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NITROSAMINE DRUG SUBSTANCE-RELATED IMPURITIES: COMPARATIVE FDA AND EMA REGULATORY FRAMEWORKS, ANALYTICAL CHALLENGES, AND SUPPLY IMPLICATIONS

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ABSTRACT

Nitrosamine drug substance-related impurities (NDSRIs) became a significant global concern after nitrosamines were found in angiotensin receptor blockers and other medicines. Both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) aim to reduce patient exposure to these potentially harmful impurities, but they differ in their legal authority, how they set acceptable intake (AI) limits, implementation timelines, reporting requirements, and flexibility. This review examines how the FDA and EMA currently manage NDSRI control, covering their legal foundations, how they set AI limits, assess structure-activity relationships, use the Carcinogenic Potency Categorization Approach (CPCA), conduct confirmatory testing, handle regulatory submissions, and manage products throughout their lifecycle. It also covers analytical method development, supply chain effects, and new mutagenicity testing methods, such as transgenic rodent assays and error-corrected next-generation sequencing. The review finds that FDA guidance allows product-specific flexibility when patient access is at risk. In contrast, the EMA uses a more formal process via the Committee for Medicinal Products for Human Use (CHMP) Article 5(3) opinion. Greater harmonization, better compound-specific data, and improved analytical

and toxicological tools could help make global standards more consistent and protect access to important medicines.

KEYWORDS: Acceptable Intake; CPCA; EMA; FDA; NDSRI; Regulatory harmonization.

INTRODUCTION

The discovery of N-nitrosodimethylamine (NDMA) in angiotensin receptor blocker (ARB) drug products in 2018 marked the beginning of a major pharmaceutical quality issue. As investigations continued, nitrosamines were detected in several other marketed medicines, prompting the FDA and EMA to establish frameworks to identify, assess risk, control, and report nitrosamine drug substance-related impurities (NDSRIs).[1,2] NDSRIs are nitrosamines structurally related to the active pharmaceutical ingredient (API). Unlike small-molecule nitrosamines such as NDMA and N-nitrosodiethylamine (NDEA), NDSRIs are usually product-specific and can form through nitrosation of the drug substance or related structural fragments.[1]

This article compares how the FDA and EMA handle NDSRI control, focusing on scientific methods, legal foundations, implementation steps, analytical challenges, mutagenicity testing, supply chain effects, and the possible future use of next-generation sequencing to help set AI limits. Here, AI refers to the daily amount of a nitrosamine impurity considered to pose an acceptable lifetime cancer risk.

Regulatory framework: U.S. vs. EU

U.S. approach (FDA)

FDA's approach is mainly based on the August 2023 guidance on Recommended Acceptable Intake Limits for NDSRIs (the RAIL Guidance) and the September 2024 Revision 2 guidance on Control of Nitrosamine Impurities in Human Drugs.[3,4] These guidances are supported by a regularly updated Center for Drug Evaluation and Research (CDER) webpage that provides current AI limits, implementation timelines, scientific updates, recommended analytical methods, and a revision history.[5]

FDA uses International Council for Harmonisation (ICH) M7 principles as the scientific basis for nitrosamine risk assessment. The assessment may include structure-activity relationship (SAR) analysis, CPCA, read-across from related compounds, and, when available, compound-specific data.[3,6] CPCA places nitrosamines into potency categories based on

structural features, allowing FDA to recommend AI limits even when compound-specific carcinogenicity data are unavailable.[3]

FDA distinguishes conventional small-molecule nitrosamines from NDSRIs based on their relationship to the API. Whereas small-molecule nitrosamines such as NDMA and NDEA are generally unrelated to the API structure, NDSRIs arise from API-related amine-containing structures and are often unique to individual products.[4]

A key feature of the FDA approach is product-specific and risk-based flexibility when patient access may be affected. During the ARB contamination issue, FDA published interim AI limits for NDMA, NDEA, and N-nitroso-N-methyl-4-aminobutyric acid (NMBA) for marketed ARBs. For example, the same 96 ng/day AI for NDMA corresponds to different ppm limits depending on each drug product's maximum daily dose.[7,8] FDA may also establish interim AI limits in specific circumstances where additional data are being generated, or immediate enforcement could affect patient access.[3,5]

EU approach (EMA)

EMA’s approach is based on the CHMP Article 5(3) opinion issued after detecting nitrosamine impurities in sartans in 2018.[9] Unlike FDA guidance, which is formally non-binding but reflects FDA’s current regulatory expectations for industry, the CHMP Article 5(3) opinion is implemented through a coordinated EU framework that assigns specific responsibilities to marketing authorization holders (MAHs). The framework applies to centrally and nationally authorized products, with implementation coordinated through the European medicines regulatory network, including the Co-ordination Group for Mutual Recognition and Decentralized Procedures - Human (CMDh) for nationally authorized products.[10]

The EMA framework follows a three-step process: Step 1, risk evaluation; Step 2, confirmatory testing; and Step 3, update of marketing authorizations where changes are required. Where confirmatory testing identifies nitrosamines above the applicable AI limit, or detects a previously unassessed nitrosamine requiring further evaluation, the MAH should notify the relevant competent authority using the applicable EMA/CMDh reporting procedures. Step 3 includes implementing corrective and preventive actions (CAPAs) and submitting any necessary marketing authorization variations.[11]

To establish AI limits, EMA uses CPCA, as described in Appendix 2, together with other evidence where appropriate, including read-across, SAR assessment, Enhanced Ames Testing (EAT), and in vivo data.[12,13] EAT is best understood as supportive evidence of mutagenicity rather than a stand-alone AI-setting method. Appendix 1 lists current AI limits.[14] Oversight and implementation involve EMA, CMDh/national competent authorities, the Nitrosamine Implementation Oversight Group (NIOG), and the European Directorate for the Quality of Medicines & HealthCare (EDQM). EDQM published the first Official Medicines Control Laboratories (OMCL) analytical procedures for NDSRIs and intermediate-related contaminants on 28 March 2024.[15]

Acceptable Intake Limits: FDA and EMA Approaches

FDA and EMA both use a risk-based approach to establish AI limits for nitrosamine impurities, including NDSRIs. The agencies are increasingly aligned through CPCA for N-nitrosamines with limited compound-specific carcinogenicity data. Under CPCA, agencies use structural features to assign a potency category linked to an AI limit. Both regulators also allow compound-specific carcinogenicity or mutagenicity data, read-across, and SAR assessments when available.[3,5,11,14]

Despite this convergence, regulatory differences remain. FDA describes AI recommendations in its 2023 NDSRI guidance, 2024 nitrosamine control guidance, and CDER nitrosamine AI webpage, and may establish interim AI limits where data are still being generated, or immediate enforcement could affect patient access.[3,5] EMA implements AI limits through the CHMP Article 5(3) framework, publishes established limits in Appendix 1 of the EMA/CMDh Q&A, and may incorporate CPCA, read-across, EAT, in vivo data, and other scientific evidence.[11,14]

FDA generally presents AI limits within a guidance-based framework. EMA incorporates AI limits into a regulatory process that includes a risk evaluation, confirmatory testing, and marketing authorization updates where necessary. Despite these procedural differences, both agencies use risk-based toxicological principles to establish and periodically revise AI limits as additional data become available.[5,6,11]

Key differences and comparative summary

Both agencies use similar scientific principles, such as ICH M7 and CPCA, but they differ in how they apply them. **Table 1** shows the main similarities and differences between the FDA

and EMA in controlling nitrosamine and NDSRI impurities, including their regulatory approaches, legal foundations, methods for setting AI limits, expectations for implementation, use of temporary limits, oversight, and reporting rules.

Table 1: Comparative summary: FDA vs. EMA regulatory approaches to nitrosamine/NDSRI control.

Dimension	FDA (US)	EMA (EU)
Regulatory philosophy	Risk-based and flexible; guidance-based; considers patient access and supply continuity[3,5]	Risk-based framework implemented through CHMP Article 5(3) opinion and coordinated EU network expectations.[9,11]
Legal/regulatory basis	FD&C Act, CGMP regulations, and FDA nitrosamine guidance documents.[4,16]	CHMP Article 5(3) opinion and EMA/CMDh implementation framework.[9,11]
AI methodology	CPCA, SAR, read-across, and mutagenicity/carcinogenicity data.[3,4,6]	CPCA, SAR/read-across, EAT, Appendix 1 AI limits, and other supporting data.[11,14]
Implementation	Risk assessment, confirmatory testing, mitigation, regulatory reporting, and flexible timelines where justified.[4,5]	Three-step review: risk evaluation, confirmatory testing, and marketing authorization update/variation.[10,11]
Temporary limits	Interim AI limits may be established case-by-case to reduce supply disruption.[5,8]	Temporary higher limits may be accepted where scientifically justified during remediation.[11]
Oversight	CDER/OPQ, with FDA compliance and drug-shortage functions involved where relevant.[4,5]	EMA, NCAs/CMDh, NIOG, and EDQM.[10,11,15]
Reporting	eCTD submissions, annual report updates, supplements/variations as applicable, and NDSRI updates.[4,5]	Structured Step 1/2 reporting and Step 3 variation submissions.[10,11]

AI, acceptable intake; CDER, Center for Drug Evaluation and Research; CGMP, current good manufacturing practice; CHMP, Committee for Medicinal Products for Human Use; CMDh, Co-ordination Group for Mutual Recognition and Decentralised Procedures - Human; CPCA, Carcinogenic Potency Categorization Approach; EAT, enhanced Ames test; EDQM, European Directorate for the Quality of Medicines & HealthCare; EMA, European Medicines Agency; eCTD, electronic common technical document; FDA, Food and Drug Administration; FD&C Act, Federal Food, Drug, and Cosmetic Act; MAH, marketing

authorization holder; NCA, national competent authority; NDSRI, nitrosamine drug substance-related impurity; NIOG, Nitrosamine Implementation Oversight Group; OPQ, Office of Pharmaceutical Quality; SAR, structure-activity relationship.

NDSRIs as a Distinct Subset of Nitrosamine Impurities

NDSRIs are a unique regulatory and analytical challenge because they are structurally related to the active pharmaceutical ingredient (API). Unlike small-molecule nitrosamines such as NDMA or NDEA, NDSRIs are usually product-specific and can form when the drug substance or its related fragments undergo nitrosation.[4] This often happens if an API with certain types of amines is exposed to nitrosating conditions, such as leftover nitrite in excipients or acidic environments during manufacturing or storage.[4,11] **Figure 1** compares how small-molecule nitrosamines and NDSRIs form, showing that NDSRIs come from API-related amine groups or breakdown products under nitrosating conditions.

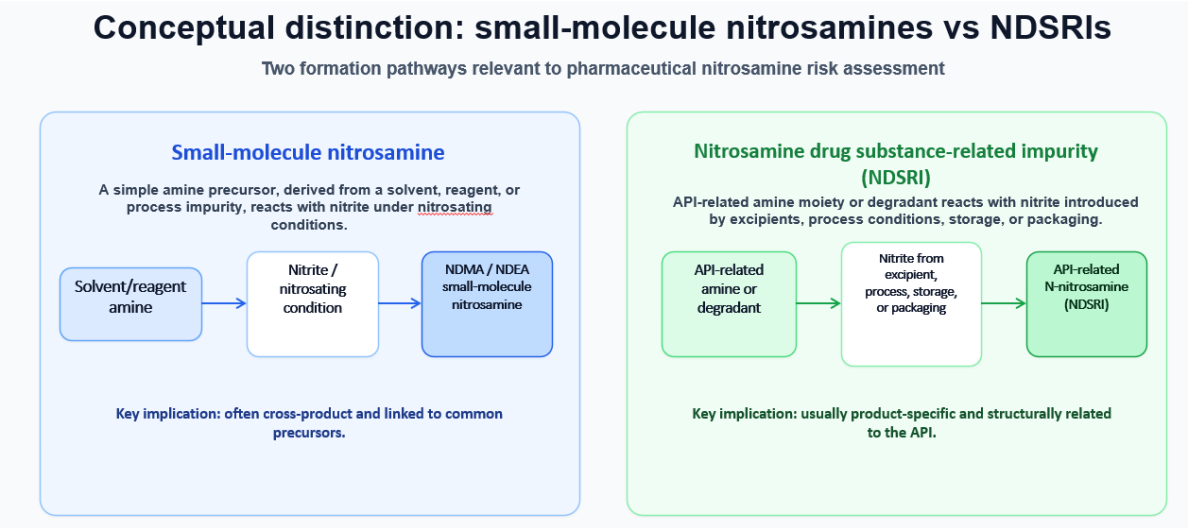


Figure 1. Conceptual comparison of conventional small-molecule nitrosamine formation and NDSRI formation.

Source: Created by the authors based on concepts described in FDA and EMA nitrosamine guidance.[3,4,11] API, active pharmaceutical ingredient; NDMA, N-nitrosodimethylamine; NDEA, N-nitrosodiethylamine; NDSRI, nitrosamine drug substance-related impurity.

The discovery of NDSRIs changed how impurities are controlled. While traditional impurity management looks at dose and duration thresholds, NDSRIs are managed under the ICH M7 framework for DNA-reactive impurities. ICH M7 sets a threshold of 1.5 micrograms per day for lifetime exposure, but many nitrosamine AI limits are even lower, based on their cancer risk.[3,6] Standard HPLC methods often cannot detect nitrosamines at these low levels. As a

result, controlling NDSRIs requires a comprehensive approach that includes API synthesis, excipient compatibility, packaging, storage, and degradation.[1,4,11]

NDSRI analytical method and control

Analytical methods

Because NDSRIs have very low AI limits, they need highly sensitive and specific analytical methods. In practice, confirmatory testing usually uses mass spectrometry techniques. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is often chosen for NDSRIs that are non-volatile, heat-unstable, polar, or complex, while gas chromatography-mass spectrometry (GC-MS) and GC-MS/MS are used more for volatile small-molecule nitrosamines when the sample is suitable for GC. The FDA's CDER nitrosamine webpage lists recommended confirmatory methods, such as LC-MS/MS, LC-HRMS, GC-MS, and GC-MS/MS, depending on the nitrosamine type, dosage form, and testing goal.[5]

As understanding of nitrosamines grew, the FDA expanded its recommended analytical methods. They moved from early headspace GC-MS for volatile nitrosamines to include direct-injection GC-MS, LC-HRMS, RapidFire-MS/MS, and LC-MS/MS for compounds that need better separation and selectivity. This is especially important for NDSRIs, which are often larger, less volatile, and more complex than NDMA or NDEA, making them harder to analyze with standard GC methods. Reviews also highlight that choosing the right method involves considering matrix interference, ion suppression, extraction recovery, nitrosamine instability, and sample preparation.[5,17]

LC-MS/MS is often preferred for NDSRIs because it can detect and measure very low levels, offers high selectivity, and works well with complex drug products. GC-MS/MS is still important for volatile nitrosamines and can measure low concentrations in the right samples. However, ranitidine can break down during GC analysis, leading to false NDMA results. The FDA asked that ranitidine products be removed from the market after finding that NDMA levels could rise during testing. For these cases, liquid chromatographic methods are usually better. The high cost and complexity of mass spectrometry equipment remain challenges, especially for smaller manufacturers and contract labs.[1,17]

Dosage-Form Considerations and Control strategies

The risk of NDSRI formation varies by dosage form. In solid oral forms, NDSRIs can come from API breakdown or nitrite impurities in excipients, and modified-release products may

have longer contact between the API and excipients during storage. In liquid forms, higher molecular movement and pH can increase nitrosamine formation. Sterile products face extra challenges because low daily doses mean lower allowed concentrations, and the FDA has found that packaging materials can also be a source of nitrosamines or nitrites.[4,5,11] The specific formulation, such as the use of polymers and how excipients interact in complex products, can also affect impurity formation, stability, and how these are controlled.[18]

To control NDSRIs, strategies include redesigning processes, managing raw materials, solvents, and excipients, controlling nitrite levels, choosing suitable packaging, and monitoring stability. The FDA focuses on risk assessment, confirmatory testing when needed, and reporting or making changes to prevent or lower nitrosamine impurities.[4,5] The EMA also includes corrective and preventive actions (CAPAs) and any needed changes to marketing authorizations in Step 3 of its framework.[11] Both agencies stress the importance of identifying the root cause, implementing robust process controls, and managing products throughout their lifecycles to keep nitrosamine levels within safe limits.[4,11]

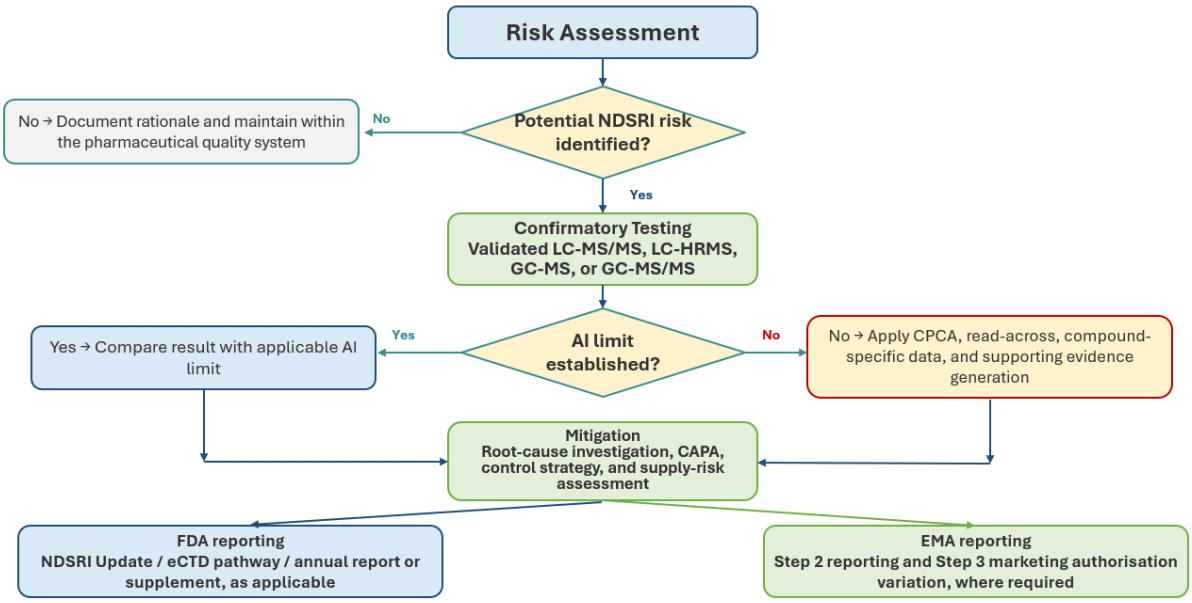
Regulatory submission expectations

New drug applications need to include nitrosamine risk assessments, reasons for any proposed AI limits, and data on controls and stability.[4,16] For products already on the market, the FDA uses the NDSRI Update in eCTD section 1.13.14 for certain updates, while in the EU, marketing authorization holders follow the required Step 1, Step 2, and Step 3 procedures.[5,11] In the future, digital tools like machine learning could help spot gaps in submissions related to impurity risk, method validation, and lifecycle reporting earlier.[19]

Planning is especially important for generic drugs, as complete and timely submissions can help avoid approval delays.[20]

Since the FDA and EMA have different implementation details, manufacturers should coordinate their global strategies to avoid duplicating work. **Figure 2** shows a simplified decision tree for handling NDSRIs in both markets, helping manufacturers move from risk assessment to confirmatory testing, set AI limits, take mitigation steps, and meet each region's specific reporting requirements.

Figure 2. Dual-market NDSRI decision tree for FDA and EMA regulatory management



Source: Created by the authors based on concepts described in FDA and EMA nitrosamine guidance and acceptable intake frameworks. [3-5,11-15]AI, acceptable intake; CAPA, corrective and preventive action; CPCA, Carcinogenic Potency Categorization Approach; eCTD, electronic common technical document; NDSRI, nitrosamine drug substance-related impurity.

Table 2 expands this decision tree into a practical compliance comparison, comparing the FDA and EMA pathways.

Table 2: The Dual-Market NDSRI Compliance and Triage Matrix.

Phase	Operational Step	FDA Pathway (U.S.)	EMA Pathway (EU)
Vulnerability assessment	Identify amine precursors and environmental nitrite risks.	Evaluate API, excipients, manufacturing process, packaging, and storage conditions in accordance with Nitrosamine Guidance Rev. 2[4]	Conduct Step 1 risk evaluation of active substances and finished products, including manufacturing processes, packaging, storage conditions, and potential sources of cross-contamination.[11]
Limit determination	Assign the acceptable intake (AI) limit.	Cross-reference the CDER AI list/webpage; if no AI exists, apply CPCA, read-across, or compound-specific data.[3,5]	Cross-reference Appendix 1; if no AI exists, apply CPCA and supporting evidence such as SAR, read-across, EAT, or other relevant data.[11,14]
Confirmatory testing	Validate ultra-trace analytical methodology.	Validate fit-for-purpose LC-MS/MS, LC-HRMS, GC-MS, or GC-MS/MS methods that align with CDER-recommended procedures,	Validate suitable LC-MS/MS, LC-HRMS, GC-MS, or GC-MS/MS methods based on analyte characteristics and product matrix; consider EDQM OMCL

		analyte volatility, and product matrix.[5]	procedures and Ph. Eur. expectations where applicable.[11,15]
Regulatory reporting	Submit or report findings when limits are exceeded or when a new nitrosamine requiring evaluation is identified.	Submit NDSRI updates and mitigation information via the appropriate regulatory submission pathway, including root cause analysis and implementation status.[5]	Report confirmed nitrosamine findings using the applicable EMA/CMDh Step 2 mechanism and submit Step 3 marketing authorization variations where changes are required.[11]
Mitigation and supply	Prevent or minimize supply disruption while implementing corrective actions.	Seek product-specific regulatory alignment when interim controls are required to maintain patient access while mitigation measures are implemented.[5,21]	Where justified, temporary limits may be accepted while CAPAs are implemented and necessary marketing authorization variations are completed.[11]

AI, acceptable intake; API, active pharmaceutical ingredient; CAPA, corrective and preventive action; CDER, Center for Drug Evaluation and Research; CPCA, Carcinogenic Potency Categorization Approach; EAT, enhanced Ames test; EDQM, European Directorate for the Quality of Medicines & HealthCare; EMA, European Medicines Agency; FDA, Food and Drug Administration; GC-MS, gas chromatography-mass spectrometry; GC-MS/MS, gas chromatography-tandem mass spectrometry; LC-HRMS, liquid chromatography-high-resolution mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MAH, marketing authorization holder; NDSRI, nitrosamine drug substance-related impurity; OMCL, Official Medicines Control Laboratory; Ph. Eur., European Pharmacopoeia; SAR, structure-activity relationship.

Supply-Chain and Implementation Implications

Controlling NDSRIs can strain the supply chain because confirmatory testing, method validation, reformulation, excipient checks, and regulatory submissions all take time and resources. The FDA sometimes uses interim or product-specific limits to balance the long-term cancer risk with the immediate risk of stopping treatment.[5,8] The EMA also allows temporary limits, if justified by science, during the remediation process in Step 3 of its framework.[11]

Manufacturers working in both regions face challenges in scientific assessment and coordinating operations. One global investigation should cover root cause analysis, analytical validation, stability checks, batch testing, and regulatory communication for both the FDA and EMA.[4,5,11]

Emerging Mutagenicity Methodologies and Future Risk Assessment

The mutagenicity assays used to support NDSRI AI limits remain an area of active scientific development. The Ames test and the transgenic rodent (TGR) gene mutation assay, described in EMA Appendix 3 and ICH M7(R2), are important tools, but their performance can vary for structurally complex NDSRIs.[6,13] A multi-sector collaboration coordinated by the Health and Environmental Sciences Institute Global Genetic Toxicology Technical Committee compared in vitro Ames and in vivo TGR results for 33 NDSRIs and reported 79% concordance in mutagenicity calls.[22] These differences have regulatory implications because a false-negative Ames result later identified as mutagenic by TGR could lead to an insufficiently protective AI, while a false-positive Ames result could trigger unnecessary reformulation, testing, cost, or supply disruption.

The Health and Environmental Sciences Institute (HESI) study also found that for NDSRIs with positive TGR results, estimates of mutagenic potency closely matched estimates of carcinogenic potency.[22] This supports using TGR benchmark dose values to set AI limits and suggests that in vivo data may provide a more accurate basis for risk assessment than relying solely on structure-based predictions. However, interpreting TGR results depends on factors such as the model, tissue, dose, study design, and assay variability.

An important new method is error-corrected next-generation sequencing (ecNGS), especially duplex sequencing (DS). Unlike the TGR assay, which detects mutations using reporter genes in rodent chromosomes, DS measures DNA mutations directly at the single-nucleotide level.[23] This could help detect rare mutagenic events more accurately and quickly, but it is not yet approved for routine regulatory use in NDSRI decision-making.

The TGR assay met Organization for Economic Co-operation and Development (OECD) and Good Laboratory Practice standards, using liver samples from MutaMouse mice exposed to NDMA. DS found dose-dependent increases in mutation frequency, helped set a no-observed genotoxic effect level, produced benchmark-dose results similar to TGR, and provided detailed mutation patterns.[23] Another study used TGR mutation data and quantum-mechanical modeling to set potency-based AI limits for NDSRIs without strong carcinogenicity data.[24] These combined in vivo and in silico methods may help establish more specific AI limits, but they remain emerging tools rather than full replacements for long-term rodent studies or current regulatory methods.

Table 3 illustrates an operational and scientific comparison of CPCA, TGR assays, and ecNGS/duplex sequencing for NDSRI risk assessment.

Table 3: Operational and scientific comparison of NDSRI risk assessment methodologies.

Operational Vector	CPCA (In Silico Structural)	TGR Assay (In Vivo Phenotypic)	ecNGS / Duplex Sequencing (In Vivo Genomic)
Estimated time to result	Immediate to days. Structure-based categorization using regulatory CPCA rules.[3]	Months. Requires in vivo dosing, tissue collection, transgene recovery, and mutation analysis.[6,22]	Weeks, depending on study design and sequencing workflow. Can analyze mutations directly from tissue DNA and may avoid reporter-gene expression/recovery steps.[23]
Relative financial cost	Low. Based mainly on structural assessment and published regulatory flowcharts.[3]	High. Requires transgenic animals, specialized tissue processing, and labor-intensive mutation analysis.[6,22]	Moderate to high. Sequencing costs are decreasing, but specialized library preparation, bioinformatics, and validation are required.[23,24]
Animal use (3Rs compliance)	None. Entirely structure- and literature-based.[3]	High. Requires transgenic animals, although study designs may be integrated with other in vivo toxicology work where scientifically appropriate.[6,22]	Potentially lower. May use tissues from existing in vivo studies, depending on study design, tissue availability, and validation expectations.[23,25]
Usefulness for AI-limit derivation	Useful when compound-specific data are absent, but may be conservative for complex NDSRIs.[3]	Provides compound-specific in vivo mutagenicity data that may support benchmark-dose modeling and potency assessment, but interpretation depends on model, tissue, dose selection, and assay variability.[6,22]	Potentially provides direct mutation-frequency data and mechanistic spectra, but regulatory acceptance of routine AI-limit derivation is still under development.[23,24]

AI, acceptable intake; BMD, benchmark dose; CPCA, Carcinogenic Potency Categorization Approach; ecNGS, error-corrected next-generation sequencing; HESI, Health and Environmental Sciences Institute; ICH, International Council for Harmonisation; NDSRI, nitrosamine drug substance-related impurity; TGR, transgenic rodent; 3Rs, replacement, reduction, and refinement of animal use.

Regulatory implications and the path forward

Current ICH M7(R2) guidance and EMA Appendix 3 do not accept ecNGS as a substitute for the TGR assay when evaluating NDSRI mutagenicity. There is no official regulatory framework for how DS or other ecNGS data should be used in NDSRI risk assessments or

compared with Ames, TGR, CPCA, and read-across evidence. The FDA's April 2025 roadmap on new methods outlines steps to reduce, refine, and replace animal testing in preclinical safety studies.[25] ecNGS fits with the 3Rs because it can use tissues from existing animal studies and provide detailed mutation data, but it will need more validation, reference data, performance standards, and guidance before regulators formally accept it.

A bigger challenge is that in silico and in vitro methods may not fully capture metabolic activation and the body's processing of chemicals. This means it is important to be careful when relying solely on CPCA-based AI limits for complex NDSRIs, especially if their metabolism differs from that of the reference nitrosamines used to build the framework.[26] Building larger compound-specific datasets and clear rules for combining different types of evidence will be important for future regulatory confidence.

More regulatory alignment could help make NDSRI expectations more consistent worldwide, especially where regional timelines differ, creating extra work for manufacturers.[27] However, alignment should encourage shared evidence standards, clear data requirements, and coordinated approaches to AI limits, analytical validation, reporting, and supply-sensitive risk management.

CONCLUSION

The FDA and EMA have created thorough frameworks for managing NDSRIs. Both use ICH M7 principles and CPCA but differ in legal authority, rule implementation, reporting methods, and flexibility. The FDA typically uses a guidance-based approach that considers product-specific risk and drug availability, while the EMA uses a more structured process through the CHMP Article 5(3) opinion and coordinated EU procedures.

New analytical methods and mutagenicity tests are improving NDSRI risk assessment. LC-MS/MS, LC-HRMS, GC-MS, and GC-MS/MS are still key for confirmatory testing, while ecNGS shows promise but is not yet used routinely in regulatory decisions. More harmonized AI limits, analytical standards, reporting processes, and methods for combining evidence could make regulations more consistent and help ensure access to safe, high-quality medicines.

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Conflict of Interest Statement

The authors declare that they have no conflicts of interest, financial or otherwise, relevant to the content of this manuscript.

REFERENCES

1. Tuesuwan B, Vongsutilers V. Nitrosamine contamination in pharmaceuticals: threat, impact, and control. J Pharm Sci., 2021; 110(9): 3118–3128. doi:10.1016/j.xphs.2021.04.021
2. Eworuke E, Shinde M, Hou L, et al. Valsartan, losartan, and irbesartan use in the USA, UK, Canada, and Denmark after the nitrosamine recalls: a descriptive cohort study. BMJ Open. 2023; 13(4): e070985. doi:10.1136/bmjopen-2022-070985
3. US Food and Drug Administration. Recommended acceptable intake limits for nitrosamine drug substance-related impurities (NDSRIs): guidance for industry. Published August 2023. https://www.fda.gov/media/170794/download. Accessed 1 June 2026.
4. US Food and Drug Administration. Control of nitrosamine impurities in human drugs: guidance for industry. Revision 2. Published September 2024. https://www.fda.gov/media/141720/download. Accessed 1 June 2026.
5. US Food and Drug Administration. CDER nitrosamine impurity acceptable intake limits. Updated 24 July 2026. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cder-nitrosamine-impurity-acceptable-intake-limits. Accessed 30 July 2026.
6. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk: ICH guideline M7(R2). Published 2023. https://database.ich.org/sites/default/files/M7_R2_Guideline.pdf. Accessed 1 June 2026.
7. US Food and Drug Administration. FDA updates and press announcements on angiotensin II receptor blocker recalls: valsartan, losartan, and irbesartan. https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-

announcements-angiotensin-ii-receptor-blocker-arb-recalls-valsartan-losartan. Accessed 1 June 2026.

8. US Food and Drug Administration. FDA is not objecting to losartan with NMBA below 9.82 ppm remaining on the market. Published 20 March 2019. https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-angiotensin-ii-receptor-blocker-arb-recalls-valsartan-losartan. Accessed 1 June 2026.
9. European Medicines Agency. Nitrosamines EMEA-H-A5(3)-1490: assessment report. EMA/369136/2020. Published 2020. https://www.ema.europa.eu/en/documents/opinion-any-scientific-matter/nitrosamines-emea-h-a53-1490-assessment-report_en.pdf. Accessed 1 June 2026.
10. European Medicines Agency. European medicines regulatory network approach to implementation of the CHMP opinion pursuant to Article 5(3) of Regulation (EC) No 726/2004 for nitrosamine impurities in human medicines. EMA/425645/2020. Published 2021. https://www.ema.europa.eu/en/documents/opinion-any-scientific-matter/european-medicines-regulatory-network-approach-implementation-chmp-opinion-pursuant-article-53-regulation-ec-no-726-2004-nitrosamine-impurities-human-medicines_en.pdf. Accessed 29 June 2026.
11. European Medicines Agency. Nitrosamines EMEA-H-A5(3)-1490: questions and answers for marketing authorisation holders/applicants on the CHMP opinion for the Article 5(3) referral on nitrosamine impurities in human medicinal products. EMA/409815/2020 Rev. 23. Updated 10 October 2025. https://www.ema.europa.eu/en/documents/opinion-any-scientific-matter/nitrosamines-emea-h-a53-1490-questions-answers-marketing-authorisation-holders-applicants-chmp-opinion-article-53-regulation-ec-no-726-2004-referral-nitrosamine-impurities-human-medicinal-products_en.pdf. Accessed 30 July 2026.
12. European Medicines Agency. Appendix 2: carcinogenic potency categorisation approach for N-nitrosamines. Published 9 November 2023. https://www.ema.europa.eu/en/documents/scientific-guideline/appendix-2-cpca-nitrosamines_en.pdf. Accessed 1 June 2026.
13. European Medicines Agency. Appendix 3: enhanced Ames test conditions for N-nitrosamines. EMA/120337/2024. Updated 26 March 2024. https://www.ema.europa.eu/en/documents/scientific-guideline/appendix-3-enhanced-ames-test_en.pdf. Accessed 1 June 2026.

14. European Medicines Agency. Appendix 1: acceptable intakes established for N-nitrosamines. EMA/42261/2025 Rev. 13. Updated 24 June 2026. https://www.ema.europa.eu/en/human-regulatory-overview/post-authorisation/pharmacovigilance-post-authorisation/referral-procedures-human-medicines/nitrosamine-impurities/nitrosamine-impurities-guidance-marketing-authorisation-holders. Accessed 30 July 2026.
15. European Directorate for the Quality of Medicines & HealthCare. First OMCL analytical procedures for determination of nitrosamine drug substance-related impurities and intermediate-related contaminants published. Published 28 March 2024. https://www.edqm.eu/en/-/first-omcl-analytical-procedures-for-determination-of-nitrosamine-drug-substance-related-impurities-ndsris-and-intermediate-related-contaminants-published. Accessed 1 June 2026.
16. US Food and Drug Administration. Control of nitrosamine impurities in human drugs; guidance for industry; availability. Fed Regist., 2024; 89: 72242. https://www.federalregister.gov/documents/2024/09/06/2024-20013/control-of-nitrosamine-impurities-in-human-drugs-guidance-for-industry-availability. Accessed 1 June 2026.
17. Manchuri KM, Shaik MA, Gopireddy VSR, et al. Analytical methodologies to detect N-nitrosamine impurities in active pharmaceutical ingredients, drug products, and other matrices. Chem Res Toxicol, 2024; 37(9): 1456–1483. doi:10.1021/acs.chemrestox.4c00234
18. Modi P, Modasiya J, Desai D. Impact of monomer selection and behavior on transdermal drug delivery systems: regulatory and formulation perspectives. Cureus. 2026; 18(3): e104819. doi:10.7759/cureus.104819
19. Modi P. A technical framework for predicting regulatory deficiencies in pharmaceutical chemistry, manufacturing, and controls submissions using machine learning. Cureus. 2026; 18(6): e110534. doi:10.7759/cureus.110534
20. Modi P, Modasiya J, Desai D. Driving first-cycle ANDA approvals through strategic regulatory planning. Int J Drug Regul Aff., 2026; 14(2): 84–89. doi:10.22270/ijdra.v14i2.921
21. US Food and Drug Administration. FDA requests removal of all ranitidine products from the market. Published 1 April 2020. https://www.fda.gov/drugs/drug-safety-and-availability/fda-requests-removal-all-ranitidine-products-zantac-market. Accessed 1 June 2026.

22. Jolly RA, Trejo-Martin A, Bercu JP, et al. Ames concordance with the in vivo transgenic rodent gene mutation assay for NDSRIs and relative in vivo TGR potency with nitrosamines with robust dose-response carcinogenicity data. Regul Toxicol Pharmacol. 2026; 167: 106051. doi:10.1016/j.yrtph.2026.106051
23. Ashford AL, Nachmanson D, Wills JW, et al. Alignment between Duplex Sequencing and transgenic rodent mutation assay data in the assessment of in vivo NDMA-induced mutagenesis. Arch Toxicol. 2025; 99(10): 4227–4242. doi:10.1007/s00204-025-04121-0
24. Roper JM, Griffin TR, Johnson GE, et al. Using N-nitrosodiethanolamine and N-nitrosopiperidine transgenic rodent gene mutation data and quantum mechanical modeling to derive potency-based acceptable intakes for NDSRIs lacking robust carcinogenicity data. Environ Mol Mutagen. 2025; 66(4): 155–171. doi:10.1002/em.70012
25. US Food and Drug Administration. Roadmap to reducing animal testing in preclinical safety studies. Published April 2025. https://www.fda.gov/media/186092/download. Accessed 30 July 2026.
26. Dande A, Dhampalwar VR, Pallaprolu N, et al. Nitrosamine drug substance-related impurities in pharmaceuticals: formation, mitigation strategies, and emphasis on mutagenicity risks. Pharm Res., 2025; 42(4): 547–578. doi:10.1007/s11095-025-03857-9
27. Modi P, Patel K, Modasiya J. Comparative pathways: reliance and convergence for medicines and medical devices. International Journal of Medical Sciences, 2026; 4(1): 9–25. doi:10.34218/IJMS_04_01_002