InSight Bulletin: A Multidisciplinary Interlink International Research Journal
Peer Reviewed International, Open Access Journal.
ISSN : 3065-7857 / Website: https://ibrj.us / Volume-2, Issue-10 / October - 2025

Original Article

A Review on Nitrosamine Impurities, Factor Affecting Formation of Nitrosamine Impurities and Systematic Approach to Mitigate Risk in Active Pharmaceutical Ingredients

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Manuscript ID:
IBMIIRJ -2025-021011

Submitted: 08 Sept. 2025

Revised: 12 Sept. 2025

Accepted: 10 Oct. 2025

Published: 31 Oct. 2025

ISSN: 3065-7857

Volume-2

Issue-10

Pp. 46-56

October 2025

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OPEN ACCESS

Quick Response Code:

Web. https://ibrj.us

DOI: 10.5281/zenodo.17620825

DOI Link:
https://doi.org/10.5281/zenodo.17620825

Abstract

The term 'Nitrosamine" or "N-Nitrosamine" are referred to the chemical substance having structure $R_2N-N=O$*, where R is usually an alkyl group. Nitrosamine are the substance produced essentially unintentionally during chemical synthesis. Most international regulatory authorities provided regulatory guidelines regarding the evaluation of nitrosamine in drug substance or drug product. The present article based on the recent discovery of nitrosamine impurities in several marketed pharmaceuticals API. This will also put light on the factor affecting for the formation, systematic evaluation of risk assessment and factor to mitigate the risk, historical approach for the nitrosamine, regulatory guidelines, and sources of Nitrosamine impurities, their generation and toxicity. This article also explains the CPCA approach for NDSRIs, and case study for Nitrosamine in Active pharmaceutical ingredient.*

Keywords: *- Impurities, Nitrosamine, Genotoxic, regulatory guidelines, drug substance, NDSRIs.*

Introduction

Impurities can be defined as presence of any foreign matter or substance in the product, it can be classified as organic impurity, inorganic impurity, further classification as volatile or non-volatile having different chemical pharmacological and toxicological effects. A description for both identified as well as unidentified substances might be included in the impurity profile. According to the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) guideline on impurities in new drug substances, an impurity is defined as 'any component of the new drug substance that is not the chemical entity defined as the new drug it is or get generated out of synthesis or undesirable chemicals that remains present with APIs. The characteristic of drug product is exceptionally altered by impurity present in API or Finished drug. Pharmaceutical product would be accomplished to serve their predetermined therapeutic activity only when they are free from impurity hence , an impurity present in an active pharmaceutical ingredient to be identified, and quantify by the of modern analytical approaches[1-3]

ICH Guidelines for impurity profiling -

Regulatory authorities, including the International Conference on Harmonization (ICH), have placed significant emphasis on addressing impurities in drug substances and drug products, as well as residual solvents. These guidelines play a vital role in ensuring the quality, safety, and efficacy of pharmaceutical products [4]

1. Q1A-"stability testing of new drug substances and products"
2. Q3A (R2) - "Impurities in New Drug Substances"
3. Q3B (R2) - "Impurities in New Drug Products"
4. Q3C (R5) - "Impurities: Guidelines for Residual Solvents"

Regulatory Guidelines on impurity: International Conference on Harmonization guidance of Technical Requirements for Registration of Pharmaceuticals for Human Use is inscribed by The United States Food and Drug Administration (FDA).

How to cite this article:
R, S. M. A., Birari, D. R., & Patil, L. N. (2025). A Review on Nitrosamine Impurities, Factor Affecting Formation of Nitrosamine Impurities and Systematic Approach to Mitigate Risk in Active Pharmaceutical Ingredients. InSight Bulletin: A Multidisciplinary Interlink International Research Journal, 2(10), 46–56. https://doi.org/10.5281/zenodo.17620825

The FDA has the assigned responsibility of ensuring the safety and efficacy of drugs. The various regulatory guidelines regarding impurities are as follows. It's important to note that the FDA's regulatory guidelines are continually updated and refined to reflect advances in scientific understanding and changing industry practices. Compliance with these guidelines is essential for pharmaceutical manufacturers seeking approval for their products and for ensuring that drugs on the market are safe and effective. The FDA plays a critical role in maintaining the high standards of the pharmaceutical industry in the United States and as well as in the other part of the world. [5]

1. ICH guidelines —stability testing of new drug substances and products"- Q1A
2. ICH guidelines —Impurities in New Drug Substances- Q3A
3. ICH guidelines —Impurities in New Drug Products- Q3B
4. ICH guidelines —Impurities: Guidelines for residual solvents- Q3C
5. US-FDA guidelines —NDAs -Impurities in New Drug Substances
6. US-FDA guidelines —ANDAs – Impurities in New Drug Substances
7. Australian regulatory guideline for prescription medicines, Therapeutic Governance Authority (TGA), Australia

Genotoxicity potential impurities: - There is considerable confusion between the term's 'genotoxicity' and 'mutagenicity'. According to ICH guideline genotoxicity is defined as "any deleterious change in genetic material regardless of mechanism by which the change is induced" [7]; and "mutagenicity is induced permanent change in the genetic material". Many of the impurities in final drug substances may possess structural alerts which could be mutagenic, wherein Ames mutagenicity test is not performed is called potentially genotoxic impurities. In 2014, ICH published the ICH M7 guideline on "DNA reactive (mutagenic) impurities" a subset of genotoxic impurities. The property ' genotoxicity' or ' mutagenicity' can be easily demonstrated by subjecting the particular impurity to standard in vitro test, the best known and worldwide accepted is Ames mutagenicity test [8-9]. The Ames test was developed by Bruce N. Ames in early 1970s. This test is an experimental procedure to evaluate the potential carcinogenicity of the materials, based on mutagenicity effect on

The drug substances manufactured by using chemical transformations with various reaction steps obviously possess traces of reagents, solvents, catalysts, metal impurities, starting materials, intermediate and side products. Out of these impurities. Compounds which are DNA-reactive and cause damage to DNA and could lead to mutation of genetic code are called genotoxic impurities, these key starting materials, intermediates, by-products, and API have been examined and compared with the structural alerts for genotoxicity as provided by Ashby and Tennant, 1989: The "Super mutagen." Wherever the structural alerts for genotoxicity were identified, such impurities are controlled as per the TTC approach or absence studies have been demonstrated for the same at adequate manufacturing step. [6-9]

N-nitrosamines potential impurities: - Nitrosamine impurities have been classified as probable human carcinogens for decades. These impurities were reported in water, food, tobacco, pesticides, and plastics, N-nitrosamines are a class of compounds that have been shown to exhibit carcinogenic and mutagenic effects in animal models at several different tissue sites and by several different routes of exposure. Several nitrosamines were classified as carcinogens 2A (probable carcinogens) and more recently in Group 1 (human carcinogens) for N-nitrosonornicotine (NNN). The REACH regulations also list N-Nitrosodimethylamine (NDMA), Methylnitronitrosoguanidine (MNNG), N-Nitrosodiethanolamine (NDELA); N-Nitrosodi-n-propylamine (NDPA) as category 1B carcinogens. Several nitrosamines are also categorized by the International Agency for Cancer Research (IARC) as 2A and 2B – Probable and Possible Carcinogens, respectively, based on study data of some species [10-12]

What is the Nitrosamine: - Nitrosamine or more correctly N-Nitrosamine denotes to any molecules having the N-nitroso functional describe in group (**Fig 1**). These Molecules concern because of their human carcinogenic properties, there present were reported in food, drinking water, but there present in medicine is intolerable even though in very low percentage too.

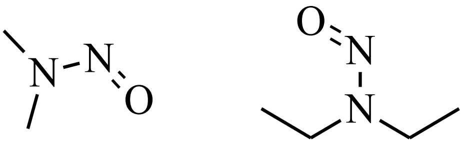

Figure 1

How they are formed: - The formation of nitrosamine is generally possible only when there is source of secondary of tertiary amine reacted with nitrous acid. Nitrous acid is unstable but can be formed in presence of nitrosetting source under acidic condition. [13]The systematic reaction mechanism is shown in (**Fig 2**)

Reaction Mechanism for the formation of Nitrosamine:-

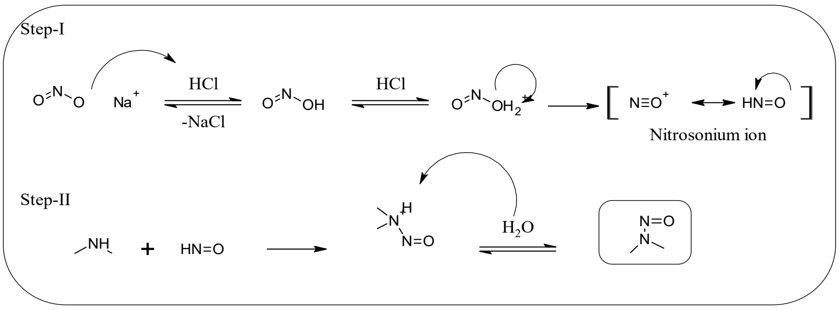

Figure 2

History of N-nitrosamines potential impurities:- The carcinogenic properties of nitrosamines have been known for more than 50 years since Peter Magee and John Barnes reported in 1956 that N-nitrosodimethylamine (NDMA), a simple water-soluble compound with only 11 atoms, readily induced liver tumours in rats and since then hundred nitrosamines have been tested for carcinogenicity, N- itrosodimethylamine (NDMA), N-nitroso-diethylamine (NDEA), N-nitroso-N-methyl-4- aminobutyric acid (NMBA), N-nitroso-diethanolamine (NDELA), nitroso morpholine NMOR), N-nitroso-N-methyl-Nethylamine, and N-nitrosopyrrolidine (NPYR), being some of the well-known among these. Of all the nitrosamines, NDMA have been researched the most. NDMA have been found to be carcinogenic in more than 20 species of animals and it is believed that no species is exempt from the carcinogenic activity of this compound. Because of this higher potency, nitrosamine impurities are considered by the ICH M7 (R2) guideline as high potency mutagenic carcinogens referred to as compounds that are part of the "cohort of concern" and as such are classified as Class 1 impurities — "known mutagenic carcinogens" — based on both rodent carcinogenicity and mutagenicity data and need to be controlled at or below compound-specific limits. Cohort of concern (CoC) is indeed defined as a group of high potency mutagenic carcinogens comprised of aflatoxin-like-, N-nitroso, and alkyl-azoxy compounds. These compound-specific limits might be much lower as compared to the limit of 1.5 µg/ day acceptable intake (AI) for other potentially mutagenic impurities which lack carcinogenicity data, as defined by ICH M7 if treatment lasts more than 10 years. The EMA and FDA in 2020 published a list of the most common volatile nitrosamines deriving from common reagents and solvents, which emerged from the learn lesson of the case sartans, with their acceptable intakes, which are 15-50 times lower than the ICH M7 TTC.[1-17]

Carcinogenicity Mechanism: - Afterwards, the subsequent in-depth analysis and risk assessments led to the discovery of a truly vast panorama of possibilities given the almost ubiquity of nitrogen's vulnerable to nitrosation in processes of drug substance and dry product manufacturers. Although a very large amount of the amines may be nitrosated, it must be considered that a consensus has been reached with the regard to the mechanism related to the high level of carcinogenicity of N-Nitrosamines, in fact to achieve a highly carcinogenic potency, metabolic activation to form a diazonium salt (e.g., methyldiazonium, ethyldiazonium, etc.) is required. In this context it would be more correct to refer to vulnerable amine rather than all nitrosatable nitrogens. The term vulnerable amine corresponds to an amine function having extractable α-proton and that could form an alkylating diazonium salt as described in below (**Figure 3**). A vulnerable amine corresponds to (but not limited to): secondary and tertiary amines, quaternary ammonium salts, N, N-dialkylamines (e.g. N-methyl-2-pyrrolidone, dimethylformamide, dimethylacetamide), and N, N-dialkyl carbamates or N, N-dialkylhydrazines. [18-20]

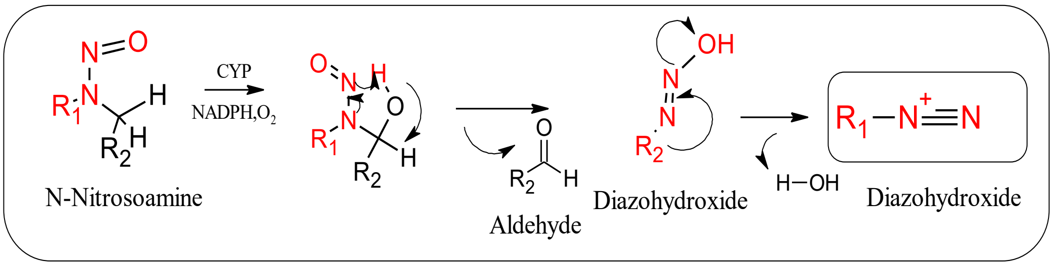

Figure 3: Formation of the DNA-reactive dizonium ion methyldiazonium ion.

N-nitrosamines impurities in medicinal products:-N-nitrosamines received attention in mid-2018 when they were also found in medicinal products. Being a risk to patients, it triggered drug recalls that left millions without treatment. Initially, the risk was found to be associated with small nitrosamines [N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), NNitrosodibutylamine (NDBA), N-Nitrosomethylphenylamine (NMPA), N-Nitrosodiisopropylamine (NDIPA), N-Nitrosoisopropylethylamine (NIPEA) and N-nitroso- N-methyl-4-aminobutanoic acid (NMBA)] for which animal toxicological data were available for establishing their Acceptable Intake (AI) limits in humans. For other small nitrosamines, such as NEIPA, NMBA, AI values were established based on read across approach. Accordingly, several analytical methods were published to control these impurities below their acceptable intake limits. Later, it was revealed that the presence of secondary amine groups in drug substances (APIs) could lead to the formation of NDSRIs under certain favourable conditions (presence of nitrosating agent and acidic pH). NDSRI was first introduced when Health Canada recalled Varenicline with levels of nitrosamine impurity, N-Nitroso varenicline in June 2021, followed by recall of several batches of Varenicline from the European region by EMA. Subsequently, FDA announced voluntary recall of Varenicline drug products in July 2021[21].

Regulatory Action:- In the European Union (EU), following an article 31 review of sartans at risk of containing nitrosamine impurities manufacturers were asked to review and make changes to their manufacturing processes to minimize nitrosamine impurities to the extent practically possible. In a similar manner, the USFDA worked to identify and recall medicines with levels above interim acceptable limits. The USFDA publishes a list of ARB products and their status with respect to nitrosamine content. Like the EMA, the USFDA emphasized that the risks (such as stroke) of abruptly discontinuing these drugs far outweigh the low risk associated with continuing the medications with these impurities. [21-24]. Many companies have initiated voluntary recalls of their products as preventative measures. Regulatory agency is very proactive about nitrosamine and updating time to time following is the revision history in table No.1

Table No.1 Revision History of EMA guidelines for nitrosamine

0	Replace obsolete Q&A published in 2019 to support the initial "call for review" with a new version reflecting the main principles agreed as part of the Article 5(3) referral which concluded in July 2020.	03rd August 2020
1	Update to Q&A 3 in order to clarify products in scope of the call for review. Update to Q&A 4 in order to add the link to the outcome of the referral under article 3 of Directive 2001/83/EC for ranitidine.	29th January 2021
2	Update to Q&A 3 on indicating testing timeline at the time of step 1 "risk identified" reporting.	24th February 2021
3	Update to Q&A 3 on the approach for non-marketed medicines. New Q&A 19 on the requirements for line extensions and variation applications.	15th April 2021
4	Update to Q&A 3 on combining step 2 response for multiple products from the same MAH.	18th May 2021
4*	Updates to Q&A 3 on when to perform step 2 confirmatory testing in order to meet the established deadline for step 3. Update and Q&A 10 to add an AI for NMOR.	29th June 2021
5	Update to Q&A 10 to add an AI for NNV.	21st September 2021
6	Guidance on confirmatory testing requirements for marketed (Q&A 8) and on-going applications (Q&A 14) to include cases where a potential nitrosamine impurity cannot be synthesised, and when a product is available in multiple strengths of the same dosage form.	14th October 2021
7	Inclusion of additional guidance on control strategies for products containing more than one nitrosamine impurity including examples (Q&A 10) and a decision tree (Annex Me).	31st January 2022
8	Update to guidance on root causes and risk factors for nitrosamine contamination (Q&A 4) and on policy for confirmatory testing (Q&A 8) and dossier requirements (Q&A 15) to allow testing of intermediates, raw materials or API under certain circumstances.	24th March 2022
9	New Q&A 20 providing clarifications on what are the regulatory steps for dealing with scenario A cases and update Q&A10 with new AIs (N-nitrosomethylphenidate, N-nitrosopiperidine, N-nitrosorasagilene, 7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3- a]pyrazine, N-nitroso-1,2,3,6-tetrahydropyridine, N-nitrosonortriptyline, N-methyl-N-nitrosophenethylamine) and guidance on use of Ames test.	20th May 2022
10	Update to Q&A 5 to provide clarifications on the expectation for MAHs to continue to re-visit risk evaluations when new information becomes available with specific reference to API-nitrosamine risk. Update to Q&A 10 to include newly adopted AI for N-nitrosodabigatran and to indicate APIs where related nitrosamines have been identified. Clarification of how to set limits for products containing salt, hydrate or solvate forms of the API. Update to Q&A 14 to reference the new risk evaluation template for use in marketing authorisation applications.	23rd June 2022
11	Update to Q&A 3 on submission of amended step 1 response and extension of Step 3 deadline for chemical medicines	29th July 2022
12	Update of Q&A 10 to add nitrosoduloxetine and introduction of Q&A 21 on approach to control presence of nitrosamine while the AI is being established	10th October 2022
13	Update of Q&A 10 to add N-nitrosofluoxetine, N-nitrosoparoxetine, N-nitrosodiphenylamine, N-nitroso-mefenamic acid, N-nitrosopyrrolidine and N-nitrosodiethanolamine.	5th December 2022
14	Introduction of Q&A 22 on approach to control presence of N-nitrosamine exceeding the AI while CAPAs are being implemented. Update of Q&A 20 to consider the possibility of an interim limit based on the LTL approach during CAPA implementation. Update of Q&A 21 for increased clarity on the application of the temporary universal limit.	22nd December 2022
15	Amendment of Q&A 22 to indicate that no variation should be submitted to implement temporary above AI limits in specifications.	30th March 2023
16	Amendment to Q&A 10 to include the Carcinogenic Potency Categorization	7th July 2023

	Approach (CPCA) and the enhanced Ames test (EAT) for establishing AIs for N-nitrosamines. Addition of Appendix 1, listing the nitrosamines for which AI have been established by the Non-clinical Working Party (NcWP), including new AIs for N-nitrosamines determined using the CPCA. Addition of Annex 2, describing the Carcinogenic Potency Categorization Approach for N-nitrosamines. Addition of Annex 3, describing the Enhanced Ames Test Conditions for N-nitrosamines.	
17	Amendment of Q&A 22 on approach to control presence of N-nitrosamine exceeding the AI while CAPAs are being implemented to extend the scope to authorised products for chronic use and clarify the applicable limits and exemptions. Amendment of Q&A 20 and Q&A 21 on approach to control presence of nitrosamine while the AI is being established to clarify that as the AIs can be established with the new carcinogenic category approach (CPCA) the approach for a universal temporary AI (t-AI) while a formal AI is established is no longer	28th July 2023

Formation of N-nitrosamines impurities in medicinal products:-

Confirmed root causes identified for Sartans: Sartans are products with tetrazole ring. In case of Sartans, solvents such as dimethylformamide (DMF), N-methylpyrrolidone (NMP), reagents such as triethylamine (TEA) diisopropylethylamine (DIPEA), the phase transfer catalysts such as triethylammonium chloride (TEA.HCl) and tetrabutylammonium bromide (TBAB) represent sources of secondary or tertiary amines such as dimethylamine (DMA), methybutylamine (MBA), diethylamine (DEA), diisopropylamine (DIPA), ethyl isopropylamine (EIPA), dibutylamine (DBA) susceptible to *N*-nitrosamine formation. According to CHMP assessment report of Sartans, Nitrosamine formation was caused by the reaction of NaNO$_2$ with different sources of secondary & tertiary amines. The different sources of secondary & tertiary amines were identified. The two different routes of N-nitrosamine generation were identified which are summarized below,

Hydrolytic and/or thermal degradation of the solvents DMF and NMP to give the secondary amines DMA and MBA respectively, followed by subsequent *N*-nitrosation by NaNO$_2$, finally yielding NDMA and NMBA.

N-nitrosative dealkylation of used triethylamine (TEA) diisopropylethylamine (DIPEA) leads to formation of diethylamine (DEA), diisopropylamine (DIPA), ethyl isopropylamine (EIPA) followed by subsequent *N*-nitrosation due to NaNO$_2$, finally yielding *N*-nitrosodiethylamine (NDEA), *N*-nitrosodiisopropylamine (NDIPA) and *N*-nitrosoethylisopropylamine (NEIPA).

Manufacturing of Sartans involved use of the phase transfer catalysts such as TEA.HCl and tetrabutylammonium bromide (TBAB) as precursors of N-nitrosamines such as NDEA and N-nitrosodibutylamine (NDBA) by considering that quaternary alkyl ammonium salts are derived from the corresponding secondary and tertiary amines and these precursors are the potential impurities also having the potential to react with nitrosating reagents NaNO$_2$, finally yielding N-nitrosamines.

In Sartans, different azides such as tributyltin azide, sodium azide alone or in combination with tributyltin chloride and bis (tributyltin) oxide [(Bu$_3$Sn) O] are frequently used as the azide source in the tetrazole forming cyclization step. Residual quantities of azide reagents were decomposed to gaseous by-products such as nitrogen (N$_2$) and dinitrogen oxide (N$_2$O) by the addition of sodium nitrite (NaNO$_2$) which are the precursor for the formation of N-nitrosamine impurity in presence of secondary or tertiary amines. [25]

Confirmed root causes identified for Pioglitazone: The Pioglitazone HCl, was observed to be contaminated with NDMA was observed due to use of sodium nitrite for nitrosative diazotization of an aniline derivative as well as use of NaNO$_2$ and HBr in an early step of manufacturing process followed by use of DMF and HCl in later step [26].

Confirmed root cause identified for Ranitidine: In the case of Ranitidine, NDMA formation is possible during storage over shelf life is being considered as a potential root cause. Preliminary findings indicate that NDMA could be generated under certain conditions when dimethylamine (DMA) is released from Ranitidine and exposed to a source of nitrite.[27]

Nitrosamine drug Substance related impurities (NDSRIs):- NDSRIs a category of nitrosamines that are structurally like the active pharmaceutical ingredient (API) in drug products). The FDA first communicated the presence of NDSRIs to industry in November 2021 with its release of an update on possible mitigation strategies to reduce the risk of nitrosamine drug substance-related impurities in drug products. NDSRIs can be formed during the synthesis, manufacture, and shelf-storage of drug products. These substances differ from the small molecule nitrosamine impurities that are identified in the FDA's Nitrosamine Guidance. Given that NDSRI formation can be triggered by part per-million levels of nitrite impurities (such as those found in commonly used excipients and in water), many drug products are now known to be at risk for nitrosamine formation [28].

On May 4th, 2023, in its first notice to industry concerning the presence of NDSRIs, the FDA directed manufacturers to use the same processes identified in the FDA's existing Nitrosamine Guidance for identifying the presence of NDSRIs. The FDA also discussed potential mitigation strategies and encouraged the development of control strategies or design of approaches to reduce NDSRIs to acceptable levels, or to eliminate these impurities. The FDA has acknowledged that "NDSRIs present unique scientific and regulatory challenges for FDA because each NDSRI is unique to the API, and there is limited compound-specific data that is available to inform safety assessments." The FDA has been working to advance the use of predictive toxicology (e.g., (Q) SAR methodologies) to assess potential mutagenicity and carcinogenicity of NDSRIs. Nevertheless, to date, the FDA has only published acceptable limits based on available safety data for a small percentage of NDSRIs. [24]

This major update on how to set limits would be particularly helpful for NDSRIs, as it is based on the ability to metabolize the α-carbon in combination with activating/deactivating features present in the molecule. EMA has therefore made available a useful list of new acceptable intakes for the most significant nitrosamines and has also made the calculation possible in a scientific and rational way for all other NDSRI Following the EMA's initial step in July 2023, the FDA also took a formal implementation in early August 2023, publishing a specific guidance document on NDSRIs. The FDA uses a predicted carcinogenic potency categorization approach to assign a recommended AI limit to an NDSRI based on the NDSRI's activating and deactivating structural features, in close alignment with the July 2023 updates of the EMA QAs. Both agencies then classify nitrosamines without experimental carcinogenicity data into 5 potency categories with identical criteria. The only difference between the agencies is the acceptable intake value for the worst-case nitrosamines that fall into potency category 1, the most potent category (18 ng/day for the EMA, 26.5 ng/day for the FDA). Additionally, the FDA also publishes a useful extended list of NDSRIs [24-27].

Carcinogenic Potency Categorization Approach for *N*-nitrosamines :-This an approach for assigning an *N*-nitrosamine impurity (including nitrosamine drug substance-related impurities [NDSRIs]) to a predicted carcinogenic potency category, with a corresponding acceptable intake (AI) limit, based on an assessment of activating or deactivating structural features present in the molecule. In the context of this document, activating or deactivating features are defined as molecular substructures that are associated with an increase or decrease, respectively, in carcinogenic potency

The Carcinogenic Potency Categorization Approach is based on structure-activity relationship (SAR) concepts described in recent scientific publications for *N*-nitrosamine compounds and also used a set of 84 *N*-nitrosamines with either rat TD50 values from the Carcinogenic Potency Database (CPDB) and/or the Lhasa Carcinogenicity Database (LCDB), relative potency classifications as defined by Rao et al. (1979), and/or AI limits based on previously-conducted surrogate analyses. The approach assumes that the α-hydroxylation mechanism of metabolic activation is responsible for the mutagenic and highly potent carcinogenic response observed for many *N*-nitrosamines. Structural features that directly increase or decrease the favourability of the activation mechanism or that increase the clearance of the nitrosamine by other biological pathways are expected to have a corresponding effect on carcinogenic potency (**Figure 4**). Therefore, a prediction of the mutagenic potential and carcinogenic potency of an *N*-nitrosamine can be generated based on its structural features. [29-30]

Flow chart to predict the potency category of N-nitrosamine

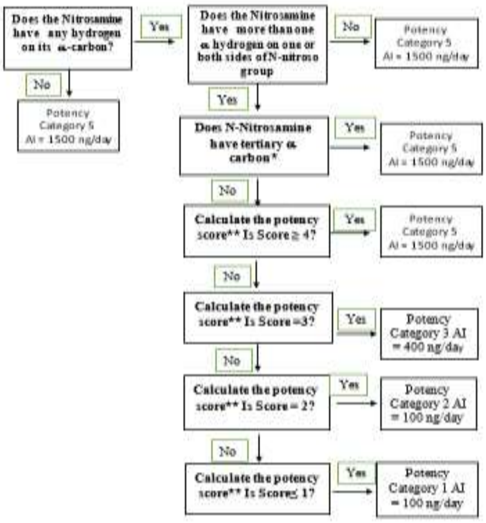

Figure 4

Evaluation of Factor affecting nitrosamine in Drug substance:

Nitrosamines have formed during the manufacturing of API unintentionally, with the understanding of how nitrosamines are formed, a risk assessment can be performed to evaluate the potential for formation within a synthetic process, following question need to evaluate

Do all the constituent parts (e.g. amine and nitrosatingagent) present within the synthesis? Stoichiometrically or sub-stoichiometrically

Do all the constituents present, to an appreciable degree, within the same stage? Assuming worst case conversion (100%), risk assessment for the control of nitrosamine in Drug substance to be performed

Risk assessment: - Risk assessment must consider reagents and materials, recovery and degradation of materials, cross contamination, manufacturing process and interaction between API, excipients and packing. Also, in situ generation of nitrosamines must be considered, in order to understand the formation and contamination of nitrosamines [27-30]

Evaluation of Factor affecting for the formation nitrosamine in Drug substance: [31-33]

- Evaluation of literature for presence and formation of *N*-nitrosamines in Medicinal Products.
- Evaluation of literature for theoretically possible root causes for *N*-nitrosamines linked with input raw material, solvents, reagents, and catalysts.
- Evaluation of route of synthesis (ROS) for manufacturing process of API, its intermediates as well as starting materials and identifying the source for formation of nitrosamine impurities.
- Evaluation of risk posed due to use of water for manufacturing process.
- Evaluation of risk posed due to use of recovered solvents, reagents, and catalysts in the manufacturing process.
- Evaluation of risk posed due to use of sharing equipment used for manufacturing of other APIs and intermediates

- Evaluation of risk posed due to degradation of solvents, reagent, catalysts, starting materials, intermediates, and drug substance.
- Evaluation of risk posed due to Physical operations of API
- Evaluation of risk posed due to primary packaging material for API.
- Evaluation of nitrosamine impurities in API.

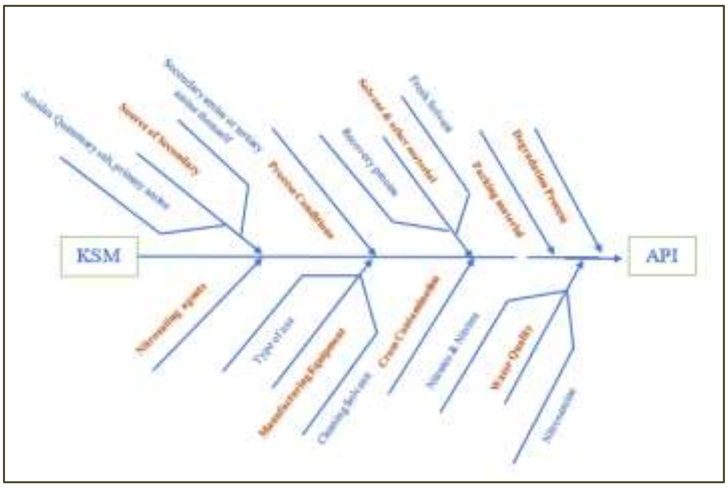

Figure 5

The designed fishbone diagram (**Figure 5**) has the huge advantage of summarizing all the currently identified root causes for the presence of nitrosamine impurities in medicines, serving therefore as a base to structure the risk assessment strategy. However, it has the fragility of being a dynamic proposal, as the list of the possible root causes discussed so far is unlikely to be exhaustive, and new root causes resulting from ongoing investigations on this topic may be identified. This will ultimately result in future updates to the diagram and, consequently, in the review of the outcome of the risk evaluations, which will be time and resource consuming [34]

Evaluation of route of synthesis (ROS) for manufacturing process of Drug substance, its intermediates as well as starting materials and identifying the source for formation of nitrosamine impurities: During risk assessment, the route of synthesis followed for manufacturing of Active pharmaceutical ingredients was reviewed to establish whether there is a possibility of nitrosamine impurity formation. The ROS has been closely evaluated to ensure that sodium nitrite ($NaNO_2$), or other nitrites are not used in presence of secondary amines like dimethylamine, diethylamine, diisopropylamine etc. or tertiary amines like triethyl amine, diisopropylethylamine (Hunig's base = DIPEA), tributylamine etc.

Further it has also been verified that sodium nitrite (NaNO2), or other nitrites are not used in presence of amide solvents like N,N-dimethylformamide (DMF), N-methylpyrrolidone (NMP), or N,N-dimethylacetamide (DMA), N-methylmorpholine etc. in the manufacturing process as they can degrade to secondary amines and also result information of nitrosamine impurity. Apart from use of sodium nitrite (NaNO2), it has also been ensured that no reagents like azide, nitric oxide (NO), nitrosyl halides/nitroso halides (e.g. ClNO, BrNO), dinitrogen trioxide (N2O3), dinitrogen tetraoxide (N2O4), organic nitrites/alkyl nitrites (e.g. t-BuONO), nitro alkanes (R-CH2-NO2, R2-CH-NO2), halogenated nitro alkanes, fremy's salt [K2NO(SO3)2], nitroso sulfonamides, chloramines (e.g. ClNH2) and quaternary ammonium salts, which are also potential source of nitrosamine, are involved in manufacturing of API. [19-24]

Source for formation of *N*-nitrosodimethylamine (NDMA):

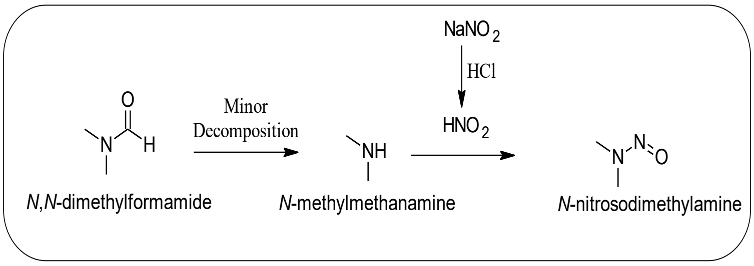

Figure 5

For the formation of NDMA, the presence of the secondary amine dimethylamine (DMA) is important. A possible route to the formation of DMA is the decomposition of dimethylformamide (DMF) at high temperature to DMA (**Figure 5**). An alternative possibility is that DMA is present as an impurity in DMF since it is a precursor in the industrial DMF synthetic process. Such contamination of dimethylamine can react with nitrous acid to form NDMA. It is also possible that recycled solvents containing NDMA are a source of cross-contamination, if solvents used in a reaction step involving azide are recovered and re-used, there is the possibility that *N*-nitrosamines could be inadvertently introduced into the manufacturing process, if the waste stream is quenched with nitrite in the presence of dimethylamine. [35]

Source for formation of *N*-nitrosodiethylamine (NDEA):

NDEA may be generated from diethylamine (DEA) (**Figure 6**) by analogy to the formation of NDMA from DMA as mentioned above.

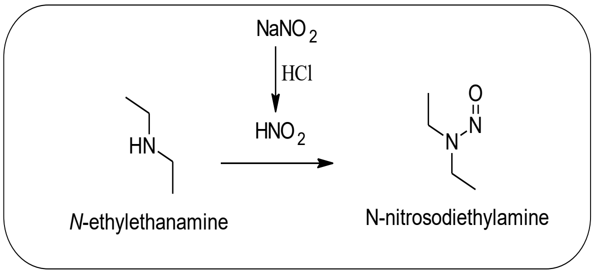

Figure 6

Analogous to DMA formation, DEA could be formed by degradation of triethylamine (TEA) or exist as impurity in TEA raw material (**Figure 7**). Alternatively, direct nitrosation of TEA may occur via a nitrosonium ion, resulting in the generation of an aldehyde and a secondary amine, which may further react with nitrous acid to form a nitrosamine as depicted below.[35-36]

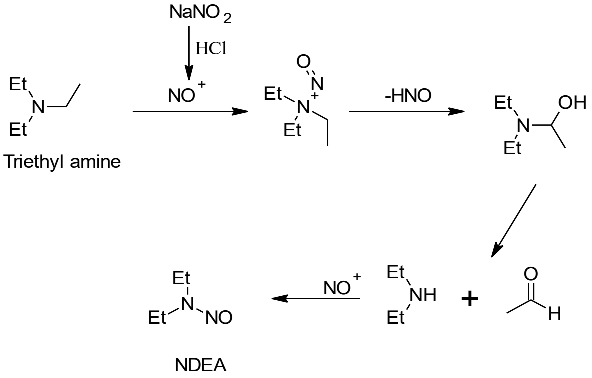

Figure 7

It is also possible that recycled solvents containing NDEA are a source of cross-contamination, if solvents used in a reaction step involving azide are recovered and re-used, there is the possibility that *N*-nitrosamines could be inadvertently introduced into the manufacturing process if the waste stream is quenched with nitrite in the presence of triethylamine or diethylamine.

Source for formation of *N*-nitrosoethylisopropylamine (NEIPA):

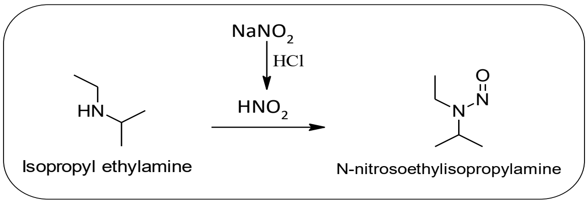

Figure 8

For the formation of NEIPA, presence of the secondary amine, ethyl isopropylamine (EIPA) is important. If ethyl isopropylamine is used in the manufacturing process in presence of nitrous acid, then this can lead to formation of NEIPA. An alternative possibility is that if ethyl isopropyl ethylamine is present as an impurity in diisopropylamine then it may react with nitrous acid to form NEIPA(**Figure 8**). It is also possible that recycled solvents containing ethyl isopropyl amine are a source of cross-contamination, if solvents used in a reaction step involving azide are recovered and re-used, there is the possibility that *N*-nitrosamines could be inadvertently introduced into the manufacturing process, if the waste stream is quenched with nitrite in the presence of ethyl isopropylamine.[25]

Source for formation of *N*-nitrosodiisopropylamine (NDIPA):

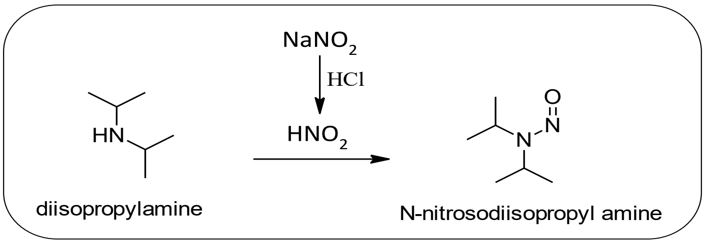

Figure 9

For the formation of NDIPA, presence of the secondary amine, diisopropylamine (DIPA) is important. If diisopropylamine is used in the manufacturing process in presence of nitrous acid, then this can lead to formation of NDIPA (**Figure 9**). An alternative possibility is that if diisopropylamine is present as an impurity in ethyl isoproylamine then it may react with nitrous acid to form

NDIPA. It is also possible that recycled solvents containing diisopropylamine are a source of cross-contamination if solvents used in a reaction step involving azide are recovered and re-used, there is the possibility that *N*-nitrosamines could be inadvertently introduced into the manufacturing process if the waste stream is quenched with nitrite in the presence of diisopropylamine. [25]

Evaluation of risk posed due to use of water in the manufacturing: water is universal solvent, and it is available in most abundant quantity**,** manufacturing process of Active pharmaceutical reagent /ingredient makes use plenty of water and considering the possibility that, water could contain low levels of nitrite contamination, during the risk assessment it has to be verified that for manufacturing of API, only purified water is used. Nitrosamines are formed through a series of mechanisms but most generally from the reaction of amines with nitrites. Nitrosamines can be found in drinking waters treated with chlorine or chloramines there are two most likely sources of nitrosamines contamination in pharmaceutical product waters. They may be present in the feed water to the treatment system potable water and not subsequently removed by a treatment process. Second, they may be unfavourably generated as a by-product of the treatment process itself when nitrogen containing compounds such as amines are present. The recent release of outlines one potential source of nitrosamine contamination when ion-exchange technology is used as part of the water treatment process. *Reaction of amines leaching from quaternary ammonium anion exchange resins with nitrosating agents present in the liquid phase. E.g. chlorination, chloro-amination and ozonisation can lead to significant N-nitrosamine generation as by-products in case vulnerable amines are present.* Further as an abundant precaution the samples of purified water, used for the manufacturing of API/intermediate, have to be evaluated for the content of nitrite (-NO_2) by ion chromatography in purified water [38].

As like nitrite, water could contain nitrosamine impurities as a contaminant as per the USFDA guideline on control of *N*-nitrosamine impurities in human drugs (February 2021, Revision-1). The most probable nitrosamine impurities which could be present in water are commonly observed nitrosamine impurities like NDMA, NDEA, NEIPA, NDIPA, NDBA, NMBA, NDPA and NMPA. Therefore, as an abundant precaution the samples of purified water, used for the manufacturing of API, have to be evaluated for the contents of commonly observed nitrosamine impurities. [38-39]

Evaluation of risk posed due to use of recovered solvents, reagents and catalysts in the manufacturing process: To evaluate the risk posed due to use of recovered solvents, reagents and catalysts, the manufacturing process of API to be reviewed to confirm if recovered solvents, reagents and catalysts are used in the manufacturing process.[39-41]

Evaluation of risk posed due to use of sharing equipment used for manufacturing of other APIs and intermediates: To evaluate the risk posed due to use of sharing equipment used for manufacturing of required API and other APIs the manufacturing stream for required API need to be evaluated. To identified that, all the other APIs and intermediates which are manufactured in the same manufacturing stream as used for manufacturing of required API does not make use of sodium nitrite or other nitrites in presence of secondary or tertiary amines

Further an abundant precaution, to be taken for assessing the equipment cleaning method (type B cleaning),

Evaluation of risk posed due to degradation of solvents, reagents, catalysts, starting materials, intermediates, and drug substance:

Degradation Product, an impurity resulting from a chemical change in the drug substance. Brought about during manufacture and/or storage of the new drug product by the effect of, for. Example, light, temperature, pH, water, or by reaction with an excipient and/or the immediate. As per the literature of Ranitidine, NDMA formation is possible during storage over shelf life is being considered as a potential root cause. Preliminary findings indicate that NDMA could be generated under certain conditions when dimethylamine (DMA) is released from Ranitidine and exposed to a source of nitrite. In case of Aminophenazone, it has a non-aromatic pyrazalone ring, substituted with a dimethylamine group at the 4-position. Hydrolytic degradation leads to the generation of the corresponding 4-hydroxypyrazol-3-one derivative with the release of dimethylamine. Formation of NDMA was caused by carryover of sodium nitrite in to the final step of Aminophenazone.

Evaluation of ROS for the API/intermediate of starting material

To be evaluated to check corresponding secondary amines anticipated as the manufacturing process & does not make use of sodium nitrite or other nitrites.

Confirmed root cause identified for Aminophenazone: Aminophenazone has a non-aromatic pyrazolone ring, substituted with a dimethylamine group at the 4-position. Hydrolytic degradation leads to the generation of the corresponding 4-hydroxypyrazol-3-one derivative with the release of DMA. Formation of NDMA was caused by carryover of sodium nitrite into the final step of Aminophenazone as represented in the scheme below (**Figure 10**).

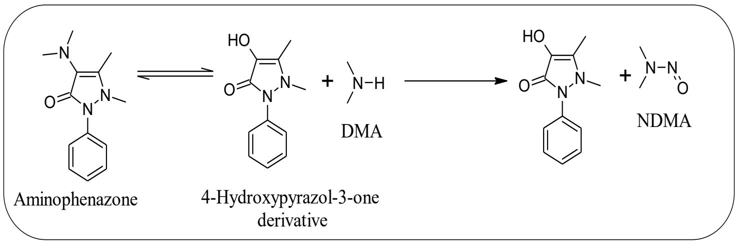

Figure 10

Source for formation of *N*-nitrosodibutylamine (NDBA):

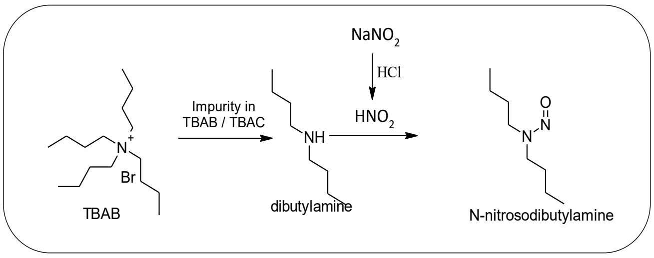

Figure 11

Secondary and tertiary amines like dibutylamine or tributylamine might also be present as impurities or degradants of quaternary ammonium compounds such as tetrabutylammonium bromide (TBAB), tetrabutylammonium chloride (TBAC) or also in primary amines such as butyl amine which may react with nitrous acid as represented in the scheme (Figure 11) if used in the manufacturing process and can lead to formation of NDBA. [42]

Source for formation of *N*-nitrosomethyl-4-amino-butyric acid (NMBA): It has been reported in the literature (**Figure 12**) that *N*-methyl-2-pyrrolidone (NMP) decomposes to 4-(methylamino) butyric acid in alkaline condition. Which may react with nitrous acid to form NMBA. [43]

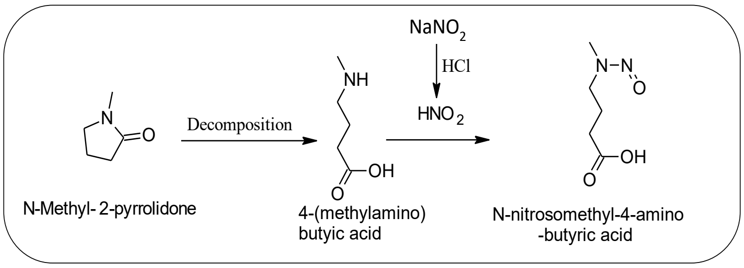

Figure 12

Source for formation of *N*-nitrosomethylphenylamine (NMPA): For the formation of NMPA, presence of the secondary amine i.e., methyl phenyl amine (MPA) is important. (**Figure 13**) MPA may be present as an impurity in dimethyl phenyl amine which may react with nitrous acid to form NMPA. [16-21]

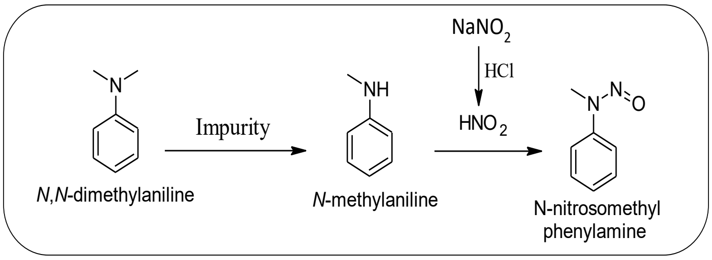

Figure 13

Evaluation of risk posed due to Physical operations of API

During the manufacturing of API and intermediate, a series Physical operation need to perform like Milling, Shifting, drying, and micronisation etc. Risk posed due to Physical operations of API is evaluated and it is observed that, Molecule having susceptible secondary amine group will degradation during Milling, Shifting, drying, and micronisation and result in to secondary or tertiary amine, (**Figure.14**)which is converted to N-nitrosamine by reacting with atmospheric oxygen. It is possible in very remote cases but while evaluating another factor this is needed to consider.

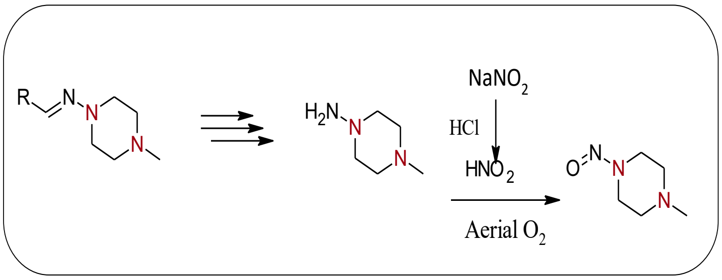

Figure 14

Evaluation of risk posed due to primary packaging material used: The primary packing material used for API/Intermediate is always complying with the food grade quality. The primary packaging material does not involve use of any nitrocellulose containing lidding foil, which could be the source of nitrite; as well as any kind of printing ink which could be the source of secondary amine in formation of corresponding nitrosamine impurities. [44]

Conclusion

Nitrosamine contamination is a carcinogenic and mutagenic contaminant leading to cancer, extremely carcinogenic and mutagenic nitrosamine contaminants must be kept to a minimum in drug ingredients and drug products. To reduce the amount of nitrosamine impurities in therapeutic substances, potential sources including raw materials, reagents, catalysts, solvents and cross contamination should be identified. Nitrosamine impurity formation can be avoided by selecting proper reagent, catalyst and solvents in the manufacturing of drug substances.

It is possible to understand that the risk of nitrosamine in medicinal product can be effectively determined and reduced, by complying with GMP requirements, and either by avoiding high risk manufacturing process or by implementation of effective mitigation measures that minimize the formation of nitrosamine as much as possible.

It is recommended that these impurities be managed appropriately to levels anticipated to impart insignificant risk to subjects and that approaches apply good science to ensure safety without unnecessarily limiting drug development. Although regulators have provided some initial guidance regarding impurities with genotoxic potential, additional challenges continue to be identified as all stakeholder gain more experience in this area and these challenges will need to be addressed. Therefore, the assessment and regulation of Nitrosamine impurities will likely evolve as technological and risk assessment approaches are further developed.

Acknowledgements

The authors thank the management of Megafine Pharma (P) Ltd. for permission to publish this work. The authors also thank colleagues of the Analytical Research and Development & Process Development team for valuable input and support for this work.
Notes: The authors declare no competing financial interest

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