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Distilling a Complex Problem into Quantitative Tools and Approaches to Address *N*-nitrosamine Formation Risk in Drug Products

CRCG-FDA workshop on NDSRIs

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An Approach to Assessing Risk of *N*-nitrosamine Presence in Drug Products?

Data to Gather and Factors to Consider for Risk Assessment

Potential reactants in NNO formation

Source and [conc.] of vulnerable amine
- In API structure
- Impurity / residual solvent in API
- Degradate
- In excipients

Properties of vulnerable amine
- Solubility
- pKa
- Physical form (e.g crystalline v amorphous)
- Particle size / surface area

Source and [conc.] of nitrosating agent
- Nitrite impurities in excipients
- Water purity
- Reagents in API synthesis
- Amide impurities in API / excipients

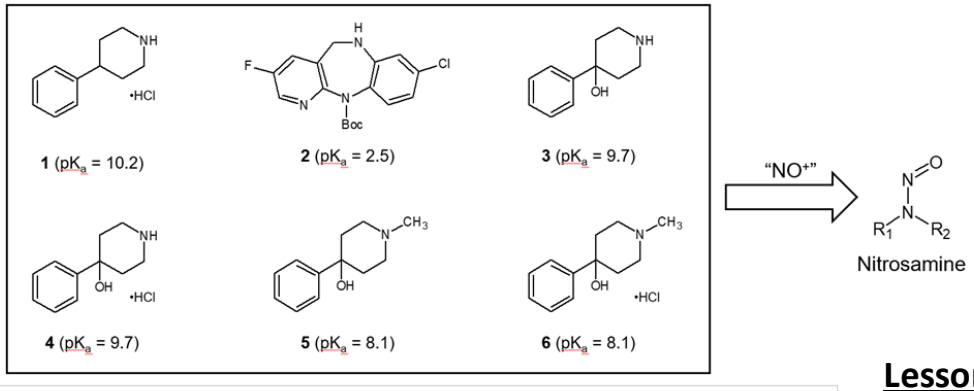
Drug Substance / Product Process

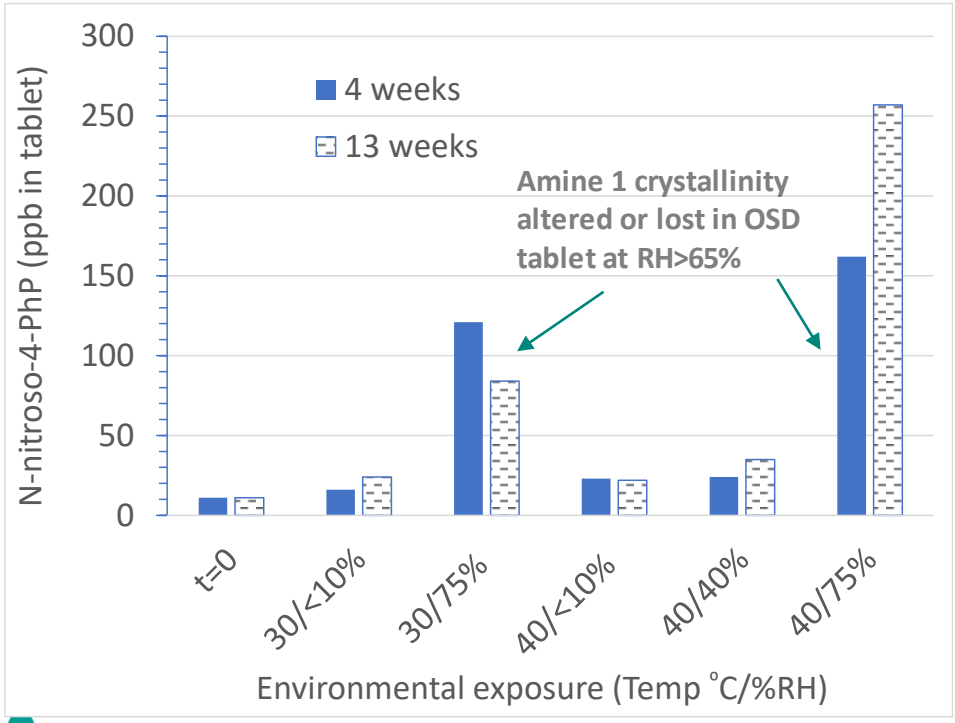
Drug substance process
- Proximity of vulnerable amine to nitrosating agent in process (purge factor)
- Temp. / pH / time

Drug product formulation
- Dosage form (solid / liquid)
- Shelf life
- Packaging type / storage conditions
- Protection from packaging (mainly for external humidity)
- Physical form of reactants (mfr. and shelf life)
- Degradation and form of degradates

Drug product process
- Addition of water / potential for reactants to dissolve
- Types of unit operations
- Temp. / pH / time
- Scale of mixing uniformity

Use of Model Amine Systems to Study Potential for NNO Formation in Oral Solid Dosage (OSD) type products





Model amine OSD studies

- Secondary and tertiary amines, salts v freebases, and varying pKa (calculated)
- Formulated using common OSD excipients using dry and wet processes for mixing. Final dosage either blend or compressed into tablets
- Formation of NNO examined after manufacture and on stressed stability

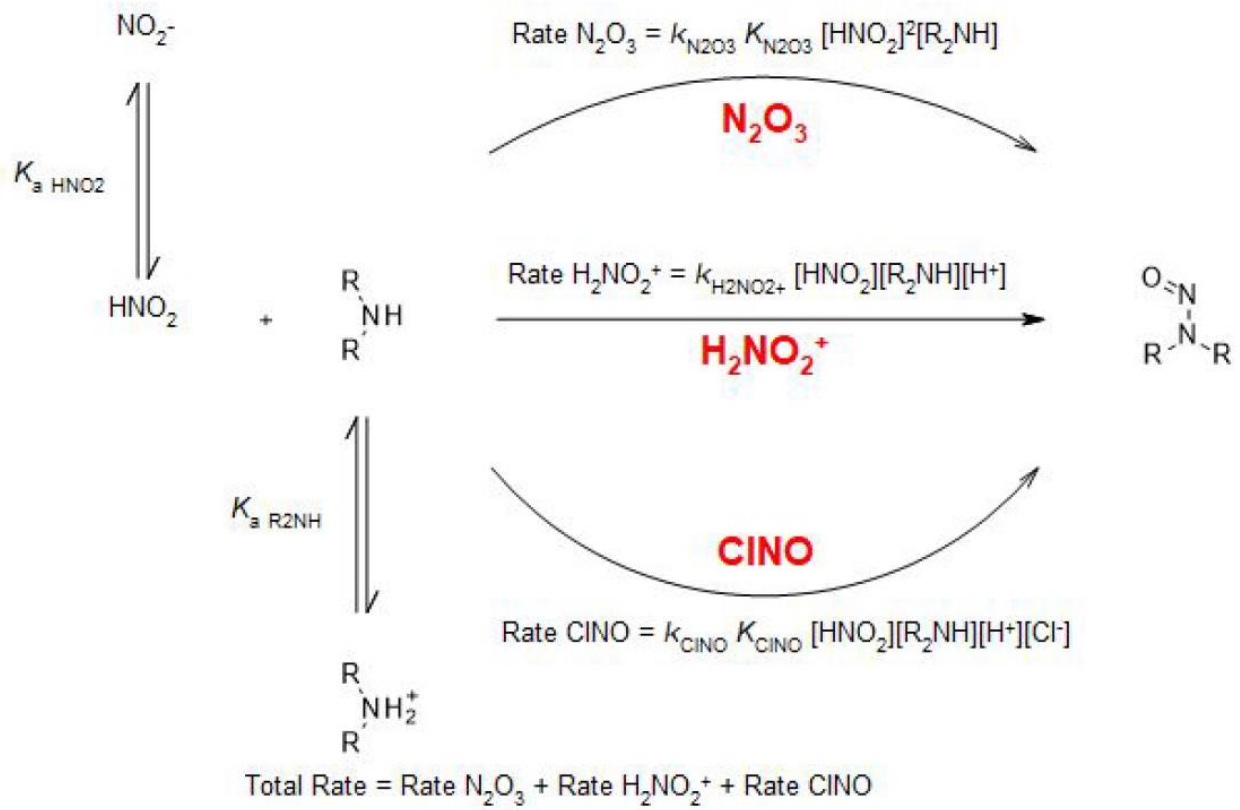
Lessons learned

- Model amine studies can be a valuable tool to inform on key factors for consideration in risk assessing products
- Model amine OSD work helped to inform the importance of the following risk factors:
  - Vulnerable amine pKa
  - Addition of water during processing (e.g wet granulation)
  - Amine form (salt, freebase)
  - Physical structure stability of vulnerable amine (e.g crystalline vs amorphous)
  - Solubility of vulnerable amine
  - Exposure T / RH% of product during storge / shelf life

Moser, J. et al. ***N*-Nitrosamine Formation in Pharmaceutical Solid Drug Products: Experimental Observations.** In print, May 2023. https://doi.org/10.1016/j.xphs.2023.01.027

Construction of a Conservative Quantification Model in Aqueous Solution

- Models have been developed which offer an effective means to quantify potential extent of NNO formation in a drug product
- Kinetics of nitrosation of dialkyl amines by N_2O_3 has been extensively studied and kinetics shown to be invariant to a wide array of amines with a wide range of pKa's[1,2]
- Authors representing contributing companies in the IQ Nitrosamine working group constructed a Berkeley Madonna model to conservatively estimate NNO formation in aqueous solutions based on equilibrium and rate equations for dimethyl amine (DMA) nitrosating to form *N*-nitrosodimethylamine (NDMA)[3]
  - This model was designed to over-predict NNO formation
    - ✓ Designed using highest reported rate constants, ignoring HNO_2 degradation
- Model can be used as a means to estimate formation of other types of *N*-nitrosamines that may form via dialkyl amines



1: Mirvish, S. *Toxicol. Appl. Pharmacology*, **1975**, *31*, 325-351
2: Williams, D. L. H. Nitrosation, Cambridge University Press, Cambridge, **1988**, Ch. 1 & 4.
3: Ashworth, I.; Dirat, O.; Teasdale, A.; Whiting, M. Potential for the Formation of *N*Nitrosamines During the Manufacture of Active Pharmaceutical Ingredients: An Assessment of the Risk Posed by Trace Nitrite in Water. Org.Process Res.Dev. DOI: 10.1021/acs.oprd.0c00224. Aug 2020

Ashworth, I. et al. ***Formation of Dialkyl-N-nitrosamines in Aqueous Solution: An Experimental Validation of a Conservative Predictive Model and a Comparison of the Rates of Dialkyl and Trialkylamine Nitrosation.*** Org.Process Res.Dev., 08-Feb-2023. https://doi.org/10.1021/acs.oprd.2c00366

Validation of the Aqueous Model Prediction Capability

- Experimental reactivity studies and simulations conducted on solutions of model secondary amine 1 (4-phenyl-piperidine HCl) and dissolved nitrite
- Model able to demonstrate expected trend of formation with pH
- As designed, model overpredicts formation of NNO and therefore when combined with appropriate assumptions in the risk assessment, it provides a tool to understand risk from NNO formation

(Not shown) Similar empirical study using trace nitrite (0.1 mM) showed much lower NNO formation (i.e 0.29% at pH=3, 0.04% at pH=4 and ND[1] at higher pH).
ND=non-detected above LOD=10 ppb (or 2 x 10^{-5} % conversion)

- This level of nitrite is closer to what may be expected in a solution drug product
- Model also overpredicted NNO formation in this case

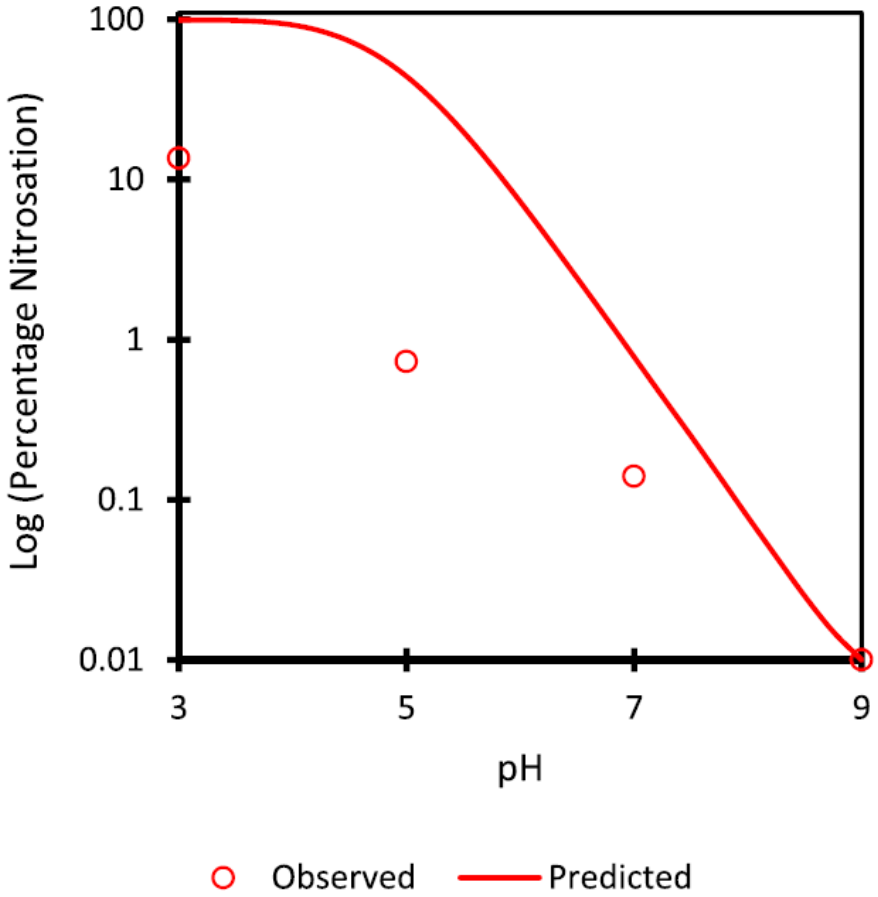


Figure 1. pH dependence of the predicted and observed extents of NPP formation from PP·HCl (0.1 M) and aqueous $NaNO_2$ (0.2 M) over 24 h at 25 °C.

Ashworth, I. et al. ***Formation of Dialkyl-N-nitrosamines in Aqueous Solution: An Experimental Validation of a Conservative Predictive Model and a Comparison of the Rates of Dialkyl and Trialkylamine Nitrosation.*** Org.Process Res.Dev., 08-Feb-2023. https://doi.org/10.1021/acs.oprd.2c00366

Example Application of Aqueous Model for Quantification
(hypothetical product for demonstration)

Model established in Dynochem for solving NNO rate equations / user interface in Excel

Amine Information	
Name	Product A
Amine MW (g/mol)	450
Amine pKa	10.2
Amine Dose (mg/unit dose)	1.000
Doses per Day	1.0

Legend
Required User Input
Optional User Input
Automatic Look-Up Value
Calculation

Simulation Information	
End Time (days)	730

Formulation Information (1 Dose Basis)

Component	Name	Mass (mg)	Nitrite Conc. (ppm)	Nitrite Contribution (mcg)
1	API (amine source)	1.0000		0.000
2				0.000
3	Unstudied excipient	10.0000	5.000	0.050
4	Blank		0.000	0.000
5	Blank		0.000	0.000
6	Blank		0.000	0.000
7	Blank		0.000	0.000
8	Blank		0.000	0.000
Total		11.00	4.55	0.05

pH	5
Total [Cl-] (M)	0.00E+00
Water [Nitrite] Spec. (ppm)	6.00E-03
Solution Volume (L)	0.001000000

Results	
5.47E-01	Max. Nitrosamine Exposure (ng/day for max. dose)
5.13E-05	Conversion (mol % from amine to Nitrosamine)
9.37E-02	Conversion (mol % from nitrite to Nitrosamine)
5.46E+02	Nitrosamine PPB (g/g, Initial amine Mass Basis)
5.46E-01	Nitrosamine PPB (g/g, Solution Mass Basis)

9/30/2021 16:12

➢ **Product information**

- API has a 2' amine functional group in structure. pKa estimated at 10.5+-0.3. *Assume conservative 10.2 pKa*
- Drug product is sterile solution for injection. Max daily dose is 1 mg supplied through 1 mL injection
- Solution contains one excipient which has unknown nitrite content. *Assume conservative 5 ppm nitrite (nitrite estimates have been further refined and >90% excipients show NMT 2 ppm nitrite content)[1].*
- Water for injection (WFI) is known to contain very little nitrite risk[1]. *Assume conservative 6 ppb nitrite.*
- Solution pH controlled to 5.0-7.0 during processing and in final product. *Assume conservative pH=5.0*

➢ **Simulate formation of N-nitroso-API over the 2 year product shelf life**

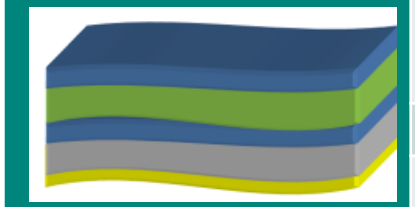
- NDMA model predicts formation of 0.547 ng of NNO over the shelf life
- This value of NNO is very low and << default of 18 ng/day for unstudied N-nitrosamines

➢ **Conclusion**

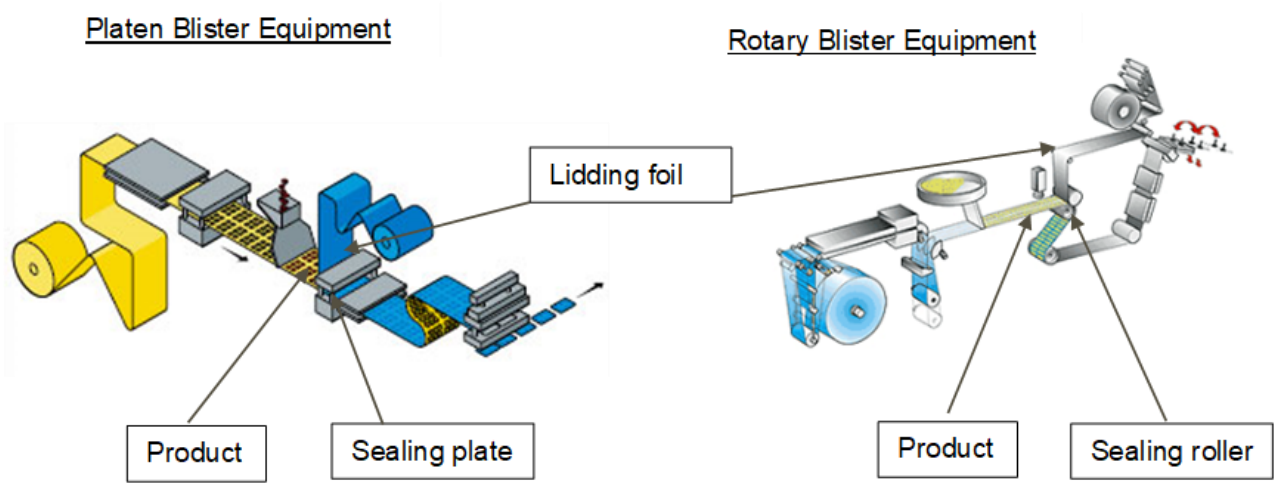
- No risk of NNO formation that would exceed the acceptable daily intake (ADI)

1: https://www.efpia.eu/media/676632/efpia-nitrosamines-quality-risk-management-workflows-sep-2022.pdf

Blister packaging process analysis

Standard lidding foil construction	Individual Components	Risk Material Contents
	Over-lacquer (optional)	Possible source of NC
	Ink/Print	Possible source of amines (NIAS)
	Ink primer	Possible source of NC
	Aluminium foil	Barrier
	Heat seal lacquer	Product contact layer

NC=nitrocellulose



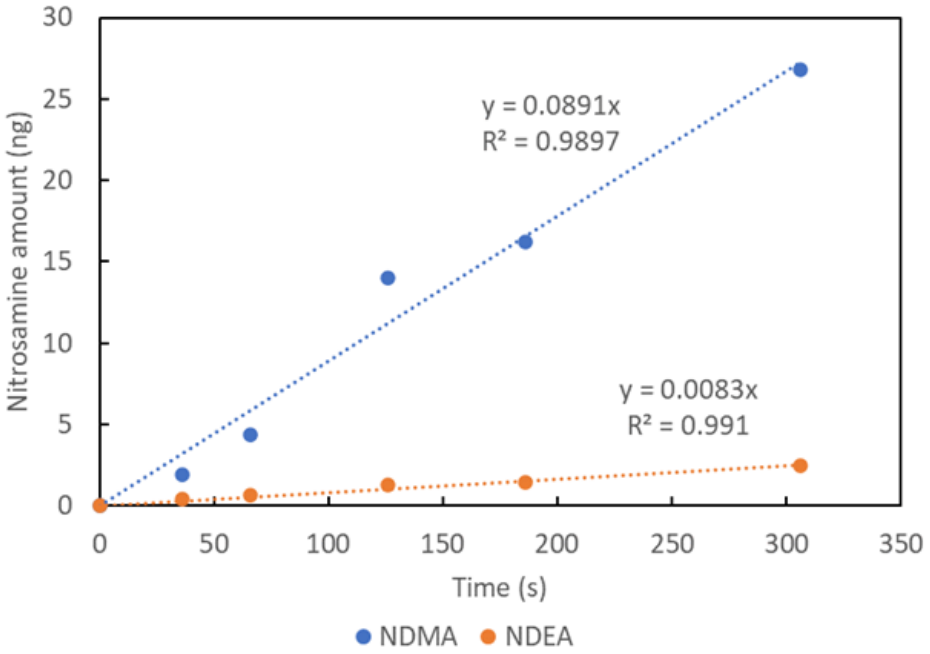
Nitrosation chemistry risk

- Nitrocellulose in print primer/inks/over-lacquer can thermally decompose and react with secondary / tertiary amines present in the ink.
- Additionally, NO-x may be present in nitrocellulose and can directly react with amines in inks to create *N*-nitrosamines prior to heat sealing

Evaluation of process and risk of contamination to product in Al foil blister line

- After sealing, the Al foil provides a barrier to direct NNO contamination of the product inside the cavity
- For contamination to occur directly from blister packaging itself:
  - NO-x from nitrocellulose and reactive amines from the ink would need to react to form *N*-nitrosamines and then volatilize, travel upstream and into open well with product

Quantitation of Risk of NNO Contamination from Al foil Blister Packaging



Zheng, J. et al. **On the Risk of Nitrosamine Contamination During Drug Product Blister Packaging.** In draft and under review for publication in J. Pharm Sci.

_N_-nitrosamine formation and volatilization experiment

- Commercial aluminum blister foil material equivalent in size for one ~400 mg tablet selected for study. Foil characterized and shown to have:
  - High amounts of nitrocellulose in over-lacquer, ink and primer
  - DMA and DEA at ppm levels. NDMA and NDEA at 349 and 3 ng, respectively in 10.3 cm^2 coupon.
- Foil sealed in vapor-impervious vial and heated at controlled temperature. NDMA and NDEA measured in headspace via nitrogen-phosphorus detection (data at left for 200°C)
- Typical blister sealing application time is ≤1 sec which would translate to <0.1 ng of either NNO released

Assessment of risk on blister packaging line

- With knowledge around conservative vaporization rates of NNO and operational elements of blister line, one can build a process model to estimate potential contamination risk.
- Assumptions for this process model example:
  - NNO vol. rate=0.851 ng/s (high T=260°C study, NDMA rate observed)
  - 1133 m^3/hr air change-over in process room
  - 360 blisters sealed per second
  - Blister volume of 0.5-cc
- Air exchange will remove NNO leading to a maximum [NNO] since linear volatilization rates observed (IN=OUT → plateau'd [NNO])
  - Using model and above assumptions along with calculated [NNO]max in surrounding room air, ~0.5 pg of NDMA contamination per blister is calculated leading to NO RISK of hitting acceptable daily limit (ADI)
  - Air exchange around sealing zone a factor in risk assessments

Conclusions

- ✓ Industry and health authority collaboration efforts have led to a process and risk assessment architecture to conduct end -to-end *N*-nitrosamine risk assessments on drug products (e.g EFPIA N-nitrosamine risk assessment workflow)
- ✓ 2' amines and other concerning nitrosable substances can be present in APIs. Nitrite impurities are common in excipients. Therefore, in some drug products, a theoretical risk of NNO formation exists
- ✓ Models, designed to over predict NNO formation, are a powerful tool to conservatively estimate risk in drug product especially when coupled with conservative constraints based on elements of the product and process control strategy
- ✓ *N*-nitrosamine formation in solid drug products can occur. Model amine studies are useful tools to help identify risk factors to consider in risk assessing drug products.
- ✓ Negligible risk of *N*-nitrosamine contamination from Al foil blister packaging in well ventilated clean rooms
- ✓ The work done to date has provided a foundation of understanding of risk in drug products. However, work remains to establish mechanistic understanding and accurate predictions particularly in solid products. Conservative estimations of risk is appropriate at this stage until the science evolves

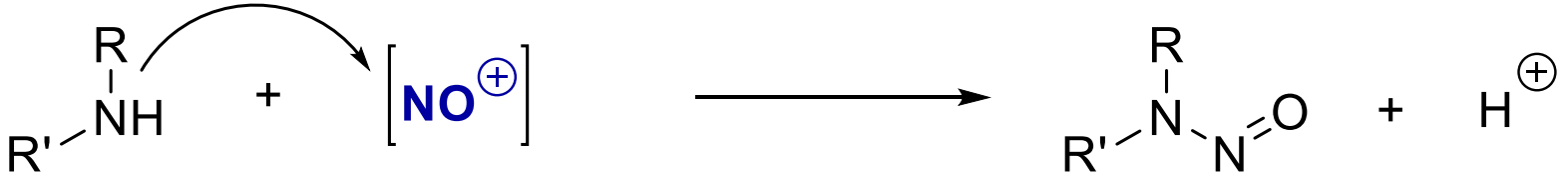
Acknowledgments

- ✓ Members of the Merck & Co., Inc. *N*-nitrosamine risk assessment team
- ✓ Numerous discussions and debates with members of the IQ *N*-nitrosamine team
- ✓ Authors of the various cited publications for their hard work and dedication to furthering the science in this important research topic
- ✓ CRCG-FDA workshop organizing committee for the invitation to speak and for putting together the workshop

Back-up

What are N-nitrosamines (NNO) and problem statement for the pharmaceutical industry?

A N-nitrosamine is formed from reaction of a secondary amine and NO+ from a nitrosating agent



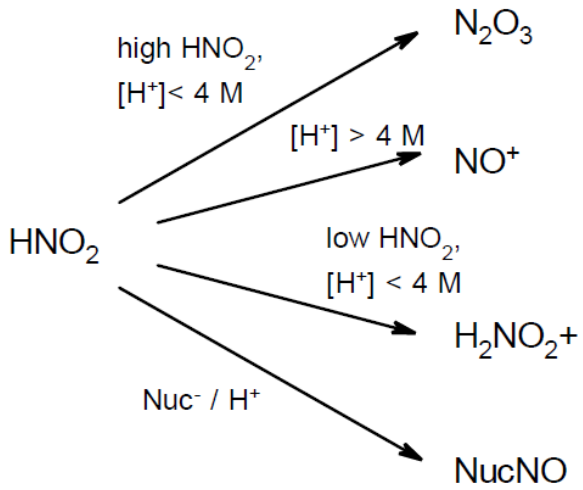
N-nitrosamine

- Literature shows various types of amines/precursors can lead to N-nitrosamines in the right conditions. Secondary (2') amines pose the greatest concern.
- Literature also shows various types of nitrosating agents (see image) that can react with said amines (ref IPEC)
  - Common excipients contain ppm levels of nitrite

- **Problem statement.** Both required reactants can be present in drug product formulations. Therefore, theoretical risk of NNO formation exists. Additionally:

  - There is a paucity of scientific literature on NNO risk in drug products
  - Low acceptable levels drives analytical challenge for detection
  - Diversity in chemical nature, morphology and physical state of drug products bring challenges in how to generalize and quantitatively predict NNO risk
    - However, approaches have been developed to calculate worst case

Active nitrosating species that may form from aqueous solutions of nitrous acid[1]



1: Ashworth, I.; Dirat, O.; Teasdale, A.; Whiting, M. Potential for the Formation of *N*Nitrosamines During the Manufacture of Active Pharmaceutical Ingredients: An Assessment of the Risk Posed by Trace Nitrite in Water. Org.Process Res.Dev. DOI: 10.1021/acs.oprd.0c00224. Aug 2020

Assessment of Excipient Risk in Drug Products

Excipient	Average nitrite (ppm)[a]	Range of values (ppm)[b]	# data points	# data points > LOQ	Min / max LOQ of methods (ppm)
Microcrystalline cellulose	0.69	0.1 – 2.38	54	29	0.1 / 1
Lactose monohydrate	0.55	0.1 – 1.7	32	12	0.07 / 1
Silicon dioxide	0.87	1.8 – 2.1	9	3	0.03 / 1
Sodium starch glycolate	0.21	0.23	3	1	0.03 / 0.2
Magnesium stearate	2.20	0.28 – 5.4	37	30	0.1 / 2
Hypromellose	0.82	0.06 – 1.4	46	9	0.01 / 1
Mannitol	0.38	No values	4	0	0.03 / 0.5
Copovidone	0.26	0.2 – 0.3	7	4	0.055 / 0.5

[a] Average of all data points listed. In the case where value < LOQ, the LOQ was assumed as a conservative estimate. Only values from methods with LOQ≤2.0 ppm were used in the average.
[b] Range of data includes points that fell above method LOQ.

- Large industry collaboration to generate nitrite impurity data on common excipients
  - Lhasa led creation of the database referenced below. Over 15 contributing companies
  - Method validation criteria enforced for included data
  - This database now contains 497 data points on 71 different excipients
  - Many data entries < method LOQ

- ***Nearly every excipient carries nitrite impurity risk***
  - Presence of water can lead to formation of nitrous acid and subsequent nitrosating agents

- A thorough review of this work is available[1]

1: Boetzel, R. et al. A Nitrite Excipient Database: A useful Tool to Support *N*-Nitrosamine Risk Assessments for Drug Products. J. Pharm Sci Apr 2022. https://doi.org/10.1016/j.xphs.2022.04.016

Data pulled from: Moser, J. et al. ***N*-Nitrosamine Formation in Pharmaceutical Solid Drug Products: Experimental Observations.** In print, May 2023. https://doi.org/10.1016/j.xphs.2023.01.027