International Journal of Zoology and Applied Biosciences
Volume 10, Issue 6, pp: 364-369, 2025 (Special Issue)
https://doi.org/10.55126/ijzab.2025.v10.i06.SP074
ISSN: 2455-9571
http://www.ijzab.com
Research Article

NITROSAMINES: EMERGING CHEMICAL CONTAMINANTS OF CONCERN IN PHARMACEUTICAL AND BIOLOGICAL SYSTEMS

***Anusha G, Hemanth A, Lalitha Srilekha R, Alekhya G, Niharika G, Likhita A and Sai Poojitha M**

Department of Pharmaceutical Analysis, Vignan Institute of Pharmaceutical Technology(A), Visakhapatnam, Andhra Pradesh-530046, India

Article History: Received 10th September 2025; Accepted 14th November 2025; Published 30th November 2025

ABSTRACT

Nitrosamines are a class of compounds characterized by a nitroso group bonded to an amine ($R_1N(-R_2)-N=O$) and are commonly found in water, food, and pharmaceutical products. Their presence in drug substances has raised significant global concern due to their genotoxic and carcinogenic potential. Nitrosamines can form through nitrosation reactions between amines (secondary, tertiary, or quaternary) and nitrous acid, often derived from nitrite salts under acidic conditions. Regulatory agencies such as the FDA, EMA, and WHO have undertaken comprehensive investigations to identify, control, and mitigate nitrosamine impurities in active pharmaceutical ingredients (APIs) and finished products. These impurities are classified as small-molecule nitrosamines (e.g., NDMA, NDEA, NMBA) and nitrosamine drug substance-related impurities (NDSRIs), which are structurally related to APIs. Root causes include the presence of amines in raw materials, cross-contamination from recovered solvents, inadequate process control, and nitrite-containing excipients. Even potable water and packaging components can contribute to contamination. To address these risks, the FDA recommends a three-step mitigation strategy: risk assessment of APIs and products, confirmatory testing of identified impurities, and regulatory submission of implemented control measures. Acceptable intake limits have been defined across major health authorities to minimize carcinogenic risk. The International Council for Harmonisation (ICH M7[R2]) also categorizes nitrosamines as "cohort of concern" compounds, requiring control at negligible risk levels. Comprehensive understanding of nitrosamine formation pathways, stringent process optimization, and supplier quality oversight are therefore essential to ensure pharmaceutical safety and regulatory compliance.

Keywords: Nitrosamines, Genotoxic impurities, NDSRI, FDA, ICH M7(R2), Pharmaceutical contamination.

INTRODUCTION

Nitrosamines are frequently found in water and food sources, such as cured and grilled meats, dairy products, and vegetables, exposing people to different levels of nitrosamines (Borikar SP *et al*., 2010). The term nitrosamine describes a class of compounds having the chemical structure of a nitroso group bonded to an amine (R1N(-R2)-N=O) The compounds can form by a nitrosation reaction between amines (secondary, tertiary, or quaternary amines) and nitrous acid (nitrite salts under acidic conditions) (Vaidehi Bhangale *et al*., 2024). The discovery of nitrosamines impurities in some drug products led FDA and other international regulators ,Such agencies include the European Medicines Agency; European Directorate for the Quality of Medicines & Healthcare; Health Canada; Therapeutic Goods Administration (Australia); Brazilian Health Regulatory Agency (Anvisa); Ministry of Health, Labour and Welfare/Pharmaceuticals and Medical Devices Agency (Japan); Health Sciences Authority (Singapore); Swissmedic (Switzerland); World Health Organization; Nitrosamine International Strategic Group; and Nitrosamines International Technical Working Group. to conduct a detailed analysis of these impurities in affected APIs and drug products.

N-nitrosamine compounds are highly genotoxic to animals, and some are identified as probable human carcinogens. These impurities were detected in drugs such as angiotensin II receptor blockers, histamine H1 receptor

*Corresponding Author: Anusha G, Department of Pharmaceutical Analysis, Vignan Institute of Pharmaceutical Technology(A), Visakhapatnam, Andhra Pradesh-530046, India. Email: anushagandi11@gmail.com.

antagonists, and synthetic antidiabetic medications. This discovery caused widespread alarm in the global pharmaceutical industry, leading to numerous international investigations. These efforts focused on identifying the root causes of nitrosamine formation in medicinal products and developing strategies to mitigate the risks of nitrosamine contamination (Gosar A *et al*., 2018).

Nitrosamine compounds are potent genotoxic agents in several animal species and some are classified as probable or possible human carcinogens by the International Agency for Research on Cancer. (International Agency for Research on Cancer Monographs). They are referred to as cohort of concern compounds in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidance for industry M7(R2) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk (July 2023). ICH M7(R2) recommends control of any known mutagenic carcinogen, such as nitroso-compounds, at or below a level such that there would be a negligible human cancer risk associated with the exposure to the compound.

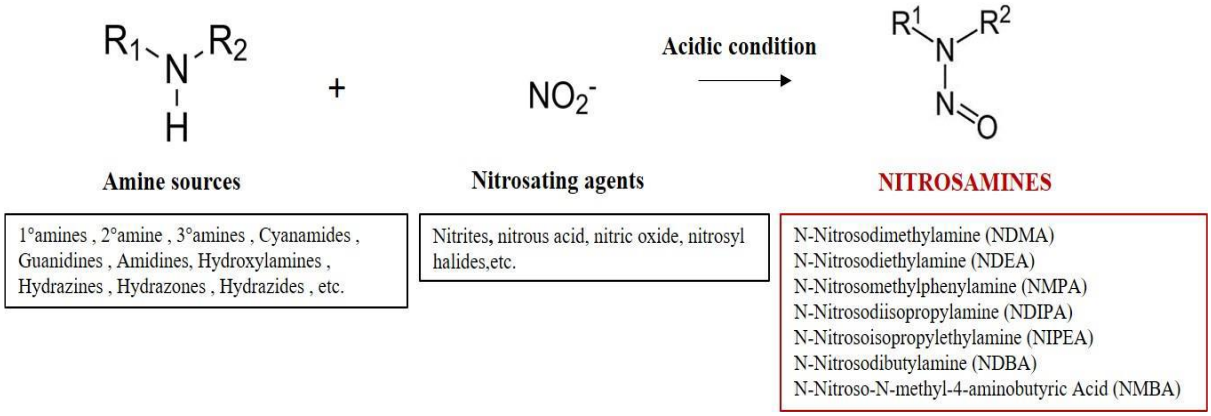

Figure 1. Generation of Nitosamines.

Source: Pharm Sci Asia 2024; 51(3), 190-203 DOI:10.29090/psa.2024.03.24.1854

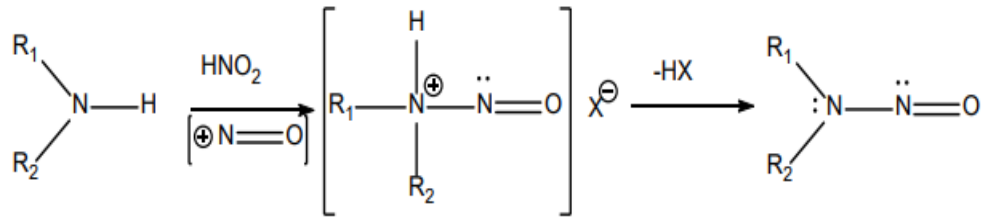

Figure 2. Representative Reaction to Form Nitrosamines.

Classification (Control of Nitrosamine Impurities, 2024)

They are two classes of nitrosamine impurities based on its structural classification Small molecules. NDSRI (Nitrosamine Drug Substance-Related Impurities).

Small molecules

N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosomethylphenylamine (NMPA), N-nitrosodiisopropylamine (NDIPA), N-nitrosoisophenylethylamine (NIPEA), N-nitrosodibutylamine (NDBA), and N-nitroso-N-methyl-4-aminobutyric acid (NMBA) are among the small-molecule nitrosamine impurities that may be found in APIs and/or pharmaceutical products.

NDSRI

NDSRIs are a class of nitrosamines sharing structural similarity to the API (having the API or an API fragment in the chemical structure) and are generally unique to each API. NDSRIs form through nitrosation of APIs (or API fragments) that have secondary, tertiary, or quaternary amines when exposed to nitrosating compounds such as nitrite impurities in excipients. Figure 4 shows the representative reaction of an API containing a secondary amine functional group in its structure with nitrite under acidic conditions.

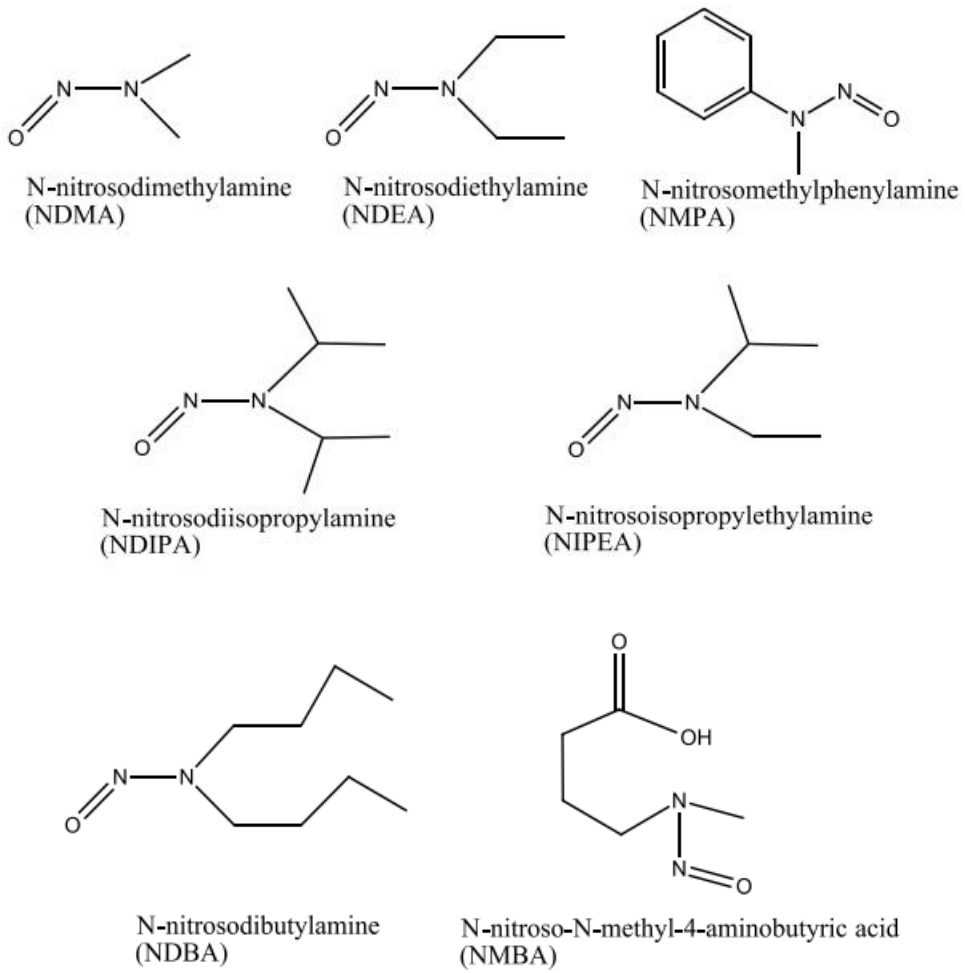

Figure 3. Chemical Structures of Potential Small-Molecule Nitrosamine Impurities in APIs and Drug Products.

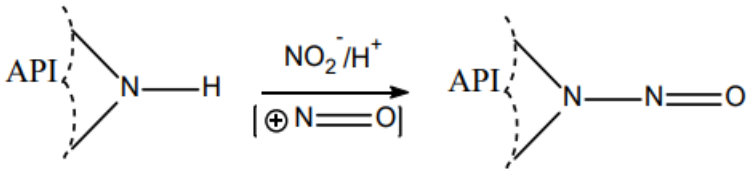

Figure 4. Representative Reaction of NDSRI Formation.

Root Causes for the Presence of Nitrosamine impurities: (Control of Nitrosamine Impurities, 2024; Horne S *et al*., 2023)

General consideration for formation of nitrosamine: Nitrosamines may form in the presence of secondary, tertiary, or quaternary amines. (Secondary and tertiary amines may be present as impurities or degradants of quaternary ammonium salts.) Under acidic reaction circumstances, secondary, tertiary, and quaternary amines, as well as nitrite, can form nitrosamine precursors. Under these circumstances, nitrite salts may generate nitrous acid, which can react with an amine to form a nitrosamine. Nitrosamine production is more likely when nitrous acid is used to quench residual azide (a reagent typically utilised in tetrazole ring formation or the insertion of an azide functional group into a molecule) in the presence of precursor amines. Despite purifying efforts, nitrites employed as reagents in one stage might cross-contaminate succeeding steps and react with amines to produce nitrosamine contaminants. As a result, anytime nitrite salts are present, carryover to following processes cannot be ruled out. In general, procedures involving nitrites in the presence of secondary, tertiary, or quaternary amines run the danger of producing nitrosamine contaminants.

Sources of Secondary, Tertiary, and Quaternary Amines for Nitrosamine Formation. Amines may appear in a manufacturing process for a variety of reasons. APIs, intermediates, and raw materials may have secondary or tertiary amine functional groups. Tertiary and quaternary amines can be purposely introduced as reagents or catalysts. Nitrosamines can be formed when these amines react with nitrous acid (Smith and Loeppky, 1967; Fiddler *et al*. 1972; Gillatt *et al*. 1984).

Secondary amines can also be derived from amide solvents, which can degrade under specific circumstances. Under high temperatures over a lengthy period, N,N-dimethylformamide can degrade into dimethylamine, which can then combine with nitrous acid to produce NDMA (refer to Figure 4). N-methylpyrrolidone, N,N-dimethylacetamide, and N,N-diethylacetamide all have comparable degradation processes, resulting in secondary amines that can react with nitrous acid to produce nitrosamine contaminants. Secondary amines can exist as impurities in amide solvents. N,N-dimethylformamide may contain impurities such as dimethylamine, which can react with nitrous acid to produce NDMA. Tertiary and quaternary amines utilised in API synthesis may have contaminants. Tertiary amines, such as triethylamine, have modest quantities of secondary amines, including dipropylamine and isopropylethylamine. Quaternary amine dealkylation can produce secondary and tertiary amines as impurities or degradation products. Tetrabutylammonium bromide, a typical phase-transfer catalyst, may include impurities such as tributyl and dibutylamine. API manufacturers should evaluate the amine impurity level that may cause nitrosamine impurities in their process.

Raw materials from sourced vendor containing nitrosamine impurity. Nitrosamine impurities can be introduced from vendor-sourced raw materials that include nitrosamines or precursors. The Agency identified the following causes of nitrosamine contamination in vendor-sourced materials: Nitrosamines have been found in fresh solvents (ortho-xylene, toluene, and methylene chloride) due to impurities carried over during storage vessel transfer. Additionally, sodium nitrite, a known impurity in some starting materials (such as sodium azide), can react with amines under acidic conditions to form nitrosamines. Nitrate-containing raw materials, such potassium nitrate, may include nitrite impurities. API manufacturers should assess the acceptable level of nitrite impurity based on their process. Impurities such as secondary or tertiary amines have been found in raw materials (see to section III.B.2) and solvents like toluene. API starting materials and intermediates may be contaminated by nitrosamine contaminants from other processes throughout production. Preventing nitrosamine impurities and cross-contamination of APIs requires understanding the supply chain of raw ingredients. Without supplier oversight, API manufacturers may be unaware of nitrosamine impurities or precursors in starting materials sourced from vendors. Even if a manufacturer's process is not typically susceptible to nitrosamine formation, vendor-sourced material may contain impurities introduced during production.

Recovered Solvents, Reagents, and Catalysts as Sources of Nitrosamine Impurities: Recovered materials, including solvents, reagents, and catalysts, may include residual amines such trimethylamine or diisopropylethylamine, which can lead to nitrosamine impurities. If nitrous acid is utilised to break down remaining azide during the recovery process, nitrosamines may develop. Nitrosamines may be entrained if their boiling points or solubility qualities match those of the recovered compounds, depending on the method of recovery and purification (e.g., aqueous wash or distillation). This further raises the possibility of nitrosamines in material recovery, these reasons, some drug products using APIs manufactured by certain “low” risk processes23 were found to contain nitrosamine impurities.

The Agency has observed the following due to this root cause: A manufacturing location may utilise many synthetic processes using common solvents to create the same API. If any of the synthetic procedures generate nitrosamines or precursor amines, the solvents used for recovery are at risk. Using recovered solvents from several processes or production lines without proper control and monitoring might result in nitrosamine contamination. If a recovered solvent containing nitrosamine impurities is used to make an API, it will still include the impurities, even if the synthetic technique is not typically prone to nitrosamine production. Third-party contractors are commonly hired to recover raw materials including solvents, reagents, and catalysts. Outsourcing procedure might be risky if the third-party recovery facility lacks precise knowledge about the materials being processed and relies on regular recovery methods. Nitrosamine impurities in raw materials might be present if equipment is not properly cleaned between customers or materials, or if it is not verified to remove all impurities. Nitrosamine impurities were found in recycled ortho-xylene and toluene due to poor cleaning and shared storage equipment among customers. Inadequate cleaning techniques might cause cross-contamination if nitrosamines are not properly avoided before combining materials from different customers for recovery. Nitrosamine impurities were introduced into catalyst tri-N-butyltin chloride (used as a source of tri-N-butyltin azide) at a third-party contractor plant after merging lots from various clients.

Lack of process control and optimization: Another potential source of formation of nitrosamine impurities is lack of optimization of the manufacturing process for APIs when reaction conditions such as temperature, pH, or the sequence, The addition of reagents, intermediates, or solvents is improper or inadequately regulated. FDA has seen. Reaction conditions vary significantly among batches and processing equipment within the same facility for the same API. Moreover, certain manufacturing Forced air procedures, such as fluid bed drying and jet milling, can lead to nitrosamine production in at-risk APIs due to the reaction of nitrogen oxides with the API. Nitrosamine impurities can arise from many primary sources throughout the API process. Multiple procedures may be required to discover all possible sources of nitrosamine production. Routine testing, such as high-performance liquid chromatography, for API purity, identification, and known contaminants may not discover nitrosamine impurities.

Different failure modes can lead to varying levels of nitrosamines in batches from the same process and API manufacturer. Impurities may be discovered in some but not all batches.

Origin of Nitrosamine impurities in Drug Product: (Umesh Dobariya *et al*., 2021)

Nitrites are prevalent nitrosating contaminants that have been identified in various excipients in parts Per million (ppm) levels. Excipients containing nitrite impurities can cause nitrosamine impurities to develop in drug products during processing. Manufacturing method and shelf-life storage duration. Manufacturers should consider the variability of nitrite impurities in excipient batches and suppliers when developing their supplier validation program. Potable water may include contaminants such as nitrite and nitrosamine, which should be considered by drug product producers and applicants. Furthermore, when nitrosamine precursors such as secondary, tertiary, and quaternary amines, including API fragments, are present as impurities in a drug substance, they can react with nitrites in excipients or nitrites from other sources used in the manufacturing process to form small-molecule nitrosamines or NDSRIs in drug products.

An example of this scenario is dimethylamine, which may exist as an impurity in a drug substance. When dimethylamine is carried over into a drug product manufacturing process, it can react with nitrite impurities in the excipients to form NDMA. The nitrosamine formation could be prevented by controlling the nitrosamine precursor, in this case dimethylamine, in the drug substance. Other secondary amine impurities in APIs also have similar risks.Container closure systems, secondary packaging components, and manufacturing equipment may include contaminants such as nitrite or nitrosamine. Impurities can leak into drug products during manufacture or storage, resulting in small molecular nitrosamine impurities or NDSRIs. Assess the risk of contaminants during extractable and leachable experiments.

Control and mitigation of Nitrosamine in drug substance and drug product

FDA Recommended three step mitigation strategy, Assessment of nitrosamine impurities in API itself like marketed product, products under approval and risk assessment should be conducted for those drugs. In case of identified any impurity during the assessment confirmatory testing shall be performed and asses them whether they are under regulatory requirements.Implemented changes shall be reported to the regulatory to prevent or reduce impurities in the API as well as Drug product. Which includes submission of DMF in accordance with CB0, CB30, PAS or annual filing.

Table 1. Regulatory requirements: (Control of Nitrosamine Impurities, 2024; Umesh Dobariya *et al*., 2021) Small molecules.

Impurities	FDA ng/day	EMA ng/day	Health Canada ng/day	TGA ng/day	PMDA ng/day	ANVISA ng/day
NDMA	96	96	96	96	96	96
NDEA	26.5	26.5	26.5	26.5	26.5	26.5
NDIPA	26.5	26.5	26.5	26.5	26.5	26.5
NDBA	26.5	26.5	26.5	26.5	26.5	26.5
NEIPA	26.5	26.5	26.5	26.5	26.5	26.5
NMBA	96	96	96	96	96	96
NMPA	26.5	34.3	-	26.5	26.5	34.3
MNP	-	26.5	96	26.5	26.5	26.5
NMOR	**-**	**127**	**-**	**-**	**-**	**-**

NDSRI

Based on the Drug maximum dose, AI limit is calculated and AI value differs with different drug products. MDD (Maximum daily dose) = Dose*Weight. Limit (PPM) = Acceptable Intake (ng/day) / Maximum Daily Dose (mg/day)

ACKNOWLEDGMENT

The authors express sincere thanks to the head of the Department of Pharmaceutical Analysis, Vignan Institute of Pharmaceutical Technology(A), Visakhapatnam, Andhra Pradesh for the facilities provided to carry out this research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

REFERENCES

Bhangale, V., & Ayre, A. (2024). Nitrosamine impurities in pharmaceuticals: Regulatory landscape and challenges. *Pharmaceutical Sciences Asia, 51*(3), 190–203.

Borikar, S. P., & Paul, V. (2010). N-nitrosation of secondary amines using p-TSA-NaNO2 as a novel nitrosating agent under mild conditions. *Synthetic Communications, 40*(5), 654–660.

Dobariya, U., Chauhan, N., Patel, H., & Pardeshi, N. (2021). Nitrosamine impurities: Origin, control and regulatory recommendations. *International Journal of Drug Regulatory Affairs, 9*(2), 77–80.

Gosar, A., Sayyed, H., & Shaikh, T. (2018). Genotoxic impurities and its risk assessment in drug compounds. *Drug Designing & Intellectual Properties International Journal, 2*(4), 224–229.

Horne, S., Vera, M. D., Nagavelli, L. R., Sayeed, V. A., Heckman, L., Johnson, D., ... Condran, G. (2023). Regulatory experiences with root causes and risk factors for nitrosamine impurities in pharmaceuticals. *Journal of Pharmaceutical Sciences, 112*(5), 1166–1182.

International Agency for Research on Cancer. (n.d.). *List of classifications*. Retrieved from https://monographs.iarc.who.int/list-of-classifications

Smith, P. A. S., & Loeppky, R. N. (1967). Nitrosative cleavage of tertiary amines. *Journal of the American Chemical Society, 89*(5), 1147–1157.

U.S. Food and Drug Administration. (2021). *Control of nitrosamine impurities in human drugs* [Guidance for industry]. Retrieved from https://www.fda.gov/media/141720/download.