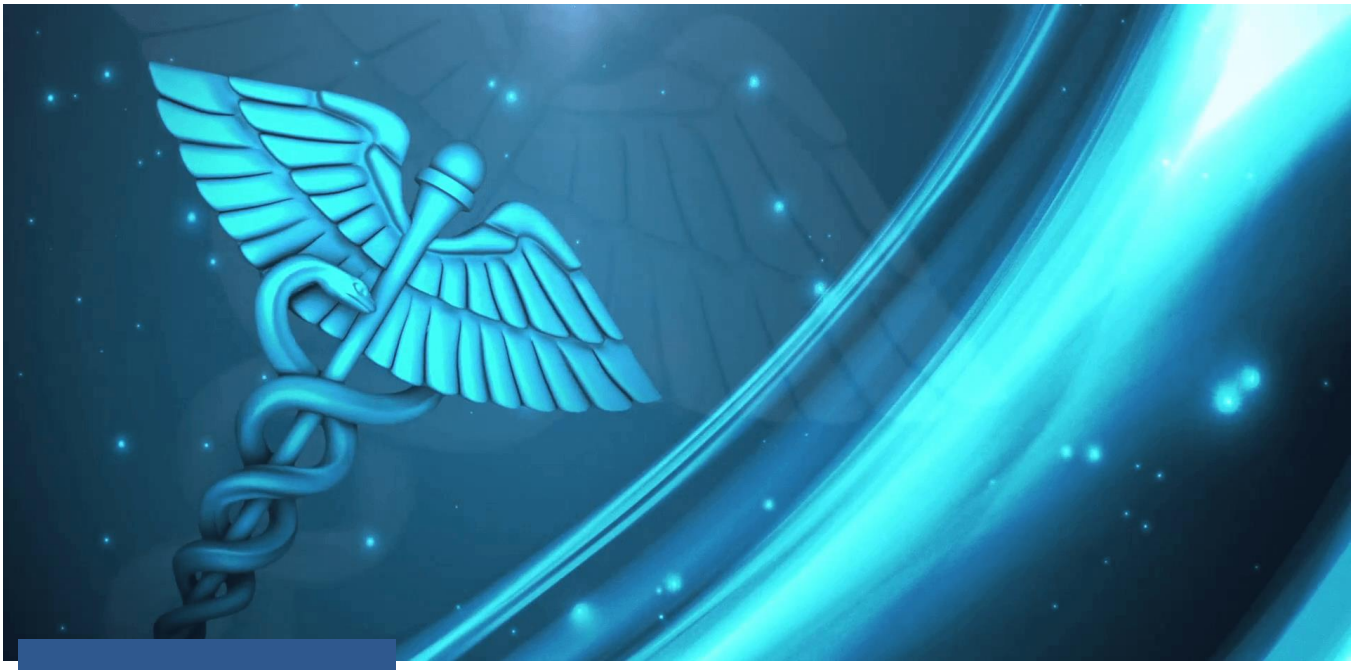
Background and Origin

Nitrosamine Drug Substance Related Impurities (NDSRIs)



PREFACE

The year 2025 marks the 10th anniversary of the Quality Forum (QF), an initiative launched by the Indian Pharmaceutical Alliance (IPA) in April 2015 to help Indian pharmaceutical manufacturers achieve parity with global quality benchmarks. Committed to a multi-year journey, the QF has actively addressed key industry challenges and developed best practice guidelines to enhance quality standards.

Over the past decade, the QF has focused on several priority areas critical to the pharmaceutical industry, including Data Reliability, Best Practices & Metrics, Culture & Capability, and Investigations. As part of its mission, the forum has undertaken the development of comprehensive Best Practices Documents covering various aspects of manufacturing, quality assurance, regulatory compliance, and engineering.

Two years ago, the IPA published a Best Practices document on Nitrosamines in pharmaceutical products. Based on feedback and insights from the leadership of IPA member companies, a new guideline was proposed focusing on Nitrosamine Drug Substance-Related Impurities (NDSRIs).

This guidance was developed with inputs from experts in R&D, analytical method development, and validation from IPA member companies. We were privileged to have Dr Mrunal Jaiwant, Senior Director, USP India; Dr B M Rao, former VP, Dr Reddy's; and Dr Rajiv Desai, Senior Technical Advisor, IPA and former Executive VP, Global Quality, Lupin, contribute their expertise in compiling and refining this document.

IPA sincerely acknowledges their dedicated efforts over the past 12 months. This Best Practices guidance incorporates insights benchmarked against existing regulatory guidelines, ensuring alignment with global standards.

Additionally, IPA extends its gratitude to the CEOs of all IPA member companies for their unwavering commitment, personal involvement, and financial support in making this initiative possible.

This document will be officially released at IPA's 10th Global Pharma Quality Summit in February 2025 and will be freely accessible to pharmaceutical companies in India and abroad via the IPA website: www.ipa-india.org.

Background and Origin – Nitrosamine Drug Substance-Related Impurities (NDSRIs) :

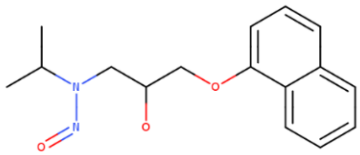
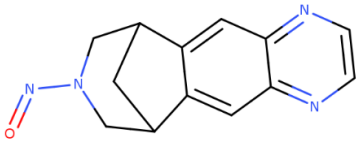
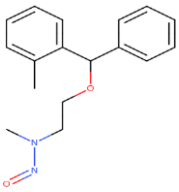
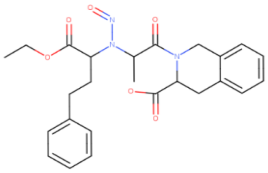
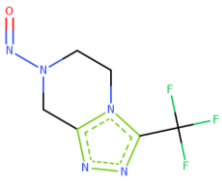
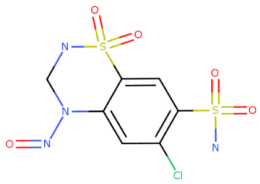
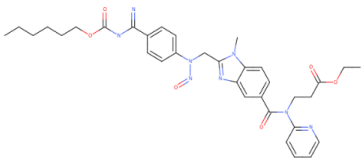
FDA (Food and Drug Administration) has been evaluating and investigating the presence of nitrosamine impurities in certain drug products since June 2018. Nitrosamines are known to be present in water and foods, including cured and grilled meats, dairy products, and vegetables. Nitrosamines have been identified as potential carcinogen, which may increase the risk of cancer if people are exposed to them above acceptable levels. The Acceptable Intake (AI) limit is a level that approximates an increased cancer risk of one additional case in 100,000 people based on a conservative assumption of daily exposure to the impurity or impurities over a lifetime (70 years) [See FDA guidance for industry "Control of Nitrosamine Impurities in Human Drug (Nitrosamine Guidance)].

Initially, the risk was found to be associated with small molecule nitrosamine impurities (such as NDMA, NDEA, etc.), for which animal toxicological data were available for establishing their acceptable intake (AI) limits in humans. Later, it was revealed that the presence of secondary amine functional groups in drug substances (APIs) could lead to the formation of Nitrosamine Drug Substance-Related Impurities (NDSRI) under certain favourable conditions (i.e. presence of nitrosating agent and acidic pH). NDSRIs was first introduced when Health Canada recalled Varenicline with levels of N-Nitroso Varenicline impurity followed by a recall of several batches of Varenicline from the Europe region by EMA. Subsequently, the FDA announced a voluntary recall of Varenicline drug products in July 2021.

In November 2021, the FDA alerted the public regarding the presence of NDSRIs in API and corresponding drug formulations and indicated that manufacturers could ascertain the presence of NDSRIs using the same three-step process identified in the Nitrosamine Guidance. As discussed further below, FDA also conveyed possible mitigation strategies and encouraged applicants to develop control strategies or design approaches to reduce NDSRIs to acceptable levels or eliminate them (where feasible). NDSRIs present unique scientific and regulatory challenges for FDA because each NDSRI is unique to the API, and there is limited compound-specific data that is available to ascertain safety assessments. Additionally, the design of validated test methods for the identification of NDSRIs and modification of existing test methods for assessment of their mutagenic potential may raise novel scientific considerations.

Nitrosamine Drug Substance-Related Impurities (NDSRIs) share structural similarities with API and are therefore unique to every APIs. Drug products containing API that have secondary and tertiary amines functional groups, when mixed with excipients that can cause the nitrosation reaction, are responsible for the formation of NDSRIs. In the year 2022, there has been a spate of market recalls caused due to the presence of Nitrosamine Drug Substance-Related Impurities (NDSRIs) in products such as Propranolol Hydrochloride Tablets, Orphenadrine Citrate, Quinapril Tablets, Hydrochlorothiazide Tablets, Sitagliptin Tablets, Dabigatran Etexilate capsules, the respective NDSRI for these products are listed below in **Table 1**.

Table 1 : NDSRIs

S.No.	Impurity Name	Structure
1.	N-Nitroso-Propranolol	
2.	N-nitroso-Varenicline	
3.	N-Nitroso-Orphenadrine	
4.	N-Nitroso-Quinapril	
5.	N-Nitroso Sitagliptin	
6.	N-Nitroso-Hydrochlorothiazide	
7.	N-Nitroso Dabigatran	

FDA has determined that certain APIs having secondary and tertiary amines are at risk of forming NDSRIs because certain excipients used in the manufacture of the drug products contain residual nitrites, and the manufacturing process may lead to the formation of the NDSRIs over a period of time. Based on chemical structure analysis, these acceptable intake (AI) limits based on predicted carcinogenic potency need to be applied to the NDSRIs, when an active ingredient contains the possible nitrosated form of secondary amines and dimethyl tertiary amine groups.

NDSRIs can be generated during manufacturing, or can form during the shelf-life storage period of the drug product. They can also be generated during the synthesis of the drug substance. In some cases, the root cause of NDSRI formation has been attributed to presence of nitrite impurities in excipients at parts per million (ppm) amounts. Nitrite impurities have been observed in a range of commonly used excipients (as well as water) and may lead to the formation of NDSRIs in certain drug products. In general, there is a risk of generating nitrosamine impurities when nitrites are in the presence of secondary, tertiary, or quaternary amines.

Degradation processes of active substances, including those induced by inherent reactivity (e.g. presence of nitro-alkyl, oxime, or other functionality) or by the presence of an exogenous nitrosating agent. This could potentially occur during both active substance and finished product manufacturing processes or during storage and could be influenced by the crystal structure, crystal habit, and storage conditions. The risk of potential Nitrosamine impurities introduced from the degradation process during finished product formulation or storage shall be evaluated.

The risk assessment should also include evaluation of any pathway (e.g., degradation and Nitrosamine precursor impurities such as dimethylamine or other secondary amine precursors) that may introduce the risk of Nitrosamine formation during drug product manufacture or during the storage.

Use of Sodium Nitrite in the presence of molecules having secondary or tertiary amines within the same or different steps of the manufacturing process.

Use of Sodium Nitrite ($NaNO_2$), or other nitro-sating agents, in combination with excipients, solvents (e.g., DMF, DMAc, and NMP), and catalysts, which are susceptible to degradation to secondary or tertiary amines, within the same or different process steps.

Possibility of Nitrosamine impurity formation during stability/storage (throughout shelf-life).

Use of certain packaging materials and components like those containing nitrocellulose or use of certain printing ink.

Reaction of amines leaching from quaternary ammonium anion exchange resins (e.g. used for purification steps) with nitrosating agents present in the liquid phase.

Accordingly, it is recommended to determine and control the levels of nitrite in excipients used in the drug formulation to mitigate the risk. There are many advanced sensitive analytical testing methods available for quantifying nitrosamines at parts per billion (ppb) level. However, there is yet, no established standard testing method for determining nitrite levels in excipients at trace levels. There are several testing methods reported using ion chromatography with conductivity detector in environmental samples. Many other test methods are reported with Griess reaction in combination with liquid chromatography and UV-Vis detection. Most of these test methods are used for nitrite content determination of water/wastewater or biological fluid matrices. Scientifically derived limits for nitrite levels should be established for Active Pharmaceutical Ingredients (APIs) having the secondary or tertiary amines. The establishment of a standard method for nitrite testing in excipients would be highly recommended to ensure comparable and meaningful values to support the excipient selection and evaluation process.

There is often a lack of mutagenicity and carcinogenicity study data for NDSRIs from which an acceptable intake (AI) limit can be determined. Indeed, several regulatory challenges arise because of uncertainty regarding the presence and acceptability of NDSRI levels in drug products. This leads to applicants conducting unnecessary studies or even discontinuing manufacturing of the drug products. Hence, there has been a rise in disruptions of supply and access to critical medicines which can lead to drug shortages and affect patient access to medications. To address these issues, the USFDA recently issued a guidance document that provides a risk-based safety assessment for NDSRIs which applies to manufacturers and applicants in identifying AI limits for NDSRIs

The 'Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities- Guidance for Industry' from the FDA provides manufacturers and drug applicants (including both prescription and over-the-counter (OTC)), a guideline for projecting the mutagenicity and carcinogenicity potential of NDSRIs. It provides a methodology that uses the molecular structural properties of NDSRIs for the determination of AI limits to produce carcinogenicity data. This FDA guidance applies to drugs (including both prescription and OTC drug products), that are in clinical development, and biological products (that have fragment and biologic-led combination products that are chemically synthesized).

The main challenge in determining the AI limits of NDSRIs is that they are unique to every API and due to this reason, there is limited or no existing safety data. Most NDSRIs do not have a recommended AI limit; the USFDA has provided recommendations for AI limits for a few NDSRIs. The new guidance is specifically focused on NDSRIs or nitrosamine impurities that have a significant structural similarity to the drug substance in a drug product. These larger molecules have long been argued to have a different, and often lower, carcinogenic potential due to their larger and more complex structure. The new NDSRI guidance acknowledges these concerns and references the work of the FDA and other health authorities to develop an approach for evaluating the predicted carcinogenic potency categorization (PCPC) based on molecular structural features. In August 2023, FDA provided its version as part of a new guidance and a dedicated webpage focused on NDSRIs. The lists of AIs provided by each health authority have some subtle differences and the FDA chose to keep the 26.5 ng/day lower limit, rather than the 18 ng/day used in Europe and Canada, but there is a much bigger difference that followers of the nitrosamine's saga should have noted.

In cases where the NDSRI mutagenic potential cannot be characterized adequately, applicants and the USFDA have turned to the Structure-Activity Relationship (SAR) methods to help support in identifying a tested surrogate that has a similar structure and reactivity as the NDSRI, to determine an estimation of carcinogenic potency to help identifying a more accurate AI limit. The rationale for choosing the surrogate is critical.

AI limits recommendations based on Predicted Carcinogenic Potency Categorization (PCPC) :

PCPC approach - It explains the techniques that use PCPC to allocate AI limit to NDSRIs which is based on structural features of NDSRIs whether it is activating / deactivating. If it is activating, then there is an increase in carcinogenic potency and vice versa. This (PCPC) approach integrates the SAR method, which assumes that mutagenic properties of NDSRIs are the result of activation of α-methylation activation. This approach applies to those NDSRIs that have C-atoms on both sides of the nitroso group, where the C-atoms are not directly bonded to the heteroatom. Also, this approach does not apply to those NDSRIs (like nitrosated indoles) whose N-nitroso group is located within an aromatic ring. This approach allows manufacturers and applicants to determine the applicable potency category and related AI limits for NDSRIs in API and drug products. If the FDA has informed the AI limits directly to manufacturers or applicants or via FDA guidance, then this approach in this guidance should not be applied to NDSRIs.

Specific applications of the PCPC approach - The FDA and EMA proposed the following five AI limits that are based on the PCPC/ CPCA approach for NDSRIs:

- For potency category 1, the recommended AI limit is 26.5 ng/day & 18 ng/day as per USFDA and EU respectively. This means NDSRIs allocated to this category have carcinogenic potency no higher than the class-specific limit for NDEA.

- For potency category 2, the recommended AI limit is 100 ng/day– which means NDSRIs allocated to this category have carcinogenic potency no higher than NDMA & NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-(butanone)).

- For potency category 3, the recommended AI limit is 400 ng/day – which means lower carcinogenic potency due to the presence of a weakly deactivating structural features.

- For potency category 4, the recommended AI limit is 1500 ng/day – which means low carcinogenic potency due to the disfavoured alpha hydroxylation pathway because of steric or electronic influences.

- For potency category 5, the recommended AI limit is 1500 ng/day due to the absence of alpha hydrogen, steric or electronic influence.

The FDA-recommended AI limits apply to individual nitrosamine impurities and are relevant only if a drug product contains a single nitrosamine. When multiple nitrosamines are present, the total limit should not exceed the AI limit for the most potent nitrosamine in the product. However, if the recommended AI limits for the individual nitrosamines differ significantly, it may be impractical to base the total limit solely on the most potent nitrosamine. In such cases, alternative approaches may be warranted.

An alternative flexible AI limit approach can be applied to establish specifications when multiple nitrosamine impurities, including both small-molecule nitrosamines and NDSRIs, are present in drug products. By following the methodology outlined in Appendix C (FDA Guidance Rev 2), manufacturers or applicants can ensure that the combined levels of nitrosamine impurities do not exceed an exposure level corresponding to an acceptable cancer risk of 1 in 100,000, as specified in ICH M7(R2).

An example of a flexible approach for total recommended AI limits for multiple nitrosamine impurities in one drug product is shown below.

Drug A has maximum daily dose 80 milligrams/day :

Nitrosamine	AI Limit (ng*/day)	Acceptance Criteria	Stability Results			
			0 Month		3 months	
		Concentration Limit (ppm)	Nitrosamine level (ppm)	% of AI Limit	Nitrosamine level (ppm)	% of AI Limit
		ppm=AI/MDD		**		**
Nitrosamine 1	26.5	NMT 0.33	0.10	30.3	0.15	45.45
Nitrosamine 2	37	NMT 0.46	0.05	10.87	0.20	43.48
Nitrosamine 3	1500	NMT 18.75	1.00	5.33	3.00	16
Total Nitrosamine		Sum of all Nitrosamine impurities NMT 100%		46.50 (Pass)		104.93 (Fail)

** Formula for % of AI limit = (Nitrosamine level (ppm) *100)/concentration limit (ppm)

Table-1: Flexible approach for total recommended AI limits for multiple nitrosamine impurities

Below is the path for USFDA and EMA recommended AI values :

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/updated-information-recommended-acceptable-intake-limits-nitrosamine-drug-substance-related#confirmatory

https://www.ema.europa.eu/en/human-regulatory-overview/post-authorisation/pharmacovigilance-post-authorisation/referral-procedures-human-medicines/nitrosamine-impurities

Confirmatory Testing :

As indicated in the NDSRI Guidance, if manufacturers and applicants identify a risk of NDSRI formation in a drug product, then confirmatory testing of batches should be conducted using sensitive and appropriately validated test methods.

Scenario-1 : Confirmatory results found not more than 10% of AI :

- If NDSRI is found not detected (below 10% of AI) in release and shelf-life samples, routine control is not warranted, provided the root cause is well understood, and manufacturing process controls are established and validated.

- If changes are introduced in the manufacturing process, excipients, API, or other materials used in the manufacturing process, Nitrosamine levels should be re-evaluated.

Scenario-2 : Confirmatory results found above 10% of acceptable intake but within the recommended AI limit :

- If NDSRI is found acceptable intake above 10% of acceptable intake but within the recommended AI limit in release and shelf-life samples, a control for nitrosamines should be established in the release and stability specifications. The source of the impurity from the process shall be identified and a suitable manufacturing control strategy shall be proposed by Formulation development as per applicability. Technical justification shall be provided if cause could not be identified during investigation. For approved drug products, this information should be submitted in a supplement as changes being effected in 30 days.

Scenario-3 : Confirmatory results found nitrosamine levels exceed the recommended AI limit

- Changes in formulation, manufacturing process, or packaging are warranted, manufacturers and applicants should implement such changes that are demonstrated to ensure that nitrosamine levels remain within the recommended AI limit.

- For approved drug products, when these changes meet the criteria as major changes, they must be submitted to the Agency in a prior approval supplement (PAS).

- If a manufacturer or applicant proposes an alternative AI limit for nitrosamine impurities or identifies an NDSRI that is not included on the nitrosamine guidance web page, a proposed AI limit or predicted carcinogenic potency category associated with an AI limit should be submitted to FDA for evaluation.

- Follow the applicable recommendations described for over-the-counter (OTC) monograph drugs and other marketed products that are not the subject of approved applications.

As per EMA guidance:

Scenario-1 : Confirmatory results found not detected :

- If NDSRI is found not detected (below 10% of AI) in release and shelf-life samples, routine control is not warranted.

Scenario-2 : Confirmatory results found below 30% of acceptable intake :

- If NDSRI is found below 30% of acceptable intake in release and shelf-life samples, skip testing should be proposed to get the periodic evaluation.

Scenario-3 : Confirmatory results found below acceptable intake limit :

- If NDSRI is found below acceptable intake and above 30% of AI in release and shelf-life samples, routine testing should be proposed as a control strategy.

Scenario-4 : Confirmatory results found more than Acceptable Intake:

- **Option-1 :** If NDSRI is found more than acceptable intake in release and shelf-life samples, then it is required to investigate the reason for the higher levels in the drug product and control strategy to be implemented based on the investigation in order to control the NDSRI below acceptable intake limit.

- **Option -2 :** If NDSRI is found more than acceptable intake in release and shelf-life samples and there is a challenge to reduce the level of nitrosamine in the drug product, Enhanced AMES study and/or comprehensive toxicology study to be performed in order to qualify the NDSRIs limit at higher acceptable intake.

For Confirmatory testing , Minimum Three Drug product (Registration or on going commercial) batches at different stability time points, different strengths (same manufacturing process), different pack sizes and within expiry (including the end of shelf life/expiry) shall be considered for confirmatory testing (retrospective and prospective) where a risk of nitroso impurities has been identified.

The pack size shall be considered based on worst case scenario (Example: Headspace volume of the pack) depends on product criticality
FDA intends to provide information on FDA-generated testing methods to provide options for regulators and industry to detect NDSRI impurities in specific drug substances and drug products. The test methods should be validated by the user if the resulting data are used to support a required quality assessment of the drug substance or drug product, or if the results are used in a regulatory submission.

Information on details of method published for NDSRIs are listed below :

LC-ESI-HRMS Method for the Determination of N-Nitroso-Bumetanide
LC-ESI-HRMS Method for the Determination of N-Nitroso-Propranolol
LC-ESI-HRMS Method for the Determination of N-Nitroso-Varenicline

Control Strategy :

As discussed earlier, both the USFDA and the EMA suggest a three-step mitigation strategy that both API and drug product manufacturers should follow. The first step includes the performance of a risk assessment, the second is the confirmation of the identified risks by testing, and the third consists of reporting changes implemented to prevent and reduce the formation of nitrosamine impurities in drug products.

A typical risk assessment involves an assessment of the chances of the formation of small nitrosamines as well as NDSRIs throughout the process of drug product manufacturing, storage, and shelf life. The nitrosamine impurities can be formed due to the presence of nitrosating agent (NO2) in excipients, processing water, solvents, packaging material, atmosphere etc.

Once the chances of the formation of nitrosamine impurities are established, it is recommended to perform a confirmatory testing to evaluate the levels of nitrosamine impurities against the respective acceptable intake (AI) values. There are several analytical methods published for confirmatory testing as listed in Table 2.

Based on the results of the confirmatory testing, manufacturers should make relevant changes (e.g. change formulation design to use inhibitors such as ascorbic acid, use nitrite-free excipient, change excipient supplier etc.) to ensure that the levels of nitrosamine impurities remain below to the acceptable intake levels of respective identified nitrosamine impurities. Any such change should be adequately evaluated and reported to the regulatory authority as a part of the successful implementation of the mitigation strategy to achieve a nitrosamine-free drug product.

Control Strategies for NDSRIs in API

API Manufacturers must:

- Optimize the synthesis process to avoid using nitrites and amine-rich solvents.
- Ensure third-party suppliers of raw materials and solvents are audited and tested.
- Consider replacing nitrites in quenching steps with other agents.

Control strategy for NDSRIs in Drug Product

Drug Product Manufacturers must:

- Test all APIs for nitrosamines during the development stage.
- Set up specifications to limit the acceptable levels of nitrosamines in APIs and drug products.
- Incorporate antioxidants like ascorbic acid into formulations to inhibit nitrosamine formation.

Based on the availability of the confirmatory testing results, the following control strategy should be considered, and risk assessment report should be prepared.

How to establish the AI value for NDSRIs?

Step - 1 : Based on the regulatory agency published values.

Step - 2 : If AI values are not available, follow PCPC as per USFDA and CPCA as per EMA approach as published by respective regulatory agency.

Step - 3 : If the results of NDSRIs found above the AI values calculated based on PCPC/ CPCA approach, pursue alternate approaches such as using safety data, obtaining compound-specific data or using read-across assessment to a suitable surrogate to support a higher AI limit.

If the results of NDSRI still found to be above the AI value, conduct EAT to establish the AI value.

If drug product batches already in distribution contain levels of NDSRIs above the FDA recommended AI limit (e.g., associated with their predicted carcinogenic potency category), and manufacturing changes or recalls are likely to lead to a disruption in the drug supply, then manufacturers and applicants should immediately contact the Center for Drug Evaluation and Research's Drug Shortage Staff at drugshortages@fda.hhs.gov.

When contacted about a potential disruption in the drug supply, FDA intends to evaluate each circumstance on a case-by-case basis. FDA may work directly with a specific manufacturer or applicant of the marketed drug and intends to consider whether it is appropriate or not appropriate to recommend an interim AI limit for a temporary period.

Mitigation strategy for NDSRIs:

To minimize disruptions in supply, the FDA advises manufacturers to implement risk management strategies :

1. **API and Raw Material Supply Chain :**

   - ❖ Conduct thorough audits of the supply chain, focusing on raw materials and APIs sourced from vendors to ensure that these materials are free from nitrosamine contamination.

   - ❖ Vendor management: Manufacturers should ensure that their suppliers maintain stringent control over solvent recovery processes and raw material transportation, as these are common sources of nitrosamine impurities.

2. **Stability Testing :**

   - ❖ Ongoing stability testing should be conducted on all drug products to monitor for nitrosamine impurities over time. This includes testing for nitrosamine formation during the product's shelf life.

   - ❖ If a product is found to form nitrosamines during storage, manufacturers must adjust the product formulation, packaging, or shelf-life duration to reduce this risk.

3. **FDA encourages manufacturers to explore other approaches to mitigate or prevent formation of NDSRIs. Examples of possible mitigation strategies related to formulation design are described below :**

   - ❖ Addition of antioxidants to formulations may significantly inhibit the formation of NDSRIs in drug products.

   - ❖ Formulation designs that incorporate excipients such as sodium carbonate that modify the micro-environment to neutral or basic pH, should in principle inhibit the formation of NDSRIs. Each manufacturer should determine the potential benefit from and demonstrate the suitability of any reformulation approach.

   FDA encourages manufacturers to consider these as well as other innovative strategies to reduce the formation of NDSRIs to acceptable levels in drug products. FDA will consider meeting requests, as appropriate, to discuss innovative mitigation strategies with prospective applicants or manufacturers.

Lifecycle Management :

- As per ICH Q10 – Pharmaceutical Quality System, and the summary in ICH M7 a set of controls based on process understanding and risk management principles (ICH Q9 – Quality Risk Management) should be defined to assure process performance (trend analysis) and product quality is defined as per Control Strategy.

- From the risk assessment and the evaluation of the level of the nitrosamine(s) impurity a specific testing frequency or any other control should be defined to assure that the level of the impurity will be kept under control and below the acceptance limit, across the product lifecycle.

- In case of change in vendor of API/Excipients, change in the manufacturing process and change in container closure, necessary impact assessment should be re-evaluated.

- Upon a change in formulation, the manufacturing site shall confirm that products continue to meet specifications, at the time of release and at the time of use as defined by the expiry date (i.e, Nitrosamine impurities/NDSRIs meet the specification limits throughout the shelf life of the products). The stability of the reformulated product shall be assessed including confirmation that the nitrosamine impurities and NDSRIs meet the specifications

- Review the outcome of risk evaluation and testing when new information on potential root causes for Nitrosamine/NDSRI formation or contamination becomes available with appropriate timelines based on risk assessment.

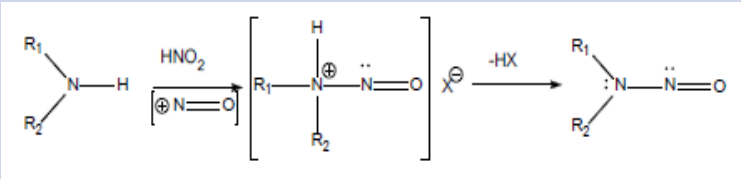
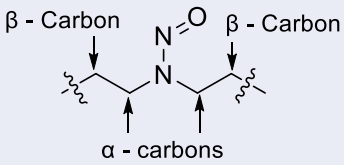
- If NDSRI is found above recommended AI in the drug product the Agency shall be informed immediately, and suitable actions shall be taken. In parallel appropriate mitigation strategies shall be developed to reduce the level of NDSRI below AI.

1 ANNEXURES :

Annexure	Template Name
Annexure-1	Risk Assessment Template
Annexure-2	One template as a best practice to characterize NDSRI impurity

2 GLOSSARY :

Term	Definitions
Acceptable Intake	An intake level that poses negligible cancer risk, or for serious/life-threatening indications where risk and benefit are appropriately balanced. An AI limit as defined in ICH M7(R2) is a level that approximates an increased cancer risk of one additional case in 100,000 subjects based on a conservative assumption of daily exposure to a mutagenic impurity in drug substances and drug products over a lifetime (70 years).
Acceptable Limit	The maximum acceptable concentration of an impurity in a drug product derived from the Acceptable Intake (AI) based on TD50 or Carcinogenicity Potency Categorization Approach (CPCA) and the maximum daily dose of the drug product.
Acceptance Criteria	Numerical limits, ranges, or other suitable measures for acceptance of the results of analytical procedures.
Control Strategy	A planned set of controls, derived from current product and process understanding, which assures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control.
Degradation Product	A molecule resulting from a chemical change in the drug molecule brought about over time and/or by the action of light, temperature, pH, water, or by reaction with an excipient and/or the immediate container/closure system.
Maximum Daily Dose	The maximum daily dose is the highest amount of allowable drug or medicine for a patient with consistent safety, as mentioned in the SmPC/Prescribing Information Leaflet

Term	Definitions
Nitrosamine Impurities	The term Nitrosamine describes a class of compounds having the chemical structure of a nitroso group bonded to an amine (R1N(-R2)-N=O), as shown in Figure 1. The compounds can form by a nitrosating reaction between amines (secondary, tertiary, or quaternary amines) and nitrous acid (nitrite salts under acidic conditions). A different class of precursor is 1,1-disubstituted hydrazine, which can be oxidized to form a Nitrosamine. The compounds e.g., 1-cyclopentyl-4-nitrosopiperazine and 1-methyl-4-nitrosopiperazine are formed via this hydrazine oxidation process. **Representative Reaction to Form Nitrosamines** 
Nitrosamine drug substance-related impurities (NDSRIs)	Nitrosamine drug substance-related impurities (NDSRIs) are a class of Nitrosamines sharing structural similarity and specific to the Drug. NDSRIs can be generated during manufacturing or during the shelf-life storage period of the drug product.  NDSRIs form through nitrosation of APIs (or API fragments) that have secondary, tertiary, or quaternary amines when exposed to nitrosating compounds such as nitrite impurities.
Permitted Daily Exposure (PDE)	A substance-specific dose that is unlikely to cause an adverse effect if an individual is exposed at or below this dose every day for a lifetime.
Possible	Cases wherein the drug product manufacturing process/component/environment is associated with the presence of secondary/tertiary amine/quaternary salts, and upon interaction with a direct/indirect source of nitrosating agent with an acidic reaction environment there is a likelihood/confirmed presence of Nitrosamine impurities/NDSRIs. It also includes cases if the API structure is associated with the presence of the secondary/tertiary amine/quaternary salts or there is a possibility of carryover of Nitrosamine impurities /NDSRIs from the API manufacturing process, that could lead to the likelihood/confirmed presence of Nitrosamine impurities.
Risk	The combination of the probability of occurrence of harm and the severity of that harm (ISO/IEC Guide 51).

Term	Definitions
Risk Assessment	A systematic process of organizing information to support a risk decision to be made within a risk management process. It consists of the identification of hazards and the analysis and evaluation of risks associated with exposure to those hazards.
Small-molecule Nitrosamine impurities	Nitrosamine impurities that do not share structural similarity to the API and are found in many different drug products. (e.g. NDMA, NDEA)
Not Possible	No possibility to form Nitrosamines/NDSRIs in the process; cases wherein the process has neither direct sources of secondary or tertiary amines or quaternary salts, nor direct sources of nitrosating agents used.

3 REFERENCES :

3.1 USFDA : Recommended Acceptable Intake Limits for Nitrosamine Drug Substance Related Impurities (NDSRIs) Guidance for Industry , August 2023 (RAIL Guidance).

3.2 USFDA : Control of Nitrosamine Impurities in Human Drugs, Guidance for Industry,September-2024 , Revision 2.

3.3 USP-NF General Chapter <1469>Nitrosamine impurities.

3.4 WHO Information Note Nov, 2019: Update on nitrosamine impurities

3.5 Guidance on nitrosamine impurities in medications (published by Health Canada).

3.6 Questions and answers for marketing authorization holders/applicants on the CHMP Opinion for the Article5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products (published by European Medicines Agency).

4 CHANGE HISTORY :

S. No.	Document Number along with Version number	Description of Changes
1	00	New Document.

Annexure I : Risk Assessment Template:

Risk assessment report for the presence of Nitrosamine Drug Substance Related Impurities (NDSRI) - Drug Product	
Product Name:	Product Strength:
Product Code:	Packing Configuration:
Document No:	Version:
Department:	Market:
Effective Date:	

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7. REFERENCES

8. CHANGE HISTORY

1.0 OBJECTIVE :

To perform Nitrosamine Drug Substance Related Impurity (NDSRI) risk assessment in drug product manufactured at respective site.

2.0 BACKGROUND :

Risk assessment was performed for small molecule nitrosamine / classical nitrosamines (such as NDMA etc.) and based on recent updates from different regulatory agencies (USFDA, EMA, Health Canada etc.) on NDSRIs, a separate risk assessment report is being prepared.

3.0 STEP -1 / RISK ASSESSMENT FOR NITROSAMINE DRUG SUBSTANCE RELATED IMPURITIES (NDSRIs)

Molecular Structure of API

3.1 Below mentioned risks are to be evaluated for the potential formation of NDSRIs in drug product.

- Check for the presence of secondary or tertiary amine in the API molecular structure and related process/degradation impurities
- Active Pharmaceutical Ingredient (Drug Substance) NDSRI risk assessment report
- Potential presence of Nitrites in excipients, primary packaging material, water used in the manufacturing of drug product
- Drug product manufacturing process parameters such as temperature, pH or any other parameter.

3.2 Based on the Active Pharmaceutical Ingredient (Drug Substance) NDSRI risk assessment report, formation of NDSRI is possible.

Table 1. Risk Assessment for formation of NDSRIs :

S.No.	Description	Possible NDSRI Structure	Published AI limit (ng/day) as per USFDA / EMA/Health Canada.	AI (ng/day) based on CPCA category
1	Drug substance			
2	Drug Substance & Product related impurities			

NA: Not applicable.

 # - If published AI values is not available, CPCA approach to be considered.

3.3 Potential presence of Nitrites in excipients, primary packaging material, water used in the manufacturing of drug product

3.4 Evaluate the documents submitted by the vendors of excipient, primary packaging material including water assessment report, for the presence of the nitrite is reported in _____, _____, _____ excipients.

3.5 Risk due to Drug Product Manufacturing and Packing process

Based on the Drug product manufacturing process, if it involves aqueous based wet granulation which comprises favourable factors for formation of NDSRI.

3.6 Conclusion for Risk Assessment (STEP-1) :

3.6.1 Based on the above risk assessment, if the NDSRI impurity formation is not possible it will be concluded that the API and the corresponding formulation does not carry any risk.

3.6.2 Based on the above risk assessment, if the NDSRI impurity formation is possible, then conduct confirmatory testing and Limits for the possible NDSRIs are derived based on Maximum Daily Dosage (MDD) for respective geographies / countries, details are provided in below Table 2.

Table 2. Geography/country wise limits for Drug Product

S. No	NDSRI	Geography wise MDD	Limits (ppm)
	NDSRI	Europe	
		Canada	
		US	

4.0 Step -2 / Confirmatory testing for Nitrosamine Drug Substance Related Impurities (NDSRIs)
Conclusion for confirmatory testing results (STEP-2) :

5.0 Step-3 / CONTROL STRATEGY :

Annexure II : One template as a best practice to characterize NDSRI impurity.

Tests to be used for characterization

S. No	Name of the Test	Test purpose
1	Appearance	Report the physical appearance (Solid/ liquid/ Semi-solid/ Gummy mass, etc.)
2	Identification by IR	Functional group identification Match with the standard
3	Identification by Mass	Molecular weight identification by m/z ratio
4	Structure confirmation by NMR	Structural analysis using various NMR experiments*: ^{1}H NMR, ^{13}C NMR, $^{1}H/^{1}H$ COSY, ^{1}H /^{13}C HSQC, $^{1}H/^{13}C$ HMBC, $^{1}H/^{15}N$-HMBC, $^{1}H/^{15}N$-HSQC, $^{1}H/^{1}H$ NOESY, ^{19}F NMR, and ^{31}P NMR.
5	Purity by HPLC/ GC (% TDA)	To determine the purity of the analyte.
6	Potency by qNMR** (% w/w)	To determine the content of the analyte (orthogonal technique to verify the chromatographic purity)
7	Loss on drying [LOD] (%w/w)	Content of water and volatile impurities
8	Residue on ignition [ROI] (% w/w)	Content of inorganic impurities

***Note-1 :**

- In general, for any impurity, experiments including ^{1}H, ^{13}C, COSY, HSQC, HMBC are to be performed to gather structural information about proton, carbon, and its correlations.

- For **NDSRIs**, the presence of a nitroso group is to be confirmed through the **$^{1}H/^{15}N$-HMBC** NMR experiment, which reveals long-range correlations (2-3 bond distances) between ^{1}H and ^{15}N nuclei. This also helps in distinguishing isomeric impurities like positional isomers and C-Nitroso compounds.

- Additional NMR experiments, including ^{19}F, ^{31}P, $^{1}H/^{1}H$ NOESY, and $^{1}H/^{15}N$-HSQC, are to be performed based on specific requirements.

****Note-2 :**

- Traditionally, impurity potency has been assessed by subtracting the percentages of total impurities determined by Chromatography, water content, volatile impurities, and inorganic impurities from 100. However, with qNMR serving as an absolute quantification method, potency can be precisely determined by employing a suitable qNMR standard. Selecting the qNMR method for determining potency can save time and resources. Loss on drying and Residue on ignition tests can be skipped if qNMR is feasible for determining potency. However, laboratories need to evaluate the most suitable method based on the compound's nature and spectral quality.

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