2025 Genetic Toxicology Association Annual Meeting

John M. Clayton Hall Conference Center

University of Delaware

Newark, Delaware

May 7-9, 2025

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2025 Annual Meeting of the GTA

John M. Clayton Hall Conference Center
University of Delaware, Newark, DE

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Annual Meeting Online Portal

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Find up-to-date information on the GTA at https://gta-us.org/

Welcome from the Chair and Chair-Elect

Dear Colleagues,

On behalf of the GTA BOD and volunteers, we welcome everyone to the 2025 Genetic Toxicology Association Meeting! We are thrilled to have attendees joining us from all corners of the globe.

This year, we have an exciting agenda lined up for you, featuring two enriching workshops and six symposia, each designed to provide valuable insights and foster stimulating discussions.

We understand that recent changes have affected some of our members and this years GTA meeting provides an opportunity to connect as a community. In these challenging times, we want to assure you that the Genetic Toxicology Association stands with you.

We value the unique perspectives and experiences our members bring and encourage you to connect with each other, share your insights, and build lasting relationships.

Once again, we are delighted to have you with us. Let's make the most of this opportunity to learn, network, and support each other. Enjoy the meeting!

Sincerely,

Wen Sun PhD
2024-2025 GTA Chair

Ashley Allemang MS
2024-2025 GTA Chair Elect

GTA BOD & Volunteer 2024 Attendees, from left to right, Dan Roberts, Leon Stankowski, Yi Yang, Elizabeth Rubitski, Penny Leavitt, Wen Sun, Ashley Allemang, Abby Myhre. Photo credit: Rajib Ghosh

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(alphabetical order)

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2025 GTA Meeting Sponsors

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2025 GTA Meeting Exhibitors

(alphabetical order)

Inotiv
2701 Kent Avenue
West Lafayette, IN 47906
USA
Phone: 800-845-4246
Email: marketing@inotiv.com
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Contact: Diane Brecha, Director, Safety Assessment Portfolio Marketing,
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The Inotiv Team welcomes you to the 2025 GTA Annual Meeting!

Your Discovery & Development Partner: Expect More with Inotiv. Inotiv delivers a comprehensive and integrated range of genetic toxicology services as well as right-size toxicology services, bioanalysis, pathology, DMPK & discovery solutions, backed by our commitment to personalized attention, insightful expertise, and on-time, quality data delivery. As your partner, let our experts bring you to your goal faster.

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- **Genetic toxicity (Q)SARs**: Complete solutions for the computational assessment of genetic toxicity, including statistical-based and expert rule-based models.

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For over 25 years, MultiCASE has developed state of the art computational toxicology software platforms for evaluating biological activity and toxicity potential for chemicals. Our software applications are used by regulatory agencies, pharmaceutical, chemical, and cosmetic companies worldwide. Consulting and licensing services are provided to fulfill REACH requirements or ICH M7 Step 2 compatible in silico evaluation for drug candidates, impurities, and reagents.

Wednesday, May 7th Program

12:00 – 1:30 PM	**Conference Registration**	**Lobby A / Registration Desk**

1:30 – 3:30 PM **Auditorium 125**

Workshop 1
Next Generation Genotoxicity Risk Assessments: AOPs as the foundation of NAM-based risk assessments for genotoxicity
Co-chairs: Giel Hendriks (Toxys) & Susanne Stalford (Lhasa)

In order to improve genotoxicity hazard prediction and develop a risk assessment strategy for genotoxicity based on in vitro data, new approach methodologies (NAMs) have been developed to enable mechanistic understanding of effects, thus predicting adverse human health outcomes. Methodologies, such as ToxTracker, multiflow and error-corrected Next Generation Sequencing (NGS), provide vast amounts of diverse data about genotoxicity and mutagenicity. Therefore, it is important to organize this evidence to provide an overview which can be used to address data gaps,and support robust decision-making when drawing a conclusion as to potential activity. Utilising adverse outcome pathways (AOPs), within tools such as Kaptis, enables the rationalisation of evidence from NAMs, alongside in silico predictions, and data from more traditional in vitro and in vivo assays. This approach helps to build a transparent, scientifically robust and reproducible picture which supports the decision-making process. The aim of this workshop is to highlight progress in this area, discussing how NAMs and AOPs can be effectively applied in tandem for genotoxicity risk assessment, and potential future work in the area.

1:30 – 1:40 PM	**Workshop introduction** Co-chairs: Giel Hendriks PhD (Toxys) & Susanne Stalford PhD (Lhasa)
1:40 – 1:55 PM	**Building weight of evidence to support indirect clastogenic effects within an AOP framework** Dan Roberts MS (Toxys)
1:55 – 2:10 PM	**Contextualising Data from New Approach Methods Using Adverse Outcome Pathways: A Recipe for Better Genotoxicity Decision Making** Tasha Jones BSc (Lhasa)
2:10 – 2:25 PM	**Assessing genotoxicity in multiple cell culture models for quantitative Adverse Outcome Pathway (qAOP) development** Caitlin Maggs MSc (Swansea University)
2:25 – 2:40 PM	**Human hepatic HepaRG cells - a new workhorse in your genetox NAM toolbox?** Ashley Allemang MS (Procter & Gamble)
2:40 – 2:55 PM	**What's in the wiki (or coming soon)? International, multi-sector efforts to build genotoxicity Adverse Outcome Pathway networks** Carole Yauk PhD (University of Ottawa)
2:55 – 3:30 PM	**Moderated panel discussion, Q&A and closing statements**

3:30 – 4:00 PM

Coffee Break

HESI Genetic Toxicology Technical Committee

4:00 – 6:00 PM **Auditorium 125**

Workshop 2

The Last Mile: Opportunities to Bridge Research and Increase Impact in Human and Environmental Health Science

Co-chairs: Connie L. Chen PhD, MPH (HESI) & Raechel Puglisi MPH (HESI)

HESI Genetic Toxicology Technical Committee

Scientific research often takes decades to progress from the conceptualization of a scientific question to application, whether that involves practice changes in research, seeing a drug to commercialization or the development (or revision) in a regulatory guideline. “The Last Mile” for human and environmental health applications can be defined as translating basic research that is targeted to a specific application and/or user groups (e.g., new tools are being used or deployed, outreach to affect behavior change, specialized regulation or legislation, development of medications to assess or increase tool, product or approach utility). This workshop will dive deeper into what “The Last Mile” means for human and environmental health, with a focus on genetic toxicology. It will highlight key efforts from the Health and Environmental Sciences Institute (HESI) that are moving research into application to yield positive change. Case studies from the Genetic Toxicology Technical Committee (GTTC), Emerging Systems Toxicology for the Assessment of Risk (eSTAR) Committee and the Botanical Safety Committee (BSC) will be highlighted. The session will close with a moderated panel discussion on improving the translation of research into impactful applications and increasing its uptake.

4:00 – 4:20 PM	**What is the Last Mile and Why Should You Care?** ***Introduction to the concept and its relevance to human and environmental health*** Raechel Puglisi MPH (HESI)
4:20 – 4:40 PM	**Advancing Genetic Toxicology: The Role of AOPs and/or in vitro NAMs in HESI GTTC** Wen Sun PhD (Pfizer)
4:40 – 5:00 PM	**HESI eSTAR OASIS: Leveraging ‘Omics Data for Next Gen Safety Assessment) development** Srijit Seal PhD (Merck Co & Inc., Broad Institute of MIT and Harvard)
5:00 – 5:20 PM	**HESI Botanical Safety Consortium: Towards a Global Genotoxicity Testing Strategy for Botanicals** Stefan Pfuhler PhD (Procter & Gamble)
5:20 – 5:40 PM	**From Discovery, to Validation and Implementation: The Journey of the TGx-DDI Biomarker** Carole Yauk PhD (University of Ottawa)
5:40 – 6:00 PM	**Moderated panel discussion, Q&A and closing statements**

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Thursday, May 8th Program

7:30 – 8:30 AM	**Conference Registration & Breakfast**	**Lobby A / Registration Desk**
8:30 – 8:35 AM	**Welcome and Introduction** Wen Sun PhD (Pfizer) 2024-2025 GTA Chair	**Lobby A / Registration Desk**

8:35 – 9:35 AM — **Auditorium 125**

Keynote Address

Integrating -omics approaches to improve genotoxicity analysis

Dr Silvia Balbo (Professor, Division of Environmental Health Sciences at the University of Minnesota)

9:35 – 10:00 AM

Coffee Break

10:00 – 11:30 AM — **Auditorium 125**

Symposium I
Regulatory Acceptance of New Approach Methodologies (NAMs)

Co-Chairs: James Kath PhD (AbbVie) & Stephanie Smith-Roe PhD (NIH/NIEHS)

10:00 – 10:25 AM	**An Overview of the Regulatory Approval Pipeline for New Approach Methodologies: Implementing Human-relevant Testing Approaches** Hans Raabe MS (Institute for In Vitro Sciences)
10:25 – 10:50 AM	**New Approach Methodologies Used in the Safety Assessment of Food Contact Substances.** Laura C. Markley PhD (HFP/FDA)
10:50 – 11:15 AM	**Validation and Implementation of the ToxTracker Assay for Mechanistic Genotoxicity Assessment** Giel Hendriks PhD (Toxys)
11:15 – 11:30 AM	**Panel discussion, Q&A** Moderated by James Kath PhD & Stephanie Smith-Roe PhD

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11:30 – 12:30 PM — **Auditorium 125**

Excellence in Science & Service Awards
Sheroy Minocherhomji PhD, ERT, FRSB (Lilly) & Penny Leavitt MS, DABT (BMS)

12:30 – 1:45 PM

Networking Lunch

1:45 – 3:15 PM — **Auditorium 125**

Symposium II
State-of-the-art for Genotoxicity and Carcinogenicity Strategies of Novel Modalities

Co-Chairs: Yi Yang PhD, DABT (AbbVie) & Sheroy Minocherhomji PhD, ERT, FRSB (Lilly)

1:45 – 2:05 PM	**Genetox Approaches for Therapeutic Peptides: Results of an Industry Survey** Yi Yang PhD DABT (AbbVie)
2:05 – 2:25 PM	**A Move Towards Tailored Genetic Toxicity Approaches for Oligonucleotide Therapeutics: Results of an Industry Survey** Natalie Holman PhD DABT (Lilly)
2:25 – 2:45 PM	**Genotoxicity Evaluation of Gene Therapy Products** Silvana Libertini PhD (Novartis)
2:45 – 3:05 PM	**Mapping CRISPR within the atlas of genotoxic risk** Jonathan Phillips PhD (Intellia)
3:05 – 3:15 PM	**Panel discussion, Q&A** Moderated by Sheroy Minocherhomji PhD, ERT, FRSB

3:15 – 3:45 PM

Coffee Break

Product Safety Labs
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3:45 – 5:30 PM — **Auditorium 125**

Symposium III
ecNGS techniques and their applicability to genotoxicity and carcinogenicity testing
Co-Chairs: Carole Yauk (University of Ottawa) and Patricia Escobar PhD (Merck & Co., Inc.)

3:45 – 4:10 PM	**Closing the gap: IWGT recommendations on the adoption of ecNGS for regulatory mutagenicity testing** Stephanie L. Smith-Roe (NIEHS)
4:10 – 4:35 PM	**Characterization of Mutational Load Using Single-molecule Mutation (SMM) Sequencing Assay** Alex Maslov PhD (Mutagentech)
4:35 – 4:55 PM	**Duplex Sequencing after Prolonged Benzo[b]fluoranthene Exposure Reveals Tissue-Specific Differences in Mutagenic Response, Chemical Potency, and Clonal Expansion of Mutations** David Schuster MS (University of Ottawa)
4:55 – 5:20 PM	**Toward more predictive and human relevant carcinogenicity testing (b): Establishing study design and approach for detecting cancer driver gene mutations using Duplex Sequencing** Patricia Escobar (Merck Co & Inc.) and Alper James Alcarez (University of Ottawa) *Patricia Escobar speaking*
5:20 – 5:30 PM	**Panel Discussion: Given the improvement of ecNGS over the transgenic rodent mutation assay, and its demonstrated superior performance, what challenges do you anticipate encountering in OECD endorsement process (for integration with other 28-day tests)? What are the best approaches to overcome these challenges?** Moderated by Carole Yauk PhD

5:30 – 7:30 PM — **Auditorium 125**

Poster Session & Cocktails

5:30 – 6:30 PM Odd numbered posters attended
6:30 – 7:30 PM Even numbered posters attended

7:30 –9:00 PM

Banquet Dinner
(Included in 2-day registration)

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2025 GTA Board of Directors Nominations

The GTA Board of Directors (BOD) will have two seats open at the conclusion of the 2025 meeting. Three candidates have been proposed, for full candidate bios and voting, please use the QR code below.

Abha Aggarwal PhD, MSPH, MS
Labcorp

Tetyana Cheairs MD, MSPH
New York Medical College

Robert Foster PhD
Lhasa Limited

Voting ends 8am Fri. May 9th 2025!

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Friday, May 9th Program

7:30 – 8:30 AM	**Conference Registration & Breakfast**	**Lobby A / Registration Desk**

8:30 – 8:35 AM	**Welcome to Day 3** *Ashley Allemang (Procter & Gamble)* *2024-2025 GTA Chair-Elect*

8:35 – 10:05 AM — **Auditorium 125**

Symposium IV
Metabolism as a Potentiator of Genetox Risk Assessment

Co-Chairs: Stefan Pfuhler (Procter & Gamble) & Tetyana Cheairs (NYMC)

8:35 – 9:05 AM	**Importance of metabolism in different NAMs to evaluate genotoxic potential for test materials** Yax Thakkar PhD (RIFM)
9:05 – 9:35 AM	**Modification of the co-factor mix for S9 treatments to improve genotoxicity predictions** Stefan Pfuhler PhD (Procter & Gamble)
9:35 – 10:05 AM	**Optimizing the in vitro metabolization protocol for the genotoxicity assessment of N-Nitrosamines in mammalian cell assays** Inger Brandsma PhD (Toxys)

10:05 – 10:35 AM

Coffee Break

10:35 – 12:05 PM **Auditorium 125**

Symposium V
Best Practices for Genetox Testing of Excipients, Impurities

Bristol Myers Squibb™

Co-Chairs: Laura Markley (FDA), John Nicolette (Johnson & Johnson), & Penny Leavitt (BMS)

10:35 – 11:05 AM	**Regulatory Framework and Case Studies for Evaluating Genotoxic Potential of Impurities in Food Contact Substances** Leighna Holt BS (Human Foods Program, US FDA)
11:05 – 11:35 AM	**Special considerations for genotoxicity testing of particles** David Kirkland BSc, PhD (Kirkland Consulting)
11:35 – 11:50 AM	**Genotoxicity testing of impurities in adjuvants** Sandy Weiner MS (Johnson & Johnson)
11:50 – 12:05 PM	**Combination Small Molecule Delivering Medical Device Considerations for Genetic Toxicity Testing** Steven Nicotra BS, MBA (Johnson & Johnson)

12:05 – 1:05 PM

Networking Lunch

1:05 – 1:15 PM

Business Meeting
Leon Stankowski Jr. (CRL) GTA Treasurer

1:15 – 1:40 PM **Auditorium 125**

Student and Early- Stage Investigator Awards
Sheroy Minocherhomji PhD, ERT, FRSB (Lilly) & Penny Leavitt MS, DABT (BMS)

2025 Annual Meeting of the GTA

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2:00 – 3:30 PM — **Auditorium 125**

Symposium VI
Nitrosamines - Current Updates, Regulatory Experience

Co-Chairs: Kevin Cross (Instem) & Leon Stankowski Jr. (CRL)

1:40 – 2:00 PM	**Current Regulatory Considerations on the Safety Assessment of Nitrosamine Impurities** Tim McGovern, PhD (White Oak Regulatory Tox, LLC)
2:00 – 2:20 PM	**Comparative Potency of N-Nitrosomorpholine and N-Nitroso Reboxetine** Shaofei Zhang PhD (Pfizer)
2:20 – 2:40 PM	**The Enhanced Ames Test – One CRO's Perspectives and Experiences** Leon Stankowski Jr. PhD (CRL)
2:40 – 3:00 PM	**In Vivo Mutation Frequency of NNK as determined in the Big Blue Rat and using error-corrected Next Generation Sequencing (ecNGS)** Jessica Noteboom BS (Eli Lilly)
3:00 – 3:20 PM	**(Q)SAR of nitrosamines and the CPCA. Where do we go from here?** Kevin Cross PhD (Instem)
3:20 – 3:30 PM	**Panel discussion, Q&A** Moderated by Leon Stankowski Jr.

3:30 – 3:40 PM	**Concluding Remarks** *Ashley Allemang (Procter & Gamble)* *2024-2025 GTA Chair-Elect*

Meeting Feedback Survey
Let us know how we did, provide suggestions for next year, volunteer and more!

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Keynote Address

Integrating Omics Approaches To Improve Genotoxicity Analysis

Silvia Balbo PhD
Professor
Division of Environmental Health Sciences
University of Minnesota

Dr Balbo got her PhD in Drug Science from the University of Torino (Italy). Her extensive laboratory experience in organic synthesis and genetic toxicology was then complemented by her training in molecular epidemiology during her post-doctoral work at the International Agency for Research on Cancer (IARC) in Lyon, France. With this background, Dr Balbo moved to the Masonic Cancer Center at the University of Minnesota with the ability to understand and propose an interdisciplinary approach to the challenging and complex questions concerning the investigation of chemical carcinogenesis. Bridging the disciplines of molecular epidemiology and chemistry, her focus became to develop methods using Mass Spectrometry to investigate the potential role of chemical genotoxicity in lung, colon and upper aerodigestive tract cancers.

Invited Speaker Abstracts

Ashley Allemang MS

Procter & Gamble, Cincinnati, Ohio, USA

Ashley is a Senior Scientist in Global Product Stewardship at Procter & Gamble. Ashley has over 10 years of industry experience in applied genetic toxicology in the context of in vitro-based safety support. Her research has primarily focused on mode of action determination and distinguishing direct and indirect genotoxicity through various in vitro methods such as the micronucleus assay, the ToxTracker assay and other genomics-based methods such as the TGx-DDI biomarker. More recently her research has employed the HepaRG micronucleus assay to develop in vitro-based genotoxicity potency rankings of pyrrolizidine alkaloids, as well as genotoxicity evaluation of mixtures. In addition to her research activities, her expertise has also expanded to include SAR based risk assessment. Ashley has been actively involved in the HESI GTTC committee since 2017 and has participated in the development of genetox-related AOPs and is currently co-leading the Indirect Genotoxicity subgroup of the In Vitro Work Group evaluating NAMs for genetic toxicity testing. Ashley is also serving as the GTA Secretary.

Workshop 1 - Next Generation Genotoxicity Risk Assessments: AOPs as the foundation of NAM-based risk assessments for genotoxicity

Human hepatic HepaRG cells - a new workhorse in your genetox NAM toolbox?

With the increasing use of the Adverse Outcome Pathway (AOP) framework, establishing a well-equipped toolbox of New Approach Methodologies (NAMs) to evaluate genotoxicity related molecular initiating events, key events and adverse outcomes is important for developing more comprehensive risk assessment strategies. The metabolically competent human hepatic cell line HepaRG is increasingly being used in genetic toxicology as it may provide a more accurate representation of human metabolism than traditional exogenous metabolism sources such as rat liver S9 or other metabolically competent cell lines like HepG2. HepaRG cells have been used in several NAM approaches applicable to the recently developed AOP for oxidative DNA damage leading to chromosomal aberrations and mutations. Oxidative DNA damage is of particular interest as it is a common cause of false or 'irrelevant' positive in vitro responses and the respective AOP framework helps to identify key NAMs that can be employed to evaluate their biological relevance. Research will be presented evaluating HepaRG cells in two NAMs relevant to the oxidative stress AOP. First, it will be demonstrated using a diverse set of chemicals, including non-genotoxic and genotoxic compounds with direct and indirect mechanisms of action, that HepaRG cells have similar sensitivity compared to TK6 cells in the MicroFlow assay evaluating micronucleus induction. Second, proof-of-principle research will be presented demonstrating the compatibility of HepaRG cells with error corrected sequencing technologies evaluating mutation. In total, the work presented will demonstrate that the HepaRG cell line is an important in vitro tool for enabling more human-relevant genotoxicity risk assessment.

Inger Brandsma PhD

Toxys, Oegstgeest, ZH, Netherlands

Inger Brandsma is the director of Genotoxicity at Toxys. She has a background in DNA repair and breast cancer research and started at Toxys as senior scientist in 2018. Since then, she has been involved in the development of new assays as well as customer projects as study director.

Symposium IV - Metabolism as a Potentiator of Genetox Risk Assessment

Optimizing the in vitro metabolization protocol for the genotoxicity assessment of N-Nitrosamines in mammalian cell assays

N-Nitrosamines (NAs) are probable human carcinogens found in tobacco, food, cosmetics, and many other products. Recently, NAs were detected as impurities in pharmaceuticals, which led to a concern for human health. NAs require metabolic activation before they become mutagenic, and not all NAs are mutagenic since their reactivity is related to their structure. While some NAs are potent mutagens *in vivo*, *in vitro* metabolization with exogenous S9 liver extract is generally less efficient. While an enhanced bacterial mutagenicity protocol was recently developed, which uses increased concentrations of S9 liver extracts, there presently is not an improved metabolization protocol suitable for mammalian cell genotoxicity assays. Therefore, we optimized a hamster S9 liver extract-based protocol for *in vitro* NA metabolization and assessed the genotoxic potential of various NAs using ToxTracker. With this enhanced

metabolization protocol (EMP), the genotoxic potency of N-nitrosodimethylamine (NDMA) increased approximately 200-fold when compared to the standard S9 liver extract-based exposure protocol in ToxTracker. The EMP was validated using a set of linear and cyclic NAs that were previously shown to be mutagenic in the Ames test, as well as nitrosamine drug substance related impurities (NDSRI), and non-mutagenic NAs. We showed that the protocol is applicable to a range of NAs. Genotoxicity could be confirmed for six out of seven mutagenic NAs using the EMP and the two non-mutagenic NAs were classified as non-genotoxic. This demonstrates that mammalian cells, and the new approach methodology (NAM) ToxTracker, may have potential when investigating NA-related genotoxicity.

Kevin Cross

Instem, Columbus, Ohio, USA

Kevin P. Cross, Ph.D., is the Senior Director of in silico Science at Instem where he is the Principal Investigator of U.S. FDA/Instem research collaborations. He has been developing chemoinformatics tools and products for over 40 years. He is involved in several collaborative efforts creating in silico protocols and procedures for performing chemical hazard and risk assessments for regulatory purposes as well as developing and assessing the performance of (Q)SAR models. He has recently been focusing on (Q)SAR risk assessment of nitrosamines and the development of in silico read-across methods. He has published over 50 papers and 3 book chapters.

Symposium VI - Nitrosamines - Current Updates, Regulatory Experience

(Q)SAR of nitrosamines and the CPCA. Where do we go from here?

The development of the Carcinogenic Potency Categorization Approach (CPCA) was a major advancement by international health authorities for the assessment of N-nitrosamine drug substance-related impurities (NDSRIs) using an algorithm based upon Structure Activity Relationships. By assessing the reactivity effects of chemical groups adjacent to the N-nitroso group (and other simple considerations), local potency contributions were defined with their summation leading to 5 different potency categories with associated Acceptable Intake limits. As limited availability of reliable animal potency data has limited the precision and accuracy of the CPCA, an investigation into using Quantum Mechanical (QM) methods to validate and refine the CPCA has been undertaken by the HESI Genetic Toxicology Technical Committee. Here QM methods (anchored to existing data) are being used to evaluate the relative potency contributions of electron withdrawing (and donating) groups adjacent to the N-nitroso group for both acyclic and cyclic nitrosamines, the stability and solubility of intermediate ions, and DNA reactivity. Preferred reaction pathways are being identified, and correlations between local reactivity steps and measure properties are being discovered. The goals of this effort are to reinforce and expand the CPCA rules and to illustrate how QM methods may be applied to estimate potency for NDSRIs.

Patricia Escobar

Merck & Co. Inc., West Point, PA, USA

Dr. Escobar is an Executive Director in the Genetic toxicology and mutagenic impurities group. Dr. Escobar is an internationally recognized genetic toxicologist with experience in academia, a contract laboratory and the pharmaceutical industry, and a frequent participant in international collaborative workshops and enterprises such as development of OECD guidelines. Dr. Escobar received a B.Sc. in Microbiology and a M.Sc. in genetic toxicology from the Universidad de los Andes in Bogotá Colombia, and her Ph.D. in Molecular Toxicology from the University of Pittsburgh. She then completed her postdoctoral training in the Gene & Environment Laboratory at University of California, Berkeley with at Martyn Smith lab. Following her post-doc, Dr. Escobar worked at BioReliance as a Genetic Toxicology Study Director. In 2008, Dr. Escobar joined Boehringer Ingelheim Pharmaceuticals, in the Nonclinical Drug Safety group in the US site, where she had a series of roles with increasing responsibility managing and leading the predictive toxicology group, while also serving as the genetic toxicology scientific lead for this group and as a toxicologist in drug discovery teams. Dr. Escobar joined Merck Co. & Inc. in 2015 and she has been responsible for screening and bringing forward the best drug candidates from Discovery; for the GLP regulatory genotoxicity testing and mutagenic impurities; and for developing follow-up strategies and application of new technologies for screening and for understanding mechanisms of genotoxicity to support compound selection and risk assessment. Dr. Escobar has contributed widely to international efforts in genetic toxicology, such as the International Working Groups on Genetic Toxicology (IWGT) and the Expert Working Group on the OECD In vivo Mammalian Alkaline Comet Assay, and the OECD group working on the Bacterial

Reverse Mutation testing guideline. Dr. Escobar is a former board chair and member of the Genetic Toxicology Association (GTA) and a council member of the Environmental Mutagenesis and Genomics Society (EMGS). For EMGS, she has chaired special interest groups on Applied Genetic Toxicology, and on New Technology, and the Alexander Hollander Committee. Currently, she is a member of the OECD Genetox expert working group, the Health and Environmental Sciences Institute (HESI) Genetic Toxicology Technical Committee (GTTC) and the Emerging Systems Toxicology for the Assessment of Risk (eSTAR) Committee. In addition, Dr Escobar is a board member of HESI. Dr. Escobar is author/co-author of more than 30 publications including peer-reviewed articles and/or book chapters.

Symposium III - ecNGS techniques and their applicability to genotoxicity and carcinogenicity testing

Toward more predictive carcinogenicity assessment: Evaluating Duplex Sequencing for Detecting Clonal Expansion as a Potential Early Carcinogenicity Biomarker

The gold-standard 2-year rodent cancer bioassay for carcinogenicity testing is costly, time-consuming, and requires ~800 rodents. Regulated sectors are now moving towards more mechanistic, resource-efficient assays. Quantifying clonal expansion (CE) of cells carrying cancer driver gene mutations (CDMs) offers a promising early biomarker of carcinogenicity. However, conventional next-generation sequencing (NGS) struggles to detect low-frequency CDMs. Duplex Sequencing (DuplexSeq), an error-corrected NGS (ecNGS) technology, enables the detection of ultra-rare mutations. The HESI eSTAR ecNGS working group is testing DuplexSeq for detecting CE of cells carrying CDMs as a potential early biomarker for tumorigenicity, including the development of study design and analytical pipelines. This study evaluated DuplexSeq assay transferability and compared tissue sampling strategies—multiple small punches versus large tissue homogenate—for quantifying CE. Liver samples were from mice fed for 90 days with either a standard diet or a diet supplemented with a non-genotoxic imidazolidine analogue that induced hepatocellular carcinoma in a 2-year bioassay. Libraries targeting hotspots from 27 cancer driver genes were generated from 1 µg of punch or homogenate DNA and sequenced to >30,000x duplex depth. High-quality libraries were obtained, demonstrating assay transferability. Preliminary results show that CE of cells carrying CDMs are evident in punches and tissue homogenates, without apparent differences between sampling strategies. Further analyses will determine if treatment differences can be detected. Current findings suggest DuplexSeq can sensitively detect CDMs in punches and homogenates, potentially enabling the use of this technology in archived samples from carcinogenicity studies.

Giel Hendriks PhD

Toxys, Oegstgeest, ZH, Netherlands

Giel Hendriks is the founder and CEO of Toxys. As CEO of Toxys, he worked to develop the company into an internationally recognized contract research organization (CRO) in chemical safety testing for industry. He has developed various in vitro assays to ensure the safety of novel medicines, chemicals and consumer products without the use of animals. He is an expert in genetic toxicology and has been co-chairing various expert working groups at the HESI GTTC and is the current president of the Dutch Environmental Mutagen Society. Giel has a PhD in molecular cell biology from Utrecht University and worked as a post-doctoral fellow in at Leiden University and Leiden University Medical Center.

Symposium I - Regulatory Acceptance of New Approach Methodologies (NAMs)

Validation and implementation of the ToxTracker assay for mechanistic genotoxicity assessment

ToxTracker is a mammalian cell reporter assay that accurately predicts the genotoxic properties of compounds. By evaluating induction of various reporter genes that play a key role in cellular pathways relevant for genetic toxicology, ToxTracker has the advantage of providing insight into chemical mode-of-action (MoA), thereby discriminating direct-acting genotoxicants from cytotoxic chemicals that induce DNA damage secondarily. To investigate how ToxTracker may complement the standard battery of *in vitro* genotoxicity assays, a comprehensive interlaboratory validation trial was conducted. The goal of this prospective validation study was to explore the applicability of ToxTracker for regulatory applications, establish the transferability and reproducibility of the assay and to explore how it can be applied to improve the in vitro genotoxicity testing strategies. In the validation trial, seven laboratories tested 64 genotoxic and non-genotoxic chemicals that together cover a broad spectrum of chemical spaces. The validation trial showed a good within-lab reproducibility (WLR) of 73-98%. The between-lab reproducibility (BLR) of ToxTracker was 83%. The interlaboratory validation confirmed the accuracy of ToxTracker to correctly predict in vivo genotoxicity of compounds with a sensitivity of 87% and a specificity of 90%. From this validation trial we concluded that ToxTracker is a robust *in vitro* assay for the accurate prediction of *in vivo* genotoxicity. With information on the MoA of chemicals

that is provided by the assay, ToxTracker is a valuable addition to the battery of genotoxicity assays that is applied for regulatory applications.

Leighna Holt BS

US FDA, College Park, MD, USA

Leighna Holt is a Toxicology Reviewer in the Division of Food Contact Substances at the Office of Food Chemical Safety, Dietary Supplements, and Innovation at HFP-FDA. She received her B.S. in Molecular Toxicology from UC Berkeley. After completion of her undergraduate degree, Ms. Holt harvested data from genetic toxicology studies to build the Chemical Evaluation and Risk Estimation System (CERES) knowledgebase. With the experience from this role, she became the Lead Database Coordinator for CERES, training interns and organizing data to be shared for the development of predictive toxicity models. Leighna is consulted for her expertise in genetic toxicology, searching internal FDA databases, and for development of in-house tools for review scientists to conduct their work more efficiently. She was a member of the OFAS Genetic Toxicology Working Group where she provided comments on OECD Test Guidelines and training on FDA's review of the mouse lymphoma assay. Ms. Holt currently serves as a HFP representative to the FDA Modeling and Simulation Working Group where she is working to foster cross-center collaboration and establish the credibility of modeling and simulation tools for regulatory decision making.

Symposium V - Best Practices for Genetox Testing of Excipients, Impurities

Regulatory Framework and Case Studies for Evaluating Genotoxic Potential of Impurities in Food Contact Substances

The Office of Food Chemical Safety, Dietary Supplements, and Innovation (OFCSDSI) in the Human Foods Program (HFP) at the Food and Drug Administration (FDA) is responsible for the pre- and post-market safety evaluation of food additives, including food contact substances (FCSs). Impurities of FCSs include residual starting materials, catalysts, adjuvants and breakdown products that are expected to result in dietary exposure from the intended use of the FCS. The Agency performs safety assessments based on identification of the FCS and its impurities, hazard identification, exposure assessment, dose response assessment, and risk characterization. The Delaney Clause of the Food, Drug, & Cosmetic Act's food additive provision prohibits the approval of a food additive if it is found to induce cancer in humans or animals when ingested or determined to be carcinogenic in toxicity studies which are appropriate for the evaluation of the safety of food additives. However, the Delaney Clause applies only to the additive itself. For potentially carcinogenic constituents or impurities of an additive FDA will derive a Lifetime Cancer Risk (LCR) and compare that LCR to established guidelines to determine if the exposure to the constituent or impurity is safe. Case examples to be presented have positive or mixed genotoxicity data, as well as a quantitative cancer risk assessment of a potentially carcinogenic impurity at the current dietary exposure level. Separately, OFCSDSI has developed technological tools to improve the efficiency of the processes of receiving regulatory submissions from external stakeholders and completion of in-house reviews.

Natalie Holman PhD, DABT

Eli Lilly and Company, Indianapolis, IN, USA

Natalie Holman, PhD, DABT is a Toxicology Project Leader at Eli Lilly and Company. Natalie is responsible for nonclinical safety assessment and regulatory strategies, supporting assets across phases of development and therapeutic areas. Prior to Lilly, she was a project toxicologist at Altavant and Roivant Sciences. Natalie is a Diplomate of the American Board of Toxicology and member of the American College of Toxicology, Society of Toxicology, and Genetic Toxicity Association. She is an active member of industry working groups for genetic toxicity and oligonucleotide safety assessment. She received her PhD in Toxicology and Certificate in Translational Medicine from the University of North Carolina Chapel Hill School of Medicine. She conducted her doctoral research in mechanistic hepatotoxicity at The Hamner Institutes for Health Sciences (formerly Chemical Industry Institute of Toxicology, CIIT).

Symposium II – Current State-of-the-art for Genotoxicity and Carcinogenicity Strategies for Novel Modalities

A Move Towards Tailored Genetic Toxicity Approaches for Oligonucleotide Therapeutics: Results of an Industry Survey

As synthesized molecules, oligonucleotide therapeutics (ONTs) are typically characterized in the standard nonclinical safety paradigm for small molecules, which includes a full battery of *in vitro* and *in vivo* genotoxicity tests. To study nonclinical safety approaches for ONT development, an Oligonucleotide Working Group (OWG) under the European Federation of Pharmaceutical Industries and Associations (EFPIA) conducted a comprehensive industry survey of historical testing results, current practices, and regulatory experience with genotoxicity assessment of ONTs (Parry et al, Nuc Acid Ther 2025). Responses from 29 pharmaceutical and biotechnology companies indicated that most conducted the standard small molecule genetic toxicity battery to support ONT development. Results showed a consistent absence of genotoxicity signals across a diverse range of ONT classes and chemistries, supporting previous observations. This extensive data set may provide precedence for well-established ONT modifications to be considered non-genotoxic. Survey results indicate the need for more tailored approaches to the assessment of ONT genotoxicity and nonclinical safety testing, providing valuable information for the recently approved ICH topic, S13: "Nonclinical Safety Evaluation of Oligonucleotide-Based Therapeutics".

Tasha Jones BSc

Lhasa Limited, Leeds, United Kingdom

Tasha Jones is a Scientist at Lhasa Limited, working on the software Kaptis to aid in decision making for carcinogenicity assessments. Tasha earned her BSc from Keele University and has experience in both clinical research and early development safety assessments working as a Research Scientist within the genotoxicity and in vitro assessment domains.

Workshop 1 - Next Generation Genotoxicity Risk Assessments: AOPs as the foundation of NAM-based risk assessments for genotoxicity

Contextualising Data from New Approach Methods Using Adverse Outcome Pathways: A Recipe for Better Genotoxicity Decision Making

Within the current regulatory paradigm for genotoxicity, there is an increasing availability of new approach methodologies (NAMs) which can be included in genotoxicity assessments to enhance testing strategies. Whilst many NAMs provide an abundance of information, it is not always easy to contextualise these outputs and identify where they can be integrated to give a more cohesive picture of genotoxicity. Adverse outcome pathways (AOPs) provide a framework to integrate NAMs alongside other evidence, allowing for the rationalisation of outputs, enabling transparent and scientifically robust conclusions to be made. To examine these benefits, a case study was performed using biomarker outputs of the in vitro ToxTracker® assay and the in silico decision support system Kaptis. To enable the integration of data from the ToxTracker®, new mechanistic knowledge was curated to link biomarker outputs to relevant key events within the AOP network housed within Kaptis. A dataset of 164 compounds was processed and genotoxicity predictions provided by Kaptis were compared in the presence and absence of data for the ToxTracker® assay. The approach of combining of ToxTracker ® and an AOP approach was able to elucidate mechanistic information for over half of the compounds analysed. Contextualisation of information from in silico systems, biomarker outputs and protein interaction assays on an AOP easily provided links between mechanisms and the adverse outcome. This enables better decision making for genotoxicity, building a more comprehensive picture for complex genotoxicity assessments and demonstrating the power how the use of NAMs can enable a more focused strategy, improving robustness and confidence in decisions made.

David Kirkland BSc, PhD

Kirkland Consulting, United Kingdom

David was Vice President of Scientific and Regulatory Consulting at Covance, Harrogate, until 2009 when became an independent genetic toxicology consultant. He has a BSc in microbiology and a PhD on cell transformation studies. Following post-doctoral fellowships at the Institute of Cancer Research he spent 30 years in contract research, at Toxicol Laboratories and Microtest Research Limited (later part of Covance). He has extensive experience with regulatory genotoxicity issues, has written numerous publications and is a regular podium speaker/chairperson.

He has received several awards:

- Fellowship of the UKEMS (2002).
- Honorary Professorship of the University of Wales, Swansea (2006)

- The first Industrial Genotoxicity Group (UKEMS) Distinguished Toxicologist Award (2010).
- The US EMGS Alexander Hollaender Award (2010) for scientific contributions to and global leadership in the field of genotoxicity testing.
- The Japanese EMS Kitashi Mochizuki Award (2014) for promotion of international harmonization of genotoxicity tests through the International Workshops on Genotoxicity Testing (IWGT) which he chaired for 20 years.
- The UKEMS Jim Parry award (2015) for outstanding contributions to the field of environmental mutagenesis.
- The EEMGS Frits Sobels Award (2022) for his contributions to regulatory genetic toxicology and guidelines development, notably founding and promoting IWGT.

He was Special Issues Editor for Mutation Research for many years, editorial board member of the Journal of Applied Toxicology, past president of the European EMGS and UKEMS, and a former councilor of US EMGS. David was a member of the UK Government Advisory Committee on Mutagenicity (UK COM) for 10 years and is an active member of the HESI Genetic Toxicology Technical Committee (GTTC).

Symposium V - Best Practices for Genetox Testing of Excipients, Impurities

Special considerations for genotoxicity testing of particles

Particulate materials such as TiO2, CaCO3, talc and kaolin are often used as excipients in pharmaceuticals. When testing reasonably soluble substances we assume cells will be exposed to more of the test substance, and more likely to induce genotoxic responses, at high than at low doses. We therefore choose a top dose based on cytotoxicity, solubility, or use the limit concentration/dose. If a soluble substance is negative at high doses, we do not usually have any concern for possible effects at lower doses. The approach with insoluble particles is different because behaviour in suspension, and ability to be taken up by cells, is different. Effects at low as well as high doses need to be studied. Evidence of uptake of particles into cells using TEM-EDX, should be included. Particle size distributions at different doses and time periods need to be measured, particularly in the vehicle used to treat the cells or animals. In vitro the top concentration for testing is lower than for soluble materials to avoid “suffocation” of the cells and interference with scoring, but exposure needs to be for at least 1 cell cycle, except if treatment with S9 is needed. Concurrent treatment with cytokinesis blockers (e.g. Cyto B) should be avoided.

Silvana Libertini PhD

Novartis Biomedical Research, Basel, Switzerland

Silvana Libertini obtained a PhD in “Molecular Oncology and Endocrinology” from the University of Naples “Federico II” (Italy) with a thesis on the use of oncolytic adenoviruses for the treatment of anaplastic thyroid carcinoma, after which she moved to the Beatson Institute for Cancer Research in Glasgow (UK), where she studied DNA damage and tumorigenicity. Since 2015, Dr Libertini has been working at the PreClinical Safety Department in Novartis (Basel) focusing on the use and development of new methods to assess the genotoxic risk associated with the use of advanced therapy products (mainly CAR-T cells, CRISPR/Cas9, AAV based gene therapy). Due to her interest in state-of-the-art in vitro models, Silvana represents Novartis in the Swiss3RCC Executive Board, in current and past NC3Rs led CRACK-IT challenges and chairs the tumorigenicity group in HESI CT-TRACS.

Symposium II - Current State-of-the-art for Genotoxicity and Carcinogenicity Strategies for Novel Modalities

Genotoxicity Evaluation of Gene Therapy Products

The main potential genotoxicity risk associated with the use of viral vectors for gene therapy is insertional oncogenesis, that is the activation of an oncogenic pathway due to the integration of the viral genome in the host genome, e.g. by disruptive integration in a cancer-related gene. Different risk levels are associated with integrating (e.g. retroviruses) and non-integrating viruses (e.g. AAVs). Guidelines from health authorities suggest a tailored approach, to be defined on a case by case basis and factoring several parameters. Examples of strategies for risk assessment at preclinical and clinical stage for integrating and non-integrating viruses will be discussed.

Caitlin Maggs MSc

Swansea University, Swansea, UK

Caitlin Maggs is a PhD student in the second year of her studies at Swansea University with Professor Gareth Jenkins. She completed her BSc in Biochemistry and Genetics at Swansea University in 2020 before undertaking an MSc studying the gut microbiome and colorectal cancer.

Caitlin then worked in the Healthcare and Technology Centre in Swansea University for ~1 year whereby she supported the collaboration of researchers between academia and industry and went on to secure a research associate position in nanotoxicology. Her current PhD research focuses on the development of quantitative Adverse Outcome Pathways for genotoxicity using 3D liver models. Caitlin sits on the NUKEMS committee which encourages and supports the progression of early career researchers.

Workshop 1 - Next Generation Genotoxicity Risk Assessments: AOPs as the foundation of NAM-based risk assessments for genotoxicity

Assessing genotoxicity in multiple cell culture models for quantitative Adverse Outcome Pathway (qAOP) development

Investigating adverse outcomes resulting from chemical toxicity supports human and biological health and DNA damage evaluation is vital as genotoxins may initiate carcinogenicity. Adverse Outcome Pathways (AOPs) link causal key events at multiple levels of biological organisation; genotoxicity AOPs are qualitative, but quantitative AOPs (qAOPs) may better support chemical safety assessment. This study aimed to provide data to develop genotoxicity qAOPs using the cytokinesis-blocked micronucleus assay, assessing the chemicals with various modes of action: electrophiles (MMS & (B[α]P)), radical species inducers (hydroquinone, sodium arsenite) & topoisomerase II inhibitors (dexrazoxane). TK6, HepG2 cells & HepG2 spheroids were dosed with compounds for 1.5 cell cycles then analysed for DNA damage. In TK6 cells, the lowest observed adverse effect levels (LOAELs) for dexrazoxane & sodium arsenite were 5μM & 3.8μM; MMS gave LOAELs of 9.1μM & 45.4μM in TK6 cells & HepG2 spheroids, respectively. The LOAELs for hydroquinone were 50μM in TK6 & HepG2 cells; B[α]P showed no genotoxicity in TK6 cells but a LOAEL of 15μM in HepG2 spheroids. This data shows the need for selection of relevant cell culture systems to support qAOP development.

Laura Markley PhD

US FDA, College Park, MD, USA

Laura C. Markley, Ph.D. is a toxicologist in the Division of Food Contact Substances within the Human Foods Program at the US FDA where she reviews premarket notifications and addresses critical post market safety questions related to food packaging. She has 20 years' experience in genetic toxicology. She holds a bachelor's degree in biology from Texas A&M University – Corpus Christi, a doctoral degree in biochemistry and molecular biology from the University of Maine in the Wise Laboratory for Genetic and Environmental Toxicology, and a postdoctoral fellowship from the US FDA in CDRH. Laura is a member of the OECD Genotoxicity Expert Group, steering committee for IWGT, Genetic Toxicology Subcommittee of the CDER PTCC, OPMAS Genetox Team, and HESI GTTC. She mentors students in genetic toxicology-related research projects at the US FDA through the UM/FDA JIFSAN program and in collaboration with NCTR.

Symposium I- Regulatory Acceptance of New Approach Methodologies (NAMs)

New Approach Methodologies Used in the Safety Assessment of Food Contact Substances

New approach methods (NAMs) have the potential to provide faster, more cost-effective, and mechanistically informative approaches to evaluate toxicological hazards while aligning with the principles of the 3Rs (replacement, reduction, and refinement of animal testing). The U.S. Food and Drug Administration (FDA) has been using NAMs for decades, particularly in the form of in vitro genetic toxicity assays and in silico assays, to assess the safety of food contact substances (FCSs). Building on this foundation, additional NAMs are being actively explored to further support the safety assessment of FCSs. This presentation will provide an overview of the advancements in NAMs at the FDA, and their potential application in evaluating FCS safety. Case examples will be discussed to illustrate how NAMs can be used in regulatory decision making, including challenges and considerations for their acceptance within the current risk assessment framework. As the regulatory landscape evolves, continued evaluation and validation of NAMs will be critical to ensuring they provide scientifically robust and reliable data to support safety determinations. This talk will highlight ongoing efforts to advance NAMs in the context of food safety assessment, and their role in modernizing regulatory science at the FDA.

Alex Maslov MD PhD

Mutagentech

Alex Maslov is the Co-Founder of Mutagentech, He received his Ph.D. in Cell biology/Biochemistry from Voronezh State University in Russia, and his M.D. from Voronezh State Medical Academy named after N.N. Burdenko in Russia. Dr. Maslov Developed a novel next generation sequencing-based approach for genome-wide assessment of somatic mutational load in normal cells and tissues and the focus of his research is on understanding the role of genome instability in the development of human disease, cancer and aging in particular. A significant part of his efforts are devoted to the development of new approaches of the identification of somatic mutations of various types.

Symposium III - ecNGS techniques and their applicability to genotoxicity and carcinogenicity testing

Characterization of Mutational Load Using Single-molecule Mutation (SMM) Sequencing Assay

Abstract coming soon

Tim McGovern

White Oak Regulatory Tox, LLC, Crownsville, MD, USA

Dr. Timothy McGovern is co-founder and a Principal Consultant at White Oak Regulatory Tox, LLC where he provides regulatory advice and nonclinical support for all aspects of nonclinical drug development. Dr. McGovern has over 22 years of regulatory experience at the US Food and Drug Administration (FDA). Most recently, he served as an Associate Director for Pharmacology and Toxicology in the Office of New Drugs (OND) at the Center for Drug Evaluation and Research (CDER). In this role, he was a member of the Pharmacology/Toxicology Senior Leadership Team within OND and a standing member of CDER's Executive Carcinogenicity Assessment Committee. Dr. McGovern was active in policy and guidance development on nonclinical and regulatory issues including FDA and International Council for Harmonization (ICH) initiatives and provided tertiary review for New Drug and Biologics License Applications (NDAs and BLAs). He was a member of the ICH Expert Working Groups for the S1B (Testing for Carcinogenicity of Pharmaceuticals), Q3C (Residual Solvents), Q3D (Elemental Impurities) and M7 (DNA reactive impurities), also serving as Rapporteur for the former three EWGs. Dr. McGovern also served as a member of CDER's Task Force on Nitrosamines in Drug Products and chaired CDER's Pharm/Tox Nitrosamines Working Group, providing nonclinical expertise in developing policies, addressing clinical safety issues, and interacting with other Drug Regulatory Agencies and industry representatives. He participated in the development of FDA's Guidances "Control of Nitrosamine Impurities in Human Drugs" and "Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs)". He was also Chair of CDER's Genetic Toxicology and Pharm/Tox Education Subcommittees. Dr. McGovern was trained in the field of inhalation toxicology and began his career at the FDA as a reviewer in the Division of Pulmonary and Allergy Products and then Supervisor in that division as well as the Division of Anesthetic, Critical Care and Addiction Products. His responsibilities included evaluating nonclinical development programs supporting Investigational New Drug (IND) applications in support of clinical development, as well as NDAs and BLAs, and providing recommendations on the safe conduct of clinical trials and eventual marketing approval and labeling. During this time, he represented FDA on a multi-stakeholder effort in developing consensus recommendations on the safety qualification of extractables and leachables in orally inhaled and nasal drug products.

Between two tenures at FDA, Dr. McGovern was a Managing Consultant at SciLucent, LLC where he provided regulatory and scientific consulting services to the healthcare product industry. He specialized in developing nonclinical testing strategies and evaluating data for small molecule pharmaceuticals, biologics, biosimilars, and medical devices as well as resolving issues related to the safety qualification of genotoxic impurities, metabolites, and product-related leachables. During this time, he also served on the US Pharmacopeia Expert Toxicology Committee.

Symposium VI - Nitrosamines - Current Updates, Regulatory Experience

Current Regulatory Considerations on the Safety Assessment of Nitrosamine Impurities

Regulatory recommendations related to the safety assessment of nitrosamine impurities have evolved as greater experience has been achieved since the issues were first identified in 2018. International regulatory agencies have actively communicated on this topic since that time with the goal of enhancing consistency in recommendations. This presentation will provide current regulatory perspectives and discuss issues still under consideration, discuss how data generated across stakeholders may inform future recommendations, and highlight next steps as the process moves to guideline development under the International Council for Harmonisation.

Steven Nicotra BS, MBA

Johnson and Johnson, Spring House, PA, USA

Steven Nicotra is a Senior Scientist in the Global Toxicology and Safety Pharmacology group at Johnson & Johnson. Steven began his career in the field of genetic toxicology 13 years ago with 17 years in the field of Toxicology. Steven holds a BSc in Animal Biotechnology and Conservation from Delaware Valley College and a Master of Business Administration from Holy Family University. Steven's areas of expertise span from performing in vivo studies and in vitro genetic toxicology studies to monitoring and directing genetic toxicology studies supporting all stages of drug development. Moreover, Steven has supported successful regulatory submissions performing the mutagenicity hazard assessment of impurities and authoring/reviewing corresponding dossier sections.

Symposium V - Best Practices for Genetox Testing of Excipients, Impurities

Combination Small Molecule Delivering Medical Device Considerations for Genetic Toxicity Testing

The requirement of genetic tox testing of medical devices uses solvent extractions of the device to assess leachates for mutagenicity and DNA damage. Inclusion of a small molecule in medical devises complicates this genetic toxicity testing. ICH guidance's, most notably ICH S2 when trying to understand the genetic toxicity risk of a small molecule, are in general the roadmap to properly derisk a small molecule pharmaceutical program for genetic toxicity. However, when you deliver this molecule via a medical device one must also consider ISO-10993-1 and other applicable regulations for medical devices. This presentation will align the audience on expectations for medical device testing, performing a risk assessment to identify any potential extractable, leachable or other impurities of genotoxicity concern, and then how to proceed to biocompatibility testing to assure your device does not contain a genetic toxicity liability for your submission. Two case studies will be shared. Case study one will discuss the testing strategy of a device delivering a known genotoxic compound for an advanced cancer indication. Case study two will discuss solvent selection as per ISO 10993-12 and its impact on genotoxicity testing.

Jessica Noteboom BS

Eli Lilly and Company, Indianapolis, IN, USA

Jessica Noteboom is a toxicologist with over 10 years of experience in genetic toxicology. She is a study manager at Eli Lilly covering genetic toxicology, hemolysis and tissue cross reactivity. She is a subject matter expert at Eli Lilly and Company on genetic toxicology. Prior to working at Eli. Lilly she spent ten years at Covance working in genetic toxicology, DART, and biodistribution. Jessica has co-authored several peer reviewed publications focused in genetic toxicity

Symposium VI - Nitrosamines - Current Updates, Regulatory Experience

Assessment of In Vivo Mutation Frequency of NNK as determined in the Big Blue Rat

Nitrosamines (NA) are chemicals found as impurities in drugs and food, associated with genotoxicity risks, but their toxicity varies based on structure, bioavailability, and potency, which can further categorize them into sub-classes of molecules that are either deemed to be a cohort of concern (CoC), weak acting mutagens (non-CoC), or non-mutagens. As part of an ongoing HESI initiative, we evaluated mutation frequency of NNK in the Big Blue (CII)® Rat Model. An evaluation of exemplar nitrosamines (NA, of which NNK is one) was done for which there are robust carcinogenicity data and compared that with in vivo mutation data. These exemplar NAs are used to set limits for nitrosamine drug substance related impurities (NDSRIs). The goal of these studies is to provide a means to better estimate the potency of NDSRIs. Here, we treated male Big Blue rats with NNK at 0, 0.001, 0.01, 0.03, 0.1, 1, 10 and 30 mg/kg for 28 days, consistent with OECD 488. A maximum tolerated dose of 30 mg/kg for NNK as judged by body weight loss and hepatic histopathology was determined in a 7-day range finding study. Mutation frequency was assessed in liver and lung, the latter being the target organ for NNK. TGR mutation data was analyzed in PROAST

using a CES of 0.5. The BMDL in liver was 1 mg/kg while the BMDL in lung was 0.1 mg/kg. These BMDL values are consistent with the NNK being more sensitive in lung and with the reported TD50 values in the Lhasa Db for these tissues (0.6 mg/kg and 0.1 mg/kg respectively). These data support the use of TGR mutation as a means of risk characterization and estimating AI for unknown NDSRIs. The same genomic DNA samples obtained from liver and lung NNK treated samples and used for TGR mutation analysis were evaluated by duplex sequencing. If available, those data will also be shared.

Stefan Pfuhler PhD

Procter & Gamble, Cincinnati, Ohio, USA

Dr. Pfuhler received his Ph.D. in Biology from the department of Pharmacology and Toxicology of The University of Ulm in 1997 and joined his current employer, Procter and Gamble, in 2000 were he serves as a Research Fellow in Global Product Stewardship. In recent years Dr. Pfuhler's research focused on alternatives to animal testing and genotoxicity testing strategies and he leads validation efforts for 3-dimensional human skin-based genotoxicity assays. He is chairing Cosmetic Europe's Genotoxicity Task Force since 2002 and serves as co-chair of HESI's Botanicals Consortium.

Workshop 2 - The Last Mile: Opportunities to Bridge Research and Increase Impact in Human and Environmental Health Science

HESI Botanical Safety Consortium: Towards a Global Genotoxicity Testing Strategy for Botanicals

Human use of botanicals to promote health and treat diseases likely dates back to pre-history. However, ensuring the safe use of chemically complex phytoconstituents is not a straightforward endeavor due to natural variability of products. HESI's Botanical Safety Consortium was established to address these challenges. Genotoxicity assessment is especially critical, because, unlike other toxicities, genotoxicity is not adequately identified by adverse event and history-of-use reports, which traditionally has been used to support the safe use of botanicals. The Consortium's Genotoxicity Technical Working Group is assessing the suitability of a genotoxicity testing strategy by testing thirteen botanicals as data-rich case studies in four standard genotoxicity assays covering mutation and chromosome damage endpoints. The four assays include the bacterial reverse mutation assay, the in vitro micronucleus (MN) assay in TK6 cells and in HepaRG cells, and the ToxTracker® assay. The results from testing all thirteen extracts in these assays will inform the selection of a fit-for-purpose genotoxicity testing strategy. Preliminary outcomes of the testing program will be shared, together with initial thoughts about a suitable testing strategy.

Symposium IV - Metabolism as a Potentiator of Genetox Risk Assessment

Modification of the co-factor mix for S9 treatments to improve genotoxicity predictions

As per OECD testing guidelines and in common practice, standard 2D in vitro genotoxicity assays utilize induced rat liver S9, which is enriched for Phase 1 reactions and the added 'S9 mix' contains cofactors for Cyp 450 Phase I enzyme pathways only. This can be looked at as a 'worst case' scenario since phase I reactions will mostly be increasing reactivity of a compound, and it is not reflective of metabolism in vivo which is represented by a balance of Phase 1 and 2 enzyme reactions. This can result in over-prediction of chemical hazard. Since Phase 2 conjugation reactions are an important and integral part of how the body detoxifies intrinsic and external chemicals, it would ideally be better reflected in genotoxicity assays. A project of the International Collaboration on Cosmetic Safety (ICCS) aims to determine whether the addition of further in vivo relevant cofactors, for both phase I and phase II, can improve the predictive capacity of in vitro genotoxicity assays. Initial results will be presented that will include an example where a regulatory agency was concerned about potential false-negative results and was requesting the addition of an additional phase I cofactor, Nicotinamide adenine dinucleotide (NAD). NAD had to be added to boost the activity of alcohol dehydrogenase, since it was thought that the lack of NAD in S9 mix may not correctly reflect in vivo metabolism. It is expected that learnings from this project addressing a key physiological attribute will favorably impact in vitro genotoxicity predictions.

Jonathan Phillips PhD

Intellia Therapeutics, United States

Dr. Jonathan Phillips leads nonclinical strategy as Vice President of Pharmacology & Toxicology at Intellia Therapeutics in Cambridge, MA. His experience developing gene editing therapies began at Vertex where he co-authored first-in-human applications for CTX001 (CASGEVY), in

partnership with CRISPR Therapeutics. He also built and led several translational and investigative safety teams during his time with Boehringer Ingelheim. Prior to industry, Dr. Phillips was a Bioastronautics Fellow at NASA Ames Research Center in Mountain View, CA investigating mechanobiology in space travel environments. He holds a PhD in cell biology from UMass-Worcester and has dozens of peer reviewed publications, book chapters, and invited presentations.

Symposium II - Current State-of-the-art for Genotoxicity and Carcinogenicity Strategies for Novel Modalities

Mapping CRISPR within the atlas of genotoxic risk

The genetic safety of CRISPR/Cas9 therapies will be examined through in the context of natural DNA damage and repair processes. CRISPR/Cas9 will be introduced as an enabling technology for multiple therapeutic approaches including gene knockout, insertion, and repair. A comprehensive off-target assessment workflow will be detailed, whereby potential off-target sites are identified through a combination of computational prediction, biochemical screening, and cell-based verification. The NTLA-2002 case study will be presented to demonstrate how this approach revealed highly specific editing with minimal off-target activity, observed only at substantially elevated doses above therapeutic levels. The presentation will conclude that properly designed CRISPR/Cas9 therapies can be considered to introduce minimal genotoxic burden relative to naturally occurring processes and common environmental exposures.

Raechel Puglisi MPH

Health and Environmental Sciences Institute (HESI), Washington, DC, USA

Raechel Puglisi joined HESI in 2019. She holds an MPH in Environmental Health Sciences and Policy from The George Washington University. Additionally, she holds a BA in Human Biology from The University of Kansas. She is interested in all aspects of environmental health, including human and ecological risk assessment, risk communication, toxicology, and special interests in both health equity and education outreach. At HESI she co-manages the Collaboration on Ototoxicity Risk Assessment (CORA), Genetic Toxicology (GTTC), and Transforming the Evaluation of Agrochemicals (TEA) committees and assists on various board and emerging issues initiatives.

Workshop 2 - The Last Mile: Opportunities to Bridge Research and Increase Impact in Human and Environmental Health Science

What is the Last Mile and Why Should You Care? Introduction to the concept and its relevance to human and environmental health

The translation of scientific research into practical application remains a persistent challenge in environmental and public health sciences. The “Last Mile” represents the critical stage where validated data and methods must be implemented to inform policy, guide decision-making, and protect health. This talk introduces the Last Mile framework through the lens of the Health and Environmental Sciences Institute (HESI), highlighting how collaborative, stakeholder-engaged approaches can enhance the usability and impact of research. By addressing barriers to adoption and prioritizing implementation from the outset, we can better ensure that scientific advances achieve their intended outcomes.

Hans Raabe MS

Institute for In Vitro Sciences, Inc., Gaithersburg, Maryland, USA

Mr. Raabe, Senior Vice President and Chief Operating Officer at the Institute for In Vitro Sciences (IIVS), has been involved in in vitro toxicology assays for over 35 years. Mr. Raabe participated extensively as Study Director for GLP-compliant in vitro bioassays, and as Chief Operating Officer he is responsible for the overall operations of the contract laboratory services, and oversees the technical transfer, optimization and validation of emerging new approach methodologies. Mr. Raabe has served as an expert on several OECD Test Guideline panels, has been an invited presenter at various regulatory workshops and review panels, and has participated in multiple ECVAM and ICCVAM validation studies. To support IIVS’ education and outreach mission, Mr. Raabe also routinely participates in international in vitro methods technical training workshops.

Symposium I - Regulatory Acceptance of New Approach Methodologies (NAMs)

An Overview of the Regulatory Approval Pipeline for New Approach Methodologies: Implementing Human-relevant Testing Approaches

The testing of chemicals, mixtures, and product formulations for regulatory hazard categorization and risk assessment is undergoing unprecedented changes in terms of the technologies applied and the approaches to integrating the data from those sources. As testing approaches move from the use of resource-intensive whole animal apical endpoint studies to rapid human-relevant key event-based in vitro methodologies predictive of downstream adverse outcomes, the process for developing, validating, approving, and implementing these new approach methods is becoming more challenging. Over the past 30+ years, numerous non-animal “alternative” test methods have been validated and approved as replacements for existing regulatory-required animal tests for chemical hazards, and the process from initial proof of principle to regulatory approval and implementation has often taken well over 10 years; a process timeline much too long to ensure that emerging technologies can be employed within their optimal lifecycle. Among the reasons are inherent in the substantial validation framework designed to evaluate the relevance and reliability of new test methods across the universe of chemicals, the conviction that existing test methods should be regarded as gold standards in the absence of full evaluation of human relevance and reliability, and the outdated expectations for a one-to-one relationship between existing and emerging test methodologies. Other explanations point to the gulf in the technological expertise of test method developers promoting the adoption of the technologies, and the regulatory personnel charged with approving them for national and international regulatory purposes. And numerous other test methods have failed to meet validation criteria or become adopted into the regulatory realm because the developers failed to prepare and optimize test methods to meet the rigors of validation, or design methods that would meet the specific regulatory needs of the agencies. This presentation will provide an overview of the sometimes tortuous journey from test method concept to the implementation by international regulatory authorities. It will highlight some of the challenges and pitfalls encountered and will provide some practical guidance through case studies of successful validations for test method developers to consider for successful test method validation.

Dan Roberts MS

Toxys, Inc, New York, NY, USA

Dan Roberts joined Toxys in 2022 and is the Director of BD and Sales in the USA. He has spent 20 years in the applied genetic toxicology field with experience in pharma, biotech, and CRO industries. Dan is presently a volunteer to the Genetic Toxicology Association and is former Chair of the BOD (2016). He also maintains activity within HESI’s Genetic Toxicology Technical Committee (GTTC) and the Applied Genotoxicity (AGT) Special Interest Group of the Environmental Mutagen and Genomics Society (EMGS). Dan’s experience from the bench and as a Study Director / PI is valuable when assessing and understanding client needs, especially with follow up strategies. Most of his career has been focused on mechanistic genetox evaluation, specifically discriminating aneugens from DNA reactive clastogens.

Workshop 1 - Next Generation Genotoxicity Risk Assessments: AOPs as the foundation of NAM-based risk assessments for genotoxicity

Building weight of evidence to support indirect clastogenic effects within an AOP framework

Adverse outcome pathways (AOPS) are descriptive networks of molecular events that lead to a meaningful toxicological effect. When faced with positive test results from a mutation or DNA strand break endpoint, AOPs can served as a guidebook for determining mechanism of action (MOA) by working backwards from the adverse outcome. While mode of action is critical for ensuring appropriate risk management, AOPs can be used to dive deeper than routine discrimination of aneugens from clastogens. With the latter, there are multiple non-DNA reactive MOAs that induce DNA strand breaks yet are considered to have margins of safe exposure. Perhaps the test chemical inhibits topoisomerase enzymes, targets replication machinery, modulates dNTP pools, or simply induces oxidative stress. This commentary will walk through the status of AOPs being developed for non-DNA reactive clastogenic MOAs and illustrate how to plan follow up testing to define specific MOAs.

David Schuster MS

University of Ottawa, Ottawa, Ontario, Canada

David Schuster is a final-year PhD candidate in the Chemical and Environmental Toxicology Program at the University of Ottawa, working with Health Canada through the GReAT Lab. His research applies Duplex Sequencing (DS), an error- corrected next-generation sequencing method, to study tissue-specific mutagenic effects of benzo[b]fluoranthene across time and dose. He compares modern ecNGS approaches like DS and SMM-seq to conventional assays such as the transgenic rodent, Pig-a, and micronucleus tests. During his MSc, David completed a thesis at BASF SE, where he established an in vitro transgenic rodent assay using primary MutaMouse hepatocytes. He later contributed to microbiome sequencing method development at Labor Bayer, expanding his expertise in NGS and applied toxicology. With experience spanning industry, academia, and government agencies, he is committed to advancing mechanistically informed, animal-free strategies for genotoxicity testing and risk assessment within the broader field of genetic toxicology.

Symposium III - ecNGS techniques and their applicability to genotoxicity and carcinogenicity testing

Duplex Sequencing after Prolonged Benzo[b]fluoranthene Exposure Reveals Tissue-Specific Differences in Mutagenic Response, Chemical Potency, and Clonal Expansion of Mutations.

Polycyclic aromatic hydrocarbons (PAHs) are known genotoxicants, and chronic exposure through ingestion or inhalation poses significant health risks. We characterized the mutagenic profile of the priority PAH benzo[b]fluoranthene (BbF) in MutaMouse males orally exposed to increasing doses over 28, 90, and 180 days. Duplex Sequencing (DS), an error-corrected next-generation sequencing technology, was applied to analyze liver and bone marrow (BM) DNA, revealing dose- and time-dependent increases in mutation frequency (MF). After 90 and 180 days the MF at 25 mg/kg-bw/day was 1.8x and 2.4x higher in BM, and 3.8x and 8.6x higher in liver, compared to 28 days. Benchmark Dose (BMD) modeling to estimate the dose required for a 50% increase in MF revealed declines in BMDs from 8.2 (28-days) to 3.7 (90-days) and 3.0 (180-days) mg/kg-bw/day in BM. In liver, the BMD50 shifted from 14.3 (28-days) to 4.3 (90-days) and 3.9 (180-days) mg/kg-bw/day. The BMD confidence intervals overlapped in liver but not BM, indicating similar potency estimates across the time points for liver but not BM. Clonal expansion of mutated cells was more pronounced in BM than in liver. These tissue-specific differences may be attributed to biological factors such as proliferation rate, metabolic activity, and cellular lifespan. BbF predominantly induced C to A transversions at all time points in both tissues. The BMDs for C to A mutations were lower than for overall MF after 180 days with 1.6 and 2.9 mg/kg-bw/day in BM and liver, respectively. These findings provide insights into the impacts of prolonged chemical exposure on mutagenicity and highlight tissue-specific differences in mutation susceptibility.

Srijit Seal PhD

Merck Co & Inc., Broad Institute of MIT and Harvard

Srijit Seal is Senior Scientist at Merck and specializes in cheminformatics. His research uses machine learning techniques to predict drug bioactivity, safety, and toxicity. His recent work at the Broad Institute of MIT and Harvard focuses particularly on the modeling and interpretation of the Cell Painting assay- a high content imaging assay to measure cellular phenotypes.

Workshop 2 - The Last Mile: Opportunities to Bridge Research and Increase Impact in Human and Environmental Health Science

HESI eSTAR OASIS: Leveraging 'Omics Data for Next Gen Safety Assessment

High-throughput, human-relevant approaches for predicting chemical toxicity are urgently needed for better decision-making in human health. In this presentation, we will show how we apply image-based profiling (the Cell Painting assay) and two cytotoxicity assays (metabolic and membrane damage readouts) to primary human hepatocytes after exposure to eight concentrations of 1085 compounds that include pharmaceuticals, pesticides, and industrial chemicals with known liver toxicity-related outcomes. Three computational methods (CellProfiler, a Cell Painting-specific convolutional neural network, and a pretrained vision transformer) were compared to extract morphology features from single cells or entire images. We used these morphology features to predict activity in the measured cytotoxicity assays, as well toxicity assays that span cytotoxicity, cell-based, and cell-free categories. We found that the morphological profiles detect compound bioactivity at lower concentrations than standard cytotoxicity assays. We envision that image-based profiling could serve as a key component of modern safety assessment.

Stephanie L. Smith-Roe PhD

Division of Translational Toxicology, NIEHS, Research Triangle Park, USA

Dr. Stephanie Smith-Roe is a genetic toxicologist in the Division of Translational Toxicology (DTT) at the NIEHS. She designs a wide range of genetic toxicity testing strategies for the DTT and serves as the Contracting Officer's Representative (COR) for the DTT's Genetic Toxicity Testing Contract. At the DTT, Dr. Smith-Roe has investigated the genotoxic potential of glyphosate (the active herbicide in Roundup products) and several other high-profile substances such as cell phone radiofrequency radiation and botanical dietary supplements. She has collaborated with scientists across sectors to assess new approaches for rapid identification of genotoxicants, and that provide information about mechanism-of-action. Altogether, Dr. Smith-Roe has published in the areas of genetic toxicology, mutagenesis, carcinogenesis, error-corrected sequencing, DNA replication and repair, DNA damage signaling, and chromatin remodeling. Dr. Smith-Roe is an enthusiastic contributor to scientific societies and organizations that focus on research related to genetic toxicology, genomic stability, and carcinogenesis, and that are also committed to supporting early career scientists. She is a Past President of the Environmental Mutagenesis and Genomics Society and a Past President of the Genetics and Environmental Mutagenesis Society. Through participation in working groups for the Health and Environmental Sciences Genetic Toxicology Technical Committee, the International Agency for Research on Cancer, the International Workshop on Genotoxicity Testing, etc., Dr. Smith-Roe has contributed to international efforts to continually improve approaches for genetic toxicity testing and to protect the public from exposures that potentially could cause cancer, birth defects, and genetic disease.

Symposium III - ecNGS techniques and their applicability to genotoxicity and carcinogenicity testing

Closing the gap: IWGT recommendations on the adoption of ecNGS for regulatory mutagenicity testing

Error-corrected sequencing (ECS) is revolutionizing genetic toxicology by enabling highly accurate detection of low-frequency, environmentally induced mutations. This transformative technology leverages consensus sequencing and advanced bioinformatics to achieve error rates comparable to baseline somatic mutation frequencies, offering significant advantages over traditional methods. The ability of ECS to define detailed mutational spectra opens new avenues for mechanistic understanding of mutagenesis. As highlighted by Marchetti et al. (2023)1,2 and the International Workshop on Genotoxicity Testing (IWGT) position paper (2025, in press), substantial progress has been made in validating ECS for regulatory use. Interlaboratory studies, including those using Duplex Sequencing (DS), have demonstrated robust concordance between ECS and established transgenic gene mutation rodent (TGR) assays (OECD TG 488). This concordance, coupled with the enhanced sensitivity and 3R benefits of ECS, supports the ongoing efforts to amend existing OECD Test Guidelines to incorporate ECS methodologies. A Detailed Review Paper (DRP), under development by international experts including USA and UK OECD national coordinators, will consolidate these findings and help guide the amendment process. This initiative is driven by a Standard Project Submission Form (SPSF) accepted by the OECD in 2024 and informed by significant contributions from the genetic toxicology community, including extensive research documented in published papers. Strategies and key considerations for the integration of ECS into regulatory mutagenicity testing paradigms will be described. Together, these efforts will provide a firm basis for integration of ECS technologies into regulatory genetic toxicology testing.

Leon F. Stankowski, Jr.

Charles River Laboratories, Ashland, OH, USA

Dr. Stankowski is the Senior Scientific Director for Genetic and In Vitro Toxicology at Charles River Laboratories. He has over 40 years of experience in genetic toxicology in various CROs as well as Pharma, and is responsible for guiding research and development for new service offerings at CRL. Dr. Stankowski provides guidance on all aspects of toxicology to help clients reach their goal of regulatory approval for new products, and acts as a consultant to clients with a lack of toxicology expertise or those who have encountered adverse toxicology issues. He is responsible for evaluating and addressing those issues when they arise, including the design of specialized protocols and mechanistic studies to resolve them. Dr. Stankowski regularly provides senior level reviews of protocols, reports and related documents for appropriate scientific content and interpretation, particularly as related to adverse outcomes for compounds being developed by pharmaceutical and other companies. He has led and or been involved in many industry work groups, including the HESI Genetic Toxicology Technical Committee and OECD Expert Working Groups for review of the Test Guidelines on Genotoxicity.

Symposium VI - Nitrosamines - Current Updates, Regulatory Experience

The Enhanced Ames Test – One CRO's Perspectives and Experiences

Charles River Laboratories had genetic toxicology laboratories operating at four sites: Den Bosch (The Netherlands), Senneville (Canada), Skokie (USA), and Vesprem (Hungary). All have performed the enhanced Ames Test (EAT) for regulatory submissions, or at least started the validation/qualification process. While no hard data can be shared at present, this presentation describes our experiences and challenges along the way. For example, one study from Skokie and another from Vesprem were submitted to separate regulatory authorities but rejected due to the use of "excessive" 100-µL DMSO dosing volumes and concern over a potential inhibitory effect on the metabolic enzymes. Repeating each study with 20- or 25-µL dose volumes yielded the same negative results as before. And the requirement to add two NDSRIs positive controls in one of those studies had no impact on the interpretation of the results. In addition, the same regulatory authority requested that the same impurity be testing using a plate incorporation treatment, even though now negative in two "standard" EAT assays. The Den Bosch site, an early adopter, evaluated six test materials as part of the HESI-GTTC ring trial site prior to performing studies for regulatory submission. However, they found that background revertant frequencies in TA100 +30% rat and hamster S9s were substantially lower than those observed –S9, +10% rat S9, and +10% hamster S9. While there was no apparent toxicity associated with this decrease, it was reproducible and considered real, and it did affect a Sponsor study. Except for the lower revertant frequencies in TA100 +30% rat and hamster S9s observed in Den Bosch, the results from all sites were similar, and they indicate DMSO up to 100 µL does not appear to adversely impact the results except for small alkyl nitrosamines.

Xiaowen (Wen) Sun PhD

Pfizer Inc, Groton, CT, USA

Wen Sun is the director of Genetic Toxicology department at Pfizer. She received her PhD in Molecular and Cellular Biology (Molecular Medicine) from the University of Iowa and worked as a post-doctoral researcher at Yale University Pharmacology department prior to joining Pfizer six years ago. Since joining Pfizer, Wen led the development, validation, and implementation of the multiplexed imaging screening platform, which enabled the delivery of regulatory endpoint and mode of action information to project teams in a single assay. Currently, Wen is responsible for the scientific and strategic development of genetox department. She provides subject matter expertise guiding teams and chemists away from genotoxicity liabilities and performs all regulatory responsibilities in compliance. Wen is the Chair of the Genetic Toxicology Association, is also an active member in the Environmental Mutagenesis and Genomics Society, along with the Health and Environmental Sciences Institute.

Workshop 2 - The Last Mile: Opportunities to Bridge Research and Increase Impact in Human and Environmental Health Science

Advancing Genetic Toxicology: The Role of AOPs and/or in vitro NAMs in HESI GTTC

Any scientific advancement requires strategic planning, exquisite execution, and most importantly, collaboration. The health and environmental sciences institute (HESI) provides a platform for participants across various sectors to exchange ideas and leverages enormous expertise and experience to drive innovation and reach solutions. HESI Genetic Toxicology Technical Committee (GTTC) consists of many working groups impacting wide range of research areas. The in vitro and mode of action groups are innovation-driven. These groups perform extensive literature evaluations to understand key molecular/cellular events leading to adverse outcomes, explore possibilities of alternative in vitro platforms, and validate the state-of-the-art technologies. These in vitro groups aim to provide future assay possibilities and accelerate the acceptance of new approach methodologies. In comparison, some other HESI groups are more specific and issue based, such as the Nitrosamine and TiO. These groups tackle the most urgent regulatory concerns, utilizing the resources of all participants to efficiently design studies and execute large-scale validations. With the help from all to reach a solution in record speed and benefit regulatory guidance modifications.

Yax Thakkar PhD

RIFM Inc.

Yax Thakkar is a Principal Scientist at RIFM. He earned his M.S. in Pharmacology and Toxicology from Long Island University and PhD in Pathology from New York Medical College and is deeply interested in advancing the development of New Approach Methodologies (NAMs) for assessing the safety of consumer product ingredients. Yax joined RIFM in 2016 and currently leads the Genotoxicity team, where he oversees safety assessment and research programs. Prior to joining RIFM he worked in various consumer product companies such (L'OREAL, COTY INC. etc.) and toxicology consulting firm.

Symposium IV - Metabolism as a Potentiator of Genetox Risk Assessment

Importance of metabolism in different NAMs to evaluate genotoxic potential for test materials

The metabolic capabilities of 3D skin and Chicken Egg Genotoxicity Assay (CEGA)/HET-MN models have been assessed, revealing adequate Phase-I and Phase-II metabolic activity, as previously documented for the Epiderm model (Gotz et al., 2012) and recently confirmed for CEGA and HET-MN. Selection of an appropriate New Approach Methodology (NAM) to validate results from standard in vitro assays (e.g., Ames, in vitro micronucleus) depends on exposure route, chemical characteristics, and metabolism. The Reconstructed Skin Micronucleus (RSMN) assay is ideal for fragrances with dermal exposure, while HET-MN is a valuable animal-free follow-up for oral exposure scenarios (e.g., lip products, flavorings) with systemic effects. Integrating *in silico* and ToxTracker data can further enhance understanding of metabolic processes. Case studies on 2-octen-4-one and methyl eugenol underscore the role of chemical-specific metabolism in NAM selection.

Sandy Weiner MS

Johnson & Johnson, Spring House, PA, USA

Distinguished Scientist in Genetic Toxicology at Johnson and Johnson for 18 years working across all modalities, including compound, metabolite, excipient, impurity and degradant hazard identification and de-risking.

Symposium V - Best Practices for Genetox Testing of Excipients, Impurities

Novel Excipients and Genetic Toxicology Qualification

Excipients are inactive ingredients in drugs and serve important functions associated with drug manufacture, delivery, palatability and stability. However, not all excipients are inert substances; some have been shown to be potential toxicants. The Federal Food, Drug, and Cosmetic Act of 1938 was enacted after the tragedy of the elixir of sulfanilamide in 1937 in which an untested excipient was responsible for the death of children. In 2005 FDA/CDER adopted the guidance, "Nonclinical Studies for Development of Pharmaceutical Excipients" which applies to any inactive ingredients that are intentionally added to therapeutic and diagnostic products, but that are not intended to exert therapeutic effects, may act to improve product delivery (e.g., enhance absorption or control release of the drug substance) and are not already qualified with regard to proposed level of exposure, duration of exposure, or route of administration. Examples of excipients include fillers, extenders, diluents, wetting agents, solvents, emulsifiers, preservatives, flavors, absorption enhancers, sustained-release matrices, carriers, and coloring agents. Assessing the genetic tox liability of excipients is required by this FDA/CDER guidance which recommends following the ICH S2(R1) guideline "Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use". There are a few hurdles to genetic tox testing of excipients including, but not limited to, impurities, interference with metabolism by CYP450s and solubility. This presentation will describe what excipients are, discuss the guidance documents, describe hurdles that might be encountered during testing and include a few case examples.

Yi Yang PhD, DABT

AbbVie Inc., North Chicago, IL, USA

Dr. Yi Yang is currently Director of Genetic, Environmental, and Occupational Toxicology at AbbVie. She is also a Therapeutic Area Leader overseeing preclinical safety portfolios in Specialty and Cell Therapy areas. Dr. Yang has over 20 years of experience in preclinical safety assessment for a variety of therapeutic modalities, including small molecules, monoclonal antibodies, degradomers, cell and gene therapy. Her areas of expertise include regulatory toxicology, predictive and mechanistic toxicology,

genetic toxicology, toxicogenomics, toxicity biomarkers, and biostatistics. Dr. Yang received her MD from Sun Yet-Sen University of Medical Sciences, her Ph.D. in Toxicology and M.S. in Biostatistics from University of Cincinnati. She is also certified as a Diplomat of the American Board of Toxicology. She authored 30 peer-reviewed publications and over 20 regulatory submissions supporting Phase 1 and Phase 2 clinical trials. She is actively involved in several industry-wide collaborations, including Predictive Safety Testing Consortium, ILSI-HESI consortium, IQ DruSafe, and EFPIA. She also served as Secretary to the American Association of Chinese in Toxicology (2010-2012), Chair to the Applied Pharmaceutical Toxicology (2015-2016), and President to the Midwest Regional Chapter of SOT (2017-2019). Dr. Yang joined GTA in 2022 and is on the Board of Directors since 2023.

Symposium II - Current State-of-the-art for Genotoxicity and Carcinogenicity Strategies for Novel Modalities

Genetox Approaches for Therapeutic Peptides: Results of an Industry Survey

Therapeutic peptides can range from simple synthetic polypeptides with natural animo acids to more complex molecules with non-natural amino acids and/or conjugated moieties. Nonclinical development programs often fall somewhere between that of small molecule and biologics programs, with aspects of both ICH M3(R2) and ICH S6(R1) being applicable. In an effort to harmonize nonclinical safety testing programs, the European Federation of Pharmaceutical Industries and Associations (EFPIA) peptide safety working group has conducted an industry-wide survey to capture the standard approaches companies are using for the development of therapeutic peptides. The presentation will focus on genotoxicity assessments of therapeutic peptides across companies. Among the 11 companies responded to the survey, genotoxicity assessment is often conducted if the peptide is considered a small molecule, contains non-natural amino acids and/or novel chemical modifications. The testing are often conducted with the entire molecule, along with the isolated non-natural structures in half of the responders. It is noted that the presence of certain amino acids can interfere with Ames testing, therefore a modified method or in vitro mammalian cell gene mutation test may be warranted.

Carole Yauk PhD

University of Ottawa, Ottawa, ON, Canada

Carole Yauk was the lead scientist of the Genomics Laboratory in the Environmental Health Science and Research Bureau at Health Canada for 18 years. She joined the University of Ottawa's Department of Biology as a professor in September 2020, where she holds the Tier 1 Canada Research Chair in Genomics and the Environment. Her research broadly focuses on multi-sector collaborative efforts to develop and implement genomic tools for human health risk assessment of environmental chemical exposures. She is involved in various international consortia to advance this area, including within the Health and Environmental Sciences Institute (a global non-profit), where she currently serves as vice-chair of the Board of Trustees. She is a Canadian Delegate to the Organisation for Economic Co-operation and Development (OECD), participating in the Advisory Group on Emerging Science in Chemicals Assessment. She is Past-President of the Environmental Mutagenesis and Genomics Society and an editorial board member of several journals focused on mutagenesis and genetic toxicology. In her spare time, you'll find her bouldering, hiking and skiing, or obsessing over her current Wordle streak.

Workshop 1 - Next Generation Genotoxicity Risk Assessments: AOPs as the foundation of NAM-based risk assessments for genotoxicity

What's in the wiki (or coming soon)? International, multi-sector efforts to build genotoxicity Adverse Outcome Pathway networks

Adverse Outcome Pathways (AOPs) are increasingly recognized as a key framework for advancing the field of genetic toxicology. By linking molecular initiating events to adverse health outcomes through defined key events, AOPs provide structured and transparent tools for describing modes of genotoxic action, evaluating weight of evidence, and integrating New Approach Methods. AOPs are also invaluable for identifying knowledge gaps, developing integrated test strategies, and applying NAMs to predict genotoxicity-associated outcomes. This presentation will review the current state of AOP development related to genetic toxicology, and highlight the contributions in the AOP-Wiki from the Health and Environmental Sciences Institute's Genetic Toxicology Technical Committee (GTTC) and the European Partnership for the Assessment of Risks from Chemicals (PARC) further expand these AOPs. A growing network of genotoxicity AOPs is emerging, encompassing pathways for DNA alkylation, oxidative DNA damage, topoisomerase inhibition, deposition of energy, and molecular events associated with aneugenicity. These emerging networks are critical for advancing mechanistic understanding and harmonizing methodologies, including dose-response modeling, temporal concordance evaluation, and the application of quantitative AOP frameworks. Some challenges that remain include overlapping or redundant key events, absence of

some critical genotoxicity molecular initiating events and pathways, and the need for standardization and collaboration across sectors to advance the field. Both PARC and the GTTC have projects on the OECD AOP workplan to address these gaps and challenges. This talk will provide an overview of endorsed and in-progress AOPs and how they connect to create a network, and outline future needs and directions for collaborative AOP development.

Workshop 2 - The Last Mile: Opportunities to Bridge Research and Increase Impact in Human and Environmental Health Science

From Discovery, to Validation and Implementation: The Journey of the TGx-DDI Biomarker

The TGx-DDI biomarker is a transcriptomic-based new approach methodology (NAM) designed to classify chemicals as DNA damage-inducing (DDI) or non-DDI using gene expression responses in cultured human cells. Its development and validation have been supported by over a decade of coordinated effort by the Health and Environmental Sciences Institute (HESI), which has led a robust research program to promote the biomarker's scientific credibility and regulatory acceptance. Initially developed using TK6 cells exposed to prototypical genotoxic and non-genotoxic agents, the 64-gene TGx-DDI panel captures transcriptional signatures regulated by p53, a central regulator of the DNA damage response. The biomarker has undergone extensive validation, including a multi-laboratory ring trial co-supported by the U.S. FDA and HESI. In this study, four laboratories implemented the standardized TGx-DDI protocol and data analysis workflow using the NanoString platform, achieving 100% sensitivity, 86% specificity, and 91% overall accuracy. TGx-DDI outperforms traditional in vitro chromosome damage assays in specificity while retaining high sensitivity, supporting its use for de-risking irrelevant in vitro positives and identifying true genotoxic hazards. This talk will outline the biomarker's development, validation, and regulatory engagement journey. TGx-DDI provides a practical NAM for mechanistic genotoxicity testing and serves as a model for advancing transcriptomic biomarkers in regulatory science.

Shaofei Zhang PhD

Pfizer

Dr. Zhang is a Senior Principal Scientist at Pfizer's Drug Safety R&D group. He specializes in utilizing both traditional and emerging scientific methodologies to assess the safety of various pharmaceutical compounds. Before joining Pfizer, Dr. Zhang contributed his expertise at BioReliance and the National Cancer Institute (NCI), where he focused his research on cancer genomics and epigenetics. In addition to his role at Pfizer, Dr. Zhang co-chairs the EMGS Mutagenic Mechanisms and Assessment Special Interest Group (SIG) and the HESI GTTC ecNGS workgroup. Through these positions, he actively promotes the use of ecNGS in safety assessments and works towards gaining regulatory acceptance for this innovative technology.

Symposium VI - Current State-of-the-art for Genotoxicity and Carcinogenicity Strategies for Novel Modalities

Comparative Potency of N-Nitrosomorpholine and N-Nitroso Reboxetine

Establishing regulatory limits for Drug Substance-Related Impurities (NDSRIs) is challenging due to the limited genotoxicity and carcinogenicity data available for many of these impurities, often leading to conservative approaches. In this study, we evaluated the genotoxic potential of two structurally related nitrosamines: N-nitrosomorpholine (NMOR) and N-nitroso reboxetine. Compared to the well-studied NMOR, there is little toxicological information available for N-nitroso reboxetine. Currently, both compounds have an acceptable intake value of 127 ng/day, based on a read-across using the available carcinogenicity data of NMOR. While both compounds tested positive in a series of in vitro and in vivo assays, we found that the mutagenic potential of N-nitroso reboxetine was significantly lower than that of NMOR. The benchmark dose (BMD) analysis of in vivo mutagenicity data supports an acceptable intake of 24,000 ng/day for N-nitroso reboxetine. Computational studies, carried out using the quantum-mechanical CADRE program, were consistent with in vitro and in vivo outcomes, suggesting an acceptable intake at or above 1500 ng/day for N-nitroso reboxetine. In comparison to NMOR, this prediction is supported by lower computed reactivity in the hydroxylation step, greater steric hindrance of the alpha carbons, and more facile proton transfer in the heterolysis toward the aldehyde metabolite. The data presented in this work can be used to refine and improve the Carcinogenic Potency Categorization Approach (CPCA). It also underscores the importance of collaboration between regulatory authorities, the pharmaceutical industry, and scientific researchers to address potential risks while avoiding overestimation of the acceptable intake limits for certain NDSRIs.

Poster Abstracts

P01
Integration of Duplex Sequencing with the Peripheral Blood Micronucleus Assay for a Comprehensive, Time Course Evaluation of Benzo[b]fluoranthene-Dependent Genotoxicity

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Benzo[b]fluoranthene (BbF) is one of 16 polycyclic aromatic hydrocarbons (PAHs) prioritized by the US Environmental Protection Agency for evaluation due to high levels of human exposure and the known mutagenic and carcinogenic effects of this chemical class. As part of a multi-stakeholder consortium, the mutagenic effects of BbF were extensively evaluated in MutaMouse males, which were exposed to 5 doses of BbF or a vehicle control via repeated oral gavage for 28, 60, 90, 120, or 180 days, with the dose range modified to account for duration of exposure (top doses were 25, 50, or 100 mg/kg/day). To comprehensively assess genotoxicity, mutagenesis was evaluated in lung tissue (n = 4) at 28-, 90- and 180-days via duplex sequencing (DuplexSeq), a targeted, error-corrected sequencing method, and chromosomal damage (n = 8) was evaluated in the peripheral blood micronucleus assay at all 5 time points. Dose-dependent increases in mutation frequency (MF) and in C:G>A:T mutations were observed after 28-, 90-, and 180-days of exposure. BbF-induced mutations were lower in genic targets, consistent with transcription-coupled repair of BbF-DNA adducts. As early as 28 days, BbF produced enrichment of cancer-associated mutational signatures linked to tobacco exposure. Mutations accumulated with time in lung tissue whereas chromosomal damage in bone marrow reached a steady state within 28 days. As a result, benchmark doses (BMDs) decreased over time for MF whereas BMDs obtained from the micronucleus assay increased. Taken together, these data indicate that BbF may potentially contribute to PAH-induced human lung carcinogenesis.

P02
Validation of the Flow Cytometry Based Pig-a Gene Mutation Assay in Rats

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As an alternative to the TGR assay, the Pig-a gene mutation assay may serve as replacement when positive signal is found in Ames assay. This abstract presents the in-house validation result of this assay in WuXi AppTec. In the study, animals were administrated with negative control or ethyl methanesulfonate (test article) for 28 consecutive days, positive control N-Nitroso-N-ethylurea (ENU) was administrated once daily from 3 consecutive days. Jackpot mutation event was eliminated on Day -7 prior to dosing. The samples were collected on Day 29 and processed following Litron's analysis kit manual. Percentage of reticulocyte among total erythrocytes (%RET), number of mutant reticulocyte (MUT RET) per million RET and number of mutant erythrocytes (MUT RBC) per million RBC were analyzed, in addition, carry-over effect, intra-day and inter-day precision and frozen sample stability were also evaluated. Data analysis revealed statistically significant ($p \leq 0.05$) and dose related increase response (Linear regression, $p \leq 0.05$) in MUT RET and MUT RBC of all test article dose groups, and the frequencies were over the literature reported negative control data range. The positive control (ENU) also induced the expected result ($p \leq 0.05$). Carryover effect of negative samples over positive samples, Intra- day and Inter-day precision were all minor and within acceptable range (carryover was 4.44%, 0.07% and -0.20%, %CV was 1.84%, 4.53%, 5.03%, 1.84%, 1.66% and 11.61% for %RET, MUT RBC and MUT RET respectively). Frozen samples stored under -85~-75℃ showed good stability for up to two months. The data demonstrated the assay is fully validated under GLP compliance in WuXi AppTec.

P03
Validation of MutaTracker, a novel approach method for the detection of gene mutations using error-corrected NGS.

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Mutagenicity is an apical endpoint in hazard identification. Traditionally, mutational events are measured by counting surviving clones phenotypically, after environmental selection of a mutation at a known genetic locus. A limitation of this approach is that mutants outside of the target locus are not enumerated, which underestimates true induced mutation frequencies (MF). With the introduction or error-corrected next generation sequencing (ecNGS), it is now possible to resolve precise MFs, without selection, across the genome. MutaTracker is a combination of the ToxTracker assay with single-molecule mutation sequencing (SMM-seq) to couple genotoxicity prediction with actual mutation detection in a rapid, compound sparing, all-in-one mammalian cell-based assay. ToxTracker is a GFP reporter-based assay that provides insight into chemical mode-of-action (MoA), thereby discriminating direct-acting genotoxicants from indirect genotoxicants. SMM-seq is a highly sensitive, ecNGS technique that detects single nucleotide variants utilizing rolling circle amplification and consensus strand calling. Here, we validated MutaTracker by testing a combination of genotoxic, non-genotoxic, and cytotoxic substances based on the revised ECVAM list. The ToxTracker assay was initially used to classify substances as genotoxic or cytotoxic and determine their mode of action (MoA). Subsequently, genomic DNA from exposed cultures was subjected to SMM-seq to determine mutation frequency and concentration-induced mutation spectra. ToxTracker correctly classified all genotoxic and non-genotoxic compounds while SMM-seq identified mutation signatures that supported the known MoA for most substances. For example, alkylating agent ENU induced a dose-dependent increase in the mutation frequency, generating a broad mutation spectrum with T>A, T>C and T>G mutations.Potassium bromate induced oxidative DNA damage represented by an increase in C>A mutations that are mainly caused by 8-oxo-guanine lesions. Together, MutaTracker can be a valuable tool for determining MoA and the combination compliments each NAM, with ToxTracker being able to identify genotoxicity, and SMM-seq identifying mutational fingerprints.

P04
New Alternative Method (NAM) for predictive genotoxicity using Microphysiological System (MPS)

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The assessment of genotoxicity for test compounds relies on a combination of tests designed to evaluate various endpoints associated with human diseases: mutagenicity, clastogenicity and aneuploidy. Currently, no single test can detect all mechanisms effectively, as existing assays involve rodent metabolic activation systems or in vivo rodent testing. This reliance can lead to discrepancies in human accuracy and predictive responses. This study aimed to develop a single in vitro assay capable of accurately addressing both in vitro and in vivo genotoxicity endpoints using a human cells metabolically competent in vitro system to improve human-relevant predictivity and replace the reliance of animal testing. Different models were evaluated, and the most promising evaluated model consists of a fluidic flow microphysiological liver-on-chip system. This system, supported by CNBio's PhysioMimix™ platform, incorporated HepaRG cell line on specialized Liver Plates (MPS-LC12), which enhanced liver cells health and functionality compared to previous tested performed on Barrier Plate (MPS-T12). Preliminary results using direct and pro-genotoxicants requiring metabolic activation, demonstrated outcomes consistent with in vivo response. These included results from the comet assay, and micronucleus test. Key measurements, such as urea production and albumin secretion confirmed appropriate liver functionality and metabolic competence. In conclusion, the human cells system using HepaRG cells in Liver Plates from CNBio represents a promising model, demonstrating appropriate metabolic properties without requiring additional metabolic activators. This system effectively addresses genotoxic adverse outcomes within a single system, i.e. induction of chromosomal damage or damage to the mitotic apparatus (micronucleus test) and DNA strand breakage (comet assay).

P05
Optimizing Automated Micronucleus Test System in Organotypic Human Airway Models

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We developed a micronucleus (MN) test system using an organotypic human airway model, capable of detecting dose-dependent MN induction by clastogens and an aneugen. Incubation with epidermal growth factor (EGF) and cytochalasin B increased the percentage of binucleated cells (%BNCs) compared to conditions without EGF. However, %BNC in vehicle controls remained around 10%, making manual MN counting labor-intensive. Automated MN analysis is essential for validating this system with various genotoxicants across concentrations to assess the dose-response relationship for MN induction. Challenges included resolving cell aggregation in suspension for proper boundary recognition and mitigating fluorescence fading of acridine orange-stained nuclei and cytoplasm. To address these issues, we optimized cell suspension preparation by refining enzyme conditions and dissociation procedures, and adopting alternative staining reagents. Optimized preparation of single cell suspensions resolved cell aggregation, facilitating the distinction between mononucleated and binucleated cells. Clear stained images without fading were obtained during long-term image acquisition. Furthermore, IMACEL, an automated image analysis platform with machine learning-based image classification, enabled dose-dependent increases in micronucleated BNCs induced by mitomycin C, comparable to manual scoring. This optimized method enhances throughput for MN analysis, even in low-proliferating cells within organotypic human tissue models, providing a robust tool for genotoxicity assessment.

P06
Key Considerations for Surrogate Selection in Nitrosamine Risk Assessments

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Regulatory guidance worldwide for nitrosamine risk assessment permits the use of read-across to determine the acceptable intake of a novel nitrosamine drug substance-related impurity (NDSRI) which lacks carcinogenicity data. While the carcinogenicity potency categorization approach (CPCA) [Kruhlak et al 2024] has provided more achievable limits for some NDSRIs than the prior default of 18 ng/day, read-across may be needed to set a limit that is both achievable via control and detectable analytically. Read-across for the determination of safety limits is established as a method across a number of fields, including agrochemicals, European REACH assessments, extractables and leachables, and various methodologies have been proposed to systematise what is currently a manual and expert process. The potential for, and problems associated with, this subjectivity have been evident in application of read-across for NDSRIs, where discrepancies exist between health authorities and indeed the acceptability of read-across for a given compound has changed over time. Critical considerations for nitrosamine read across include the following: 1. Robustness of the carcinogenicity data of the proposed surrogate. 2. Structural features in the NDSRI and surrogate. 3. DMPK-relevant properties such as molecular weight and logP. 4. Overall similarity and localised similarity. The use of the considerations above allows for more robust selection of read-across surrogates for nitrosamines for many NDSRIs for which the CPCA-derived limit is unachievable. This allows for the continued marketing of the affected drugs with confidence that there is no exposure of patients to unacceptable increases in carcinogenic risk.

P07
The Use of In Silico Methods to Model Nitrosamine Formation and Hazard in Cosmetics and Personal-Care Products

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Most nitrosamines are classified as carcinogens that are formed in a reaction between a vulnerable amine and a nitrite compound. Although originally found in pharmaceuticals, nitrosamines can also form in cosmetic and personal care products, thus impacting a large group of consumers. Raw materials, preservatives, reaction conditions, and production habits can all be related to the presence/formation of nitrosating agents, such as the nitronium ion, nitrogen trioxide, or NOx in a cosmetic formulation. In silico modeling using Density Functional Theory (DFT) can provide a cost-effective way to accurately screen potential transformations and compute the kinetics and thermodynamics of nitrosamine formation

potential, including their transition states. Calculated energetics can, in turn, be leveraged to best understand the underlying structural drivers of nitrosamine formation. Our approach considers a range of secondary amines, nitrosating agents and commonly used solvents, which have not been extensively studied. Since not all nitrosamines are the same, the mutagenic/carcinogenic potential of the impurity will be concurrently assessed using the regulator-approved Carcinogenic Potency Categorization Approach (CPCA). This in-silico approach aims to enhance the evaluation of both existing and future formulations, enabling concerns to be addressed at early stages of the design process to limit the adverse effects of cosmetics and personal care products on human health. Ultimately, the goal of this project is to demonstrate its application on commercial products and provide industry with guidelines on how to limit the formation of hazardous nitrosamines in their formulations.

P08
Validation of Fluorescence In Situ Hybridization (FISH) Method for Identifying the Mechanism of Micronucleus Formation

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Micronuclei are small chromatin-containing bodies arising from chromosome fragments or whole chromosomes that are not incorporated into daughter nuclei following mitosis. To identify the aneugenic or clastogenic modes of action (MOA) for micronucleus formation, FISH is the most frequently used technique, which is both available under in vitro and in vivo conditions. This abstract presents the FISH validation data in in vitro TK6 micronucleus and in vivo mouse micronucleus assays. For in vitro assay, TK6 cells were treated with Colchicine (COL) with ten concentrations including with negative and positive controls, then harvested to prepare slides. For in vivo assay, the mouse was treated with cyclophosphamide (CP) and vinblastine (VIN) including with negative control, and the bone marrow cells were filtered through a self-made cellulose column to prepared slides. Then the slides were hybridized with pan-centromeric probes, incubated, washed, and finally stained with DAPI and PI (in vivo only). For FISH analysis, preferably 100 micronuclei were scored to discriminate the micronucleus with centromeric positive (C+) and negative (C-). The results showed that: for in vitro assay, the C+ rates at 0.004, 0.0063, and 0.0073 μg/mL with 4%, 21%, and 53% cytotoxicity were 33.0%, 77.0%, and 71.0%, respectively. The C+ rates induced at doses of 0.0063 and 0.0073 μg/mL showed a significant increase compared with negative control; for in vivo assay, statistically significant increase in C+ MN-PCE (74.22%±1.67%) was found in the VIN dose group, which was comparable with the literature reported data range (67.9%); the CP dose group did not induce the notable increase in centromeric positive micronuclei (21.86%±6.59%) over the control group (40.97%±25.34%), but was similar to literature reported range (25.3±1.3%). The results indicated that FISH methods were successfully validated at our lab, which can identify the aneugenic or clastogenic MOA for micronucleus formation.

P09
The Fertilized Avian Egg Fetal Liver Assays for Assessing DNA Damaging Potential of Chemicals

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The ability to produce direct DNA damage (genotoxicity) underlies the carcinogenic mode of action of various chemicals. As such, genotoxicity endpoints are typically evaluated in a regulatory-approved battery of in vitro tests with potential in vivo follow-up. Growing concern for animal welfare and implementation of new regulations which restrict the use of laboratory animals necessitated the introduction of New Approach Methodologies (NAMs). The avian egg-based (in ovo) models, the Chicken and related Turkey Egg Fetal Liver DNA Damage Assays, were developed as metabolically competent NAMs to potentially replace short-term in vivo genotoxicity assays for chemicals that are genotoxic in vitro. Both models utilize avian fetal livers for the evaluation of endpoints indicative of DNA damage produced by either direct or indirect mechanisms, specifically, the formation of nuclear DNA adducts and strand breaks. Moreover, avian embryos carry genetic and morphologic resemblance to mammals and can be used for an extensive evaluation of other endpoints including histopathology and tissue-specific genomic profiling. Avian fetal livers contain a full complement of metabolizing enzymes and are capable of bioactivation, detoxication, and elimination of xenobiotics. The comprehensive analysis of 87 and 59 chemicals assessed in the chicken and turkey models, respectively, revealed a stronger correlation with the results from in vivo assays demonstrating that in ovo models can detect the genotoxic potential of a broader range of compounds compared to in vitro assays with S9 supplementation. In conclusion, fertilized avian egg fetal liver assays offer a promising alternative to traditional in vivo genotoxicity assays.

P10
Importance of Extraction Vessel Shape in Medical Device Testing: A GeneTox Case Report

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Introduction: Extraction is a critical factor for biological testing/risk assessment of a new device. The device is extracted at specific temperatures/conditions. Extraction vessels are specified as clean, inert, closed with least dead space, but their shape is not considered. We present a case report where the shape of the extraction vessel made a significant difference in the results for Chromosomal Aberration Test (CAT).
Methods: Two CAT-Confirmation Assays conducted using Chinese Hamster Ovary cells. Device was absorbable white powder, an implant. Extracted in Ham's/F12 at 37±1°C/72±2 hours, using 0.2g/mL ratio, beyond its absorptive capacity. Vessel used was flat-bottomed jar and conical-centrifuge-tubes for first and second assays respectively. Post-extraction, extracts were kept at room/ambient temperature for at least 8 hours and used within 24 hours. Cells exposed (>20 hours) without metabolic activation, dosed at 100%, 50%, 25%, 12.5%, and 6.25% concentrations Negative/positive controls parallelly prepared. Two-hundred metaphases/condition analyzed. Chi-squared performed ($p=\leq 0.05$).
Results: Device dissolved partially; biphasic extract. Validity was confirmed with significant increase in aberrations in positive versus negative control ($p=0.000$). Device extracted in the flat-bottomed jar induced a significant increase in aberrations versus the negative control ($p=0.000$, clastogenic), but the test article extracted in the conical-centrifuge-tube did not ($p>0.411$, non-clastogenic).
Discussion: Under similar extraction conditions and solvent, our results show that the shape of the extraction vessel can produce clastogenic versus non-clastogenic response. This report gives us insights on whether the shape of the extraction vessel should be another factor to consider while performing extractions of medical devices in a powder form.

P11
Constructing a Database of Nitrosation Reactions to Confirm and Expand (Q)SAR Model Predictions

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While tools like the CPCA exist to assess the mutagenic risk of nitrosamines once they are known, there is still a gap in predicting the formation of nitrosamines, which can occur during synthesis, storage, or even digestion. Our goal was to build a (Q)SAR model from the available experimental data to accurately predict if an amine is likely to be nitrosated and what nitrosamine(s) would be produced. We also sought to create a database of the existing nitrosation reaction data to support the predictions made by the model through comparison with structurally similar compounds. We constructed two (Q)SAR models, one statistical and one expert rule-based. The statistical model used graph convolution neural networks which were trained on 207 compounds. The expert rule-based model consisted of 15 rules, covering both activating and deactivating features. The rule-based system was built into a nitrosation tool that also included a database of nearly 700 nitrosation reactions. When a query compound is submitted to the nitrosation tool, the rule-based model generates the plausible nitrosamines for each amine, along with their likelihood of formation based on the expert rules. Then, the reaction database is searched for the most similar parent/nitrosamine pairs, including exact hits, if they exist. Since it is impossible to have database containing every possible parent compound, searching for compounds that are structurally similar near the amine provides far more robust results. The reaction database proved to be extremely useful in confirming the predicted nitrosamines.

P12
Genotoxicity Evaluation of Fragrances and Botanicals using ToxTracker® assay

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ToxTracker® assay is designed to identify genotoxic and non-genotoxic carcinogens, thus providing industry and regulatory toxicologists with highly useful information for hazard and risk assessment purposes. Whereas ToxTracker® can be used as a confirmatory assay when chemicals show positive or equivocal results in Ames and/or micronucleus (MN) tests, the test method is also used for rapid and cost-effective screening of chemicals to identify potential DNA mutagens. The test method is reported to have a wide chemical applicability domain; accordingly, this study was focused on evaluating chemicals found to have genotoxic potential either from classic genetox test methods or have compelling

evidence of in vivo carcinogenic activity. We tested four chemicals in the ToxTracker® assay: Aristolochic acid (AA: mutagenic; positive in Ames test) and Pyrrolizidine (PZD: positive Ames and IVMN tests) in the botanical category and 2-octen-4-one (OCT: positive IVMN but negative in RSMN and in vivo tests) and Veratraldehyde (VTA: negative Ames but positive IVMN test) in the fragrance category. The results demonstrated that the AA and VTA were genotoxic while OCT and PZD were identified as non-genotoxic, under the test conditions of the experiment. The OCT was positive for oxidative stress induction and p53 activation. Although PZD was negative or equivocal for the DNA damage marker induction, it did show a dose-dependent increasing trend. While OCT and VTA have been tested in the ToxTracker® and our results show reproducibility of the data, there are no published reports of AA or PZD being tested in the assay. Our results show that the ToxTracker® assay may be a valuable tool in the genotoxicity assessment of both fragrances and botanicals.

P13
Consistently confident: key questions to review mutagenicity negative predictions

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Stringent testing requirements are set across all industries to ensure the safety to human health of chemicals brought to the market. Although testing classically requires in vivo and in vitro assays, in silico predictions of mutagenicity are accepted under ICH M7 in lieu of assay data; regulatory acceptance is a result of predictive accuracy being comparable to the Ames test. False negative predictions present the greatest concern, potentially allowing a mutagenic chemical into the population without confirmatory testing. Fortunately, the negative predictivity of (Q)SAR models for mutagenicity is high (>80%), increasing further upon application of expert review (>90%). Derek Nexus, an expert in silico prediction system, provides an indication of the uncertainty in negative predictions by highlighting if features in the query chemical are misclassified (chemical is similar to experimentally active chemicals) or unclassified (chemical contains features outside the model). Such predictions require additional scrutiny to conclude on mutagenic potential; however, analysis of predictions for 5,898 chemicals showed that they represent a small portion of datasets (<10%) and still retain high accuracy (up to 91% for misclassified and 96% for unclassified features respectively). Two key questions need to be answered when reviewing these predictions. 1. Is the misclassified feature relevant to the query? 2. Is the unclassified feature likely to be mutagenic? Identifying key features requiring review guides assessors to answer these questions, supporting a consistent approach that increases confidence in concluding negative during mutagenicity assessments using in silico approaches.

P14

Investigating limit dose and identifying genotoxic hazard of common pharmaceutical solvents with ToxTracker

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When testing substances in early phases of development it is not always possible to avoid residual solvent in API. To determine tolerability, we assessed 19 commonly used solvents in ToxTracker and then compared observations to published toxicological findings. ToxTracker is a new approach method that provides insight into MOA using GFP-gene expression and can discriminate direct-acting genotoxicants from non DNA-reactive genotoxicants. The selected solvents were tested as high as possible (up to 5% v/v) using a 24h exposure in the absence or presence of S9. GFP reporter activation and cell survival were assessed using flow cytometry and ~half (10/19) of the solvents induced genotoxic effects (Rtkn-GFP) with concomitant increases in oxidative stress (Srxn1-GFP) in the absence of S9. To investigate causality of oxidative stress, solvents were retested in the absence and presence of ROS scavengers (NAC and GSH) and 3/10 became non-genotoxic. For the remaining solvents, we back calculated molarity and evaluated dose-response using benchmark dose (BMD) analysis in PROAST. A benchmark response (BMR) of 100 was used and BMD limits (95% CI) showed that most (9/10) of the solvents had been tested far above 10 mM, indicating they would be deemed negative if applying this OECD recommended limit dose, and aligning with reported in vivo genotoxicity and carcinogenicity findings. Finally, using BMRs up to -20%, cell count data were used to quantitively assess reasonable limits of these solvent in ToxTracker.

P15

Off-target PARP1 inhibition as an indirect mechanism of clastogenicity

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Small molecule drugs are estimated to interact with up to several dozen “off-target” proteins at pharmacologically relevant concentrations, and even more in the higher free concentrations evaluated in in vitro assays. Commonly described off-target interactions with potentially genotoxic effects are tubulin binding and Aurora kinase inhibition, both of which are associated with aneugenicity. Here we describe a chemical series with poly(ADP-ribose) polymerase 1 (PARP1) as the key off-target of concern with indirect clastogenic effects, and the development of improved compounds lacking these genotoxic effects. Early nonselective tool compounds, and a key competitor molecule, generate robust micronucleus induction, with a similar response and mechanistic profile to disclosed data for approved PARP1/2 inhibitors. Although development of this chemical series is focused on ICH S9 indications, greatly increased selectivity against PARP1 was achieved through a structure-activity relationship-based screening campaign to reduce the risk of off-target toxicity. The resulting selective tool compound lacked any significant clastogenic signal in “hazard ID” evaluations. A quantitative analysis suggests a potential threshold for this indirect mechanism, as would be expected for a protein-small molecule interaction exhibiting a sigmoidal concentration response.

P16
Validating Duplex Sequencing for Mutagenicity Assessment: Strong Correlation with the TGR Assay Across Tissues

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The transgenic rodent (TGR) gene mutation assay is the current gold standard for in vivo mutagenicity assessment; however, error-corrected next-generation sequencing approaches, such as Duplex Sequencing (DS), now offer enhanced resolution and mechanistic insights. We compared mutation detection between DS and the TGR assay in MutaMouse males exposed to benzo[b]fluoranthene (BbF), a priority polycyclic aromatic hydrocarbon. We administered BbF (0, 6.25, 12.5, 50, 100 mg/kg/day) via oral gavage for 28 days, and sampled liver and bone marrow three days post-exposure. Mutation analysis was performed using DS on the mouse mutagenesis panel (MMP) and lacZ transgene, alongside the TGR assay. We detected a significant dose-response with all three approaches. DS mutation frequency minimum (unique mutations) of the MMP strongly correlated with the TGR assay (Pearson’s r: 0.98 in bone marrow, 0.99 in liver). The correlation decreased to 0.73 in bone marrow but remained 0.99 in liver for mutation frequency maximum (all mutations). DS on lacZ also showed a robust concordance with DS on MMP and the TGR assay. Benchmark dose (BMD) confidence intervals (mg/kg/day) to induce a 50% increase in mutation frequency overlapped across methods in both tissues: 10.1–18.4 and 3.6–12.8 for DS on MMP; 10.6–29.7 and 7.62–32.5 for DS on lacZ; and 5.08–15.4 and 1.44–8.06 for the TGR assay in liver and bone marrow, respectively. These findings support DS as a viable alternative for mutagenicity assessment, providing comparable results to the TGR assay while offering additional insights into the location, type, and clonal expansion of mutations.

P17
Multi-endpoint Genotoxicity Assessment of N-Nitroso morpholine in four-week-old Wistar Han Rats

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Error-corrected next generation sequencing (ecNGS) technology revolutionized mutation detection with its high sensitivity and reproducibility. Duplex Sequencing (DS) being one of the most validated ecNGS platform gained popularity rapidly, with many incorporating DS as an additional endpoint to the traditional transgenic rodent mutation assay. Unfortunately,

the recent shortage in DS kits hindered the usage of the technology and prompted our multi-endpoint study to explore the possibilities of using alternative in vivo endpoints. Leveraging the knowledge of key events leading up to mutation formation caused by nitrosamines, we assessed the potential of N-nitroso morpholine (NMOR) to induce micronuclei (MN), DNA damage (using Comet assay), and mutations in hepatocytes. Groups of 4-week-old male Wister Han rats were given 10 daily doses of vehicle control, 15 mg/kg N-Nitrosodiethylamine, as positive control, or 0.1, 1, 5, 10, 15 and 20 mg/kg of NMOR. Liver was collected from animals and used for the liver micronucleus, Comet Assay, and DS. Benchmark dose analysis was performed to compare the sensitivities between endpoints. Our data demonstrated while all assays were able to detect positive responses, DS was the most sensitive endpoint with the smallest BMDU/BMDL ratio, followed by the Comet assay, and the Liver MN being the least sensitive.

P18
An Assessment of Five Small N-nitrosamines as Positive Controls for the Enhanced Ames Test (EAT)

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Current guidance for testing of N-nitrosamine Impurities in drugs requires using an Enhanced Ames Test (EAT) to assess the impurity's mutagenic potential. The design calls for use of a 30 min preincubation method, 30% induced rat and hamster liver S9, and requires two to three nitrosamine specific positive controls. The current study was designed to assess several small N-nitrosamine molecules for their use as positive controls in the EAT. These include Nitrosodimetylamine (NDMA), Nitrosodipropylamine (NDPA), Nitrosodibutylamine (NDBA), Nitrosodiisopropylamine (NDIPA), 1-Cyclopentyl-4-nitrosopiperazine (CPNP). All the compounds were tested under standard EAT conditions. None of the compounds were positive with tester strains TA98 or TA1537. NDIPA was considered weakly positive based on a 3.1-fold increase with tester strain TA1535 in the presence of hamster S9 at 500 µg/plate. All other compounds induced mutagenic responses in multiple strains as well with either rat or hamster S9. For instance, NDPA yielded positive results in tester strains TA100, TA1535, and WP2 uvrA (pKM101) in the presence of hamster S9 as well as TA1535 and WP2 uvrA (pKM101) with rat S9. Similarly, NDMA was positive in the same strains as NDPA in the presence of hamster S9, although only positive in WP2 uvrA (pKM101) with rat S9. NDBA was also positive in the same strains with hamster S9, but negative in all strains with rat S9. Lastly CPNP was positive in TA100 and TA1535. While none of the nitrosamines tested yielded positive results across the board for tester strains TA100, TA1535, and WP2 uvrA (pKM101) in the presence of both rat and hamster S9, an EAT study design incorporating a combination of two to three small n-nitrosamines such as NDMA, NDPA, and CPNP may provide ample evidence that the test system and metabolic activation systems are sensitive and optimized for detecting an unknown mutagenic nitrosamine.

P19
Age Dictates Vulnerability to N-Nitrosodimethylamine-Induced Liver Damage in a DNA Repair Deficient Mouse Model

Lindsay Volk, Monet Norales, Callie Karjane, Joshua Corrigan, Lee Pribyl, Sebastian Carrasco, Megan Blawas, Natalya Yakimchuk, Emily Michelsen, Ella Dulski, Nicolette Bugher, Matilda Swanson, Desiree Plata, Robert Croy, John Essigmann, Bevin Engelward
MIT, Cambridge, MA, USA

This presentation explores how age influences the effects of N-Nitrosodimethylamine (NDMA), a probable human carcinogen, using a Genetically Modified Mouse Model deficient in DNA repair (Aag-/-;Mgmt-/-). We exposed juvenile and adult mice to NDMA through drinking water and assessed DNA damage, inflammation, mutagenesis, and long-term pathological changes. Juvenile mice were exquisitely sensitive to NDMA, with enhanced DNA damage, inflammation, and mutations persisting long after exposure, culminating in increased liver pathology and tumor incidence. In stark contrast, adult mice were highly resistant to the negative effects of NDMA exposure. As juveniles and adults start with similar DNA adduct levels, the heightened liver proliferation rate in juveniles likely promotes the downstream consequences of unrepaired NDMA adducts. Indeed, T3-induced cellular proliferation promoted the deleterious effects of DNA adducts, including increased mutations, in adult mice co-exposed to NDMA. Our findings highlight age as a critical risk factor for NDMA exposure, emphasizing the importance of developmental stage in toxicological assessments. With regard to regulatory decision making, for some endpoints, the magnitude of the impact on susceptibility for the very young is far greater than the 10X correction normally applied.

P20
Genetic Toxicity Evaluation of Melatonin in the Bacterial Reverse Mutation Assay

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Background: Melatonin is a neurohormone commonly used as a supplement to manage sleep disturbance. It also exhibits neuroprotective properties and is a key active pharmaceutical ingredient in IGC-AD1 - IGC Pharma's Investigational Drug targeting agitation in Alzheimer's disease. While melatonin is Generally Recognized As Safe ("GRAS"), its non-clinical safety profile remains inadequately characterized due to the lack of formal FDA approval. This study evaluated the mutagenic potential of melatonin using the Bacterial Reverse Mutation Test ("Ames test"), an established assay for assessing genetic toxicity.
Methods: Melatonin was tested at concentrations ranging from 1.6 to 5000 µg/plate using dimethyl sulfoxide ("DMSO") as the vehicle. The assay employed multiple histidine- or tryptophan-dependent bacterial strains, including Salmonella typhimurium (TA1535, TA1537, TA98, and TA100) and Escherichia coli (WP2 trp uvrA), both with and without an S9 metabolic activation system. Using the plate incorporation method, revertant colony counts were assessed in triplicate for each condition.
Results: Melatonin did not induce a significant or dose-dependent increase in the number of revertant colonies in any of the strains tested under either metabolic condition. Positive and negative controls met the acceptance criteria, confirming assay validity.
Conclusions: Melatonin exhibited no evidence of mutagenic activity under the conditions of this study, supporting its safety profile at the tested dose range. These findings contribute to the non-clinical safety assessment of melatonin as part of the IGC-AD1 development program."

P21
A375 Human Melanoma Cell Line: A Novel Platform for Genotoxicity Assessment Using In Vitro Micronucleus Assay with Flow Cytometric Analysis

Brian Yard, Emily Liptock, Meira Simolin, Javed Bhalli
Frontage Laboratories Concord OH USA

The in vitro Micronucleus (MN) assay is widely used for genotoxicity evaluation, with flow cytometric-based approaches enhancing assay throughput and objectivity. TK6 lymphoblastoid cells are commonly used due to their p53 competence, but they often exhibit reduced proliferative capacity following chemical exposure, complicating data interpretation. In contrast, A375 human melanoma cells proliferate robustly and display enhanced resistance to apoptosis, making them a promising alternative. This study evaluated A375 cells in the flow cytometric-based in vitro MN assay with and without S9 using three reference genotoxicants: Cyclophosphamide (CP; metabolically activated clastogen), Methyl Methanesulfonate (MMS; direct-acting clastogen) and Vinblastine (VB; aneugen). A375 cells were seeded at 5 × 104 cells/well in 12-well plates and then treated approximately 24 hours later with CP (10–15 µM) or MMS (75–100 µM) for 4 hours with or without S9 respectively, or with VB (1.5–2.0 nM) for 30 hours without S9. Cells were harvested 30 hours post-treatment and MN frequency and percent cytotoxicity (%RICC) were measured using flow cytometric analysis. All three compounds induced statistically significant increases in %MN frequency compared to the vehicle controls, with baseline MN frequency ranged from 1.13% to 2.71%. A375 cells also exhibited lower relative cytotoxicity compared to historical TK6 data at the same or higher concentrations of the positive controls. These findings support that A375 cell line can be effectively used as an alternative for flow cytometry-based in vitro MN assays. Future studies will expand compound diversity, microscopic scoring, and explore further standardization for regulatory use.

P22

DNA Methylation Damage Induces Persistent Interferon Activation, Clonal Expansion, and Cancer in Mgmt Null Mouse Livers

Lee Pribyl[1], Jennifer Kay[2] Josh Corrigan[1], Norah Owiti[1], Monet Norales[1], Ilana Nazari[1], Matilda Swansen[1], Evan Kowal[1], Aimee Moise[1], Ishwar Kohale[1], Ben Ryan[1], Bob Croy[1], Duanduan Ma[1], Stuart Levine[1], Tim Ragan[3], Steve Dertinger[4], Sebastian Carrasco[5], Dushan Wadduwage[6], John Essigmann[1], Bevin Engelward [1]
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[6]Harvard University Cambridge MA USA

Alkylating agents are abundant in our environment, produced endogenously, and are used as chemotherapeutics. One such agent is N-nitrosodimethylamine (NDMA), a Group 2A probable human carcinogen, which is a common contaminant in drinking water and arises as a breakdown product of prescription drugs. Here, we have explored the role of a key DNA repair protein, namely O6-methylguanine methyltransferase (MGMT), in modulating molecular, cellular, and physiological responses as they unfold from days to weeks to months following exposure to NDMA in a mouse model. Over time, we monitored transcriptomics, phosphoproteomics, protein expression levels, apoptosis, cell proliferation, homologous recombination, pathophysiological changes, and clonal expansion within the liver. Phosphoproteomic analysis revealed a strong DNA damage response quickly after exposure. Transcriptomic profiling revealed a striking contrast: a robust interferon response involving hundreds of genes sharply diverged from the limited DNA repair activity, which implicated fewer than a dozen protein-coding genes. Remarkably, an acute exposure to NDMA as a neonate lead to permanent dysregulation of gene expression, and a persistent interferon response, culminating in pathophysiological responses and cancer. Spatial transcriptomics indicates that clonal expansion of physiologically normal tissue precedes tumor formation, coinciding with anti-inflammatory gene expression. Several genes are expressed in both growth-advantaged cells and tumors, suggesting that early-stage aberrant gene expression may contribute to cancer development months later. This work provides a comprehensive and holistic perspective on disease initiation and progression, shedding light on underlying mechanisms, providing potential biomarkers predictive of downstream disease, and raising the possibility of novel disease mitigation strategies.

P23

Error-Corrected Sequencing Reveals that Alpha-Pinene, a Chemical with Widespread Human Exposure, is Mutagenic in Rat Mammary Tissue

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[1]NIEHS, Research Triangle Park, NC, USA
[2]Inotiv RTP, Research Triangle Park, NC, USA

The Division of Translational Toxicology (DTT) is conducting an extensive toxicological evaluation of alpha-pinene (AP). AP is released by pine trees and is present in cannabis plants, and it is present in food, dietary supplements, and consumer products. It is also the main component of turpentine, which is used in occupational settings. AP is negative in the Ames assay, but its metabolite, alpha-pinene oxide (APO), is positive. A 13-week inhalation study of 0, 50, 100, or 200 ppm AP in male and female Hsd:Sprague Dawley SD rats conducted at the DTT offered the opportunity to evaluate whether duplex sequencing (DuplexSeq), an error-corrected sequencing (ECS) approach, could detect AP-dependent mutagenesis in mammary tissue, a target for AP and APO. DuplexSeq uses a strategy of tagging fragmented DNA duplexes and deep sequencing of twenty, 2.4 kb target sites to detect true mutations at the level of somatic cell mutagenesis. The overall mutation frequency did not reach statistical significance for male or female rats; however, one of the 20 target sites was especially mutable in response to AP exposure in both sexes. Additionally, significant increases in the proportion of T>C mutations were observed in both sexes. In female rats, mutational signatures obtained from AP-exposed mammary tissue were similar to those observed in mammary tumors from a 2-year rodent cancer bioassay for AP. These findings support the use of DuplexSeq for evaluation of mutagenesis in subchronic studies and suggest that ECS can detect early emergence of mutations associated with AP-dependent tumorigenesis.

P24
Evaluation of Potential Causes for the Lower Response of Cyclophosphamide in the In Vitro Micronucleus Assay
Stephanie Kellum
Corteva Agriscience, Newark, DE, USA

Cyclophosphamide (CP), an in vitro micronucleus positive control for the metabolically activated test condition, has demonstrated lower inductions in micronuclei (MN) compared to the positive controls for the non-activated test conditions (mitomycin-C and vinblastine). This can lead to instances where the CP historical control ranges overlap the corresponding vehicle control ranges, as well as instances where the test condition fails due to the CP response not resulting in a statistical increase compared to the concurrent vehicle control. Since the highest concentration of CP used in our design, 15 µg/mL, was selected during development as it typically results in cytotoxicity within the OECD 487 recommended limit of 55±5%, other factors were explored that may influence MN induction, including CP storage duration, CP and S9 lots, and donor variation. The results demonstrated that the lower MN induction observed after treatment with CP is not influenced by any of the evaluated factors. Since the maximum concentration of CP being used is at the OECD 487 recommended cytotoxicity limit of 55±5%, it is evident that the lower response is an inherent response of the assay. This may result in the upper portion of the VC historical range overlapping with the lower portion of the CP historical control range and potential repeat testing if CP does not meet assay acceptability criteria. Future considerations would include evaluation of Benzo(a)pyrene for comparison to CP. Evaluation of the acceptability of a run will continue to follow the criteria outlined in the OECD 487 test guideline.

P25
The Enhanced Ames Test – One CRO, Four Labs, What Could Possibly Go Wrong?
Leon F. Stankowski, Jr.
Charles River Laboratories, Ashland, OH, USA

Charles River Laboratories had genetic toxicology laboratories operating at four sites: Den Bosch (The Netherlands), Senneville (Quebec, Canada), Skokie (IL, USA), and Vesprem (Hungary). Three have performed the enhanced Ames Test (EAT) for regulatory submissions, and one is currently in the validation/qualification process. However, the first three initiated testing independently, without the benefit of coordinated planning or review. Generally, the five activation conditions (–S9, +10 and 30% rat and hamster S9s) had little effect on negative control values. The lone exception was +30% rat and hamster S9s in TA100 at Den Bosch, where values were substantially lower than historical controls. There was no apparent toxicity associated with this decrease and the cause is yet to be determined, but it was reproducible and considered real. While no hard data regarding client studies can be shared, one study from Skokie and another from Vesprem, submitted to separate regulatory authorities, were rejected due to the use of “excessive” 100-µL DMSO dosing volumes and concern over potential inhibition of metabolic enzymes. Repeating each study with 20- or 25-µL dose volumes yielded the same (negative) results. The requirement to add two NDSRIs positive controls in one study had no impact on the interpretation of the results. In addition, the same regulatory authority requested a third test using plate incorporation treatment, even though already negative in two “standard” EAT assays. Unfortunately, the low background values in TA100 +30% rat S9 in Den Bosch negatively impacted this third test. Except for the low values in TA100 +30% rat and hamster S9s, positive and negative control results were similar across all sites. Comparisons of DMSO dosing volumes made in Skokie and Vesprem indicate DMSO, up to 100 µL, does not appear to adversely impact the results except for small alkyl nitrosamines like NDMA.

2024 GTA Excellence in Science Award Recipient

Congratulations Francesco Marchetti, PhD!

Francesco Marchetti (left) and Sheroy Minocherhomji (right, 2024 ESA[2] Committee Member and 2023 GTA Chair)

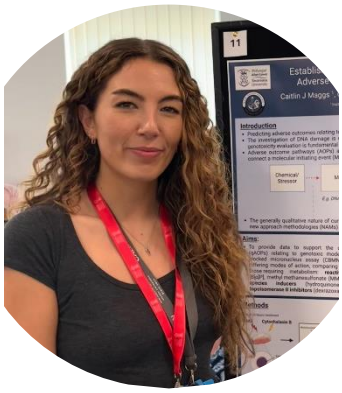
The 2024 GTA Excellence in Science Award was proudly presented to Dr. Francesco Marchetti, a distinguished senior research scientist at Health Canada's Environmental Health Science and Research Bureau and an Adjunct Research Professor at Carleton University. Renowned globally for his expertise in genetic toxicology and germ cell mutagenesis, Dr. Marchetti has significantly advanced our understanding of environmental impacts on somatic and germ cell mutagenesis.

Dr. Marchetti's has authored over 130 peer-reviewed publications during his career and played a key role in refining OECD TG 488: the transgenic rodent somatic and germ cell mutation assay. In addition, his groundbreaking work with next-generation sequencing technologies has led to the first whole genome sequence of a TGR model. He has applied ecNGS technology to demonstrate that germ cell mutations are transmitted to offspring. His work also revealed how detailed mutation spectra from ecNGS studies in rodents can be used to identify mutational mechanisms operating in human cancers.

A dedicated member of the Environmental Mutagenesis and Genomic Society, Dr. Marchetti has served as its President and Editor-In-Chief of the EMM journal. His accolades include the Health Canada Deputy Minister’s Award for Excellence in Science and honorary membership in the Italian EMGS.

Congratulations to Dr. Francesco Marchetti for his exceptional contributions to science and genetic toxicology!

2025 Student Platform Presenter Awards



Caitlin Maggs MSc
Swansea University

Assessing genotoxicity in multiple cell culture models for quantitative Adverse Outcome Pathway (qAOP) development

David Schuster MS
University of Ottawa

Duplex Sequencing after Prolonged Benzo[b]fluoranthene Exposure Reveals Tissue-Specific Differences in Mutagenic Response, Chemical Potency, and Clonal Expansion of Mutations

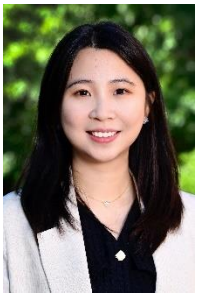
2025 GTA Abstract Award Winners

Student

Jillian Brejnik
The George Washington University

The Use of In Silico Methods to Model Nitrosamine Formation and Hazard in Cosmetics and Personal-Care Products

Early-Stage Investigator



Xinwen Zhang
National Institute of Environmental Health Sciences

Integration of Duplex Sequencing with the Peripheral Blood Micronucleus Assay for a Comprehensive, Time Course Evaluation of Benzo[b]fluoranthene-Dependent Genotoxicity

Alicia Predom
Pfizer

Multi-endpoint Genotoxicity Assessment of N-Nitroso morpholine in four-week-old Wistar Han Rats.

Krystle Reiss
MultiCASE Inc.

Constructing a Database of Nitrosation Reactions to Confirm and Expand (Q)SAR Model Predictions

2024 GTA Emerging Scientist Award Recipient

Krystle Reiss PhD
MultiCASE Inc.

Predicting Nitrosation of Secondary and Tertiary Amines Using Statistical (Q)SAR Models

GTA Membership Information

The Genetic Toxicology Association is organized exclusively to meet educational and scientific goals. The primary purpose is to foster the exchange and dissemination of information regarding genetic toxicology and to promote the development of the science of genetic toxicology. Membership is open to anyone interested in the field of genetic toxicology and the annual dues are currently $50. Student memberships are $25 per year. Dues are for the calendar year.

Benefits of membership include discounted conference registration and access to previous meeting materials

Renew and pay your membership on-line at www.gta-us.org.

@ genetoxtesting

genetic-toxicology-association

@gta_genetox

2026 GTA Excellence in Science & Service Awards

Call for Nominations!

This award recognizes the contributions of a member who has made particularly notable contributions to the field of genetic toxicology. **All GTA members are invited to submit a nomination.** One awardee each is selected for the Science and Service awards by a committee of past GTA Board Members, specifically former Chairs.

The nomination package consists of a short (up to 1 page) description of the nominee's contributions to GTA and the field of Genetic Toxicology. The nominee must be a current member of GTA, and nominators are encouraged to discuss potential nominations with the nominee to make sure (s)he is aware of the nomination and will be able to attend the GTA meeting in 2025. Nominations should be sent to Stephanie Kellum, incoming Chair of the ESA selection committee.

Nominations for the 2026 GTA Excellence in Science & Service Awards are due by December 1, 2025.

Please email your nominations to: stephanie.n.kellum@corteva.com

Past ESA Winners

2024	Francesco Marchetti, Ph.D.
2023	Dan Levy, Ph.D. (Excellence in Science)
2023	Ofelia Olivero (Excellence in Service)
2022	Stephen Dertinger, Ph.D
2020	Krista L. Dobo, Ph.D.
2019	Gary Williams, M.D.
2018	Maria Donner, Ph.D.
2017	Leon F. Stankowski, Jr, Ph.D.
2016	Kerry L. Dearfield, Ph.D.
2015	Miriam C. Poirier, Ph.D.
2014	David Jacobson-Kram, Ph.D., D.A.B.T.
2013	Rosalie Elespuru, Ph.D.
2012	Marilyn J. Aardema, Ph. D.
2010	James T. MacGregor
2008	Ronald B. Snyder, Ph.D.
2007	R. Daniel Benz, Ph.D.
2006	Sheila Galloway

GTA Board of Directors 2024-2025

Chair (2024)
Wen Sun

Wen Sun is a Principal Scientist in the Genetic Toxicology Department at Pfizer. She received her PhD in Molecular and Cellular Biology (molecular medicine) from the University of Iowa and worked as a post-doctoral researcher at Yale University Pharmacology department prior to joining Pfizer three and half years ago. Since joining Pfizer, Wen lead the development, validation, and implementation of the multiplexed imaging screening platform, which enabled the delivery of regulatory endpoint and mode of action information to project teams in a single assay. The platform also incorporated computational predictive modeling and quantitative dose-response assessment to support pharmaceutical development. Currently, Wen oversees the screening laboratory, provides subject matter expertise guiding teams and chemists away from genotoxicity liabilities. In addition, she serves as the drug safety team lead on projects and participates in genetic toxicology impurity assessment. Wen is an active member of the Genetic Toxicology Association, the Environmental Mutagenesis and Genomics Society, and Health and Environmental Sciences Institute. She has presented her work at numerous conferences and currently contributing to manuscript and AOP preparation. Wen has a particular passion in in vitro assays, adverse outcome pathways, innovative technologies, and alternative testing methods.

Chair-Elect (2024)
Ashley Allemang

Ashley has over 10 years of industry experience in applied genetic toxicology in the context of in vitro-based safety support. Her research has primarily focused on mode of action determination and distinguishing direct and indirect genotoxicity through various in vitro methods such as the micronucleus assay, the ToxTracker assay and other genomics-based methods such as the TGx-DDI biomarker. More recently her research has employed the HepaRG micronucleus assay to develop in vitro-based genotoxicity potency rankings of pyrrolizidine alkaloids, as well as genotoxicity evaluation of mixtures. In addition to her research activities, her expertise has also expanded to include SAR based risk assessment. Ashley has been actively involved in the HESI GTTC committee since 2017 and has participated in the development of genetox-related AOPs and is currently co-leading the Indirect Genotoxicity subgroup of the In Vitro Work Group evaluating NAMs for genetic toxicity testing.

2025 Annual Meeting of the GTA

John M. Clayton Hall Conference Center
University of Delaware, Newark, DE

James Kath

James Kath is a Principal Scientist in the Genetic Toxicology group at AbbVie. He received his PhD in Biophysics from Harvard University where he studied bacterial translesion DNA polymerases, and was an active participant in the academic communities studying DNA replication, repair, and mutagenesis.
In 2017, James joined AbbVie's Chemical Biology group, where he supported small molecule programs through the development of target engagement assays, identification of off-targets, and evaluation of new therapeutic modalities. Over the past six years in AbbVie Discovery, James developed a strong interest in toxicology through a close collaboration with AbbVie's Investigative Toxicology and Pathology group, and leading two cross-functional working groups on new modalities. Since transitioning to the Genetic Toxicology group in 2023, James's focus has been on the implementing new approach modalities and supporting regulatory filings. He is excited to participate the GTA and HESI GTTC committee and build further connections between the genetic toxicology and DNA replication and repair communities.

Steven Nicotra

Steven Nicotra is a Senior Scientist in the Global Toxicology and Safety Pharmacology group at Johnson & Johnson. Steven began his career in the field toxicology in 2008 and started a focus on genetic toxicology in 2011. Steven holds a BSc in Animal Biotechnology and Conservation from Delaware Valley College and a Master of Business Administration from Holy Family University.
Steven's areas of expertise span from performing in vivo studies and in vitro genetic toxicology studies to monitoring and directing genetic toxicology studies supporting all stages of drug development. Moreover, Steven has supported successful regulatory submissions performing the mutagenicity hazard assessment of impurities and authoring/reviewing corresponding dossier sections. Steven has been active volunteer for the Genetic Toxicology Association since 2020. His volunteer responsibilities have focused on meeting preparation with input, collating and reviewing Student/Early Investigator submitted abstracts, and chairing symposia.

Liz Rubitski

Liz is a Senior Scientist at Pfizer, holds a B.S. in Diagnostic Genetic Sciences from the University of Connecticut and has been working in Genetic Toxicology for over 25 years. Current portfolio responsibilities include serving as technical lead for the high content iScreen assay providing micronucleus results with mechanism of action to project teams and as a POA for the ex vivo portion of the Big Blue Assay. Liz also works on developmental projects involving data management, 6-well Ames imaging, continuous improvement of routine assays and serves as the image analysis specialist to groups within Drug Safety and Development. As well as roles on the board for the Society of Biomolecular Imaging and Informatics (SBI2) since its founding, she has been a member of GTA for over 20 years, serving as a board member and scientific program chair in years past.

Yi Yang

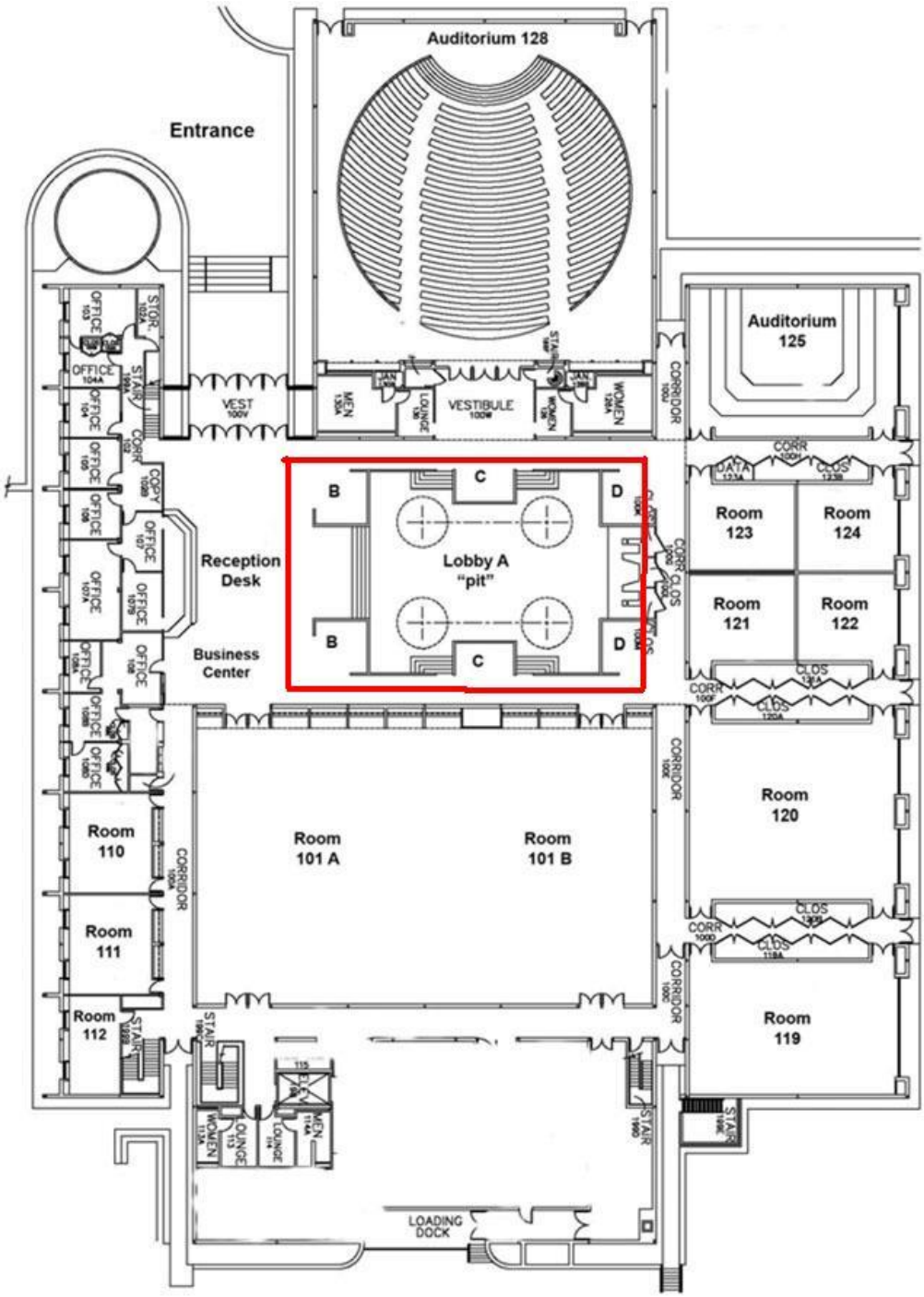
Dr. Yi Yang is currently Director of Genetic, Environmental, and Occupational Toxicology at AbbVie. She is also a Therapeutic Area Leader overseeing preclinical safety portfolios in Specialty and Cell Therapy area. Dr. Yang has 20 years of experience in preclinical safety assessment for a variety of therapeutic modalities, including small molecules, monoclonal antibodies, degradomers, cell and gene therapy. Her areas of expertise include regulatory toxicology, predictive and mechanistic toxicology, genetic toxicology, toxicogenomics, toxicity biomarkers, and biostatistics. Dr. Yang received her MD from Sun Yet-Sen University of Medical Sciences, her Ph.D. in Toxicology and M.S. in Biostatistics from University of Cincinnati. She is also certified as a Diplomat of the American Board of Toxicology. She authored 25 peer-reviewed publications and over 20 regulatory submissions supporting Phase 1 and Phase 2 clinical trials. She is actively involved in several industry-wide collaborations, including the Predictive Safety Testing Consortium and the ILSI-HESI consortium. She also served as Secretary to the American Association of Chinese in Toxicology (2010-2012), Chair to the Applied Pharmaceutical Toxicology (2015-2016), and President to the Midwest Regional Chapter of SOT (2017-2019). Dr. Yang joined GTA in 2022 and is co-chair of the Scientific Program Committee for the 2023 annual meeting.

Zhiying Ji

Dr. Zhiying Ji is currently a toxicologist at Incyte Corporation in Wilmington, DE. He manages toxicology programs in accordance with global regulatory requirements to support drug discovery and development.
Prior to joining Incyte, Dr. Ji worked for Bristol Myers Squibb Company (BMS) in New Brunswick, NJ from 2017 to 2022. He provided scientific leadership in developing genotoxicity testing strategies in support of drug discovery and development of different modalities; conducted mutagenicity hazard assessment for intermediates/impurities in accordance with ICH M7 guideline; and leaded genetic toxicology innovation activities.
He also served as Project Toxicologist for multiple programs to support drug discovery and development. Dr. Ji was a Lead Scientist – Genetic Toxicology at Dow Chemical Company from 2012 to 2017. He provided science leadership in genetic toxicology studies to support product development and global registration; acted as Study Director for in-house GLP and non-GLP genetic toxicology studies; acted as Study monitor for GLP and non-GLP genetic toxicology studies conducted at CROs; leaded capability development of innovative genotoxicity techniques. Dr. Ji received his Ph.D. degree in Toxicology from Chinese Center for Disease Control and Prevention in 2004 and his post-doctoral training under the supervision of Prof. Martyn Smith at University of California, Berkeley. He applied fluorescence in situ hybridization (FISH) in the development of early effect biomarkers for benzene and formaldehyde exposure and investigated the genetic and epigenetic mechanisms of chemical mutagenesis and carcinogenesis. Dr. Ji has authored or co-authored over 20 peer-reviewed publications. He is an active member of GTA, EMGS and SOT.

John M. Clayton Hall Conference Center – Map

University of Delaware, Newark, DE



The Genetic Toxicology Association (GTA) is a tax-exempt 501c3 educational and scientific organization that was founded in 1975 and incorporated in 1981 under the laws of the state of Delaware. Its primary purpose is to promote the development of the science of genetic toxicology and to foster the exchange and dissemination of information concerning the field.