials or intermediates used in the manufacturing process? YES <input checked="" type="checkbox"/> NO <input type="checkbox"/> - Known to be used in the preparation of reagents/catalysts/processing aids used in the manufacturing process? YES <input type="checkbox"/> NO <input checked="" type="checkbox"/> - Known to be generated as impurities during the manufacturing process? YES <input checked="" type="checkbox"/> NO <input type="checkbox"/> <p>BioSpectra’s Approved Tris Raw Material Supplier has stated that Nitric Acid, Nitrous Acid, Nitric Oxide, and Organic Nitrites are involved in the raw material manufacturing process. Nitric Acid is used by BioSpectra’s Approved Raw Material Supplier in the initial synthesis step. BioSpectra uses Tris and HCl from approved Raw Material Suppliers to synthesize and purify Tris Hydrochloride. BioSpectra does not intentionally add any additional Nitric Acid, Nitrous Acid, Nitric Oxide, and Organic Nitrites during the BioSpectra manufacturing process to synthesize Tris Hydrochloride.</p> <p>.....</p>			<p><i>Information not available</i></p> <p><input type="checkbox"/></p> <p><input type="checkbox"/></p> <p><input type="checkbox"/></p>
<p>2) Have you analysed, and are the results available for the excipient for:</p> <ul style="list-style-type: none"> - Nitrites? YES <input type="checkbox"/> NO <input checked="" type="checkbox"/> - Nitrates? YES <input type="checkbox"/> NO <input checked="" type="checkbox"/> - Nitrosamines? YES <input type="checkbox"/> NO <input checked="" type="checkbox"/> <p>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.</p> <p>BioSpectra has not analyzed Tris Hydrochloride for Nitrites, Nitrates, or Nitrosamines. BioSpectra’s Approved Tris Raw Material Supplier has analyzed Tris raw material with analytical data confirming the lack of nitrosamines found in any quantifiable level with a 20ppb limit of detection, and has stated that they have analytical data that supports and shows that the Tris raw material utilized in BioSpectra’s manufacturing process to synthesize Tris Hydrochloride does not contain the nitrosamines principally attributed to the presence of the specific nitrosating species indicated. BioSpectra uses Tris and HCl from approved Raw Material</p>			<p><i>Test result, if available</i></p>

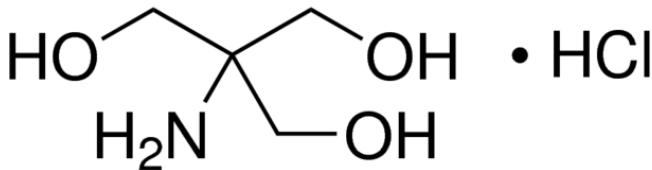
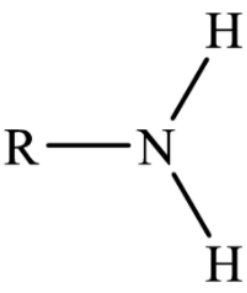
⁶ See Guidance 1 in Annex

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.

<p>Suppliers to synthesize and purify Tris Hydrochloride.</p>			
<p>3) If water is used in the manufacturing process⁷, is it prepared by distillation, by ion exchange or by reverse osmosis? If “No”, please inform about the maximum level of: - Nitrites - Nitrates (Note: Purified water according Ph. Eur. Complies with a nitrates level of maximum 0.2 ppm)</p>	<p>YES <input checked="" type="checkbox"/> ____ ppm ____ ppm</p>	<p>NO <input type="checkbox"/> <i>Not Specified</i> <input type="checkbox"/> <input type="checkbox"/></p>	<p><i>Not applicable</i> <input type="checkbox"/> <input checked="" type="checkbox"/> <input checked="" type="checkbox"/></p>
<p>4) Is there any secondary and/or tertiary amine⁸ present in the manufacturing process as⁴: - Raw material⁹? - Intermediate? - Reagent? - Processing aids? - Catalyst / Base? - Solvent? If yes, are those amines present in the - Same - Previous - Subsequent step as any nitrosating agent mentioned in section 1? Information about the chemical name / structure of amine(s): BioSpectra’s Approved Tris Raw Material Supplier has shown that secondary and tertiary amines may be generated as impurities during the raw material manufacturing process. BioSpectra uses Tris and HCl from approved Raw Material Suppliers to synthesize and purify Tris Hydrochloride. BioSpectra does not intentionally add any secondary or tertiary amines during the BioSpectra manufacturing process to synthesize Tris Hydrochloride. </p>	<p>YES <input checked="" type="checkbox"/> YES <input type="checkbox"/> YES <input type="checkbox"/> YES <input type="checkbox"/> YES <input type="checkbox"/> YES <input type="checkbox"/> YES <input type="checkbox"/> YES <input type="checkbox"/> YES <input type="checkbox"/> YES <input checked="" type="checkbox"/></p>	<p>NO <input type="checkbox"/> NO <input checked="" type="checkbox"/> NO <input checked="" type="checkbox"/> NO <input checked="" type="checkbox"/> NO <input checked="" type="checkbox"/> NO <input checked="" type="checkbox"/> NO <input checked="" type="checkbox"/> NO <input checked="" type="checkbox"/> NO <input checked="" type="checkbox"/> NO <input type="checkbox"/></p>	<p><i>Not applicable</i> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/></p>

⁸ see Guidance 2 in Annex

⁹ 2014 IPEC General Glossary of Terms and Acronyms

<p>5) Is there any amide, primary amine or ammonium salt¹⁰ used or present in the substance manufacturing process as:</p> <ul style="list-style-type: none"> - Raw material - Intermediate - Reagent - Processing aid - Catalyst / Base - Solvent - Washing Fluid <p>Information about the chemical name / structure:</p> <p>Tris Hydrochloride chemical structure contains a primary amine. Solvent refers to approved Tris Hydrochloride recycled mother liquor used in the BioSpectra manufacturing process. BioSpectra's Approved Tris Raw Material Supplier has shown that primary amines may be generated as impurities during the raw material manufacturing process. BioSpectra uses Tris and HCl from approved Raw Material Suppliers to synthesize and purify Tris Hydrochloride. BioSpectra does not intentionally add any additional Amides, Primary Amines, or Ammonium Salts during the BioSpectra manufacturing process to synthesize Tris Hydrochloride.</p> <p>Tris Hydrochloride Chemical Structure:</p>  <p>Primary Amine Chemical Structure:</p>  <p>.....</p>	<p>YES <input checked="" type="checkbox"/></p> <p>YES <input checked="" type="checkbox"/></p> <p>YES <input type="checkbox"/></p> <p>YES <input type="checkbox"/></p> <p>YES <input type="checkbox"/></p> <p>YES <input checked="" type="checkbox"/></p> <p>YES <input checked="" type="checkbox"/></p> <p>YES <input type="checkbox"/></p>	<p>NO <input type="checkbox"/></p> <p>NO <input type="checkbox"/></p> <p>NO <input checked="" type="checkbox"/></p> <p>NO <input checked="" type="checkbox"/></p> <p>NO <input checked="" type="checkbox"/></p> <p>NO <input type="checkbox"/></p> <p>NO <input type="checkbox"/></p> <p>NO <input checked="" type="checkbox"/></p>	
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¹⁰ see Guidance 2 in Annex

Product Code: Reference the Tris Hydrochloride Stroudsburg Manufactured Compliance Statements Cover Letter

Product Name: Tris Hydrochloride, Bio Excipient Grade and Bio Pharma Grade

6) Recycled/recovered Solvents: - Are recycled / recovered nitrogen containing solvents used in the manufacturing process?	YES <input checked="" type="checkbox"/>	NO <input type="checkbox"/>	
7) Multipurpose Equipment ⁷ : - Is the substance 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?	YES <input type="checkbox"/>	NO <input checked="" type="checkbox"/>	<i>Not applicable</i>
	YES <input type="checkbox"/>	NO <input type="checkbox"/>	<input checked="" type="checkbox"/>

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Annex¹¹:

Guidance 1 (Sources of nitrosating agents)

Nitrosating agents to be considered include; nitrites (e.g. sodium nitrite, NaNO₂) and nitrous acid (HNO₂), nitric oxide (NO), nitrosyl halides (e.g. ClNO, BrNO), dinitrogen trioxide (N₂O₃), dinitrogen tetroxide (N₂O₄) and organic nitrites (e.g. t-BuONO).

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 considered¹²
- Ozone may lead to the formation of N-nitrosamines by initial oxidation of amines to nitrite⁹
- Use of azide salts and azide compounds is commonly followed by quenching with nitrous acid or nitrites and may lead to nitrite residues.¹
- Nitric acid and nitrates under reducing conditions may result in by-products with nitrosating activity.¹⁰

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)¹³

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-methylmorpholine) are known to degrade to secondary amines and have been implicated in N-nitrosamine formation.

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-methylpyrrolidinone (NMP)
- Of quaternary ammonium salts such as tetrabutylammonium bromide (TBAB)
- Of primary amines such as monoethylamine
- 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.

Guidance 3 (Potential contamination risks)

¹¹ This information is transferred from the EFPIA decision tree for drug substances, published 1 Nov 2019

¹² Nawrocki, J et al. Nitrosamines and Water, J. Hazard. Mater. 2011, 189, 1-18.

¹³ SCCS (Scientific Committee on Consumer Safety), Opinion on Nitrosamines and Secondary Amines in Cosmetic Products, 27 March 2012.

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Consider all potential sources of contamination 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 considered to be 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 reference¹⁴ 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.¹⁵

These levels should be adjusted for less than lifetime exposures as described in ICH M7.¹⁶

Calculate acceptable limits in ppm relative to the substance using the maximum daily dose. Higher limits may be justified for ICH S9 indications.¹⁷

Guidance 5 (Conducting purge assessments)¹⁸

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).^{10,19}

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

¹⁴ EMA, Temporary interim limits for NMBA, DIPNA and EIPNA impurities in sartan blood pressure medicines, 20 August 20, 2019.

¹⁵ EFPIA position with respect to safety related aspects of EMA and Health Canada requests for N-nitrosamine evaluations, 2019.

¹⁶ ICH M7, Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, 31 March 2017.

¹⁷ ICH S9, Nonclinical Evaluation for Anticancer Pharmaceuticals, 29 October 2009.

¹⁸ 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.

¹⁹ Williams, D. L. H. Nitrosation reactions and the chemistry of nitric oxide. 2004, Amsterdam, Elsevier.

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- Strong oxidants (H_2O_2 ; $KMNO_4$)