World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(REVIEW ARTICLE)

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N-NITROSAMINE IMPURITIES IN PHARMACEUTICALS: FORMATION PATHWAYS, TOXICOLOGICAL CONCERNS, REGULATORY APPROACHES AND RISK MITIGATION STRATEGIES

Safae Alaoui [1, 2, *], Ali Cherif Chefchaouni [1, 2], Badreddine Moukafih [1, 2], Soufiane El Marrakchi [1, 2], Abdeslam El Kartouti [1] and Ismail Bennani [1, 2]

[1] Laboratory of drug sciences, Department of Pharmacy, Faculty of Medicine, Pharmacy, and Dental Medicine of Fez, Sidi Mohamed Ben Abdellah University, Fez, Morocco.
[2] Department of Pharmacy, Hassan II University Hospital of Fez, Morocco.
* Corresponding Author; Email: safae.alaoui@usmba.ac.ma

ORCID Details
Safae Alaoui: https://orcid.org/0009-0005-9292-5864
Ali Cherif Chefchaouni: https://orcid.org/0000-0001-8142-1218
Badreddine Moukafih: https://orcid.org/0000-0002-6726-0670
Soufiane El Marrakchi: https://orcid.org/0009-0001-8741-6257
Abdeslam El Kartouti: https://orcid.org/0009-0000-3057-6834
Ismail Bennani: https: //orcid.org/0000-0002-3299-5490

World Journal of Biology Pharmacy and Health Sciences, 2026, 28(01), 198–203

Publication history: Received on 15 September 2026; revised on 20 October 2026; accepted on 23 October 2026

Article DOI: https://doi.org/10.30574/wjbphs.2026.28.1.0517

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ABSTRACT

Background: N-nitrosamine impurities represent an important pharmaceutical quality and patient safety concern because several members of this chemical class exhibit mutagenic and carcinogenic potential.

Objective: To synthesize scientific and regulatory evidence concerning their formation, toxicological assessment, and risk control in medicines.

Methods: A narrative literature review and bibliographic synthesis was undertaken using selected scientific publications and international guidance, particularly from EMA, FDA and ICH.

Results and discussion: The literature identifies nitrosatable amines, nitrosating species and favourable manufacturing or storage conditions as key determinants of formation. Risks arise from active substances, excipients, solvents, processing operations, and packaging-related factors. Evaluation of nitrosamine drug substance-related impurities requires structure-informed approaches, including carcinogenic potency categorization and justified read-across when compound-specific data are limited. Risk management combines systematic assessment, targeted confirmatory testing, preventive controls and lifecycle monitoring. Conclusion: An integrated, science-based approach encompassing formulation, manufacturing, and regulatory requirements is essential to reduce patient exposure to N-nitrosamine impurities.

Keywords: N-nitrosamines; NDSRIs; Mutagenic Impurities; Risk Assessment; Carcinogenic Potency; Pharmaceutical Quality

1 INTRODUCTION

The discovery of N-nitrosodimethylamine (NDMA) in valsartan in 2018 triggered an international reassessment of impurity control in medicines. Subsequent investigations identified additional nitrosamines in sartans and other medicinal products, including ranitidine, metformin and varenicline. The concern is no longer restricted to a particular synthetic route: nitrosamines may enter or form in drug substances and finished products, sometimes during shelf life [1–4, 9, 11].

Regulatory expectations have consequently evolved toward systematic evaluation of potentially nitrosatable structures, nitrosating sources, and relevant processing or storage conditions. This narrative review summarizes the chemical, toxicological and regulatory basis of that evaluation and discusses practical prevention and control options. The product-specific case study in the underlying dissertation is outside the scope of this article.

2 MATERIALS AND METHODS

This article is a narrative literature review and bibliographic synthesis of scientific publications and international regulatory guidance on N-nitrosamine impurities in medicinal products. The review was developed from the theoretical component of a pharmaceutical sciences dissertation, with emphasis on chemical formation pathways, toxicological considerations, acceptable intake derivation, risk assessment and mitigation throughout the product lifecycle.

Relevant literature was identified through a topic-oriented review of scientific publications and regulatory documents, including guidance from the European Medicines Agency (EMA), the United States Food and Drug Administration (FDA) and the International Council for Harmonization (ICH). Publications and guidance addressing low-molecular-weight nitrosamines, nitrosamine drug substance-related impurities (NDSRIs), excipient nitrites, carcinogenic potency categorization, and manufacturing or storage-related risks were prioritized. Evidence was organized thematically and compared qualitatively to identify areas of convergence, practical implications, and outstanding challenges.

As a narrative rather than a systematic review, this synthesis did not use a preregistered protocol, exhaustive database screening, a formal study-quality scoring system, or quantitative meta-analysis. Accordingly, the findings should be interpreted as a structured overview of the selected literature and regulatory framework, not as an exhaustive evidence synthesis.

3 RESULTS AND DISCUSSION

3.1 Chemical nature and pharmaceutical classification

N-nitrosamines are compounds containing a nitroso group bonded to nitrogen, generally represented as $R^1R^2N–N=O$. They may occur in environmental and consumer-product matrices, but their presence in medicines is especially consequential because exposure can be prolonged. Although several members of this chemical class are potent experimental carcinogens, hazard and potency cannot be assumed to be identical for all structures [8, 9, 13].

3.1.1 Small-molecule nitrosamines

Low-molecular-weight examples include NDMA, N-nitrosodiethylamine (NDEA), N-nitroso-N-methyl-4-aminobutyric acid (NMBA), N-nitrosodiisopropylamine (NDIPA), N-nitrosoethylisopropylamine and N-nitrosomethylphenylamine. These impurities are generally not structurally related to a single active pharmaceutical ingredient (API) and may originate from common solvents, reagents or contamination pathways [9, 11].

3.1.2 Nitrosamine drug substance-related impurities

NDSRIs share structural features with an API or an API-related fragment. Their formation may occur when a nitrosatable API, impurity or degradation product encounters nitrosating species, including nitrite-derived species in a formulation. Their chemical diversity and the limited availability of compound-specific carcinogenicity data make toxicological assessment more challenging [2, 7, 9, 16].

3.2 Sources and mechanisms of N-nitrosamine formation

3.2.1 Nitrosating agents and nitrosatable precursors

A common pathway involves three elements: a nitrosatable amine, a source of nitrosating species and physicochemical conditions permitting reaction. Nitrite can generate nitrous acid under acidic conditions, followed by reactive nitrosating species such as dinitrogen trioxide. Secondary amines are direct substrates for N-nitrosation, whereas tertiary amines can contribute through degradation or dealkylation to secondary amines [9,10].

Potential precursor sources include API starting materials, synthetic intermediates, amine-containing reagents, solvent impurities, recovered solvents, degradation products and, in certain circumstances, excipients. The historical valsartan example illustrates the conjunction of sodium nitrite and dimethylamine associated with dimethylformamide under favorable conditions [9,11].

3.2.2 Process and formulation conditions

Acidity often promotes formation of nitrosating species, with some systems showing pronounced nitrosation around pH 3-4. However, a neutral bulk pH does not establish the absence of risk, because local environments and additional reaction pathways may matter. Temperature, moisture, water activity, liquid-phase contact and reaction time influence kinetics and precursor mobility. Wet granulation, drying and storage therefore deserve evaluation where chemically plausible [9, 10].

3.2.3 Nitrite contributions from excipients

Excipients may contain trace nitrites, with substantial variation between materials, suppliers and lots. The nitrite contribution of a component depends both on its measured nitrite level and its mass fraction in the formulation. Consequently, a widely used diluent with a modest nitrite concentration can contribute meaningfully to the total nitrite burden. Excipients should be assessed quantitatively where relevant, rather than assumed inert for nitrosation [12].

3.2.4 Formation during shelf life

An initially low or undetectable nitrosamine level does not exclude later formation. In formulations containing nitrosatable APIs or degradation products, trace nitrites and suitable moisture or temperature conditions may permit gradual NDSRI generation. Stability studies and container-closure considerations should therefore be aligned with the identified chemical risk [2, 9].

Table 1: Major sources and relevant controls.

Risk domain	Potential mechanism	Risk-management focus
API synthesis	Amine precursors and nitrosating reagents or impurities	Route review, reagent specifications and process controls
Excipients	Trace nitrite burden, supplier and lot variability	Supplier qualification and targeted nitrite testing
Manufacturing	Contact, moisture, temperature and pH conditions	Process parameter assessment and mitigation
Storage and packaging	Continued nitrosation or introduced contaminants	Stability monitoring and packaging assessment
Shared equipment	Carryover from other products or operations	Validated cleaning and contamination control

3.3 Toxicological significance

Several N-nitrosamines undergo metabolic activation, frequently catalyzed by cytochrome P450 enzymes. α-Hydroxylation can produce unstable intermediates that generate electrophilic species capable of alkylating DNA. DNA adducts, including O6-methylguanine following NDMA exposure, may produce mutations if not appropriately repaired. The balance between activation, detoxification, and DNA repair helps explain differences in potency across structures [13, 14].

Human exposure–response data for individual pharmaceutical nitrosamines remain limited, and hazards should be distinguished from the magnitude of patient risk at a given exposure. ICH M7(R2) identifies N-nitroso compounds among the “cohort of concern”; therefore, the generic threshold of toxicological concern used for many other mutagenic impurities should not automatically be applied to these compounds [8].

3.4 Determination of acceptable intakes

An acceptable intake (AI), usually expressed in ng/day, provides a compound-specific or scientifically justified basis for controlling patient exposure. When adequate carcinogenicity evidence exists, AI derivation can use cancer potency data, including TD50-based approaches. The underlying ICH M7 framework commonly uses a theoretical excess lifetime cancer risk of approximately one in 100,000 as a reference for mutagenic impurities, while nitrosamines require particular consideration [8, 15].

3.4.1 Read-across and the CPCA framework

For NDSRIs lacking robust experimental data, a justified read-across may draw on an appropriate structural analogue, with attention to metabolic activation and relevant molecular features rather than superficial structural resemblance alone. The Carcinogenic Potency Categorization Approach (CPCA) classifies applicable nitrosamines into five predicted potency categories, considering α-hydrogen availability and activating or deactivating structural features. Compound-specific evidence can supersede a purely predicted category-based limit where scientifically justified [7, 16–18].

3.4.2 Conversion to product specifications

For an oral product, the AI can be translated into a concentration limit using the maximum daily dose of the medicinal product or API on a consistent mass basis: concentration limit (ng/mg) = AI (ng/day) ÷ maximum daily dose (mg/day). Limits for shorter-than-lifetime exposure and for multiple nitrosamines require case-specific interpretation of the applicable regulatory guidance; a single universal value is inappropriate [7, 8].

3.5 Regulatory risk assessment and analytical confirmation

EMA and FDA guidance emphasize a structured sequence of risk evaluation, confirmatory testing where indicated and implementation of changes or controls. The assessment should cover API structure and synthesis, raw materials, recovered materials, excipients, manufacturing steps, equipment, packaging and stability. A plausible chemical pathway is a reason for investigation, not proof that an impurity is present [2, 3, 7].

Where testing is needed, appropriately validated or qualified sensitive methods are essential. Gas chromatography–mass spectrometry can be suitable for certain volatile small nitrosamines, while liquid chromatography coupled to tandem mass spectrometry is often used for less volatile and structurally complex compounds. Method suitability must address specificity, sensitivity, matrix effects and possible analytical artefacts, including unintended nitrosamine generation during sample preparation [19, 20].

3.6 Risk mitigation and lifecycle control

Prevention should preferentially address the source of nitrosation. Depending on the product, strategies may include eliminating or substituting nitrosating reagents, controlling amine impurities, selecting low-nitrite excipients, modifying formulation or processing conditions, strengthening solvent-recovery controls and qualifying suppliers. Nitrite scavengers may be investigated where formulation compatibility and product quality are demonstrated; they should not be regarded as universally suitable [10, 12].

A documented control strategy should link identified hazards to analytical evidence, product specifications, supplier controls, change management, and ongoing stability surveillance. Reassessment is warranted following changes to API routes, raw materials, suppliers, excipients, manufacturing equipment, packaging or storage conditions. Importantly, the absence of intentionally added nitrites does not establish the absence of trace nitrite or of nitrosamine formation risk [2, 3, 7].

Table 2: Contrasts key regulatory and scientific instruments.

Instrument	Principal contribution
ICH M7(R2)	Risk-based framework for DNA-reactive mutagenic impurities and special concern for N-nitroso compounds
EMA nitrosamine guidance and Q&A	Marketing authorisation holder risk assessment, testing, acceptable intakes and lifecycle actions
FDA nitrosamine guidance	Sources, testing, mitigation and consideration of small nitrosamines and NDSRIs
CPCA and read-across	Scientifically supported AI estimation when compound-specific evidence is insufficient

3.7 Critical discussion and remaining challenges

The transition from an API-synthesis-centered model to a finished-product and lifecycle model is the most important practical development in nitrosamine control. Three uncertainties recur: incomplete knowledge of trace nitrite content, uncertain reactivity of structurally diverse amine-containing APIs and insufficient carcinogenicity data for many NDSRIs. These uncertainties argue for prioritization rather than undifferentiated testing of every hypothetical compound. Mechanistic plausibility, formulation-specific exposure and analytical evidence should be integrated before deciding on corrective measures [9, 12, 16–18].

CPCA and read-across improve decision-making but remain prediction tools requiring scientific justification. Likewise, a negative result at release may not address storage-related formation. Effective quality systems therefore combine sound chemistry, appropriate testing, preventive design and periodic review. This review is narrative and based on the literature selected for the underlying dissertation; it is not a systematic review or meta-analysis.

4 CONCLUSION

Nitrosamine control is a multidimensional pharmaceutical quality challenge involving precursor chemistry, formulation composition, manufacturing conditions, toxicological potency, and patient exposure. A product-specific, lifecycle-based strategy that integrates risk assessment, confirmatory analysis and preventive controls provides the strongest basis for minimizing these impurities. Attention should be paid to NDSRIs, excipient-derived nitrites, and potential formation during storage.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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