U.S. FOOD & DRUG ADMINISTRATION

Nitrosamines in Drugs: A Regulatory Perspective on Research Progress and Opportunities

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

Introduction

- The presence of nitrosamines (NAs) in drugs continues to present challenges and impacts drug supply
- Today's session will address two areas of nitrosamine risk assessment:

1. Safety assessment of nitrosamines: small molecule nitrosamines (e.g., NDMA) and complex nitrosamines (e.g., NDSRIs)

   Challenge: Carcinogenicity data are limited to small molecule nitrosamines so alternate methods are needed to assess risk of NDSRIs

2. Nitrosamine formation during manufacturing and in drug product formulations

   Challenge: Nitrosamine formation may occur at multiple steps, making root cause determinations and mitigation efforts complex

Safety Assessment of Nitrosamines

- FDA recommends a step-wise approach to determine Acceptable Intakes (AI) for nitrosamines. This is the product of research and collaboration
  1. **Carcinogenicity Potency Categorization Approach (CPCA)** – AI determined based on chemical structure without additional data
  2. **In vitro characterization** – Enhanced Ames Assay, in vitro mammalian assay and metabolism assessment to obtain up to 1500 ng/day
  3. **In vivo mutagenicity assay** – To qualify levels above 1500 ng/day

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Determining recommended acceptable intake limits for *N*-nitrosamine impurities in pharmaceuticals: Development and application of the Carcinogenic Potency Categorization Approach (CPCA)

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Optimizing the detection of *N*-nitrosamine mutagenicity in the Ames test☆

Robert H. Heflich [a,*], Michelle E. Bishop [a], Roberta A. Mittelstaedt [a,1], Jian Yan [a], Sharon K. Guerrero [a], Audrey M. Sims [a], Kamela Mitchell [a], Nyosha Moore [a,1], Xilin Li [a], Nan Mei [a], Rosalie K. Elespuru [b,1], Sruthi T. King [c], David A. Keire [d], Naomi L. Kruhlak [e], Robert T. Dorsam [c], Andre S. Raw [d], Karen L. Davis Bruno [f], Timothy J. McGovern [f], Aisar H. Atrakchi [f]

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HESI GTTC ring trial: Concordance between Ames and rodent carcinogenicity outcomes for *N*-nitrosamines (NAs) with rat and hamster metabolic conditions

Joel Bercu [a], Alejandra Trejo-Martin [a], Connie Chen [b], Maik Schuler [c], Jennifer Cheung [c], Tetyana Cheairs [d], Anthony M. Lynch [e], Dean Thomas [e], Andreas Czich [f], Aisar Atrakchi [g], Timothy J. McGovern [g], Robert H. Heflich [h], Alisa Vespa [i], Roland Froetschl [j], Yi Yang [k], Raj D. Gandhi [l], Joanne Elloway [l], Verena Ziegler [m], Anna Hellmann [n], Michelle Schaefer [o], Rachael E. Tennant [p], Walter Westerink [q], Roy Hoffmans [q], Robert Jolly [r], Jessica Noteboom [r], Pavan Gollapudi [s], Zhanna Sobol [t], Katherine K. McGettigan [t], Joel S. Christensen [t], Stephanie Simon [u], Jessica Dieckhoff [u], Andreas Zeller [v], Christine Marchand [v], Kerstin Waese [f], Michelle E. Bishop [h], Penny Leavitt [w], Victoria Hargreaves [x], Connor Glick [y], Yang Liao [y], Rosalie Elespuru [z], Raechel Puglisi [b,*]

CPCA and Adjustments to AIs

- Exploring enhancements to CPCA through collaboration
  - Refinement of CPCA Category AIs based on new and existing data
  - Inclusion of new activating and deactivating features for CPCA scoring, including consideration of molecular weight
- Significant ongoing efforts through HESI to advance Structure Activity Relationship (SAR) research to inform CPCA changes using quantum mechanics (QM)-based methods
- Challenges remain:
  - Access to computational resources and expertise to use them
  - Lack of new carcinogenicity data to validate QM/(Q)SAR methods and expand SAR knowledge of complex nitrosamines
    - Advancing alternative testing methods to assess carcinogenic potency will expand SAR knowledge and inform CPCA updates
  - Knowledge gaps in how competing metabolic pathways in complex nitrosamines impact carcinogenic potency

Potency Category	Recommended AI (ng/day)
1	26.5
2	100
3	400
4	1500
5	1500

Less Than Lifetime (LTL)

- Challenge: Is the ICH M7 LTL framework adequately protective for nitrosamines?
  - Carcinogenicity data cancer potency is a function of the lifetime cumulative dose (LCD) vs daily dose

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Evaluating the lifetime cumulative dose as a basis for carcinogenic potency of nitrosamines – a key tenet underpinning less-than-lifetime approaches for establishing acceptable intake limits

Susan P. Felter [a,*], Ashley M. Mudd [a], David J. Ponting [b], Rob Thomas [b], Alisa Vespa [c], Timothy J. McGovern [d], Andreas Zeller [e], Roland Froetschl [f], Bodo Haas [f], Yi Yang [g], Anthony Lynch [h], Angela White [h], Matthew Schmitz [i], Raechel Puglisi [j], Joel P. Bercu [k]

- Regulatory concerns/data gaps:
  - Is the ICH M7 LTL framework adequately protective for nitrosamines?
    - Potential to overwhelm DNA repair with high daily exposures
    - Potential for polypharmacy to result in exposure to multiple NAs
    - Findings that some NAs have been found to induce tumors in rodents following a single or few exposures
- Continued research and data analyses focused on addressing data gaps for nitrosamines and non-nitrosamines are ongoing

In Vitro Mutagenicity Assessment

Ongoing research and opportunities

- Refinement of in vitro assays for mutagenicity assessment
  - Enhanced Ames Assay conditions and positive controls
  - In vitro mammalian assays and utility of a battery of studies
  - Mechanistic studies on metabolism of NDSRIs
- Challenge: in vitro data is positive or negative, but does not define carcinogenic potency

Environmental and Molecular Mutagenesis

RESEARCH ARTICLE | Free Access

Adjusting the Preincubation Conditions to Enhance the Ames Test for Detecting the Mutagenicity of *N*-Nitrosamines

Michelle E. Bishop, Audrey M. Sims, Sharon K. Guerrero, Kamela Mitchell, Nan Mei, Hannah Xu, Naomi L. Kruhlak, Sruthi T. King, Robert T. Dorsam, Aisar H. Atrakchi ... See all authors

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Mutagenicity and genotoxicity evaluation of 15 nitrosamine drug substance-related impurities in human TK6 cells☆

Xilin Li [a,*,1], Yuan Le [a,1], Xiaoqing Guo [a], Sruthi T. King [b], Robert T. Dorsam [b], Aisar H. Atrakchi [b], Timothy J. McGovern [b], Karen L. Davis-Bruno [b], David A. Keire [b], Robert H. Heflich [a], Nan Mei [a,**]

Mutagenesis. 2026 Feb 23:geag012. doi: 10.1093/mutage/geag012. Online ahead of print.

Mutagenicity of N-nitroso-fluoxetine and N-nitroso-varenicline in human HepaRG cell models

Ji-Eun Seo [1], Hannah Xu [1], Javier Revollo [1], Jaime Miranda-Colon [1], Aisar H Atrakchi [2], Timothy J McGovern [2], Karen L Davis Bruno [2], David A Keire [2], Robert H Heflich [1], Xiaoqing Guo [1]

In Vivo Mutagenicity Assessment

- Leveraging in vivo mutation assay results for quantitative assessment
  - Transgenic rodent (TGR) assay is used for hazard ID
  - Studies underway to determine whether output from this assay can be used to assess potency across chemical classes, including nitrosamines
  - Study design elements are critical to support quantitative assessment
- Ongoing work:
  - Correlate mutation response to carcinogenic potency using Benchmark Dose (BMD) analysis
  - Assess utility of error-corrected Next Generation Sequencing (ecNGS) in quantifying mutation response, compared to established traditional tissue evaluation
  - Standardize TGR study design elements for nitrosamines
  - Standardize and validate models for ecNGS

Nitrosamine Formation and Mitigation

- Nitrosamines may form at various stages in drug manufacturing or during shelf-life

  In Active Pharmaceutical Ingredients (APIs)
  - E.g., Contaminated raw materials or recovered solvents

  In Drug Products
  - Nitrite impurities in excipients reacting with APIs containing amine groups
  - Container closure systems and manufacturing processes
- Opportunity: Continued research on root cause and mitigation strategies
  - Addition of anti-oxidants (e.g., ascorbic acid, α-tocopherol, cysteine HCl) can mitigate formation of some NDSRIs
  - Modification of pH to neutral or basic using excipients like sodium carbonate
  - Processes and container closures that minimize nitrosamine formation

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Pharmaceutics, Drug Delivery and Pharmaceutical Technology

Bumetanide as a Model NDSRI Substrate: *N*-nitrosobumetanide Impurity Formation and its Inhibition in Bumetanide Tablets

Diaa Shakleya[a,*], Bethel Asmelash[b], Alaadin Alayoubi[a], Nicolas Abrigo[a], Adil Mohammad[a], Jiang Wang[a], Jinhui Zhang[a], Jingyue Yang[a], Tim Andres Marzan[a], David Li[a], Maha Shaklah[a], Fahd M. Alsharif[a], Saaniya Desai[a], Patrick J. Faustino[a], Muhammad Ashraf[a], Thomas O'Connor[a], Matthew Vera[b], Andre Raw[b], Vilayat A. Sayeed[b], David Keire[a]

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journal homepage: www.elsevier.com/locate/ijpharm

Nitrosamine mitigation: *NDMA* impurity formation and its inhibition in metformin hydrochloride tablets

Diaa Shakleya [a,*], Alaadin Alayoubi [a], Dustin Brown [a], Alaa Mokbel [a], Nicolas Abrigo [a], Adil Mohammad [a], Jiang Wang [a], David Li [a], Maha Shaklah [a], Fahd M. Alsharif [a], Saaniya Desai [a], Martha Essandoh [a], Patrick J. Faustino [a], Muhammad Ashraf [a], Thomas O' Connor [a], Matthew Vera [b], Andre Raw [b], Vilayat A. Sayeed [b], David Keire [a]

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Pharmaceutics, Drug Delivery and Pharmaceutical Technology

Antioxidants had No Effects on the In-Vitro Permeability of BCS III Model Drug Substances

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Conclusion

- Nitrosamines continue to present challenges, but research and collaboration resulted in substantial progress
- Continued collaboration and research efforts are vital to progress
  - Safety assessment: CPCA enhancement and maximizing value of in vitro and in vivo assays for hazard and risk assessment approaches
  - Nitrosamine formation: Progress has been made via research on the effects of antioxidants, pH control, and management of heat and moisture throughout manufacturing process
  - Continued efforts are needed to understand how manufacturing process and container closures contribute to nitrosamine formation

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Nitrosamine Int'l Technical Working Group
HESI Collaboration
ICH M7 Expert Working Group

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