Overview Of HESI Nitrosamine (NA) Research Program

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Gilead Sciences Inc.

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QR code for the HESI Nitrosamine Research Program (NRP)

Quantitative Analysis

Error Corrected Sequencing

Mode of Action

Mechanism-based Genotoxicity Risk Assessment (MGRA)

Integrating Risk Assessment and Decision Making

HESI Genetic Toxicology Technical Committee

- **Improve** the scientific basis of the interpretation of results from genetic toxicology tests for purposes of more accurate hazard identification and assessment of human risk
- **Develop** follow-up strategies for determining the relevance of test results to human health; to provide a framework for integration of testing results into a risk-based assessment of the effects of chemical exposures on human health
- **Promote** the integration use of new techniques and scientific knowledge in the evaluation of genetic toxicology; and to monitor and promote the development of innovative test and testing strategies.

In Vivo Follow-Up

In Vitro

Germ Cells

Historical Control Distribution

Improved Testing Guidelines & Strategies

Education Outreach

PRDV Awards

Project Awards

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Nitrosamines Research Program

- Launched initially under the MGRA working group in 2022 to develop a new mechanism-based risk assessment paradigm for genotoxicity, that uses a genetic toxicology testing strategy designed from a “clean slate”, incorporating new science and technology to allow for a greater diversity of genomic damage to be addressed, and genotoxicity Adverse Outcome Pathways (AOP)s.
- Nitrosamines was selected as a case study to demonstrate usefulness of innovative, mechanism-based testing approaches using data-rich compounds
- **Currently the central convening point for the collective prioritization, design, resourcing, execution, and analysis of nitrosamine safety studies. The research has been at the forefront of regulatory science and is directly shaping global regulatory guidance (ICH M7 addendum) on nitrosamine impurities.**

11 Government/ Regulatory

- Agence nationale de sécurité du médicament et des produits de santé (ANSM)
- Danish Medicines Agency
- European Medicines Agency
- Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte, (BfArM)
- Health Canada
- Institut National de lar recharge agronomique (INRA)
- National Institute of Health Sciences (NIHS), Japan
- National Institute for Public Health and the Environment (RIVM)
- SwissMedic
- US Food and Drug Administration

31 Private Industry

- AbbVie
- Amgen
- AstraZeneca
- BASF
- Baxter
- Bayer
- Boehringer Ingelheim
- Bristol Myers Squibb
- Charles River Laboratories
- Corteva Agriscience
- Eli Lilly
- Gilead
- Gentronix
- GSK
- Haleon
- Inotiv
- Janssen
- Labcorp
- Leadscope/Instem
- Litron Laboratories
- Merck & Co. Inc.
- Merck kGA
- MultiCASE, Inc.
- Novartis
- Perrigo
- Pfizer
- Procter & Gamble
- Roche
- Sanofi
- Teva Pharmaceuticals
- Xenometrix

10 Academic, NGO, Consultants

- FSTox Consulting
- Lhasa Limited
- Nudelman ChemTox Consulting
- White Oak Regulatory Tox., LLC
- George Washington University
- New York Medical College
- St. George’s Medical School, University of London
- Swansea University
- University of Colorado
- University of Minnesota

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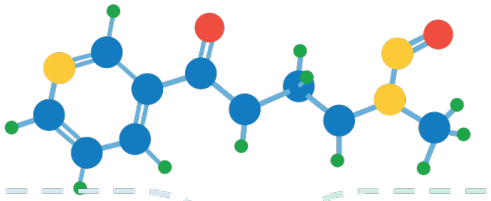

NRP Working Groups

Ames Optimization

Develop an Ames protocol predictive for carcinogenic potential of Nitrosamines

Phase 1 (complete):

- Conducted ring-trial on 32 nitrosamines ran in duplicates across 16 labs
- Generated data on sensitivity, specificity, and BMD modeling (FDA grant NoA: 3U01FD006676-03S2)

Phase 2:

- Refining protocol with positive controls (PCs) and strain condition analysis;
- Preparing for a second ring-trial

In vitro test strategy

Identification & verification of in vitro assays with alternative cell models supporting Nitrosamines risk identification

Phase 1 (complete):

- Developed a protocol using 2D HepaRG cells;
- Included initial testing and data sharing from Pfizer, P&G, and AbbVie

Phase 2:

- Launching experimental work in Q3–Q4 2025 testing NAs/NDSRIs;
- Conducting a landscape analysis of alternative cell lines and endpoints for testing

In vivo Ames follow-up

Develop in vivo strategy to verify Ames data within the frame of ICH M7

Phase 1 (complete):

- Assessed in vitro/in vivo concordance for exemplar nitrosamines and NDSRIs;
- Completed literature review and analysis on less-than-lifetime (LTL) exposure relevance

Phase 2:

- Advancing testing of additional N-nitroso compounds using Pig-a;
- Initiating LTL experimental work and quantitative dose-response analysis;
- Comet and ecNGS testing to proceed as resources allow

(Q)SAR/QM

Refine and extend the CPCA using (Q)SAR and QM for more predictive and accurate predictions

Phase 1:

- Defined approach to evaluate existing CPCA features (e.g., sterics, beta-substitution) using QM and statistical tools

Phase 2:

- Expanding models to include new electronic and metabolic features;
- developing confidence metrics and applicability domains

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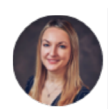


HESI GTTC MGRA

Nitrosamines Research Program

Leadership:
Joel Bercu
Tetyana Chears

HESI Staff Contact:

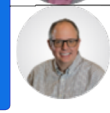
Raechel Puglisi

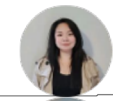
Ring-Trial
Leads: Anthony Lynch, Joel Bercu

Positive Controls
Leads: Alejandra Trejo-Martin
Rhys Whomsley

AMES OPTIMIZATION

Leadership:
Anthony Lynch (GSK)
Joel Bercu



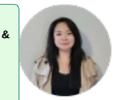


IN VITRO APPROACHES

Leadership:
Xiaowen Sun
Xiaoqing (Carol) Guo

HepaRG Protocol Design & Experimental Trial

Lead: Wen Sun



Landscape Analysis of Alt. Cell Lines
Leads: Carol Guo, Wen Sun

HESI Staff Contact:

Raechel Puglisi

HESI Staff Contact:

Raechel Puglisi

Less-than-Lifetime Liteature Search

Lead: Susan Felter

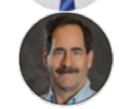
Less-than-Lifetime: Experimental Study Design

Lead: Zhanna Sobol, Bob Jolly

Carcinogenicity Potency Analysis
(Quantitative Analysis WG)

IN VIVO FOLLOW-UP

Leadership:
Bob Jolly
Joel Bercu
Sheroy Minocherjohmji
Timothy McGovern

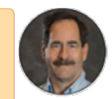


(Q)SAR-QM

Leadership:
David Ponting
Jakub Kostal
Kevin Cross

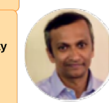
EWG/EDG

Lead: Kevin Cross



Ion Stability and Reactivity

Lead: Suman Chakravarti

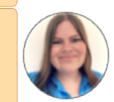


Ring Size

Lead: David Ponting

CYPs Metabolism

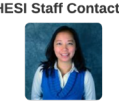
Leads: Clarence Wybon



HESI Staff Contact:

Alexandra Taraboletti

HESI Staff Contact:



Connie Chen

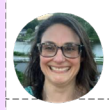
Step 1a: Safety Literature Review
Leads: Michelle Kenyon, Crystal D'Silva,

Step 1b: Mechanism Literature Review
Leads: Raphy Nudelman, Graham Smith

Step 2: Experimental Work
Step 3: QSAR-QM

N-NITROSO ASSESSMENT

Leadership:
Michelle Kenyon



ALTERNATE EXPOSURES

Leadership:
TBD

HESI Staff Contact:

Alexandra Taraboletti

LEGEND

Active

Scoping, Not Yet Launched

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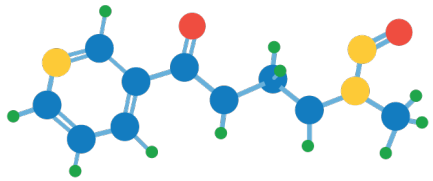
NRP Initiatives Informing the ICH Guidelines



Nitrosamines Research Program

Research Initiatives

- **Enhance Ames Test (EAT)**
- In vivo mutagenicity
- **Less than lifetime**
- CPCA development
- Quantum Mechanics

ICH harmonisation for better health

Final Concept Paper

Addendum to ICH M7 on Risk Assessment and Control of *N*-Nitrosamine Impurities

Dated 24 April 2025

Endorsed by the Management Committee on 07 May 2025

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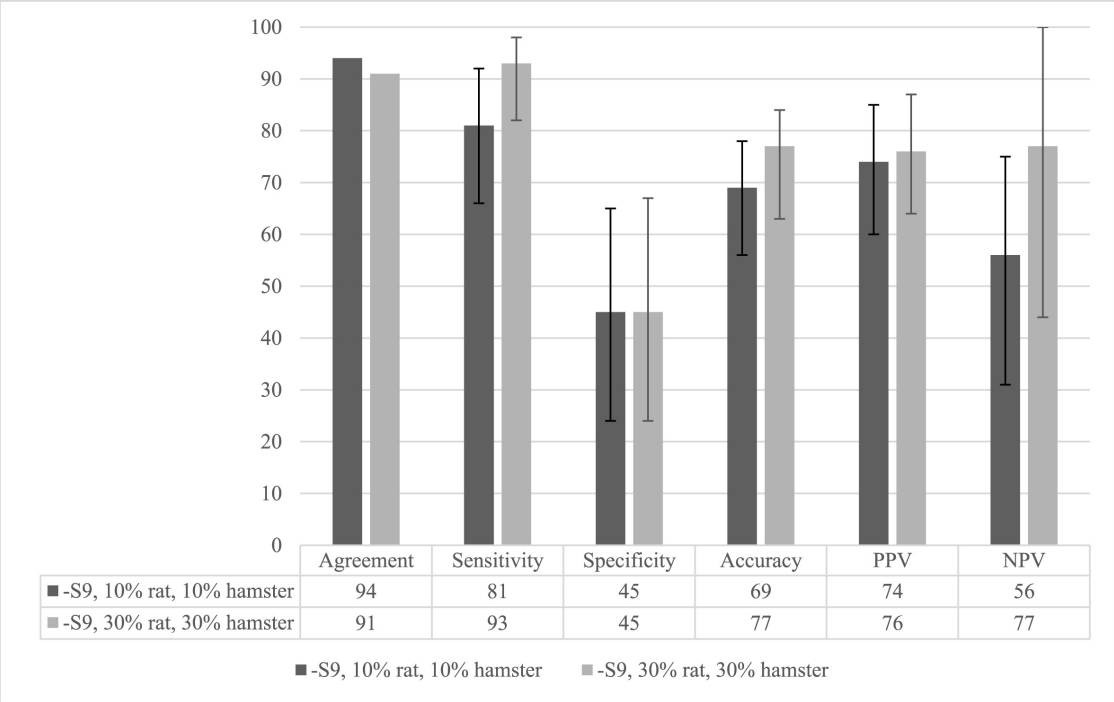
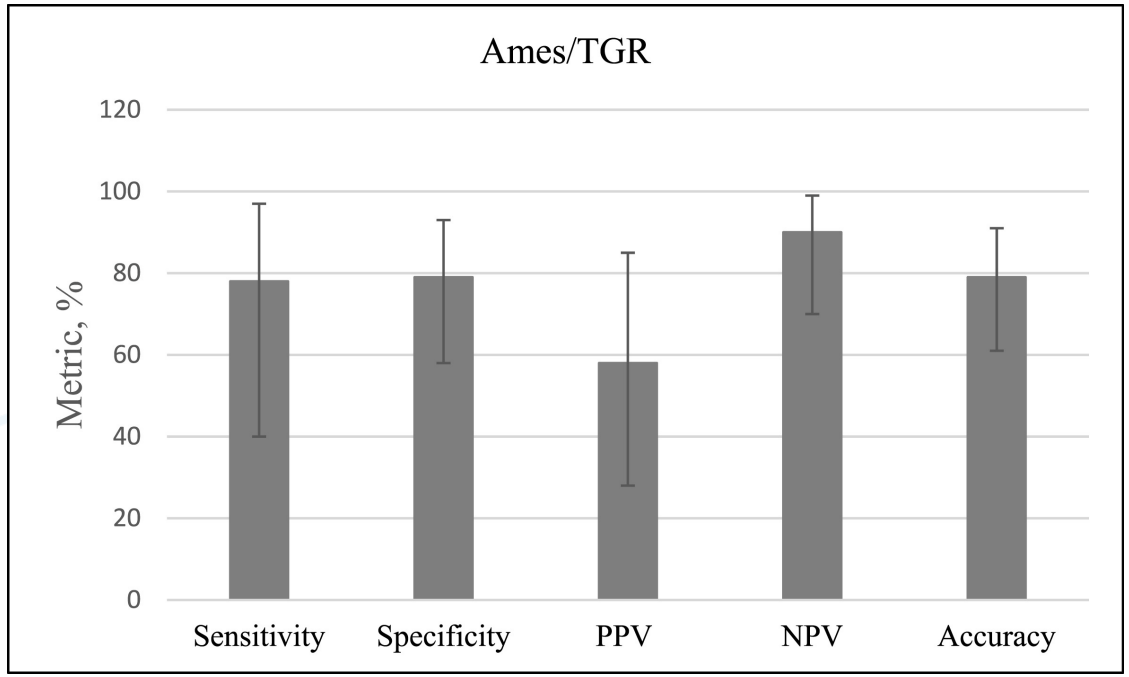
Ames performance versus carcinogenicity and transgenic rodent mutation assay



	Agreement	Sensitivity	Specificity	Accuracy	PPV	NPV
-S9, 10% rat, 10% hamster	94	81	45	69	74	56
-S9, 30% rat, 30% hamster	91	93	45	77	76	77

Ames vs carcinogenicity for 29 NAs, 3 N-nitroso compounds (64 tests)

Bercu et al., 2025. Regul Toxicol Pharmacol. 161:105835.



Ames vs Transgenic Rodent Mutation Assay for 33 NA drug substance related impurities

Jolly et al., 2026. Regul Toxicol Pharmacol. 167:106501.

Data supports design of Enhanced Ames Test

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EAT Positive Control Experimental Work

Goal: Identify appropriate nitrosamine-related positive controls for the optimized Ames protocol
Activities: The subteam will perform a multi-site experimental trial, where 5 compounds will be tested at 3 labs/compound across 15 lab sites. Work will also include in vitro metabolism characterization of the positive controls (Met ID Studies).

Organizations	Compound	CAS
AbbVie	NNK (N-methyl-N-(4-oxo-4-(pyridin-3-yl)butyl)nitrous amide)	64091-91-4
GSK	N-Nitroso Varenicline	2755871-02-2
Novartis	N-nitroso nortriptyline	555855-42-0
CRL	N-nitroso-lorcaserin	2518136-84-8
Eli lilly	N-Nitroso duloxetine	2680527-91-5
Inotiv	NNK (N-methyl-N-(4-oxo-4-(pyridin-3-yl)butyl)nitrous amide)	64091-91-4
Gilead	N-nitroso nortriptyline	555855-42-0
Sanofi	N-nitroso-lorcaserin	2518136-84-8
Merck & Co, Inc.	N-Nitroso duloxetine	2680527-91-5
GenEvolutioN	N-Nitroso Varenicline	2755871-02-2
NCTR	NNK (N-methyl-N-(4-oxo-4-(pyridin-3-yl)butyl)nitrous amide)	64091-91-4
Merck Healthcare KGaA	N-nitroso nortriptyline	555855-42-0
Perrigo	N-nitroso-lorcaserin	2518136-84-8
Boehringer Ingelheim	N-Nitroso duloxetine	2680527-91-5
Gentronix	N-Nitroso Varenicline	2755871-02-2

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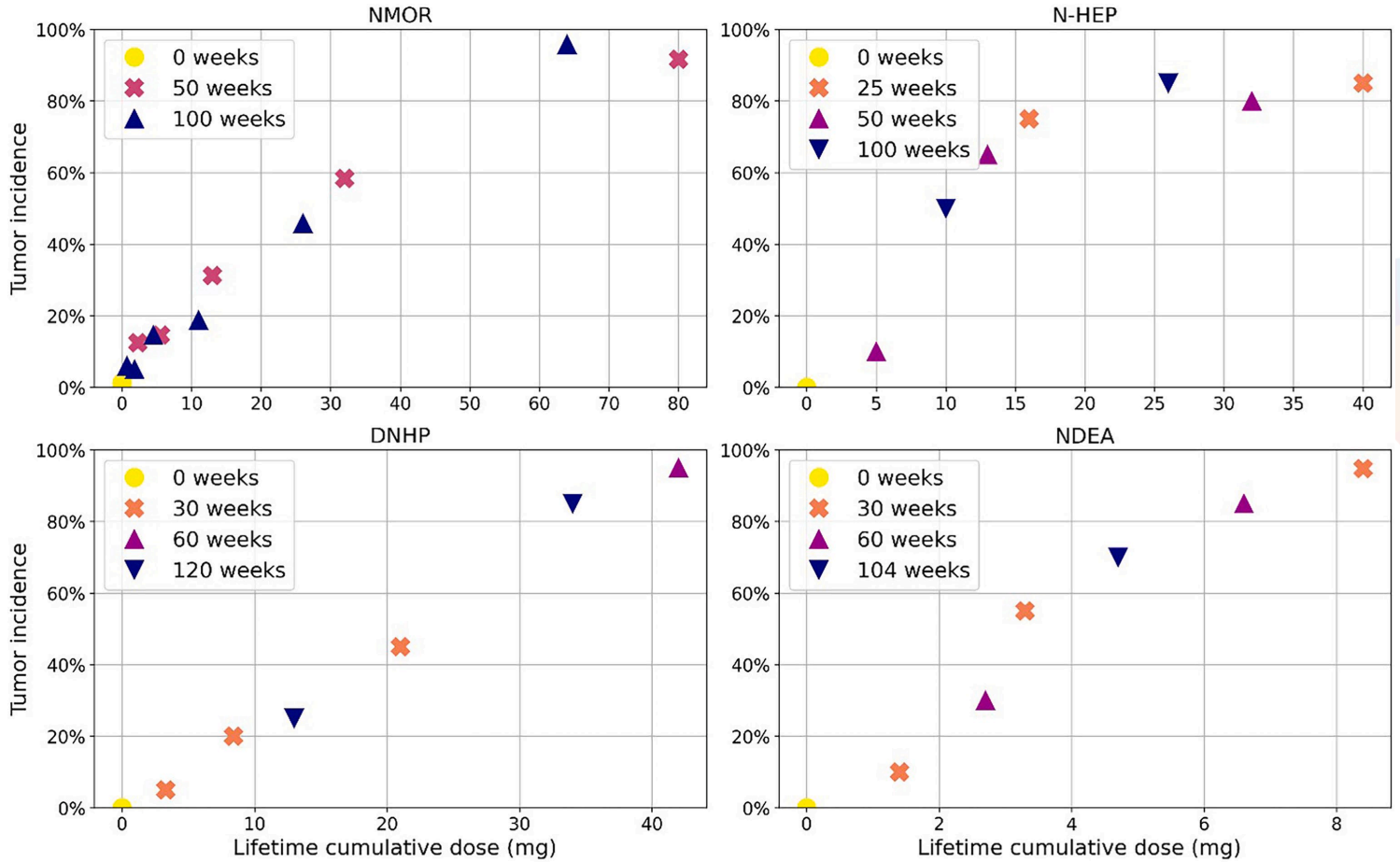
Lifetime cumulative dose is a critical aspect of NA carcinogenicity supporting the ICH M7 LTL concept



Supported by evaluation of carcinogenicity data spanning 21-120 weeks for 8 NAs plus aflatoxin B1.

Felter et al., 2025. Regul Toxicol Pharmacol. 162:105903

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Future work less than lifetime

- Literature review
  - Evaluate the relevance of single, intermittent, and less-than-lifetime exposures in nitrosamine risk assessment, including whether acute exposures may exceed DNA repair capacity.
  - Examine the suitability of current ICH M7 adjustment factors using insights from exemplar compounds, polypharmacy scenarios, and intermittent dosing data.
- Experimental Work
  - Develop and implement a study design to evaluate how exposure duration influences mutagenic outcomes for NAs to inform future LTL risk assessment approaches.

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Conclusions

- HESI NRP has been advancing scientific initiatives to support ICH M7 guidance on N-nitrosamines
- Ames Optimization
  - Milestone – achieved optimal performance with carcinogenic and in vivo mutagenicity outcomes
  - Ongoing work on positive controls
- Less-than-lifetime
  - Milestone – Data supporting the impact of cumulative dose on carcinogenic outcome, supporting the LTL principles for NAs
  - Ongoing work – Literature analysis to address key unresolved issues. In vivo TGR studies to determine the impact of cumulative dose on mutagenicity

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