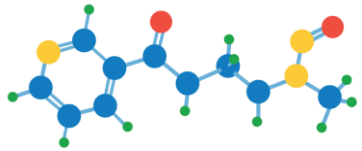
Public Comments for Session 1
Nitrosamine Drug Substance-Related Impurities (NDSRIs)

In Person Comments:

- Connie L. Chen, PhD, MPH, Senior Scientific Program Manager, Health and Environmental Sciences Institute (HESI)
- Pali De Silva Indrasekara, PhD, Technical Director, Advanced Manufacturing Technologies, USP
- Amar G. Chittiboyina, PhD, Assistant Director , National Center for Natural Products Research, School of Pharmacy, The University of Mississippi
- Eric J. Munson, PhD, Professor, Purdue University

Virtual Comments:

- Raphael Nudelman, PhD, ERT, Senior Director Impurity Expert, R&D Operation, Teva
- Marko Trampuž, PhD, Scientist Early Development, SANDOZ DEVELOPMENT CENTER, SLOVENIA



Nitrosamines Research Program

Opportunities to Leverage Public-Private Partnerships to Address Nitrosamine Safety Liabilities

Connie L. Chen, PhD, MPH
Health and Environmental Sciences Institute (HESI)
Senior Scientific Program Manager
www.hesiglobal.org

Health and Environmental Sciences Institute (HESI)

- Non-profit scientific institute based in Washington DC USA
- Operating internationally for 35 years.
- Expert convener of PPPs
- Recognized collaborator with FDA

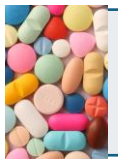
The HESI Model:

Bridging Research to Translation

IMPROVED

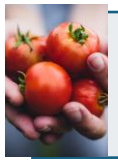
Safety and Innovation for Human Health and Environmental Health

ACCURATE AND EFFICIENT CHEMICAL RISK ASSESSMENT



SAFE AND EFFECTIVE MEDICINES

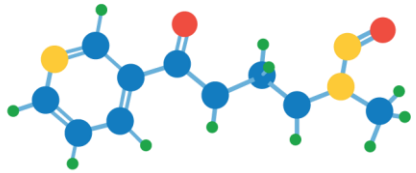
ENVIRONMENTAL QUALITY AND SUSTAINABILITY



FOOD SAFETY

Pooled Expertise and Resources Across 300+ Organizations and 18 scientific committees Committed to Protecting Public Health

TODAY'S FOCUS



Nitrosamines Research Program

Nitrosamine Research Program

Pooled Industry and FDA Grant Funding

FDA grant:
NoA 3U01FD006676-03S2

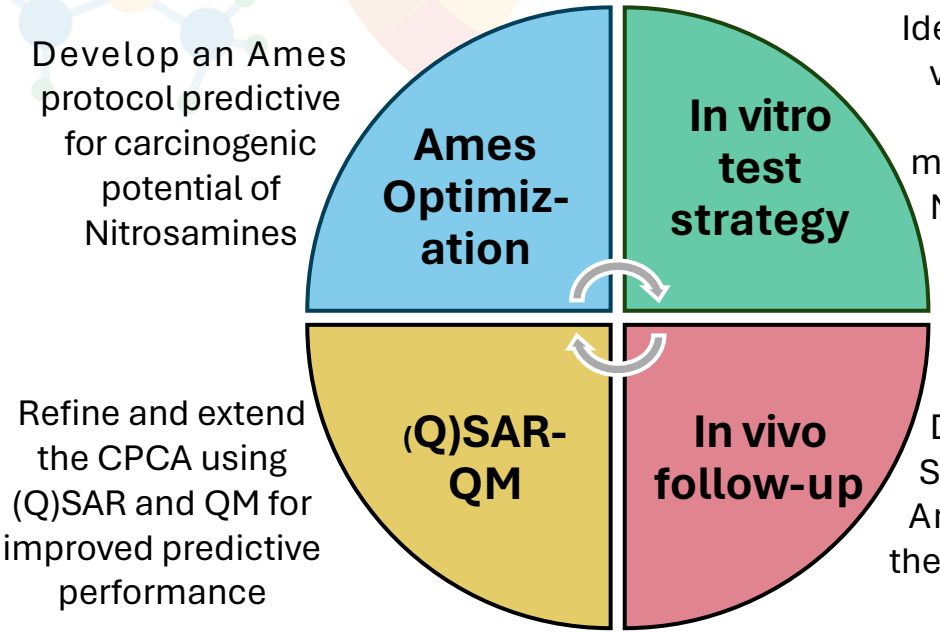
Expertise and Data from Pharma, Generics, CROs, Chemical, Foods, Academe, Govt

Global Participation from

- 10 regulatory/government (including FDA CDER/OND, NCTR)
- 26 private companies
- 11 academic, consulting, NGO/not-for-profit

Shared Data and Methods Development

Novel Experimental Studies to Build Best Practices



WORKSHOP | IN PERSON

FDA/HESI Research Roadmap Planning on Hazard and Risk Assessment of Nitrosamine Impurities in Drugs

MAY 31, 2023 - JUNE 1, 2023

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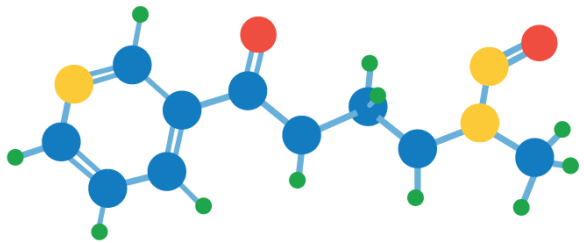
- **Stakeholder exchange** on ongoing research
- **Identify research needs and gaps** for new work



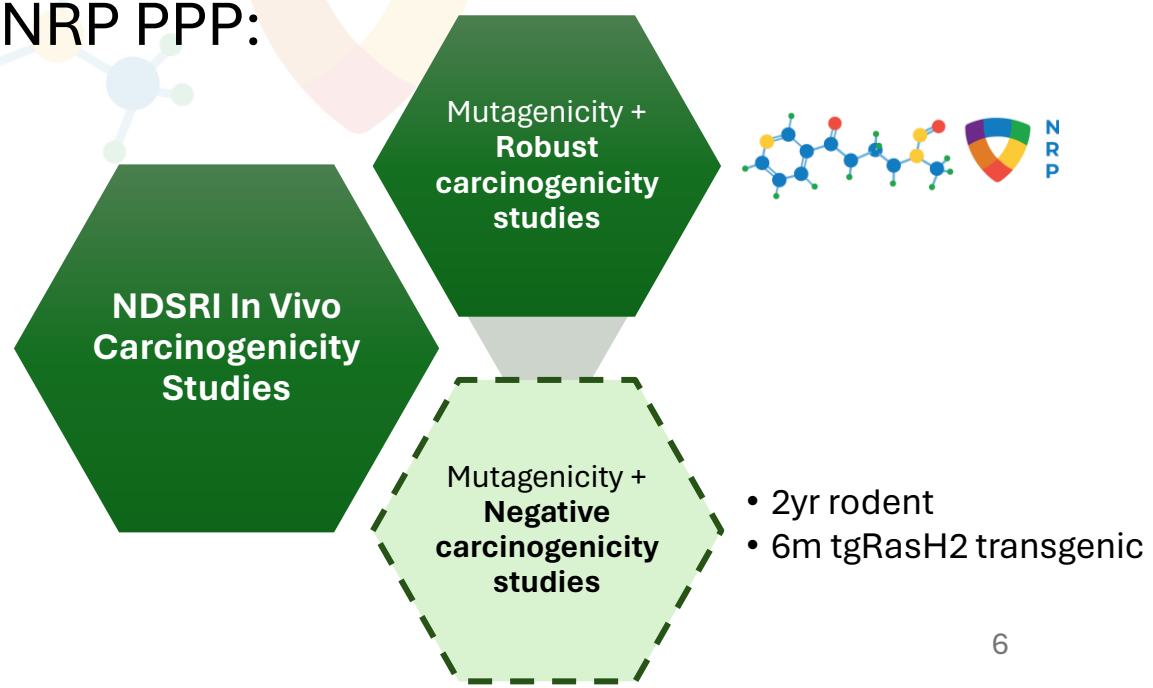
Nitrosamines Research Program

Active and Growing Portfolio Seeking Added Participation and Support

HESI

Opportunities for GDUFA (and OGD)

- Participation of FDA Office of Generic Drugs in ongoing NRP Programs
- GDUFA funding to HESI NRP work streams in progress to advance depth, breadth, and speed
- Financial support from GDUFA for NEW efforts not funded by/planned to be conducted under the auspices of HESI's NRP PPP:
  - Chronic rodent studies
  - Polypharmacy
  - Exogenous exposure assessment



Thank you!

Connie L. Chen, PhD, MPH

cchen@hesiglobal.org

https://hesiglobal.org/gttc-nitrosamines/

Emerging & Advanced Manufacturing Technologies to Support Generics Industry

FY 2024 Generic Drug Science and Research Initiatives Public Workshop
May 20th 2024

Pali De Silva, Ph.D.
Technical Director, Global Health and Manufacturing Services

(1) Priority area: Facilitate adoption of advanced manufacturing technologies (AMT) for generic drug products

Background:

- > 80% drugs currently made using AMT*
- **AMT adoption by generics is lower than branded**
- AMT may reduce per unit production cost

AMT adoption → Generic cost

Input from generic industry stakeholders:

"need platform technologies to manufacture more than 1 drug product"

"lack of use cases and studies to demonstrate benefits vs. return on investment"

" require concerted effort between many industry partners"

Areas to focus on:

- Funding opportunities targeted to generic industry to explore and facilitate adoption of AMT
- Facilitating industry collaboration to develop business cases for successful AMT implementation
- Accelerating development of technical guidelines for validating alternative methods using process analytical techniques to ease adoption by generics

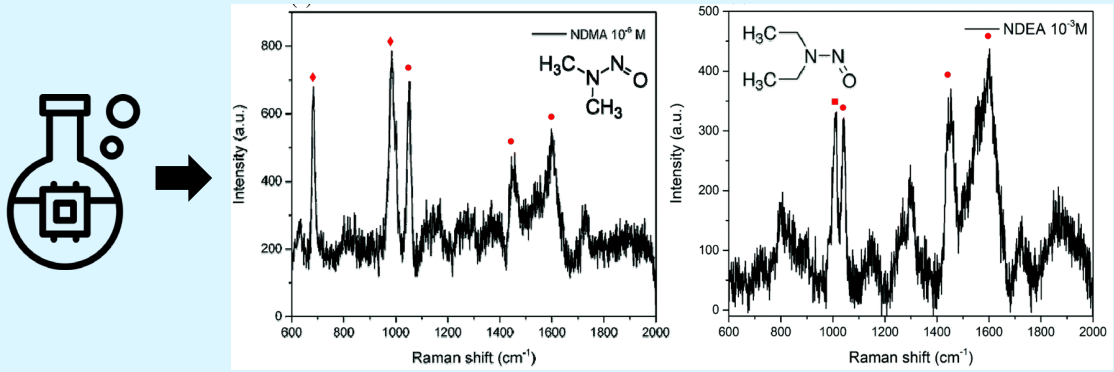
**Remarks by Acting Commissioner Janet Woodcock to the FDA Pharmaceutical Quality Symposium 2021: Innovations in a Changing World - 10/26/2021 | FDA*

(2) Emerging analytical technologies to detect & monitor nitrosamine formation

Limitation of current analytical methods: liquid chromatography-mass spectrometry methods;

- Matrix effect impacting method accuracy
- Indirect detection interfering accurate nitrosamine origin determination
- Total cost and time to perform the analysis

Molecular sensors & imaging technologies from other industries:



Nanoscale, 2020,12, 1075-1082

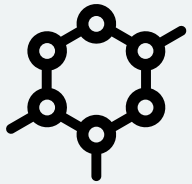
Areas to focus on:

- Exploring & evaluating spectroscopy technologies, optical sensors, and portable process analytical technologies for trace impurity detection
- *In situ* analytical technologies for mechanistic understanding of nitrosamine formation

(3) Holistic mechanistic understanding of impurity formation for risk assessment

Gap in knowledge: Mechanistic knowledge of risks associated during drug product manufacturing

Challenges: Controlling NDSRI in drug products



Molecular structure -focused

+

Manufacturing process-focused

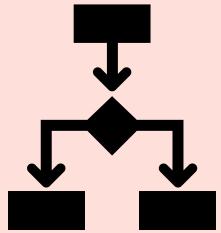
→ Formulation-based

→ Product type-based

→ Batch vs. continuous process–dependent

Areas to focus on:

- Developing risk-based decision trees, guidelines & strategies pertaining to drug product manufacturing
- Developing framework to reduce the burden of specific & extensive testing for nitrosamine

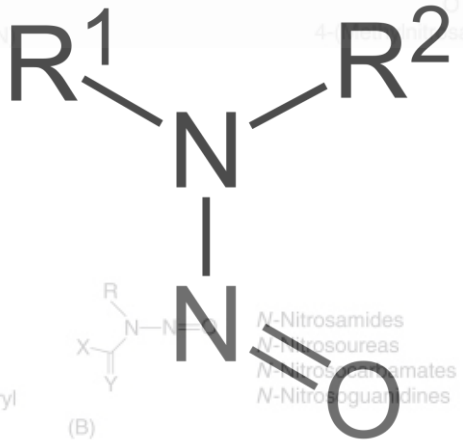


Thank You

Empowering a healthy tomorrow

Unmasking Nitrosamines

– Analytical Insights and Challenges

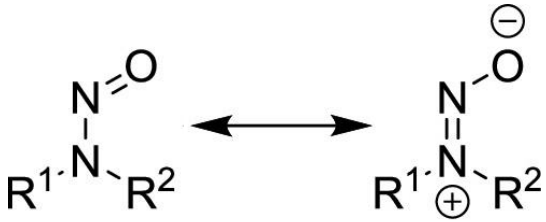


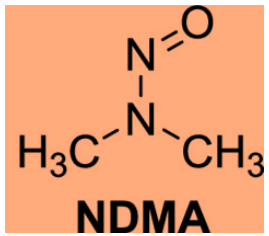
Amar Chittiboyina

05/21/2024

Nitrosamines

Structure, formation, and significance



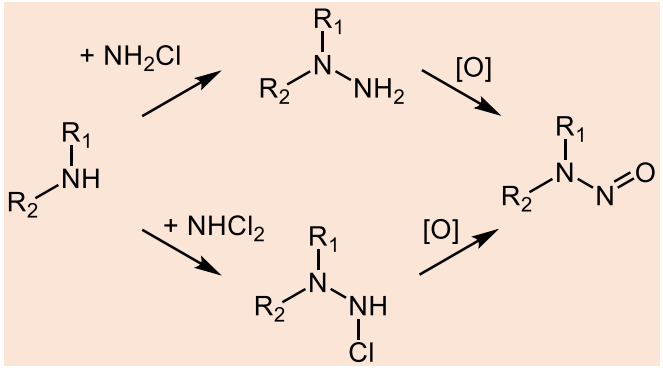


Over 300 nitrosamines have been identified, with about 90% being carcinogenic in animals. As of February 2021, five specific nitrosamines were discovered in certain medications.

- N-Nitrosodimethylamine (NDMA)
- N-Nitrosodiethylamine (NDEA)
- N-Nitroso-N-methyl-4-aminobutanoic acid (NMBA)
- N-Nitrosoisopropylethyl amine (NIPEA)
- N-Nitrosomethylphenylamine (NMPA)

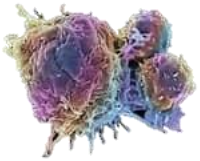
J. Org. Chem. 2021, 86, 3, 2037-2057

Nitrosamines can form unintentionally under various conditions and enter the environment through industrial waste or from environmental precursors via biological or chemical reactions.



Nitrosamine formation during chloramination.

Carcinogenesis: Known to produce DNA alkylating agents, viz., diazomethane from NDMA



The FDA recalled more than 1,400 product lots due to carcinogenic N-nitrosamine impurities exceeding the acceptable intake limit of 26.5 ng/day.

Recalled drugs:
Valsartan and losartan
Ranitidine
Metformin
Sitagliptin
Rifampin and Rifapentine
Varenicline (Chantix)
And others

The FDA issued a final guidance – AIL for NDSRIs

FDA-2020-D-1530

Nitrosamines
Detection methods

Remediation and mitigation of nitrosamines in water

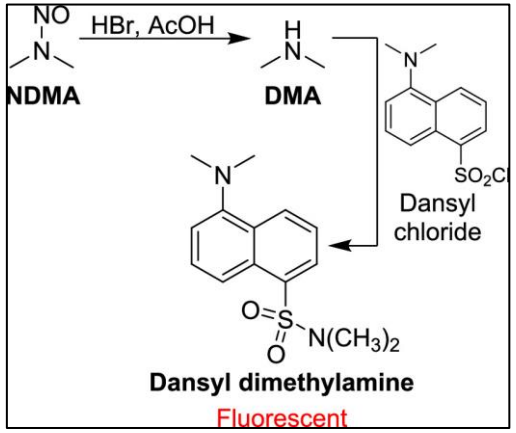
- Destruction
- Physical removal (i.e., filtration)
- Prevention of the formation

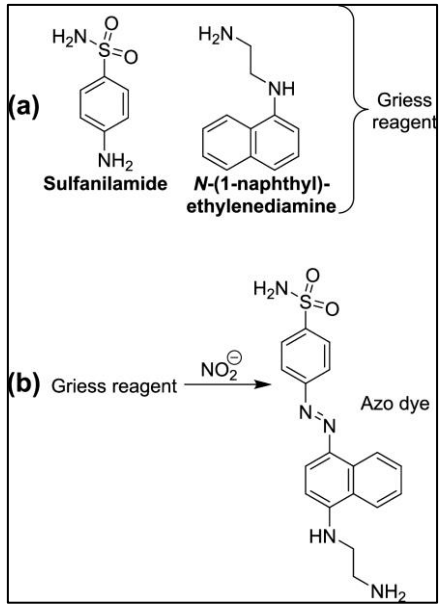
Detection methods –

Several widely used techniques (GC/LC-MS) for highly sensitive detection (in the range of ng/L) of aqueous nitrosamines share a common approach:

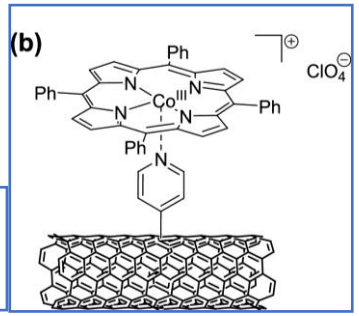
Extraction: Nitrosamines are extracted from water.
Concentration: The sample is significantly concentrated using an organic solvent.
Chromatographic Separation: Components are separated using chromatography.
Detection: The individual components are then detected, often employing mass spectrometry.

Classical reagents for detection





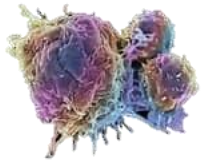
carbon nanotube-based sensor for NO detection

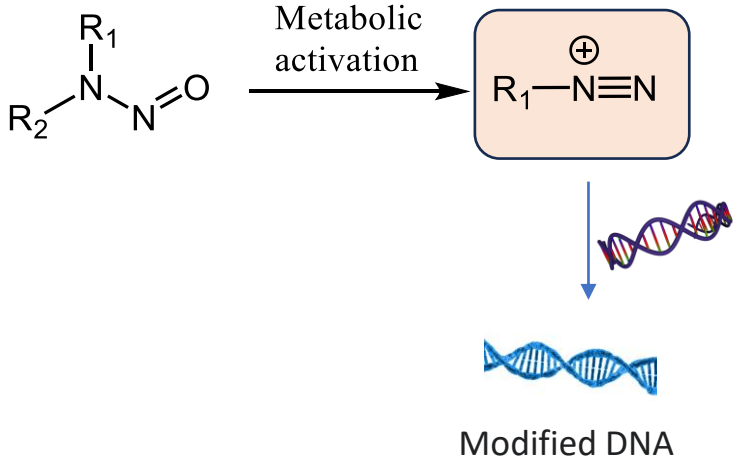


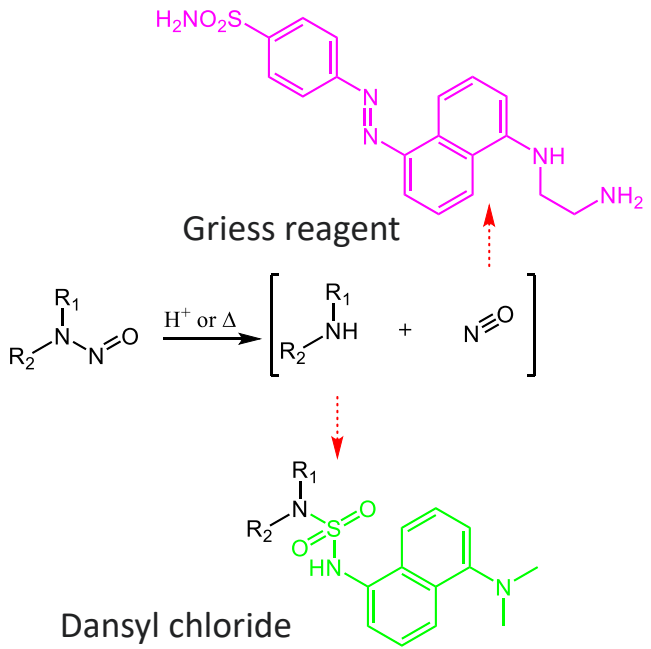
Nitrosamines

Major issues with current methodologies

Carcinogenesis: Known to produce DNA alkylating agents, viz., diazomethane from NDMA







Indirect methods to quantify the actual nitrosamines?

What if the composition includes other NO or amine species? Do these methods exhibit selectivity and specificity towards nitrosamine impurities?

Have we obtained the necessary information about alkylating agents within NDSRIs, or were we misled by these indirect findings?

Nitrosamines

Major issues with current methodologies

Gas chromatography (GC)-based methods exhibit high sensitivity in measuring volatile nitrosamines.

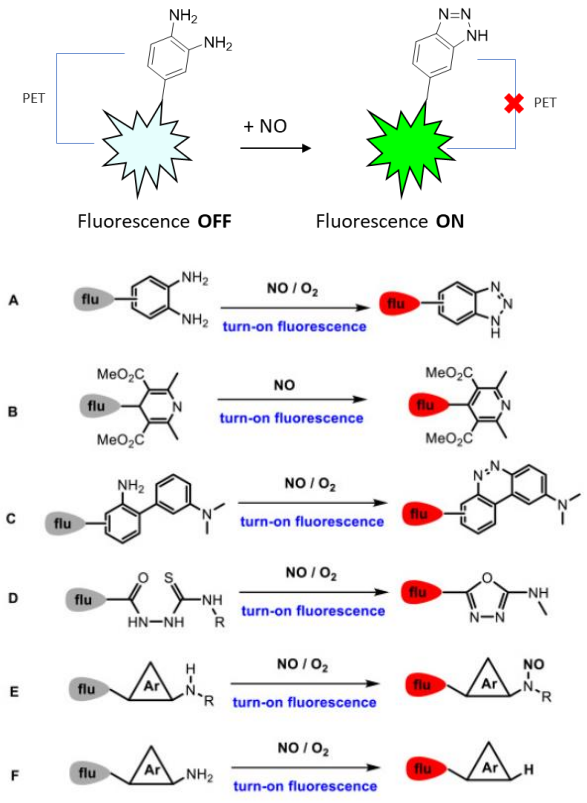
- Addressing nonvolatile or thermally unstable nitrosamines, such as N-nitrosodiphenylamine, presents a challenge.

Various liquid chromatography-mass spectrometry (LC-MS) methods, particularly LC-MS/MS, have been developed to accommodate a broader spectrum of nitrosamines.

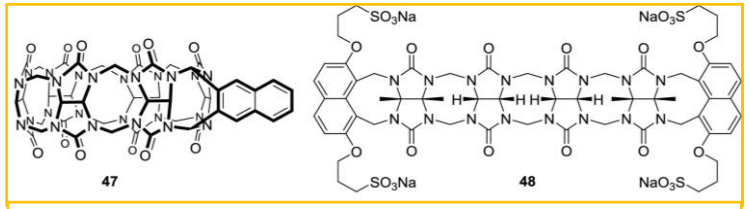
- Highly sensitive and specific to the analyte(s) of interest.
- UV detection is preferred over mass spectrometry (MS).
- Prior knowledge about the analyte's information is beneficial

Nitrosamines

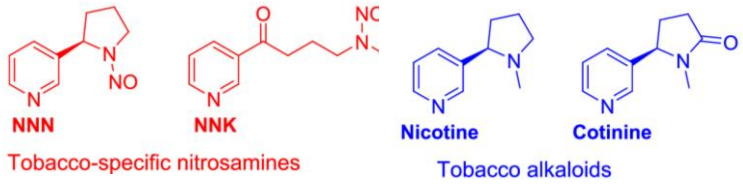
Potential solutions

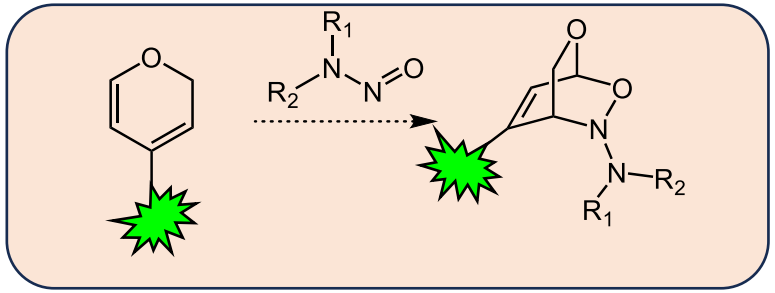


https://doi.org/10.1016/j.niox.2020.02.002



Cucurbituril-based fluorescent probes for detecting nitrosamines in tobacco





- Indirect Detection Methods for Nitrosamines
  The applicability of indirect detection methods to determine the presence or absence of nitrosamines in drugs and APIs remains a topic of investigation.
- Fluorescence Technology Modification:
  Can the off-on fluorescence technology be adapted into a sensitive, high-throughput methodology? Further research is needed to explore this possibility.
- Chemical Probes for Alkylating Agents:
  Developing specific and selective chemical probes could yield critical chemical information about real alkylating agents. These probes may also aid in the rapid dereplication of reactive intermediates.

Thank you!

R1, R2, R = alkyl, aryl
(A)
(B)
N-Nitrosamides X: Alkyl, aryl Y: O
N-Nitrosoureas X: NH2, NHR, NR2 Y: O
N-Nitrosocarbamates X: RO Y: O
N-Nitrosoguanidines X: NH2, NHR, NR2 Y: NH
N-Nitrosodimethylamine (NDMA)
N-Nitrosopyrolidine (NPYR)
N-Nitrosodiethanolamine (NDELA)
N-Nitrosomethyl urea
N-Nitrosonornicotine (NNN)
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)

FDA Recalls Nitrosamine-Containing Products

FDA Recalls Marketed Drugs Containing Nitrosamines

2018
- Recalls of Angiotensin II Receptor Blockers (ARBs) such as Valsartan®, Losartan® & Irbesartan®

2019
- Recalls of **Ranitidine HCl** (Zantac®) & Nizatidine Products

2020
- Recalls of certain Metformin® Products

FDA NEWS RELEASE

FDA Requests Removal of All Ranitidine Products (Zantac) from the Market

FDA Advises Consumers, Patients and Health Care Professionals After New FDA Studies Show Risk to Public Health

Share | Post | Linkedin | Email | Print

For Immediate Release: April 01, 2020

FDA NEWS RELEASE

FDA Alerts Patients and Health Care Professionals to Nitrosamine Impurity Findings in Certain Metformin Extended-Release Products

Agency Continues Investigations of Nitrosamine Impurities in Drug Products

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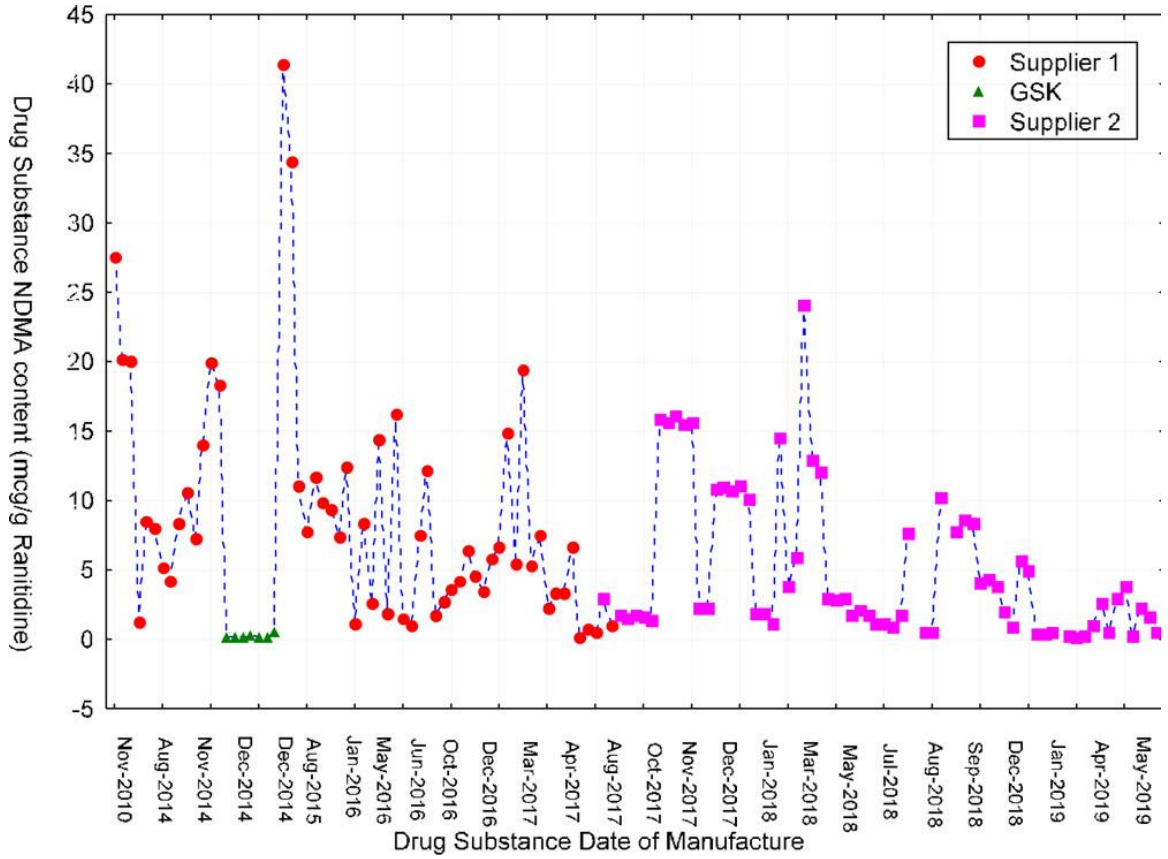
For Immediate Release: May 28, 2020

https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-ndma-zantac-ranitidine
https://www.1800law1010.com/defective-drugs/zantac-lawsuit/

NDMA Content From Different Suppliers of Ranitidine HCl Varied Depending Upon Storage

NDMA Content from Three Suppliers of Ranitidine HCl, Manufactured from 2010 - 2019

WHY?



- GSK tested in late 2019 different lots of Ranitidine HCl drug substance manufactured from 2010-2019.
- Ranitidine HCl drug substance manufactured by GSK had low levels of NDMA compared to two suppliers.[5]

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King, F. J., et al. (2020). "Ranitidine—investigations into the root cause for the presence of N-nitroso-N, N-dimethylamine in ranitidine hydrochloride drug substances and associated drug products." Organic process research & development **24**(12): 2915-2926.

Stability Differences Between Samples

- Drug substance was stored at 60 ℃ in closed vials
  - Supplier 2, Process 1 Ranitidine HCl
  - Supplier 2, Process 2 Ranitidine HCl
  - GSK Ranitidine HCl

WHY?

NDMA Content (mcg/g) of Ranitidine HCl stored at 60℃			
Days	Process 1 (mcg/g)	Process 2 (mcg/g)	GSK (mcg/g)
0	ND	ND	0.2
3	3.6	<LOQ (0.17)	0.36
7	7.4	0.28	0.39
46	38	2	not tested
115	66	3.3	not tested
Rate of formation over 7 days (mcg/g per day)	1.06	0.04	0.04

King, F. J., et al. (2020). "Ranitidine—investigations into the root cause for the presence of N-nitroso-N, N-dimethylamine in ranitidine hydrochloride drug substances and associated drug products." Organic process research & development **24**(12): 2915-2926.

Differences Between Process 1 and Process 2

Parameter	GSK	Process 1	Process 2
solvent composition	IPA	IPA/MeOH (70:30)	IPA/MeOH (40:60)
total solvent volume	8.7	3.5	2.5
water concentration	0.036	Not Added	0.034
crystallization temperature	50 ℃	10-25 ℃	10-25 ℃
observed rate of NDMA formation	Slow	Fast	Slow

*Solvent volume: Volume of the solvent as a ratio to the input weight of the ranitidine free base
Water concentration: % v/v water in the overall crystallization mixture.

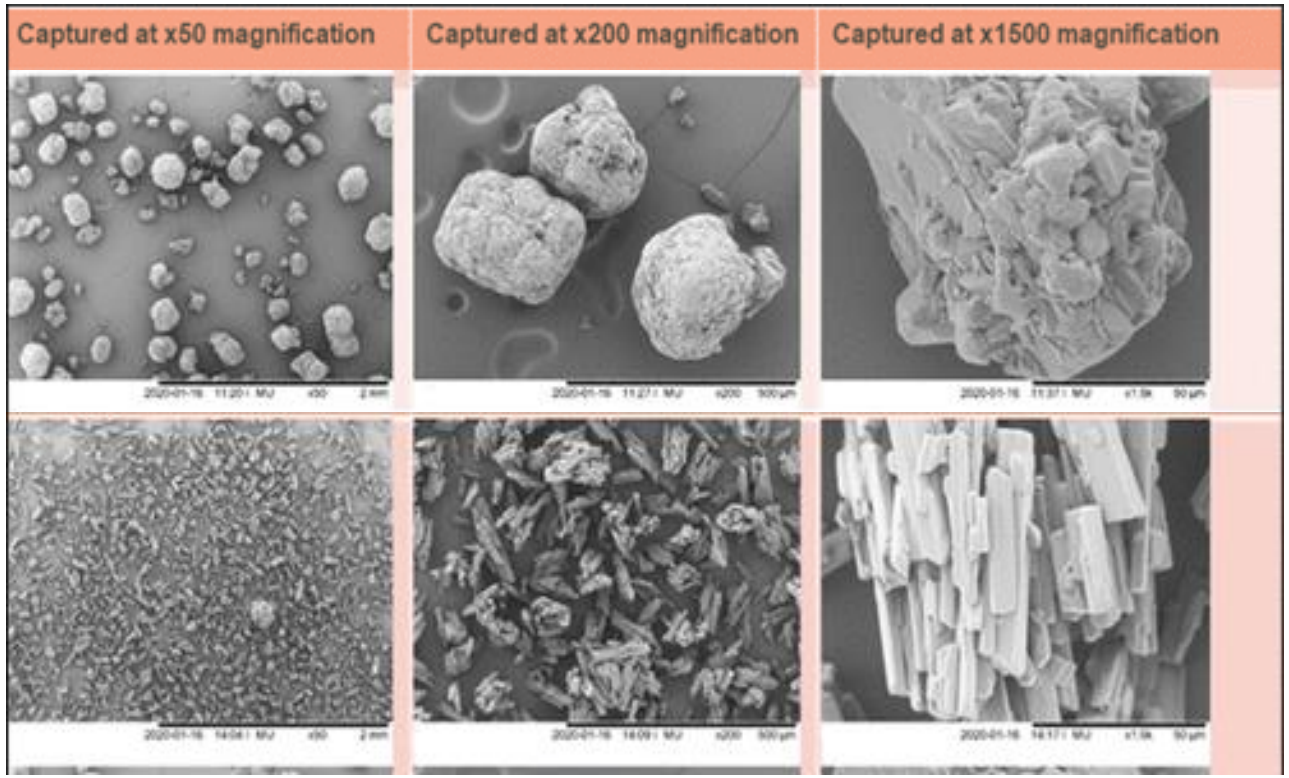
- **Manufacturing steps for Process 1 and Process 2 were identical except for the *final salt formation/ crystallization step***

King, F. J., et al. (2020). "Ranitidine—investigations into the root cause for the presence of N-nitroso-N, N-dimethylamine in ranitidine hydrochloride drug substances and associated drug products." Organic process research & development **24**(12): 2915-2926.

GSK Research Shows No Difference in Measured Physical Properties Between Process 1 and Process 2

Process 1
Less Stable

Process 2
More Stable



Physical property Testing of Ranitidine HCl		
Test	**Process 1**	**Process 2**
DVS (mositure uptake at 70%RH)	0.063% w/w	0.043% w/w
TGA Analysis (decomposition initiation)	<150 ℃	>150 ℃
Specific Surface Area	0.24 m^2/g	0.40m^2/g
Ture Density	1.32 g/cm^3	1.32 g/cm^3
PXRD, Raman and FTIR	Form 2, No difference	

No differences were observed between samples produced using Process 1 and Process 2 except slight differences in TGA data and morphology differences

King, F. J., et al. (2020). "Ranitidine—investigations into the root cause for the presence of N-nitroso-N, N-dimethylamine in ranitidine hydrochloride drug substances and associated drug products." Organic process research & development **24**(12): 2915-2926.

Why do Samples Degrade in the Solid State?

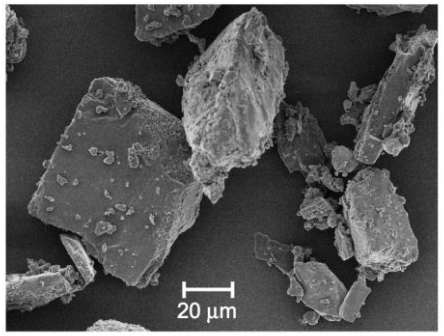
- **Crystal defects** are the primary source of degradation for crystalline drugs
- **Crystal defects** can be produced by physical transformations (milling, grinding, compression) and by crystallization
- Extensive research has shown that materials containing more **crystal defects** have faster degradation rates compared to materials having fewer defects.
- Solid-state NMR spectroscopy can be used to determine the number of **crystal defects** in both drug substances and in drug products

Ranitidine HCl NDMA Amount After Cryogrinding

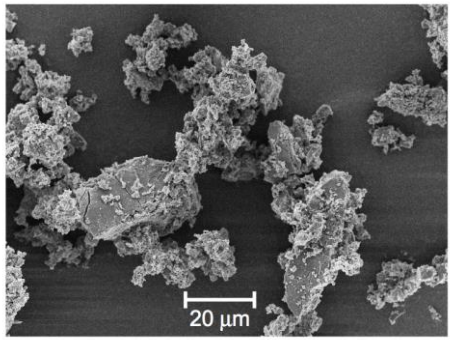
- Cryogrinding produces **crystal defects**
- Samples cryoground for 2 and 10 minutes produced significantly more NDMA than unground sample
- Surface area also increases for materials that were cryoground

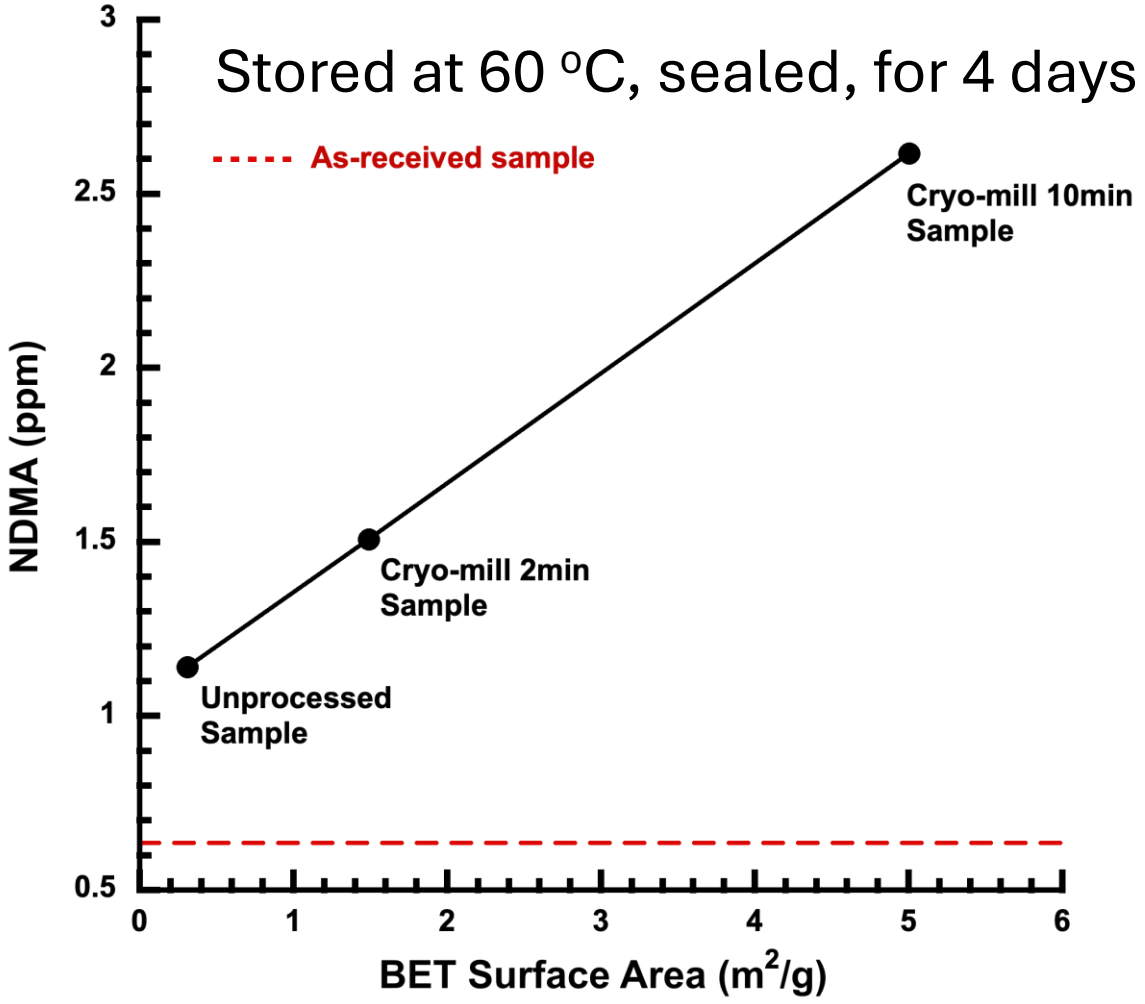
Impact of cryogrinding on lactose:

No grinding



60 min cryogrinding





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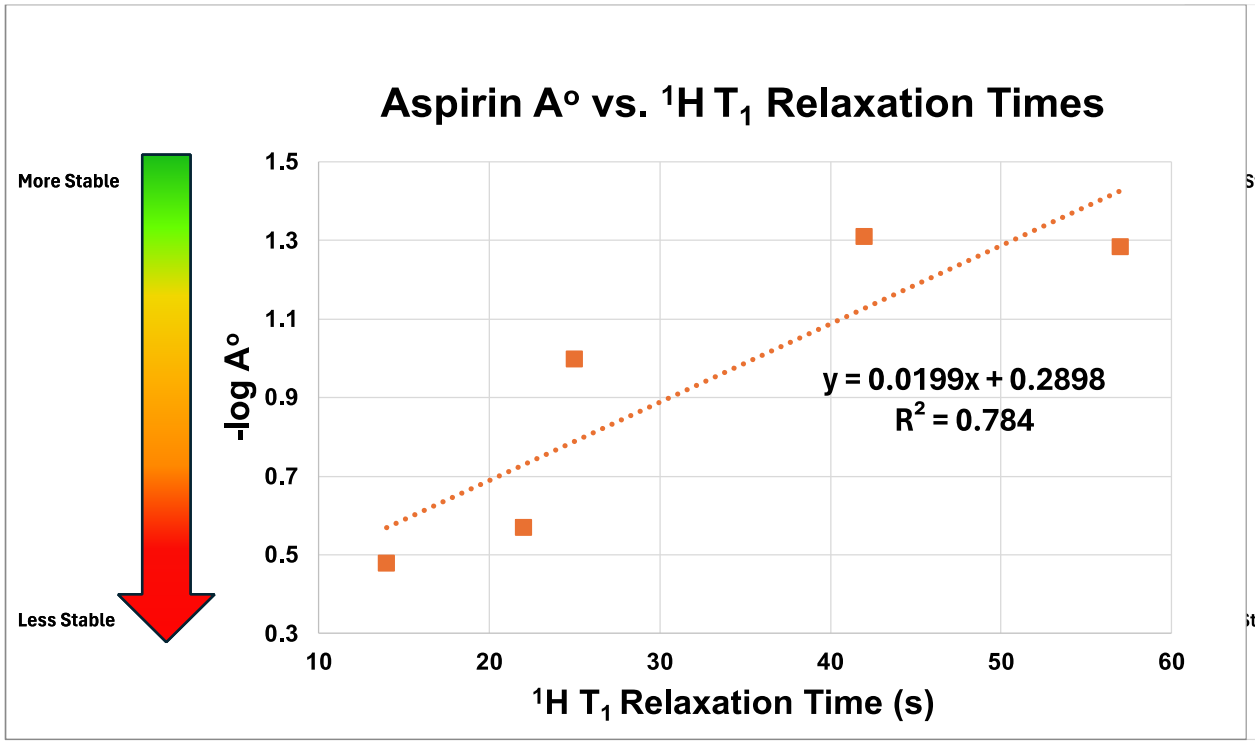
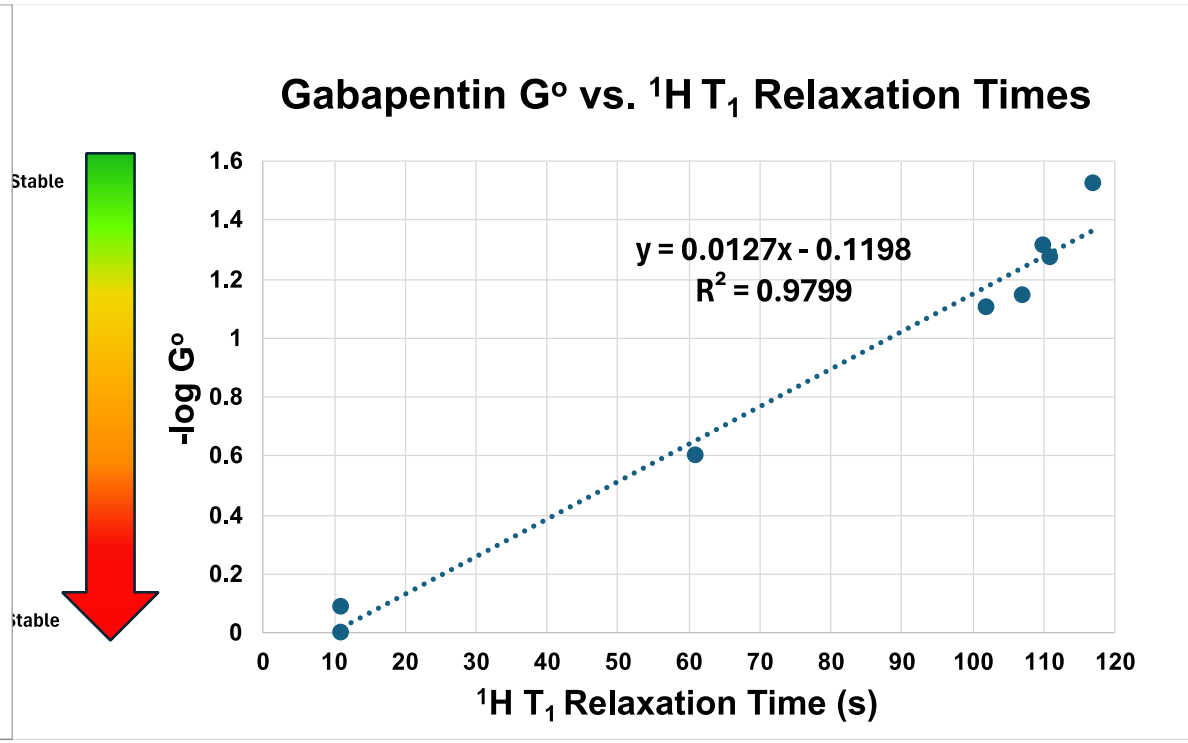
Correlating Solid-State NMR Relaxation Times with Degradation Rates of Compounds After Processing





- Numerous examples exist of solid-state NMR relaxation times changing upon either processing (milling, etc.) or crystallization

Thank you!

Public Comments for Session 1
Nitrosamine Drug Substance-Related Impurities (NDSRIs)

In Person Comments:

- Connie L. Chen, PhD, MPH, Senior Scientific Program Manager, Health and Environmental Sciences Institute (HESI)
- Pali De Silva Indrasekara, PhD, Technical Director, Advanced Manufacturing Technologies, USP
- Amar G. Chittiboyina, PhD, Assistant Director , National Center for Natural Products Research, School of Pharmacy, The University of Mississippi
- Eric J. Munson, PhD, Professor, Purdue University

Virtual Comments:

- Raphael Nudelman, PhD, ERT, Senior Director Impurity Expert, R&D Operation, Teva
- Marko Trampuž, PhD, Scientist Early Development, SANDOZ DEVELOPMENT CENTER, SLOVENIA

Public Comment Presentation on NDSRIs – Confirming Correlation Between Mutagenicity and Carcinogenicity Potencies of NDSRIs

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FDA - Fiscal Year (FY) 2024 Generic Drug Science and Research Initiatives Public Workshop
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Positive *in vivo* mutagenicity

- NDSRIs do not have carcinogenicity data (and will most probably not have such data generated).
- To date approx. 25% of NDSRIs (in Lhasa Complex Nitrosamines data sharing initiative) are positive *in vitro* mutagens and are likely to be positive also *in vivo.*
- Can positive *in vivo* mutagenicity data be used to set limits for NDSRIs?

ICH M7 Q&A 7.2

Question 7.2	Answer
If an Ames positive impurity cannot be controlled to an acceptable limit and is subsequently tested in an appropriate in vivo assay and the results are positive, does that support setting compound-specific impurity limits?	When a mutagenic impurity cannot be controlled to the TTC (or less than lifetime-based limit), results from an appropriate in vivo assay could complement the available data for a weight of evidence approach to support a higher limit on a case by case basis. However, in vivo gene mutation assays alone are currently not validated to directly assess cancer risk because the endpoint is mutation and not carcinogenicity (i.e., they are used for hazard identification).

Current Status

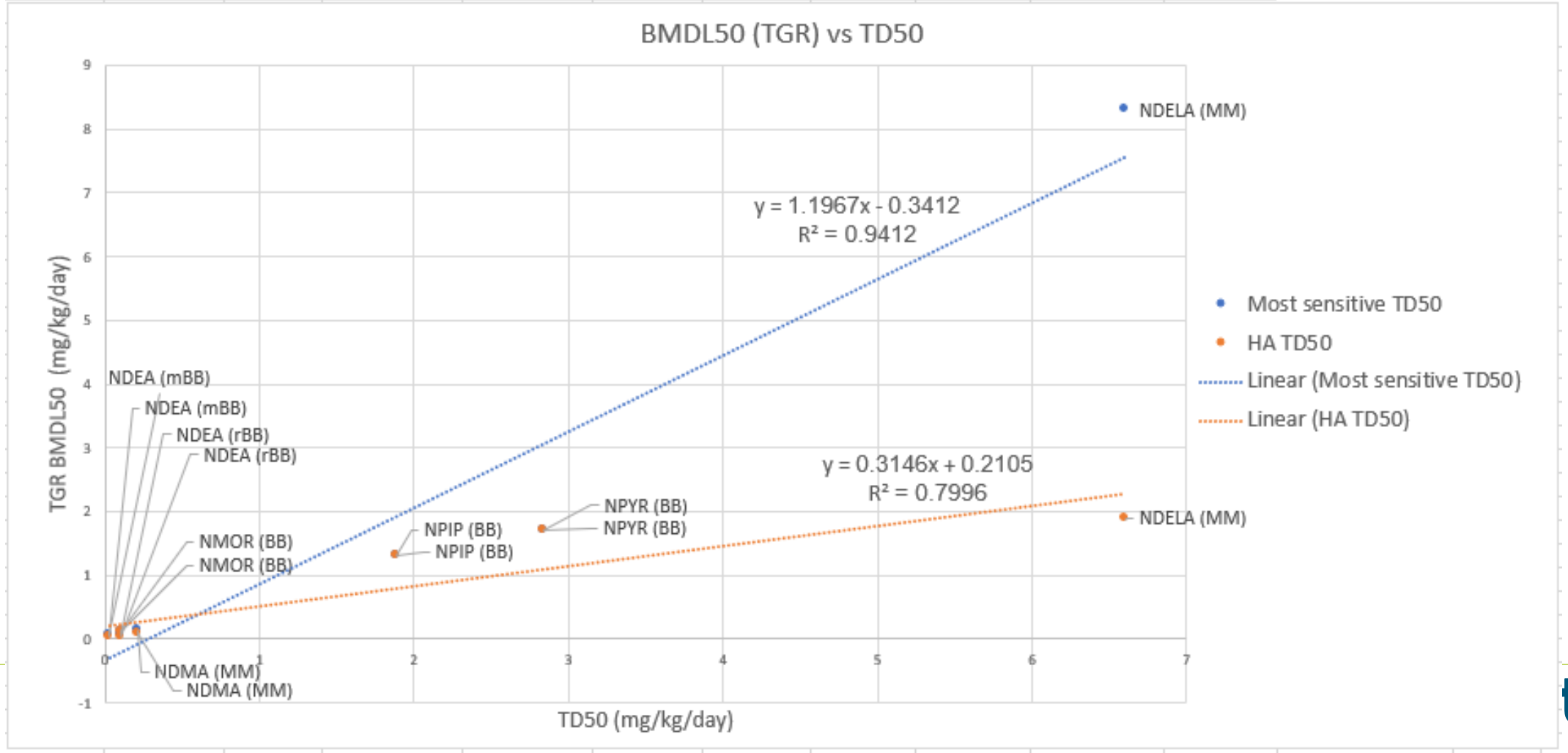
- Currently, positive *in vivo* mutagenicity data has no use...
- Possible consequence:
  - NDSRIs that are positive in the EAT will not be tested *in vivo*
  - ***May lead to serious market shortages***

HESI Genetic Toxicology Technical Committee (GTTC) Mechanis based Genotoxicity Risk Assessment (MGRA) Working Group (2 Annual meeting – Apr 2024)

- *In vivo* follow-up working group
  - Goals
    - Generate *in vivo* mutation data for 7 model nitrosamines, with robust carcinogenicity data, confirming the TGR assay as a surrogate for carcinogenicity for known nitrosamines
    - Provide information and comparators for assessment of relative potency, i.e. assess mutation frequency relative to the TD50
    - Concurrently, collect tissues for ecNGS to show consistency with TGR for that endpoint and provide data to support a more amenable methodology.

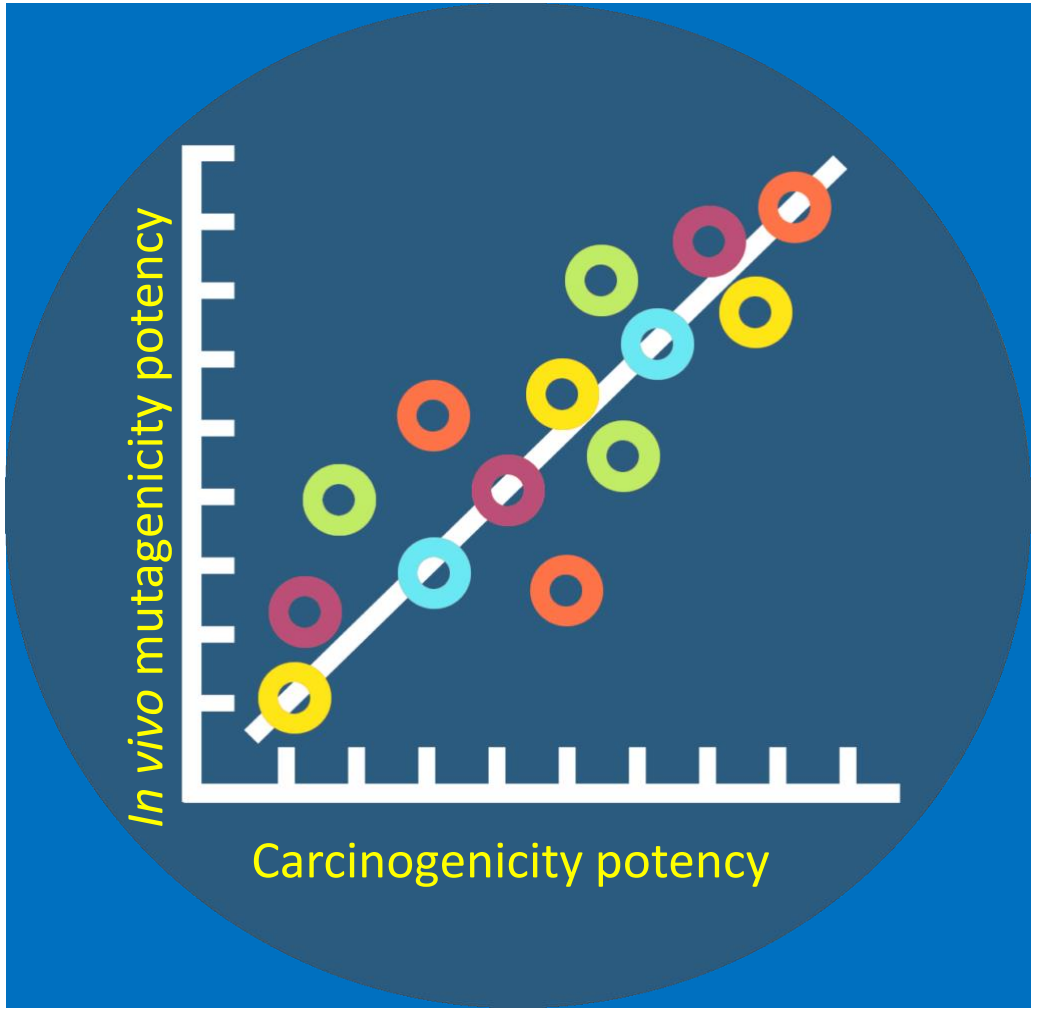
Exemplar Nitrosamine Data Contributed to HESI GTTC

Substance	Type of Endpoint	Species	Most Sensitive Tissue	Label	NOGEL (mg/kg/day)	LOGEL (mg/kg/day)	BMDU50 (mg/kg/day)	BMDL50 (mg/kg/day)	Most sensitive TD50	HA TD50
NDMA	Muta™Mice	Mouse	Liver	NDMA (MM)	0.36	1.1	0.46	0.21	0.145	0.096
NDEA	Muta™Mice	Mouse	Liver	NDEA (rBB)	0.1	1	1	0.1	0.062	0.0265
NDEA	Big Blue	Mouse	Liver	NDEA (mBB)	0.1		0.58	0.03	0.062	0.0265
NMOR	Big Blue	Mouse	Liver	NMOR (BB)	0.3	1	0.707	0.101	0.129	0.127
NPI	Big Blue	Rat	Liver	NPIP (BB)	1	3	3.61	1.89	1.3	1.3
NPYR	Big Blue	Rat	Liver	NPYR (BB)	0.5	5	8.42	2.84	1.7	1.7
NDELA	Muta™Mice	Mouse	Liver	NDELA (MM)	2.5	25	17.3	6.6	8.3	1.9



Proposal

- Strong correlation between in vivo mutagenicity and carcinogenicity has been established for exemplar small nitrosamines
- Concurrently, tissues are being collected from the in vivo studies of the exemplar nitrosamines for ecNGS to show consistency with TGR for that endpoint and provide data to support a more amenable methodology
- Proposal to GDUFA: select exemplar NDSRIs that tested positive in *in vivo* mutagenicity and test using rasH2 Transgenic Mouse Carcinogenicity to further establish the correlation between mutagenicity and carcinogenicity potencies



Thank You

In vitro studies on metabolic stability of NDSRIs and properties of reactive metabolites

Marko Trampuž, Vesna Gorenjak, Zdenko Časar

FY 2024 GDUFA Public Workshop
May 20th, 2024

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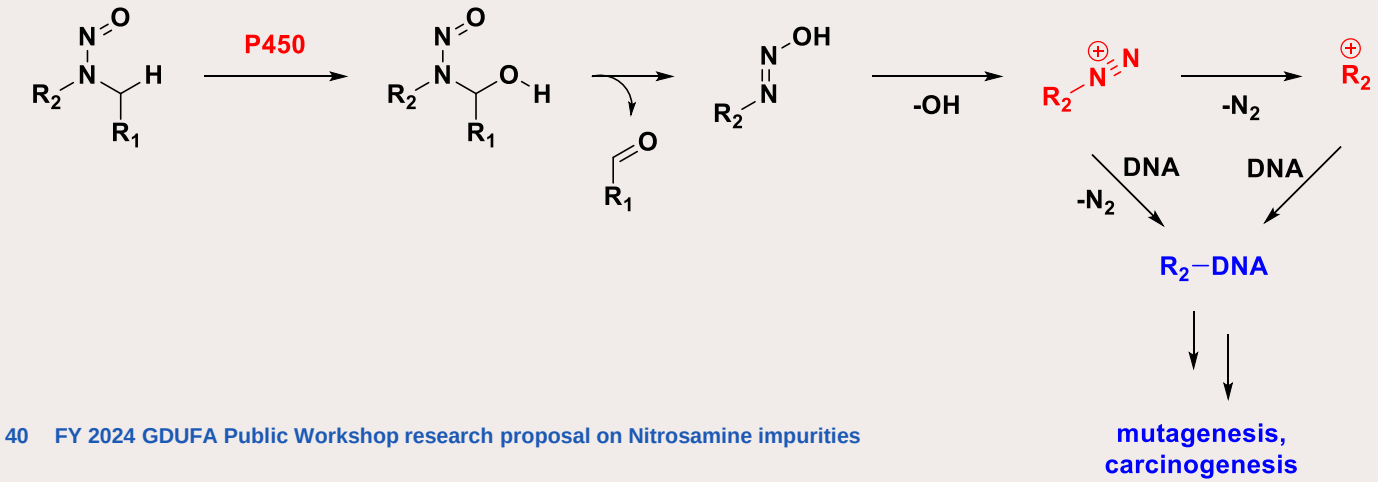
Introduction

To support a higher AI limit for a Nitrosamine Drug Substance-Related Impurity (NDSRI) than the one associated with Carcinogenic Potency Categorization Approach (CPCA), the Agency requests:[1]

- **compound-specific data,** such as mutagenicity assessment via an **Enhanced Ames Test (EAT) or**
- a **read-across approach** based on a **surrogate with robust carcinogenicity data**

Additional safety data may be required by the Agency to support higher AI, meaning that **a negative EAT or read-across approach** are **not considered sufficient evidence**!

The mutagenicity of *N*-nitrosamines is dependent upon **metabolic activation** by cytochrome P450 (CYP450) drug metabolizing enzymes, largely via hydroxylation on the alpha-carbon.[2] Furthermore, the **DNA-alkylating reactivity of metabolites** depends greatly upon the structure (R_2 substitutent).[3]



1: Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs) Guidance for Industry, FDA, August 2023.
2: Cross et al., *Comput. Toxicol.* **2021**, 20, 100186.
3: Li et al, *Int. J. Mol. Sci.* **2022**, 23(9), 4559.

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Model NDSRI study

In 2023, the Agency published the results of an investigation aiming to evaluate the risk of a model NDSRI and **to identify optimized testing conditions** for its **safety assessment *in vitro***[4]

The model NDSRI was studied using the following experimental systems:

- **Five bacterial strains** with **rat / hamster S9 preincubation** to evaluate mutagenicity
- **Human lymphoblastoid TK6 cells** with **hamster S9 preincubation** to evaluate genotoxicity
- **TK6 cell lines with stable CYP expression** to identify specific human enzymes involved in bioactivation
- **Human HepG2** & **HepaRG cells** to evaluate genotoxicity

Mutagenicity and genotoxicity were evaluated via ***in vitro* micronucleus assay**, **Comet assay**, **MultiFlow DNA damage assay**, and **gene mutation assays** (**TK**, **HPRT**).

The study did not address:

- Chemical analysis of the metabolically activated NDSRI (extent of metabolism, identity of metabolites)
- Stability/reactivity of the formed metabolites

4: Li et al., *Regul. Toxicol. Pharmacol.* **2023**, 141, 105410.

Proposal

We propose to the Agency to prioritize research and provide guidance on *in vitro* studies on **NDSRI metabolic stability**, **generation of reactive metabolites**, and their **reactivity**.

- What type of experimental systems (S9 / cell lines / spheroids / organoids) would need to be used to evaluate **whether alpha-hydroxylation metabolism occurs for an NDSRI**?
- Would the **absence of detection of potentially mutagenic metabolites** be seemed as valid evidence to justify higher AI? What would be the required analytical **limit of detection**?
- Would a **quantitative comparison of generation of reactive metabolites between an NDSRI and a surrogate** complement the read-across approch to justify higher AI limit?

The Agency could also provide guidance on better understanding the **stability of these potentially reactive mutagenic metabolites** and their **reaction kinetics with DNA**, as these larger molecules may have completely different properties compared to small diazoniums (both sterical and electronic differences) which are formed from small *N*-nitrosamines.[3]

The formed metabolites may be:

- Very unstable and would **immediately degrade** with water.
- Very stable and would **not react with surrounding moleculs** (including DNA).

} **Low mutagenicity risk**

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