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Nitrosamine Impurities: FDA Guidance & AI Risk Assessment

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Published by IntuitionLabs · 2026-05-10 · nitrosamine impurities · fda guidance · risk assessment · ai software · acceptable intake · qsar models · ndsris · in silico modeling

Original article: https://intuitionlabs.ai/articles/nitrosamine-impurities-fda-guidance-ai-risk-assessment



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Nitrosamine Impurities in Pharma (2026): FDA Guidance, AI Risk Assessment & Mitigation Software

Executive Summary: Nitrosamines are a class of potent mutagens and probable human carcinogens that can inadvertently arise in pharmaceuticals. Nitrosamine findings in some medicines since 2018 have highlighted the ubiquity and risk of such impurities. Regulatory agencies worldwide (FDA, EMA, Health Canada, TGA, WHO, etc.) have responded with guidelines and enforceable limits. In September 2024 the FDA issued a *final* Guidance ("Control of Nitrosamine Impurities in Human Drugs") detailing preventive steps – risk assessments, testing, and controls – for both routinizing detection of nitrosamines and redesigning synthetic routes if needed (www.fda.gov). Health Canada, EMA and others have issued similar risk-management frameworks. All these guidance documents set extremely low **acceptable intake (AI)** levels for common nitrosamines (e.g. FDA AI for NDMA is *96 ng/day* (www.fda.gov), NDEA *26.5 ng/day* (www.fda.gov), NMBA *1500 ng/day* (www.fda.gov), NEIPA *400 ng/day* (www.fda.gov), etc.), corresponding to a lifetime excess cancer risk on the order of 1 in 100,000.

Nitrosamines can form via several pathways (e.g. reaction between amines and nitrosating agents like nitrite under certain pH conditions (www.sciencedirect.com)), and some pharmaceutical processes (e.g. use of contaminated reagents, degradative reactions of some APIs) are especially prone. Digital tools and software are increasingly employed to manage these risks. QSAR and in silico models (e.g. MultiCASE modules) can predict nitrosation propensity and carcinogenic potency of novel nitrosamines (multicase.com). Purge- and fate-calculation software (e.g. Lhasa's Mirabilis) quantifies how well synthetic steps can remove nitrosamine impurities (pubs.acs.org). Contemporary reviews emphasize that **digital risk assessment** – integrating structure-activity relationships, kinetic modeling, and process simulations – is vital for quantifying formation rates and purge efficiencies (www.sciencedirect.com) (www.jstage.jst.go.jp). Going forward, integration of AI-driven analytics into Quality Risk Management (QRM) pipelines is expected to advance. Preliminary

applications include machine learning algorithms for predicting nitrosamine formation from synthetic route parameters, and generative analytics to prioritize testing resources based on risk. While these tools are still evolving, stakeholders are already deploying them for proactive impurity control.

This report provides a detailed, data-driven analysis of nitrosamine impurities: its historical context; chemical and toxicological background; regulatory frameworks (U.S. FDA, EMA/CHMP, Health Canada, ICH M7, WHO); analytical methods and recommended limits; notable case studies (API and product recalls); and the emerging role of AI and in silico software in risk assessment and mitigation.

Introduction and Background

N-nitrosamines (general formula R$_2$N–N=O) are small organic compounds that are well established as highly mutagenic and probable carcinogens (pmc.ncbi.nlm.nih.gov). The IARC has classified many nitrosamines in Group 1 or 2A carcinogens (e.g. NDMA, NDEA are “probably carcinogenic” to humans (pmc.ncbi.nlm.nih.gov)). Crucially, regulatory guidelines (ICH M7(R1), 2017) list nitrosamines as a *“cohort of concern”* in mutagenic impurities – meaning that **any presence** must be avoided or stringently controlled (pmc.ncbi.nlm.nih.gov). For “known” nitrosamines (with positive animal carcinogenicity data), ICH M7 treats them as Class 1 impurities (no safety threshold allowed) (pmc.ncbi.nlm.nih.gov). In practice, however, regulators have set very low acceptable intake (“AI”) limits, based on lifetime cancer risk (typically targeting ~1×10^{-5} or 1×10^{-6} excess cancer risk). For example, FDA’s recommended AI for NDMA is 96 ng/day (www.fda.gov) (based on extrapolated carcinogenic potency), and NDEA 26.5 ng/day (www.fda.gov). Health Canada, EMA and WHO similarly define ng/day limits for nitrosamines in oral and other products. These levels correspond to an excess lifetime cancer risk on the order of ~10^{-5} (1 in 100,000) when a patient takes the medicine daily (see Section *Risk Assessment and AI Limits*).

Nitrosamines can form inadvertently in drug substances or products through classic nitrosation chemistry. In the typical mechanism, secondary (or sometimes tertiary) amines react with nitrosating agents (e.g. nitrite, nitrous acid) under acidic conditions, yielding N-nitrosamines (www.sciencedirect.com). Many drug syntheses involve amines (common building blocks) and potential nitrosating species (e.g. nitrite in water, contamination in solvents or reagents). High concentrations of amines and nitrosating agents in a reaction mix can produce significant nitrosamine quantities, but even trace amounts can matter given extremely low acceptable thresholds. For example, a trace amine impurity like secondary amine in the API may nitrosate under some conditions, or nitrite in water can nitrosate excipients or amine-derived side-products. Notably, certain N-nitrosamine contaminants are “drug substance-related impurities” (NDSRIs) unique to a given API’s structure (for example, nitrosation of a drug intermediate) whereas others are small-molecule residues from reagents or solvents (e.g. NDMA from dimethylamine). **FDA guidance explicitly distinguishes** “small-molecule nitrosamine impurities” from “nitrosamine drug substance-related impurities (NDSRIs)” (www.fda.gov). Identifying which class applies is key to targeting controls.

Since mid-2018, nitrosamine contamination has emerged as a major safety crisis for many medicines. Nitrosamine impurities have since been reported at trace levels in several classes of medicines. These findings have strained drug supplies and underscored that nitrosamine risk is *insidious* – stemming from

manufacturing changes, degraded reagents, packaging, and even in situ transformation of the API or dosage form over time.

Faced with this persistent problem, authorities have moved from ad hoc recalls to formal policies. Key international guidelines were released: ICH M7(R1) (2017) highlighted nitrosamines as mutagenic impurity *cohorts of concern*, but after the 2018 outbreaks regulators issued more specific criteria. The EU's CHMP adopted an Article 5(3) referral (Resolution in Sept. 2019) covering all nitrosamines in marketing authorisation products (www.ema.europa.eu). In Japan, Korea, and Asia, equivalent guidelines were promulgated. The WHO organized international meetings and issued a Medical Product Alert in July 2018 followed by a November 2019 information note on nitrosamine impurities. By 2024–2025 standalone nitrosamine guidances emerged: e.g. FDA's final guidance (Sep 2024) (www.fda.gov), Health Canada's guidance (Apr 2022), EMA's Q&A updates (2021–2024), and WHO's GMP guidance (draft in 2024, finalized 2025 (www.who.int) (www.raps.org)).

This report comprehensively examines the nitrosamine issue as of 2026. It includes historical context and case studies, chemical/toxicological background, regulatory and industry responses (including guidance and acceptable intake limits), analytical testing requirements, data-driven risk assessment, and novel tools. Special emphasis is given to **EMERGING AI-driven software** for nitrosamine risk management. These tools (QSAR models, reaction simulators, machine learning algorithms, etc.) are an evolving frontier for proactive impurity control. We review the current state of such digital approaches, their demonstrated applications, and future directions.

Chemical and Toxicological Overview of Nitrosamine Impurities

N-Nitrosamines are organic compounds with the general structure R$_2$N–N=O. As a class they have a long history of being recognized as carcinogens. IARC lists several nitrosamines as known or probable human carcinogens (NDMA, NDEA, NMBA, etc. are Group 2A or even Group 1 in some cases (pmc.ncbi.nlm.nih.gov)). Biochemically, nitrosamines are believed to cause cancer by metabolic activation: the nitrosamine is metabolized to a diazonium ion (an electrophile) that can alkylate DNA (www.sciencedirect.com). Key risk factors include small molecular size and the presence of an α-hydrogen (enabling metabolic cyclization). For example, nitrosamines with α-hydrogens (like NDMA) are highly mutagenic, whereas tertiary amine nitrosamines may also pose risk if they contain labile hydrogens (www.sciencedirect.com).

Mechanisms of Formation.

The classic chemical route is nitrosation of secondary amines by nitrous acid (HONO), which is in equilibrium with nitrate/nitrite under acidic conditions. (The fundamental mechanism – amine + NO$^+$ → N-nitrosamine – is well established (www.sciencedirect.com).) In pharmaceutical contexts, plausible pathways include:

- **Reaction of amine reagents or intermediates with nitrite.** Many syntheses use amines (e.g. triethylamine, dimethylamine, piperazines). If trace nitrite (NO$_2^-$) is present in inputs (water, excipients, or reagents), these can convert amines to nitrosamines, especially under acidic or warm

conditions (www.sciencedirect.com) (www.jstage.jst.go.jp). Water for injection containing nitrites, or nitrosating impurities, has been implicated. The solvent DMF itself can degrade to dimethylamine, which can be nitrosated.

- **Degradation of drugs yielding nitrosatable fragments.** Some pharmaceuticals contain tertiary amines or amides that can degrade to nitrosating species. Degradation pathways of an API can also be relevant to nitrosamine risk assessment, so these should be evaluated case by case.
- **Use of nitroso-containing impurities or reagents.** Direct contamination (e.g. using a nitrosamine as an intermediate) can lead to residues. Some older literature has examples of nitrosamines formed via rearrangements (the “diazonium” route), but these are less common in modern APIs.
- **Post-manufacturing transformations.** Even after production, nitrosamines may evolve. For example, compounding or tablets stored for long times in air with nitrites can slowly form nitrosamines.

Because of these diverse pathways, risk assessment requires evaluating *all* materials and steps: active drug, intermediates, excipients, solvents, reagents, water, lubricants, cleaning agents, and even packaging. Regulatory guidance instructs manufacturers to consider “all sources of nitrosating agents and amines” (www.sciencedirect.com) and to implement controls (e.g. replacing nitrite-contaminated materials, adding scavengers, or altering pH) to prevent nitrosation. Notably, in many cases nitrosamine formation follows known chemistry: for example, Health Canada notes that common nitrosation conditions involve the combination of amines and nitrite ion under acidic conditions, and separately lists a carbonyl-catalysed pathway at pH >7 (www.canada.ca).

Toxicological Potency and Acceptable Intake.

Due to their genotoxicity, nitrosamines are handled with a near-zero-tolerance approach. The FDA and other agencies estimate a *cancer potency factor* from animal studies (e.g. T25, TD50 values) to derive acceptable intake limits (AIs). In practice, agencies establish AI such that lifetime cancer risk is $\leq 1\times10^{-5}$ or similar. Notably, the *Thresholds of Toxicological Concern (TTC)* approach for genotoxic carcinogens usually sets an AI of 1.5 µg/day (1.5 parts per billion in a 1-gram dose) for general mutagens; however, nitrosamines are treated more stringently given specific carcinogenic data. For instance, FDA’s derived AI for NDMA is 96 ng/day (www.fda.gov) (†0.096 µg/d), roughly 15× lower than 1.5 µg/d; for NDEA it is only 26.5 ng/d (www.fda.gov). These values align with international practice (see Regulatory Guidance section). Importantly, if a nitrosamine lacks direct animal data, FDA and Health Canada apply a **carcinogenic potency categorization approach (CPCA)**: potency categories (e.g., “extremely potent”) are assigned based on structure–activity similarity to known model compounds, and corresponding AI defaults are given. For example, a nitrosamine predicted to be among the most potent might get a 26.5 ng/day limit (Category 1 in FDA’s CPCA scheme (www.fda.gov)). Notably, Canada’s Q&A sets out less-than-lifetime (LTL) adjustment factors that can be applied to established AIs for eligible products (www.canada.ca).

Because multiple nitrosamines can accumulate additively, guidance also addresses combined risk: the risk from multiple nitrosamines should be evaluated together; consult the regulator's current guidance for the applicable approach.

Analytical Detection.

Detecting nitrosamines is challenging because allowed levels are near the limits of modern instrumentation. Approved methods typically use GC-MS (often with high-resolution or tandem MS) or LC-MS. The FDA and pharmacopeias have released numerous validated methods for NDMA, NDEA, NMBA, etc. (see Appendix in FDA guidance). Key points: sample preparation must prevent artifactual nitrosation (no nitrite in reagents, low temperature). Methods often achieve LOQs in the low-ng/g range for dosage forms. Tarafder *et al.* (2023) stress that “analytical methods capable of detecting low levels [ng/g] of impurities” are essential (pubs.acs.org). Methods must be “phase-appropriate” – e.g., development of QC methods for marketed products requires full validation at trace levels (pubs.acs.org). The FDA has also issued “recommended testing methods” for specific products with required dates (www.fda.gov). All in all, an analytical laboratory must demonstrate sufficient sensitivity and robustness to reliably meet or exceed the guidance’s detection requirements (pubs.acs.org).

Regulatory Standards and Guidance (2026)

Regulators around the globe have crafted guidance to manage nitrosamine risk. These provide manufacturers with risk-assessment and control frameworks, target AI limits, and timelines for compliance. Key documents include:

- **FDA (USA):** The FDA’s *Control of Nitrosamine Impurities in Human Drugs* Guidance (final, Sept. 2024) revises the final guidance of the same name issued on February 24, 2021. It "recommends steps" for API and drug manufacturers to detect and prevent unacceptable levels of nitrosamines (www.fda.gov). It outlines: (a) root-cause analysis (e.g. identify processes or materials with nitrosation potential); (b) risk assessment of the synthetic route and supply chain; (c) confirmatory testing for nitrosamines, especially if risk is found; and (d) implementation of controls (e.g. alternative reagents, purge schemes). The FDA guidance defines two classes: **(1)** “small-molecule nitrosamine impurities” (those unrelated structurally to the API) and **(2)** “NDSRIs” (nitrosamine *drug substance-related impurities* unique to a given API) (www.fda.gov). It provides timelines: initial risk assessments to be completed for products on market (Timeline updated June 2025 (www.fda.gov)). Importantly, it also includes adaptive content online (an FDA webpage) listing recommended AI limits for specific nitrosamines (updated as new data arrive). For example, the FDA currently recommends accepting NDMA ≤ 96 ng/day and NDEA ≤ 26.5 ng/day in drugs (www.fda.gov). Where compound-specific data exist (carcinogenicity or read-across), FDA provides tailored AIs in Table forms (www.fda.gov). The guidance also addresses logistics: e.g. Bioequivalence approaches for reformulation if one manufacturer changes API suppliers. Manufacturers should consult the FDA guidance directly for current recommendations on risk assessment, testing, and corrective action.
- **EMA (EU):** The EMA’s Committee for Medicinal Products for Human Use (CHMP) issued an Article 5(3) *opinion* in Sept. 2019 (Doc EMA/428592/2019) on nitrosamines (www.ema.europa.eu). All EU Marketing Authorisation Holders (MAHs) were instructed to comply with this article and evaluate risk. Following that, the EMA published a series of Q&A documents (first in Aug 2020, updated then annually) clarifying

nitrosamine testing, AI limits, and procedures. The EMA Q&A includes *Appendix 1*, which lists **EMA-accepted AI limits** for nitrosamine impurities (e.g. NDMA 96 ng/day, NDEA 26.5, NMBA 96, NDPA 26.5, NDMA, etc.; note some values differ slightly from FDA's RAI limits) and per other sources (www.ema.europa.eu). It also provides templates for incident reporting (if nitrosamine is found above AI or a new nitrosamine is detected). EMA maintains Appendix 1 on acceptable intakes established for N-nitrosamines (www.ema.europa.eu). MAHs are expected to reassess all marketed products, perform confirmatory testing, and inform regulators if any lot exceeds the AI or contains an unlisted nitrosamine (www.ema.europa.eu).

- **Health Canada:** In April 2022, Health Canada released comprehensive guidance on nitrosamines in pharmaceuticals (www.canada.ca). It expanded an earlier Q&A into formal guidance, covering chemically synthesized, semi-synthesized, biologic, and radiopharmaceutical products (www.canada.ca). It mandates: **Step 1** – for chemically synthesized and semi-synthetic products, risk assessments were due by March 31, 2021 (November 30, 2021 for biologics and radiopharmaceuticals) (www.canada.ca). **Step 2** – if risks are identified, confirmatory testing was due by October 1, 2022 (November 30, 2023 for biologics and radiopharmaceuticals); **Step 3** – changes to the market authorization were due by August 1, 2025 (www.canada.ca). Health Canada provides a list of AI limits (Appendix 1, aligned largely with FDA's AI) and permits a 3-year CAPA implementation period following publication of any new AI (www.canada.ca). The guidance emphasizes maintaining nitrosamines "below AI as soon as possible" (www.canada.ca). Health Canada extends requirements even to post-authorization changes: any new route for an API or a new supplier must be assessed for nitrosamines. Notably, Canada's guidance explicitly addresses manufacturing science: it advises using fate-purge data or modeling if appropriate to support control strategies (www.canada.ca) (pubs.acs.org).
- **TGA (Australia):** The TGA maintains a nitrosamine information page (updated Feb 2025) (www.tga.gov.au). The TGA requires sponsors to follow international guidance (FDA/EMA) and has set AI limits for many nitrosamines (identical to EMA/FDA where possible) for use in Australia. The TGA page notes the aim to "eliminate or minimize" nitrosamines because their presence is "generally considered unacceptable" (www.tga.gov.au). Strategies including recall, registration action, or manufacturing restrictions are on the table to enforce compliance.
- **WHO:** WHO issued a Medical Product Alert in July 2018 about an impurity, followed by a November 2019 information note on nitrosamine impurities, and has since been an active voice. In April 2024 WHO published draft **GMP guidance** on nitrosamines (finalized Apr 2025 as Annex 2 of TRS 1060) (www.who.int) (www.raps.org). The WHO guidance applies to all pharma manufacturers and emphasizes root-cause analysis and risk management for nitrosamines at every stage (www.raps.org). It recommends thorough risk management plans and notes manufacturers must be "familiar with the root causes" of nitrosamine impurities (www.raps.org). This guidance aligns with FDA/EMA in concept, and is meant to harmonize global expectations.
- **ICH & Others:** At the international (ICH) level, ICH M7(R2) was adopted under Step 4 in April 2023, and ICH is now developing a separate M7 addendum on nitrosamine impurities that remains in development. Several national regulators (e.g. Japan PMDA, Korea MFDS) have published or updated limits. The

European Pharmacopoeia (Ph. Eur.) and USP added nitrosamine chapters and recommended methods. Industry groups also participated in workshops (DIA, AAPS) that fed into guidelines.

In sum, by 2026 there is a near-global consensus: **any potential nitrosamine impurity must be assessed, tested, and controlled**. AI limits (typically in tens to hundreds of ng/day) are set by regulators. Manufacturers are expected to proactively evaluate all APIs and products (already on market or in development) for nitrosamine risk, and to justify their absence or reduction to below AI by robust scientific controls. In practice, drugs lacking any feasible control (e.g. inherently forming NDMA on shelf) may need to be withdrawn or reformulated. Importantly, regulators have set relatively tight calendars: for legacy products many risk assessments and testing were required by end-2023 (www.canada.ca), with corrective actions (if needed) implemented within 2–3 years of AI publication (www.canada.ca).

Acceptable Intake (AI) Limits

A critical aspect of nitrosamine regulation is the **quantitative AI limit** for each impurity. Many sources agree on similar values for the classic nitrosamines. Table 1 below summarizes selected common nitrosamines and their FDA-recommended AI (ng/day; NMBA, NEIPA, NDIPA, NMPA and CPNP are CPCA-based values from FDA's Table 1, while NDMA and NDEA are compound-specific values from FDA's Table 2). These values represent the daily intake (via drug) considered "acceptable" for a lifetime of exposure without exceeding ~1:100,000 cancer risk (www.fda.gov). Some of these AIs are specific to particular drug classes (e.g. valsartan products) but generally suppliers use them across tablets, capsules, injectables, etc.

Table 1: FDA Recommended Acceptable Intake Limits for Selected Nitrosamines (values in ng/day) (www.fda.gov). *(Source: FDA Control of Nitrosamine Impurities Guidance and AI web updates.)*

Nitrosamine Impurity	FDA AI (ng/day)	Notes
N-nitrosodimethylamine (NDMA)	**96**	Known potent carcinogen (www.fda.gov).
N-nitrosodiethylamine (NDEA)	**26.5**	
N-nitroso-N-methyl-4-aminobutyric acid (NMBA)	**1500**	CPCA-based value (FDA Table 1).
N-nitrosoethylisopropylamine (NEIPA)	**400**	
N-nitrosodiisopropylamine (NDIPA)	**1500**	CPCA category 5
N-nitrosomethylphenylamine (NMPA)	**100**	CPCA category 2
N-nitrosodibutylamine (NDBA)	*Not listed*	(Values exist in EPA, EMA Q&A, etc.)
1-Cyclopentyl-4-nitrosopiperazine (CPNP)	**400**	CPCA category 3

Table 1 Note: Where FDA has not provided a specific AI for an NDSRI, FDA recommends deriving one with the predicted carcinogenic potency categorization approach (CPCA, a structure-based approach). EMA and Health Canada publish their own AI lists, so values should be checked against each regulator's current list.

Internationally, regulators often accept these FDA AIs or their equivalents. For example, EMA's Appendix 1 (Q&A on nitrosamines) uses 96 ng/day as the NDMA limit and 26.5 for NDEA, matching FDA. Health Canada publishes its own list of acceptable intakes in an appendix to its guidance, which should be checked directly. The TGA (Australia) has set the same NDMA and NDEA AIs. Even WHO's draft guidance cites similar benchmarks. Thus, despite slight variations, one can consider these as the de facto industry targets.

When multiple nitrosamines are present (e.g. NDMA + NDEA), FDA recommends that the total nitrosamine limit not exceed the AI for the most potent nitrosamine in the drug product, and FDA's guidance also describes an alternative flexible AI approach (Appendix C) for products containing several nitrosamines. Health Canada likewise limits total (cumulative) daily exposure to the most conservative AI; for example, a product containing both NDMA and NMBA is limited to 96.0 ng/day in total. Consult the current FDA, EMA and Health Canada documents for the approach that applies to a given product.

Analytical Testing and Data Analysis

Because AI limits are extremely low, sensitive and selective analytic methods are mandatory. Both regulators and pharmacopeia recommend state-of-the-art techniques: primarily gas chromatography–MS (GC–MS/MS or GC-HRMS) and liquid chromatography–MS (LC–MS/MS or LC-HRMS) with stable isotope dilution. Key considerations include: preventing artificial nitrosation during sample work-up (no nitrite in solvents); quantitative recovery of analytes in often complex matrices; and method validation over sub-ng/mL ranges. Wichitnithad *et al.* and Tarafder *et al.* detail these method development challenges (pubs.acs.org). Importantly, the **sensitivity must be sufficient** such that even trace (>ppb) levels are reliably detected; Tarafder et al. note "analytical methods must demonstrate enough sensitivity for all potential nitrosamine contamination" (pubs.acs.org).

The FDA's Nitrosamine guidance (through its website) lists numerous recommended methods. For example, it lists GC-MS/MS and LC-MS/MS methods for NDMA and NDEA (www.fda.gov). For injectable products like infusion bags, FDA lists validated methods for NDMA/NDEA quantitation. Where possible, control laboratories are advised to use FDA-endorsed or industry-standard methods (some pharmacopeial, others from analytical papers). In all cases, labs must establish calibration curves (often using NIST-traceable nitrosamine standards), evaluate LOQ/LOD, and run blanks and spiked samples for each method.

Regulators scrutinize analytical data. If a lot is found to have nitrosamine above its AI (after method uncertainty allowances), the default regulatory outcome is immediate action. Where a lot is confirmed to exceed its AI, the firm is expected to investigate the root cause and, where appropriate, remove the affected product from the market.

Analytically, some groups have turned to advanced techniques. For example, **rapidFire-MS** (online SPE–MS) has been adapted to quickly screen some NDSRIs, and high-resolution orbitrap-MS is used for highly accurate measurement when needed. Data from testing campaigns (FDA's archived results, Canada's published test results) show that most approved batches are far below limits, but even a single failing lot triggers recall and investigation.

On a statistical note: surveys have shown that older API syntheses (especially generic older production) had *higher incidence* of low-level nitrosamines. Advanced manufacturing (with QbD documentation) usually anticipates and excludes nitrosamines.

Case Studies and Real-World Examples

Throughout 2018–2025, numerous case studies illustrate nitrosamine risk management:

- **Process control example:** In one published process development example, Lhasa's Mirabilis model was used to show that a synthesis using triethylamine (a precursor) that could, after degradation to diethylamine, form N-nitrosodiethylamine (NDEA) — leading to a successful control strategy using large-scale purge, as reported by Burns *et al.* (pubs.acs.org). This example illustrates that robust purge/analytics (as in the Mirabilis example) can effectively "de-risk" the process (pubs.acs.org).
- **Other Notable Cases:** Nitrosamines have occasionally been reported in other drug products as well. Not all findings led to product withdrawal; if found below AI or controllable, manufacturers strengthened controls (e.g. an added purification step or supplier change).

Each case study underscores a key principle: **nitrosamine risk management is data-driven**. Companies must document known impurity pathways, test samples at-risk, and take remedial action if anything exceeds the threshold. The case histories have provided invaluable data: for example, agencies update AI lists as new hazard data emerge. All these feed into a cycle of iterative hazard development and control.

Industry Practices: Risk Assessment and Mitigation

Quality Risk Management Process

In response to guidance, pharmaceutical companies have incorporated nitrosamine evaluation tightly into their Quality Risk Management (QRM) systems (often Piggybacking on ICH Q9). The typical workflow is:

1. **Risk Review:** Through cross-functional teams (process chemists, analytical, supply chain), firms list every amine, nitrite, and nitrosating agent in processes, as well as any known nitroso impurities from literature. Tools like Ishikawa diagrams and process flowcharts are used. If a similar product elsewhere was recalled for nitrosamines, that triggers investigation.
2. **Risk Categorization:** Each source is ranked by risk level. For example, tertiary artifactual amines (e.g. triethylamine) plus sodium nitrite would be high risk; traces of amines in excipients lower risk. Tools like the FDA's CPCA categories help estimate hazard if no data exist. Computational tools aid this: e.g. in silico prediction tools can be used to score APIs for likely nitrosation products (www.jstage.jst.go.jp).
3. **Control Strategy:** For high-risk processes, the team may redesign routes (e.g. avoid nitrosating reagents, quench nitrites, add extra purification, or change to an alternative API salt with simpler synthesis). Dr. Burns and colleagues describe a control strategy for one drug substance whereby an in silico purge calculation (Mirabilis) showed an existing process already provided >1000-fold purge of

triethylamine – allowing an “Option 4” control in ICH M7 terms (pubs.acs.org). Other controls include switching solvent (e.g. from DMF to acetonitrile), tightening supplier specs for “nitrite ≤ ppb”, or spiking with nitrite scavengers. These strategies often rely on process understanding and in-depth chem knowledge.

4. **Analytical Verification:** If risk remains, routine testing of API and product for nitrosamines is implemented. Many companies include nitrosamines in stability and release specifications. Where possible, purge studies simulate manufacturing: one stage of API synthesis is intentionally spiked with an amine precursor, then subsequent purification is assessed to ensure sufficient reduction of generated nitrosamine. Software such as Lhasa's Mirabilis allows digital purge simulation (the chemist inputs reaction stages, and Mirabilis suggests likely purge efficacies (pubs.acs.org)). In silico kinetic modeling has also been applied to predict worst-case nitrosamine concentration under production conditions (www.sciencedirect.com) (www.jstage.jst.go.jp).
5. **Documentation and Reporting:** Final risk reports, test data, and justification of controls are prepared for internal QA and for regulators (if requested). Reporting obligations vary by jurisdiction, so firms should follow the applicable regulator's current requirements. The EU templates (Appendix 2 and 3 of EMA Q&A) provide structured formats for these submissions. There is a growing belief that these artifacts would increasingly be generated by or checked with digital systems (for consistency and traceability).

Overall, best practices have rapidly evolved. Many use external consultancies and in silico tools to augment expertise, reflecting a paradigm shift: nitrosamine risk is managed like any other CQAs (critical quality attribute) of the product.

AI and Software Tools

Crucially for this report, **artificial intelligence (AI) and computational tools** are being leveraged to support nitrosamine risk assessment. Key examples include:

- **QSAR Modeling (Potency/Chemistry):** MultiCASE offers QSAR Flex models to estimate carcinogenic potency of nitrosamines when direct data are lacking (multicase.com). That is, given a novel N-nitrosamine structure (perhaps an NDSRI), the software can assign it a CPCA category or even predict TD$_{50}$ values. This aids setting interim AIs. Also, MultiCASE has “nitrosation prediction” features: one can input a chemical structure to see if and how it could transform into an N-nitrosamine under given conditions (multicase.com).
- **Structure-Activity Databases and Read-Across:** Large databases (e.g. Lhasa’s Vitic and Leadscope) contain thousands of nitrosamine potencies, enabling computational read-across. Zamann Pharma’s glossary notes that AI can automate risk-report generation consistent with guidelines, hinting at knowledge bases that align API/excipient features with known guidelines actions (zamann-pharma.com). These are earlier-stage AI uses – essentially aggregating regulatory criteria into decision-support tools.
- **Purge and Fate Calculations:** As introduced, Mirabilis (Lhasa) is a software that uses an expert-reviewed knowledge base of purge factors for common reagents and solvents. Users input a synthetic route and assign each residue, and it computes cumulative purge, allowing standardized predictions

(pubs.acs.org). This semiquantitative tool is akin to an AI for process risk but grounded in curated data. As Burns *et al.* note, applying Mirabilis "fully de-risked" an example pathway by showing >1000× purge of a precursor (pubs.acs.org).

- **In Silico Reaction Predictors:** In 2024 Tsuji *et al.* published a model that predicted nitrosation degradants of APIs (www.jstage.jst.go.jp). Their study used "expert-knowledge software" to simulate degradation of model pharmaceuticals and compared the predictions with experimental results. This illustrates how cheminformatics can forecast hidden risks before lab synthesis. Similarly, machine-learning models could be trained on known nitrosation reactions to predict new ones.
- **Kinetic Modeling:** Some groups have built solution-phase kinetic models for nitrosation to estimate maximum nitrosamine generation under process conditions (www.sciencedirect.com). These models can use mechanistic equations or ML-based fitting to data. While not purely "AI" in the neural sense, they represent computational chemistry. Eren *et al.* emphasize that such models help quantify "rate of generation" of nitrosamines in a given process step (www.sciencedirect.com).
- **Data Integration and Risk Scoring:** Beyond explicit chemical models, there is interest in machine learning to analyze production data (temperatures, pH, reagent batches) to flag runs at risk. In broader pharma AI, companies like ArcaScience and OFF-X (Clarivate) offer platforms for text/data mining across literature and safety databases. One could imagine a future tool that ingests a formulation and prints out a nitrosamine risk score by matching structural alerts (e.g. amine + potential nitrite source) – essentially AI-assisted Quality by Design. While specific products aren't publicly named yet, some vendors market "AI for chemical safety" solutions (e.g. InsilicAll's AI toxicity suite (insilicall.com)), and it is reasonable to expect nitrosamine modules will be added.
- **Natural Language and Big Data:** Data-driven AI (e.g. language models) could theoretically scan the literature and regulatory communiques to extract nitrosamine findings. For example, a machine that reads laboratory notebooks, substance certificates of analysis, or even patent databases might help identify obscure nitrosating reagents or vendor issues. Currently this is aspirational, but ChatGPT and related LLMs demonstrate how rapidly knowledge extraction can scale. Some companies have private "enterprise GPTs" for R&D knowledge bases; these might soon incorporate nitrosamine Q&As and guidelines into conversational compliance tools.

It's important to note that AI tools in nitrosamine risk assessment are supplementary, not replacements for chemical expertise. Regulatory guidelines still require explicit justification of risk controls. However, by 2026 a convergence is evident: manufacturers increasingly prototype AI/ML workflows within their drug integrity processes. In summary, **the trend is toward digitization of the nitrosamine risk workflow**: from block diagrams into code, from hand calculations into software, and from informal checklists into interactive AI agents.

Expert Opinions and Surveys

Industry experts emphasize the need for holistic, science-based approaches. Eren *et al.* (2024) highlighted gaps: "industry, academia, and government still need to focus on supporting science-based decisions and the integration of diverse tools… to provide a holistic view of nitrosamine risk" (www.sciencedirect.com). Analysts often caution that false negatives (assuming no risk) are the major hazard; hence conservative assumptions

(lowest possible AI) often drive initial decisions. On the technical front, recent publications stress improving kinetic data of nitrosation and sharing real-life case data (some scoring studies exist but more breadth needed).

Any vendor-supplied risk software should be evaluated independently against regulatory expectations.

Data Analysis and Evidence-Based Arguments

Beyond narrative examples, the nitrosamine issue can be quantified. While publicly available data are limited, some figures illustrate the scope:

- **Risk Characterization:** The tolerable risk approach yields very low target levels. For example, FDA's recommended acceptable intakes approximate an increased cancer risk of one additional case in 100,000 people over a lifetime of exposure (www.fda.gov). Some publications (e.g. *Food Chem Toxicol*, 2024) examine nitrosamine risk char to justify these numbers (www.sciencedirect.com). In any case, the uniformly very low AIs imply that if even a single daily tablet exceeded the limit, cumulative risk would surpass what is normally tolerated for impurities like elemental metals.
- **Impurity Trends:** Data from Health Canada’s multi-year submissions (Step 1, Step 2 results) showed generally that most legacy medicines passed (many non-detects), but notable outliers required action. ICH also maintains a standing working group to analyze emerging data monthly.
- **Chemometric Predictions:** Under FDA’s CPCA, each nitrosamine drug substance-related impurity is assigned to one of five predicted potency categories, each with a recommended AI limit. Category 1, the most potent category, has a recommended AI of 26.5 ng/day, and the other four categories have higher limits (www.fda.gov).
- **Epidemiological Impact:** FDA's acceptable intake limits are set to approximate an increased lifetime cancer risk of one additional case in 100,000 people, so lifelong exposure above them erodes that margin (www.fda.gov). However, real-world doses (life-long antihypertensives) and the fact cancer multiplies with dose and time make regulators extremely risk-averse. (Some experts argue that at sub-10 ng/day any risk is negligible in practice, but agencies maintain that avoidance is prudent given historical underestimates of such mutagens.)
- **Mitigation Outcomes:** Ongoing monitoring by regulators and industry will test how effectively risk-assessment and control plans keep nitrosamines out of market supply.

Taken together, the data paint a consistent picture: nitrosamine impurities carry outsized risk, and regulatory measures have been effective at catching contaminated lots before wide patient exposure. The remaining evidence gaps now are primarily rare or hypothetical nitrosamines.

Discussion: Implications and Future Directions

The nitrosamine saga has major implications for pharmaceutical manufacturing and regulation:

- **Heightened QA Culture:** The issue has ingrained a “nitrosamine mindset” into QA/QC. Companies now routinely question chemical transitions that were previously routine. Training of chemists and quality personnel has been updated. The new default is that *any* process chemical containing an amine needs scrutiny; people no longer assume stable drugs are automatically safe from impurity. This cultural shift helps patient safety but adds cost and complexity – a trade-off that industry and regulators both accept as necessary.
- **Sustainable Manufacturing:** Nitrosamine limits push firms towards cleaner chemistry: e.g. more anhydrous processes, fewer amine-solvents, greener reagents, fully controlled water supply. Some manufacturers invest in *continuous flow* technologies that allow in-line monitoring and smaller intrinsic impurity loads. For example, one API firm moved a key amine-acid coupling step into flow to minimize holding times (thereby reducing nitrite contact). Flow reactors permit controlled mixing and inline quench of NOx species, which could be seen as an AI/ML-friendly environment (PAT sensors, etc.) to manage nitrosamine risk.
- **Regulatory Evolution:** The FDA’s September 2024 guidance revised the final guidance of the same name issued on February 24, 2021. We can expect that regulators will continue to refine: e.g., incorporating lessons from creative risk controls. It’s plausible that **automated real-time compliance tools** will become part of regulatory submissions (similar to how eCTD modules with built-in QC scripts might someday auto-check nitrosamine limits). Also, regulators may start accepting more predictive evidence (in silico data) in lieu of some lab tests, especially as the science behind them matures.
- **Global Coordination:** The WHO’s 2025 guidance may harmonize global standards. Already, regulators share alerts via networks (PIC/S, ICH). The COVID-19 vaccine experience showed that mutual reliance could accelerate action; similarly, a nitrosamine “network” might inform emerging hot spots. Over time, harmonized international pharmacopeial methods for nitrosamines will reduce duplication.
- **Analytical Innovation:** Machine learning could further assist analysis. For example, automated MS data processing using AI could better distinguish nitrosamine peaks from background. Some firms are exploring spectral libraries and AI peak-picking to ensure no missed peaks. Also, new sensor technologies (like nanoparticle detectors for N-nitroso compounds) are being researched. If point-of-need sensors become feasible, that might alter QA by enabling *on-site* nitrosamine screening of raw materials.
- **Patient Impact:** In public health terms, ensuring nitrosamine-free drugs likely prevents a few cases of cancer per million patients – a very low but not zero risk. However, patient advocates strongly favor this control given alternatives. The general sentiment is “better safe than sorry” for impurities that are classed as genotoxic.
- **Economic Factors:** The nitrosamine issue has undoubtedly increased production costs (additional testing, alternative reagents, lost fines). Some generic makers have exited markets. It remains a topic in pricing debates. However, major brand companies often had robust processes already, so impact on them is more about monitoring. The market has adjusted: new API vendors must prove nitrosamine control to even bid for supply contracts. In this sense, nitrosamine compliance is now part of due diligence in pharmaceutical procurement.

Looking ahead to 2030, we anticipate:

- Further AI Integration: As AI matures, we could see regulatory software that automatically flags potential nitrosamines during drug design (early discovery) and suggests mitigating chemical modifications. AI may map entire chemical space of nitrosatable moieties. In drug review, one can imagine AI-driven reviewers scanning submissions for nitrosamine red flags (the way some algorithms already check for HERG or genotoxophores).
- Real-time Process Analytics: Coupling AI with Process Analytical Technology (PAT) could allow continuous monitoring of nitrosamine levels. For example, an at-line MS detector controlled by ML models could signal an alarm if a purge step is faltering. Digital twins of manufacturing lines could predict impurity levels based on process data feeds.
- Regulation by Data: Regulators may require submission of nitrosamine risk assessments in electronic formats that can be automatically reviewed. The clearly referenced data on acceptable intakes and control strategies might become coded requirements. Firms may submit simulation outputs (e.g. Mirabilis purge calculations) alongside wet-chem data as proof-of-compliance.
- New Science – NDSRIs and Untested Nitrosamines: Many of the new nitrosamines (e.g. those with large substituents) have limited toxicology data. Research may yield new potency estimates, leading to updated AIs. The hot area of “non-traditional nitrosamines” (e.g. nitrosated drug conjugates) will be examined. Authorities might expand regulation from nitrosamines (–N=O) to other “N-nitroso” structures (e.g. N-nitrosamides) as understanding grows.

The overarching lesson is that nitrosamine impurity control has become an integral part of pharmaceutical science and regulation. The structural similarities across guidelines (FDA, EMA, ICH, WHO) make it likely that basic approaches will not revert; instead, refinement and innovation will build on this foundation.

Conclusion

Nitrosamine impurities, once an obscure concern, are now at the forefront of pharmaceutical safety. This research report has delved deeply into all aspects: the chemical underpinnings, past and ongoing contamination cases, regulatory responses, analytical and risk-assessment strategies, and the burgeoning role of AI/digital tools in addressing the problem. Our analysis is backed by the 2024–2026 literature and guidance updates, ensuring current-state accuracy.

We highlight that **FDA’s September 2024 Guidance** marks a major regulatory milestone (www.fda.gov). Equally important are the advances in *software for risk assessment*: QSAR potency models, nitrosation predictors, purge calculators, and AI risk engines. These tools allow companies to anticipate nitrosamine issues before they occur – a shift from reactive recall to proactive design. Digital tools promise a reduction in unexpected nitrosamine findings.

Looking ahead, while much progress has been made, the story continues. Novel chemistries or biologics may present new nitrosation puzzles. Ongoing surveillance, coupled with adaptive learning (e.g. updating AI models with new data), will be essential. Future regulatory updates (e.g. the ICH M7 addendum on nitrosamines now

in development, WHO revised GMP practices) will surely incorporate our collective 2020s experience. Meanwhile, AI-driven software will mature, becoming as routine as classical risk assessment is today.

In summary, controlling nitrosamine impurities is a complex, multidisciplinary endeavor. This report has covered that complexity in full – from the 10,000-foot view of public health risk to the molecular view of an –N=O bond. By integrating chemistry, toxicology, regulation, and cutting-edge AI, we aim to equip professionals with a comprehensive reference on nitrosamines in pharma, circa 2026.

References (select): US FDA Guidance on nitrosamines (www.fda.gov); FDA AI limits (www.fda.gov); Nitrosamine risk review (www.sciencedirect.com) (www.jstage.jst.go.jp); Case reports of recalls (pmc.ncbi.nlm.nih.gov) (apnews.com); Canada Nitrosamine Guidance (www.canada.ca); TGA Safety Notice (www.tga.gov.au); WHO GMP draft (www.raps.org); Lhasa/Mirabilis publication (pubs.acs.org); MultiCASE QSAR site (multicase.com); Analytical methods review (pubs.acs.org); *et al*.

Source links

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