Nitrosamine Impurities
An Overview

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Agenda

- Chemical Background
- Overview of Regulatory Actions
  - Small/common Nitrosamines
  - Complex Nitrosamines – Nitrosamines Drug Substance Related Impurities (NDSRIs)
- Defining Limits for Nitrosamine Impurities
- EMA Q&A - Article 5(3) of Regulation (EC) on nitrosamine impurities
- FDA Guidance on NDSRIs
- Risk Assessment – Case Study
- USP Perspectives on Nitrosamines

Background

N-Nitrosamine impurities

- *N*-Nitrosamines are a class of chemical compounds with the general structure shown in Figure 1. The essential feature of *N*-nitroso compounds is the N–N=O structure;

- They are part of a group of high potency mutagenic carcinogens (DNA Reactive Impurities) referred to as the “cohort of concern” in ICH M7* (aflatoxin-like, N-nitroso-, and alkyl-azoxy compounds)

*“Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk”

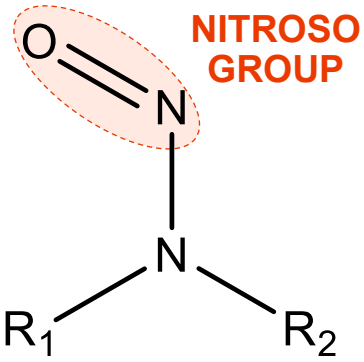
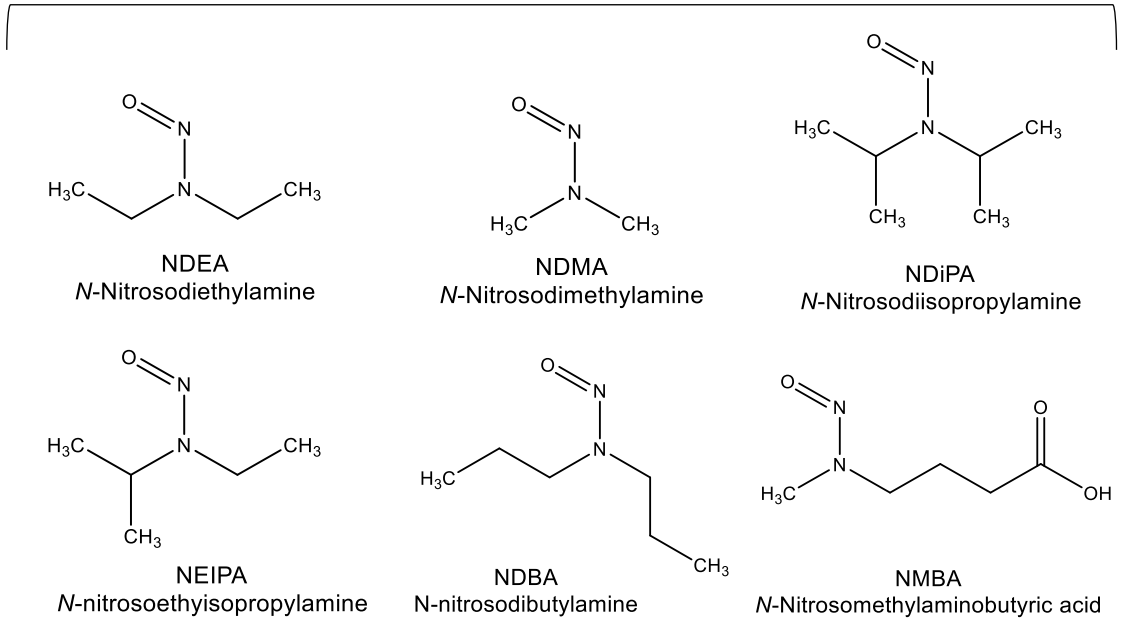


Figure 1. Generic *N*-nitrosamine structure



Background

N-Nitrosamine impurities

- *N*-Nitrosamines are classified by the ICH M7(R1) Guideline as Class 1 impurities ("known mutagenic carcinogens")[3]

Table 1: Impurities Classification with Respect to Mutagenic and Carcinogenic Potential and Resulting Control Actions

Class	Definition	Proposed action for control (details in Section 7 and 8)
1	Known mutagenic carcinogens	Control at or below compound-specific acceptable limit
2	Known mutagens with unknown carcinogenic potential (bacterial mutagenicity positive*, no rodent carcinogenicity data)	Control at or below acceptable limits (appropriate TTC)
3	Alerting structure, unrelated to the structure of the drug substance; no mutagenicity data	Control at or below acceptable limits (appropriate TTC) or conduct bacterial mutagenicity assay; If non-mutagenic = Class 5 If mutagenic = Class 2
4	Alerting structure, same alert in drug substance or compounds related to the drug substance (e.g., process intermediates) which have been tested and are non-mutagenic	Treat as non-mutagenic impurity
5	No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity	Treat as non-mutagenic impurity

ICH M7(R1) Guideline "Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk"[2]

- Many *N*-nitrosamines are classified as probable carcinogens (group 2A) by International Agency for Research on Cancer [IARC][1,2]

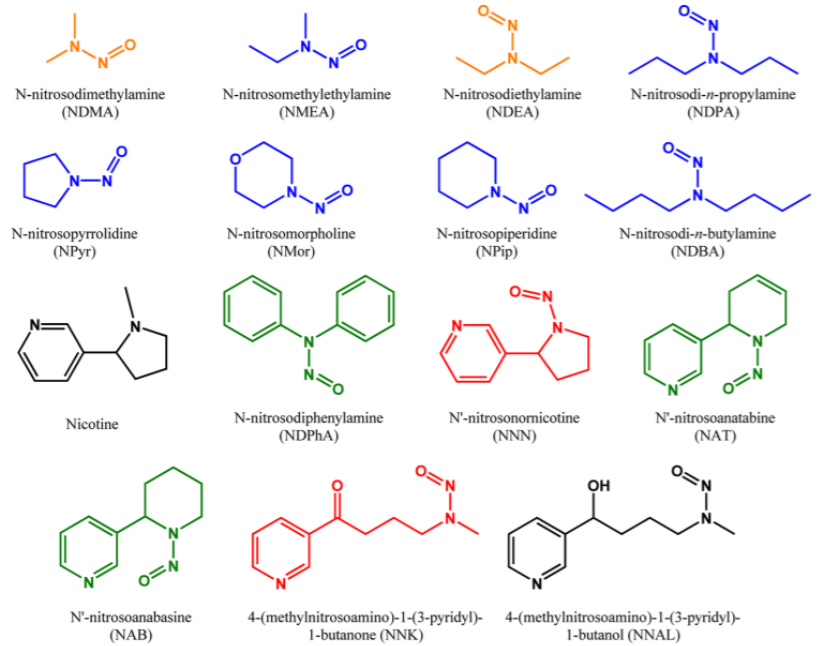


Figure 1. Chemical structures of the target N-nitrosamines, TSNAs and nicotine and their respective IARC classifications: (red) group 1, known carcinogen to humans; (orange) group 2A, probable carcinogen to humans; (blue) group 2B, possible carcinogen to humans; and (green) group 3, not classifiable as to its carcinogenicity to humans.[11]

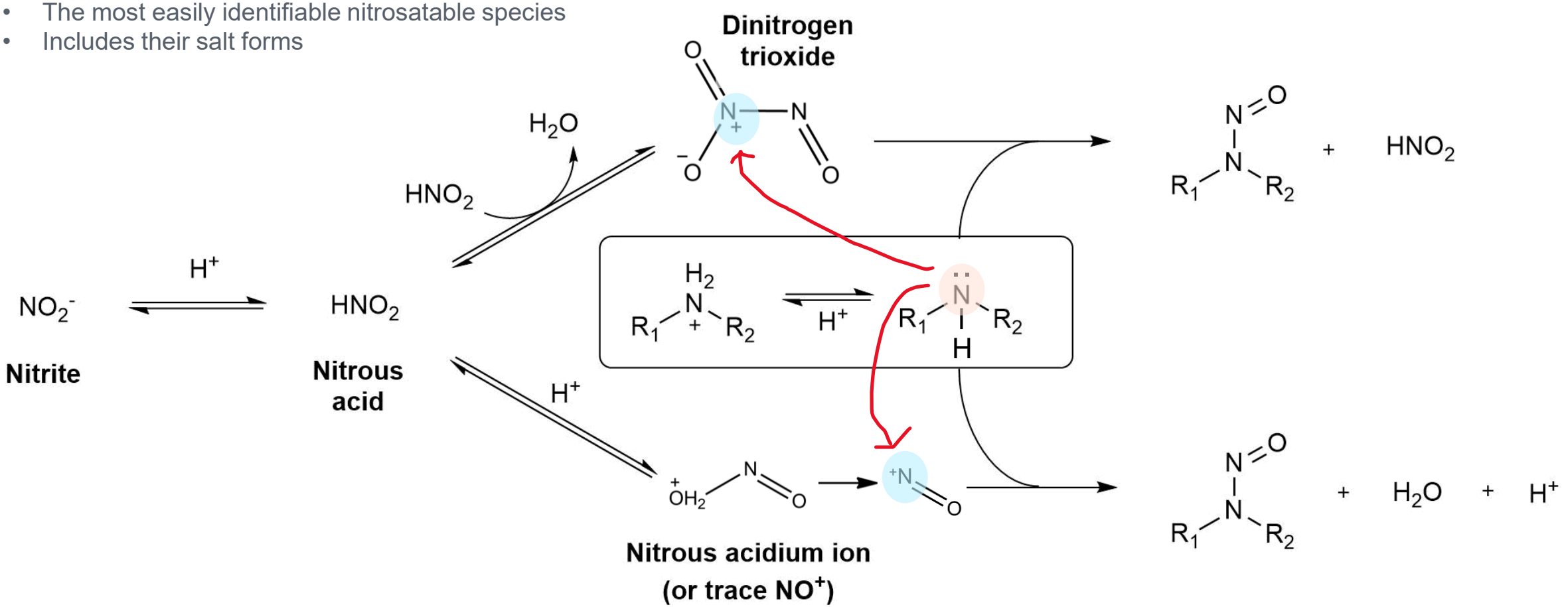
[2]Farren, J.F. et al. Estimated Exposure Risks from Carcinogenic Nitrosamines in Urban Airborne Particulate Matter. Environ. Sci. Technol. 2015, 49, 9648−9656

Chemical Background

Nitrosation of secondary amine

All secondary amines

- The most easily identifiable nitrosatable species
- Includes their salt forms

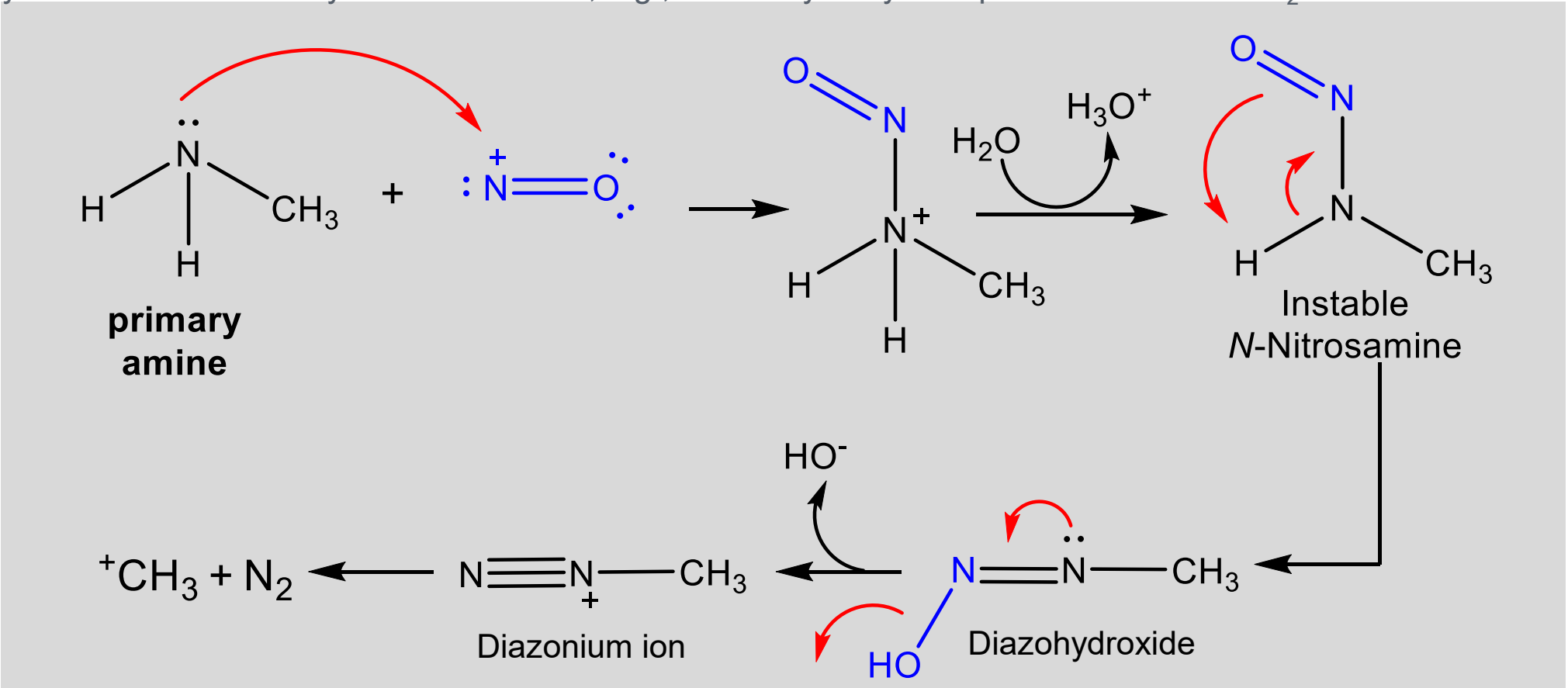


https://doi.org/10.1016/j.xphs.2022.11.013

Chemical Background

Nitrosation of primary amines

- Primary amines are no nitrosamine precursors. The nitrosation of an aliphatic primary amine yields an alkyl diazonium ion and water, not a nitrosamine.
- The alkyl diazonium ion is very reactive and will, e.g., form a hydroxyl compound and release N_2 in a reaction with water

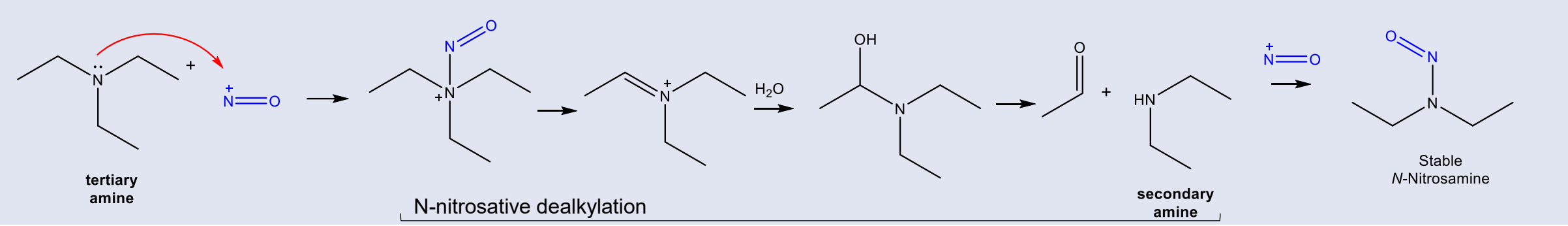


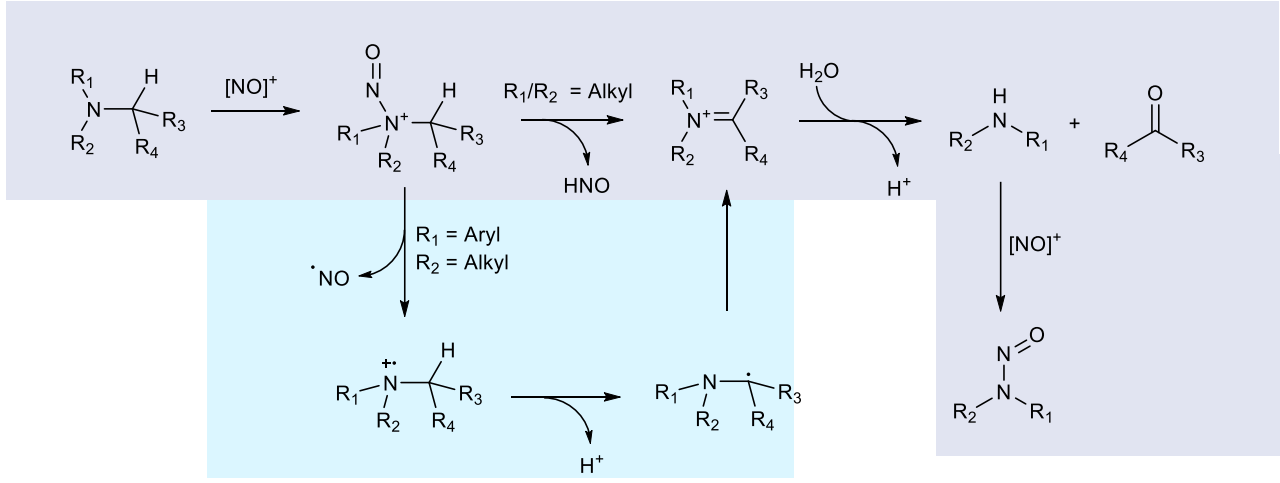
Chemical Background

Nitrosation of tertiary amines

Tertiary amines

- Presence of an α-hydrogen is (usually) necessary for initial dealkylation
- Two mechanisms of formation believed to exist
- Structural features can impact which mechanism is favoured



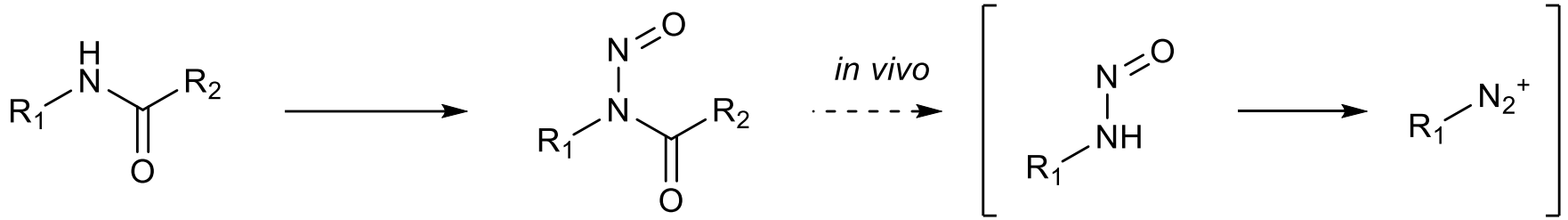


Chemical Background

Nitrosation of amides

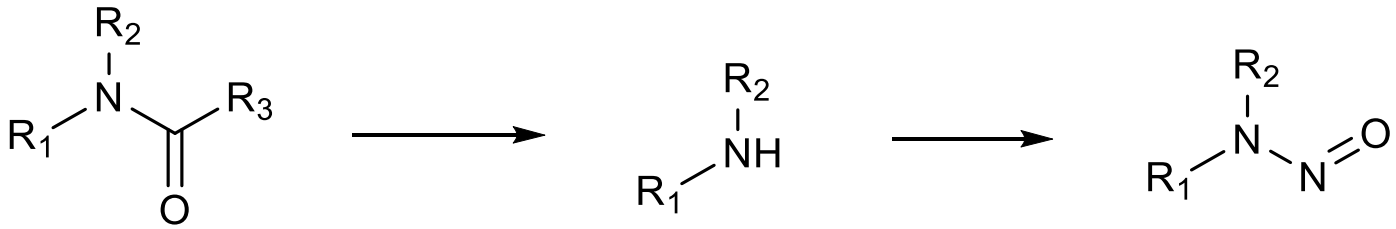
Secondary Amide

- Subsequent in vivo hydrolysis could release the key mutagen
- Nitrosation appears more difficult and therefore discarded



Tertiary Amides

- No direct nitrosation possible, requires initial hydrolysis which is not always facile

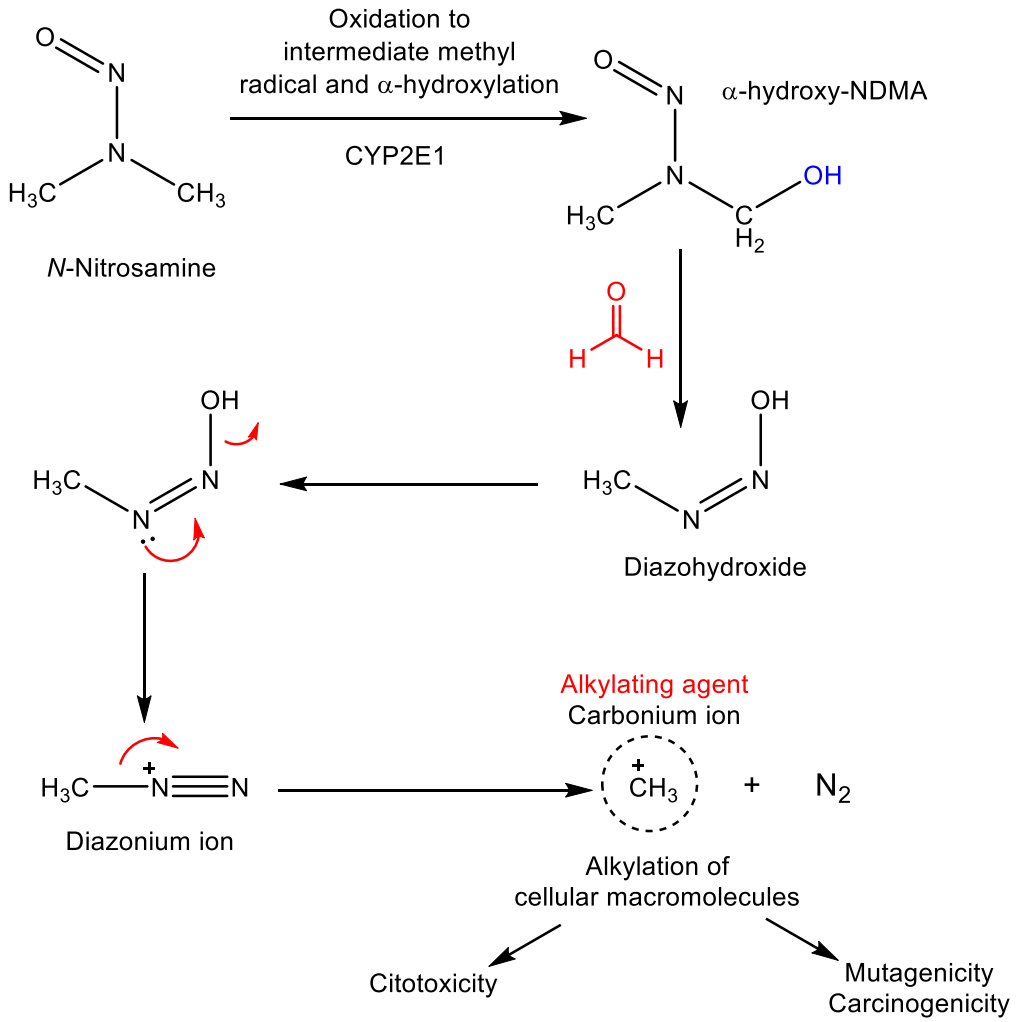


https://doi.org/10.1016/j.xphs.2022.11.013

Background

Nitrosamine-mediated mutagenesis/carcinogenesis

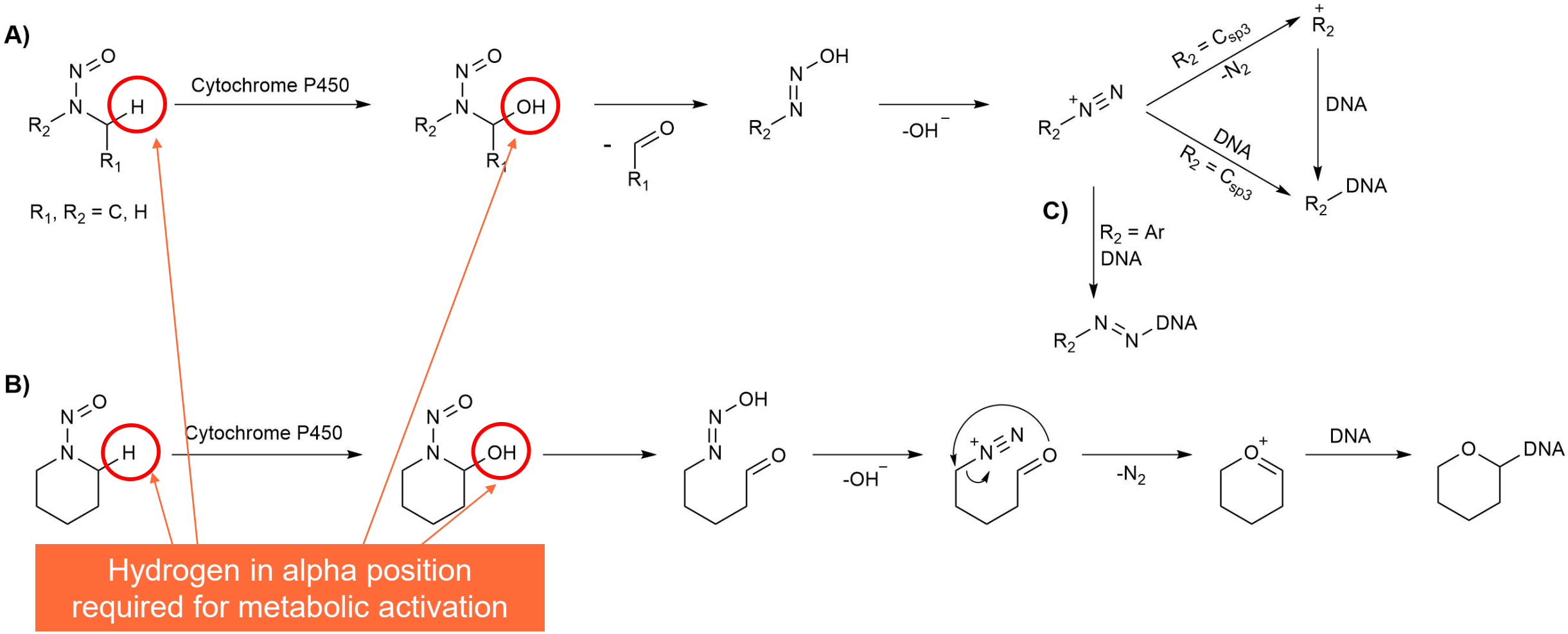
- Nitrosamines are metabolized in liver and the metabolism of some of them can produce DNA reacting agent



Cheung, P.C.W. A Historical Review of the Benefits and Hypothetical Risks of Disinfecting Drinking Water by Chlorination. Journal of Environment and Ecology 2017, Vol. 8, No. 1

Chemical Background

Metabolic activation and DNA adduct formation of nitrosamines



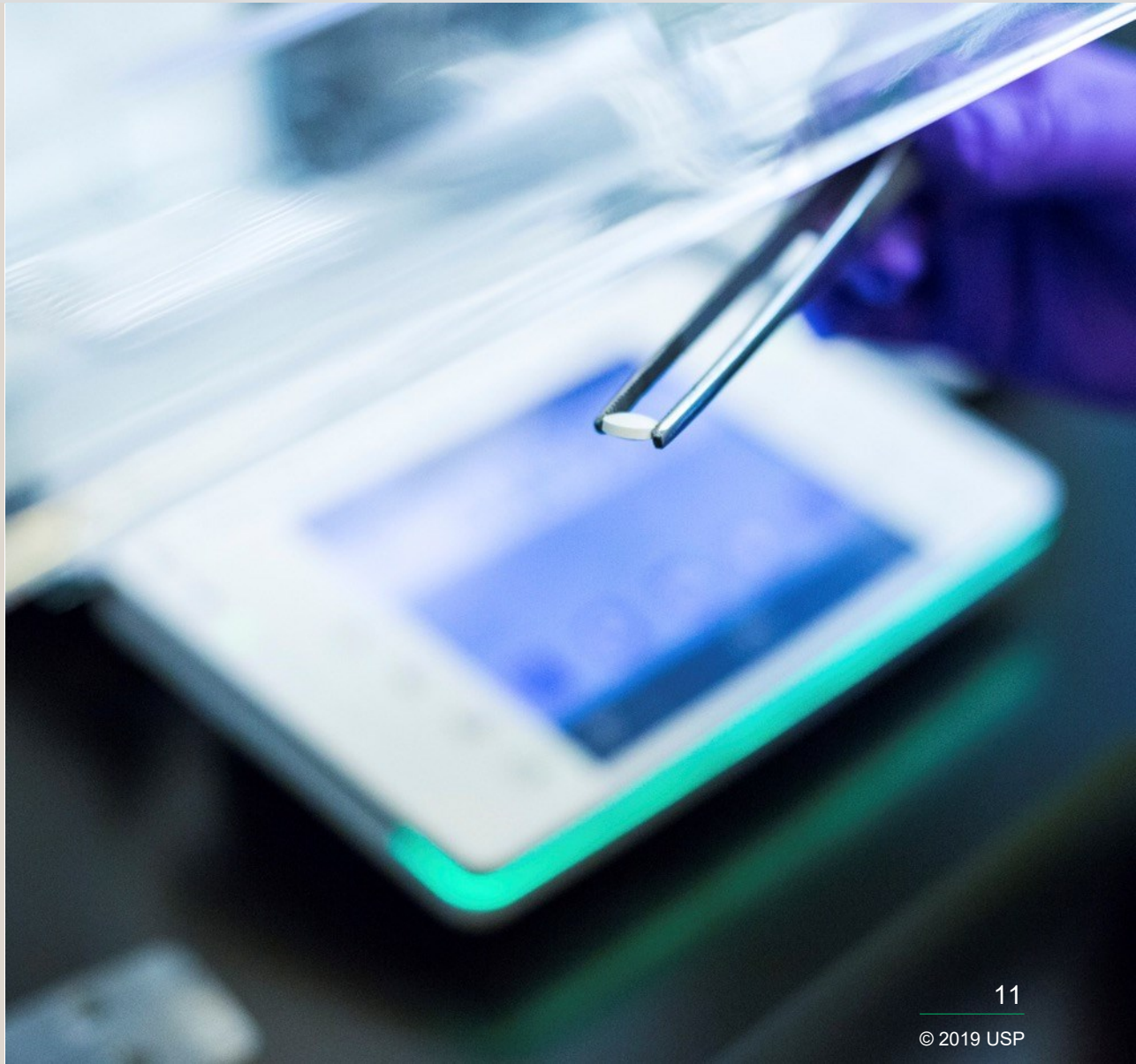
https://doi.org/10.1016/j.xphs.2022.11.013

Regulatory Concerns & Awareness

N-Nitrosamine impurities

- The nitrosamine presence in pharmaceutical products emerged as a public health concern in 2018 after reports that harmful levels of nitrosamine impurity, *N*-nitrosodimethylamine (NDMA), were observed in angiotensin II receptor blockers (ARBs) (Sartan products).

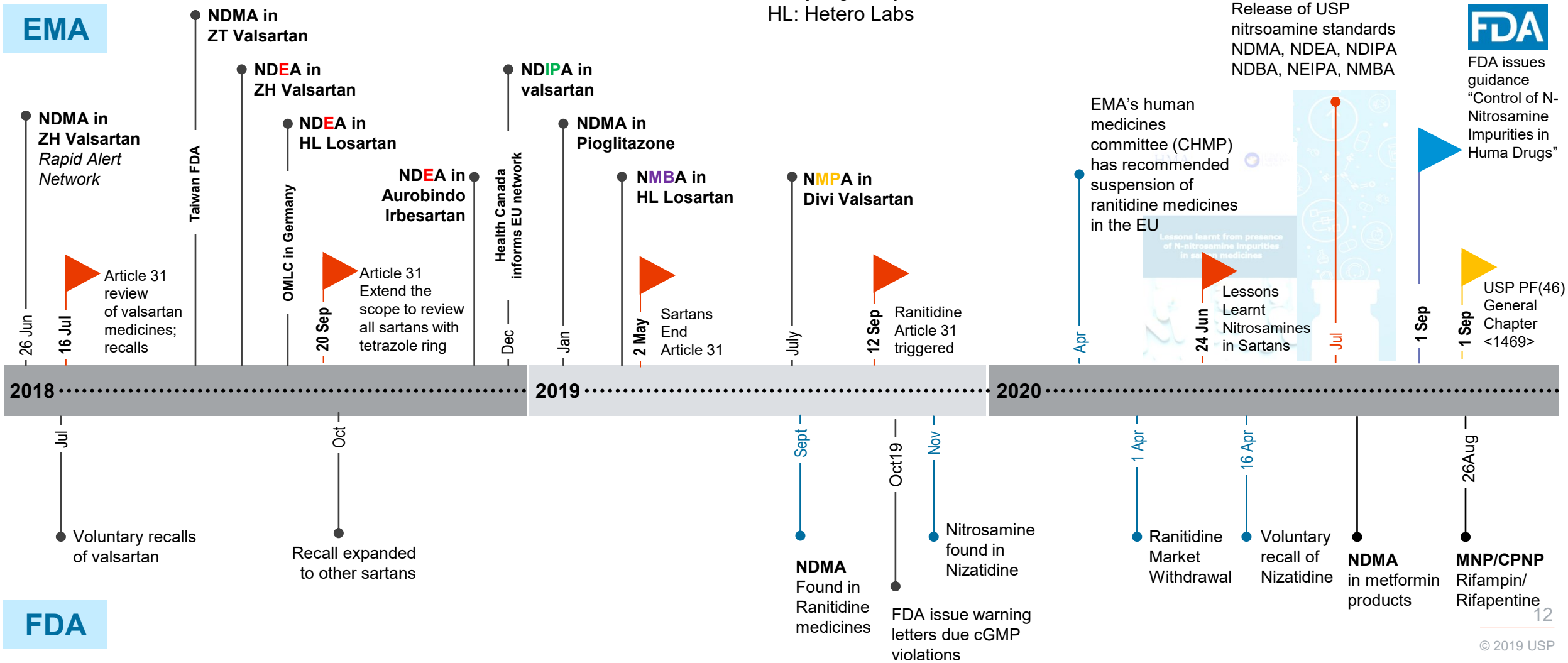
- Following these reports and after further investigation, agencies issued public health alerts and guidance documents, which have limits, regarding the presence of nitrosamine impurities in several drug products:
  - World Health Organization (WHO),
  - US Food and Drug Administration (FDA),
  - European Directorate for the Quality of Medicines (EDQM), and other agencies.



Regulatory and Other Actions

Timeline

Legend:
ZH: Zhejiang Huahai Pharmaceuticals
ZT: Zhejiang Tianyu
HL: Hetero Labs



Nitrosamine Timeline - NDSRIs

2021

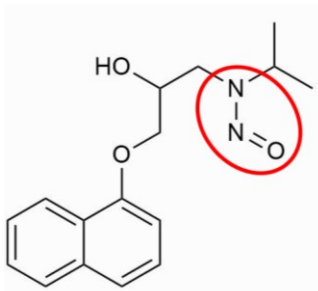
- N-nitroso-Varenicline
- Oct — FDA: Updates on possible mitigation strategies to reduce the risk of nitrosamine drug substance-related impurities in drug products

2022

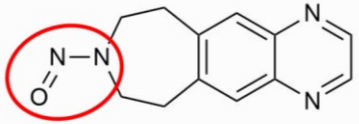
- N-Nitroso-irbesartan
- N-Nitroso-Desloratadine
- N-Nitroso-Folic Acid
- N-Nitroso-Quinapril
- N-Nitroso-Orphenadrine
- N-Nitroso-Propranolol

2023

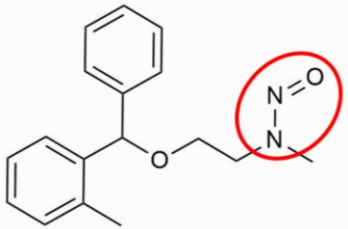
- Jul — EMA: Q&A on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal product
- Aug — FDA: Recommended Acceptable Intake Limits for Nitrosamine Drug Substance Related Impurities (NDSRIs) Guidance for Industry



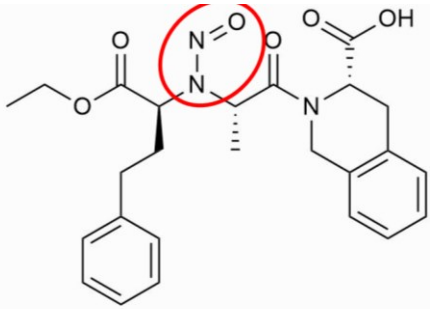
N-Nitrosopropranolol



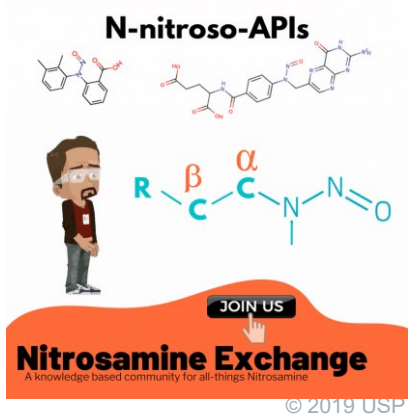
N-Nitrosovarenicline



N-Nitrosoorphenadrine



N-Nitrosoquinapril



N-Nitroso-Propranolol

Evolution…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Abstract

Drug interactions. III. Formation of nitrosamines from therapeutic drugs. Formation, mutagenic properties and safety assessment of propranolol hydrochloride with respect to the intragastric formation of N-nitrosopropranolol under conditions found in patients.

I H Raisfeld-Danse and J Chen

Journal of Pharmacology and Experimental Therapeutics June 1983, 225 (3) 713-719;


Français

Government of Canada / Gouvernement du Canada

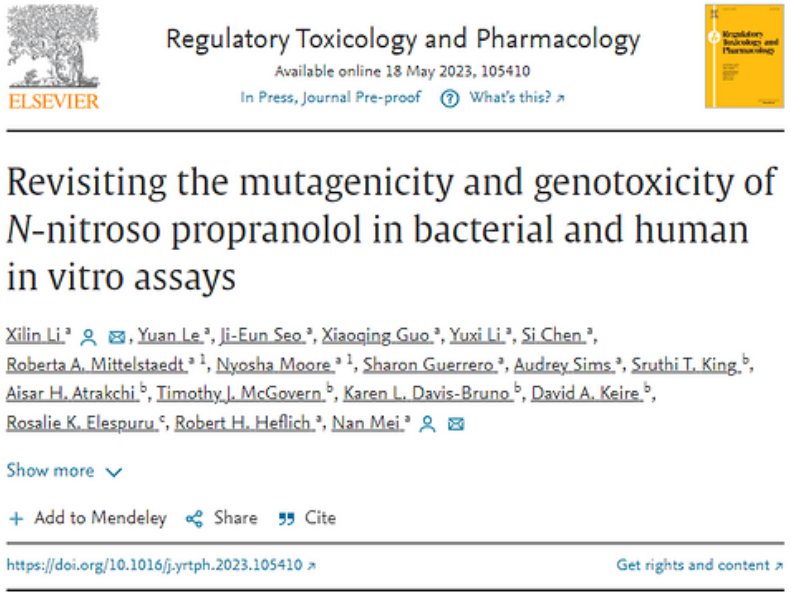
Search website

Canada.ca > Health > Recalls and safety alerts

Recalls and safety alerts

Public advisory

Pfizer recalls Inderal-LA (propranolol hydrochloride) capsules due to a nitrosamine impurity


ELSEVIER

Regulatory Toxicology and Pharmacology

Available online 18 May 2023, 105410

In Press, Journal Pre-proof What's this?

Revisiting the mutagenicity and genotoxicity of *N*-nitroso propranolol in bacterial and human in vitro assays

Xilin Li, Yuan Le, Ji-Eun Seo, Xiaoqing Guo, Yuxi Li, Si Chen, Roberta A. Mittelstaedt, Nyosha Moore, Sharon Guerrero, Audrey Sims, Sruthi T. King, Aisar H. Atrakchi, Timothy J. McGovern, Karen L. Davis-Bruno, David A. Keire, Rosalie K. Elespuru, Robert H. Heflich, Nan Mei

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https://doi.org/10.1016/j.yrtph.2023.105410

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- NNP has been reported to be **negative** in the bacterial reverse mutation test (the Ames test) but genotoxic in other in vitro assays
- Systematically examined the in vitro mutagenicity and genotoxicity of NNP using several modifications of the Ames test known to affect the mutagenicity of nitrosamines
- This study indicates that **NNP is genotoxic** in a variety of bacterial and mammalian systems. Thus, NNP is a mutagenic and genotoxic nitrosamine and a potential human carcinogen

Regulatory Approach (Risk & Control)

FDA Recommendations

Assess the risk

- In a timely manner, within 6 months (March 2021)
- Based on prioritization of drugs

Conduct confirmatory testing

- When there is any risk for the presence of nitrosamine
- Specific and sensitive analytical methods

Report changes

- DMF amendments or drug applications
- Within 3 years of publication of guidance (Sept. 2023)

Source: https://www.fda.gov/media/141720/download

Defining Limits for Nitrosamine Impurities

Defining Limits for Impurities

- NDMA
  - classified as “Probable carcinogenic to humans” [IARC, WHO]
  - NDMA belongs to *N*-nitroso compounds > “Cohort of Concern” [ICH M7]

- Acceptable intake (AI) must be derived from specific carcinogenicity data
  - 50% tumor incidence (TD50) > NDMA: 0.096 mg/kg/day [Carcinogenic Potency Database]
    - ✓ This approach assumes a lifelong daily administration of the maximum daily dose of the medicinal product and is based on the approach outlined in the ICH M7(R1) guideline

Acceptable nitrosamine content = AI/Max Daily Dose

Acceptable nitrosamine content (NDMA) = 96 ng/day / 320 mg/day [Valsartan]

Acceptable nitrosamine content (NDMA) = 0.3 ppm [Valsartan]

Source: https://www.americanpharmaceuticalreview.com/Featured-Articles/561484-Nitrosamines-in-Pharmaceuticals-Toxicity-Risk-Analysis-Chemistry-and-Test-Methods/

Defining Limits for Impurities

Table 1: FDA-Published Interim Limits for NDMA, N-nitrosodiethylamine (NDEA), and nitroso-N-methylaminobutyric acid (NMBA) in Angiotensin II Receptor Blockers

Drug	Maximum Daily Dose (mg/day)	Acceptable Intake NDMA (ng/day)[a]	Acceptable Intake NDMA (ppm)[b]	Acceptable Intake NDEA (ng/day)[a]	Acceptable Intake NDEA (ppm)[b]	Acceptable Intake NMBA (ng/day)[a]	Acceptable Intake NMBA (ppm)[b]
Valsartan	320	96	0.3	26.5	0.083	96	0.3
Lorsartan	100	96	0.96	26.5	0.27	96	0.96[c]
Irbesartan	300	96	0.32	26.5	0.088	96	0.32
Azilsartan	80	96	1.2	26.5	0.33	96	1.2
Olmesartan	40	96	2.4	26.5	0.66	96	2.4
Eprosartan	800	96	0.12	26.5	0.033	96	0.12
Candesartan	32	96	3.0	26.5	0.83	96	3.0
Telmisartan	80	96	1.2	26.5	0.33	96	1.2

a. The acceptable intake is a daily exposure to a compound such as NDMA, NDEA, or NMBA that approximates a 1:100,000 cancer risk after 70 years exposure.
b. These values are based on a drug's maximum daily dose as reflected in the drug label.
c. FDA is temporarily not objecting to losartan with NMBA below 9.82 ppm remaining on the market.

Source: https://www.americanpharmaceuticalreview.com/Featured-Articles/561484-Nitrosamines-in-Pharmaceuticals-Toxicity-Risk-Analysis-Chemistry-and-Test-Methods/

FDA and EMA Guidance on Nitrosamine Impurities

FDA Approach	EMA Approach
• All chemically synthesized APIs in human drugs (including drug products)	All human medicinal products, • Chemically synthesized APIs • And Biological products
• Risk-based approach	• Risk-based approach
• AI limits for NDMA, NDEA, NMBA, NMPA, NIPEA, NDIPA	• Limits for NDMA, NDEA, EIPNA, DIPNA, NMBA, MeNP, and NDBA

Source: https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-assessment-report_en.pdf

Source: https://www.fda.gov/media/141720/download

Table 1. AI Limits for NDMA, NDEA, NMBA, NMPA, NIPEA, and NDIPA in Drug Products

Nitrosamine	AI Limit (ng/day)[1,2]
NDMA	96
NDEA	26.5
NMBA	96
NMPA	26.5
NIPEA	26.5
NDIPA	26.5

[1] The AI limit is a daily exposure to a compound such as NDMA, NDEA, NMBA,NMPA, NIPEA, or NDIPA that approximates a 1:100,000 cancer risk after 70 years of exposure. Appendix B includes a description of the AI derivation for NDMA, which is an example of how FDA applied ICH M7(R1) to set a limit.

[2] The conversion of AI limit into ppm varies by product and is calculated based on a drug's maximum daily dose (MDD) as reflected in the drug label (ppm = AI (ng)/MDD (mg)).

The following limits have been established for some specific N-nitrosamines and should be applied:

***N*-Nitrosamine (CAS number)**	**ng/day*** **
NDMA* (62-75-9)	96.0
NDEA*(55-18-5)	26.5
EIPNA**(16339-04-1)	26.5
DIPNA**(601-77-4)	26.5
NMBA**(61445-55-4)	96.0
MeNP**(16339-07-4)	26.5
NDBA**(924-16-3)	26.5

These limits are applicable only if a finished product contains a single N-nitrosamine.

Less than Life (LTL) Time Approach

10. Which limits apply for nitrosamines in medicinal products?

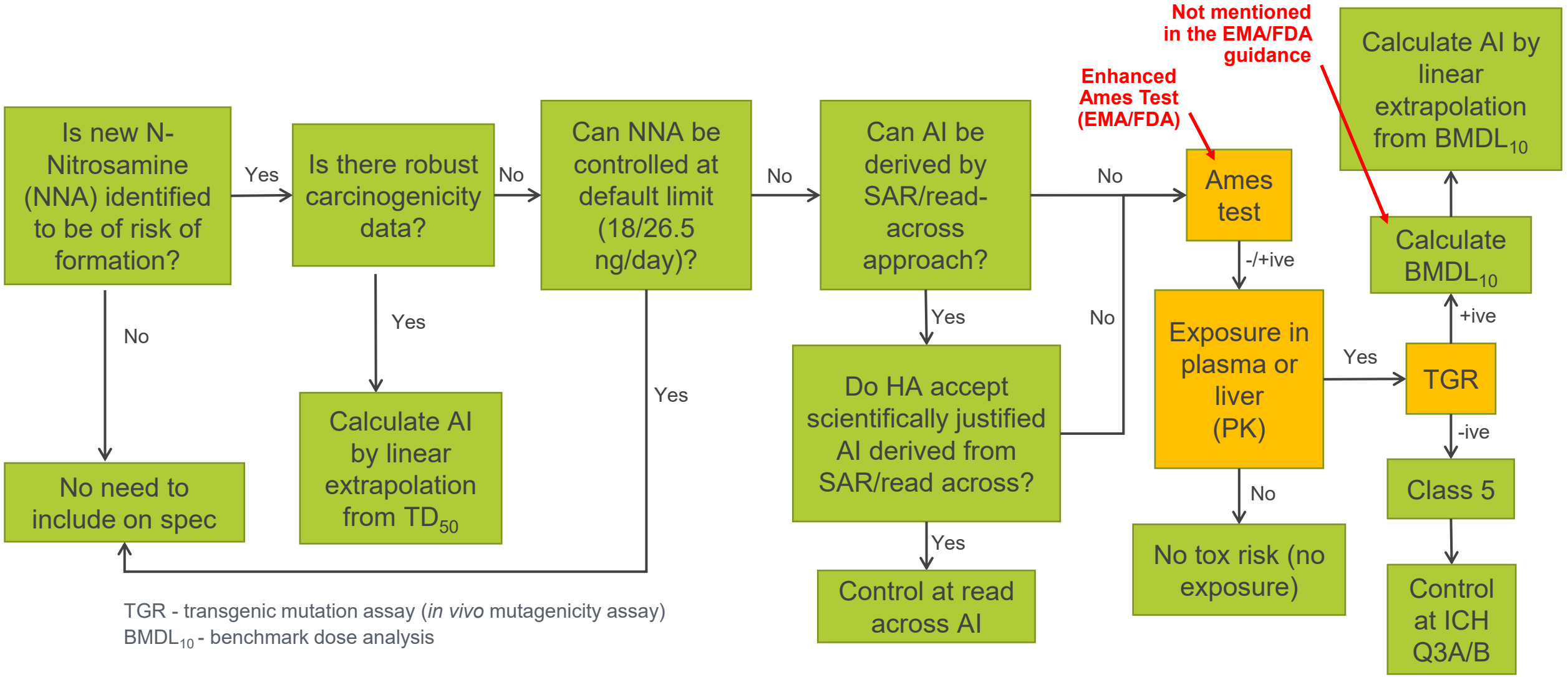
ICH M7(R1) guideline defines N-nitrosamines as substances of the "cohort of concern" for which limits in medicinal products refer to the so-called substance-specific acceptable intake (AI) (the Threshold of Toxicological Concern, TTC, value of 1.5 ug/day cannot be routinely applied) which is associated with a negligible risk (theoretical excess cancer risk of <1 in 100,000 over a lifetime of exposure). The calculation of AI assumes a lifelong daily administration of the maximum daily dose of the medicinal product and is based on the approach outlined in the ICH M7(R1) guideline as well as the principles described in relation to the toxicological evaluation in the assessment report of the CHMP's Article 5(3) opinion on nitrosamine impurities in human medicinal products.

The 'less than lifetime' (LTL) approach should not be applied in calculating the limits as described above but can only be considered after consultation with competent authorities as a temporary measure until further measures can be implemented to reduce the contaminant at or below the limits defined above.

EMA Q&A - Article 5(3) of Regulation (EC) on nitrosamine impurities - July 2023

Defining Limits

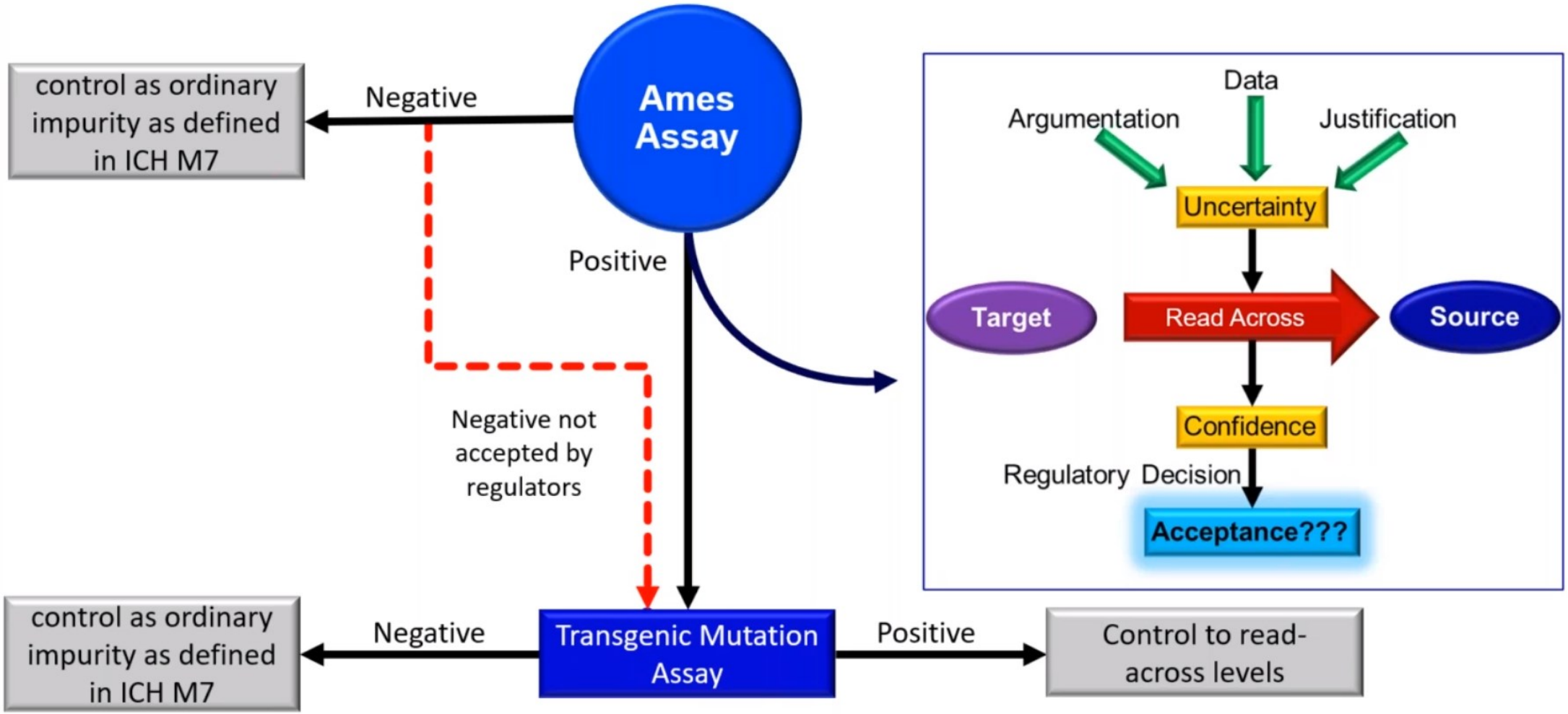
New Approach Jul/Aug 2023: Carcinogenic Potency Categorization Approach (CPCA) – FDA/EMA



TGR - transgenic mutation assay (*in vivo* mutagenicity assay)
$BMDL_{10}$ - benchmark dose analysis

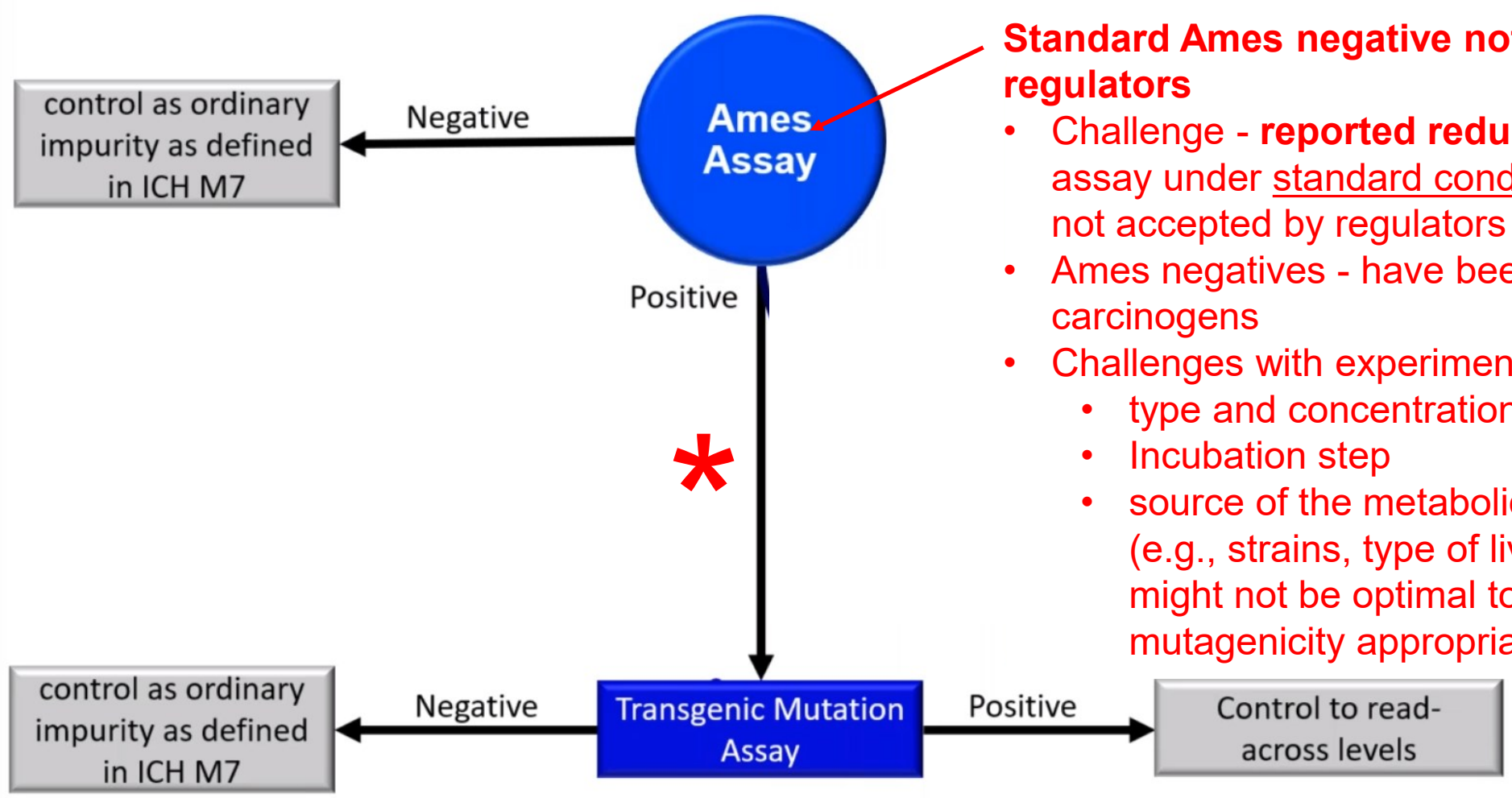
Source from: Dr. Nudelman (TEVA Pharmaceuticals)
Nudelman, R., et al. (2023). The Nitrosamine "Saga": Lessons Learned from Five Years of Scrutiny. Organic Process Research & Development. https://doi.org/10.1021/acs.oprd.3c00100

ICH M7 – Framework (Safety)



Source from: Dr. Schuler (Pfizer)

ICH M7 – Framework (Safety)

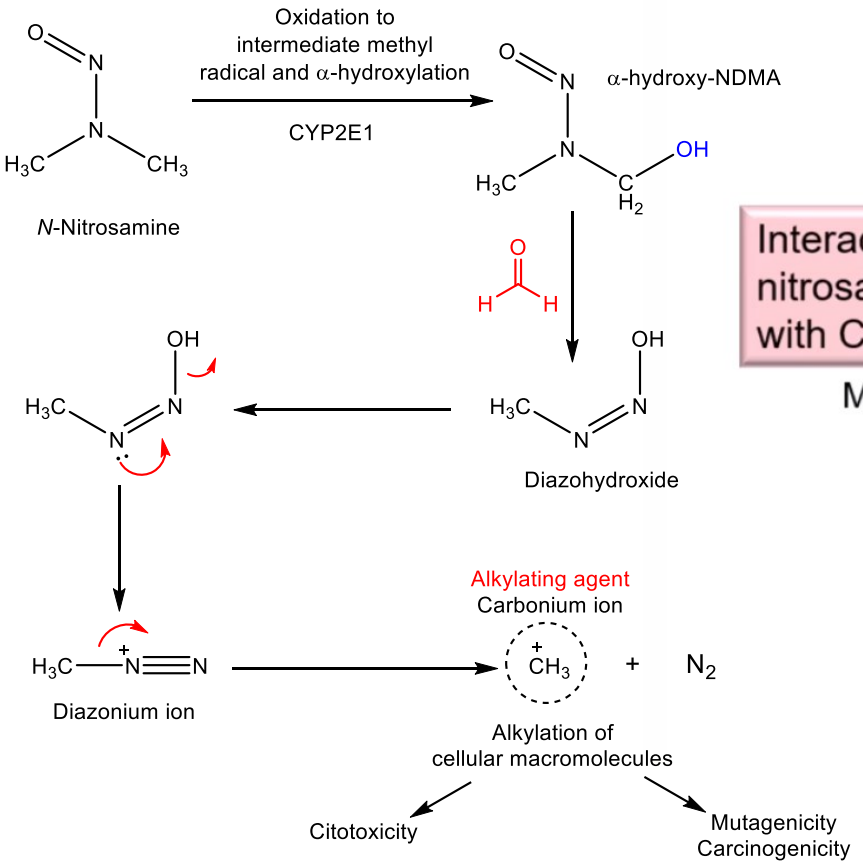


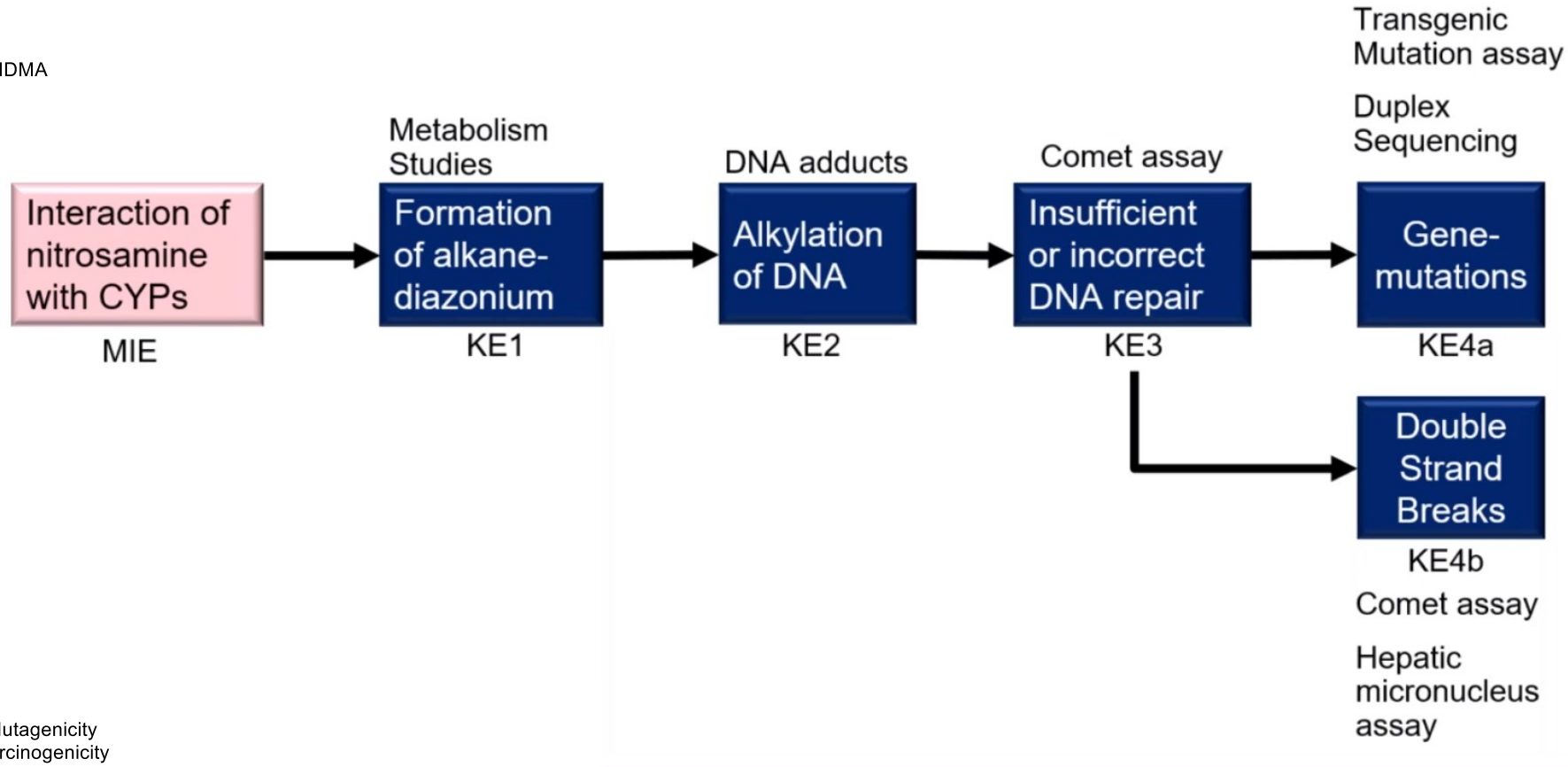
Standard Ames negative not widely accepted by regulators

- Challenge - **reported reduced sensitivity** of the assay under standard conditions – often (-) test is not accepted by regulators
- Ames negatives - have been shown to be in vivo carcinogens
- Challenges with experimental conditions
  - type and concentration of solvent
  - Incubation step
  - source of the metabolic activation system (e.g., strains, type of liver extract used etc) might not be optimal to detect NA mutagenicity appropriately

Source from: Dr. Schuler (Pfizer)

Industry Proposal (Safety)





Source from: Dr. Schuler (Pfizer)

EMA Q&A - Article 5(3) of Regulation (EC) on nitrosamine impurities

Appendix 1 – AIs
Annex 2 – CPCA
Annex 3 – Enhanced Ames Test Conditions

EMA Q&A - Article 5(3) of Regulation (EC) on nitrosamine

Establishment of the AIs

Two scenarios are foreseen for detection of new nitrosamines:

A. If N-nitrosamines are identified with sufficient substance specific animal carcinogenicity data, the TD50 should be calculated and used to derive a substance specific limit for lifetime exposure as recommended in ICH M7(R1) guideline.

B. If N-nitrosamines are identified without sufficient substance specific data to derive a substance specific limit for lifetime exposure as recommended in ICH M7(R1) guideline,

   1. The Carcinogenic Potency Categorization Approach (CPCA) for N-nitrosamines (Annex 2) should be used to establish the AI, unless other robust data are available that would override this AI.

   2. A negative result in an GLP-compliant enhanced Ames test (EAT, Annex 3) allows control of the N-nitrosamine at 1.5 µg/day. For substances testing positive, the AI should be established using options 1 or 3.

   3. If a surrogate nitrosamine is available with sufficiently robust carcinogenicity data, the TD50 from the surrogate substance can serve as a point of departure for derivation of AI by SAR and read across.

   4. A negative result in a relevant well-conducted in vivo mutagenicity study can allow control of the N-nitrosamine as a non-mutagenic impurity, i.e. according to Q3A/B limits, irrespective of the limit calculated through option 1, 2 or 3. For substances testing positive, the AI should be established using options 1 or 3.

EMA Q&A - Article 5(3) of Regulation (EC) on nitrosamine

When more than one nitrosamine is identified in the same product

- Two approaches are considered acceptable in order not to exceed the acceptable risk level of 1:100,000 as outlined in ICH M7(R1) guideline:
  1. The total daily intake of all identified N-nitrosamines not to exceed the AI of the most potent N-nitrosamine identified, or
  2. Total risk level calculated for all identified N-nitrosamines not to exceed 1 in 100,000.
- Specifications for individual *N*-nitrosamines should generally include an AI limit expressed in ppm or ppb.
- It is considered that the presence of one or more N-nitrosamines at ≤10% of their respective AI constitutes a negligible toxicological risk, and as such, they do not need to be specified.
- *N*-Nitrosamines present below 10% of their respective AI do not need to be factored into the calculation of limits for individual or total N-nitrosamine(s)

EMA Q&A - Article 5(3) of Regulation (EC) on nitrosamine

When more than one nitrosamine is identified in the same product

Example:
NDMA and NDEA are both detected at or above 10% of their respective AI) in a finished product with maximum daily dose of 300 mg.

AI limit

- NDEA: 26.5 ng/day / 300 mg/day = 0.088 ppm or 88 ppb = most potent N-nitrosamine
- NDMA: 96.0 ng/day / 300 mg/day = 0.32 ppm or 320 ppb

Specification possibilities for different control options:

Nitrosamine	**Option 1**	**Option 2 - Fixed** **Example 20:80 ratio**[2]	**Option 2 - Flexible**
NDMA	Not needed	NMT 64 ppb *(320 ppb x 0.2)*	NMT 320 ppb
NDEA	Not needed	NMT 70 ppb *(88 ppb x 0.8)*	NMT 88 ppb
Total Nitrosamines	NMT 88 ppb	Not needed	NMT 100%[1]

[1] $\left(\frac{[NDMA]\ ppb}{320\ ppb} + \frac{[NDEA]\ ppb}{88\ ppb}\right) x\ 100\% \leq 100\%$
NMT 100% = 1:100,000 theoretical excess cancer risk.
[2] For option 2 fixed approach, a ratio of 20% NDMA to 80% NDEA (20:80) is used as an example only. Different ratios could be used in different situations dependent on relative amounts present, provided that the sum of the % AI limits for each specified nitrosamine does not exceed 100%.

EMA Q&A - Article 5(3) of Regulation (EC) on nitrosamine

Appendix 1

- AI have been established by the Nonclinical Working Party (NcWP), including new AIs for N-nitrosamines determined using the Carcinogenic Potency Categorization Approach (CPCA)
- Link to Appendix 1

Appendix 1: Acceptable intakes established for N-nitrosamines

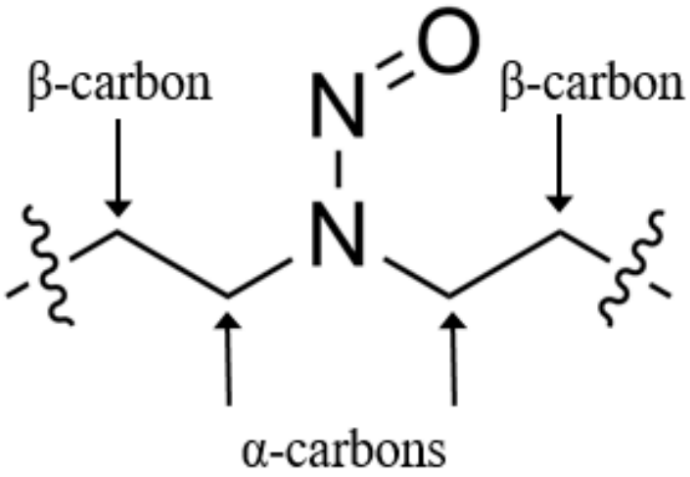
N-Nitrosamine (CAS number)	Source[2]	CPCA Category	ng/day[1,*]	Publication date
1-cyclopropylmethyl-4-nitrosopiperazine		3	400	01/07/2023
1-methyl-4-nitrosopiperazine, MeNP[5] (16339-07-4)	Rifampicin	3	400	01/07/2023
1-nitroso-pyrrolopiperidine		4	1500	01/07/2023
2-nitroso-octahydrocyclopenta(c)pyrrole[17]	Gliclazide		1700	01/07/2023
4-(methylnitrosoamino)-1-(3-pyridinyl)-1-butanone (NNK)[7]			100	01/07/2023
7-nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3- a]pyrazine[11]	Sitagliptin		37	01/05/2022
nitroso impurity C" [N-(2,6-dimethylphenyl)-2-(4-nitrosopiperazin-1-yl)acetamide]		3	400	01/07/2023
nitroso-orphenadrine	Orphenadrine	1	18	01/07/2023
nitroso-praziquanamine [2-nitroso-3,6,7,11b-tetrahydro-1H-pyrazino[2,1-a]isoquinolin-4-one]	Arpraziquantel	4	1500	01/07/2023
N-methyl-N-nitrosophenethylamine, NMPEA[3,7] (13256-11-6)			8	01/05/2022
N-nitroso-1,2,3,6-tetrahydropyridine, NTHP[3] (55556-92-8)			37	01/05/2022
N-nitroso-2,6-pipecoloxilidide	Ropivacaine	4	1500	01/07/2023
N-nitroso-ambroxol	Ambroxol	3	400	01/07/2023
N-nitroso-aryl piperazine / N-nitroso-desalkylquetiapine (NDAQ)	Quetiapine	3	400	01/07/2023
N-nitroso-Atemoxetine[13]	Atomoxetine		100	01/07/2023
N-nitroso-atenolol	Atenolol	4	1500	01/07/2023
N-nitroso-azaerythromycin	Azithromycin		NMI[16]	01/07/2023
N-nitroso-benazepril	Benazepril	5	1500	01/07/2023
N-nitroso-betahistine	Betahistine	1	18	01/07/2023
N-nitroso-bisoprolol (NBP)	Bisoprolol	4	1500	01/07/2023
N-nitroso-bumetanide (NNB)	Bumetanide	4	1500	01/07/2023
N-nitroso-bupropion	Bupropion	5	1500	01/07/2023
N-nitroso-cilazapril	Cilazapril	5	1500	01/07/2023
N-nitroso-ciprofloxacin	Ciprofloxacin	4	1500	01/07/2023
N-nitroso-dabigatran[10]	Dabigatran	3	400	01/07/2023
N-nitroso-desloratadine	Desloratadine	3	400	01/07/2023
N-nitroso-desmethyl trimebutine	Trimebutine	5	1500	01/07/2023
N-nitroso-desmethylazithromycin	Azithromycin		NMI[16]	01/07/2023
N-nitroso-desmethyl-chloropyramine (N-DMCP)	Chloropyramine	1	18	01/07/2023

EMA Q&A - Article 5(3) of Regulation (EC) on nitrosamine

Annex 2 - Carcinogenic Potency Categorization Approach for N-nitrosamines.

- CPCA - based on an assessment of activating or deactivating structural features present in the molecule.
- These features are defined as molecular substructures that are associated with an increase or decrease, respectively, in carcinogenic potency

Figure 1. Structural Representation of α- and β-carbons on an *N*-nitrosamine



EMA Q&A - Article 5(3) of Regulation (EC) on nitrosamine

Annex 2 - Carcinogenic Potency Categorization Approach for N-nitrosamines.

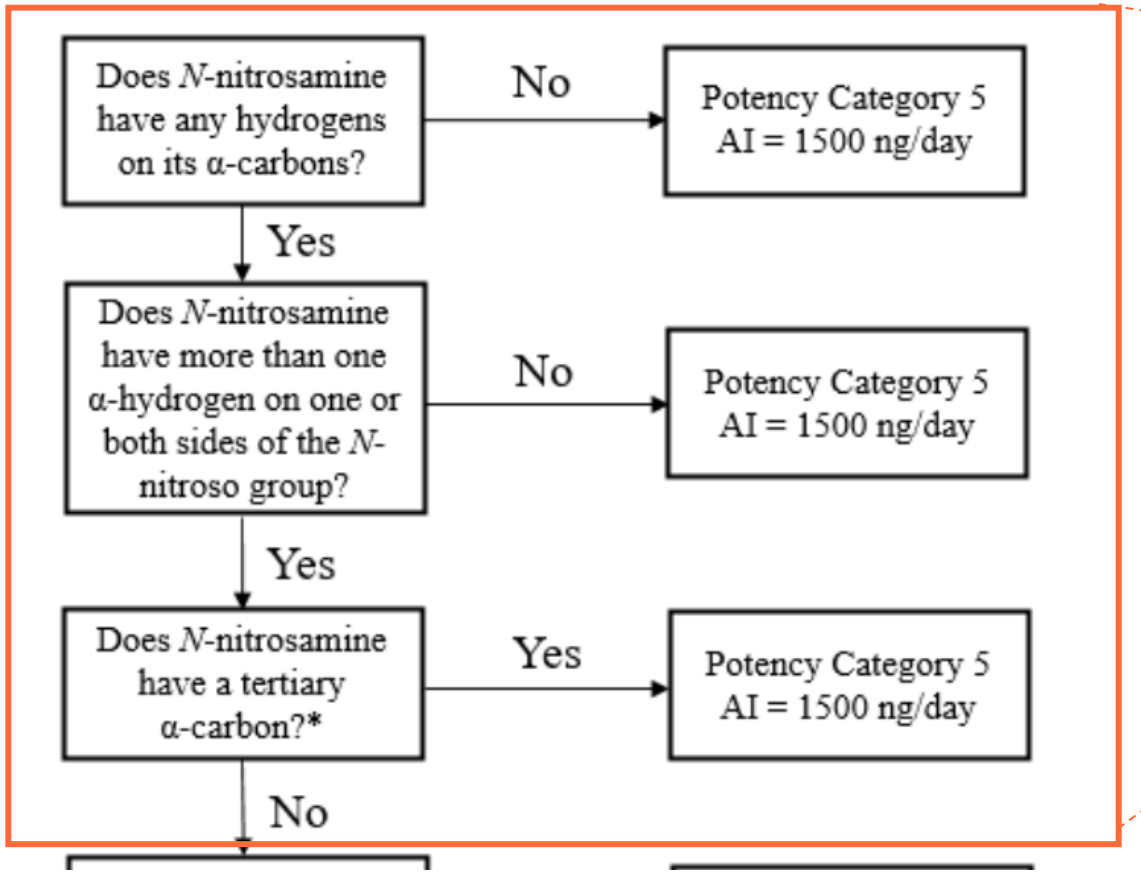
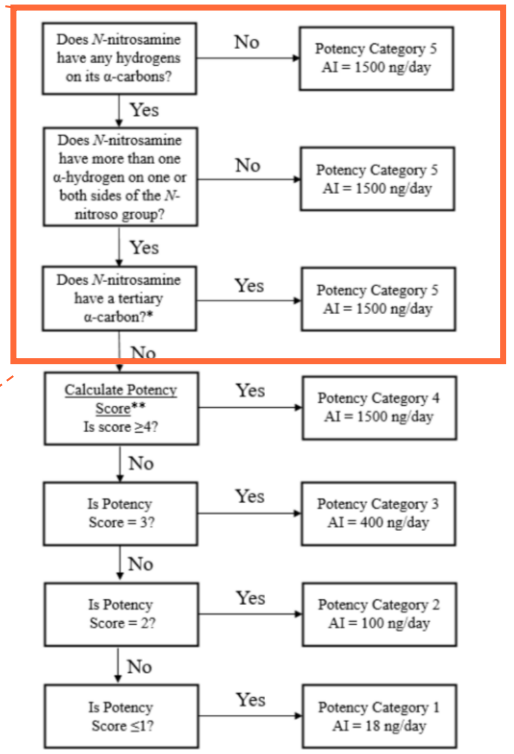


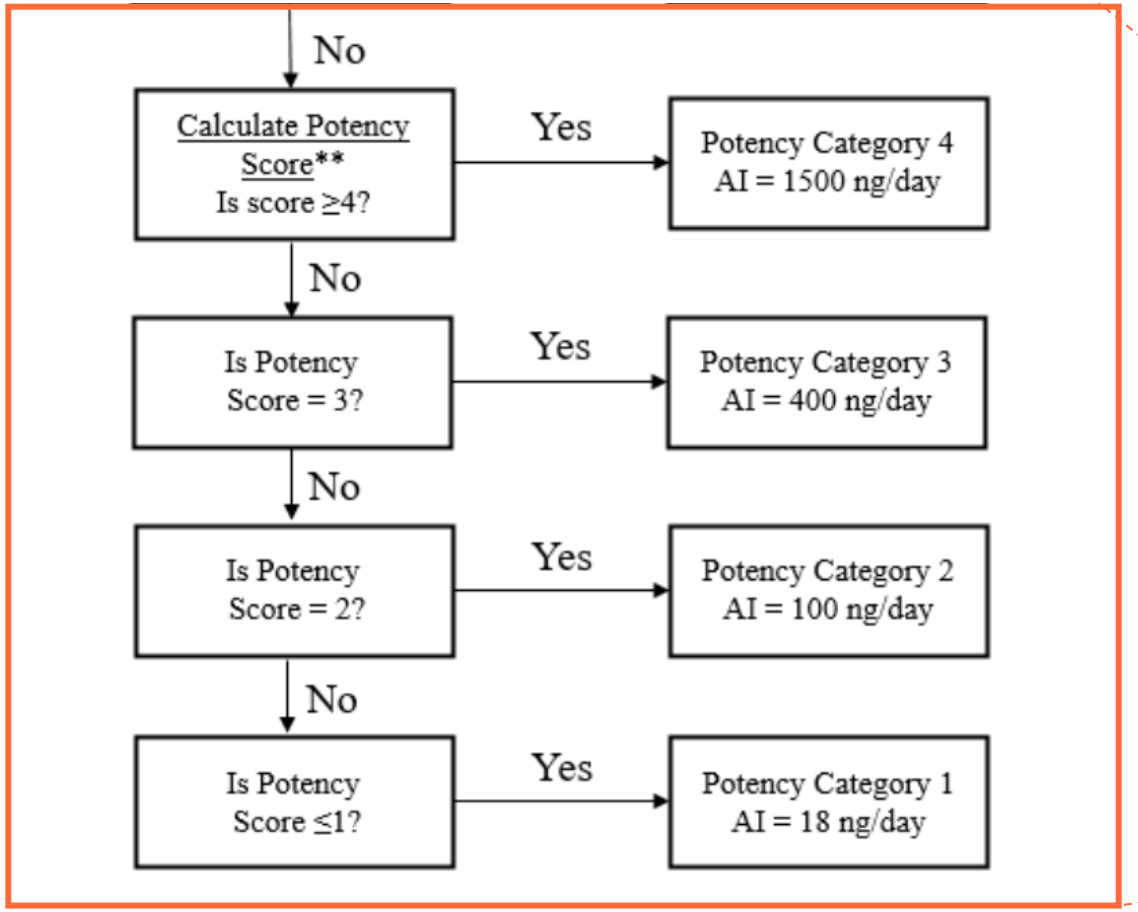
Figure 2. Flowchart to Predict the Potency Category of an *N*-nitrosamine

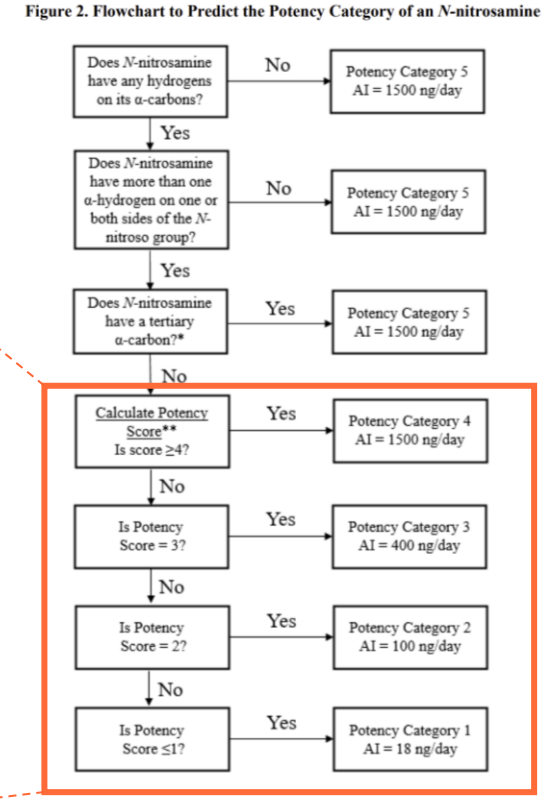


* A tertiary α-carbon is defined as an α-carbon atom in an sp3 hybridization state, bonded to three other carbon atoms.
** To calculate Potency Score, see Appendix A.

EMA Q&A - Article 5(3) of Regulation (EC) on nitrosamine

Annex 2 - Carcinogenic Potency Categorization Approach for N-nitrosamines.



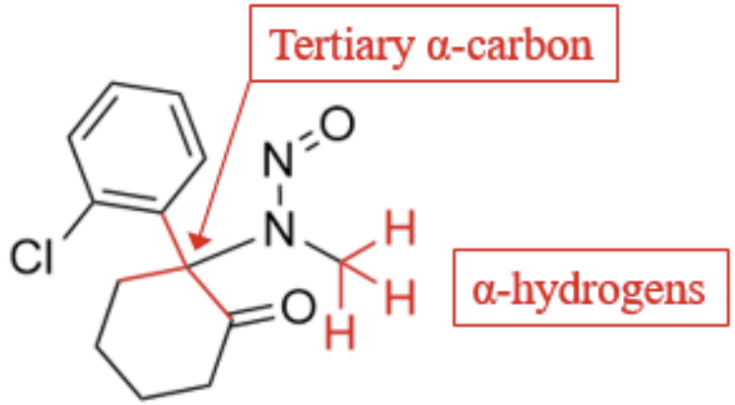


* A tertiary α-carbon is defined as an α-carbon atom in an sp3 hybridization state, bonded to three other carbon atoms.
** To calculate Potency Score, see Appendix A.

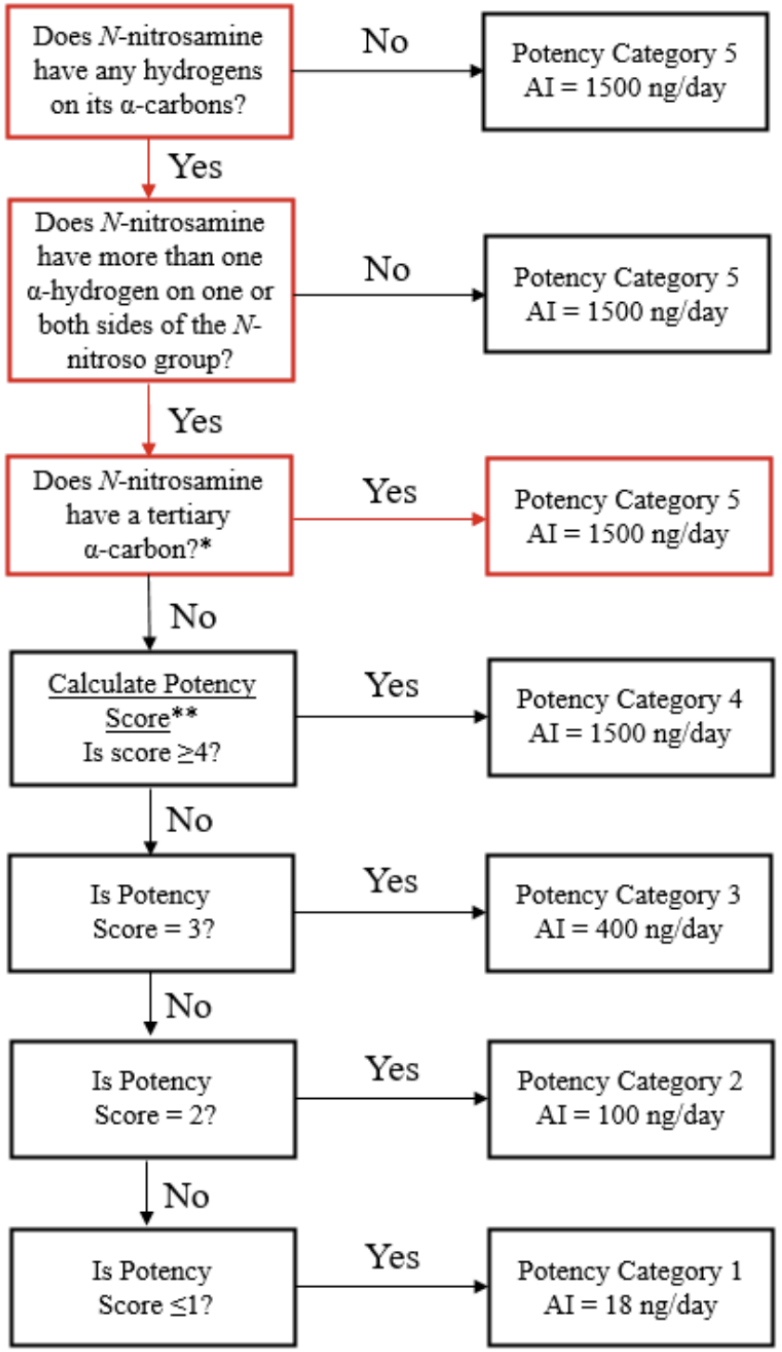
CPCA - Example

Example 3 – *N*-Nitroso-ketamine

Example 3 shows how the potency categorization approach flow chart (Figure 2) can be applied to the *N*-nitrosamine, *N*-nitroso-ketamine. *N*-Nitroso-ketamine is placed in Potency Category 5 with an associated AI limit of 1500 ng/day.



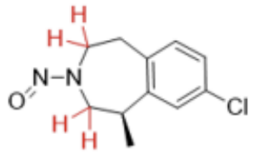
Potency Category 5	AI = 1500 ng/day

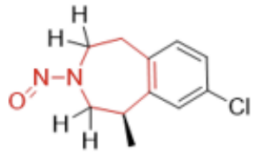


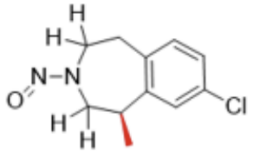
CPCA - Example

Example 8 – *N*-Nitroso-lorcaserin

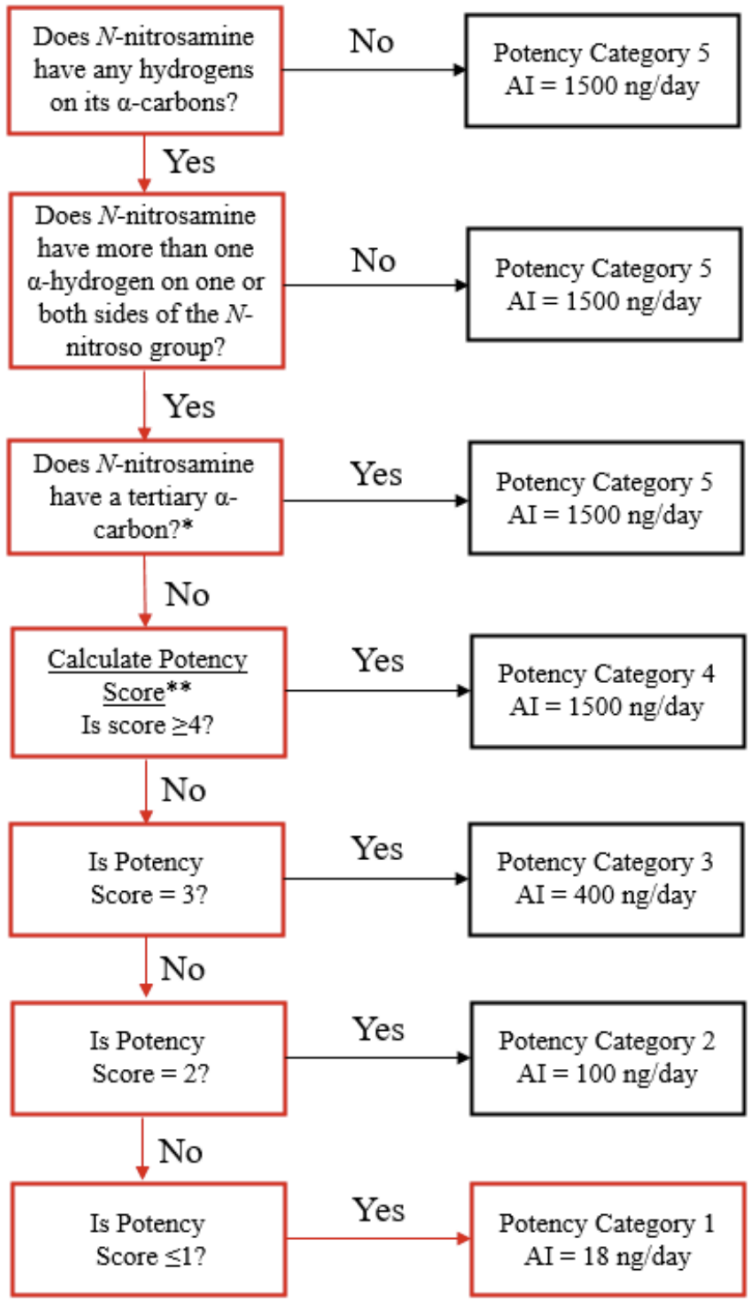
Example 8 shows how the potency categorization approach flow chart (Figure 2) can be applied to the *N*-nitrosamine, *N*-nitroso-lorcaserin. A Potency Score of 1 is calculated for *N*-nitroso-lorcaserin, resulting in its placement in Potency Category 1 with an associated AI limit of 18 ng/day.

Count of α-Hydrogens	Score	Feature Highlighted in Red
2,2	1	

Deactivating Features	Score	Feature Highlighted in Red
N-nitroso group in a 7-membered ring	+1	

Activating Features	Score	Feature Highlighted in Red
Methyl group bonded to β-carbon (cyclic or acyclic)	-1	

Potency Score = 1 + 1 - 1 = 1	Potency Category 1	AI = 18 ng/day



New FDA Guidance on NDRSIs

FDA Guide on NDSRIs

- Introduction to Predicted Carcinogenic Potency Categorization Approach – same as presented in the EMA Q&A – July 2023
- “FDA recommends that if NDSRIs were not considered in previous risk assessments, manufacturers and applicants reevaluate the risk within 3 months of publication of this guidance, with a recommended completion date by November 1, 2023, as part of overall risk management.”
- “Confirmatory testing using sensitive and appropriately validated methods should start as soon as the risk of an NDSRI is identified and should begin immediately for drug products considered to be at high risk.”

FDA Guide on NDSRIs

C. Approaches to Justify or Qualify a Proposed Alternative AI Limit

If the observed level of an NDSRI in a drug product exceeds the FDA-recommended AI limit (e.g., associated with the predicted carcinogenic potency category for that NDSRI), the Agency recommends that manufacturers and applicants pursue mitigation efforts to reduce or remove the NDSRI.[52] A manufacturer or applicant should submit a scientifically justified rationale to pursue an AI limit higher than the FDA-recommended limit associated with the predicted carcinogenic potency category for that NDSRI. Alternative approaches using safety data, such as obtaining compound-specific data or using read-across assessment to a suitable surrogate, could be used to support a higher AI limit. Importantly, manufacturers and applicants should note that the Agency may request additional safety data, beyond what is described here, to support alternative AI limits. If compound-specific data or a read-across approach is pursued, we recommend the following:

a. Mutagenicity assessment:[53,54] When testing the NDSRI using a bacterial mutagenicity assay (Ames assay), FDA recommends use of the full complement of testing strains described in Organisation for Economic Co-operation and Development (OECD) 471,[55] utilizing the preincubation method and using both rat and hamster S9 at concentrations of 30 percent. FDA recommends using S9 fractions typically prepared from animals treated with an inducer of CYP450s, like a combination of phenobarbital and beta naphthyl flavone. Manufacturers and applicants should use the recommended pre-incubation time of 30 minutes as part of the optimal conditions for detecting a signal for mutagenicity.

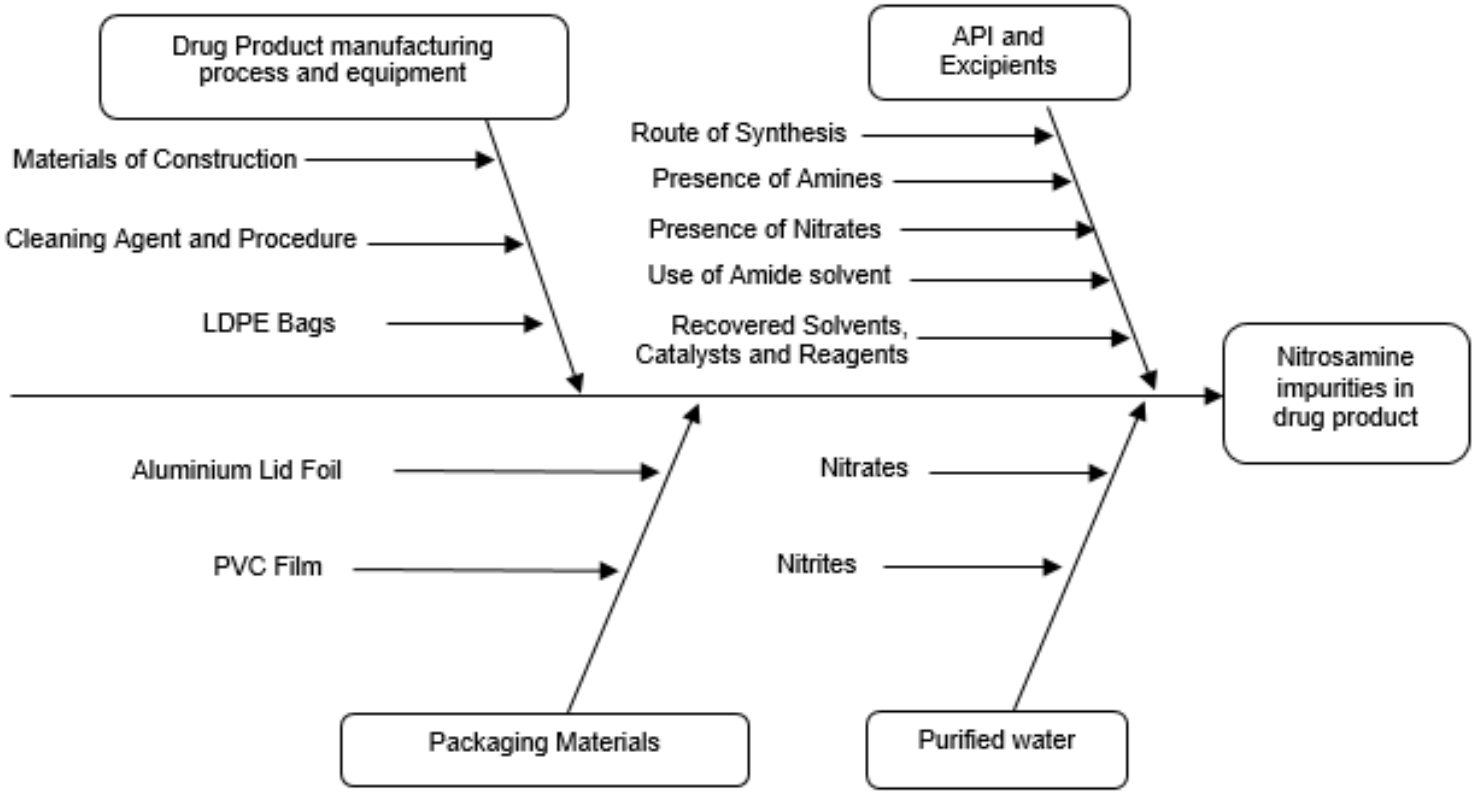
b. Read-across assessment based on surrogate: An NDSRI may have a well-justified AI limit based on a surrogate that maintains a 1 in 100,000 cancer risk estimate and is higher than the recommended AI limit associated with the predicted carcinogenic

Risk Assessment

The Why and How

Risk Assessment

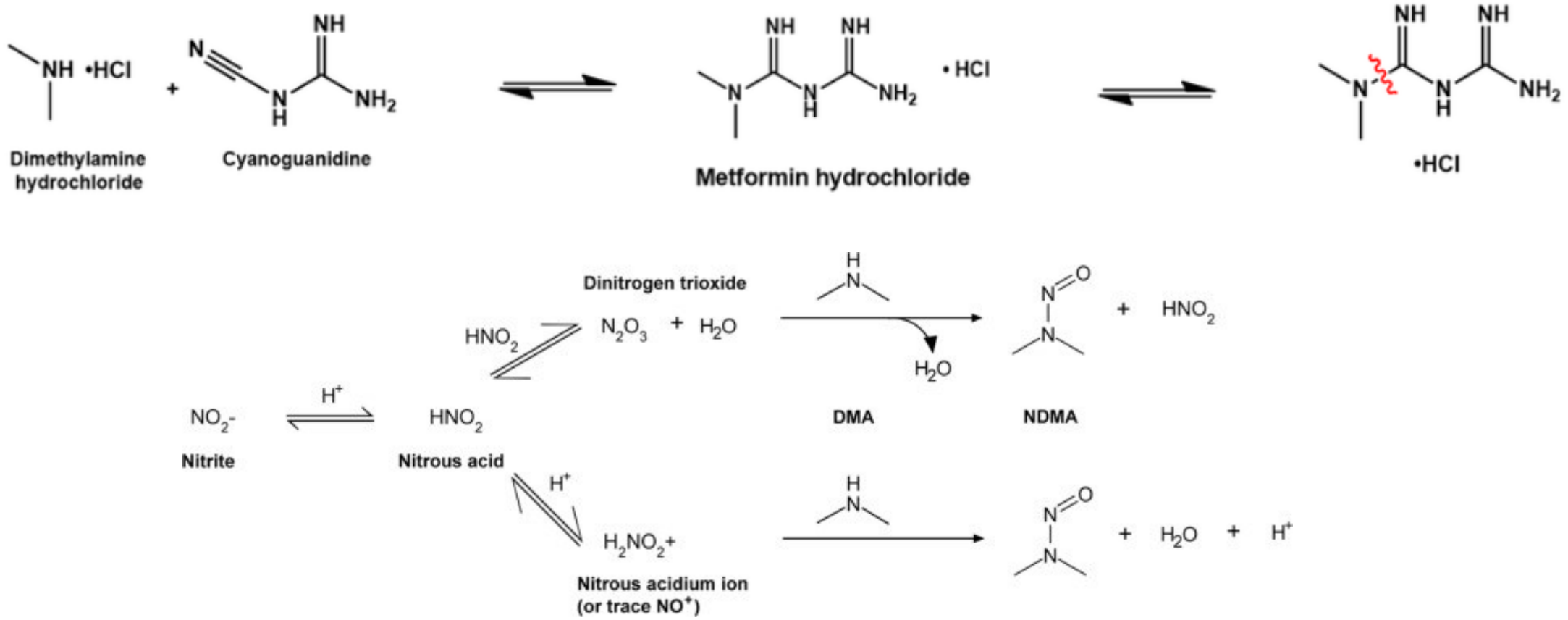
Figure 1: Potential sources of nitrosamine impurities for a drug product



Risk Assessment Case Study - NDMA in Metformin products

Risk Assessment

Avoiding N-nitrosodimethylamine (NDMA) formation in metformin pharmaceuticals by limiting dimethylamine and nitrite



Schlingemann, J. et al. Avoiding N-nitrosodimethylamine formation in metformin pharmaceuticals by limiting dimethylamine and nitrite. Int. J. of Pharmaceutics. 2022. https://doi.org/10.1016/j.ijph

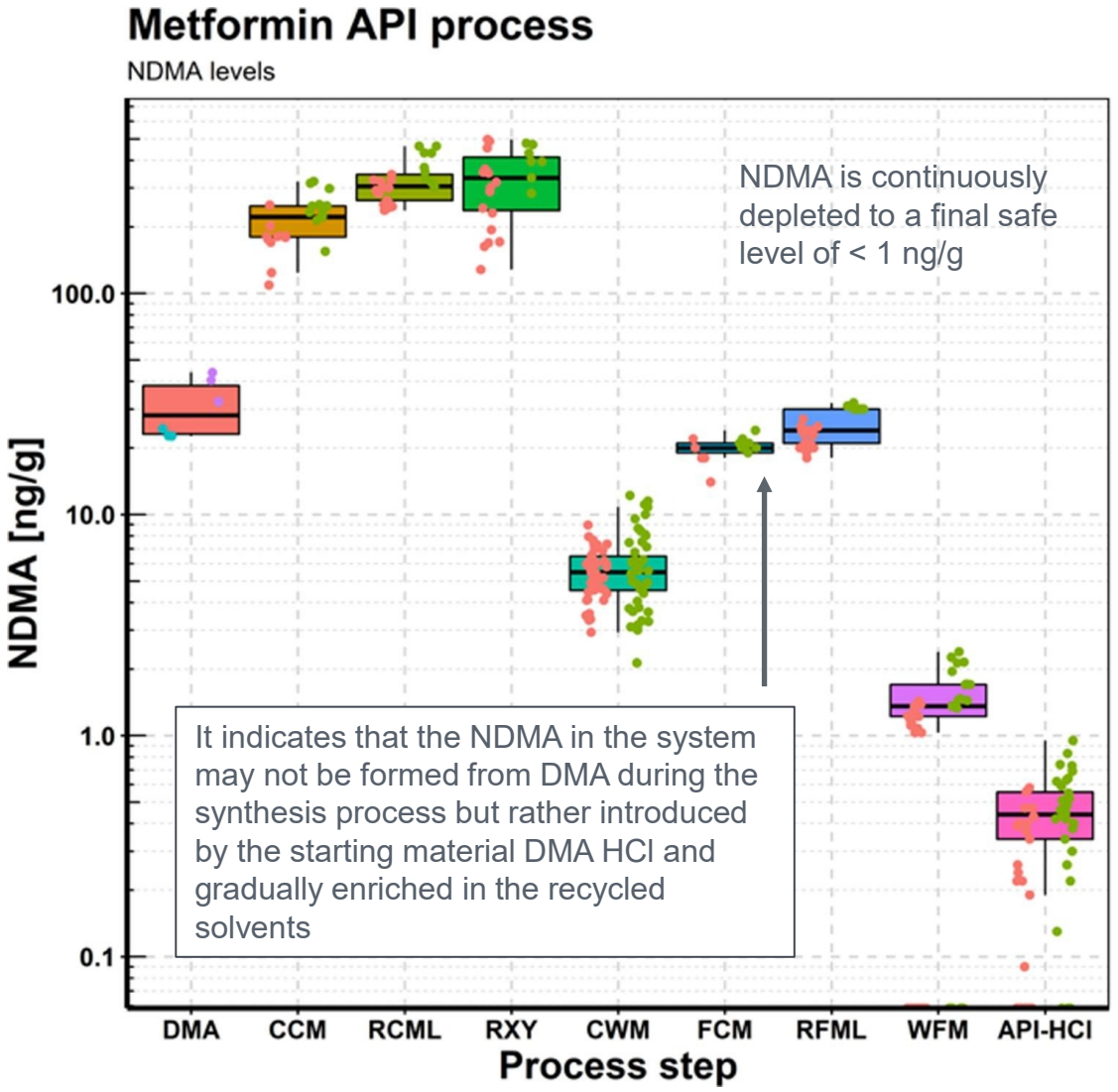
Risk Assessment

Root cause investigation for the presence of NDMA in Metformin drug products

- Analysis of NDMA content (GC–MS/MS and LC-MS/MS) to identify the process steps during which NDMA is either created or depleted
  - the API manufacturing process and
  - for 2 different drug product manufacturing processes
    - Glucophage® (IR - Immediate Release)
    - Glucophage® XR (XR - Extended Release)
- NDMA analysis of > 2000 historical batches - to identify key factors impacting the extent of NDMA formation in the drug product.
- NDMA formation monitoring in different packaging systems under stress, accelerated and long-term conditions.

Schlingemann, J. et al. Avoiding N-nitrosodimethylamine formation in metformin pharmaceuticals by limiting dimethylamine and nitrite. Int. J. of Pharmaceutics. 2022. https://doi.org/10.1016/j.ijph

Risk Assessment - API Manufacturing Process



https://doi.org/10.1016/j.ijpharm.2022.121740

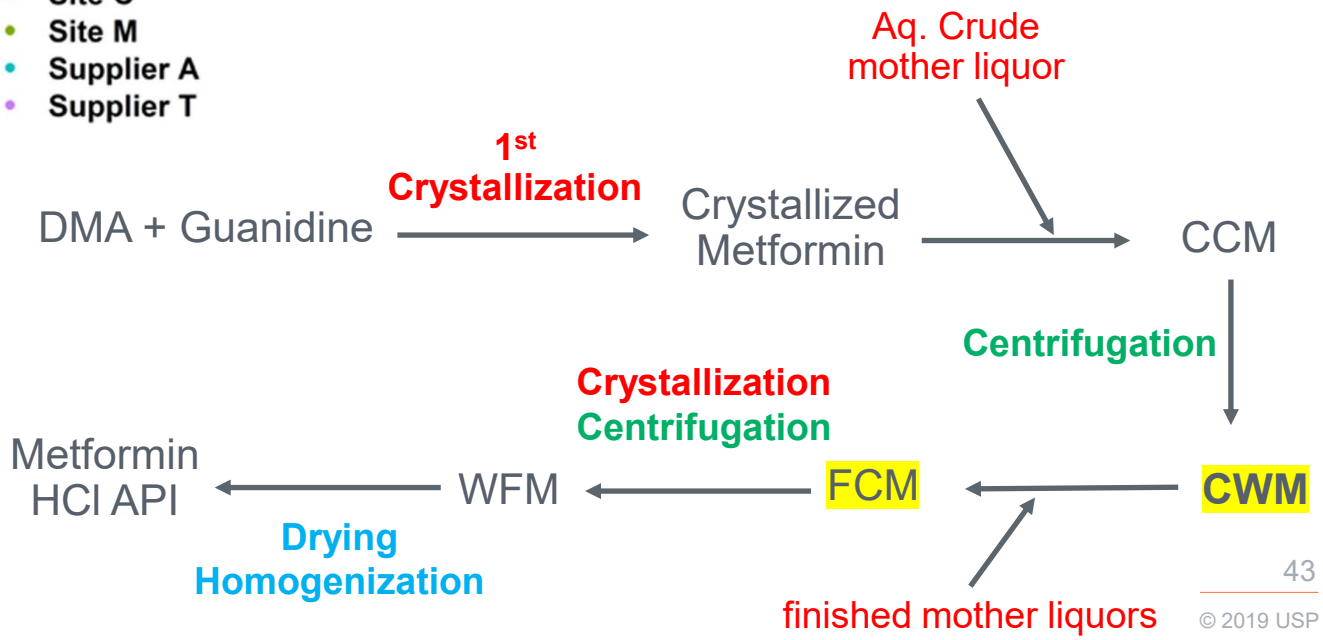
Process step

- DMA | DMA Hydrochloride
- CCM | Crude Crystallization Mix
- RCML | Recycled Crude Mother Liquors
- RXY | Recycled Xylene
- CWM | Crude Wet Metformin
- FCM | Finished Crystallization Mix
- RFML | Recycled Finished Mother Liquors
- WFM | Wet Finished Metformin
- API-HCl | Metformin Hydrochloride

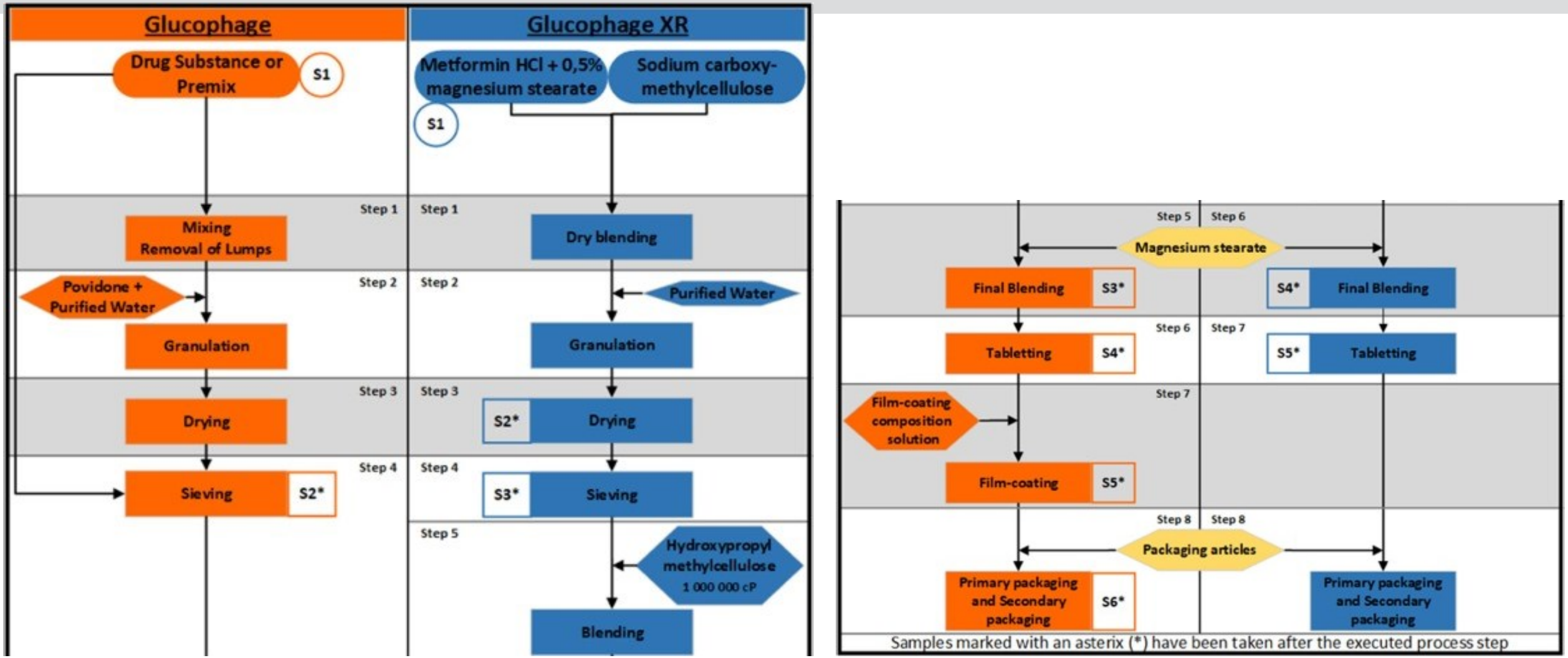
Site / Supplier

- Site C
- Site M
- Supplier A
- Supplier T

Samples from these steps were collected and analyzed for NDMA by LC-MS/MS



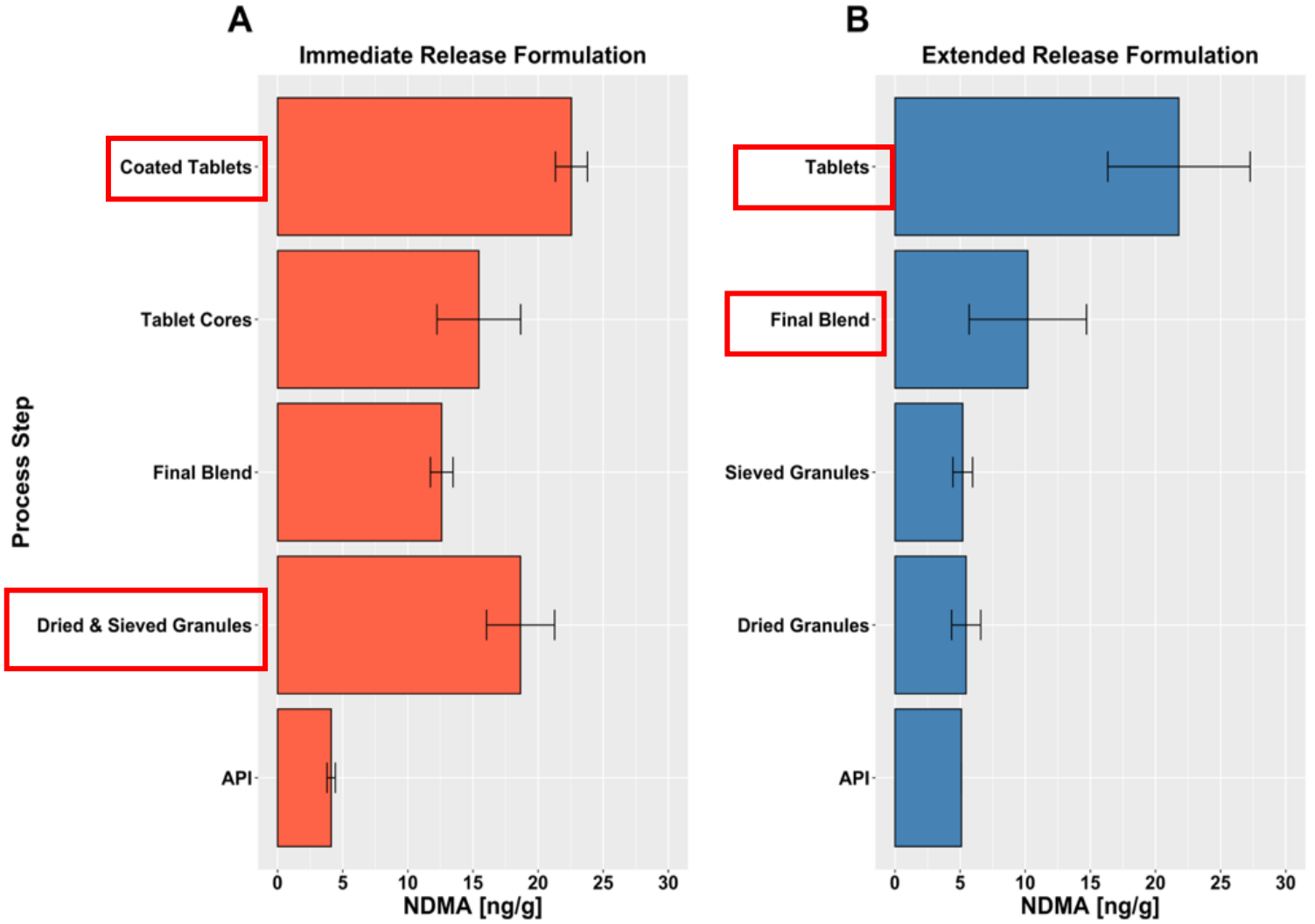
Risk Assessment - Drug Product Manufacturing Process



https://doi.org/10.1016/j.ijpharm.2022.121740

Risk Assessment - NDMA is formed during DP manufacturing

- NDMA content increased during wet granulation and coating.
- The wet granulation step introduces heat and PVP K30 as a significant source of nitrite
- The coating process introduces heat and moisture.

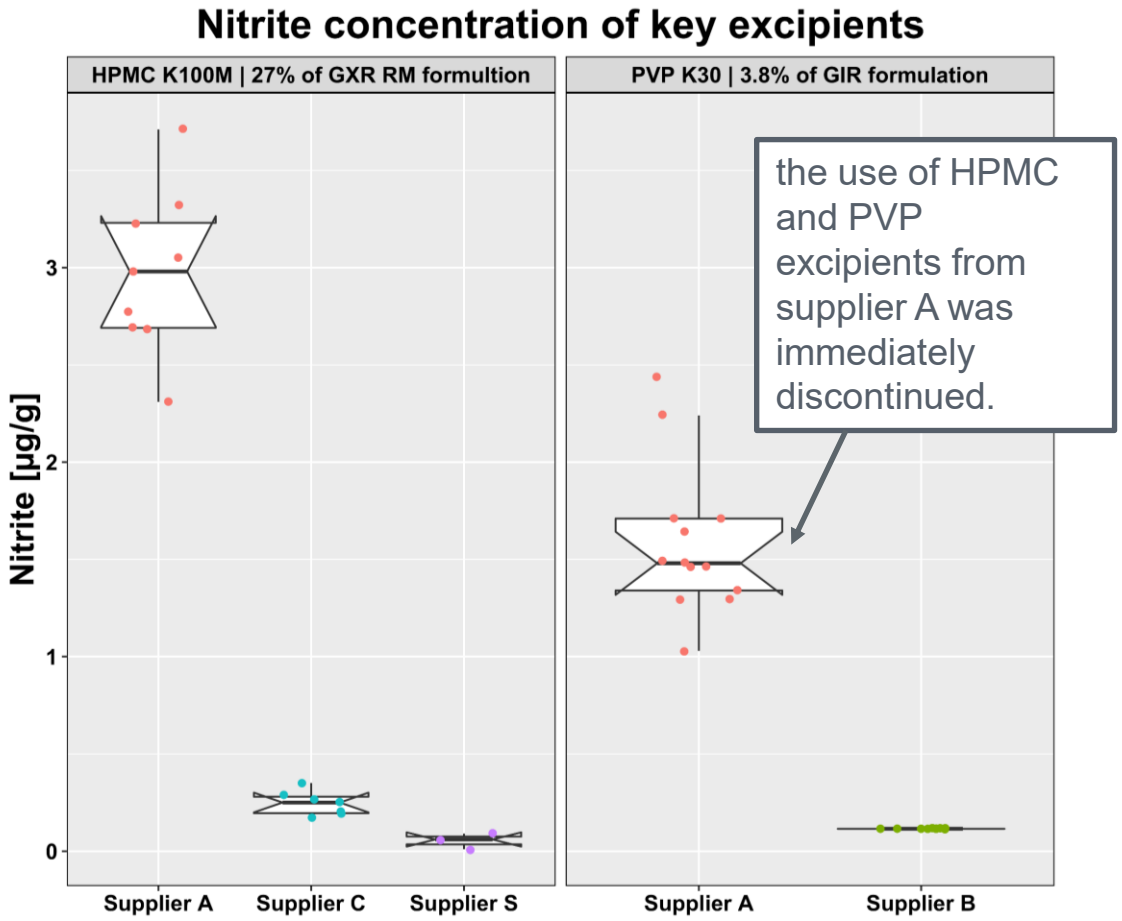


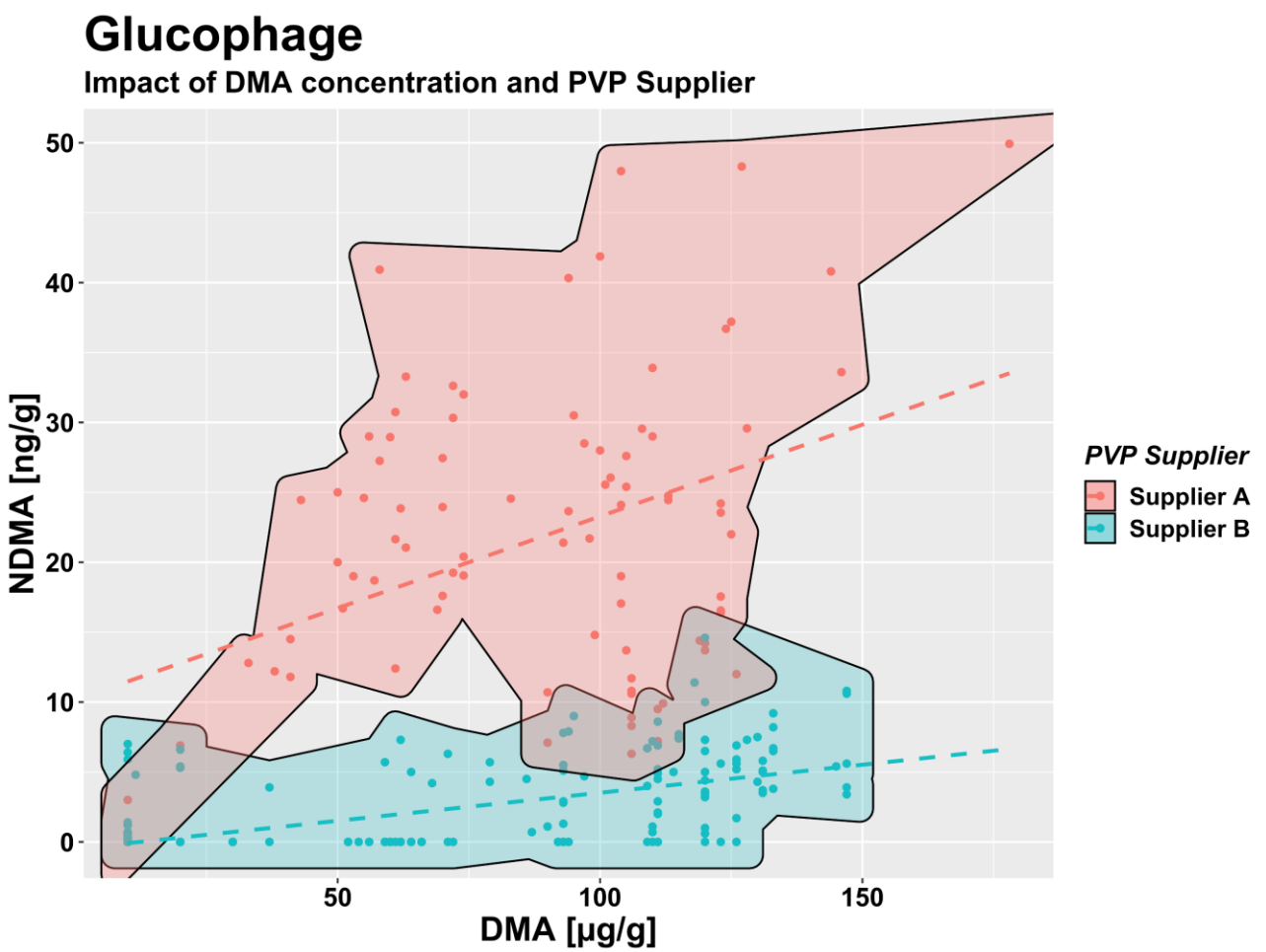
- NDMA content increases after introduction of HPMC 100 k
- HPMC had been identified as the main contributor of nitrite to Glucophage® XR tablets.
- Tableting of the granules led to a further increase of NDMA, possibly due to the generation of heat and mechanical stress through the compression

https://doi.org/10.1016/j.ijpharm.2022.121740

Each graph shows the average from the production of three commercial-scale batches made before implementation of any improvements described in the paper

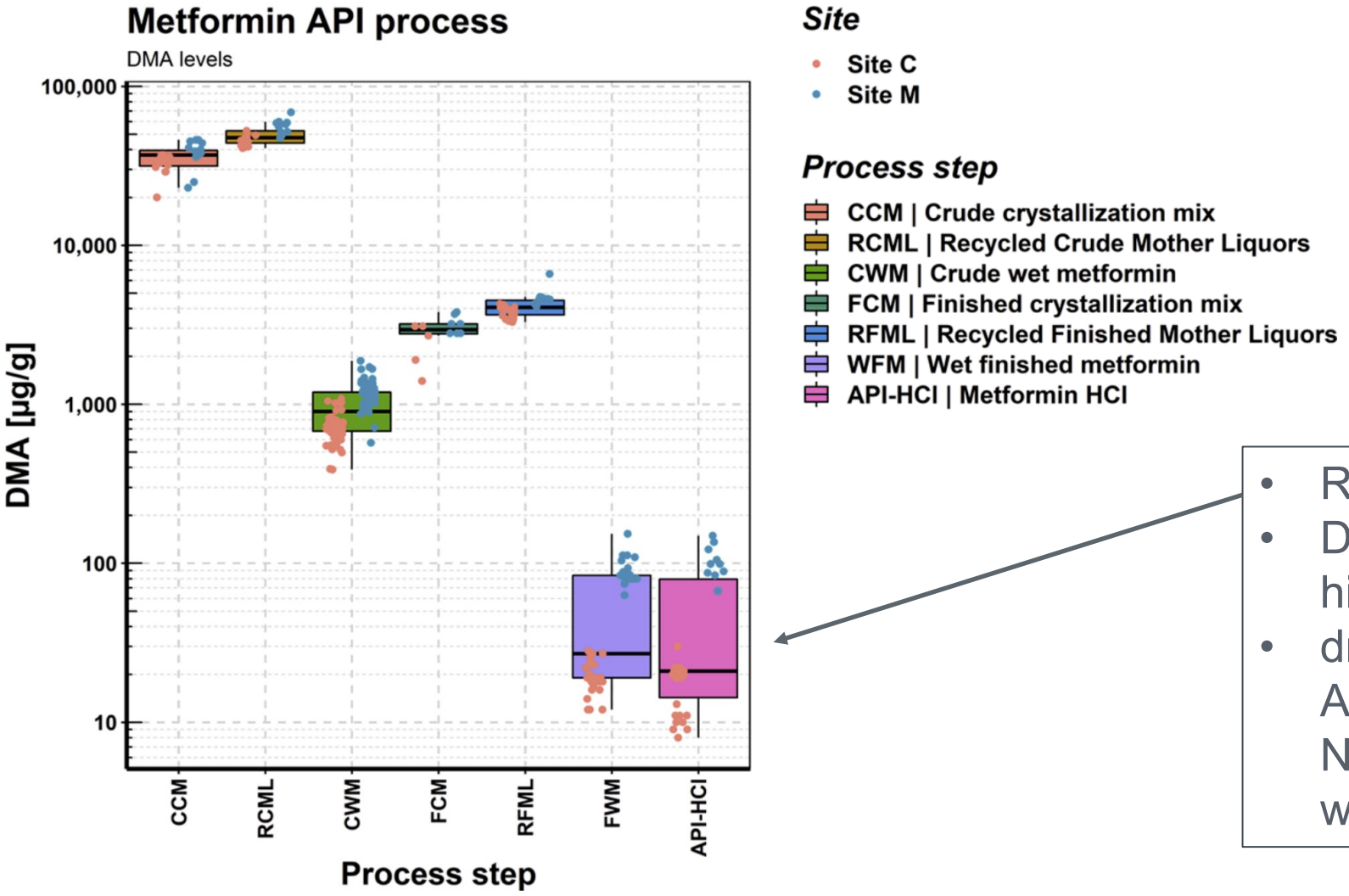
Risk Assessment - Nitrite from excipients





https://doi.org/10.1016/j.ijpharm.2022.121740

Risk Assessment - API Manufacturing Process

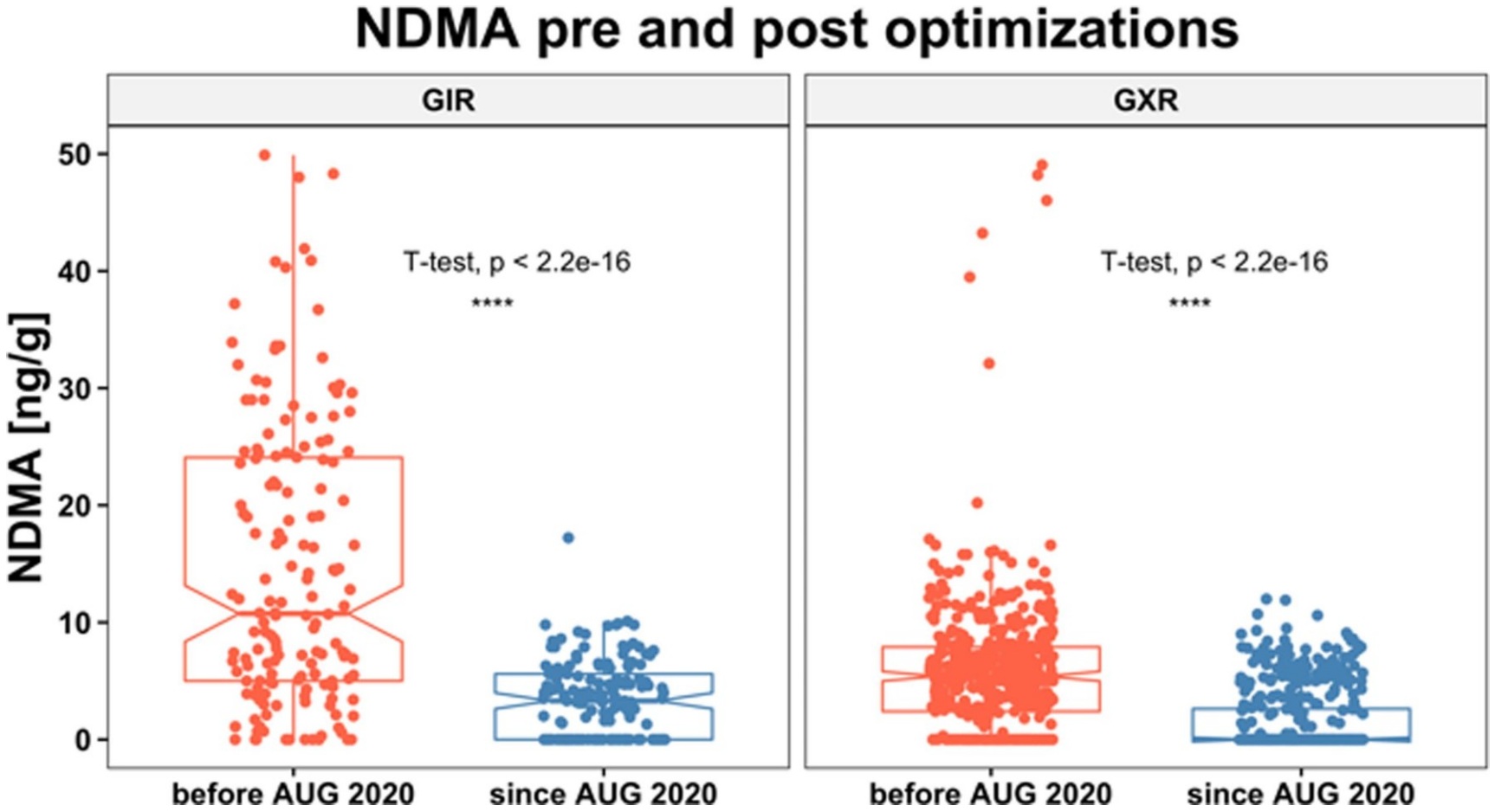


Control Strategy:
process optimization started at site M during - reduce residual DMA by 50%

- Residual DMA remains in the API
- DMA content in API from site M is higher than in API from site C
- drug product batches made from API of site M tend to be higher in NDMA than those batches made with API from site C.

https://doi.org/10.1016/j.ijpharm.2022.121740

After process controls



The box plots display NDMA results for 158 Glucophage® batches and 449 Glucophage® XR batches produced before August 2020 as well as 168 Glucophage batches and 516 Glucophage XR batches produced since August 2020.

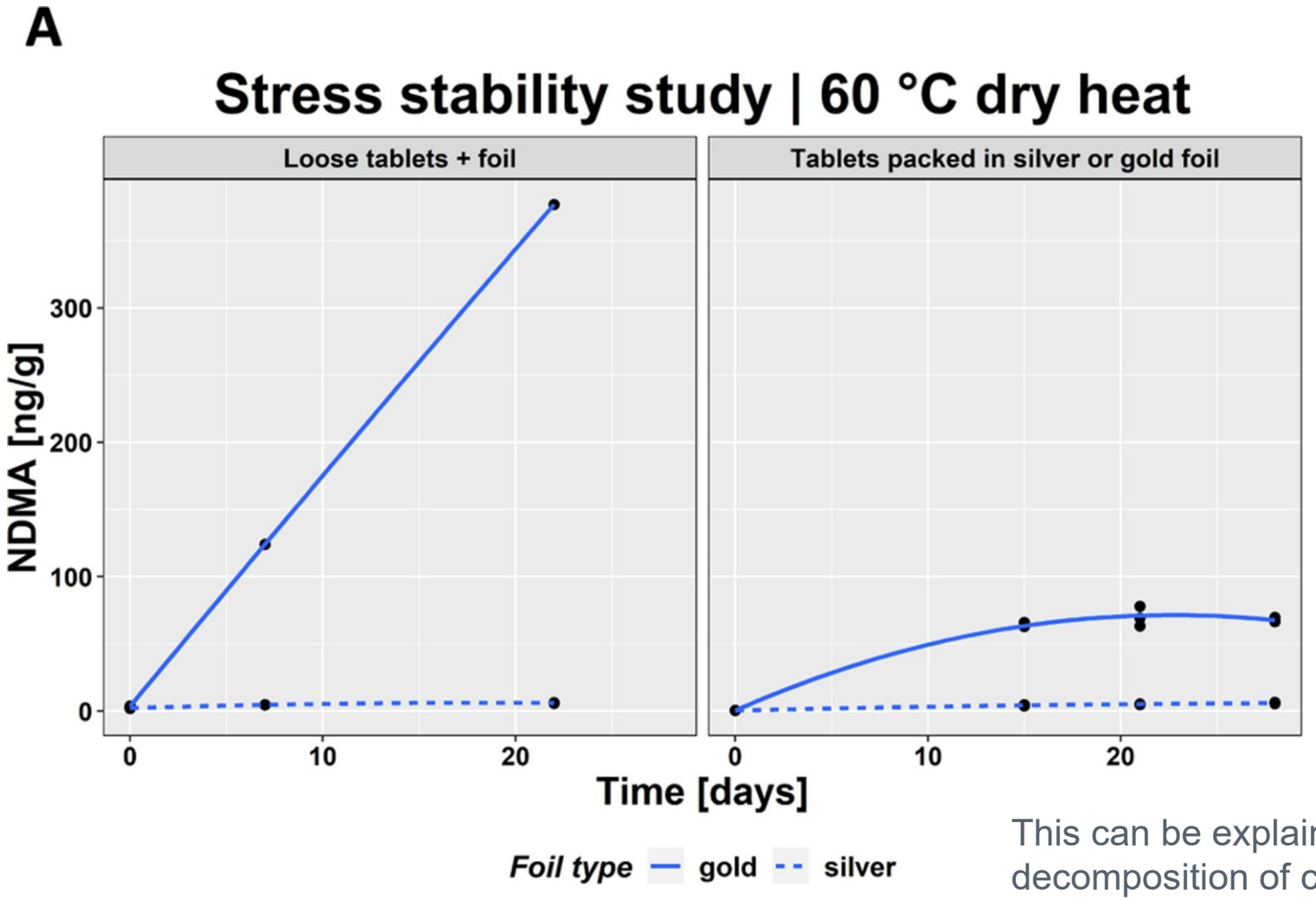
- **Control strategies:**
  - high-nitrite PVP from supplier A was discontinued
  - high-nitrite HPMC was discontinued
  - DMA reduction in freshly drug product batches and inclusion of limits
- The effectiveness of the measures was confirmed by continuous monitoring of NDMA in drug product batches via implementation of a skip testing program.

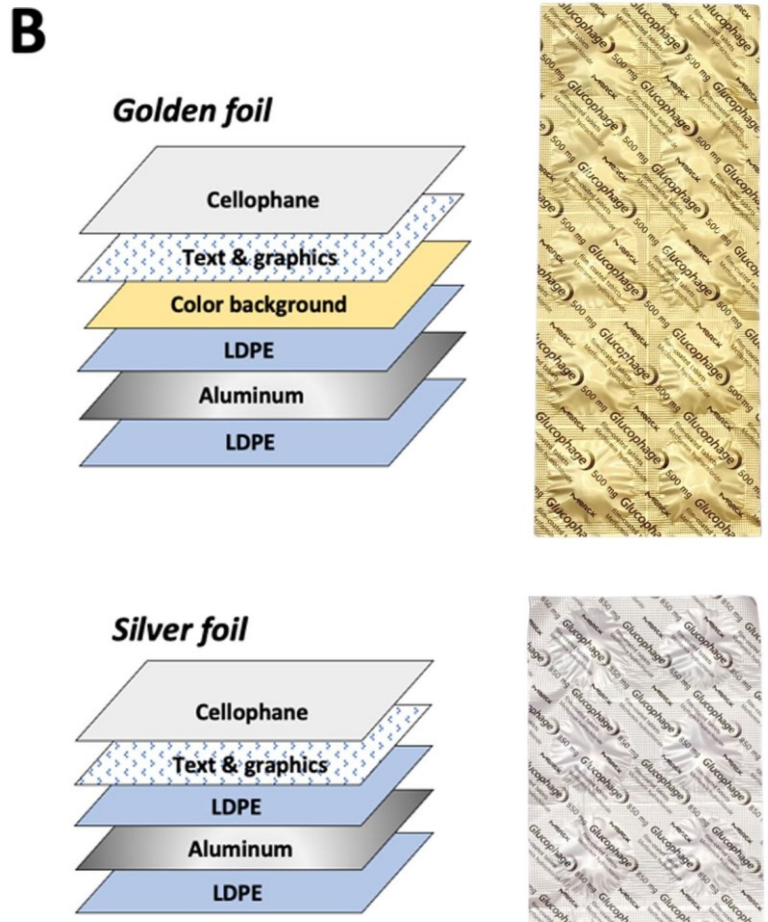
https://doi.org/10.1016/j.ijpharm.2022.121740

NDMA on packaging

- Cellulose nitrate (“nitrocellulose”) is used as a key component/matrix of printing primers, printing inks commonly used for pharmaceutical packaging materials.
- The thermal decomposition of cellulose nitrate releases nitrogen oxides (NOx i.e., NO and NO2)
- Nitric oxide (NO) can react with water to form nitrous acid (HNO2), a precursor of nitrosating agents as one component.
- Hyphothesis:
  - the nitrocellulose decomposition could lead to the nitrosation of DMA or DEA amine components in the printing ink used on blister lidding foil,
  - Nitrosamines could be transferred to the product through vaporization during heat sealing –
  - No confirmatory experimental data have been published so far.

NDMA on packaging



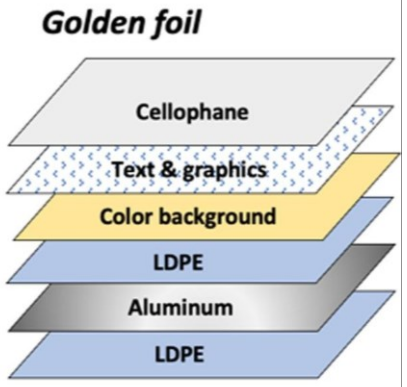


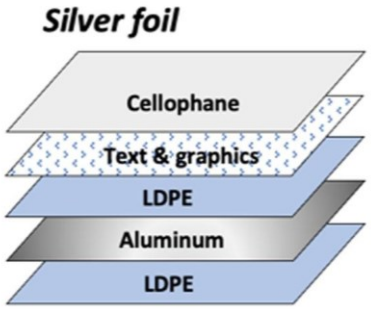
This can be explained by NOx created by the decomposition of cellulose nitrate from the **golden ink** migrating into the tablets, where it caused nitrosation of DMA

https://doi.org/10.1016/j.ijpharm.2022.121740

NDMA on packaging

- Tablets sealed inside the aluminum strip packs
  - It can be considered impermeable to both cellulose nitrate and NOx
  - NDMA formation in combination with the golden foil, even though the tablets were sealed inside the aluminum strip packs
  - In this case, the NDMA formation is likely to be caused by cellulose nitrate deposition on the inner surface of the foil, which is supplied as a role,
    - small amounts of cellulose nitrate are likely to permeate through the cellophane layer and attach to the inner lowdensity-polyethylene (LDP)





https://doi.org/10.1016/j.ijpharm.2022.121740

NDMA on packaging

Confirmatory testing

NDMA 👍

NDMA 👎

Nitrocellulose

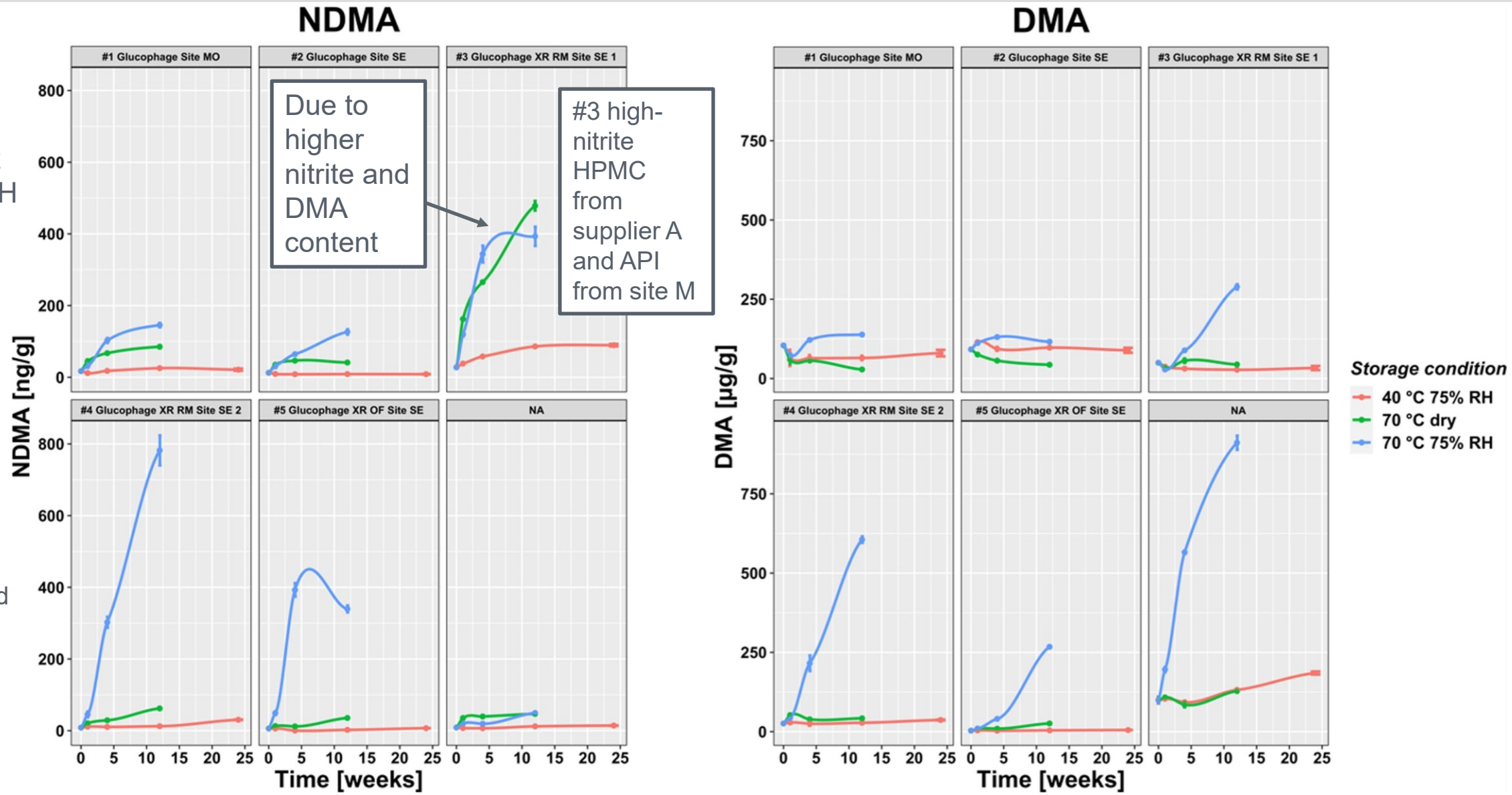
NDMA 👍

Source from: Dr. Schlingemann (Merck KGaA)

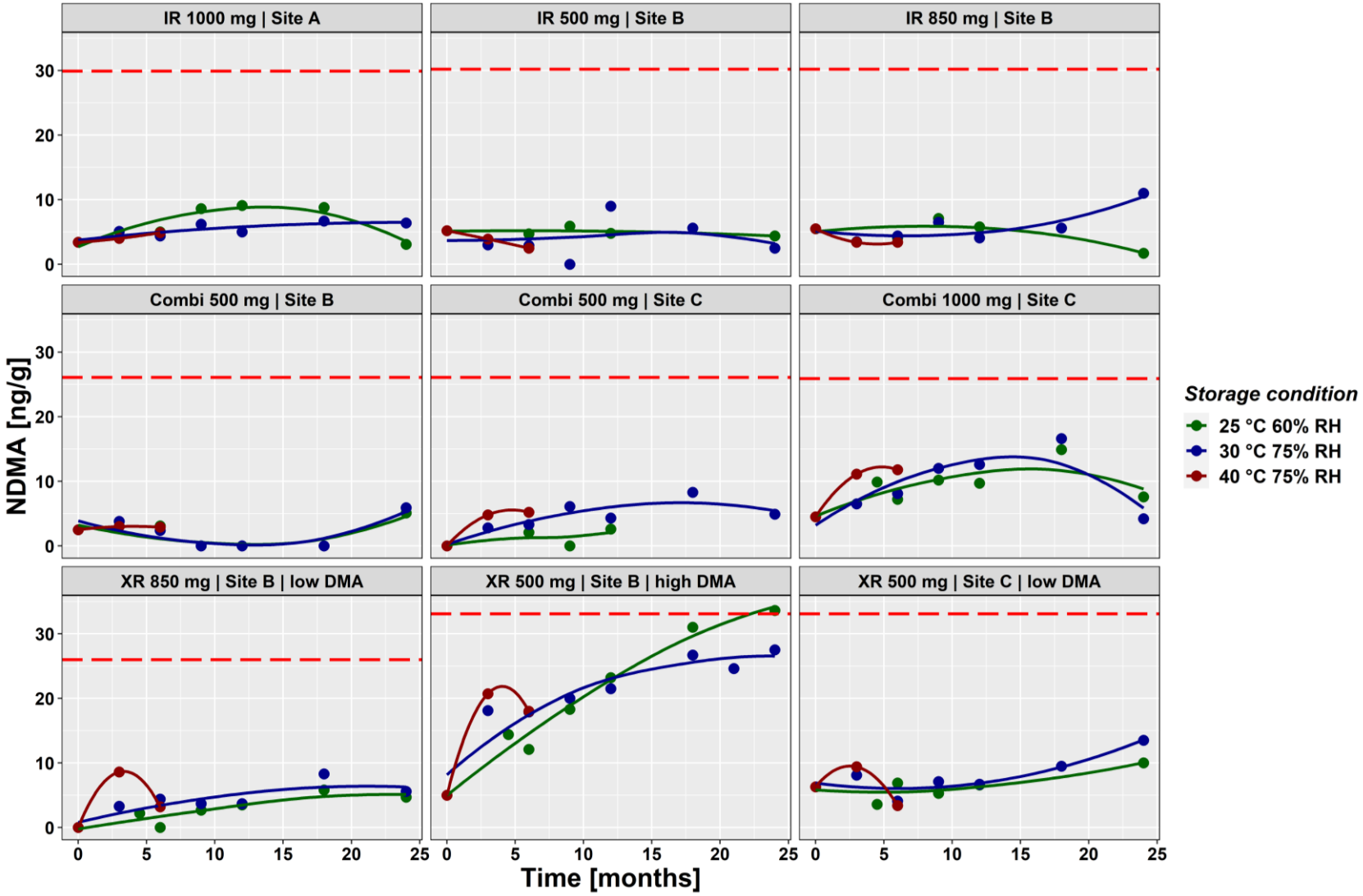
NDMA formation during stress-conditions

Very little NDMA formation at 40C-75% RH and little at 70C dry heat.

#4 low-nitrite HPMC from supplier B and API from site C



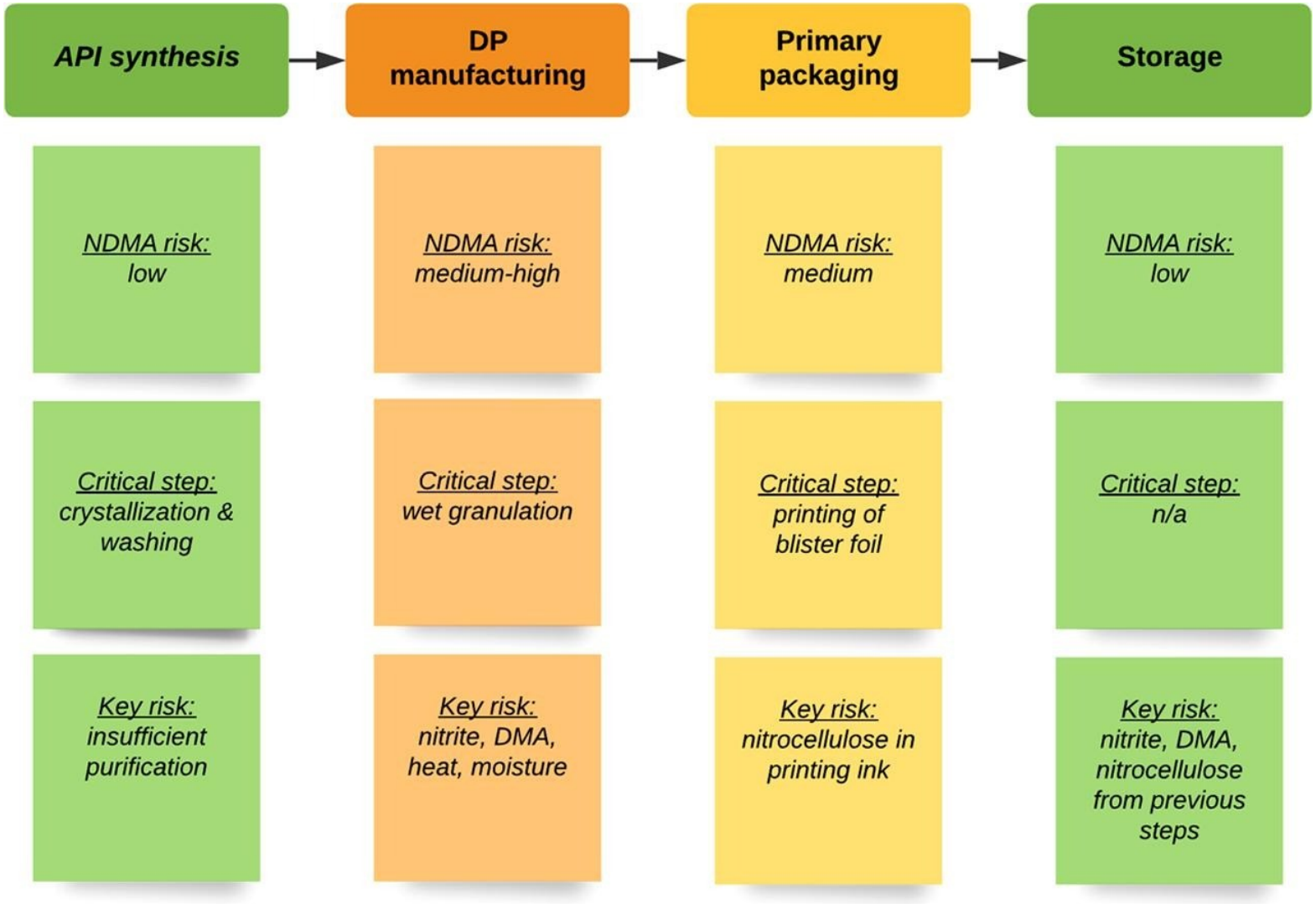
NDMA in Metformin - Stability Data



Findings from Risk Assessment (Validation)

- Depletion of NDMA introduced during API manufacturing by double crystallization
- Comparison of measured NDMA & DMA purge factors and respective in-silico predictions. Mirabilis Consortium that predicts purge factors for potentially mutagenic impurities based on physicochemical parameters and process conditions.
- NDMA formation in the drug product manufacturing process
  - Reduction of DMA in the API
  - Discontinuation of suppliers of PCP and HPMC with high concentration of nitrite
- nitrocellulose ink is particularly problematic in combination with Metformin products, as they contain DMA as nitrosatable amine component, so that NDMA can be formed easily even if the ink is free from DMA –selection of appropriate ink

Risk Assessment



Schlingemann, J. et al. Avoiding N-nitrosodimethylamine formation in metformin pharmaceuticals by limiting dimethylamine and nitrite. Int. J. of Pharmaceutics. 2022. https://doi.org/10.1016/j.ijph

Risk Assessment (Formulation)

ELSEVIER

Journal of Pharmaceutical Sciences

Available online 2 February 2023

In Press, Corrected Proof ⓘ What's this? ↗

Pharmaceutics, Drug Delivery and Pharmaceutical Technology

N-Nitrosamine Formation in Pharmaceutical Solid Drug Products: Experimental Observations

Justin Moser [a], Ian W. Ashworth [b], Laurence Harris [c], Michael C. Hillier [d], Kausik K. Nanda [e], Garry Scrivens [c]

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https://doi.org/10.1016/j.xphs.2023.01.027 ↗

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Monitoring the formation of the *N*-nitrosamines after processing and upon stressed stability conditions showed that *N*-nitrosamine formation **can occur** in **solid drug** product formulations.

USP Perspectives on Nitrosamines

Nitrosamine Activities

1 Documentary Standard

General Chapter <1469>
Nitrosamine Impurities

2 Reference Standard

- 8 USP Reference Standards (NDMA, NDEA, NDIPA, NDBA, NEIPA, NMBA, NMPA and NDMA-d6)

- Pharmaceutical Analytical Impurities
  34 impurities to be released in 2023
  (e.g., CPNP, MNP, NPYR, NDELA, NDPA, NMOR, **NDSRI** etc)

3 Analytical Procedures

- In-house procedure development and validation
- External Collaborations
- Analytical Hub

4 Education

- USP Education Course
- Tutorial Videos

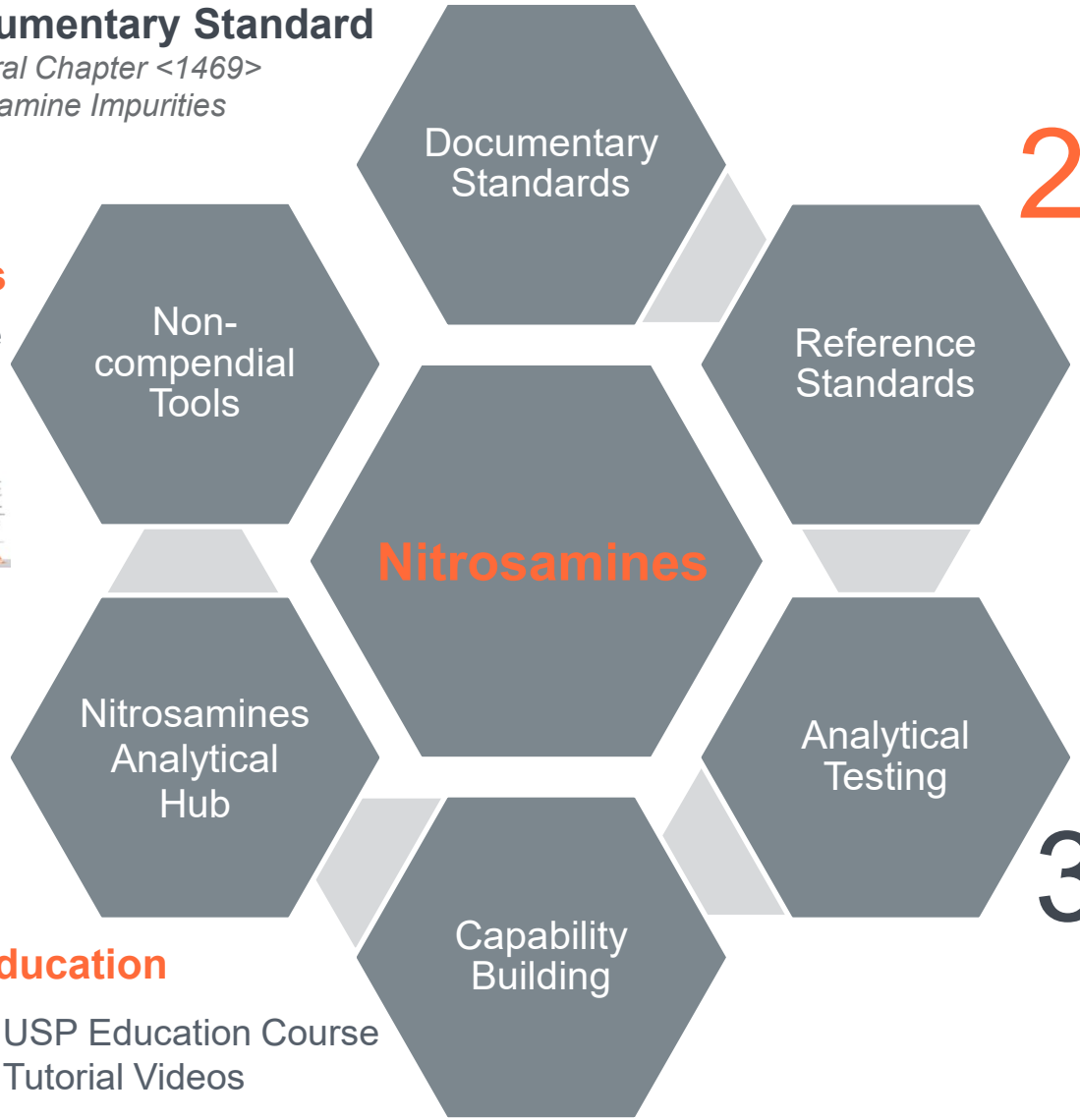
5 Capability Building

6 Stakeholder Engagement

- Support regulators and industry
- Collaborations

7 Non-compendial tools

- Nitrosamines Exchange Community
- Analytical Hub
- Publications



Overview of USP Nitrosamine activities

1 **Documentary Standard**
To address the nitrosamine impurities safety concern from a pharmacopeia perspective, USP Joint Expert Subcommittee (JSC) was convened since February 2020 to develop *General Chapter <1469> Nitrosamine Impurities.*

USP's Nitrosamine Program

2 **Reference Standard**
Eight USP Reference Standards have been established to support *General Chapter <1469> Nitrosamine impurities*

- *N*-Nitrosodimethylamine (NDMA) (1 mg/mL in MeOH)
- *N*-Nitrosodiethylamine (NDEA) (1 mg/mL in MeOH)
- *N*-Nitrosodiisopropylamine (NDIPA) (1 mg/mL in MeOH)
- *N*-Nitrosodibutylamine (NDBA) (1 mg/mL in MeOH)
- *N*-Nitrosoethylisopropylamine (NEIPA) (1 mg/mL in MeOH)
- *N*-Nitrosomethylaminobutyric Acid (NMBA) (1 mg/mL in ACN)
- *N*-Nitrosomethylphenylamine (NMPA)
- Deutero *N*-Nitrosodimethylamine (NDMA-d_6)

- Additional Impurities to be developed
  - 1-Cyclopentyl-4-NitrosPiperazine (CPNP)
  - 1-Methyl-4-NitrosoPiperazine (MNP)
  - *N*-Nitroso pyrrolidine (NPYR)
  - *N*-Nitrosodiethanolamine (NDELA)
  - *N*-Nitrosodipropylamine (NDPA)
  - *N*-Nitrosomethylethylamine (NMEA)
  - *N*-Nitrosomorphpline (NMOR)
  - *N*-Nitrosopiperidine (NPIP)

Pharmaceutical Analytical Impurities (PAI)

Released in June, 2023

Item number	Name	API	CAS
1A04020	1-Methyl-4-nitrosopiperazine (MNP) Solution (1 mL (1 mg/mL)) (1-Methyl-4-nitrosopiperazine)	Rifampicin	16339-07-4
1A04060	N-Nitrosopyrrolidine (NPYR) Solution (1 mL (1 mg/mL) (1-Nitrosopyrrolidine))	NA/54 APIs	930-55-2
1A04190	1-Cyclopentyl-4-nitrosopiperazine (CPNP) Solution (1 mL (1 mg/mL)) (1-Cyclopentyl-4-nitrosopiperazine)	Rifapentine	61379-66-6
1A03930	N-Nitrosodipropylamine (NDPA) Solution (1 mL (1 mg/mL)) (N,N-dipropylnitrous amide)	NA	621-64-7
1A04400	N-Nitrosoiminodiacetic acid (10 mg) (2,2'-(nitrosoazanediyl)diacetic acid)	NA	25081-31-6
1A04080	1-(2-Methoxyphenyl)-4-nitrosopiperazine Solution (1 mL (1 mg/mL)) (1-(2-Methoxyphenyl)-4-nitrosopiperazine)	Piperazine/19 APIs	221933 9-64-5

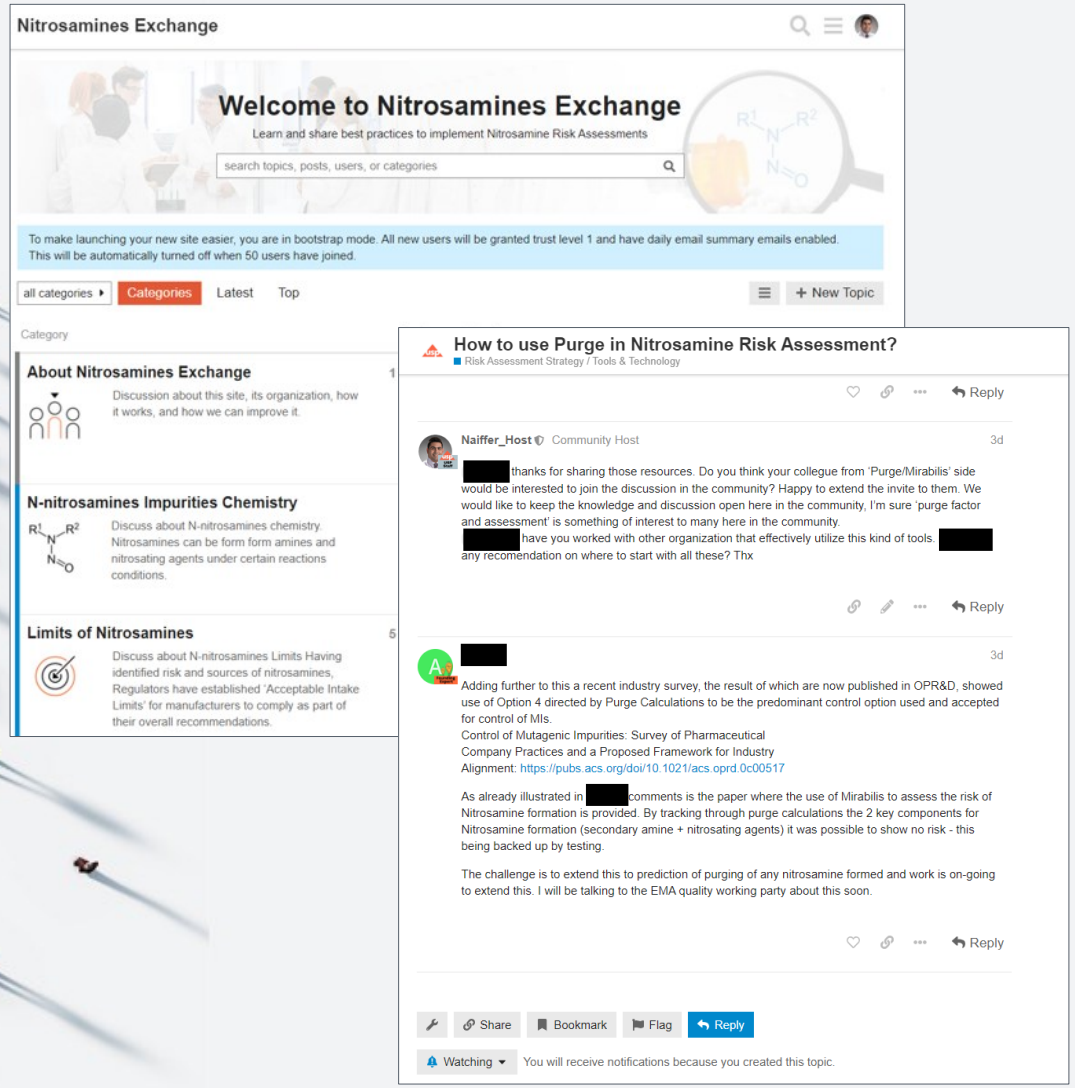
Pharmaceutical Analytical Impurities (PAI)

To be released in July, 2023

Item number	name	API	CAS
1A04420	1-Benzhydryl-4-nitrosopiperazine (10 mg) (1-Benzhydryl-4-nitrosopiperazine)	NA	1698-25-5
1A04130	1-Benzhydryl-4-nitrosopiperazine Solution (1 mL (1 mg/mL))	Piperazine/16 APIs	1698-25-5
1A04440	2-(4-Nitrosopiperazin-1-yl)pyrimidine (10 mg) (2-(4-nitrosopiperazin-1-yl)pyrimidine)	NA	872826-80-7
1A04140	4-Nitroso-1-(4-fluorobenzoyl)piperazine Solution (1 mL (1 mg/mL))	Piperazine/15 APIs	N/A
1A04410	4-Nitrosopiperazine-1-ethanol (10 mg) (2-(4-Nitrosopiperazin-1-yl)ethan-1-ol)	NA	48121-20-6
1A04100	4-Nitrosopiperazine-1-ethanol Solution (1 mL (1 mg/mL))	NA/17 APIs	48121-20-6
1A04220	N-Nitroso Atenolol Solution (1 mL (1 mg/mL))	Atenolol	134720-04-0
1A04250	N-Nitroso Bisoprolol Solution (1 mL (1 mg/mL))	Bisoprolol	2820170-76-9
1A04380	N-Nitroso Dabigatran Etexilate	Dabigatran	N/A
1A04000	N-Nitroso Duloxetine Solution (1 mL (1 mg/mL))	Duloxetine	2680527-91-5
1A04240	N-Nitroso Labetalol Solution (1 mL (1 mg/mL))	Labetalol	2820170-74-7
1A04200	N-Nitroso Metoprolol Solution (1 mL (1 mg/mL))	Metoprolol	138768-62-4
1A03980	N-Nitroso Nortriptyline Solution (1 mL (1 mg/mL))	Nortriptyline/ Amitriptyline	55855-42-0
1A04230	N-Nitroso Propranolol Solution (1 mL (1 mg/mL))	Propranolol	84418-35-9
1A04030	N-Nitroso Rasagiline Solution (1 mL (1 mg/mL))	Rasagline	2470278-90-9
1A03950	N-Nitrosopiperidine Solution (1 mL (1 mg/mL))	NA/88 APIs	100-75-4

Nitrosamine Exchange Community

Nitrosamine Exchange Knowledge Community

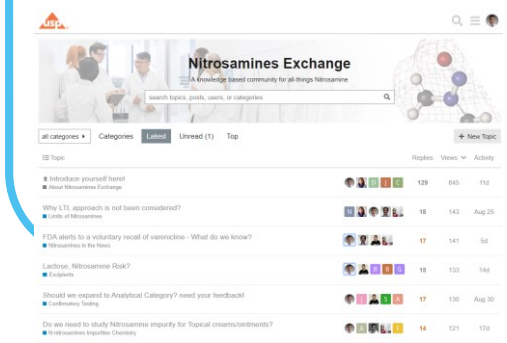


Join http://nitrosamines.usp.org

Learnings Nitrosamines Exchange – Can we do it?

Apr'21

Launch

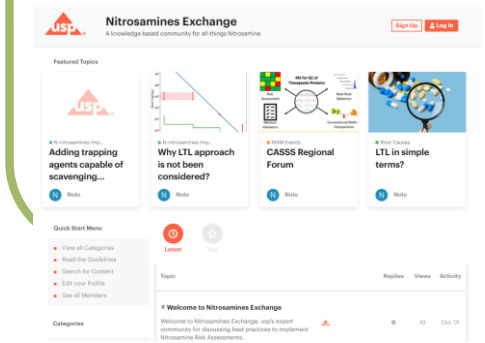


Oct'21

Analytical expansion

Feb'22

Redesign & Multi-language



Today

Collaboration Hub

- 2800+ members, 90 countries
- 70% new to USP; 86% outside U.S.
  - *new in 2022: ability to translate text between 22 languages*
- ~500K page views
- 60% give 4 or 5 on usefulness (scale 1-5)

Non-compendial solutions: USP Analytical Hub

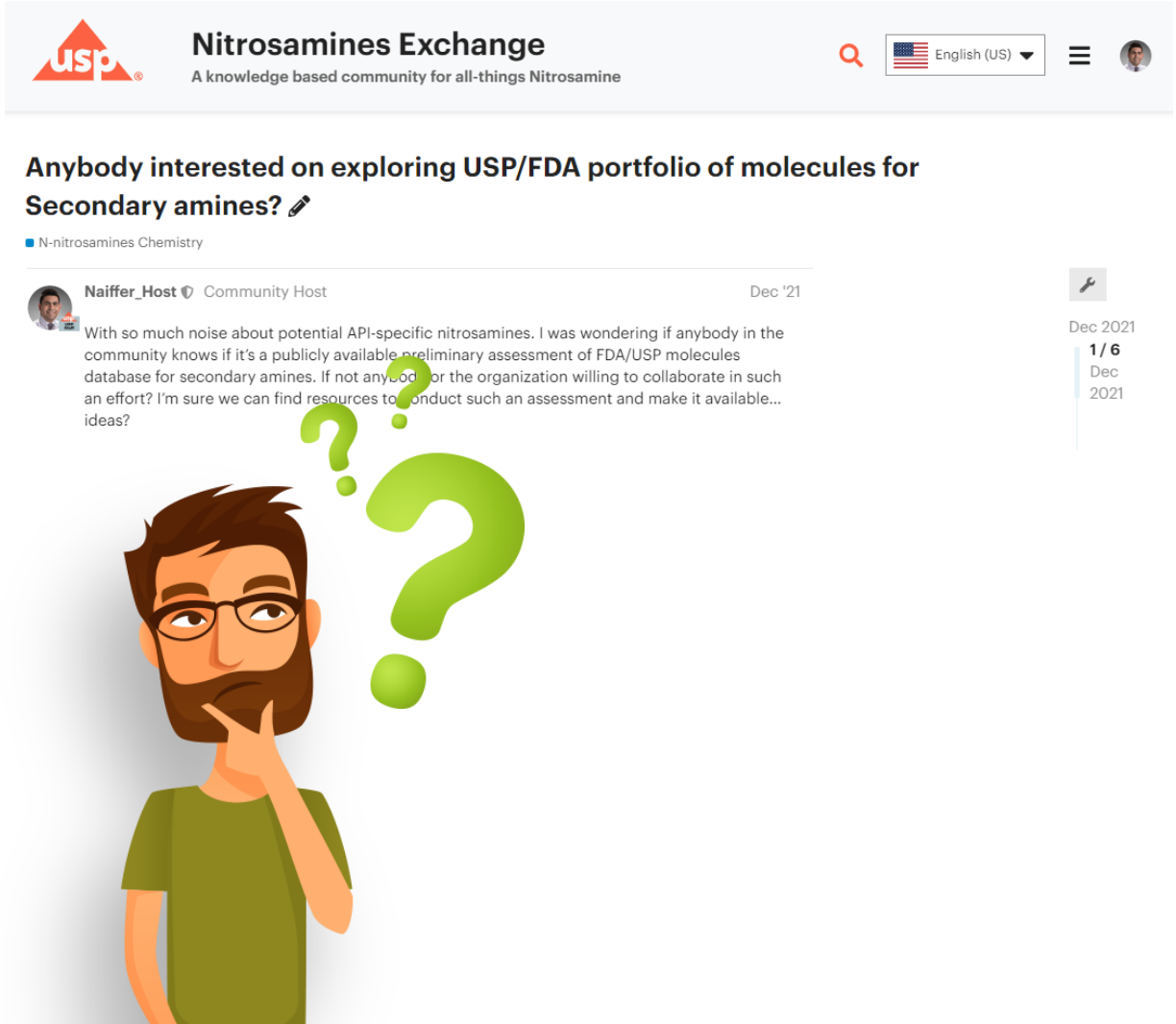
- **Launched in December 2022**

[USP App Note - Nitrosamines analysis in Solvents by GC-MS-MS V2.pdf]

- Public **online repository** containing **non-compendial** analytical procedures (analytical notes) for the testing of nitrosamine impurities and related substances.
- USP's scientists curate these analytical procedures through **internal development/validation** or through scientific review of non-compendial donations. They are **NOT** compendial standards.
- The **procedures** contained in the analytical notes should be **validated** by the user. USP is **not** and will not be responsible for the use or implementation of the procedures.
- Hosted in **The Nitrosamine Exchange**. The Analytical Hub allows keyword searches and the view of key analytical procedure parameters and chromatograms.

Publications

Scale of the problem?



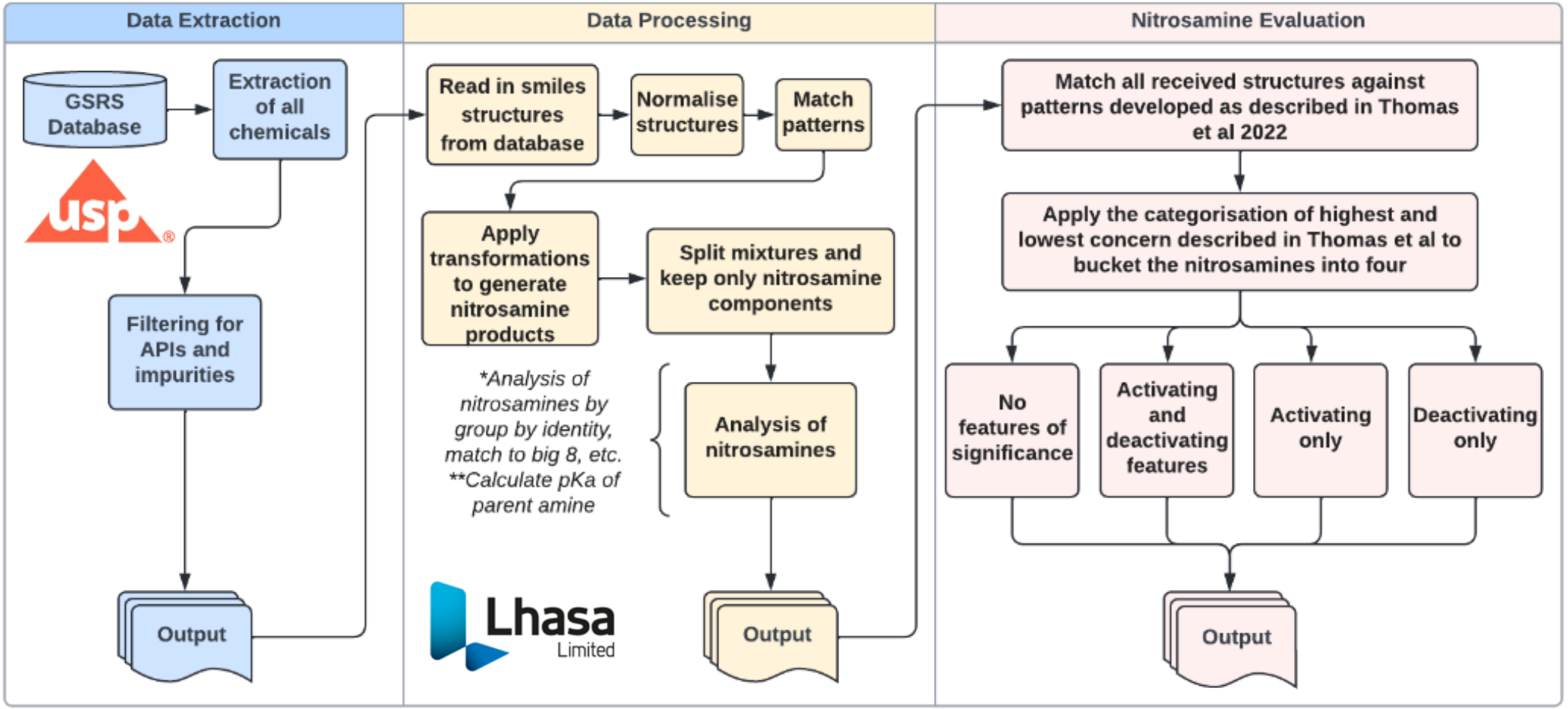
Data Set available:

- United States Pharmacopeia – Global Substance Registration System APIs and registered impurities
- FDA Orange book
- Top200 small molecule drugs by sales
- WHO Essential Medicines list

Plan:

- Analyze the databases and identify amines that could present a nitrosamine risk
- Identify trends in structural properties of the nitrosamines
- Assess likely toxicology trends based on current understanding

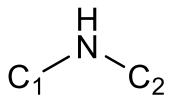
How to approach the data?



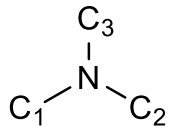
Theoretically generate Nitrosamines

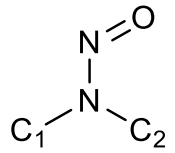
- Identify all amines at risk of forming nitrosamines
  - The focus is on chemical potential, not the toxicity
- Generate all the unique nitrosamines which may theoretically form
  - Identify all at-risk amines
  - Transform to the corresponding nitrosamines

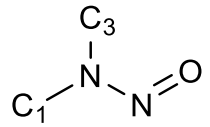
Secondary Amine

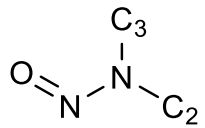


Tertiary Amine

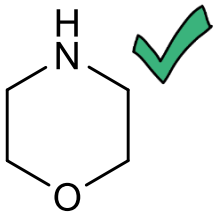


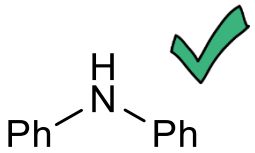


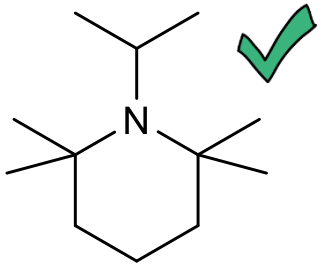


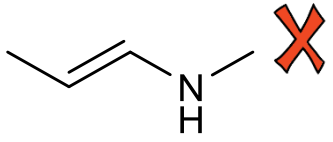


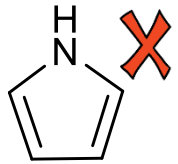
C_1/C_2 = aromatic or single bonds only
C_3 = single bonds only, hydrogen count > 0
N = single bonds only (excludes aromatic)

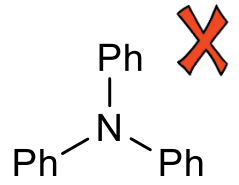




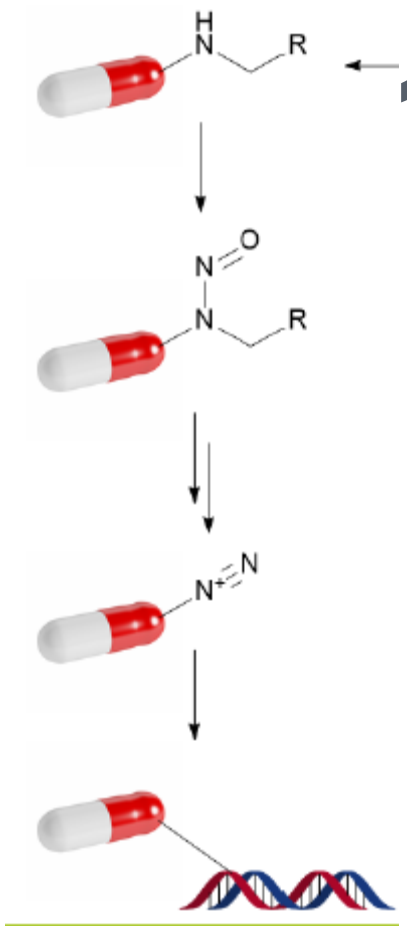








Outcome…

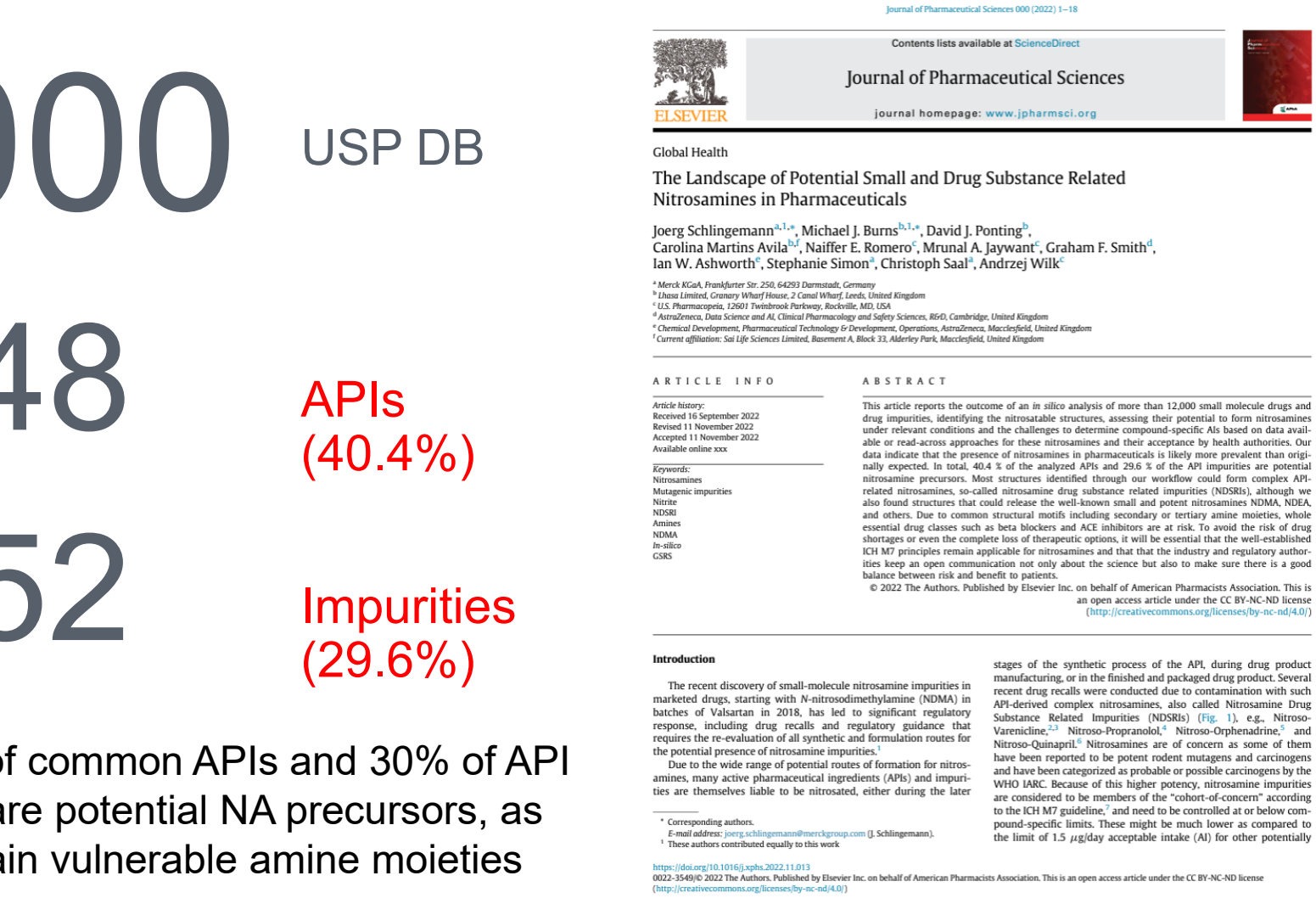


12,000 USP DB

4,848 APIs (40.4%)

3,552 Impurities (29.6%)

About 40% of common APIs and 30% of API impurities are potential NA precursors, as they contain vulnerable amine moieties



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Global Health

The Landscape of Potential Small and Drug Substance Related Nitrosamines in Pharmaceuticals

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NDSRI
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NDMA
In-silico
GSRS

ABSTRACT

This article reports the outcome of an *in silico* analysis of more than 12,000 small molecule drugs and drug impurities, identifying the nitrosatable structures, assessing their potential to form nitrosamines under relevant conditions and the challenges to determine compound-specific AIs based on data available or read-across approaches for these nitrosamines and their acceptance by health authorities. Our data indicate that the presence of nitrosamines in pharmaceuticals is likely more prevalent than originally expected. In total, 40.4 % of the analyzed APIs and 29.6 % of the API impurities are potential nitrosamine precursors. Most structures identified through our workflow could form complex API-related nitrosamines, so-called nitrosamine drug substance related impurities (NDSRIs), although we also found structures that could release the well-known small and potent nitrosamines NDMA, NDEA, and others. Due to common structural motifs including secondary or tertiary amine moieties, whole essential drug classes such as beta blockers and ACE inhibitors are at risk. To avoid the risk of drug shortages or even the complete loss of therapeutic options, it will be essential that the well-established ICH M7 principles remain applicable for nitrosamines and that that the industry and regulatory authorities keep an open communication not only about the science but also to make sure there is a good balance between risk and benefit to patients.

Introduction

The recent discovery of small-molecule nitrosamine impurities in marketed drugs, starting with *N*-nitrosodimethylamine (NDMA) in batches of Valsartan in 2018, has led to significant regulatory response, including drug recalls and regulatory guidance that requires the re-evaluation of all synthetic and formulation routes for the potential presence of nitrosamine impurities.[1]

Due to the wide range of potential routes of formation for nitrosamines, many active pharmaceutical ingredients (APIs) and impurities are themselves liable to be nitrosated, either during the later stages of the synthetic process of the API, during drug product manufacturing, or in the finished and packaged drug product. Several recent drug recalls were conducted due to contamination with such API-derived complex nitrosamines, also called Nitrosamine Drug Substance Related Impurities (NDSRIs) (Fig. 1), e.g., Nitroso-Varenicline,[2,3] Nitroso-Propranolol,[4] Nitroso-Orphenadrine,[5] and Nitroso-Quinapril.[6] Nitrosamines are of concern as some of them have been reported to be potent rodent mutagens and carcinogens and have been categorized as probable or possible carcinogens by the WHO IARC. Because of this higher potency, nitrosamine impurities are considered to be members of the "cohort-of-concern" according to the ICH M7 guideline,[7] and need to be controlled at or below compound-specific limits. These might be much lower as compared to the limit of 1.5 μg/day acceptable intake (AI) for other potentially

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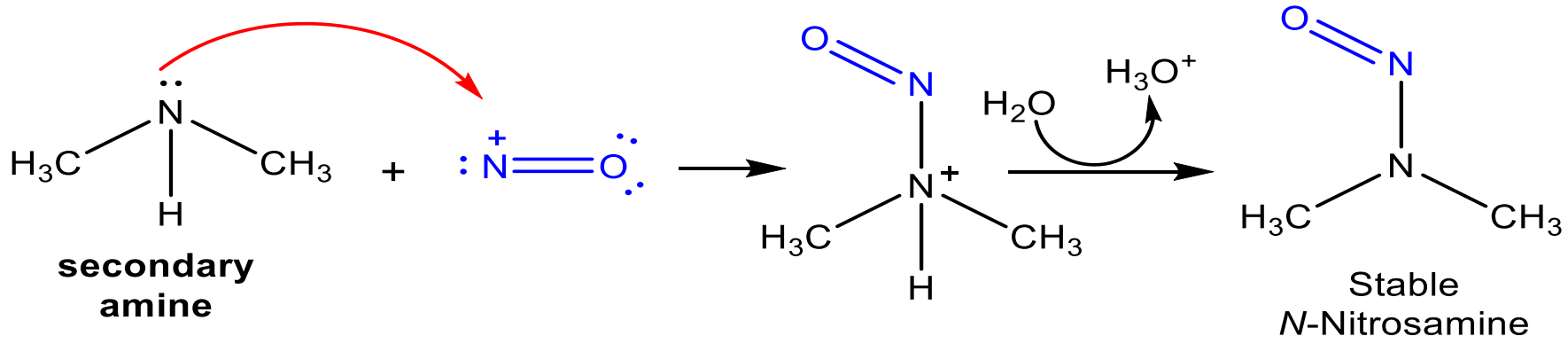
[1] These authors contributed equally to this work

https://doi.org/10.1016/j.xphs.2022.11.013
0022-3549/

The Landscape of Potential Small and Drug Substance Related Nitrosamines in Pharmaceutical. Journal of Pharmaceutical Science Nov'23 - https://doi.org/10.1016/j.xphs.2022.11.013

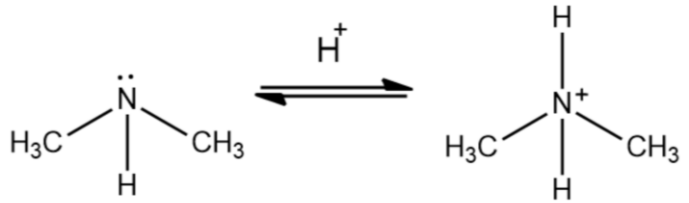
Susceptibility of Nitrosation

The susceptibility of amines to nitrosation relates to the basicity of the amine moiety.



For simple aliphatic amines, nitrosation involves nucleophilic attack of the amine lone pair to the electrophilic nitrosating agent

The free-base form of an amine enables nitrosation to occur.
This reaction cannot occur if the 2ry amine is protonated as in this case the lone pair is not available for reaction with the nitrosating species.



Susceptibility of Nitrosation

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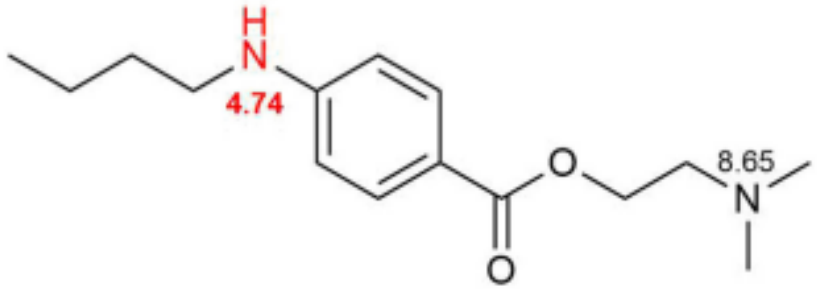
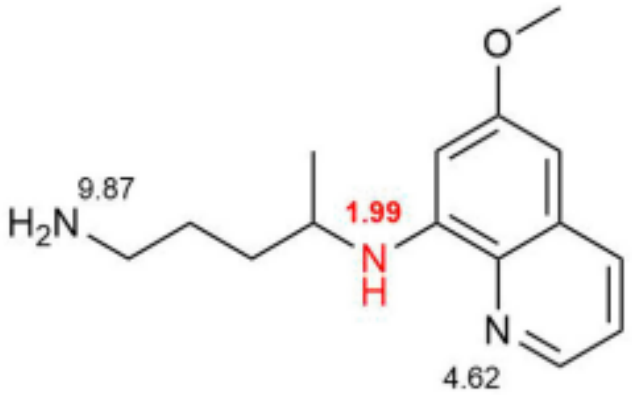


Tetracaine

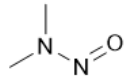
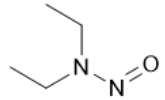
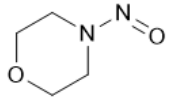
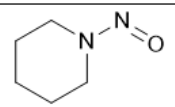
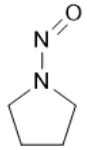
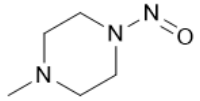
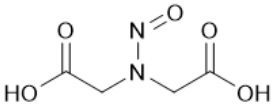


Primaquine

Considering the most reactive secondary amines: 13−15% of APIs/Imp are potentially at risk.

- Given the acidic nature of conditions required to generate the active nitrosating species, the protonation state of the amine has an important bearing on the rate of nitrosation.
- Less basic amines are **easier to nitrosate** as there will be a lower fraction in the protonated state at an acidic / neutral pH compared to more basic amines.
- In the case of secondary aromatic amines, the mechanism becomes more complicated, with p-orbital interactions suggested as alternative initiating event. Therefore, changes in steric and electronic properties of the aromatic ring may impact susceptibility to nitrosation

Recurring Nitrosamines in APIs

Rank	APIs	IUPAC name or common name	Synonym	Structure
1	373	*N*-Nitrosodimethylamine	NDMA	
2	175	*N*-Nitrosodiethylamine	NDEA	
3	89	*N*-Nitrosomorpholine	NMOR	
4	88	*N*-Nitrosopiperidine	NPIP	
5	54	*N*-Nitrosopyrrolidine	NPYR	
6	36	1-Methyl-4-Nitrosopiperazine	MeNP	
7	26	*N*-Nitrosoiminodiacetic acid	NIDA	

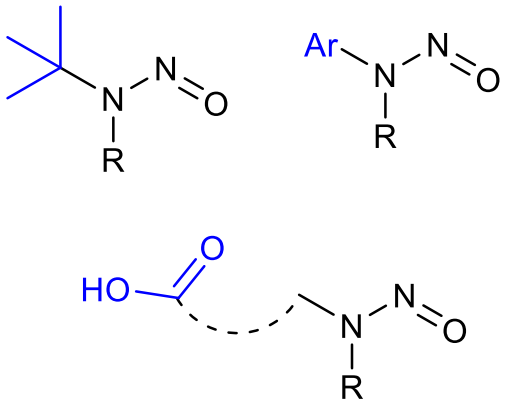
- Some common Nitrosamines can form across multiple drugs
  R-NMe_2, R-NEt_2 etc are common functional groups
- Incident rate drops off rapidly
- >6100 unique nitrosamines
- In comparison with FDA/EMA guidances, the absence of any potential formation of NMPA, NEIPA, NMBA ∴ Likely form within API synthesis itself rather than in the Drug Product

Assessing Toxicological Potential

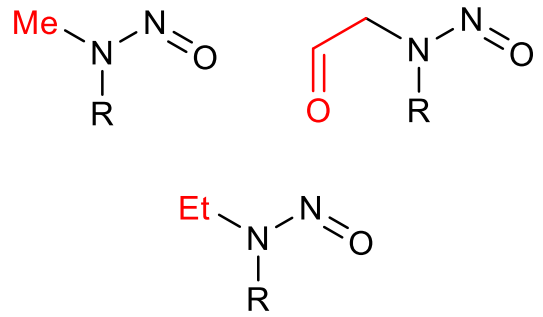
Nitrosamines were binned according to structural features associated with their impact on potency

Rules derived from existing publications*

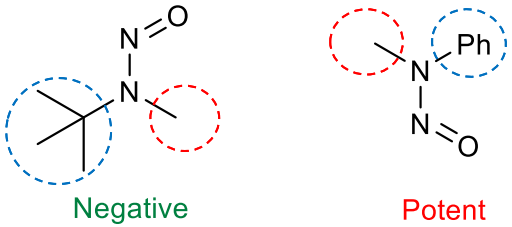
Potency reducing features



Potency increasing features

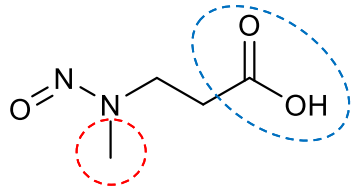


Contains both features



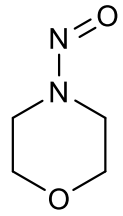
Negative

Potent

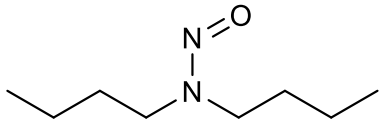


Medium

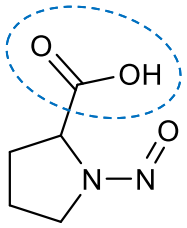
Contains neither



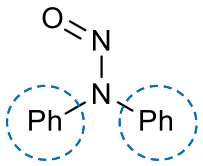
Potent



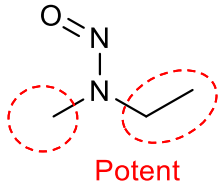
Weak



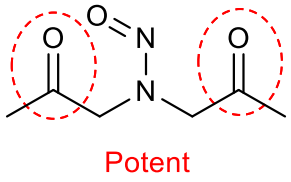
Negative



Weak



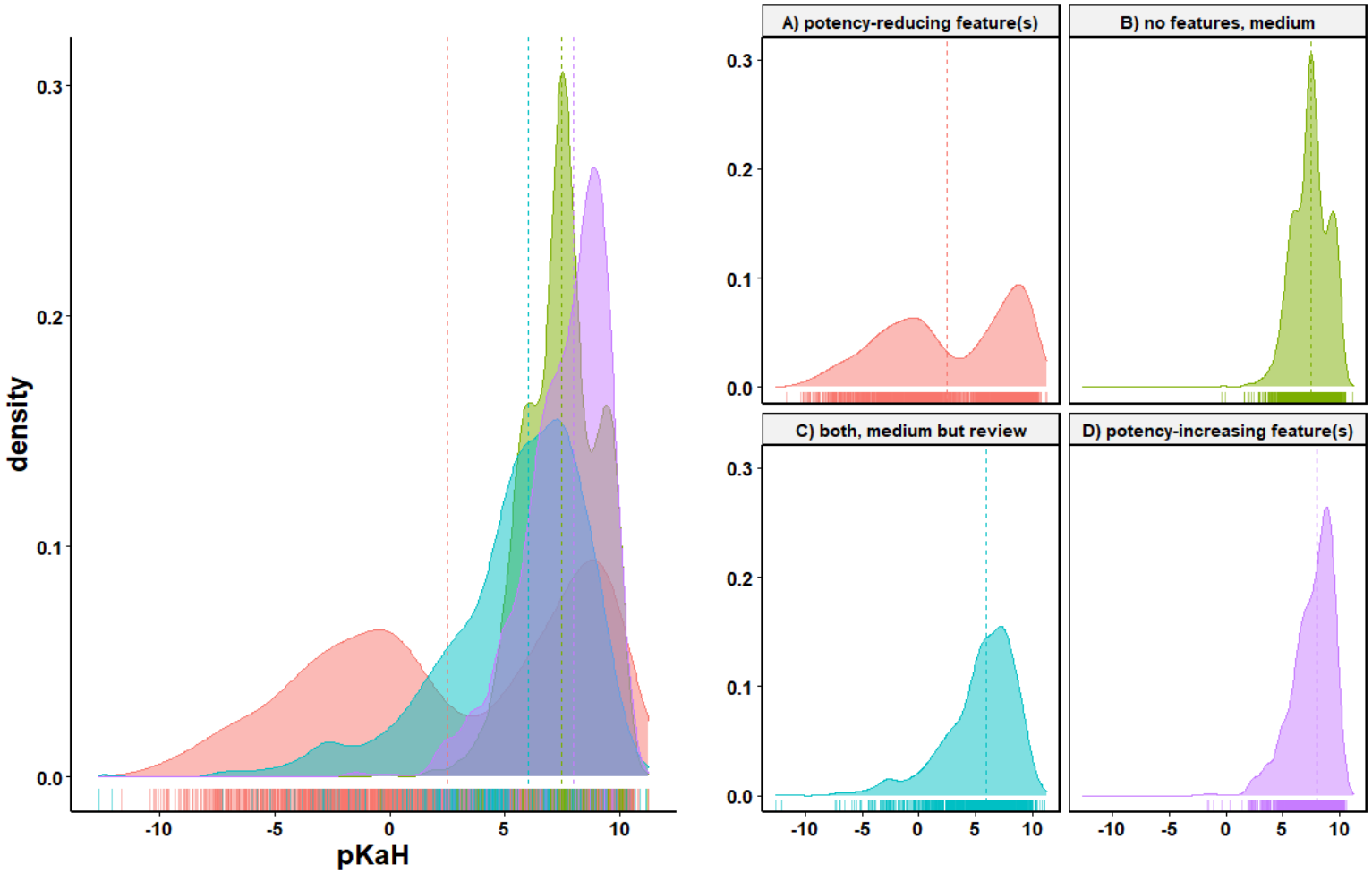
Potent



Potent

*Cross and Ponting, Comput. Toxicol. 2021, 20, 100186; Thomas et al., Chem. Res. Toxicol., 2022, *ASAP;* Ponting et al., J. Med. Chem.

Likelihood of Formation VS Estimated Potency



Considering that the rate of nitrosation is higher for amines with lower pKaH, this means that it's more likely to find a low-potency nitrosamine at high concentration than a high-potency nitrosamine

Flagged Challenges

Synthesis

- Many risks are only theoretical
- Synthesis is necessary for analytical and toxicity testing
- Tertiary amine likelihood is generally lower, yet these account for most significant proportion of risk
- Most Nitrosamines highlighted are unique

Toxicity

- Acceptance of Ames
- Long term study capability
- Read across and *in silico* for limit setting is not clear cut
- Regulators want a lot more toxicity data

Analytical

- Each NA needs a bespoke analytical test developing and certifying at ppb level
- Reliable Material to perform analytical testing

USP Nitrosamines Roadmap

- Method for NDSRIs
- Stakeholders Collaboration
- Risk Assessment Tool
- Additional Nitrosamine RS (PAI)
- Hands-on Training: Lab Demonstration
- Strengthening collaboration with Regulatory Agencies
- Launch of Analytical Hub part of Nitrosamine Exchange

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