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# Computational Chemistry and Data-Driven Pharmaceutical Quality Monitoring: Nitrosamine Detection, Impurity Risk Assessment, and Preventive Process Control

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***Abstract***: *N-nitrosamine impurities in pharmaceutical products have resulted in product recalls, supply disruptions and increased regulatory scrutiny since 2018. Given their potential carcinogenicity and the nanogram-per-day range of established acceptable daily intake (ADI) limits, their detection, risk assessment and control require an integrated and preventive approach. This study reviews evidence on nitrosamine toxicology, formation mechanisms, analytical detection and regulatory control, evaluates the application of computational chemistry and data-driven modelling, and proposes an integrated framework for preventive pharmaceutical quality monitoring. The review draws on a published PRISMA-based systematic review that screened 180 articles and retained 143 for synthesis, supplemented by a targeted literature search covering structure–activity modelling, quantum-chemical potency assessment, nitrosamine formation kinetics and machine learning in pharmaceutical process monitoring. The reviewed evidence identifies cytochrome P450-mediated metabolic activation, particularly alpha-hydroxylation, as an important mechanism underlying nitrosamine genotoxicity and carcinogenicity. It also establishes vulnerable amines, nitrite-containing materials, trace aldehydes, packaging components and storage conditions as relevant factors influencing nitrosamine formation. LC-MS/MS and GC-MS/MS remain important techniques for trace impurity detection, while structure–activity relationships, the Carcinogenic Potency Categorisation Approach, quantum-chemical modelling and kinetic prediction provide complementary tools for risk assessment. Based on these findings, a conceptual data-driven framework is proposed, integrating six information layers, a five-stage monitoring workflow and three risk-classification tiers to connect molecular screening, material and process information, analytical results and regulatory requirements. The framework provides a proposed basis for prioritising confirmatory testing and implementing preventive process controls. However, its effectiveness remains to be established through retrospective and prospective validation using real pharmaceutical manufacturing, stability and analytical datasets.*

***Keywords:*** *nitrosamine impurities; NDSRI; computational chemistry; QSAR; risk assessment; LC-MS/MS; quality monitoring; process control; genotoxicity*

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## 1.0 Introduction

Nitrosamine impurities have emerged as a major pharmaceutical quality and patient-safety concern because of their mutagenic and carcinogenic potential, particularly following the detection of N-nitrosodimethylamine (NDMA) and related compounds in several widely used medicines. Nitrosamines are a group of N-nitroso compounds that can arise through the reaction of susceptible amines with nitrosating agents under favourable chemical and environmental conditions. Depending on their molecular structures, metabolic activation and exposure levels, some nitrosamines can

induce DNA damage and contribute to carcinogenesis. Experimental evidence has demonstrated that nitrosamine-induced DNA lesions can interfere with normal cellular processes and promote mutagenesis, thereby increasing cancer risk (Kay *et al*., 2021; Li & Hecht, 2022). The identification of these impurities in pharmaceutical products has therefore raised substantial concerns regarding the safety, quality and reliability of medicinal products, particularly where contamination occurs at trace concentrations.

The pharmaceutical nitrosamine crisis became prominent in 2018 following the detection of NDMA in valsartan-containing medicines, which subsequently triggered recalls and regulatory investigations involving other angiotensin receptor blockers, ranitidine, nizatidine, metformin and varenicline (Bharate, 2021; Ruepp *et al*., 2021). These incidents exposed weaknesses in the control of pharmaceutical impurities and demonstrated that nitrosamine contamination could originate from active pharmaceutical ingredient (API) synthesis, raw materials, recovered solvents, excipients, packaging components and drug-product storage conditions (Ashworth *et al*., 2020; Vikram *et al*., 2024). The European regulatory response included a comprehensive review of nitrosamine contamination in human medicines, with recommendations for risk evaluation, confirmatory testing and the implementation of appropriate preventive controls (European Medicines Agency [EMA], 2020). Similarly, the United States Food and Drug Administration (FDA) issued guidance requiring manufacturers to evaluate potential sources of nitrosamine contamination, undertake appropriate analytical investigations and implement strategies to prevent or minimise impurity formation. The FDA subsequently revised its guidance in September 2024 to incorporate updated recommendations addressing both small-molecule nitrosamines and nitrosamine drug substance-related impurities (NDSRIs) (FDA, 2021, 2024).

European Medicines Agency (EMA)+1

An important development in the understanding of pharmaceutical nitrosamine contamination is the recognition of two major structural categories: small-molecule nitrosamines and NDSRIs. Small-molecule nitrosamines, such as NDMA and N-nitrosodiethylamine (NDEA), are relatively simple compounds that may enter pharmaceutical products through manufacturing-related contamination or degradation processes. In contrast, NDSRIs are structurally related to the parent API and may arise from the nitrosation of susceptible amine functionalities within the drug molecule. Their structural complexity presents additional challenges because compound-specific toxicological information and analytical reference standards may be limited, making conventional impurity assessment more difficult (Schlingemann *et al*., 2023; Wichitnithad *et al*., 2023). The International Council for Harmonisation (ICH) guideline M7(R2) provides a risk-based framework for assessing and controlling DNA-reactive (mutagenic) impurities in pharmaceuticals, while the FDA has introduced specific recommendations for determining acceptable intake limits for NDSRIs using structural characteristics and predicted carcinogenic potency (ICH, 2023a; FDA, 2023). These regulatory developments demonstrate the increasing importance of integrating chemical structure, toxicological evidence and analytical measurements into pharmaceutical impurity assessment.

The formation and carcinogenic potential of nitrosamines have been extensively investigated through experimental toxicology, analytical chemistry and pharmaceutical process studies. Mechanistically, nitrosamine carcinogenicity is associated with metabolic activation, particularly cytochrome P450 (CYP)-mediated alpha-hydroxylation, which produces reactive intermediates capable of interacting with cellular DNA (Li & Hecht, 2022; Yamazaki *et al*., 1992). NDMA and NDEA have been associated with carcinogenic effects in experimental models, particularly involving the liver, although other organs and tissues may also be affected (Vikram *et al*., 2024). From a pharmaceutical manufacturing

perspective, Ashworth *et al*. (2020) demonstrated the importance of trace nitrite contamination in water and manufacturing materials as a potential source of nitrosamine formation. Similarly, Schlingemann *et al*. (2022) investigated the contribution of dimethylamine and nitrite to NDMA formation in metformin pharmaceuticals, highlighting the importance of controlling both susceptible amines and nitrosating agents. Boetzel *et al*. (2023) further demonstrated the value of excipient-specific nitrite information in supporting pharmaceutical nitrosamine risk assessments. These studies indicate that nitrosamine formation is not attributable to a single contaminant but may result from interactions among molecular, material, manufacturing and environmental factors.

Despite advances in pharmaceutical analytical chemistry, the reliable detection and quantification of nitrosamine impurities remain challenging. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) are among the principal techniques used for detecting and quantifying nitrosamines at trace concentrations. However, analytical challenges associated with matrix effects, background interference, isobaric compounds, limited availability of reference standards and the structural diversity of NDSRIs can affect method sensitivity, specificity and reliability (Wichitnithad *et al*., 2023). The review by Wichitnithad *et al*. (2023) on nitrosamine analytical methods also highlights the importance of developing sensitive and selective procedures capable of minimising false-positive results and improving the identification of emerging N-nitroso impurities. Consequently, analytical testing alone may not provide a sufficiently comprehensive basis for anticipating contamination risks across complex pharmaceutical product portfolios.

Computational chemistry has consequently become an important complementary approach for identifying potential nitrosamine impurities, estimating their carcinogenic potency and predicting their formation. Structure–activity relationship (SAR) models and read-across approaches provide opportunities to identify structural features associated with nitrosamine activity and estimate potential toxicological risks where compound-specific experimental data are unavailable (Cross & Ponting, 2021). Schlingemann *et al*. (2023) further demonstrated the importance of systematic structural screening in identifying potential small-molecule nitrosamines and NDSRIs associated with pharmaceutical substances. More recently, the Carcinogenic Potency Categorisation Approach (CPCA) has provided a structured method for assigning potency categories and corresponding acceptable intake recommendations to NDSRIs using relevant molecular characteristics and structural alerts (FDA, 2023; Kruhlak *et al*., 2024). Quantum mechanical modelling and mechanistic computational approaches also offer opportunities to investigate metabolic activation pathways and refine potency predictions, particularly for compounds whose structures fall outside established classification domains (De *et al*., 2024; Göller *et al*., 2024). These developments suggest that computational approaches can improve the prioritisation of compounds for experimental investigation and support more informed pharmaceutical risk-assessment decisions.

In addition to molecular screening and potency prediction, quantitative modelling of nitrosamine formation provides an opportunity to anticipate contamination under different manufacturing and storage conditions. Experimental studies have demonstrated that nitrosamine formation in pharmaceutical solid dosage forms depends on factors such as amine concentration, nitrite availability, local acidity, moisture and processing conditions (Moser *et al*., 2023). Ashworth *et al*. (2025) subsequently developed a conservative kinetic model for predicting secondary-amine nitrosation, demonstrating the potential application of mechanistic modelling in pharmaceutical formation-risk assessment. Nevertheless, the availability of molecular prediction tools and formation models does not automatically translate into effective preventive quality

control. Their practical application requires the integration of computational outputs with excipient composition, supplier information, manufacturing records, packaging characteristics, stability data and confirmatory analytical results.

A further development in pharmaceutical quality management is the increasing application of data-driven techniques, particularly machine learning, process analytical technology (PAT) and continued process verification (CPV). Artificial neural networks and other machine-learning approaches have demonstrated potential for identifying complex relationships among manufacturing variables, monitoring process performance and supporting data-based quality decisions (Nagy *et al*., 2022). Similarly, recommendations for artificial intelligence applications in pharmaceutical manufacturing emphasise model transparency, interpretability, validation and appropriate human oversight (Stassen *et al*., 2025). The ICH Q9(R1) guideline also provides principles for systematic quality risk management throughout the pharmaceutical product lifecycle, including manufacturing and distribution (ICH, 2023b). Although these approaches provide a suitable foundation for predictive pharmaceutical quality management, their specific integration with nitrosamine structural screening, formation prediction and analytical monitoring remains insufficiently developed.

Against this background, three interconnected knowledge gaps can be identified. The first is the analytical detection gap, arising from the difficulty of identifying and quantifying trace nitrosamine impurities, particularly structurally complex NDSRIs for which suitable reference standards and validated analytical procedures may be limited (Wichitnithad *et al*., 2023). The second is the impurity risk-assessment gap, which results from the fragmented evaluation of molecular structures, vulnerable amine groups, nitrosating agents, excipient composition, manufacturing conditions, packaging and storage characteristics, despite their combined influence on nitrosamine formation (Ashworth *et al*., 2020; Boetzel *et al*., 2023). The third is the preventive process-control gap, reflecting the continued reliance on retrospective analytical investigations, corrective actions and product recalls rather than integrated systems capable of identifying and prioritising potential contamination risks before they become significant quality problems (Bharate, 2021; Ruepp *et al*., 2021; FDA, 2021, 2024).

Although previous investigations have addressed individual aspects of these challenges, an important methodological gap remains in the integration of computational chemistry, analytical detection, material information and manufacturing-process data within a unified pharmaceutical quality-monitoring system. Existing SAR and CPCA approaches primarily support molecular screening and potency assessment, while kinetic models estimate the likelihood of nitrosamine formation under specified conditions. Analytical techniques provide experimental confirmation, whereas PAT, CPV and machine-learning systems support process monitoring. However, these approaches have not yet been sufficiently integrated into a single, continuously updated framework specifically designed to connect nitrosamine identification, impurity risk assessment, confirmatory testing and preventive process control. This limitation provides the central justification for the present study.

The aim of this study is to critically review existing evidence on nitrosamine toxicology, formation mechanisms, analytical detection and regulatory control; evaluate the application of computational chemistry and data-driven modelling in pharmaceutical impurity risk assessment; and develop a conceptual framework for integrating nitrosamine detection, impurity risk assessment and preventive process control within pharmaceutical quality-management systems. Specifically, the study examines the toxicological mechanisms and factors responsible for nitrosamine formation, evaluates the applicability of structure-based screening, CPCA, quantum mechanical modelling and kinetic prediction approaches,

and proposes a multilayer data-driven framework incorporating molecular, material, manufacturing, packaging, analytical and regulatory information. It also establishes proposed risk-classification criteria, performance indicators and a roadmap for future empirical validation.

The significance of this study lies in its proposed integration of previously separate computational, analytical and manufacturing-quality approaches into a coordinated preventive monitoring framework. Scientifically, it provides a basis for linking molecular characteristics and carcinogenic potency predictions with the chemical and environmental conditions responsible for nitrosamine formation. Technologically, it demonstrates how computational chemistry, analytical measurements and machine-learning-supported process monitoring could complement one another in identifying and prioritising pharmaceutical impurity risks. Practically, the framework could assist pharmaceutical manufacturers in improving supplier assessment, selecting appropriate excipients and packaging materials, prioritising confirmatory testing, identifying potential manufacturing-related contamination sources and implementing preventive controls. It could also support regulatory agencies and pharmaceutical quality-assurance professionals in developing more systematic, transparent and evidence-based approaches to nitrosamine management. Importantly, the proposed framework is conceptual and requires retrospective and prospective validation using real pharmaceutical manufacturing, stability and analytical datasets before its effectiveness can be established.

## 2. 0 Materials and Methods

The evidence base for Sections 3 to 7 is the systematic review of Vikram *et al*. (2024), which searched PubMed, Medline, eMedicine, the National Library of Medicine, and ReleMed (1960 to date) according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Page *et al*., 2021). That review screened 180 articles and retained 143 for synthesis, grouped into three themes: toxicity and physiological effects, analytical detection, and regulatory updates (Vikram *et al*., 2024).

For Sections 8 and 9, a targeted search addressed (i) carcinogenic potency categorization and regulatory guidance, (ii) quantum-chemical potency modelling, (iii) formation kinetics and excipient nitrite data, and (iv) machine learning in process analytical technology and continued process verification. The authors should record the databases, dates, search strings, and counts in Fig. 1, panel B. Sources comprised peer-reviewed articles, regulatory guidance, and, where marked, preprints; preprints and non-peer-reviewed material were used only to indicate emerging methods, not to support firm conclusions.

## 3.0 Nitrosamines in medicines: chemistry, formation, and acceptable intake

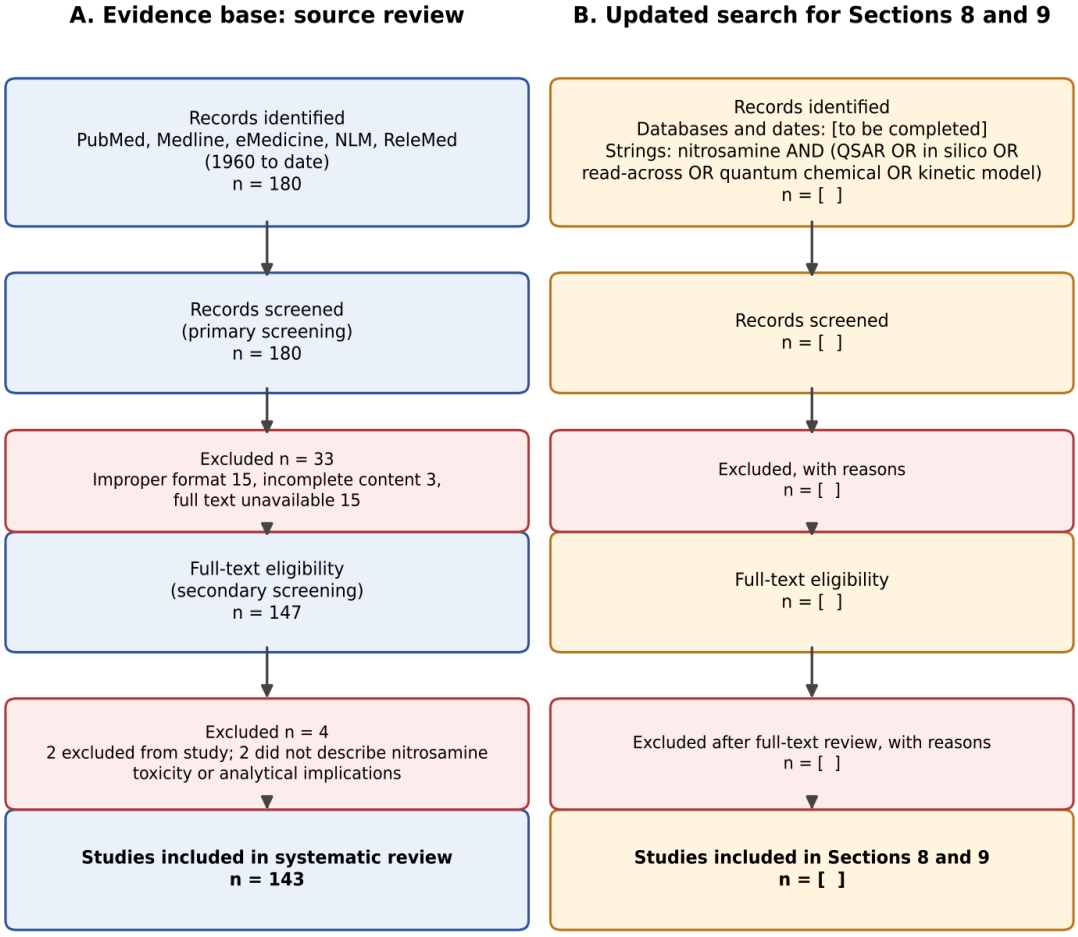
Nitrosamine impurities in pharmaceutical products comprise a diverse group of N-nitroso compounds whose formation, toxicological properties and regulatory significance depend on their molecular structures and the conditions under which they are generated. Understanding their structural characteristics is essential for identifying susceptible pharmaceutical ingredients, predicting potential formation pathways and evaluating their carcinogenic risks. In addition, knowledge of the chemical conditions that promote nitrosation and the acceptable daily intake limits established by regulatory authorities provides a scientific basis for designing effective impurity control strategies. This section therefore examines the structural classification, formation chemistry and acceptable intake limits of nitrosamines in pharmaceutical products.

### *3.1 Structural classes*

Fig. 2 illustrates the structures of small molecule nitrosamines (NDMA, NDEA, NMBA, NDBA, NDSRIs (N-nitroso-quinapril, N-nitroso-orphenadrine, N-nitroso-varenicline), and NMPA, MeNP and NMOR). Unlike small nitrosamines, NDSRIs are linked to an amine in a larger molecule with a scaffold similar to an API, making it difficult to

extrapolate their carcinogenic activity from simple analogues (Schlingemann *et al*., 2023). An important distinction exists between small-molecule nitrosamines and NDSRIs in terms of their molecular complexity and the implications for toxicological assessment. Small-molecule nitrosamines generally possess relatively simple structures, allowing their properties to be evaluated using established toxicological data and comparisons with structurally related compounds. In contrast, NDSRIs contain structural features derived from the active pharmaceutical ingredient, which may influence their metabolic activation, molecular reactivity and potential carcinogenic potency. Consequently, the toxicological assessment of NDSRIs may require compound-specific evaluation using structure–activity relationships, read-across approaches and computational prediction methods rather than direct extrapolation from simple nitrosamine analogues (Schlingemann *et al*., 2023).

The structural differences between these classes are particularly important for computational risk assessment because molecular descriptors, functional groups and metabolic activation pathways can influence the predicted toxicity and formation potential of individual nitrosamine impurities.




*Panel A reproduces the counts in the text of Vikram et al. (2024) (Scheme 1 of that paper shows different totals).*
*Panel B follows the PRISMA 2020 flow structure (Page et al., 2021). Adapted from Vikram et al. (2024).*

**Fig. 1: PRISMA-style flow diagram. Panel A shows the screening counts reported in the text of the source review; panel B is the template for the updated search used for Sections 8 and 9. Adapted from Vikram *et al*. (2024)**

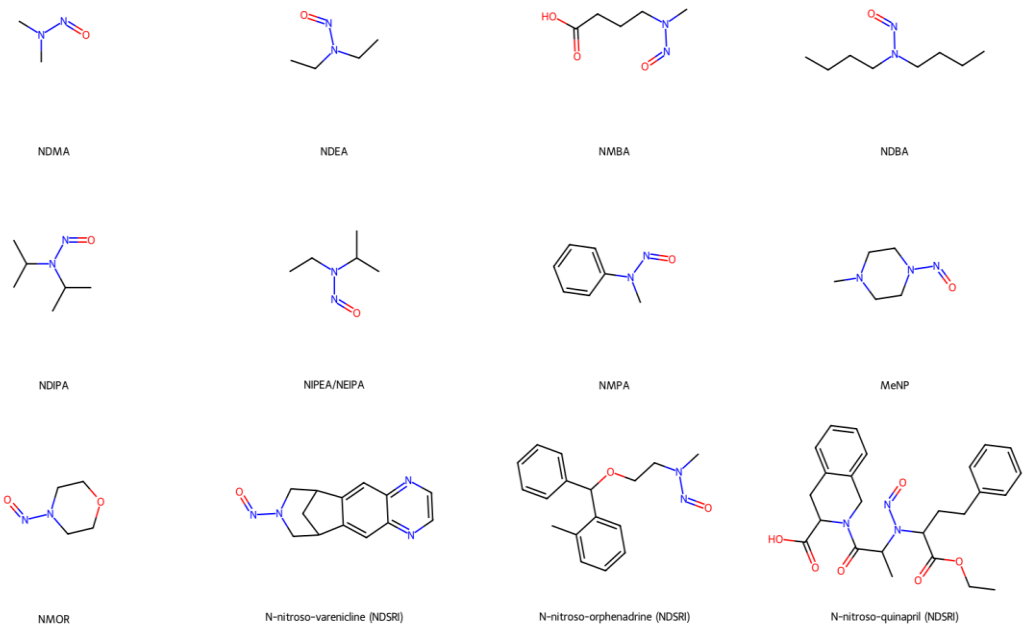



**Fig. 2: Structures of nitrosamines (first nine) and NDSRIs (last three). Redrawn from Vikram *et al.* (2024) using RDKit and extended with the three NDSRI**

### *3.2 Formation chemistry*

Nitrosamines are primarily formed through the reaction of susceptible amine-containing compounds with nitrosating species generated from nitrite under acidic or otherwise favourable chemical conditions. In aqueous environments, nitrite can be converted into nitrous acid, which may generate reactive nitrosating intermediates capable of transferring a nitroso group to susceptible secondary amines. Although secondary amines are common precursors, certain tertiary amines may also contribute to nitrosamine formation through reaction pathways involving dealkylation or other chemical transformations (Ashworth *et al*., 2020).

Nitrite can enter through starting materials, reagents, recovered solvents, catalysts, and excipients (Ashworth *et al*., 2020; Boetzel *et al*., 2023). The extent of nitrosamine formation is influenced by several interacting factors, including the concentration of nitrite and susceptible amines, pH, temperature, moisture content and reaction time. Acidic conditions generally favour the generation of nitrosating species from nitrite, while elevated temperatures and prolonged exposure may increase the rate of relevant chemical reactions. The presence of suitable amine precursors and nitrosating agents within the same formulation or manufacturing environment is therefore a critical determinant of impurity formation. These factors should be considered collectively during pharmaceutical risk assessment rather than evaluating the presence of nitrite or amines independently.

Trace aldehydes present in pharmaceutical excipients may promote nitrosamine formation by facilitating reactions between secondary amines and nitrosating species. Their presence is therefore relevant to pharmaceutical risk assessment, particularly where excipients contain residual aldehydes and nitrite or other nitrosating agents are also present (Harmon, 2023). Also, nitrocellulose blister or lidding materials can act as nitrosating agents when they contact amines such as dimethylamine from printing ink (Golob *et al*., 2022). Nitrosation may also occur in the stomach when amines and nitrite are ingested together (Vikram *et al*., 2024). Fig. 3 summarises the root causes.

This pathway demonstrates that nitrosamine formation is not restricted to the synthesis or formulation stages, as interactions between pharmaceutical ingredients and packaging materials may also contribute to impurity

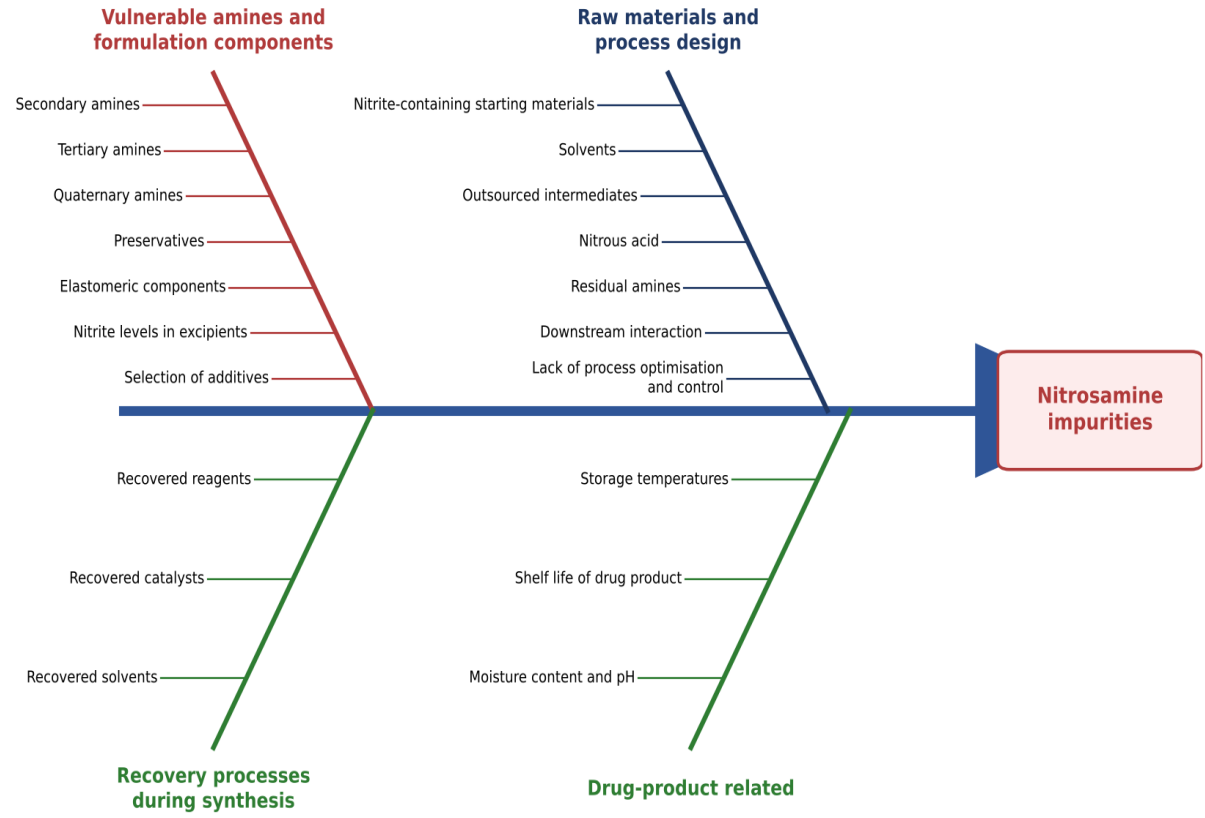
development during product storage (Golob *et al*., 2022).

These formation pathways indicate that nitrosamine contamination may originate from multiple stages of the pharmaceutical product lifecycle, including raw-material sourcing, active pharmaceutical ingredient synthesis, formulation, packaging and storage. Consequently, effective preventive control requires the identification of both direct chemical precursors and indirect sources of nitrosating agents. Information on excipient composition, supplier specifications, recovered solvents, manufacturing conditions and packaging materials should therefore be incorporated into pharmaceutical impurity risk assessment.

The identification of these chemical and process-related variables provides important input parameters for computational models designed to predict nitrosamine formation and identify manufacturing conditions requiring preventive intervention.

### *3.3 Acceptable daily intake limits*

Regulators have set ADI limits in the ng/day range (Table 1, Fig. 4). NDMA and NMBA carry the highest limits among the common small nitrosamines (96 ng/day), NDEA, NDIPA, NIPEA, NDBA, and MeNP are set at 26.5 ng/day, and N-nitrosomorpholine (NMOR) at 127 ng/day (Vikram *et al*., 2024). A sensitive method should quantify an impurity at 10% of its ADI-derived limit (Schlingemann *et al*., 2023).




*Adapted from Vikram et al. (2024)*

**Fig. 3: Root causes and sources of nitrosamine impurities in APIs and medicinal products. Adapted from Vikram *et al*. (2024)**

**Table 1. Acceptable Daily Intakes (ADIs) and Regulatory Agencies for Selected Nitrosamine Impurities**

| Impurity | Type | ADI (ng/day) | Agencies |
|---|---|---|---|
| NDMA | Small nitrosamine | 96.0 | FDA, EMA, WHO |
| NMBA | Small nitrosamine | 96.0 | FDA, EMA, WHO |
| NDEA | Small nitrosamine | 26.5 | FDA, EMA, WHO |
| NDIPA | Small nitrosamine | 26.5 | FDA, EMA, WHO |
| NDBA | Small nitrosamine | 26.5 | EMA |
| EIPNA/NEIPA | Small nitrosamine | 26.5 | EMA |
| MeNP | Small nitrosamine | 26.5 | FDA, EMA, WHO |
| NMPA | Small nitrosamine | 34.3 | EMA |
| NNV | Nitrosamine drug substance-related impurity (NDSRI) | 37.0 | EMA |
| NMOR | Small nitrosamine | 127.0 | EMA |

*****ADI = acceptable daily intake; FDA = United States Food and Drug Administration; EMA = European Medicines Agency; WHO = World Health Organization; NDSRI = nitrosamine drug substance-related impurity. Values are presented as supplied.***

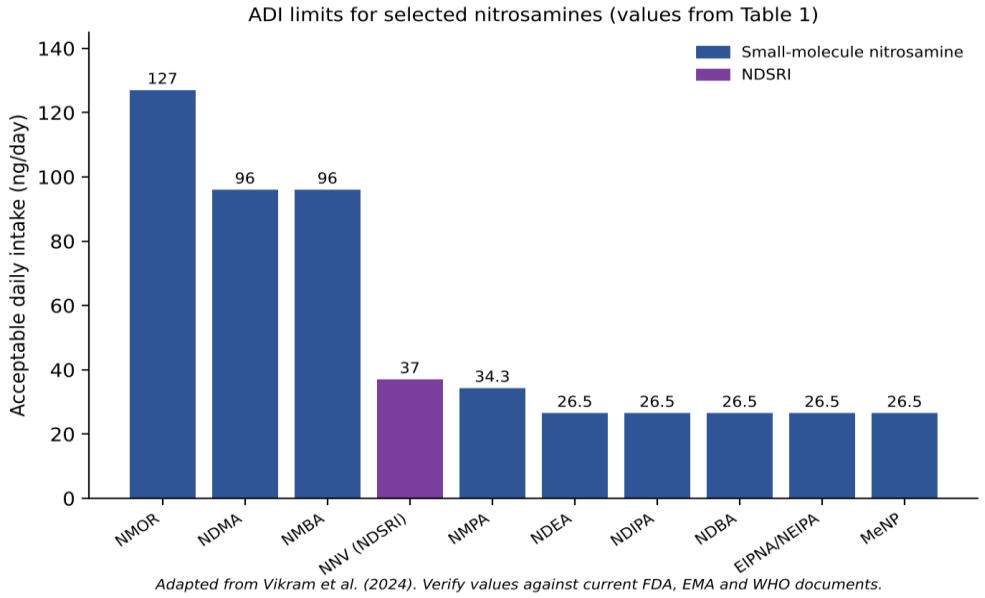



**Fig. 4: Acceptable daily intake limits for selected nitrosamines and NDSRIs (values from Table 1). Adapted from Vikram *et al*. (2024)**

## 4. 0 Toxicology and carcinogenicity

### *4.1 Metabolic activation and genotoxicity*

Nitrosamines are pro-mutagens. CYP2E1 and CYP2A6 are the major human catalysts for activation of N-nitrosodialkylamines, and CYP3A4 contributes to activation of larger nitrosamines (Cross & Ponting, 2021; Yamazaki *et al*., 1992). Hydroxylation at the alpha-carbon yields unstable alpha-hydroxy nitrosamines that decompose into reactive alkylating species: formaldehyde and methyl diazohydroxide for NDMA, and ethyl

diazohydroxide for NDEA (Li & Hecht, 2022; Yamazaki *et al*., 1992). These electrophiles form DNA adducts such as O6-methylguanine and N7-methylguanine, and unrepaired lesions promote mutagenesis (Vikram *et al*., 2024). Because activation depends on enzyme-substrate fit, modelling of alpha-hydroxylation is a logical route to potency estimation (Section 8).

### *4.2 Carcinogenicity across species and sites*

Table 2 condenses the carcinogenicity evidence. Liver tumours dominate for NDMA and NDEA across many routes and species, with lung, nasal, vascular, and digestive-tract tumours also reported (IARC, 1983; Peto *et al*., 1991).

**Table 2. Routes of Exposure, Experimental Models and Principal Tumour Sites Associated with Selected Nitrosamines**

| Compound | Routes and Models | Principal Tumour Sites |
|---|---|---|
| NDMA | Oral, inhalation, subcutaneous, intraperitoneal and intramuscular administration; mice, rats, hamsters, rabbits, fish, amphibians and foxes | Liver, lung, nasal cavity, blood vessels, haematopoietic system and ovary |
| NDMA (human data) | Epidemiological studies | Oropharynx, stomach, oesophagus and colorectum |
| NDEA | Inhalation, rectal, intramuscular, intratracheal and oral administration; rats, mice, hamsters, rabbits, chickens, cats and fish | Liver, lung, trachea, pancreas and larynx |
| NMOR | Oral, intravesical and intravenous administration; rats, mice and hamsters | Respiratory and digestive tracts, oesophagus and liver |

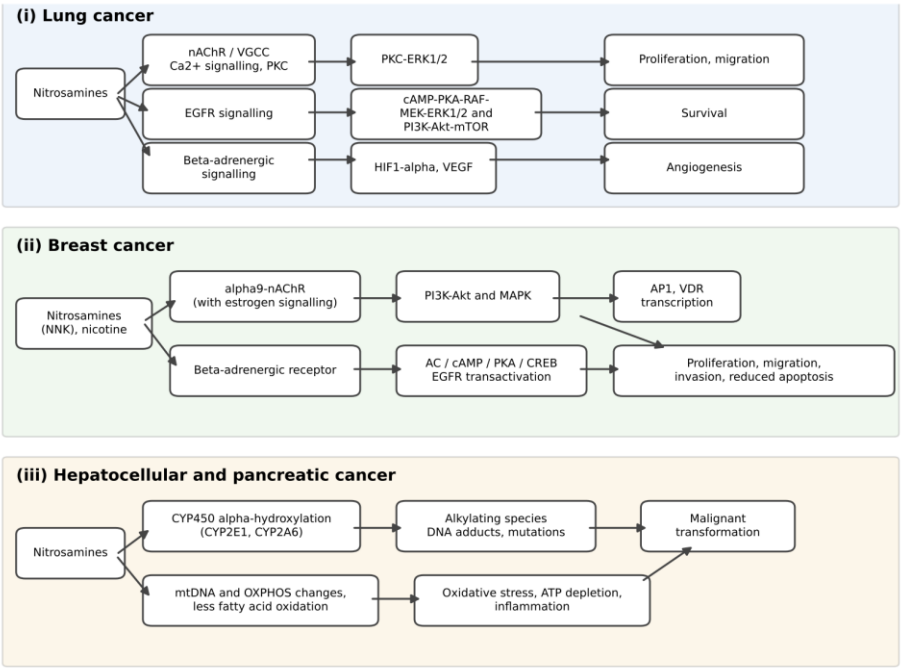
***Note. NDMA = N-nitrosodimethylamine; NDEA = N-nitrosodiethylamine; NMOR = N-nitrosomorpholine. Routes and experimental models refer to reported exposure studies, while tumour sites indicate the principal organs or tissues in which neoplastic effects have been observed (Vikram et al. (2024), IARC (1983) and Peto et al. (1991).***

### *4.3 Organ-specific and pathway-level effects*

Fig. 5 summarises the signalling pathways implicated in nitrosamine-associated carcinogenesis across different cancer types. In oesophageal cancer, urinary concentrations of NMEA, NDBA, nitrosopyrrolidine and NMOR were reported to be higher in patients with oesophageal squamous cell carcinoma than in healthy controls, suggesting a possible dose-response relationship between nitrosamine exposure and disease development (Zhao *et al*., 2020). In the liver, higher dietary intake of N-nitroso compounds has been associated with an increased risk of hepatocellular carcinoma, particularly among individuals with hepatitis B virus (HBV) or hepatitis C virus (HCV) infections or a history of alcohol consumption (Zheng *et al*., 2021). Furthermore, nitrosamine exposure may disrupt hepatic lipid metabolism and mitochondrial function, potentially contributing to liver injury and carcinogenesis (Vikram *et al*., 2024).

In lung, breast and pancreatic cancers, tobacco-specific NNK has been shown to activate nicotinic acetylcholine receptor, beta-adrenergic receptor and epidermal growth factor receptor (EGFR) signalling pathways, thereby promoting cellular proliferation, migration and survival. Dietary exposure to N-nitroso compounds has also been associated with pancreatic cancer (Vikram *et al*., 2024). However, the extent to which nitrosamine impurities identified in pharmaceutical products influence these specific signalling pathways remains insufficiently investigated (Vikram *et al*., 2024).

Beyond their potential carcinogenic effects, nitrosamine contamination has raised significant pharmaceutical safety concerns. Angiotensin receptor blockers (ARBs), particularly valsartan and losartan, have been subject to product recalls following the detection of nitrosamine impurities, with contamination primarily attributed to manufacturing-related deficiencies (Shephard & Nawarskas, 2020).




*Simplified schematic of pathways described in section 4.3. Adapted from Vikram et al. (2024), Fig. 4. Pathway involvement for NNK is from tobacco studies; effects of pharmaceutical impurities remain to be tested (Vikram et al., 2024).*

**Fig. 5. Simplified molecular signalling pathways of nitrosamines in lung, breast, and hepatic cancers. Adapted from Vikram *et al*. (2024).**

## 5.0 Root causes and risk factors for nitrosamine formation

Root causes fall into four groups (Fig. 3, Table 3): vulnerable amines, nitrosating agents and nitrite sources, process and recovery practices, and drug-product factors including excipients, packaging, moisture, pH, and storage (Ashworth *et al*., 2020; Vikram *et al*., 2024). Ranitidine illustrates storage dependence: N-nitrosodimethylamine levels rose with temperature, and the root cause was linked to storage and degradation chemistry (King *et al*., 2020). In metformin, NDMA formation has been studied in relation to dimethylamine and nitrite levels (Schlingemann *et al*., 2022).

**Table 3. Root Causes of Nitrosamine Formation and Contamination in Pharmaceutical Products and Recommended Mitigation Strategies**

| Root Cause Category | Examples | Mitigation Strategies | Key Sources |
|---|---|---|---|
| Vulnerable amines | Secondary or tertiary amines present in active pharmaceutical ingredients (APIs), reagents and solvents | Avoid amine-containing reagents where possible and introduce additional purification steps | Ashworth *et al*. (2020); Schlingemann *et al*. (2022) |
| Nitrosating agents | Nitrite contamination in water, raw materials and excipients | Control nitrite levels in raw materials and water and routinely screen excipients for nitrite contamination | Ashworth *et al*. (2020); Boetzel *et al*. (2023) |
| Process and material recovery | Use of recovered solvents, catalysts and reagents, as well as outsourced intermediates | Qualify recovered materials through appropriate testing and optimise manufacturing process parameters | Vikram *et al*. (2024) |

| | | | |
|---|---|---|---|
| Excipients | Trace quantities of nitrite and formaldehyde in pharmaceutical excipients | Select low-nitrite excipients and implement appropriate controls for aldehyde contamination | Boetzel *et al*. (2023); Harmon (2023) |
| Packaging materials | Nitrocellulose-containing lidding materials, blister primers and elastomers | Select alternative packaging materials and conduct appropriate migration and compatibility assessments | Boltres (2022); Golob *et al*. (2022) |
| Storage and shelf life | Exposure to unfavourable temperature, moisture and pH conditions | Maintain stringent storage conditions, use oxygen-scavenging packaging where appropriate and evaluate shelf-life limits | King *et al*. (2020); Vikram *et al*. (2024) |
| In-vivo formation | Nitrosamine formation through gastric nitrosation following exposure to susceptible amines and nitrosating agents | Incorporate nitrosation inhibitors, such as ascorbic acid and alpha-tocopherol, and consider basic-pH excipients where formulation compatibility permits | Vikram *et al*. (2024) |

NDSRIs add difficulty because of structural complexity, lack of standards, and the need for expensive LC-MS/MS or GC-MS/MS instrumentation (Wichitnithad *et al*., 2023). Analytical results are the ground-truth data stream for the framework in Section 9.

## 6. 0 Regulatory response

After NDMA was found in sartans in mid-2018, FDA, the European Medicines Agency (EMA), and other agencies coordinated alerts and asked manufacturers to review their processes (FDA, 2021; Vikram *et al*., 2024). Valsartan products were recalled in 2018, and ranitidine, nizatidine, and extended-release metformin followed in 2019 to 2020 (Bharate, 2021).

The FDA strategy has three steps: a risk assessment of each product portfolio, confirmatory testing with sensitive methods, and reporting of root causes with process changes (FDA, 2021). The EMA set parallel steps and timelines (Ruepp *et al*., 2021). Industry resources include a database of nitrite in excipients (Boetzel *et al*., 2023). For NDSRIs, FDA published acceptable-intake recommendations in 2023 (FDA, 2023). Vikram *et al*. (2024) caution that recalls so far may be only the tip of the iceberg and call for a unified global task force.

## 7.0 Computational chemistry for nitrosamine risk

Regulators and industry already use computational tools for potency categorisation, mechanistic modelling, and formation prediction. This section reviews these tools and notes where this manuscript extends them.

### *7.1 Structure-activity relationships, read-across, and the CPCA*

Structure-activity relationships have been developed specifically for N-nitrosamine activity (Cross & Ponting, 2021), and a landscape analysis has catalogued potential small and drug-substance-related nitrosamines in pharmaceuticals (Schlingemann *et al*., 2023). Regulators have since formalised an SAR-based method. The Carcinogenic Potency Categorization Approach (CPCA) assigns a nitrosamine to one of five categories, each with an acceptable intake, using the number of alpha-hydrogens at the N-nitroso centre and other activating or deactivating structural features (Kruhlak *et al*., 2024). In the FDA guidance, limits run from 26.5 ng/day for category 1 to 1,500 ng/day for category 5 (FDA, 2023). The developers describe the approach as conservative and open to refinement as data accumulate (Kruhlak *et al*.,

2024), and it sits alongside (Q)SAR assessment of mutagenic impurities under ICH M7(R2) (ICH, 2023a). Here the CPCA is the first computational filter in the framework (layer L1, Table 4).

### *7.2 Mechanistic modelling of metabolic activation*

Because cytochrome P450 (CYP)-mediated alpha-hydroxylation is a major determinant of nitrosamine carcinogenic potency (Li & Hecht, 2022; Yamazaki *et al*., 1992), mechanistic modelling approaches offer opportunities to evaluate and refine the Carcinogenic Potency Categorisation Approach (CPCA). De *et al*. (2024) employed quantum mechanical (QM) potency modelling to calculate the activation energies associated with reactions progressing from alpha-hydroxylation to DNA interaction. Their findings showed that QM predictions agreed with CPCA classifications for some nitrosamines, whereas, in other cases, the CPCA either overestimated or underestimated the acceptable intake values. Similarly, Göller *et al*. (2024) applied semi-empirical and density functional theory calculations to nine nitrosamines with available in vivo carcinogenicity data to investigate their activation and deactivation pathways. Their results indicated that carcinogenic nitrosamines generally formed reactive diazonium ions that preferentially interacted with DNA, whereas non-carcinogenic compounds tended to produce more stable carbocations that reacted preferentially with water. These findings demonstrate the potential contribution of quantum chemical calculations to weight-of-evidence assessments of nitrosamine carcinogenicity.

Experimental evidence also provides an important basis for developing and validating mechanistic prediction models. In this regard, genotoxicity data obtained from human TK6 cells expressing CYP enzymes, as reported by Li *et al*. (2022), can provide useful training and validation datasets for computational models. Collectively, these studies support the application of quantum mechanical descriptors as a complementary, second-tier assessment tool for evaluating CPCA predictions, particularly when high carcinogenic potency is predicted or when nitrosamine structures fall outside the established CPCA classification rules (De *et al*., 2024; Göller *et al*., 2024). Nevertheless, computational predictions should complement rather than replace experimental evidence in regulatory risk assessment and decision-making.

### *7.3 Predicting formation risk*

The prediction of nitrosamine formation has evolved from qualitative structural alerts to more quantitative and mechanistic modelling approaches. Experimental investigations using model solid dosage forms have demonstrated that nitrosamine formation can occur but generally reaches a plateau considerably below the theoretical yield expected from the available nitrite. This behaviour depends on several factors, including amine solubility, nitrite concentration, local acidity and processing conditions. Furthermore, trace secondary-amine impurities have been shown to present substantially lower nitrosamine formation risks than secondary amines present at drug-substance levels (Moser *et al*., 2023).

Building on these experimental findings, Ashworth *et al*. (2025) developed a solution-phase kinetic model to predict nitrosamine formation through secondary-amine nitrosation. The model incorporates conservative assumptions that tend to overestimate nitrosamine formation, making it suitable for precautionary risk assessment. Its predictions were subsequently evaluated against experimental product-testing data, including data for two nitrosamine drug substance-related impurities (NDSRIs), providing support for its application in conservative pharmaceutical risk assessment. In addition, excipient nitrite databases developed by Boetzel *et al*. (2023) provide essential material-specific information for estimating the potential contribution of pharmaceutical excipients to nitrosamine formation.

The principal model inputs, including amine reactivity, nitrite concentration, pH, moisture content and temperature, correspond directly to the different assessment layers (L1–L4) of the proposed framework, as presented in Table 6. Despite these advances, an integrated framework that simultaneously combines API structural characteristics, excipient nitrite content, manufacturing process parameters and analytical testing data into a unified and continuously updated decision-support system remains insufficiently developed in the published literature. Addressing this methodological gap is the central objective of the framework proposed in Section 9.8.4, *Computational support for analytical development*

Ionisation behaviour and fragmentation are central to LC-MS/MS sensitivity (Wichitnithad *et al*., 2023). Predicting this behaviour in silico, and selecting precursor and product ions accordingly, is a sensible use of computational

support for NDSRIs where reference standards are scarce, but systematic model-based prediction for NDSRIs is not yet established in the literature reviewed. It is therefore treated as a development need.

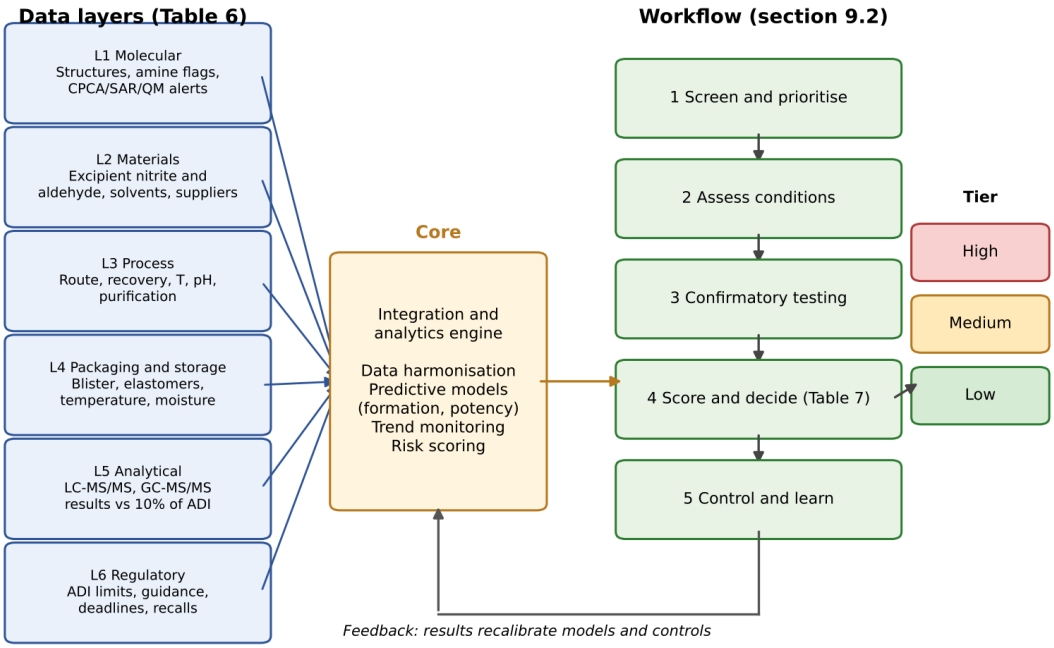
### *7.5 Data-driven quality monitoring in pharmaceutical manufacturing*

Machine learning has been reviewed for process analytical technology, where neural networks support real-time monitoring and release testing (Nagy *et al*., 2022). Recommendations for artificial intelligence in continued process verification emphasise transparency, interpretability, and risk management (Stassen *et al*., 2025), and ICH Q9(R1) provides the regulatory frame for risk-based decisions (ICH, 2023b). These sources support the architecture proposed in Section 9, but none addresses nitrosamines specifically.

**Table 4: Computational Approaches for Nitrosamine Risk Assessment and Their Current Evidence Status**

| Need | Computational Approach | Key Evidence | Status |
|---|---|---|---|
| Identification of at-risk active pharmaceutical ingredients (APIs) and intermediates | Structure-based screening of potential nitrosamine formation pathways and structural alerts | Schlingemann *et al*. (2023) | Supported |
| Prediction of carcinogenic potency categories and acceptable intake limits for nitrosamine drug substance-related impurities (NDSRIs) | Carcinogenic Potency Categorisation Approach (CPCA), structure–activity relationship (SAR) analysis and read-across methods | Cross and Ponting (2021); FDA (2023); Kruhlak *et al*. (2024) | Supported by regulatory guidance |
| Refinement of potency predictions for highly potent compounds or structures outside established prediction domains | Quantum mechanical (QM) modelling of activation energies and metabolic activation pathways | De *et al*. (2024); Göller *et al*. (2024) | Supported; methodology still evolving |
| Prediction of nitrosamine formation under manufacturing, processing and storage conditions | Kinetic and knowledge-based modelling incorporating excipient nitrite data | Ashworth *et al*. (2025); Boetzel *et al*. (2023); Moser *et al*. (2023) | Supported by conservative predictive models |
| Analytical method development for NDSRIs | In silico prediction of mass spectrometric (MS) fragmentation and analytical behaviour | Wichitnithad *et al*. (2023) | Proposed |
| Portfolio-level monitoring and risk scoring | Machine learning applications in process analytical technology (PAT) and continued process verification (CPV), supported by ICH Q9(R1) principles | ICH (2023b); Stassen *et al*. (2025); Nagy *et al*. (2022) | Supported in general; application to nitrosamine risk assessment proposed |

***Note.*** **API = active pharmaceutical ingredient; CPCA = Carcinogenic Potency Categorisation Approach; CPV = continued process verification; ICH = International Council for Harmonisation; MS = mass spectrometry; NDSRI = nitrosamine drug substance-related impurity; PAT = process analytical technology; QM = quantum mechanical; SAR = structure–activity relationship. The status column distinguishes approaches supported by existing experimental or regulatory evidence from those proposed for further development and integration.**




*Conceptual (proposed) framework. Not yet validated.*

**Fig. 6. Architecture of the proposed monitoring framework: data layers L1 to L6 (Table 5) feed an integration and analytics core,**

## 8.0 Proposed data-driven quality monitoring framework

This section is the conceptual contribution of the manuscript. It organises the evidence from Sections 3 to 8 and has not yet been validated.

### *8.1 Design principles*

The framework follows the three-step regulatory logic of risk assessment, confirmatory testing, and process change (FDA, 2021) but turns it into a continuous cycle with shared data. Its components are individually supported by the literature: CPCA and QM tools for the molecular layer (De *et al*., 2024; Göller *et al*., 2024; Kruhlak *et al*., 2024), formation models and excipient nitrite data for the material, process, and packaging layers (Ashworth *et al*., 2025; Boetzel *et al*., 2023; Moser *et al*., 2023), validated chromatographic methods for the analytical layer (Wichitnithad *et al*., 2023), and machine-learning-enabled monitoring with risk management for the core (ICH, 2023b; Stassen *et al*., 2025; Nagy *et al*., 2022). Their integration for nitrosamines is the proposed contribution. Figure 5 shows the architecture and Table 5 shows the data layers.

**Table 5. Multilayer Framework for Integrated Nitrosamine Risk Assessment and Decision-Making**

| Layer | Content | Data Sources | Key Evidence |
|---|---|---|---|
| L1: Molecular | API and intermediate structures, identification of vulnerable | SAR, CPCA and QM modelling outputs | Cross and Ponting (2021); De *et al*. |

| | | | |
|---|---|---|---|
| | amine groups, CPCA categories, and quantum mechanical (QM) or structure–activity relationship (SAR) alerts | | (2024); Kruhlak *et al*. (2024) |
| L2: Materials | Nitrite and aldehyde content of excipients, solvent and reagent histories, and supplier-specific information | Excipient databases and supplier certificates of analysis | Boetzel *et al*. (2023); Harmon (2023) |
| L3: Process | Synthesis routes, recovery of solvents and catalysts, processing temperature, pH and purification procedures | Batch manufacturing records and process analytical data | Ashworth *et al*. (2020); Nagy *et al*. (2022) |
| L4: Packaging and Storage | Primary packaging materials, elastomers, storage temperature and moisture conditions | Stability studies and packaging compatibility assessments | Boltres (2022); Golob *et al*. (2022); King *et al*. (2020) |
| L5: Analytical | LC-MS/MS and GC-MS/MS results evaluated against 10% of the acceptable daily intake (ADI), including analytical matrix and method metadata | Quality control (QC) laboratory records and analytical testing reports | Schlingemann *et al*. (2023); Wichitnithad *et al*. (2023) |
| L6: Regulatory | ADI limits, CPCA classifications, regulatory guidance, compliance deadlines and product recalls | Regulatory agency publications and official safety communications | FDA (2021, 2023) |

****ADI = acceptable daily intake; API = active pharmaceutical ingredient; CPCA = Carcinogenic Potency Categorisation Approach; GC-MS/MS = gas chromatography–tandem mass spectrometry; LC-MS/MS = liquid chromatography–tandem mass spectrometry; QC = quality control; QM = quantum mechanical; SAR = structure–activity relationship. The six layers provide an integrated structure for combining molecular, material, manufacturing, packaging, analytical and regulatory information in pharmaceutical nitrosamine risk assessment.**

### *8.2 Workflow*

The proposed framework begins with the screening and prioritisation of active pharmaceutical ingredients (APIs) and intermediates by identifying vulnerable amine groups and assigning appropriate Carcinogenic Potency Categorisation Approach (CPCA) classifications. For compounds predicted to exhibit high carcinogenic potency or fall outside the established prediction domain, additional quantum mechanical (QM) assessments should be undertaken using information from Layers L1 and L2 (Kruhlak *et al*., 2024; Schlingemann *et al*., 2023).

The next stage involves assessing the conditions that may promote nitrosamine formation using information from Layers L2–L4. This assessment incorporates relevant factors such as nitrite and aldehyde concentrations, pH, moisture content, packaging materials and storage conditions. Conservative kinetic and knowledge-based models can then be applied to estimate the potential for nitrosamine formation under the anticipated manufacturing and storage conditions (Ashworth *et al*., 2025; Moser *et al*., 2023).

Following the predictive assessment, high-priority pharmaceutical products should undergo targeted confirmatory analytical testing using appropriate methods and representative product matrices. Testing should consider the 10% acceptable daily intake (ADI)

threshold as an early-warning screening benchmark, consistent with the recommendations of Schlingemann *et al*. (2023) and Wichitnithad *et al*. (2023).

The results obtained from computational predictions, process assessments and confirmatory analytical investigations should subsequently be integrated to determine the overall risk scores and assign appropriate risk classifications in accordance with the tiering criteria presented in Table 8. This classification will guide the selection of suitable risk-management interventions and the prioritisation of regulatory and quality-control activities. The final stage involves implementing preventive measures, as outlined in Table 3 and Figure 7, while continuously improving the predictive capability of the framework. Measured analytical results should be fed back into the system to facilitate the periodic recalibration and refinement of predictive models, with appropriate human oversight and expert review of model outputs (ICH, 2023b; Stassen *et al*., 2025). Importantly, computational predictions should remain conservative and serve as supplementary risk-assessment tools rather than substitutes for experimentally measured nitrosamine concentrations, which should take precedence in regulatory and quality-assurance decisions.

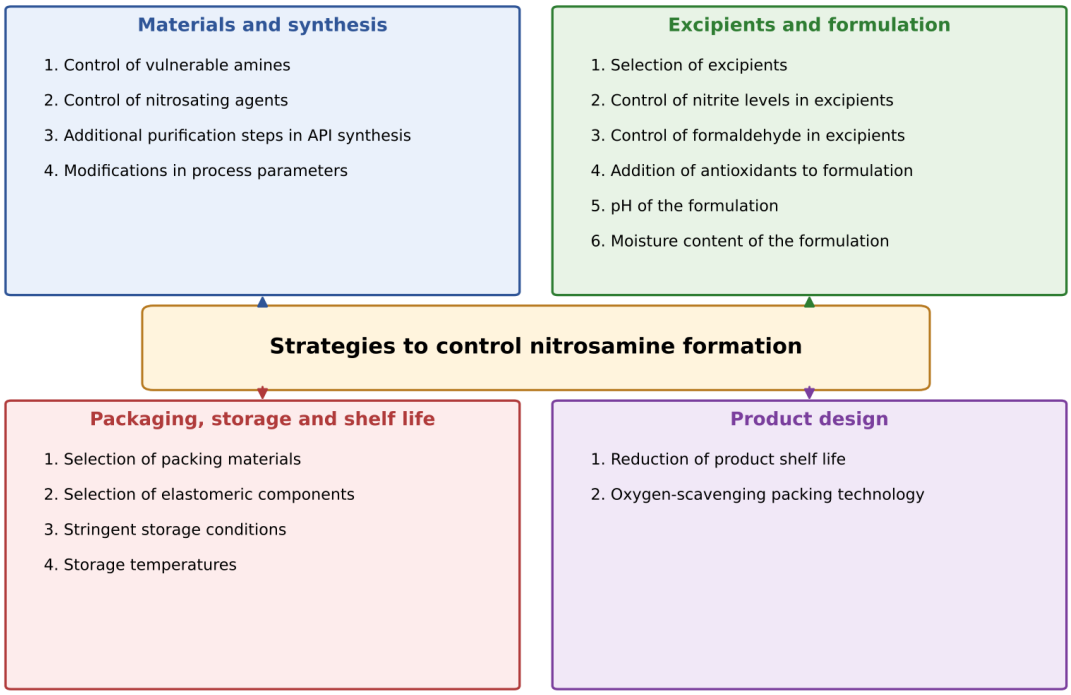
**Table 6. Risk Classification Tiers, Triggering Conditions and Recommended Management Actions for Nitrosamine Contamination**

| Risk Tier | Typical Triggers | Recommended Actions |
|---|---|---|
| High | Presence of vulnerable amine groups associated with high CPCA potency categories (e.g., Categories 1 or 2), or high potency indicated by quantum mechanical (QM) modelling; availability of nitrite or other nitrosating agents; analytical results approaching or exceeding the acceptable daily intake (ADI) limit | Conduct confirmatory analytical testing, perform root-cause investigations, implement appropriate manufacturing process or formulation modifications, and notify relevant regulatory authorities where required |
| Medium | Presence of vulnerable amine groups with a plausible but unconfirmed source of nitrosating agents, or borderline trends observed during stability studies | Conduct targeted analytical testing, review suppliers and excipient compositions, and strengthen stability monitoring |
| Low | Absence of vulnerable amine groups or plausible nitrosation pathways, together with adequately controlled raw materials and manufacturing processes | Conduct periodic risk assessments and routine trend monitoring |

***Note.*** **CPCA = Carcinogenic Potency Categorisation Approach; ADI = acceptable daily intake; QM = quantum mechanical. Risk classification should consider the combined evidence from molecular structure, material composition, manufacturing conditions, stability data and analytical results.**

### *8.3 Preventive process control*

Control options map onto the root causes in Table 3 and are grouped in Figure 7: control vulnerable amines and nitrosating agents, set stringent storage conditions, add purification steps, choose low-nitrite excipients and suitable elastomers or blister materials, control moisture and pH, include antioxidants such as ascorbic acid or alpha-tocopherol, use basic-pH excipients, shorten shelf life where needed, and consider oxygen-scavenging packaging (Boltres, 2022; Golob *et al*., 2022; Harmon, 2023; Vikram *et al*., 2024).




*Adapted from Vikram et al. (2024), Fig. 5 (16 strategies regrouped by theme).*

**Fig. 7: Reformulation and process control strategies to mitigate nitrosamine impurities (16 strategies regrouped by theme). Adapted from Vikram *et al.* (2024).**

### *8.4 Performance indicators*

The performance of the proposed framework can be evaluated using several key indicators. These include the proportion of the active pharmaceutical ingredient (API) and finished-product portfolio with documented and up-to-date risk classifications, as well as analytical detection results expressed as a percentage of the acceptable daily intake (ADI) and monitored across different products and manufacturing sites. Additional indicators include the level of agreement between in silico predictions and confirmatory experimental tests, particularly the sensitivity and specificity of computational alerts, and the time required to investigate identified risks and achieve root-cause resolution. Furthermore, the number of excipient and packaging material batches with documented nitrite and aldehyde content data can serve as an indicator of the completeness and effectiveness of material traceability and supplier quality management within the framework.

## 9.0 Discussion

### *9.1 Principal findings and implications*

The documents reviewed suggest that the key components of a preventive nitrosamine system are available but are implemented separately. Potency categorisation (Boetzel *et al.*, 2023), quantum-chemical refinement (De *et al.*, 2024), formation modelling (Kruhlak *et al.*, 2024), and excipient nitrite data (Wichitnithad *et al.*, 2023) each take care of one aspect of the problem. The proposed framework considers them as a "layered" structure of one system and provides for structural alerts, material and process information, and analytical results to all contribute to deciding where to test, and which controls to implement. This is consistent with risk-based quality management (ICH, 2023b) and with the shift towards data-driven process monitoring (Nagy *et al.*, 2022).

### *9.2 Limitations*

First, the framework is conceptual and has not been validated against real portfolios. Second, the CPCA is deliberately conservative and expected to change (Kruhlak *et al.*, 2024), the kinetic formation model over-predicts by design (Ashworth *et al.*, 2025), and QM models agree with the CPCA only in part (De *et al.*, 2024; Göller *et al.*, 2024). Third, the evidence base for toxicology and analysis rests largely on a single systematic review (Vikram *et al.*, 2024) whose reported counts and some intake values are internally inconsistent. Fourth, the effects of pharmaceutical nitrosamine impurities on

cancer signalling pathways remain untested (Vikram *et al*., 2024), and some sources used for emerging methods are preprints or conference material.

### *9.3 Research agenda*

Priorities are (a) retrospective testing of the framework on recall cases (sartans, ranitidine, metformin, varenicline, and quinapril; Bharate, 2021), (b) prospective pilots using the indicators in Section 9.4, (c) compound-specific potency and formation data for NDSRIs, (d) model-based prediction of NDSRI mass-spectrometric behaviour, and (e) shared, harmonised data on excipient nitrite and analytical performance.

## 10.0 Conclusion

This review examined the integration of computational chemistry, nitrosamine detection, impurity risk assessment and preventive process control within pharmaceutical quality-management systems. The findings demonstrate that nitrosamine contamination is influenced by interconnected molecular, material, manufacturing, packaging and storage factors, requiring a comprehensive approach to risk identification and control. The carcinogenic potential of nitrosamines is primarily associated with cytochrome P450-mediated metabolic activation, which produces reactive intermediates capable of inducing DNA damage. Their formation can arise from identifiable sources, including vulnerable amines, nitrite-containing excipients, residual aldehydes, manufacturing reagents, packaging materials and unfavourable storage conditions.

Advances in LC-MS/MS and GC-MS/MS have improved the detection of trace nitrosamine impurities, while structure–activity relationships, the Carcinogenic Potency Categorisation Approach, quantum-chemical modelling and kinetic prediction provide complementary tools for evaluating carcinogenic potency and formation risks. However, these approaches are frequently applied independently, limiting their capacity to support continuous and preventive quality management.

To address this limitation, the study proposes a conceptual data-driven framework comprising six interconnected information layers, a five-stage monitoring workflow and three risk-classification tiers. The framework integrates molecular screening, material and process information, packaging and storage conditions, analytical measurements and regulatory requirements to support risk prioritisation, confirmatory testing and preventive intervention. Its implementation could improve impurity traceability, strengthen supplier and process controls, and reduce reliance on retrospective investigations and product recalls.

Nevertheless, the framework has not been empirically validated. Future research should prioritise its retrospective assessment using documented pharmaceutical recall cases and prospective validation with real manufacturing, stability and analytical datasets. Further development of compound-specific NDSRI toxicity data, predictive models and harmonised material databases will also be essential. Ultimately, integrating computational predictions with validated analytical measurements and risk-based process controls offers a promising direction towards more proactive, transparent and reliable pharmaceutical quality assurance.

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## Declarations

### Declarations

**Ethical Approval and Consent to Participate**

Not applicable

**Availability of Data and Materials**

Data shall be made available upon request

No new datasets were generated. Figures 1 to 7 were produced for this manuscript; those adapted from Vikram *et al*. (2024) are reused under CC BY 4.0 with attribution.

**Competing Interests**

The authors declare that they have no competing interests.

**Funding**

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**Authors' Contributions**