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Nitrosamine Impurities in Pharmaceutical Products: Risk Assessment Strategies, Regulatory Mandates and Quality System Implications

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Abstract

The detection of N-nitrosodimethylamine in valsartan in 2018 exposed a systemic gap in pharmaceutical impurity risk management that extended rapidly to multiple drug classes globally. Nitrosamine impurities arise through two pathways: small-molecule nitrosamines generated during API synthesis, and nitrosamine drug substance-related impurities formed by excipient-derived nitrite nitrosating the drug substance's amine groups during manufacture or storage. Carcinogenicity proceeds through cytochrome P450-mediated alpha-hydroxylation generating reactive diazonium ions that alkylate DNA, with carcinogenic potency codified in the carcinogenic potency categorization approach assigning acceptable intake limits of 18 to 1500 nanograms per day across five structural categories. Global regulatory frameworks from the FDA, European Medicines Agency, and ICH have evolved substantially, with the FDA's September 2024 Revision 2 guidance and a formally initiated ICH M7 addendum representing the current frontier. Confirmatory testing demands mass spectrometric methods at sub-parts-per-billion sensitivity, while mitigation spans API process redesign, excipient nitrite management, and nitrite scavenger incorporation. Embedding nitrosamine risk across corrective action, change control, supplier qualification, and annual product review transforms reactive compliance into proactive quality governance. This review synthesizes the mechanistic, toxicological, regulatory, analytical, and quality system dimensions of nitrosamine impurity management to provide a comprehensive integrated evidence-based framework for pharmaceutical scientists and quality professionals worldwide.

Keywords: acceptable intake limits, drug contamination, genotoxic impurities, nitrosamines, pharmaceutical quality assurance, quality risk management

1. Introduction

The European Medicines Agency declared a public health alert on 5 July 2018 after detecting N-nitrosodimethylamine in the valsartan active pharmaceutical ingredient (API) produced by Zhejiang Huahai Pharmaceutical Co. Ltd (ZHP), China [1]. What started off as a local quality incident quickly escalated to a global recall involving several manufacturers across multiple ARB drug classes valsartan (July 2018), irbesartan (October 2018) and losartan (November 2018) showcasing structural weaknesses in how risk is managed across the pharmaceutical supply chain that the pharmaceutical quality community had never been challenged to address on this level [2]. Some batches of valsartan were found to contain levels of NDMAs up to 200 times higher than the FDA's established acceptable intake limit of 96 nanograms/day, with the most severely impacted batches found to contain the highest concentrations of such compounds, in animal studies associated with tumors in the liver, lungs, kidneys, gastrointestinal tract, and nasal cavity, and as a Group 2A probable human carcinogen by the International Agency for Research on Cancer [3]. The contamination could be linked to a change in the process around 2012, when dimethylformamide (DMF) was used as a solvent in the formation of tetrazole rings, resulting in the formation of NDMA as a byproduct of the process, a reaction pathway that, with the benefit of hindsight, was chemically predictable [4].

The crisis was far from over when the ARBs stopped. Further investigations into other drug classes found that NDMA had been found in ranitidine, caused in this instance by the inherent chemical instability of the ranitidine molecule itself; in metformin, nizatidine, rifampin, rifapentine, and pioglitazone and showed that nitrosamine contamination was not limited to any one synthetic route or therapeutic class [5]. As a result, the expanded scope led regulatory agencies all over the world to provide extensive guidance for conducting systematic nitrosamine risk evaluations for all manufacturers of chemically synthesised APIs and finished drug products. The discovery of nitrosamine drug substance-related impurities, a structurally different and mechanistically more complex impurity class due to the nature of the API, presented a new regulatory and scientific challenge that keeps growing [6].

The urgency and complexity of the nitrosamine issue is compounded by the fact that it spans the domains of genotoxicology, synthetic organic chemistry, formulation science, and pharmaceutical quality management, disciplines seldom encountered in the same regulatory risk management [7]. Since 2018, FDA, EMA, ICH, Health Canada, and multiple other health authorities have issued more and more regulatory guidance, however, no review has been performed that brings all of this together in one place including the mechanistic chemistry behind the formation of nitrosamines, the toxicology basis of existing acceptable intake levels, analytical requirements for detection at sub-ppb levels, and the quality system considerations for embedding nitrosamine risk intelligence into the pharmaceutical manufacturing environment as a routine practice, not as a crisis response plan [8].

This review fills that void. It outlines the chemistry of nitrosamine formation and structural classification, discusses the frameworks for the evaluation of the mutagenic and carcinogenic risk posed by nitrosamines, presents an overview of the evolving global regulatory environment, including the FDA's recently released guidance that expands upon *FDA Guidance for Industry Analytical Procedure for the Identification of Impurities in Drug Products, and considers the need for pharmaceutical quality systems to structurally adapt in order to address nitrosamine risk as a permanent part of quality management, not an episodic issue. The article is written with pharmaceutical scientists, quality assurance professionals and regulatory strategists in mind for their need to navigate the demands of nitrosamine compliance in the present and the future.

2. Chemistry of Nitrosamine Formation: Structural Classification and Mechanistic Pathways

N-nitroso compounds are nitrosamines, in which a nitroso group (–N=O) is attached to a nitrogen atom that has been formed from an amine precursor. In pharmaceutical systems these are formed when three chemical ingredients converge: a nitrosatable amine, a nitrosating agent, and a catalyst (acidic pH, high temperature, or catalytic species) that promotes the reaction. In the pharmaceutical manufacturing context, the main nitrosating species is nitrous acid, which is thermodynamically unstable but can easily be formed in situ from nitrite salts in acidic environments [9]. Secondary amines are the most reactive class towards nitrosation, the N-nitroso product is stable, while primary amines can react with nitrosating agents but the product formed is a diazonium which is not stable [10]. The risk of N-nitrosation for tertiary amines is more complex: they will not directly undergo N-nitrosation in the classical way, but can break down or undergo hydrolysis under acidic or oxidative conditions, which can release secondary amine species capable of subsequent nitrosation, as with metformin for which NDMA has been found experimentally during the production and storage of drug products [11].

The process-chemistry aspect of the problem is illustrated by the mechanistic pathway by which valsartan is contaminated. Sodium azide is employed as an azidating reagent in the synthesis of the majority of sartan APIs, and is subsequently quenched by sodium nitrite, under acidic conditions, into nitrogen gas and nitrous oxide gas [12]. This quenching step produces nitrous acid in situ, while the presence of dimethylamine (in many solvents, such as N,N dimethylformamide, a known residual contaminant in the solvent) will lead to rapid formation of NDMA, and will be further stimulated by the presence of Lewis acid species such as zinc chloride, which may be present in the process. The realization that dimethylamine, a ubiquitous impurity for

API synthesis in DMF a solvent that was both used at trace levels, was also a potential NDMA (N-Nitrosodimethylamine) precursor, marked a paradigm shift in impurity risk thinking [13].

In addition to process chemistry, another class of impurities has taken center stage in the regulatory and scientific agenda since around 2021: the nitrosamine drug substance-related impurities. Unlike the small dialkylnitrosamines, which are completely different from the API, NDSRIs form through the nitrosation of the functional group of the API's secondary amine or a secondary amine degradant, resulting in an N-nitroso derivative with a high degree of similarity to the API [14]. This closeness of structure makes analytical problems particularly noteworthy, one of which is co-elution with the parent compound, and raises toxicological questions because the carcinogenic and mutagenic nature of the highly complex, structurally elaborate nitrosamines cannot be reliably predicted based on the reference compounds used for simple dialkylnitrosamines. Drug products identified to have NDSRIs and linked to voluntary recalls range from cardiovascular therapeutics (varenicline, propranolol, quinapril), neurological therapeutics (orphenadrine), antibiotic therapeutics (rifampin, rifapentine), antidiabetic therapeutics (sitagliptin), and anticoagulant therapeutics (dabigatran etexilate) [15].

Water is not only a solvent, it also facilitates molecular mobility and, therefore, a mechanistically important part in the formation of NDSRs in solid dosage forms. Experimental evidence published in the literature shows that during accelerated stability testing the level of NDSRI consistently and proportionally rises with increasing humidity of the solid product; the humidity solubilizes the trace amounts of nitrite present in excipients and the proximal secondary amine whose formation of NDSRI is accelerated, thus generating local aqueous microenvironments in which nitrosation takes place efficiently in the apparent absence of water [16]. Wet granulation is a process that can form the most favorable environment for nitrosamine formation, while other manufacturing processes, such as fluid bed drying and tablet coating, involve NOx gases that have been linked to NDSRI and NDMA formation in metformin and atomoxetine formulations, respectively. From these mechanistic considerations, the classification is drawn out and summarized in Table 1, which outlines the two main impurity classes, their structural basis, their formation pathway, the drug categories that they affect, and the default acceptable intake limits as defined in current regulatory guidance [17].

Table 1. Structural Classification of Nitrosamine Impurities in Pharmaceutical Products: Common Small-Molecule Nitrosamines vs. Nitrosamine Drug Substance-Related Impurities (NDSRIs) [18–20]

Parameter	**Small-Molecule Nitrosamines**	**Nitrosamine Drug Substance-Related Impurities (NDSRIs)**
Structural definition	Simple dialkyl or alkylaryl N-nitroso compounds; molecular mass typically below 150 Da	N-nitroso analogues of the API or its degradants; structurally complex, high molecular weight
Representative examples	NDMA, NDEA, NMBA, NDIPA, NEIPA, NDBA	N-nitroso-varenicline, N-nitroso-propranolol, N-nitroso-quinapril, N-nitroso-atomoxetine, N-nitroso-sitagliptin
Primary amine precursor	Dimethylamine, diethylamine, diisopropylamine from solvents (DMF), reagents, or process impurities	Secondary amine group within the API molecular structure, or secondary amine degradant of a tertiary amine API
Principal formation locus	API manufacturing process (synthesis, quench steps, recovered solvent)	Drug product during manufacturing (wet granulation, tablet coating) or long-term storage

Key contributing conditions	Acidic pH, elevated temperature, Lewis acid catalysts ($ZnCl_2$), sodium nitrite-based quench steps	Trace nitrite from excipients, moisture (humidity), NOx in process air, extended shelf life
Therapeutic categories reported	ARBs (valsartan, losartan, irbesartan), ranitidine class, rifamycins, pioglitazone	Beta-blockers, ACE inhibitors, smoking cessation agents, anticoagulants, antidiabetics, antidepressants
Default acceptable intake (AI) limit	Compound-specific, range 26.5–180 ng/day; NDMA AI = 96 ng/day; NDEA AI = 26.5 ng/day	18 ng/day (conservative default in absence of compound-specific data); CPCA-based limits range 18–1500 ng/day
Toxicological data availability	Robust carcinogenicity data from animal studies; TD50 values established for NDMA, NDEA	Limited; in silico CPCA classification used as surrogate; compound-specific in vivo data emerging
Regulatory classification	ICH M7 cohort of concern; well-defined class	ICH M7 cohort of concern; structural complexity limits standard SAR prediction accuracy

3. Sources and Root Causes of Nitrosamine Contamination Across Drug Product Systems

The systematic examination of the pharmaceutical supply chain from the synthesis of starting materials to the container closure system of the finished product is required to trace the origin of the nitrosamine, depending on the chemistry and manufacturing context. The first tier includes API manufacturing related contamination, where nitrosamines are a byproduct of synthetic chemistry processes. The confirmed pathway in early sartan recalls (where dimethylamine was carried over as a residual impurity from DMF and reacted with nitrous acid produced during the azide-quenching step) has since been recognized as typical of a wider class of risks where there is a presence of any secondary amine in solvents, reagents, raw materials, or upstream intermediates [21]. One risk that is not highly valued in multi-product API manufacturing facilities is the presence of trace secondary amines in recovered solvents that may have been manufactured in previous batches but not highly appreciated. Perhaps the highest-risk unit operation in API synthesis is a quench step designed to eliminate excess azide, oxidant, and/or other potentially hazardous species at the end of a synthesis, resulting in the intentional production of nitrous acid under controlled acidic conditions that, in the event of a coincidental amine contaminant, will always lead to a nitrosamine product [22].

The second tier is the drug product associated nitrite contributions caused by excipients, which became a separate and significant root cause class with increased knowledge of the NDSRI formation, from 2021 to 2023. Trace levels of nitrite (sub-ppm - low ppm) are found in common excipients used in the manufacture of oral solid dosage forms: microcrystalline cellulose, lactose monohydrate, crospovidone, hypromellose, sodium starch glycolate, and magnesium stearate [23]. The mean nitrite content for crospovidone was ~8.3 µg/g with a peak of 14 µg/g from samples collected from five suppliers, whereas the mean for the other tablet excipients ranged from 0.2 to 4.5 µg/g.Crospovidone had one of the highest mean nitrite levels of all the commonly used tablet excipients (around 8.3 µg/g), with a maximum of 14 µg/g found across tested samples for five different suppliers, compared to the other excipients, which ranged between 0.2 and 4.5 µg/g. The range and mean values for lactose monohydrate were more restricted, with a range of 0.07 to 1.7 µg/g across eight suppliers, and a mean value of around 0.70 µg/g for microcrystalline cellulose. While having a moderate amount of nitrite per gram of excipient, they often make up the bulk of the mass fraction of a solid oral product, so that

the total amount of the nitrite presented to the secondary amine API or its degradants is significant. Published case studies have shown that supplier qualification programs that require nitrite specifications on certificates of incoming materials can lead to up to a 150% increase in predicted nitrite formation as compared to the average nitrite scenario [24].

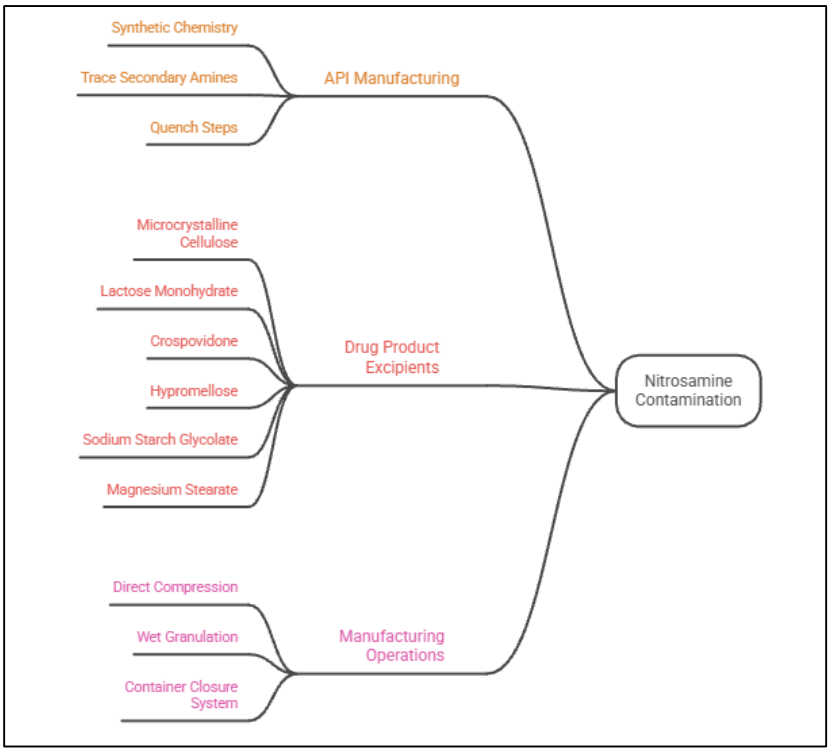

Figure 1. Nitrosamine Contamination in Pharmaceutical Products

The third level is related to drug product manufacturing operations and long term stability as sources of NDSRIs. Direct compression methods do not offer the same degree of protection against nitrosamine formation as wet granulation does, because they do not involve the presence of water, but are reported to be NDSRI-generation events when NOx gases in the process air supply react with secondary amines in the drug product matrix during the fluid bed drying and tablet coating processes. The container closure system should also be considered, as some of the container closure components, in particular, those containing rubber or plastic, can contain contaminants derived from amines which can migrate into the drug product under high humidity and temperature conditions and can participate in nitrosation reactions during the shelf life [25,26].

4. Genotoxicity, Mutagenicity and Carcinogenicity Assessment: From Mechanism to Acceptable Intake Limits

The carcinogenic and mutagenic properties of nitrosamines come from a bioactivation sequence that has been extensively studied since the 1950s and is well characterized as taking place by the action of cytochrome P450. The most important and essential initiating step is the CYP-mediated α-hydroxylation of the carbon atom next to the nitroso group, which is mainly carried out by CYP2E1 in the liver, and to a lesser extent by CYP2A6 and other isoforms depending on the complexity of the structure of the nitrosamine [27]. The enzymatic hydroxylation creates an intermediate α-hydroxynitrosamine that breaks down to an aldehyde co-product and a very reactive alkyl diazonium ion. The electrophilic character of the diazonium ion will cause it to alkylate DNA; it will attack nucleophilic centers in DNA bases by SN1 or SN2 mechanisms to form covalent DNA adducts [28]. The principal adducts formed initially are the N7-methylguanine adducts (~65% of all adducts) while O6-methylguanine is formed in significantly lower amounts (~7% of all adducts), but it is the adduct with the greatest miscoding potential and is comparatively poorly repaired by cellular mechanisms, which is why it is the adduct most directly implicated in carcinogenicity and mutagenicity. The mutagenic effect, and

the eventual carcinogenesis, is believed to be due to the persistence of O6-methylguanine adducts, which are not efficiently removed by the O6-methylguanine-DNA methyltransferase [29].

This mechanistic understanding leads to the structural determinants of the carcinogenic potency of nitrosamines. Nitrosamines with freely accessible α-hydrogen atoms adjacent to the nitroso group are more potent carcinogens, as are those that do not contain features in the molecular structure that hinder hydroxylation or the resulting diazonium ion from undergoing metabolic decomposition. On the other hand, structural features like bulky α-substituents, α-carbon substitution with electron-withdrawing groups, aromatic groups adjacent to the nitrogen, or the presence of nitrogen in a ring system may decrease metabolic activation efficiency and/or predicted potency, which is the scientific basis for the CPCA framework [30].

The CPCA, developed under the global Nitrosamines Implementation Technical Working Group and formally adopted by the EMA, FDA, Health Canada and the Japanese MHLW, categorizes each nitrosamine into one of five categories of potency (PC1 to PC5) that corresponds to a specific AI limit. Importantly, the EMA has assigned an AI of 18 ng/day for PC1 compounds while the FDA has assigned 26.5 ng/day for the same class, which are based on the same toxicological data but on agency specific modelling conventions [31]. The entire CPCA tier structure, structural scoring basis and the corresponding AI limits are given in Table 2. Sixty-six percent of nitrosamines were assigned to PC1 or PC2 in a published training set of 81 nitrosamines with 16 percent assigned to PC4 or PC5. Significantly, if the same categorization scheme was applied to 263 hypothetical (simulated) NDSRIs derived from drug substances with secondary amine or dimethyl tertiary amine centers, 39% would be classified as PC1 or PC2 while 48% would be assigned to PC4 or PC5, indicating that the structural complexity of NDSRIs often leads to a lower predicted carcinogenic potency than the structurally simpler dialkylnitrosamine class, but not a diminished regulatory concern in the absence of compound-specific empirical data [32].

The toolbox of mutagenicity evaluation covers three methodological levels, each having its own advantages and disadvantages. The in silico approaches (Derek Nexus, and the statistical Sarah Nexus) can meet the ICH M7 requirement of two complementary computational approaches and offer initial hazard identification in a timely fashion, however they possess limited predictive power for structurally complex NDSRIs that lie outside of the chemical domain of their training sets [33]. Currently, the preferred in vitro test for NDSRIs is the Enhanced Ames Test which has been developed to incorporate reverse mutation bacterial strains with an optimized metabolic activation system that includes S9 fractions added to the test system to enhance the ability to detect bioactivation of the test compound by CYP. Published EAT results for seventeen NDSRIs of varied structure showed that eight were mutagenic with a general trend of good agreement of the results with the CPCA category predictions, with PC1 and PC2 compounds generally positive and the pattern of the remaining compounds (PC4 and PC5) being mixed or negative [34]. The transgenic rodent gene mutation assay, usually performed in the Muta™Mouse model, can be used to generate in vivo mutagenicity data that encompasses complex metabolic, toxicokinetic, and DNA repair pathways not accessible to cell-based assays, and is the most confident tier of data needed to set AI limits for a compound or to support read-across arguments. In order to support an increase in an AI from the default conservative value, FDA's 2025 supplementary guidance also required a second in vitro mammalian cell mutation assay and in vitro metabolism data from human hepatocytes or microsomes for a negative Enhanced Ames Test result [35].

Table 2. Carcinogenic Potency Categorization Approach (CPCA) Framework Potency Categories, Structural Determinants, Acceptable Intake Limits and Representative NDSRIs [36–38]

CPCA Potency Category	EMA AI Limit (ng/day)	FDA AI Limit (ng/day)	Key Structural Activating Features	Key Structural	Representative NDSRI Examples	Predominant Mutagenicity in EAT

				Deactivating Features		
PC1 (Most potent)	18	26.5	Multiple accessible α-H atoms; no deactivating groups; linear dialkyl or monosubstituted structures	None	N-nitroso-nortriptyline, N-nitroso-lorcaserin, N-nitroso-desmethyl-diphenhydramine	Positive
PC2	100	100	One or more α-H atoms; mild structural constraints; moderate electronic influence	Partial steric or electronic deactivation	N-nitroso-atomoxetine, selected acyclic α-substituted NDSRIs	Mixed
PC3	400	400	Limited α-H access; strong β-carbon electron-withdrawing groups present	Significant electronic deactivation	Selected cyclic NDSRIs with ring constraints limiting CYP activation	Mixed to negative
PC4	1500	1500	Minimal metabolic activation potential; α-carbons constrained by adjacent electron-withdrawing groups or aromaticity	Multiple deactivating structural features	N-nitroso-diclofenac, select aryl NDSRIs	Negative
PC5	1500	1500	Same as PC4; designated for NDSRIs with structural features predicting non-activation	Strong deactivation (amide, aromatic, carbamate flanking groups)	NDSRIs bearing flanking amide or carbamate nitrogen-adjacent groups	Negative

5. Global Regulatory Landscape: A Comparative Analysis of FDA, EMA, ICH, and Health Canada Frameworks

There is no uniformity, nor a simultaneity, in the regulatory approach to nitrosamine impurities, and this necessitates understanding the differences if a pharmaceutical quality system is used in a multi-market context. Overall, Figure 2 shows where key regulatory milestones have occurred for the main authorities between 2018 and 2025, with convergence on the science and significant differences on requirements for implementation. In July 2018, the FDA initiated a preliminary investigation and coordinated with the voluntary withdrawals, followed by several interim guidance communications until its original Guidance for Industry on Control of Nitrosamine Impurities in Human Drugs, published in September 2020, and revised in February 2021 [39]. The initial guidance for the first revision focused on a risk assessment to determine if the formation of small molecule nitrosamines was a potential theoretical risk to the product, and a three-step investigation process for all synthetic drug products: 1) Risk Assessment – determine if the formation of small molecule nitrosamines was a potential theoretical risk to the product; 2) Confirmatory Testing if risk was identified, confirmatory test was to be performed; 3) Controls or Specification Change if the confirmatory test detected small molecule nitrosamine impurities at concentrations exceeding the AI, the product was to be brought into compliance by implementing controls or specification changes [40]. The FDA has called upon manufacturers to finish the three-step process for small-molecule nitrosamines by October 1, 2023. In August 2023, a separate guidance was issued recommending AI limits for NDSRIs, which endorsed the CPCA framework, and in September 2024, the Revision 2 of the control guidance, which the pharmaceutical industry deemed a paradigm shift from previous versions, finally separated the two impurity classes and pulled together the agency's updated expectations, including the August 1, 2025 deadline for completion of the confirmatory testing of NDSRIs and notification of required application changes. To acknowledge the practical challenges of NDSRI mitigation strategies, the FDA has updated their position on June 23, 2025 to allow detailed progress reports in lieu of full implementation by August 1, 2025, as long as confirmatory testing results, mitigation actions and estimated timeframes for full resolution were documented [41].

The EMA has been pursuing an approach that accords with its scientific principles, but has taken a different procedural structure. The first Article 31 process, only for sartan-class products, was launched in July 2018, but the second, under Article 5(3), was started in September 2019 and has been expanded to all chemically synthesized APIs in human medicines. The EMA's Q&A document, which was updated many times from 2019 through 2024, has been the key living guidance document, adding new risk factors, clarifying CPCA categories, and anticipating changing expectations on how much confirmatory testing is required for each threshold [42]. By January 2024, the European Pharmacopoeia has switched to a new approach for the management of nitrosamine impurities in individual monographs, which included the first official analytical procedures for the detection of NDSRI. As of the EMA's extensive progress report, more than 170 compound-specific AI limits had been posted on the EMA's website, and the agency estimated that only a small number of products had been withdrawn and that patients had not been unnecessarily exposed to high levels of the products [43].

One key area of difference between FDA and EMA is willingness to accept in vivo negative data in ICH Q3A and Q3B as a basis for NDSRI de-risking. The EMA and Health Canada have accepted a well designed negative in vivo mutagenicity study to support higher AI limits, while FDA's guidance (Revision 2) stated that the agency would not accept such studies in either Q3A or Q3B frameworks, regardless of EMA/Health Canada de-risking conclusions (operational implications for companies with global product portfolios where only a single regulatory strategy is available) [44]. The global Nitrosamines Implementation Technical Working Group (ITWG) has provided the broad coordination throughout the period: The ITWG is a group of staff from the FDA, EMA, Health Canada and several other health authorities that were involved in the joint development and timing of the CPCA. At the ICH plenary meeting in Fukuoka in June 2024, the creation of a formal ICH implementation working group to develop an addendum to ICH M7 specifically focused on the safety and toxicology aspects of nitrosamines was agreed, signaling that the scientific and regulatory

community now regards harmonized global nitrosamine standards as a long-term institutional priority rather than an emergency response measure [45].

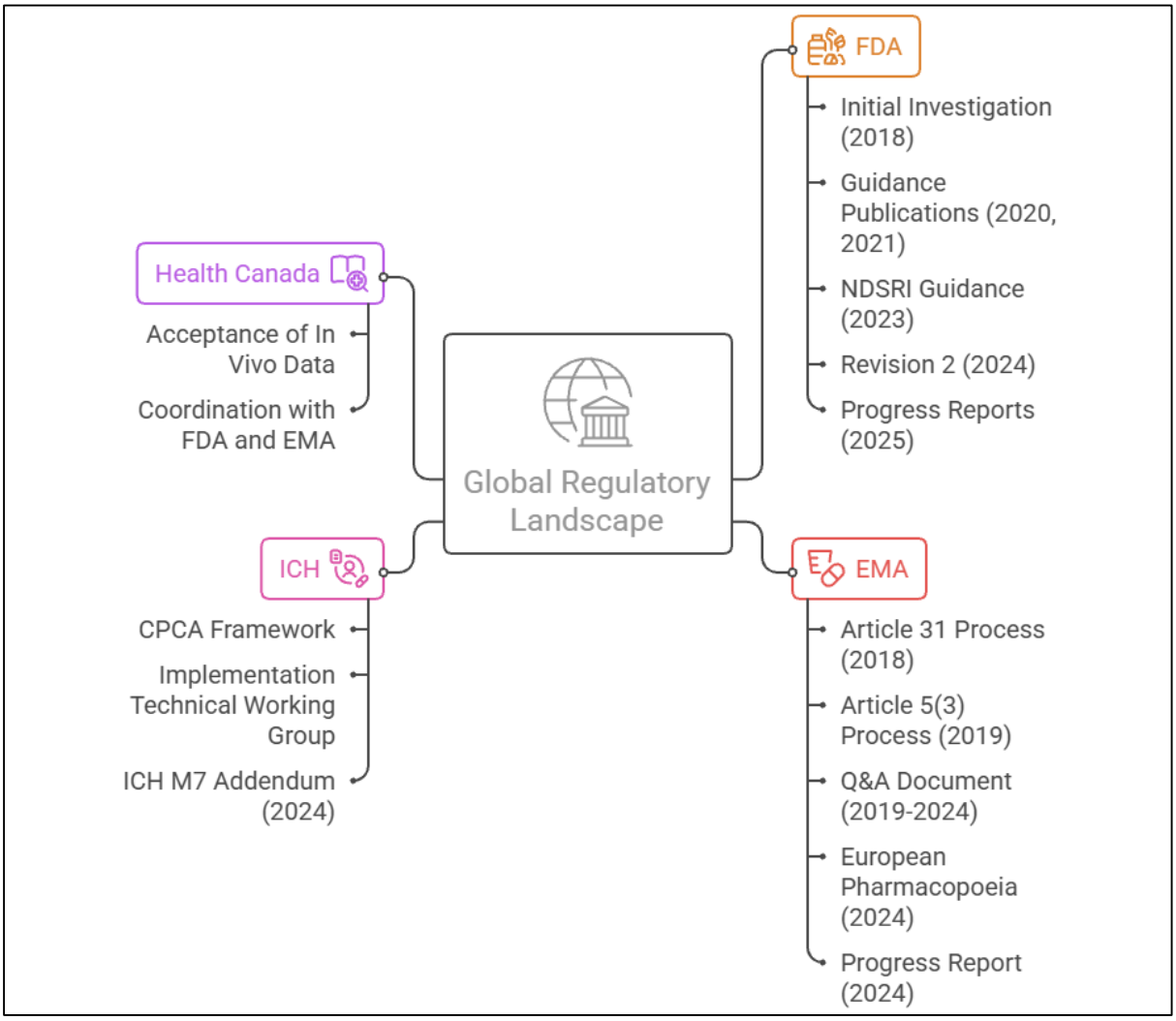

Figure 2. Global Regulatory Landscape of Nitrosamine Impurities.

6. Pharmaceutical Quality System Response: CAPA, Change Control, and Risk-Based Quality Management

The nitrosamine impurity crisis has been an inadvertent but revealing test of the pharmaceutical Quality Management Systems, not only because of the analytical problem, but through the 'lack of' as well as the 'lacking' of risk intelligence in the system, its generation, documentation, communication and action. Applying the fundamental problem, it was not that individual risk assessments were not possible, it was that there was no system in place in most organizations' QMS frameworks to encourage systematic hazard identification for nitrosamines as a routine part of formulation development, raw material qualification or change control review. It is not enough to incorporate nitrosamine risk intelligence into one pillar of the QMS at a time, however, but to embed it into the daily operations of multiple pillars at once, in order to correct this [46].

The ICH Q9 (R1) Quality Risk Management guideline was revised in 2023 and is the best suited structural framework for this integration. Nitrosamine formation potential must be considered a standing hazard category throughout the QRM cycle (hazard identification, risk analysis, risk evaluation, risk control) at all stages of the formulation lifecycle where formulation chemistry, API structural features, excipient selection or manufacturing process design decisions are made. The FDA's Revision 2 guidance invokes the concept of ICH Q9(R1) as the basis for the prioritization of risk assessment and suggests that chronic medications with high maximum daily dose (MDD) should be considered first for risk assessment attention over low dose or short

duration products, reflecting a rational pharmacotoxicological hierarchy. Supplier qualification programmes are a QMS function that is directly affected by the guidance, as they are required to be periodically re-assessed whenever there have been changes in the raw material sources or excipients suppliers [47].

The CAPA system that has always been triggered by deviation, OOS or external events like regulatory alerts now faces significant disruption from the nitrosamine problem, as the presence of the impurity is frequently not known until specifically identified with the use of a sensitive analytical method. Confirmatory testing results that trigger CAPA workflows now must not only encompass a decision to either increase or decrease the batch disposition but also include a consideration for root cause investigation at the formulation level and process level, NDSRI and submission of change control for specification changes or reformulation. Organizations that did not have a nitrosamine specific risk assessment in their dossiers at the date of regulatory implementation deadlines have been faced with the burden of having to assess the risks of the compound, on top of the burden of executing CAPAs [48].

When nitrosamine mitigation requires a formulation change for an approved marketed product, it will have a strong impact on change control as that is the QMS function most impacted. The change of excipients or reformulation to decrease the amount of nitrite in the product, such as changing the source of MCC to a source that contains less nitrite, or switching from sodium starch glycolate to a product that contains less nitrite in the same excipient type, would be a change in formulation that should be notified or approved depending on the magnitude of the change and the country in which the product is used. The product change control package should not only include the technical basis for the change but also confirmatory stability data showing that NDSRI levels are maintained below the AI limits for the intended shelf life for the new formulation. If there is no alternative to excipient substitution, and the formation of nitrosamines cannot be sufficiently reduced by process optimization, the reformulation strategy may involve the addition of substances with nitrosation inhibitory properties, such as ascorbic acid or alpha tocopherol, which also require compatibility studies, stability studies and regulatory change management [49].

Annual Product Review, a recurring requirement of the QMS as per 21 CFR 211.180(e) and EU GMP Chapter 1, is an excellent and untapped tool for pro-active nitrosamine trend monitoring. Organizations that have integrated results of nitrosamine confirmatory testing (including trend data across multiple commercial batches) into their APR structure have produced actionable intelligence about NDSRI formation rates as a function of the variability of excipients from batch to batch, storage conditions, and cumulative shelf life. The addition of nitrosamine risk as an APR data domain in addition to the traditional quality parameters like OOS rate, complaint trends, and validation status is a systems-level development that makes the QA department a proactive, quality intelligence function, not just a compliance mechanism [50].

7. Detection, Analytical Control, and Confirmatory Testing Strategies

The concentrations necessary for detection of the nitrosamine impurities specified for current AI limits (some as low as 18 nanograms per day for PC1 NDSRIs) require analytical methods with the ability to accurately quantify target impurities at sub-ppb levels in complex pharmaceutical matrices where there may be significant excess of analyte in the matrix (API, excipients, degradation products that can cause matrix suppression or spectral interference). Traditional HPLC-UV analysis, the standard method for decades for the analysis of impurities in pharmaceuticals, is not ideally suited for this task, as nitrosamines have no chromophore that absorbs strongly in the UV region and they can only be detected by HPLC-AI at concentrations that are orders of magnitude lower than those available for HPLC-UV detection [51].

Mass spectrometric methods have thus become the standard in the analytical world for the detection of nitrosamines, as outlined in the FDA formal publication of USP General Chapter <1469> which covers four types of mass spectrometric methods: LC-HRMS, LC-MS/MS, GC-MS, and headspace GC-MS/MS. Headspace GC-MS methods can be used for volatile small-molecule nitrosamines (such as NDMA) that have

high vapor pressure enough to allow extraction from solid dosage forms into the headspace above the heated sample, which reduces matrix interferences. It is one of the first methods released by the FDA in August 2018 dedicated to the quantification of NDMA in valsartan API and finished products. But NDSRIs are structurally complex, high molecular weight, and low volatility compounds and are not easily extracted into the vapor under any practically obtainable headspace conditions [52].

Multiple reaction monitoring (MRM) is the mode of operation for LC-MS/MS that has become the platform of choice for routine confirmatory quantitation of small-molecule nitrosamines and NDSRIs in solid and liquid pharmaceutical matrices. The technique combines chromatographic retention and characteristic product ion transitions to give the sensitivity and selectivity to discriminate target nitrosamines from isobaric matrix components. Using validated LC-MS/MS with sample preparation optimisation using tandem solid-phase extraction (SPE) to enhance the analyte concentration and to remove co-extractant matrix components, quantification at the parts per trillion (ppt) level has been achieved for NDMA, allowing compliance to be verified even under the most stringent AI limits. One unique analytical challenge for NDSRIs is that the close structural similarity to the parent API can give rise to chromatographic co-elution, which is very much an active area of method development to develop orthogonal conditions using optimized chemistry of the stationary phase and gradient profiles. Another limitation applicable to NDSRIs is the availability of authentic reference standards, which are needed for calibration and system suitability (required for confirmatory testing program for each drug product); many NDSRI compounds are novel compounds and must be synthesized de novo for use in each drug product's confirmatory testing program [53].

The use of UHPLC-HRMS instrumentation, like high resolution quadrupole time-of-flight or Orbitrap platforms, provides an extra dimension of data, based on the ability to accurately measure mass, allowing retrospective data mining for unforeseen NDSRIs in samples that were not initially suspected. This has gained more importance as science has been learning more about the routes of NDSRI formation, which can create NDSRI structures that were not initially considered during the risk assessment; thus, there is a need for a structural space wider than that covered by targeted MRM methods to be covered by analytical surveillance. The decision process for technique selection, from the classification of impurity type to the selection of technique and the choice of validation strategy, which can be helpful to QA laboratories and analytical scientists dealing with confirmatory testing obligations. It is important to emphasize the importance of sample preparation, since it is the most critical step between analytical success or failure. Sample solutions with added methanol extract containing NDMA have been shown to undergo measurable concentration loss if the sample is not analyzed within defined holding times, further highlighting the need for sample stability data as a required method validation parameter in the context of nitrosamines, as defined by ICH Q2(R2) requirements, with consideration of the unique physicochemical instability of NDMA [54].

Table 3. Comparative Assessment of Analytical Techniques for Nitrosamine Detection in Pharmaceutical Products [55–57]

Technique	LOQ Range	Applicability	Key Strength	Key Limitation	Regulatory References
Headspace GC-MS	1–10 ppb (volatile NAs)	Small-molecule volatile nitrosamines (NDMA, NDEA, NDBA) in solid and liquid dosage forms	Minimal matrix interference; no complex sample preparation needed	Inapplicable to NDSRIs; limited to volatile analytes	USP <1469> Procedure 4; FDA Aug 2018 valsartan methods

GC-MS/MS (direct injection or extraction)	0.15–1 ppb	Small-molecule nitrosamines in APIs and finished products; nine simultaneous nitrosamines reported	High selectivity through MRM transitions; simultaneous multi-compound quantification	Requires extensive sample preparation; solvent interference possible	USP <1469> Procedure 2; EMA OMCL GC-MS/MS methods
LC-MS/MS (MRM mode)	0.5–3 ppb	Small-molecule nitrosamines and NDSRIs in APIs, tablets, capsules, liquids	Gold-standard selectivity and sensitivity; suitable for NDSRIs; regulatory benchmark	Co-elution with API possible for NDSRIs; reference standards required; matrix suppression risk	USP <1469> Procedure 3; FDA LC-MS/MS NDSRI methods
UHPLC-HRMS (Q-TOF or Orbitrap)	0.5–2 ppb; accurate mass	Small-molecule NAs and NDSRIs; retrospective mining for unanticipated impurities	Accurate mass data supports structural confirmation; enables data mining for unknown NDSRIs	Instrument platform-dependent performance; higher cost and complexity	USP <1469> Procedure 1; EMA EDQM LC-ESI-HRMS procedures
UHPLC-APCI-MS/MS	Sub-ppb (0.1–1 ppb range)	NDMA, NDEA in sartan drug substances and products; nizatidine formulations	Atmospheric pressure chemical ionization avoids electrospray suppression in apolar matrices	Less universal ionization mode; limited database of NDSRI-specific APCI conditions	CVUA Karlsruhe OMCL method; EMA GEON network publications

8. Mitigation Strategies: Formulation Redesign, Process Optimization, and Excipient Management

There are three basic ways to achieve pharmaceutical mitigation of nitrosamine impurities, both at the API manufacturing stage and at the drug product stage: eliminate or minimize the amount of one of the two necessary reactants (amine precursor or the nitrosating agent), alter the reaction environment to reduce the rate of nitrosation and chemically react with the nitrosating agent to prevent it from reacting with the amine precursor. The principles are manifested in interventions that are organised at three strategic levels, each having different implications for regulatory, formulation and quality management. The most effective and enduring mitigation at the API synthesis level is process redesign to eliminate nitrosating steps or to substitute nitrosamine-forming secondary amine solvents with alternatives that do not pose a risk for nitrosamine formation. Systematic control of the secondary amine impurities in the synthesis by tighten specification and qualification of suppliers is a practical measure, which is also applicable for the synthesis of APIs containing tetrazole where quench steps are inevitable as in the synthesis of sartans. The most obvious risk management result from a facility that recovers solvents that involves secondary amines and nitrous acid is to exclude the solvents from the processes that present a risk of nitrosamines, or to expressly test the solvents for nitrosamines before reusing them in processes that do involve secondary amines and nitrous acid. Where relevant, secondary amine impurities that are identified as nitrosamine precursors during process understanding activities carried out in accordance to ICH Q11 should be controlled in API specifications [58].

For drugs at the drug product formulation level, the choice of excipients and excipient supplier management are the most modifiable parameters that can be utilized to control NDSRI formation. Published modeling studies have shown that using croscarmellose sodium as superdisintegrant, which has the lowest measured levels of nitrite among tablet excipients, in place of crospovidone (with a mean nitrite of 0.55 µg/g) can lead to an approximately 36% reduction in the theoretical nitrite formation; substitution of the superdisintegrant alone with the same excipient formulation is not sufficient and the simultaneous replacement of the same supplier of MCC and lactose with excipients with the lowest reported levels of nitrite in the superdisintegrants can result in up to an 89% reduction in the theoretical formation of nitrites per dose. This is a big operational hurdle for manufacturers to face and a change in formulation must be controlled, even if it has been approved by the regulatory authorities for a marketed product [59].

The most widely discussed formulation-level mitigation strategies for NDSRIs is the incorporation of nitrite scavengers. The FDA Revision 2 guidance explicitly states ascorbic acid and alpha-tocopherol as acceptable antioxidant excipients for this use, and both are included in the FDA Inactive Ingredient Database. They function by selectively reducing the nitrous acid and other reactive nitrogen species present, thus competing with the secondary amine of the API for the available nitrosating agent, which means the fraction of nitrous acid reacting is reduced to form the NDSRI. Published studies proved that ascorbic acid, up to 1%, in a placebo tablet (direct compression or wet granulation) measurably decreases the level of nitrite, and that the effect of ascorbic acid is proportional to its concentration up to this limit of 1%. BCS Class I, II, and III drugs that are reformulated to include ascorbic acid at doses up to 10 mg per dose (or max. daily dose, whichever is less) can be shown to be bioequivalent without in vivo studies, as outlined specifically in FDA's Revision 2 guidance, a regulatory accommodation that significantly reduces the developmental burden of implementing this mitigation. Container closure system changes such as the use of low-NOx packaging conditions, either by purging with nitrogen gas or by packaging with a desiccant to reduce humidity during shelf life, are another type of change that does not affect excipients, thereby reducing the change control burden in the event of an excipient level change [60].

9. Future Perspectives

The nitrosamine impurity management scientific and regulatory landscape since 2018 has been transformative but will continue to evolve and go through periods of development in its next chapter, with several clear scientific frontiers to be defined. The key scientific question is the fundamental restriction of the CPCA as a risk quantification tool. It is operationally essential that it is a regulatory default, but what empirical data have always revealed is that it is rather coarse as illustrated by the range of acceptable intake values published for each of the categories, with the category limit being rather conservative for most of the structures placed in it. A complementary approach that has emerged is quantum mechanical modelling that can help narrow the distinctions, by calculating activation energies for the entire carcinogenic activation sequence, from alpha-hydroxylation to the formation of a diazonium intermediate, to DNA base alkylation and competing hydrolysis pathways, for structurally diverse nitrosamines for which experimental carcinogenicity data does not exist. Previous published quantum mechanical evaluations have shown examples where CPCA over- and under-estimated the actual potency and have suggested that it should be possible to differentiate biologically inert nitrosamines from potent nitrosamines more effectively than is currently possible by using a mechanistically resolved energy barrier analysis. The combination of two-step modeling utilizing linear discriminant analysis of quantum mechanical descriptors and three-dimensional QSAR regression (QSAR) has proved to be an early success in predicting carcinogenic potency for NDSRIs with structural complexity that is beyond the confident range of currently available expert rule-based systems like Derek Nexus. The next ICH M7(R3) addendum, which is expected to incorporate a set of globally harmonized and unified values acceptable to all regulators based on the most advanced mechanistic science, is the institutional mechanism that will make this computational progress meaningful in terms of applicable regulatory standards [60].

The second frontier is the application of predictive nitrosamine risk modelling in early pharmaceutical development, and inclusion of the formation potential of NDSRI as a designed property. These ever-increasing datasets of excipient nitrite profile, API amine structural features, manufacturing process platform conditions and confirmed observations of the generation of NDSRI are being used to create machine learning models that have theoretical potential to give formulation stage risk estimates prior to any excipient and process platform commitment. The confidence-scoring frameworks applied to CPCA predictions, in one case using a reference set of 7,679 hypothetical NDSRIs with structural neighbourhood analysis to identify CPCA predictions with weak neighbour support, are early steps toward making the risk assessment itself uncertainty-aware, so that low-confidence predictions automatically prompt experimental follow-up, rather than being treated as equally reliable as those with strong structural precedent. This has led to the need for inter-industry data sharing, and only in a consortium structure is this density possible, making collaborative platforms a scientific and quality governance imperative in the ongoing development of the industry [61].

10. Conclusion

The nitrosamine impurity affair has uncovered more than just known tainted manufacturing paths for drugs. It has exposed the extent to which the institutional structures, regulatory science, and analytical power to address a class of risk had not been prepared by the underlying chemistry, and had in fact been theoretically predicted for decades. The findings from the mechanistic, toxicological, regulatory, and formulation areas presented in this review clearly indicate that the conditions for the formation of nitrosamines were present in many products well before 2018, and detection only happened after an external event rather than the internal quality intelligence. That this contamination was chemically predictable from the first principles, and that secondary amines, nitrite sources, and acidic conditions were universally found in pharmaceutical manufacturing makes the systemic failure of the prediction a more important lesson than the chemistry of any individual recall. The most weighty continuing restriction is not technical, it is epistemic. The development of the approach for categorizing carcinogenic potencies is an important step in bringing structure-activity relationships into play for use in formulating reasonable regulatory limits, but the boundaries between the categories are only approximate, covering several orders of magnitude in actual potency. With the increasing accumulation of the toxicological data base for nitrosamine drug substance-related impurities, including quantum chemical modeling, expanded in vivo transgenic rodent data, and machine learning-assisted QSAR refinements, it is time to no longer use tier-zero linear approximations, but rather to establish limits that are scientifically defensible as they carry regulatory weight. The ICH M7 addendum process, which started in June 2024, is the right institutional process to be used for this consolidation and it is only through continued commitment on the part of the regulatory authorities and the pharmaceutical industry to sharing nitrosamine toxicological data, rather than keeping it as proprietary information, that this will be achieved; in a domain where collective scientific knowledge is the chief scientific safeguard to patient health, information silos are a failure of the regulatory authorities and cannot be fixed through guidance revisions.

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