NITROSAMINE IMPURITIES IN MEDICINES: FORMATION, TOXICITY, REGULATIONS, AND CONTROL

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Abstract: Nitrosamines are a class of N-nitroso compounds with high thermal and aqueous stability, considered potential carcinogenic and mutagenic substances. They are formed when secondary or tertiary amines react with nitrosating agents (nitrites) under acidic conditions. The detection of N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), and other nitrosamines in drugs such as valsartan, ranitidine, and metformin has led to product recalls and strict regulatory measures by the FDA, EMA, and WHO.
This paper analyzes the mechanisms of formation, toxicity, sources of contamination, regulatory frameworks, and best practices for risk management, with the aim of ensuring the safety of pharmaceutical products and protecting patient health.
Keywords: nitrosamines; pharmaceuticals; pharmaceutical regulation; quality control, risk assessment, toxicity

1. INTRODUCTION

In the past decade, the pharmaceutical industry has faced a new and highly significant challenge: the presence of nitrosamine impurities in medicines. These compounds, which belong to the class of N-nitrosamines, are known for their potential to cause carcinogenic effects in humans. N-nitrosamines are highly mutagenic organic impurities, categorized as probable human carcinogens (IARC, 1980). Some of them, such as N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), have been classified by the IARC as probable human carcinogens (IARC, 1978). Although these compounds are also present in other sources such as food and tobacco, their occurrence in pharmaceutical products — where their presence is not expected — has triggered a global response by regulatory authorities. In recent years, the detection of NDMA, NDEA, and related compounds in certain groups of medicines such as sartans (valsartan, losartan), ranitidine, and metformin, has led to numerous product recalls and raised serious concerns about drug safety and the reliability of the manufacturing supply chain (European Medicines Agency [EMA], 2019).

Although isolated instances of these impurities in pharmaceuticals were noted earlier (Eisenbrand et al., 1979), the detection of N-nitrosodimethylamine (NDMA) in valsartan in mid-2018 prompted large-scale recalls and regulatory actions, including an Article 31 referral under EU Directive 2001/83/EC (EMA, 2021). This triggered broader investigations into the "sartan" class of medicines due to structural and synthetic similarities, eventually revealing N-nitrosodiethylamine (NDEA) in some products. As a result, marketing authorization holders (MAHs) were required to implement strict impurity limits in product specifications.

A similar regulatory response occurred in 2019, when elevated NDMA levels were found in ranitidine (EMA, 2020). An Article 31 review concluded that ranitidine products should be suspended until MAHs could guarantee acceptable impurity levels throughout the product's shelf life and demonstrate that NDMA is not formed post-ingestion (Florian et al., 2021). Later studies showed NDMA formation could occur via degradation of ranitidine itself, even without external nitrites (King et al., 2020).

Considering these findings, EU regulators conducted a more comprehensive scientific review (Article 5(3) procedure) to assess N-nitrosamine risks across all medicines. This review evaluated root causes, toxicological limits, analytical requirements, patient risk, and the need for further nonclinical and epidemiological research. Most known nitrosamine contamination was traced back to the active substance manufacturing stage, though the potential for formation in drug products via excipients, water, or packaging was also acknowledged (Bharate, 2021).

Recommendations from the review included minimizing nitrosamine levels, establishing control strategies for active pharmaceutical ingredients (APIs) and finished products, conducting risk assessments, setting specification limits, and using highly sensitive analytical methods. When data for specific nitrosamines is lacking, a default class-based limit of 18 ng/day was introduced. Temporary exceptions may be made for cancer drugs or cases where benefits outweigh risks. Continued studies on patient exposure remain a regulatory priority (EMA, 2020).

Given the serious public health concerns posed by nitrosamine impurities in medicines, this study examines their origin, toxicology, and regulatory control, offering practical risk management approaches to support safe pharmaceutical manufacturing and patient protection.

2. MECHANISMS OF NITROSAMINE FORMATION IN MEDICINES

The formation of nitrosamines is a chemical process that requires the presence of secondary or tertiary amines and a nitrosating agent (usually sodium nitrite), resulting in the creation of an N-nitroso functional group (–N=O) (Rojsitthisak et al., 2023). In an acidic environment, sodium nitrite transforms into a nitroso agent (HNO_2), which readily reacts with organic amines to form stable nitrosamine structures. A particular risk exists during the synthesis of active pharmaceutical ingredients (APIs), such as in the case of angiotensin II receptor blockers (the so-called sartans), where sodium nitrite is used to form tetrazole rings. If secondary amines such as dimethylamine are used simultaneously or introduced accidentally, NDMA can form as an impurity (EMA, 2019). Furthermore, the migration of nitrosamine precursors from packaging materials (e.g., rubber containing nitroso stabilizers) and impurities in excipients further exacerbate the issue (EMA, 2019). Nitrosamine precursors can migrate from packaging materials into pharmaceutical products, contributing to impurity formation. This typically occurs when packaging components such as rubber stoppers or liners contain nitrosatable substances like secondary amines or nitroso stabilizers. Under certain conditions—such as heat, humidity, or acidic environments — these compounds can react with nitrosating agents, leading to the formation of nitrosamines within the drug product. Materials like nitrile rubber or neoprene are of particular concern. Regulatory authorities now require thorough assessment of packaging materials as part of the overall nitrosamine risk evaluation to ensure patient safety and product stability throughout the shelf life. (EMA, 2020).

Thus, the formation profile is closely linked to the presence of high-risk substances (nitrites, amines, azide compounds) and production conditions (acidity, temperature). The challenges lie in identifying and eliminating these risks within synthetic schemes.

3. TOXICOLOGICAL PROFILE AND CARCINOGENIC POTENTIAL

Nitrosamines are potent genotoxic substances with well-documented carcinogenic potential. Upon entering the body, they undergo metabolic activation primarily by cytochrome P450 enzymes, forming reactive intermediates capable of alkylating DNA. Nitrosamines are considered highly reactive compounds that, through metabolic activation (mainly via cytochrome P450 enzymes), can produce alkylating agents that bind to DNA and cause mutations (Chourasiya & Ranbhan, 2022). This leads to mutations that may initiate tumor development, particularly in the liver, gastrointestinal tract, and lungs. This explains their carcinogenic potential, which has been observed in various animal species, particularly in hepatic and gastrointestinal tissues (IARC, 1978). Compounds such as N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) have shown carcinogenicity in multiple animal studies and are classified as probable human carcinogens by the International Agency for Research on Cancer (IARC). Due to their high potency, acceptable intake limits are set extremely low, typically in the nanogram per day range, to protect public health. tissues (IARC, 1978).

According to ICH M7(R1), NDMA and NDEA fall into the so-called “cohort of concern,” in which even minimal levels of exposure (in the nano- or picogram range) are considered hazardous (ICH, 2018). For this reason, acceptable daily intake limits set by regulators are extremely low — for example, 96 ng/day for NDMA (U.S. Food and Drug Administration [FDA], 2020).

These limits are based on toxicological risk assessments using lifetime cancer risk models (e.g., 1 in 100,000 risk level). Below are some of the key ADI values set by regulatory authorities such as the EMA, FDA, and ICH M7(R1) guideline:

Table 1. Common Nitrosamines and Their ADI Limits

Nitrosamine	Acceptable Daily Intake (ADI)
NDMA (N-Nitrosodimethylamine)	96 nanograms/day
NDEA (N-Nitrosodiethylamine)	26.5 nanograms/day
NMBA (N-Nitroso-N-methyl-4-aminobutyric acid)	96 nanograms/day
NDIPA (N-Nitrosodiisopropylamine)	26.5 nanograms/day
NEIPA (N-Nitrosoethylisopropylamine)	26.5 nanograms/day
NPIP (N-Nitrosopiperidine)	26.5 nanograms/day
NMPA (N-Nitrosomethylphenylamine)	26.5 nanograms/day
Class-specific limit (when data is lacking)	18 nanograms/day

Source: Author research

4. IDENTIFIED NITROSAMINE CONTAMINATIONS IN MEDICINES

Nitrosamine contamination has been identified in several widely used medicines, raising serious safety concerns. The issue first gained attention in 2018 when NDMA and NDEA were detected in batches of valsartan, an angiotensin II receptor blocker (ARB). Subsequent testing revealed similar impurities in other "sartan" drugs, including losartan and irbesartan. In 2019, similar concerns emerged for the H_2-receptor antagonist ranitidine, due to molecular instability that allows for endogenous NDMA formation under certain storage conditions (Rojsitthisak et al., 2023). In the months that followed, the FDA and EMA recalled hundreds of medicine batches and initiated comprehensive reviews of synthetic processes and compositions of all drugs at risk of forming these compounds. Additionally, other medicines such as metformin and rifampicin were subject to investigation and further analysis. Metformin, a first-line treatment for type 2 diabetes, also showed trace levels of NDMA in some samples. Rifampicin, an antibiotic used for tuberculosis, was likewise found to contain nitrosamine impurities. (EMA, 2019; FDA, 2020; WHO, 2019).

5. REGULATORY RESPONSE (EMA, FDA)

Following these discoveries, regulatory bodies took action to prevent and control nitrosamine contamination. In 2019, the European Medicines Agency (EMA) published a "Questions and Answers" document for marketing authorization holders, providing guidance on assessing each drug for possible nitrosamine presence and reporting findings by set deadlines (World Health Organization [WHO], 2019). EMA established strict control measures: setting limit values, requiring proof of absence or mitigation plans for nitrosamines, and periodic progress reporting. Most regulatory guidance documents were issued in 2020–2021, including instructions for determining thresholds when multiple nitrosamines are present.

Similarly, the U.S. FDA issued a revised industry guidance: Control of Nitrosamine Impurities in Human Drugs (2020), recommending maximum acceptable daily intake limits (e.g., 96 ng/day for NDMA). All new and already approved products were subjected to rigorous testing and evaluation. The FDA continued reviewing permitted drugs and allowed temporary exemptions only after thorough risk assessment. Other regulatory agencies (such as WHO, PMDA, and EMA's CHMP) also published similar documents. In short, the response included standardized risk assessment procedures and control plans based on scientific principles and the ICH M7 framework.

6. RISK ASSESSMENT AND CONTROL

Risk assessment and control of nitrosamine impurities are essential steps to ensure the safety of pharmaceutical products. The process begins with identifying potential sources of nitrosamine formation throughout the drug manufacturing lifecycle, including raw materials, reagents, solvents, intermediates, excipients, and packaging. Key risk factors include the presence of secondary or tertiary amines, nitrosating agents such as nitrites, acidic conditions, and high temperatures (EMA, 2020). According to the ICH M7(R1) guideline, a structured approach must be applied, including a thorough evaluation of synthetic routes and storage conditions (ICH, 2018). Methods include composition analysis, chemical inventorying, and analytical testing of intermediates and finished drugs using highly sensitive GC-MS or LC-MS/MS methods.

Nitrosamine control involves several key measures:

- Process changes: avoiding the use of nitrite-containing compounds when possible or substituting with alternative methods. For example, developing new synthetic routes that do not require sodium nitrite for tetrazole formation.
- Raw material control: strict oversight of reagent and solvent contamination.
- Equipment cleanliness: preventing contamination by thorough cleaning of vessels and equipment, especially when recycled solvents or catalysts are used.
- Testing and monitoring: implementing routine analyses of final products and critical control points to determine nitrosamine levels are below established thresholds. If detected, corrective actions are taken (reprocessing, rejection, or formulation change).
- Dosing and acceptable intake: according to ICH M7(R1), thresholds for carcinogenic risk (1 in 100,000) are used to define "acceptable daily intakes" (e.g., 96 ng/day for NDMA). If a patient is taking multiple medications, the total exposure is monitored. Special attention is given to minimizing cumulative exposure risks, as patients often take multiple drugs simultaneously, potentially exceeding the AI limits (FDA, 2020).

Once a risk is identified, confirmatory testing using highly sensitive analytical methods—such as gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS)—is performed to quantify nitrosamine levels (FDA, 2020). If impurities are detected above acceptable intake limits,

control measures must be implemented. These include modifying synthesis processes, replacing problematic raw materials, improving equipment cleaning procedures, and enhancing packaging controls.

Acceptable daily intakes are established based on toxicological risk, usually in the nanogram per day range, and are applied to both single and multiple nitrosamine exposures. If multiple impurities are present, a combined limit is calculated. These measures, together with improved analytical testing, ensure that nitrosamine contamination is minimized and kept below regulatory limits. Risk mitigation strategies must be integrated into the manufacturer's quality management system, aligned with Good Manufacturing Practices (GMP) and regulatory expectations.

7. CONCLUSION

Nitrosamine impurities in medicinal products represent a significant challenge for modern pharmaceutical manufacturing and public health protection. The discovery of genotoxic compounds such as NDMA and NDEA in widely used medications — initially in valsartan, and subsequently in ranitidine, metformin, and rifampicin — has prompted global concern and regulatory intervention (EMA, 2019; FDA, 2020; WHO, 2019). These findings highlighted previously underestimated risks in synthetic routes, storage conditions, and packaging interactions that can lead to the formation of N-nitrosamines.

Nitrosamines are potent carcinogens that undergo metabolic activation in the human body, resulting in DNA alkylation and mutation (Chourasiya & Ranbhan, 2022; IARC, 1978). Due to their toxicity, extremely low acceptable daily intake (ADI) levels have been established — for example, 96 ng/day for NDMA — based on lifetime cancer risk models (FDA, 2020). Regulatory authorities such as the EMA and FDA have issued detailed guidelines for risk assessment and control, mandating comprehensive evaluations of synthesis, materials, and final product stability (EMA, 2020; ICH, 2018).

Risk mitigation strategies now include the redesign of chemical processes to avoid nitrosating agents, careful selection and testing of excipients and packaging components, and the use of advanced analytical techniques like GC-MS and LC-MS/MS to detect impurities at trace levels (Rojsitthisak et al., 2023). Manufacturers are also required to implement control strategies and integrate nitrosamine testing into their quality management systems in accordance with Good Manufacturing Practices (GMP) (ICH, 2018; EMA, 2020).

This review brings together the key elements of the issue: the chemical formation mechanisms, the high carcinogenicity of nitrosamines, real-world drug cases, and the regulatory response. Through the establishment of science-based strategies for risk assessment and control, the implementation of ICH guidelines, and the active involvement of manufacturers in self-assessment, the occurrence of contaminated medicines can be prevented. Prevention, transparency, and continuous monitoring of trends in synthesis and packaging remain the pillars of safe pharmaceutical delivery.

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