Session 4
Nitrosamines: Known Issues and Practical Advice

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Nitrosamine-Impacted Drug Products Containing BCS IV Drug Substances

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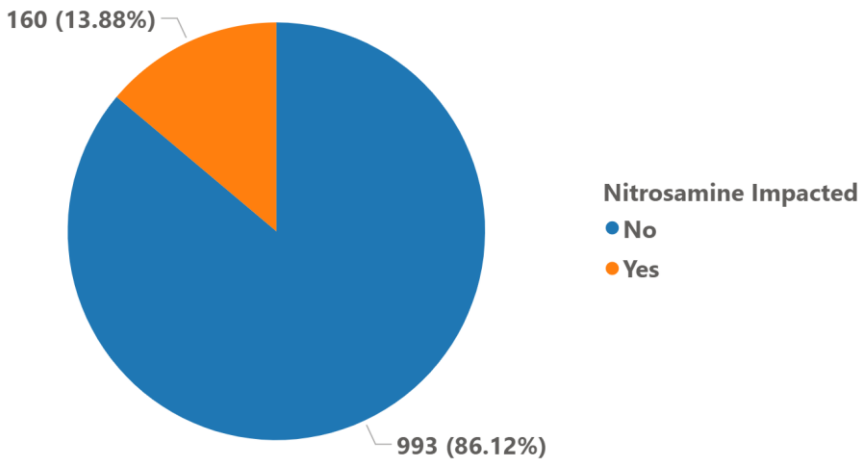
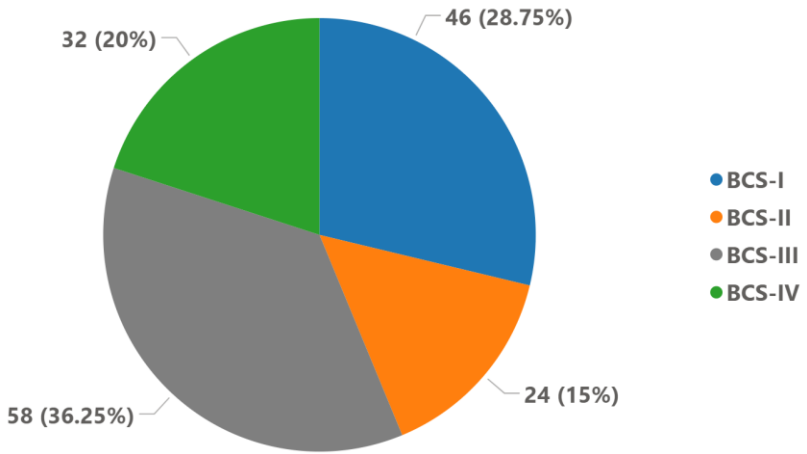
Outline

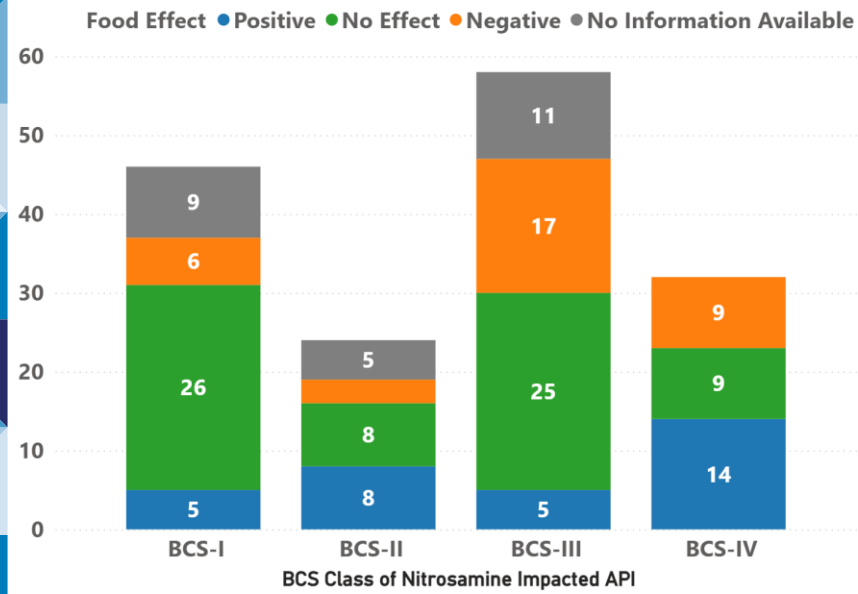
- Current nitrosamine challenges across immediate-release oral products with various BCS classifications
- FDA's alternative bioequivalence (BE) framework and BCS Class IV limitations
- Proposed risk-based subcategorization for BCS Class IV drugs
- Case study validation and regulatory implications

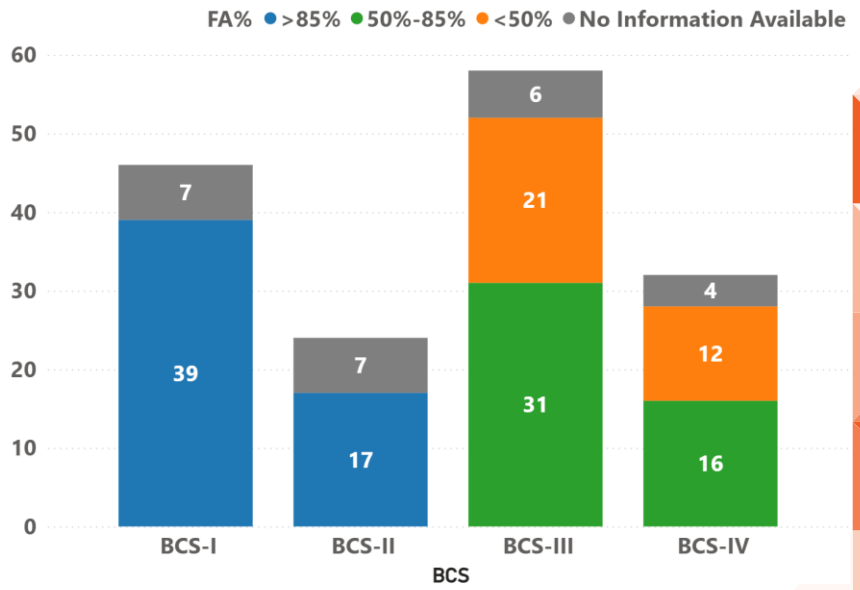
Nitrosamine-impacted Immediate-Release (IR) Oral Drug Products





Nitrosamine-impacted IR products across BCS: Food Effect and Fa%[1]





[1] Fraction absorbed%: estimated based on absolute bioavailability and the results of mass balance studies from labeling information.

FDA Guidance on alternative BE approaches for nitrosamine-impacted drug products[2]

- **Focus on alternative BE approaches for products with added antioxidants and pH modifiers**
  - For reformulated products to include excipients such as antioxidants or pH modifiers aimed at reducing nitrosamine impurities, the FDA guidance allows for the use of alternative BE approaches to demonstrate BE without the need for a traditional in vivo (fasting or fed) BE study.
- **Importance of BCS classification in determining alternative BE approaches**
  - **BCS I, II, III**: **Comparative dissolution testing (multi-pH dissolution profiles) may be used as an alternative BE approach**, if the reformulated IR product uses pH modifiers or specific antioxidants (like ascorbic acid, alpha-tocopherol, cysteine hydrochloride, and propyl gallate) within acceptable limits (typically ≤ 10 mg per dose).
  - **BCS IV**: **Supported by studies such as a validated in vitro-in vivo correlation, physiologically based pharmacokinetic modeling (PBPK) or in vivo BE studies.**
- **Scientific justification and risk-based approach when selecting alternative BE approaches**
  - A thorough risk assessment considering factors such as drug properties, nitrosamine formation pathways, and the extent of formulation changes

[2]Guidance For Industry: Control of Nitrosamine Impurities in Human Drugs (September 2024)

BCS IV challenges

Absorption Complexity

- BCS IV drug substances are characterized by both low solubility and permeability, making bioavailability/bioequivalence unpredictable and challenging.
- Comparative dissolution tests may not reliably predict complex absorption behavior, particularly compared to their effectiveness with more soluble and permeable drugs.

Formulation Sensitivity

- Due to intrinsic challenges to BCS IV drugs, even small formulation changes may necessitate rigorous BE evaluation methods to ensure that the therapeutic performance is maintained.

Pathway Forward

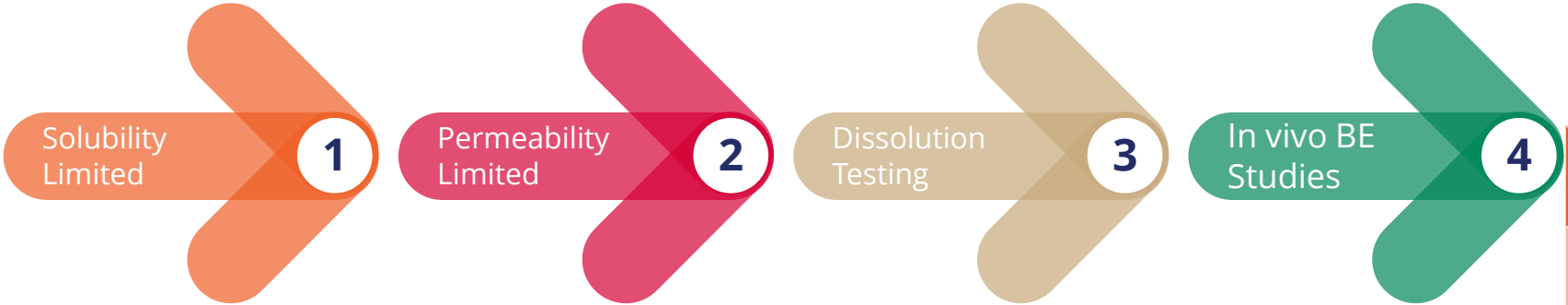
- **A robust risk-based approach — including rigorous dissolution testing and PBPK modeling methods when necessary — can provide a pathway to waive in vivo BE studies.**
- This approach is particularly applicable for BCS IV drugs when changes involve antioxidants or pH modifiers with limited impact on overall absorption.

BCS IV subcategories and risk factors

This table presents a classification of BCS IV subcategories along with their associated risk factors and critical excipients. It breaks down BCS IV drugs into different subcategories based on their dominant limitations – whether solubility or permeability – and identifies the corresponding challenges relevant to each category.

Category	Solubility limited (BCS II like)			Dual limited	Permeability limited (BCS III like)		
Risk factors	pH-dependent solubility (weak acid and base)	Precipitation (weak base)	Low solubility and poor dissolution	Low solubility and low passive permeability	Low passive permeability	Efflux transporter	Short absorption window
Food effects	Positive effects with higher Fa%			Unpredictable	No effects or negative effects with lower Fa%		
Critical excipients	pH modifiers	Polymers, nanoparticles, solid dispersions	Solubilizers, lipid based	Solubilizers, permeability enhancers	Surfactants, lipid based	Permeability enhancers	Lipid excipients

4 Tier risk evaluation for BCS IV reformulated drug products[3]



Evaluation: Conduct solubility-pH testing
Key indicators: Positive food effects. pH-dependent solubility

Evaluation: Estimate Fa by comparing the reformulated drug to known model drugs
Key indicators: No or negative food effects.

Evaluation: Focus on product design and critical excipients; conduct comparative dissolution testing in QC medium and multi-pH media
Key indicators: Dissolution not similar

Evaluation: Perform in vivo BE study if dissolution results or absorption predictions (PBPK) suggest high risk
Key indicators: Non-BE

[3]Qi Zhang, Managing Bioequivalence Risks for Nitrosamine Impacted Drug Products Containing BCS IV Drug Substances, CRCG, Nov 2024

Antioxidants: Rationale to extend BCS III findings to BCS IV

BCS III Model Drugs	Estimated BCS Class	Dosage form	Estimated Fa%	Estimated food effect AUC	Estimated food effect Cmax
CIMETIDINE	BCS-III	TABLET	56-68%	0.9	1.1
ACYCLOVIR	BCS-III/IV[4]	CAPSULE (200 mg)	10-15%	1	1
ACYCLOVIR	BCS-III/IV	SUSPENSION (800 mg)		1.3	1
ACYCLOVIR	BCS-III/IV	TABLET (800 mg)		~1.5	~1.5
ATENOLOL	BCS-III	TABLET	50%	~0.9	~0.9
RANITIDINE	BCS-III	TABLET	50-60%	1	1
RANITIDINE	BCS-III	CAPSULE		1	1
RANITIDINE	BCS-III	TABLET, EFFERVESCENT		1	1

Research[5] on the BCS III model drugs show that antioxidants do not alter the permeability.

For BCS IV drugs with comparable or better permeability/absorption than BCS III model drugs (Fa >10%), antioxidants are expected to have minimal or no effect on the permeability.

For BCS IV drugs where permeability is not the primary limiting factor, antioxidants is not expected to affect drug permeability and absorption.

In more complex BCS IV formulations, it's essential to confirm that the antioxidant amount threshold (e.g., above 10 mg) do not interfere with other excipients (solubilizers, surfactants, etc.) or introduce permeability alterations.

[4]Solubility is estimated for acyclovir with the highest single dose of 800 mg

[5]Lu, D., et al. (2024). Antioxidants had No Effects on the In-Vitro Permeability of BCS III Model Drug Substances. *Journal of Pharmaceutical Sciences*, *113*(9), 2708-2714.

Case Study

- IR tablet with multiple strengths
- Antibiotic
- Nitrosamine impurities
  - Detected in all tested batches of marketed product
  - Exceeded acceptable intake limit of 1500 ng/day
- Root cause
  - Nitrite impurities in excipients
  - Manufacturing process conditions
  - Storage conditions during shelf life

Case Study – Cont'd

- Nitrosamine risk mitigation strategy:
  - Reformulation by adding antioxidant (less than 10 mg) as nitrite scavenger through prior approval supplement

Ingredients	Approved Composition	Revised Composition
API	✓	✓
Diluent	✓	Adjusted
Disintegrant	✓	✓
Nitrate scavenger	X	✓
Binder	✓	✓
Extragranular	✓	✓

- Risk assessment:
  - Low risk per ICH M13A guidance

Case Study – Cont'd

- Maximum single dose is 780 mg
- BCS Class IV
- pH-dependent solubility:
  - pH 1.2: 18 mg/mL
  - pH 4.5: 68 mg/mL
  - pH 6: 4 mg/mL
  - pH 6.8: precipitated
- Moderate to high permeability:
  - 68%-72% absolute bioavailability
  - Food effect (Fed/Fast): AUC: 0.75 -0.80, Cmax: 0.70 -0.75

Case Study – Cont'd

- Comparative dissolution profile (QC media) – Before reformulation

Collection Time	10 min	20 min	30 min
Cumulative average % Dissolved	98	101	104

- Comparative dissolution profile (QC media) – After reformulation

Collection Time	10 min	15 min	20 min	30 min
Cumulative average % Dissolved	93	94	98	100

- Very rapid dissolution: >85% dissolved within 15 minutes (all strengths)

Case Study – Cont'd

- PK profile – Before reformulation

Parameter	T/R Ratio (Fasting)	T/R Ratio (Fed)
AUC_{0-t} Ratio	95.74%	104.93%
$AUC_{0-\infty}$ Ratio	95.23%	104.73%
C_{max} Ratio	90.48%	101.88%

- PK profile – After reformulation

Parameter	T/R Ratio (Fasting)	T/R Ratio (Fed)
AUC_{0-t} Ratio	99.76%	100.27%
$AUC_{0-\infty}$ Ratio	98.82%	101.15%
C_{max} Ratio	99.34%	100.58%

- Antioxidant addition did not impact BE, confirming predictable antioxidant effects similar to established BCS I/II/III approaches

Framework Application Potential

- Case study demonstrates key principles:
  - Antioxidant addition (less than 10 mg) maintained BE
  - Supports theoretical basis for predictable antioxidant impact
- Framework relevance:
  - Case shows high solubility up to pH 6 and moderate to high permeability (68-72% Fa) with no significant food effects, supports the 4-tier risk-based framework leveraging the established BCS I/II/III approaches.
  - Suggests feasibility for BCS IV drugs with similar absorption characteristics and reformulation strategies
- Future research needs:
  - Additional BCS IV cases to refine and validate the proposed framework
  - Evidence generation for alternative BE approaches

Final thoughts and future direction

- Key Considerations
  - Formulation changes for nitrosamine mitigation must not compromise solubility, permeability, or bioavailability/bioequivalence.
  - BCS Class IV drugs present unique BE challenges due to dual solubility and permeability limitations.
- Strategic Approach
  - Food effects guide BCS IV subclassification:
    - Positive food effects → solubility-limited
    - Negative/minimal food effects → permeability-limited
  - Leverage existing knowledge: Antioxidants minimally impact permeability; established BCS I/II/III approaches can guide BCS IV assessments.
- Future Research Focus
  - For complex BCS IV formulations with antioxidants and other excipients:
    - Assess existing PK data
    - Evaluate dissolution across pH ranges
    - PBPK modeling and cross-validate in vitro findings with in vivo data

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FDA U.S. FOOD & DRUG ADMINISTRATION

Thank you!

Addressing Potential Pitfalls in Nitrosamine Risk Assessment and Control

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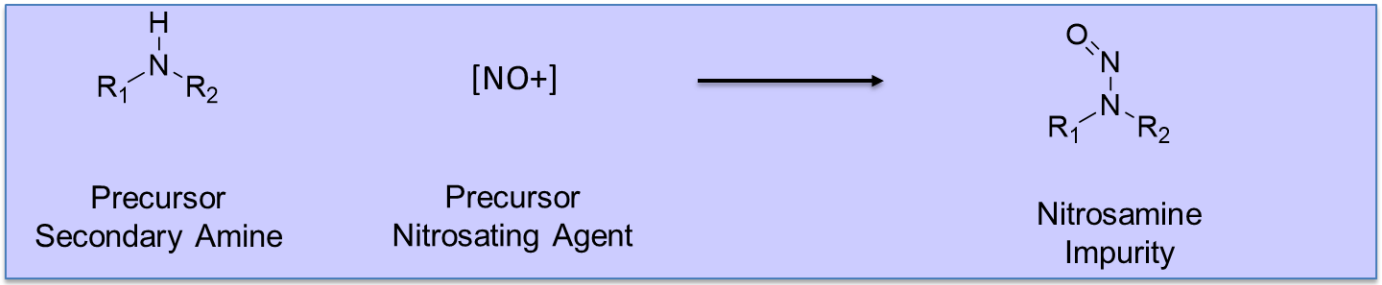
Introduction to the Nitrosamine Issue

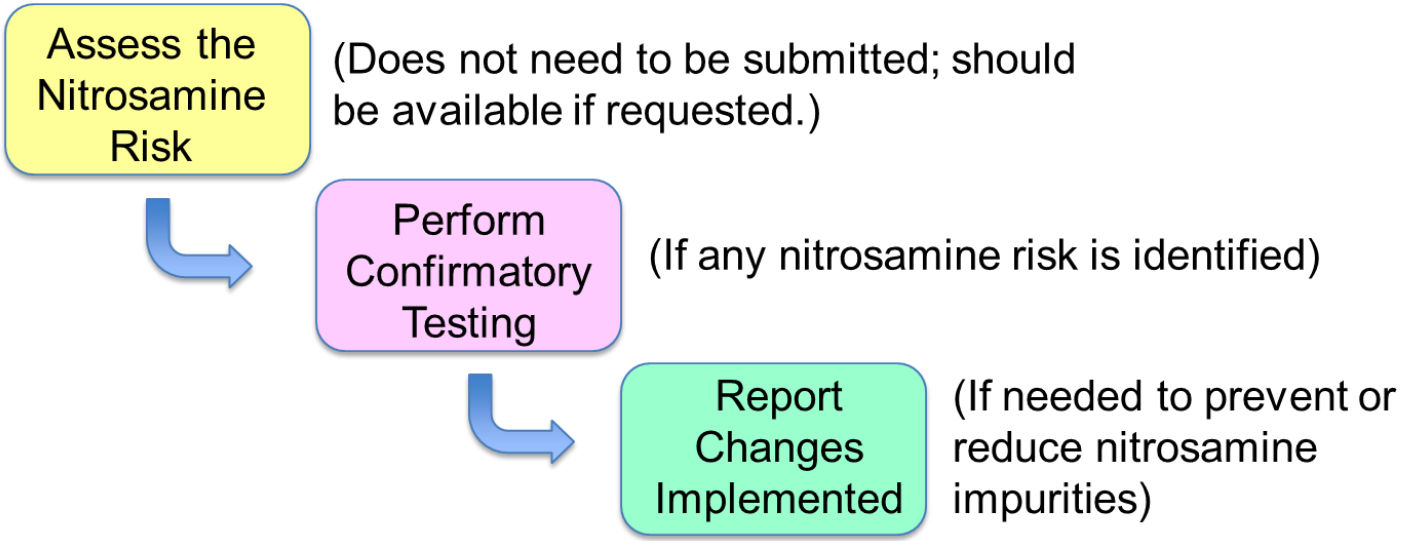
- Nitrosamines can be present as impurities in both drug substances and drug products

- Nitrosamines are potent genotoxic agents in several animal species and some are classified as probable or possible human carcinogens by the International Agency for Research on Cancer.



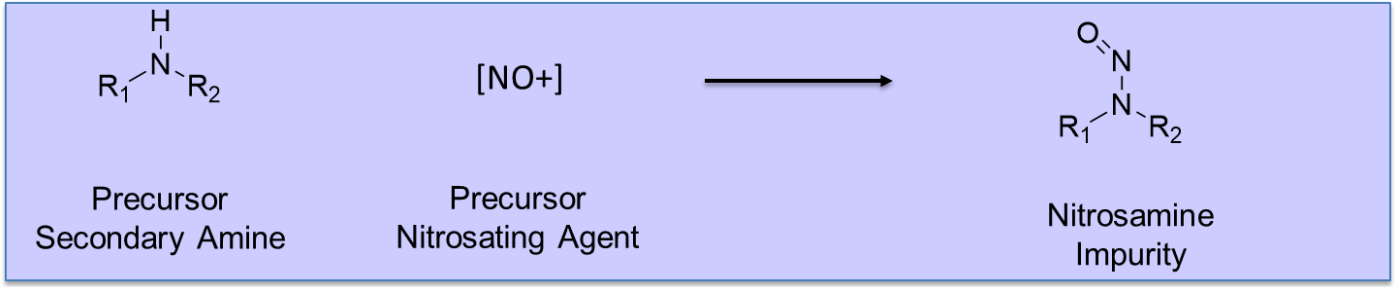
- FDA Guidance for Industry: "Control of Nitrosamine Impurities in Human Drugs," September 2024.
- CDER Nitrosamine Impurity Acceptable Intake Limits also includes updated information

Recommended Three-Step Nitrosamine Mitigation Strategy



- FDA Guidance for Industry: "Control of Nitrosamine Impurities in Human Drugs," September 2024.

Types of Nitrosamine Risk



Contamination Risk:

An intact nitrosamine is introduced into a pharmaceutical product

- Cross-contamination from recycled solvents
- Introduction of a nitrosamine from a container-closure component or raw material

Formation Risk:

The chemical precursors encounter each other to form a nitrosamine during the manufacture or shelf-life of a drug substance or drug product.

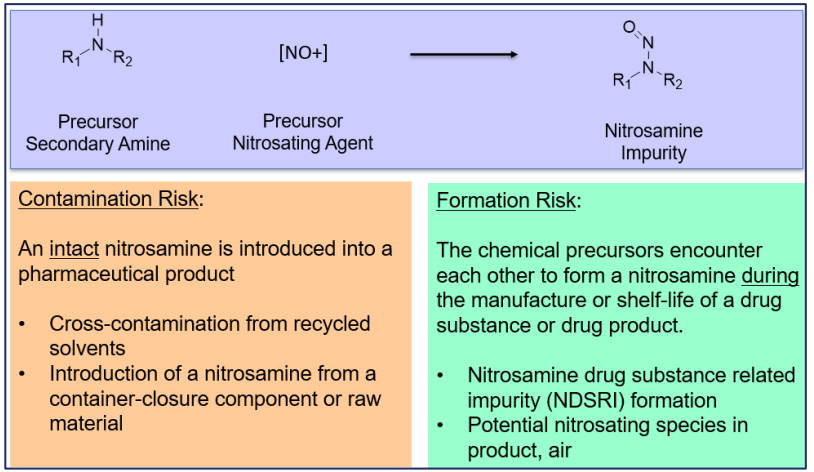
- Nitrosamine drug substance related impurity (NDSRI) formation
- Potential nitrosating species in product, air

Common Pitfalls

- Not considering nitrosamine risk holistically
- Issues with reference standards
- Acceptable Intake (AI) limits not endorsed by FDA
- Misunderstanding interim limits
- Multiple nitrosamines in the same product
- Potential options when a formulation exceeds the AI limit
- Evolving landscape

Pitfall 1: Not Considering Risk Holistically

- The risk question (see ICH Q9[1]) or problem statement is too narrow
  - Considers only contamination risk.
  - Considers only formation risk
  - Does not consider both NDSRI scenarios
    - "Classical" NDSRI
    - "Fragment" NDSRI

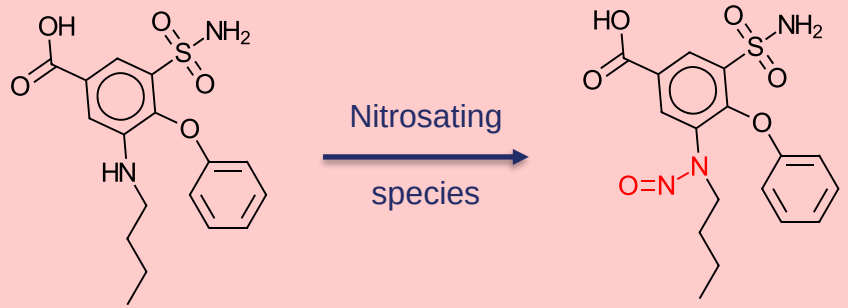


- CDER Nitrosamine Impurity Acceptable Intake Limits website provides a list of APIs having a hypothetical risk of nitrosamine formation

(1) FDA Guidance for Industry: "Q9(R1) Quality Risk Management," May 2023.

The “Classical” NDSRI Scenario

The pure API undergoes nitrosation:



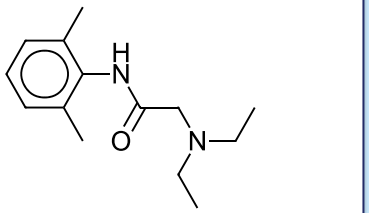
Bumetanide API
(A secondary amine)

Nitrosamine
(“Classical” NDSRI)

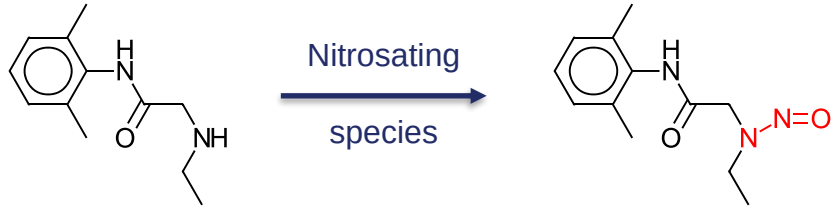
Shakleya, et al. *J. Pharm. Sci.* 112 (**2023**) 3075. https://doi.org/10.1016/j.xphs.2023.06.013.

The “Fragment” NDSRI Scenario

A fragment impurity of the API undergoes nitrosation:



Lidocaine API
(Not a secondary amine)



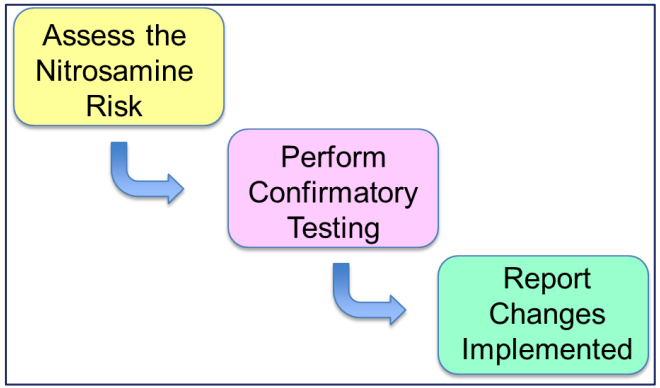
Process or degradation impurity
(Ph. Eur. Impurity D)

Nitrosamine
(“Fragment” NDSRI)

Kalauz, et al. *J. Pharm. Sci.* 114 (**2025**) 103921. https://doi.org/10.1016/j.xphs.2025.103921.

Pitfall 2: Issues with Reference Standards

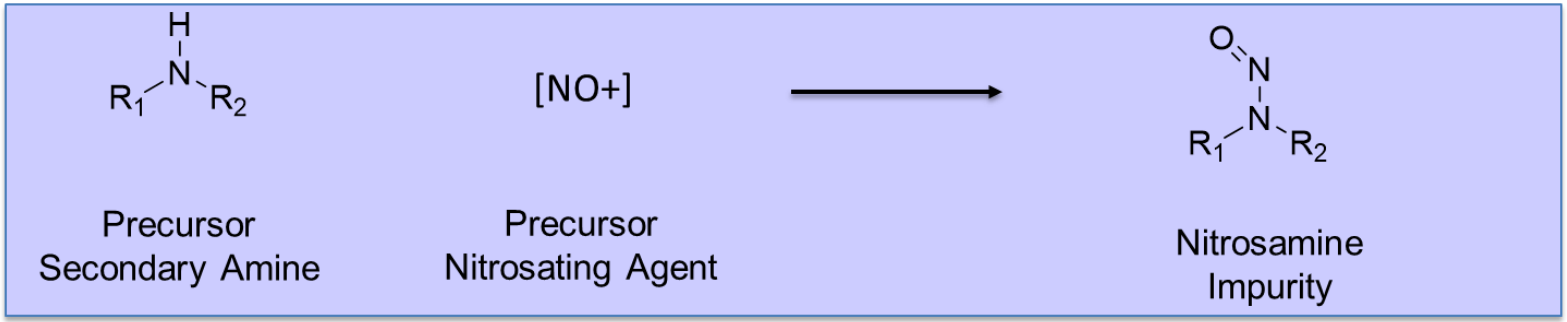
- Reference standards for confirmatory testing may not be available



- Commercial reference standards may not be the correct structure
  - Importance of characterization
- Some secondary amines are difficult or impossible to nitrosate
  - Includes some hypothetical NDSRIs listed on the CDER Nitrosamine Impurity Acceptable Intake Limits website.

- Experimental evidence that a drug substance or impurity is chemically resistant to nitrosation can be an important part of the risk assessment.
  - May justify omission of confirmatory testing
  - FDA Guidance for Industry: "Control of Nitrosamine Impurities in Human Drugs," September 2024.

Evaluating Susceptibility to Nitrosation



- Important factors to consider:
  - Reaction solvent
  - pH and basicity of amine
  - Nitrosating agent
  - A reaction other than nitrosamine formation may occur
  - The nitrosamine may form transiently and then react further
- Lack of nitrosation in a single experiment is generally not sufficient

Evaluating Susceptibility to Nitrosation: Useful Scientific Literature

General conditions for synthesis of nitrosamines:

Chaudhary, et al. “An Efficient Synthesis of N-nitrosamines Under Solvent, Metal and Acid Free Conditions using *tert-B*utyl Nitrite,” *Green Chem*., **2016**,18, 2323. https://doi.org/10.1039/c5gc02880a

Optimized procedure for screening drugs for nitrosation susceptibility:

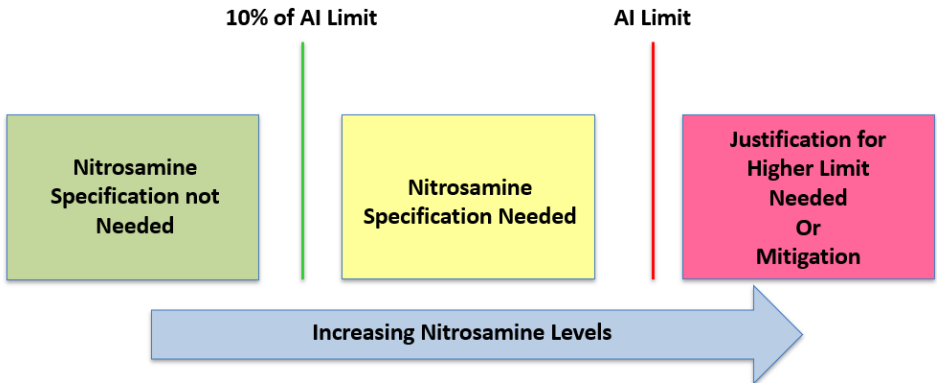
Sharma, et al. “Modified NAP test: A Simple and Responsive Nitrosating Methodology for Risk Evaluation of NDSRIs,” *J. Pharm. Sci*., 112 (**2023)** 1333. https://doi.org/10.1016/j.xphs.2023.02.024

Three standard, complementary forced degradation conditions to evaluate NDSRI formation; discussion of chemical reactivity considerations:

Ashworth, et al. “Approaches and Considerations for the Investigation and Synthesis of N-Nitrosamine Drug Substance-Related Impurities (NDSRIs),” *Org. Process Res. Dev.* ***2023****, 27, 1784−1791*. https://doi.org/10.1021/acs.oprd.3c00084

Pitfall 3: AI Limits Not Endorsed by FDA

- Acceptable Intake (AI) limits not listed on the CDER Nitrosamine Impurity Acceptable Intake Limits website need to be evaluated by FDA
  - Includes any AI limit for nitrosamines which are not listed on the website
  - Includes higher AI limits accepted by other regulatory agencies
  - Prior Approval Supplement vs Changes Being Effected Supplement



Pitfall 4: Misunderstanding Interim Limits

Recommended Interim AI Limits for Certain Nitrosamine Impurities

If drug product batches already in distribution contain nitrosamine impurities at levels above the FDA recommended AI limit, and manufacturing changes or recalls are likely to lead to a disruption in the drug supply, then manufacturers and applicants should immediately contact CDER'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 to recommend an interim AI limit for a temporary period. If FDA recommends an interim AI limit, it generally does not intend to object, for example based on applicable underlying CGMP violations, to distribution of such drug product batches that contain nitrosamine impurity levels at or below the recommended interim AI limit during the specified period under certain circumstances which will be determined on a case-by-case basis. In certain cases where FDA does not intend to object to the distribution of drug products from multiple drug manufacturers that contain nitrosamine levels at or below the recommended interim AI limit, FDA intends to post such recommended interim AI limit on this website.

- Interim AI limits cannot be approved in a specification.
- AI limits not listed on the CDER Nitrosamine Impurity Acceptable Intake Limits website need to be evaluated by FDA

Pitfall 5: Multiple Nitrosamines

- FDA-recommended AI limits:
  - correspond to individual nitrosamine impurities;
  - are applicable only if a drug product contains a single nitrosamine

- When more than one nitrosamine is identified, a limit for total nitrosamines is recommended.

> Each individual nitrosamine should be calculated as a percentage of its AI limit, such that the sum of all the nitrosamines does not exceed 100 percent. The calculation for total nitrosamines is described as below:
>
> $$\sum_{i=2}^{n} \frac{Xi}{AIi} * 100\% \leq 100\%$$
>
> Where Xi is the amount of each single nitrosamine i in parts per million (ppm), AIi is the AI limit of each nitrosamine in ppm, and n is not more than 3.

- FDA Guidance for Industry: "Control of Nitrosamine Impurities in Human Drugs," September 2024. See Section A (p.13) and Appendix C (p. 40.)

Pitfall 5: Multiple Nitrosamines

Acceptable Intake (AI) Limit

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).

Control of Total Nitrosamine Levels

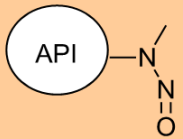
- The acceptable level of risk can be distributed among more than one nitrosamine.
- The risk from multiple nitrosamine is additive
- The aggregate risk from multiple nitrosamine should be NMT 1 in 100,000
- Think in terms of percentages of acceptable risk: NMT 100% overall

2024 FDA Guidance: "Control of Nitrosamine Impurities in Human Drugs" Revision 2. See Section A (p.13) and Appendix C (p. 40.)

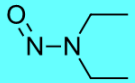
Pitfall 5: Multiple Nitrosamines

Hypothetical drug product which could contain both and NDSRI (nitrosated API) and a small-molecule nitrosamine (NDEA)

The MDD is 100 mg/day



NDSRI
AI = 1500 ng/day
Concentration limit = 15 ppm
(Result = 12 ppm)



NDEA
AI = 26.5 ng/day
Concentration limit = 0.265 ppm
(Result = 0.212 ppm)

- Each result is 80% of its AI.
- Each nitrosamine contributes 80% of the overall acceptable risk to the product
- This product would have 160% of the acceptable risk.

Pitfall 5: Multiple Nitrosamines

Example of flexible approach to total nitrosamine control :

Drug A has maximum daily dose 80 milligrams/day

		Acceptance Criteria	Stability Results			
			0 month		3 months	
Nitrosamine	AI Limit (ng*/day)	Concentration Limit (ppm)[2]	Nitrosamine level (ppm)	% of AI Limit	Nitrosamine level (ppm)	% of AI Limit
Nitrosamine 1	26.5	NMT* 0.33	0.10	30.30	0.15	45.45
Nitrosamine 2	37	NMT 0.46	0.05	10.87	0.20	43.48
Nitrosamine 3	1,500	NMT 18.75	1.00	5.33	3.00	16.00
Total nitrosamine[1]		Sum of all nitrosamines NMT 100%		46.50 (pass)		104.93 (fail)

* ng = nanogram; NMT = no more than

[1] Total nitrosamine is the sum of the percentage of AI limit for each individual nitrosamine. No more than 100 percent means the theoretical cancer risk is no more than 1:100,000.

[2] The concentration limit here is the AI limit for the nitrosamine in ppm which is calculated based on a drug's maximum daily dose (ppm = AI (ng/day)/maximum daily dose (milligrams/day)).

2024 FDA Guidance: "Control of Nitrosamine Impurities in Human Drugs" Revision 2. See Appendix C (p. 40.)

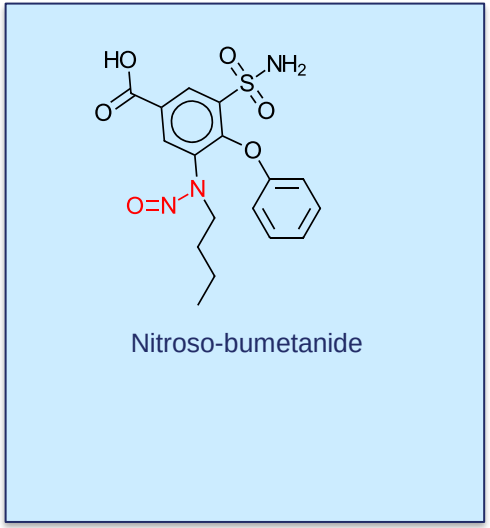
Pitfall 6: Exceeding the AI Limit

Options:

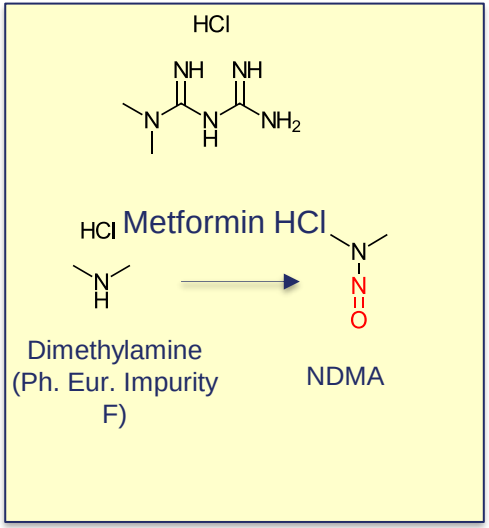
- Justify a higher AI limit
- Make changes to inhibit/reduce nitrosamine levels

➢ Changes in raw materials or manufacturing process

➢ Formulation changes: use of antioxidants, pH modifiers



Nitroso-bumetanide



Dimethylamine (Ph. Eur. Impurity F)

NDMA

Shakleya, et al. *J. Pharm. Sci.* 112 (**2023**) 3075. https://doi.org/10.1016/j.xphs.2023.06.013.

Shakleya, et al. *Int.J. Pharmaceutics,.666* (**2024**) 124832. https://doi.org/10.1016/j.ijpharm.2024.124832

Pitfall 7: Evolving Landscape

Useful published literature:

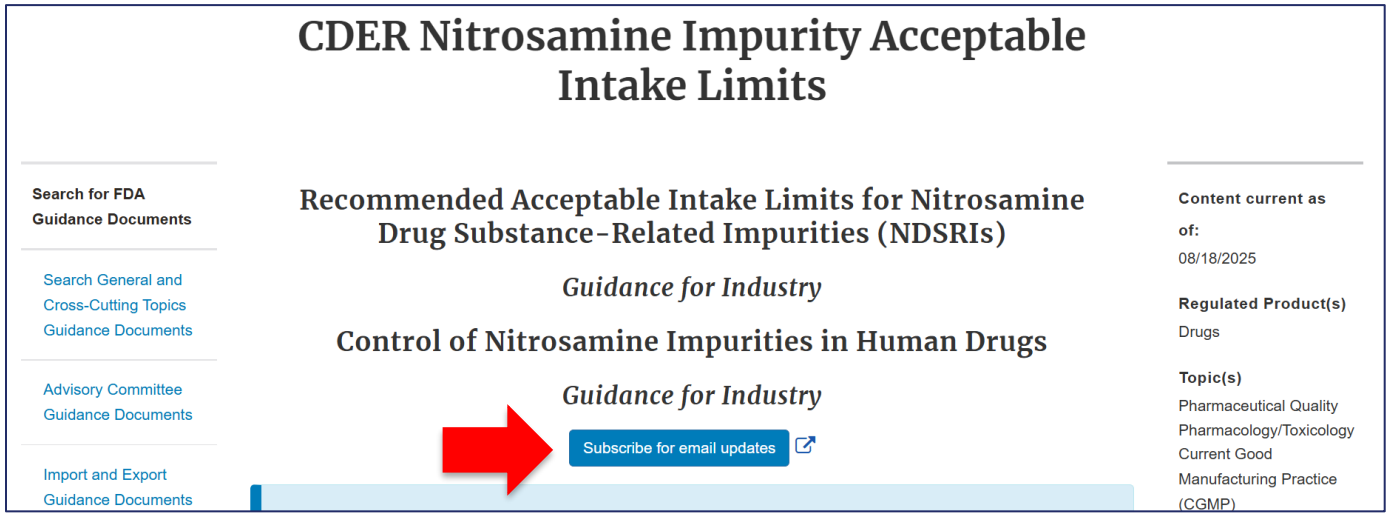
“N-Nitrosamine Formation in Pharmaceutical Solid Drug Products: Experimental Observations,” Moser, et al. *J. Pharm. Sci*., 112 (**2023**) 1255−1267. https://doi.org/10.1016/j.xphs.2023.01.027

“Drug Substance and Drug Product Workflows for Quality Risk Management for the Presence of Nitrosamines in Medicines,” Dirat, et al. *Org. Process Res. Dev.* **2025**, 29, 1538−1553, https://doi.org/10.1021/acs.oprd.5c00097

“Regulatory Experiences with Root Causes and Risk Factors for Nitrosamine Impurities in Pharmaceuticals,” Horne, et al. *J. Pharm. Sci*., 112 **(2023**) 1166-1182. https://doi.org/10.1016/j.xphs.2022.12.022

Pitfall 7: Evolving Landscape

[CDER Nitrosamine Impurity Acceptable Intake Limits](#) Website:



Pitfall 7: Evolving Landscape

CDER Nitrosamine Impurity Acceptable Intake Limits Website: Updated Implementation Timeline for Approved Products

† June 23, 2025 Update

While FDA reiterates that applicants should conclude confirmatory testing for products with a risk of nitrosamine drug substance-related impurity (NDSRI) formation and submit necessary changes by August 1, 2025, **FDA is allowing additional time for submission of required changes**. Many applicants have submitted NDSRI mitigation proposals, but FDA acknowledges that timeframes for full implementation of NDSRI control or mitigation strategies may vary depending upon the specific strategy, for example adding a specification or reformulation. Therefore, FDA acknowledges that the original August 1, 2025 timeline is not achievable for all approved applications for at-risk products.* **For those applicants unable to submit the necessary changes within this timeline, provide a progress update as described below by August 1, 2025**.

Pitfall 7: Evolving Landscape

CDER Nitrosamine Impurity Acceptable Intake Limits Website: Updated Implementation Timeline for Approved Products

Recommendations on what to provide and how:

- The progress update should include the following information:
  - determination of whether NDSRIs can form under targeted forced degradation,**
  - NDSRI(s) detected,
  - nitrosamine test method with validation,
  - product batch(es) analyzed, and date analyzed relative to date of manufacture,
  - confirmatory test results for NDSRI(s) in the drug product (in ng/day or ppm),
  - root cause (if known),
  - a description of attempts to mitigate identified NDSRIs, if mitigation is necessary, and
  - an estimate of the timeframe when mitigation will be completed

    Note: If you cannot provide responses to the information requested above, submit justification.
- Applicants should include a description of their progress in addressing NDSRIs that includes the recommended information above titled as “NDSRI Update” in the Log of Outstanding Regulatory Business section (eCTD 1.13.14) of the Annual Report (AR)***;

Pitfall 7: Evolving Landscape

CDER Nitrosamine Impurity Acceptable Intake Limits Website:

Other Emerging Scientific and Technical Issues

As FDA becomes aware of new and emerging information on nitrosamine impurities, it may communicate new information on nitrosamine impurities and FDA's understanding of the root cause of such impurities and their formations. It may also communicate recommendations for mitigation to address such nitrosamine impurities.

- August 18, 2025: Emerging Scientific and Technical Information on Leachable NDBA and Other Small-Molecule Nitrosamines in Infusion Bags
- October 28, 2024: Emerging Scientific and Technical Information on Ritonavir

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Thank you!

Pharm/Tox considerations for safety evaluation of nitrosamine impurities

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CDER | US FDA

Advancing Generic Drug Development Workshop: Translating Science to Approval
October 7-8, 2025

Disclaimer

This presentation reflects the views of the author and should not be construed to represent FDA's views or policies.

Nitrosamines and safety assessment overview

- Nitrosamines (NA) are potent rodent carcinogens, probable human carcinogens.
  - **Small molecule nitrosamines**: lower molecular weight (MW), e.g., *N*-nitrosodiethylamine (NDEA), *N*-nitrosodimethylamine (NDMA)
  - **Complex nitrosamines or nitrosamine drug substance-related impurities [NDSRIs])**: unique to drug substance, larger nitrosamines containing activating and deactivating features, lack of carcinogenicity data.
- Nitrosamines belong to "cohort of concern" (CoC) in International Council for Harmonisation (ICH) M7 guidance
  - Carcinogenic potency of NAs varies
  - ICH M7 recommends setting compound-specific acceptable intake (AI)
  - AI limit is the allowable exposure for a 1:100,000 cancer risk estimate
  - AI limits are generally less than the threshold of toxicological concern (TTC) which is 1.5 mcg/day

Control limits for nitrosamine impurities: guidance

- FDA recommends the following methods for setting AI limits
  - Carcinogenic Potency Categorization Approach (CPCA)
  - AI limit based on compound-specific data
  - Read-across from a surrogate with robust carcinogenicity data

- FDA published several guidance to support nitrosamine risk assessments.
  - Quality guidance: Control of Nitrosamine Impurities in Human Drugs, September 2024
  - Safety guidance: Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs), August 2023 (“RAIL” Guidance)
  - FDA Nitrosamine guidance webpage: CDER Nitrosamine Impurity Acceptable Intake Limits

“RAIL” guidance and FDA webpage

- Describes framework for setting AI limits for NDSRIs using CPCA as a starting point to set a limit without compound-specific data.
- Describes test for nitrosamine in product and encourages mitigation.
- Provides options to support alternate AI and clarifies expectations for data needed.
  - **To support AI limit up to 1500 ng/day**: negative enhanced Ames assay, confirmatory *in vitro* mammalian cell mutation assay, and *in vitro* metabolism data
  - **To support AI limit > 1500 ng/day**: additional negative *in vivo* mutagenicity data, which may not be supportive of an AI limit equal to the qualification threshold (QT) of ICH Q3A/Q3B
  - **To support a surrogate-based AI limit**: surrogate should be structurally relevant with robust carcinogenicity data
- Encourages communication and includes section on emerging issues, timeline for implementation, and options for approved products when there are drug supply concerns.

Nitrosamine Safety Assessment: guidance through collaboration

- FDA's multi-disciplinary experts support risk assessments, advance nitrosamine science, and develop nitrosamine guidance
  - Formed the Nitrosamine Safety Team to coordinate among Pharm/Tox (in OGD and Office of New Drugs (OND)) and computational toxicologists
  - Participate in Nitrosamine International Technical Working Group and ICH M7 nitrosamine addendum development
  - Collaborate with National Center for Toxicological Research (NCTR) on research needs for nitrosamine risk assessment
  - Support FDA nitrosamine research and collaborations through Health and Environmental Science Institute (HESI)
- Safety assessment of nitrosamines in new and generic drugs follows FDA's guidance on nitrosamines
  - OGD Pharm/Tox collaborates with OND to align on assessment approaches
  - Pharm/Tox assessors work closely with other disciplines to support risk assessments and maintain access to medically necessary drugs

Common pitfalls in nitrosamine safety review

- Inadequate enhanced Ames test (EAT)
  - Low test dose, solvent concentration interfering with metabolic activation, lack of appropriate nitrosamine positive controls, insufficient S9 activity
  - Preincubation method is recommended for EAT of nitrosamines, along with use of hamster S9 at 30%
- Justifying an alternate AI limit of 1500 ng/day solely based on negative EAT
  - FDA recommends additional studies (in vitro mammalian mutagenicity assay and in vitro metabolism study with focus on alpha-hydroxylation and formation of DNA-reactive intermediates) along with EAT
- Selection of inappropriate surrogate to establish AI
  - Surrogate structural considerations: N-nitroso structural alert in the same chemical environments, degree of substitution, steric bulk, electronic influences, potential for metabolic activation, stability/reactivity of the resulting metabolites, and overall MW
  - Surrogate data considerations: should have robust carcinogenicity data

Common pitfalls in nitrosamine safety review (continued)

- Inappropriate application of MW or less-than-lifetime (LTL) adjustments to set AI limits
- Derivation of AI limit from in vivo mutagenicity studies: current area of investigation for nitrosamine impurities
  - ICH M7 does not support this approach for mutagenic impurities
  - Importantly, a positive mutagenicity result warrants a CPCA-based AI limit
- Justifying proposed AI limit with a repeated dose general toxicology study
  - Not appropriate to support AI limit for a nitrosamine
  - Transgenic rodent assay (OECD 488) is recommended to evaluate mutagenicity of nitrosamines in vivo

Common pitfalls in nitrosamine safety review (continued)

- Application of ICH Q3A/Q3B qualification threshold to nitrosamines which test negative in an in vivo mutagenicity study
  - FDA considers outcome of compound-specific assessments and batch testing results to recommend an allowable limit
- Other inadequate justifications
  - Citing AIs recommended by other regulators, without submitting the supporting data or letter of authorization for right of reference
  - Submitting comparative impurity analysis with RLD
  - Citing interim AI limit as final product control limit

Hypothetical Case study 1: Alternate AI using a surrogate compound

Hypothetical Case 1: Alternate AI limit based on surrogate

NDSRI-1: Potency category (PC)3 of CPCA with AI of 400 ng/day, MW >300 g/mol

Alternate AI limit of 1000 ng/day was based on:

- Proposed Surrogate X and cited FDA-recommended AI of 1000 ng/day for Surrogate X
  - N-nitroso structural alert is in the same chemical environment as NDSRI-1
  - Surrogate X has a MW of ~100 g/mol
  - Justification addressed the degree of substitution, steric bulk, electronic influences, potential for metabolic activation, stability/reactivity of the resulting metabolites, and overall molecular weight.

Proposed AI limit is acceptable because:

- Agreed that Surrogate X was an acceptable surrogate for NDSRI-1
  - FDA's Computational Toxicology Consultation Services identified three potential surrogates with structural similarity, but only Surrogate X has robust carcinogenicity data and was accepted as a valid surrogate.
- Key Point: A successful surrogate-based AI proposal is thorough and is supported by robust carcinogenicity data

Hypothetical Case study 2: Alternate AI using compound-specific data

Hypothetical Case 2: Alternate AI limit based on compound-specific data

NDSRI-2: PC2 of CPCA with AI of 100 ng/day, MW >300 g/mol

An alternate AI limit of 1500 ng/day was based on:

- Submitted a positive 28-day in vivo transgenic rodent (TGR) mutation assay using an acceptable rodent (oral dosing).
- Derived lower confidence limit of benchmark doses (BMD) that increase liver X gene mutant frequency from control, which was used to adjust AI.

A proposed AI based on this type of data cannot be accepted because:

- The NDSRI is mutagenic: dose-dependent increase in mutation frequency in relevant tissue at top doses in the in vivo study.
- As impurity is mutagenic in vivo, CPCA-based limit is recommended.
- Recommended applying mitigation approaches to control at CPCA AI limit
- Key point: Derivation of AI limit for nitrosamines from in vivo mutagenicity studies is a current area of investigation for nitrosamine impurities.

Summary

- FDA's nitrosamine guidances provide a framework to conduct risk assessments of nitrosamine impurities to set safe limits.
- To establish AI limits in the absence of compound-specific data, use the CPCA as described in FDA's RAIL guidance
- Monitor FDA's guidance webpage for updates on control limits, safety testing approaches and other updated information.
- OGD Pharm/Tox works closely with key internal and external stakeholders to support ANDA assessments, guidance development, and discussions to achieve international harmonization on nitrosamine safety risk assessment approaches, and advance nitrosamine science.
- For recommendations on AI limits or approaches to support alternate limits, submit a Controlled Correspondence to OGD.

Resources

- FDA Guidance for Industry Control of Nitrosamine Impurities in Human Drugs (Revision 2) September 2024; https://www.fda.gov/media/141720/download
- FDA Guidance for Industry Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs), August 2023; https://www.fda.gov/media/170794/download
- CDER Webpage, CDER Nitrosamine Impurity Acceptable Intake Limits; Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs); Control of Nitrosamine Impurities in Human Drugs, Content current as of 06/30/2025; https://www.fda.gov/regulatory-information/search-fda-guidance-documents/updated-information-recommended-acceptable-intake-limits-nitrosamine-drug-substance-related
- Guidance for industry M7(R2) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk (July 2023); https://www.fda.gov/media/170461/download
- Guidance for industry Good ANDA Submission Practices (January 2022); https://www.fda.gov/media/110689/download
- Guidance for industry Controlled Correspondence Related to Generic Drug Development (March 2024); https://www.fda.gov/media/164111/download
- HESI nitrosamine research; https://hesiglobal.org/gttc-nitrosamines/

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Thank you!