White Paper

Nitrosamines

Extinguishing Nitrosamine Risk: How Dipharma Safeguards Your Product Quality

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Extinguishing Nitrosamine Risk: How Dipharma Safeguards Your Product Quality

The blaze of nitrosamine impurities in pharmaceuticals first ignited in June 2018 with the detection of N-nitrosodimethylamine (NDMA) in valsartan, a widely used antihypertensive drug. Years later, **this fire still smolders, requiring constant vigilance and expertise to keep it under control**. This discovery led to immediate regulatory actions and widespread recalls. **Dipharma quickly implemented rigorous testing and mitigation strategies to address contamination risks and ensure patient safety.**

From the outset, **Dipharma embarked on a rigorous journey of research and process optimization**. By meticulously reviewing and refining every aspect of our operations, **we have successfully developed a comprehensive protocol that underpins our innovative Nitrosamine program**.

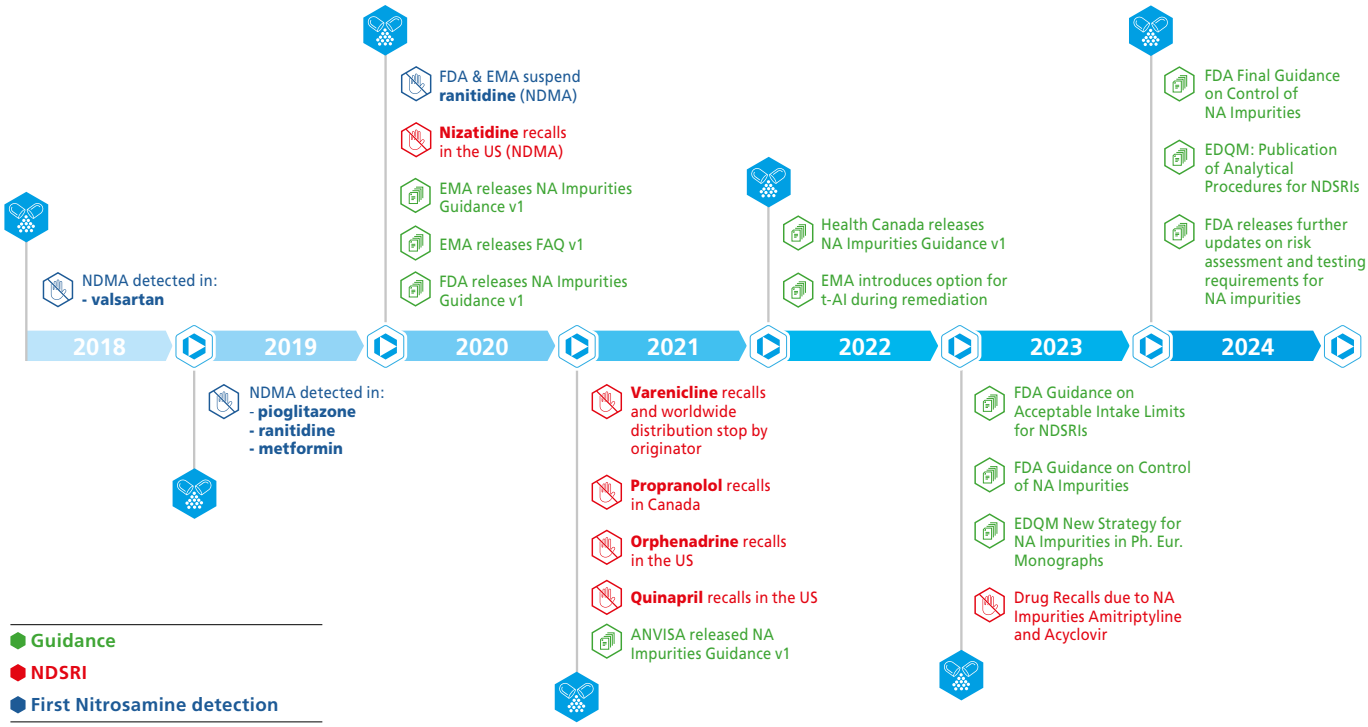


FIGURE 1: Key events in pharmaceuticals related to Nitrosamines (NAs), including the detection of potent NAs and Nitrosamine Drug Substance Related Impurities (NDSRIs), industry and regulatory actions (recalls, suspensions), and the release of regulatory guidance. The list is not exhaustive but captures the main events.

What are Nitrosamines

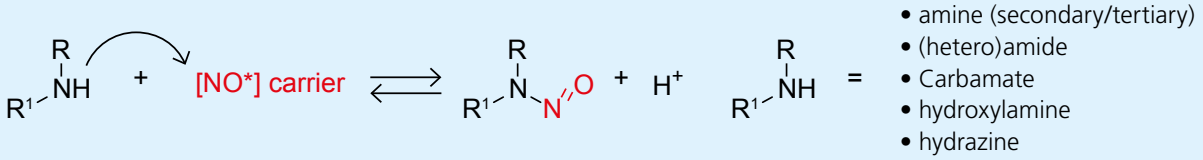
N-nitrosamines are a class of chemical compounds that belong to a larger group of carcinogenic molecules classified as N-nitroso compounds.

Nitrosamines are present in trace amounts in air, water, food, tobacco, cosmetics etc.

Nitrosamines are known to be mutagenic and potent animal carcinogens (classified as cohort of concern in ICH M7). However, if a drug contains NAs below the acceptable limits, defined as Acceptable Intake (AI), an increased cancer risk is not expected even with chronic dosing.

How do they form?

A well-known chemical reaction occurs between a nitrosation agent (e.g., nitrite) and an amine source (e.g., secondary or tertiary amines) under acidic conditions, leading to nitrosamine formation. (Figure 2)



Reactive [NO*] carriers: 6 main species

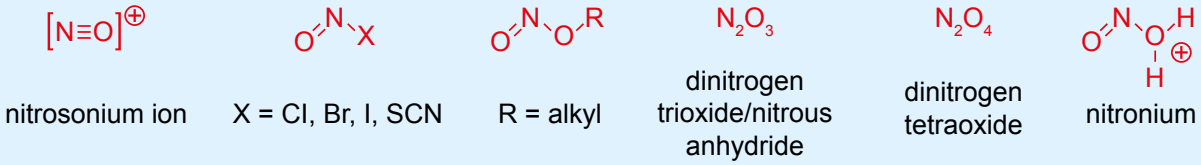


FIGURE 2: Nitrosamine mechanism of formation from a secondary amine.

N-nitrosamines can undergo several organic-synthetic transformations (Figure 3).

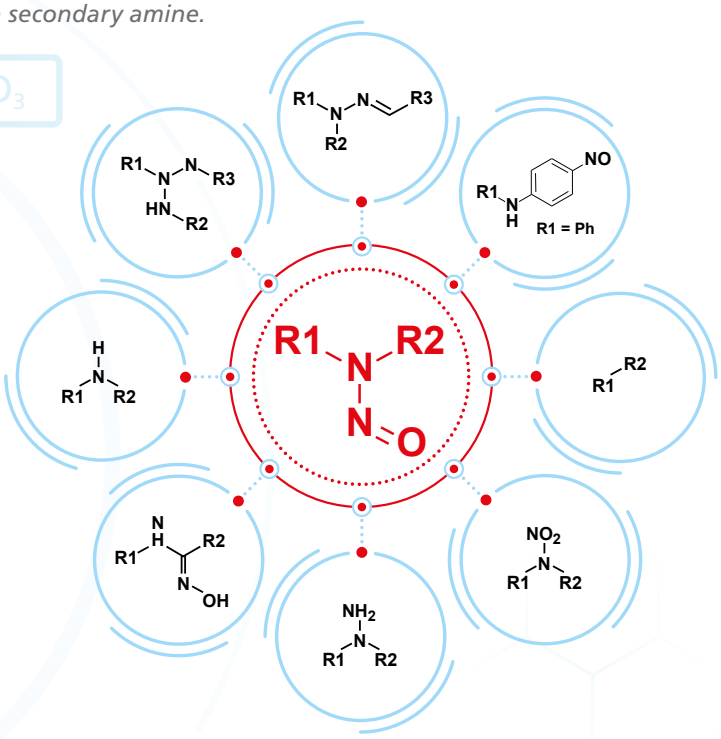


FIGURE 3:
Possible organic synthetic transformation of a nitrosamine

Dipharma's Nitrosamines Risk Assessment Protocol

Dipharma has proactively addressed nitrosamine concerns by developing a robust risk assessment protocol aligned with EMA and FDA guidelines.

This protocol consists of three essential steps:

1 **Risk Evaluation**
A detailed assessment of the potential presence of nitrosamines in each product, leveraging a structured risk matrix to determine sources and severity.

2 **Risk Assessment & Confirmatory Testing**
A thorough investigation into nitrosamine formation mechanisms backed by R&D evaluations, setting limits following EMA and FDA guidelines, and targeted QC testing.

3 **Risk Mitigation Measures**
Implementation of effective corrective and preventive strategies to eliminate or minimize nitrosamine risks.

Dipharma's Actions in Nitrosamine Control

From the onset of the nitrosamine crisis, **Dipharma adopted a proactive and comprehensive approach. The company initiated a multi-phase strategy to evaluate, assess, and control nitrosamine contamination**.

In an extreme case of a product with a daily dose of 3.2 g/day, the NDEA limit is a miniscule 8.3 ppb. Dipharma was able to successfully quantify 0.8 ppb, an amount 10-fold less than the extremely low limit.

Assessment Phase

Dipharma's R&D and QA teams undertook an extensive review of existing scientific literature and regulatory guidance. This enabled the creation of a risk analysis matrix for evaluation of all APIs and manufacturing processes, including the starting materials and their impurities, reagents and catalysts used in the process, solvents employed and equipment used. Each aspect was evaluated to determine its priority level based on its potential nitrosamine risk.

Testing Phase

The company performed detailed examinations of high-risk and moderate-risk processes. Potential nitrosamines were identified, and analytical methods were developed and validated to quantify their presence. This included:

- Targeted synthetic route evaluations.
- Analytical validation of probable process-related nitrosamines.
- Implementation of robust detection methods.

Control Strategy

Dipharma employs a variety of strategies to control nitrosamine formation:

- Denitrosation Reactions: Acid-catalyzed denitrosation processes can be introduced to minimize nitrosamine risks. These reactions are typically conducted using aqueous acids such as HCl, AcOH, or HBr, with alternative methods involving CuCl/HCl.

- Reduction Methods: Certain nitrosamines can be degraded using reduction agents like zinc and lithium aluminium hydride, demonstrating a strong purge effect in risk mitigation.
- Process Optimization: Careful evaluation of reaction conditions to avoid pathways that favor nitrosamine formation.

Additionally, Dipharma meticulously analyzes various sources that contribute to nitrosamine contamination, including solvents, water, excipients, and equipment.

Risk Assessment Model

To ensure a structured evaluation, Dipharma has developed a proprietary risk matrix that categorizes APIs into three levels:

- High Risk: Immediate action required, including analytical verification and customer notification.
- Moderate Risk: Controlled assessment and potential process refinements.
- Negligible Risk: No further mitigation needed beyond standard GMP compliance.

For high-risk APIs, additional actions are mandated:

- Customer notifications via risk assessment reports.
- Mandatory batch testing in accordance with EMA guidelines.
- Analytical method validation and establishment of detection limits (Table 1).
- Root cause investigations and corrective action planning.
- Full compliance with the latest EMA and FDA regulatory updates.

Dipharma Detection Platform	Chemical/Commercial name	Acronym	Acceptable Intake ng/day (EMA)
The WATERS ARC + QDa single quadrupole system is a versatile and robust liquid chromatography-mass spectrometry (LC-MS) solution that seamlessly integrates HPLC and UHPLC methods, offering high sensitivity and reliability for diverse analytical applications.	N-nitrosodimethylamine solution	NDMA	96
	N-nitrosodiethylamine solution	NDEA	26,5
	N-nitrosoethylisopropylamine	NEIPA	26,5
	N-nitrosodiisopropylamine	NDIPA	26,5
	n-methyl-n-nitrosoaniline	NMPA	34,3
	N-nitrosodi-n-butylamine	NDBA	26,5
	n-nitrosomorpholine solution	NMOR	127
	lithium(1+) ion (E)-(5-methylpyridin-2-yl) diazenolate	PIRZERONAM*	18
	4-methylene-1-nitroso-piperidine	EFINAM	18
	N-nitrosoisonipecotamide	MPZNAM*	18
	N-nitroso DOXEPIN	DOX NAM	18
	N-nitroso NORTRIPTYLINE	NORNAM	18
	N-nitrosoiminodiacetic acid	NIDA	18
	N-nitroso ethylene diamino triacetic Acid	NED3A	18
	N-Nnitroso bupropione	BUP NAM	1500
	methyl 2-[[4-(2-cyanophenyl)phenyl] methyl-nitroso-amino]-3-nitro-benzoate	MBN-NNO	400

*Internal acronym

***TABLE 1**: Examples of the well-known and specific N-nitrosamines analyzed in Dipharma's processes; Acceptable Intakes (AI) established.*

Considering the Maximum Daily Dose (MDD) of Dipharma products, which ranges from 8 mg to 3.2 g, the average MDD (excluding outliers) is 0.25 g/day. For instance, the FDA recommends a daily intake limit of no more than 26.5 ng/day for NDEA, which corresponds to an average concentration of 106 ppb (26.5/0.25). By setting our internal limit at 10% of this value, we have consistently demonstrated the capability to achieve 10 ppb, ensuring a significant safety margin.

In an extreme case of a product with a daily dose of 3.2 g/day, the NDEA limit is a miniscule 8.3 ppb. Dipharma was able to successfully quantify 0.8 ppb, an amount 10-fold less than the extremely low limit. An example chromatogram of this case is shown below (Figure 4).

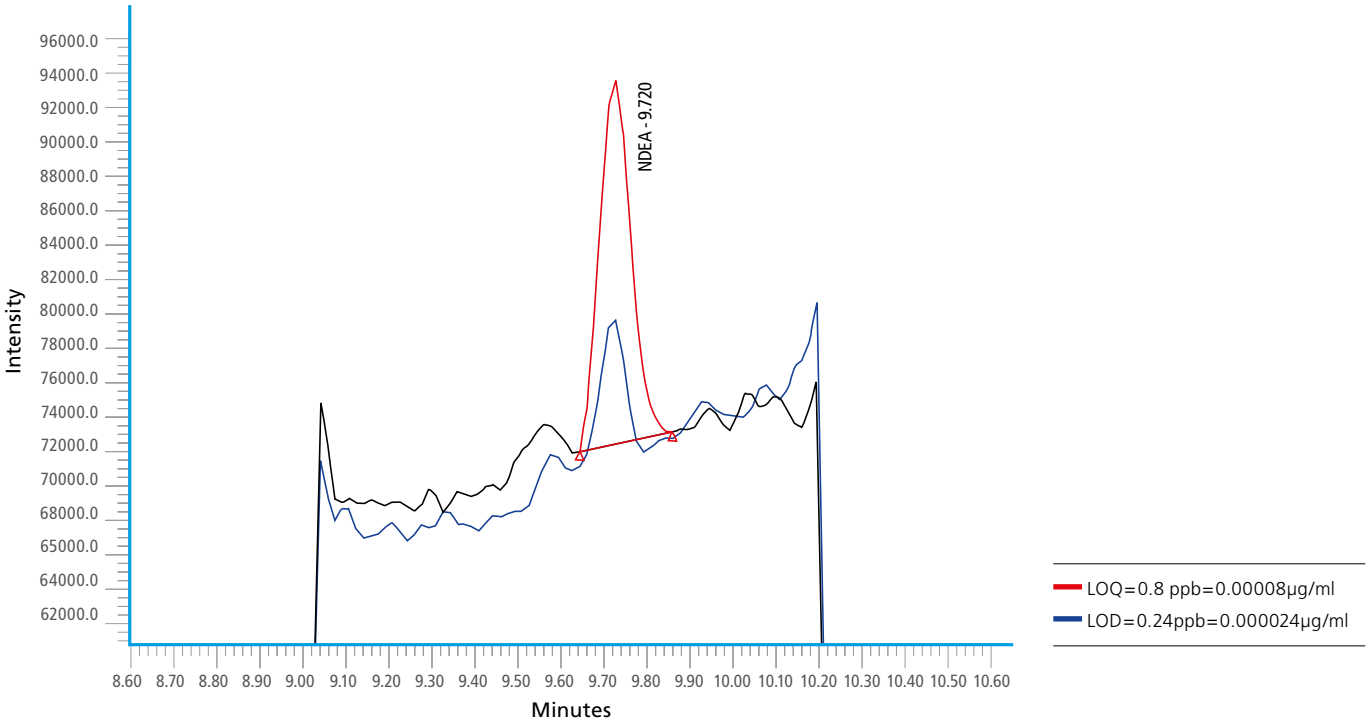


FIGURE 4: NDEA LOQ and LOD peaks at 0.8 and 0.24 ppb.

Innovative ^{15}N-NMR Approach for Nitrosamine Risk Assessment: A Breakthrough by Dipharma

Authorities often require additional release analyses to verify whether an API or intermediate (typically containing an amine or amide group) includes its corresponding nitrosamine. To address this, the strategy involves synthesizing the nitrosamine in the laboratory, characterizing it with appropriate analytical methods, and ensuring that purification steps effectively remove the nitrosamine from the API or keep it below established limits.

When a secondary amine is present, the approach is to synthesize a standard of the corresponding nitrosamine and analyze its content in random production batches. In borderline cases, where a secondary amine is absent or its formation is unlikely, a Nitrosation Assay Procedure (NAP) test can be performed. This simple test provides scientific support for risk mitigation.

Dipharma Francis has taken a significant step forward in the detection and risk assessment of nitrosamines, a critical concern in the pharmaceutical industry. **Dipharma's focus on the application of ^{15}N-NMR spectroscopy, a cutting-edge technique that enhances the identification and quantification of nitrosamine impurities with remarkable sensitivity, allows for the precise tracking of nitrosation reactions using ^{15}N enriched nitrite, ensuring that even trace amounts of nitrosamines can be detected and controlled.** The ^{15}N-NMR technique is highly effective for characterizing nitrogen-containing compounds, especially for detecting N-nitrosamines.

When using a ^{15}N-enriched nitrosating agent like $Na^{15}NO_2$, the N-^{15}N=O signal can be quickly and easily identified within the diagnostic range of 120 to 190 ppm. This signal indicates the presence of an NO group bonded to a nitrogen atom of a secondary amine, forming an N-nitrosamine. Moreover, in cases where the molecule contains multiple secondary amine groups, this technique can provide information on which specific group is actually involved in the formation of the nitrosamine, thereby identifying the correct structure. For a preliminary test, acquiring the ^{15}N-NMR spectrum only within this range is sufficient. If no signal is detected, further investigation in other areas of the spectrum may be necessary to understand the molecule's behavior under nitrosation conditions.

One of the many key case studies involves pirfenidone, an antifibrotic agent with anti-inflammatory and antioxidant properties that is used to treat idiopathic pulmonary fibrosis. The study aimed to determine if one intermediate (5-methyl-2-pyridinamine) could react with nitrosating agents to form N-nitroso compounds using the NAP test with ^{15}N-enriched sodium nitrite ($Na^{15}NO_2$).
After four hours, no diagnostic signal of ^{15}N-enriched N-nitrosamines was detected in the ^{15}N-NMR spectrum, indicating that no N-nitrosamine formation occurs.

With this breakthrough, Dipharma solidifies its position as a reliable partner in pharmaceutical manufacturing, offering advanced analytical solutions to meet the industry's growing regulatory requirements. This innovation is not only a testament to the company's scientific expertise but also a game-changer for ensuring drug safety in a rapidly evolving landscape.

Regulatory Landscape and Mitigation Strategies

EMA & FDA Guidelines

1 EMA
July 2024 Revision Mitigation Measures

Measure	Description
Control Strategy Implementation	Minimization of nitrosamine formation and contamination by: • Changing manufacturing processes. • Improving raw material quality. • Introducing appropriate specifications. • Developing analytical methods.
Control of Nitrosamine Levels	Ensuring compliance with defined limits.

2 FDA
Recommendations Risk Reduction Strategy

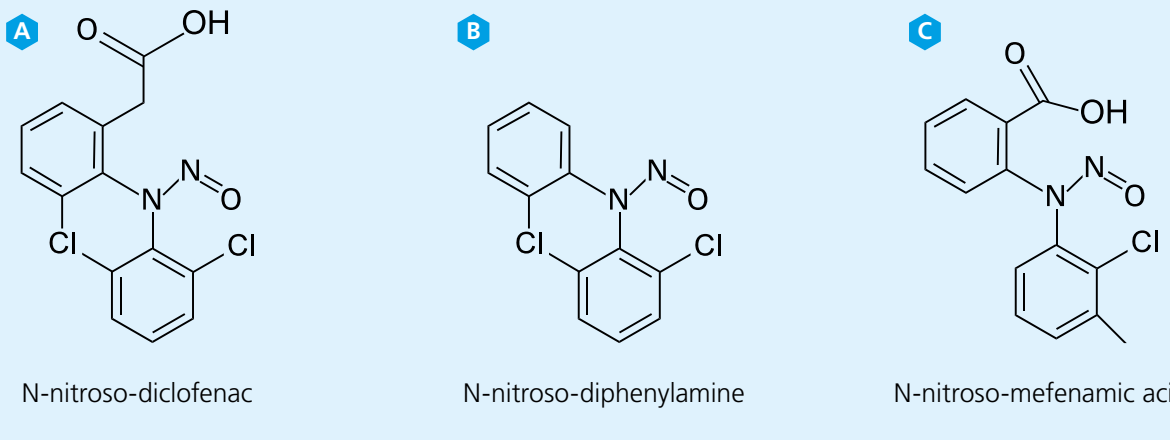
Strategy	Description
Excipient Qualification	Strengthening qualification to minimize nitrite impurities.
Formulation Adjustments	Reformulating drug products with antioxidants to inhibit nitrosamine formation.
pH Adjustment	Modifying formulation pH to neutral or basic conditions to slow reaction kinetics.

EMA - AI Establishment

Approach	Description
TD50 method	Use of specific animal carcinogenicity data to calculate a TD50 and further define acceptable intake.
When TD50 is not known	
CPCA	Carcinogenic Potency Categorization Approach to define AI.
Negative EAT Result	If Enhanced Ames Test (EAT) is negative, nitrosamine can be controlled at 1.5 mcg/day (All Ames assays after August 2023 must follow EAT protocol).
Read-Across Approach	Using TD50 from a surrogate substance as a point of departure if robust carcinogenicity data is available *(See below example in Figure 5)*.
In Vivo Mutagenicity Study	If negative, nitrosamine can be treated as a non-mutagenic impurity, allowing ICH Q3A/B limits to be applied.
Higher AI limits Justification	The use of an interim limit based on Less Thean Lifetime (LTL) approach may be considered on a temporary basis for market action purposes. The two most conservative adjustment factors (6.7 and 13.3 x AI for up to 12 and >12 months of treatment duration) should be used.

Read-Across Approach calculation for the limit of specific NDSRI

N-nitroso-diclofenac is a diaryl N-nitrosamine lacking α-hydrogens just as like N-nitroso-mefenamic and N-nitroso-diphenylamine. For the purpose of Read-Across calculation, N-nitroso-diphenylamine can be considered as a surrogate nitrosamine with robust carcinogenicity data (TD50=167 mg/kg/day) for both molecules.



N-nitroso-diclofenac | N-nitroso-diphenylamine | N-nitroso-mefenamic acid

FIGURE 5: N-nitroso-diphenylamine as a surrogate for N-nitroso-diclofenac and N-nitroso-mefenamic acid AI calculation.

AI for N-nitroso-diclofenac proposed and justified by SAR and read-across approach based on surrogate was calculated considering variation in molecular weight [3] and following the below calculation:

$$\text{AI (N-Nistroso-Diclofenac)} = \frac{325.1}{198.22} \times \left(\frac{167 \times 50}{50000}\right) = 273890 \ \frac{ng}{day}$$

Where:
325.1 = Molecular weight of N-nitroso-diclofenac
198.2 = Molecular weight of N-nitroso-diphenylamine
167 mg/kg/day = Gold TD50 of N-nitroso-diphenylamine
50.000 = risk factor for TD50 extrapolation
50 = body weight (Kg)

Dipharma Read-Across calculation was proposed to EMA, at that time, no AI were yet published, and the approach and results were accepted.
Later in 2023, EMA published an AI for N-nitroso-diclofenac equivalent to 78000 ng/day, where N-nitroso-diphenylamine TD50 data was used as surrogate.

FDA Guidelines for Establishing Nitrosamine Impurity Limits (NDSRIs & Others)

Criteria	Description
CPCA-Based Limits	FDA provides recommended AI limits for nitrosamines (including NDSRIs) based on CPC *(See below example in Table 2).*
Compound-Specific Data	AI limits based on carcinogenicity/ mutagenicity data or read-across from a surrogate.
Higher AI Limits Justification	A higher AI limit than FDA's recommendation must be scientifically justified: Negative EAT[2] result can support a higher limit. Second *in vitro* mammalian cell mutation assay + *in vitro* metabolism data (hepatocyte/ microsome) required for AI limit of 1500 ng/day.

CPCA approach calculation of the limit of specific NDSRI

For potency score the following formula is used:
Potency Score = α-Hydrogen Score + Deactivating Feature Score (sum all scores for features present in the N-nitrosamine) **+ Activating Feature Score** (sum all scores for features present in the N-nitrosamine).

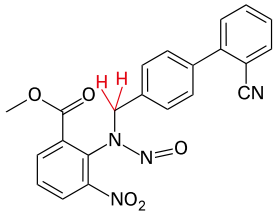
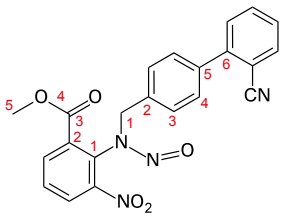
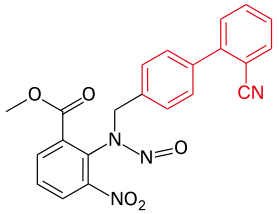
Count of α-Hydrogens	Score	Feature Highlighted in red MBN-NNO
0,2	3	
Deactivating Features		**Feature Highlighted in red**
Chains of ≥25 consecutive non-hydrogen atoms (cyclic or acyclic) on both side of acyclic N-nitroso group. Not more than 4 atoms in each chain may be in the same ring.	+1	
Activating Features		**Feature Highlighted in red**
Aryl group bonded to a-carbon (i.e., benzylic or pseudo-benzylic substituent on N- nitroso group).	-1	
Potency Score = 3	Potency Category =3	**AI = 400 ng/day**

TABLE 2: Example of CPCA calculation.

Case Studies in Nitrosamine Risk Management

Dipharma has successfully implemented its nitrosamine control strategies across multiple APIs. The complete list is far more comprehensive; below, we present just a few examples:

- Triamterene: Addressed customer concerns through comprehensive technical justifications.
- Selegiline: Identified overlooked packaging risks that had been underestimated by the customer.
- Isosorbide-5-mononitrate (IS5MN): Gained EDQM CEP approval in December 2021.
- Pirfenidone: Demonstrated through R&D testing that nitrosamine formation was not a risk.

Ensuring Customer Compliance and Product Safety

Customer Relations and Support

Even commonly used excipients in drug formulations may contain nitrosamines or reactive nitrites at trace levels, presenting additional contamination risks. Such excipients include sodium starch glycolate, croscarmellose sodium, pre-gelatinized starch, polyvinylpyrrolidone (PVP), and lactose. The formulation process itself can contribute to impurity formation.

Dipharma serves as a strategic partner by supplying APIs with the lowest possible nitrosamine content. Additionally, the company assists customers in understanding potential formation mechanisms, guiding them toward the best formulation choices. With its well-equipped R&D laboratory, Dipharma also conducts formulation trials to help customers minimize risks and ensure compliance.

Dipharma works closely with clients to ensure comprehensive risk management. Beyond high-purity API production, the company provides:

- Technical support in understanding nitrosamine formation mechanisms.
- Assistance in formulation optimization to prevent impurity generation.
- Stability studies to continuously monitor nitrosamine formation over time.

Dipharma's Contributors

- **Roberto Fanelli** *API Catalogue Business Unit Leader*
- **Elena Gavrilova** *R&D Scientist - Impurity & RA Documentation Managment*
- **Mauro Atzori** *Corporate QC Laboratory Responsible*
- **Anna Simonetto** *R&D Researcher*
- **Riccardo Carcano** *Corporate QA & RA Manager*

Conclusion

As regulatory expectations for nitrosamine control continue to evolve, API manufacturers must proactively design robust synthetic routes to minimize impurity formation. **Dipharma has long been at the forefront of this approach, integrating risk mitigation strategies from the earliest feasibility studies and throughout process development.**
By leveraging expertise in synthetic route design, Dipharma optimizes reaction conditions to prevent nitrosamine formation, adhering to ICH M7(R2), Q7, and Q11 guidelines.

Since 1949 Dipharma

The Trusted API Partner

PROACTIVE PROCESS DESIGN

Avoiding conditions that promote nitrosamine formation, utilizing alternative bases and solvents, and implementing rigorous fate and purge studies.

SOLVENT RECOVERY CONTROLS

Preventing cross-contamination by strictly managing recovered solvents, reagents, and catalysts.

SUPPLY CHAIN OVERSIGHT

Auditing raw material suppliers, ensuring traceability, and setting stringent specifications for at-risk intermediates.

WATER QUALITY MANAGEMENT

Monitoring nitrite and nitrosamine levels in processing water to eliminate exogenous contamination risks.

ADVANCED PURIFICATION STRATEGIES

Optimizing reaction sequences to enhance nitrosamine purge while ensuring compliance with GMP standards.

REPROCESSING COMPLIANCE

Ensuring that any batch reprocessing aligns with ICH Q7 guidelines under rigorous quality oversight.

With Dipharma as your API manufacturing partner,

you gain a proactive ally committed to delivering high-purity APIs while staying ahead of regulatory expectations for nitrosamine control.
By choosing Dipharma as your partner, you gain access to cutting-edge technology, deep industry expertise, and innovative problem-solving strategies.
Our team is dedicated to upholding the highest quality standards, ensuring your products achieve the required specifications and smoothly progress through regulatory and commercial milestones.

References:
[1] EMA /409815/2020 Rev. 21, 19 July 2024
[2] CDER Nitrosamine Impurity Acceptable Intake Limits, January 2025
[3] J.Fine et al., Regulatory Toxicology and Pharmacology, 145, 2023, 105505

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