Genetic Toxicology Association
Translating Genetox

The GTA Newsletter

Looking back at our 2022 virtual GTA meeting

Year-end greetings and Happy December -- how this year has flown by!

Due to the continued uncertainties of the COVID, the 2022 Annual Meeting was held as a virtual event. Despite not being able to see each other in person, the event was a great success with attendees from 24 US States, Washington DC and Puerto Rico, and 30 foreign countries. Highlights of the strong scientific content of our virtual gathering are featured throughout this issue.

We are hoping to host an in-person meeting in 2023 but for now, please enjoy this summary of the meeting's scientific sessions, workshops, and awardees.

As always, thank you for your continued interest and support of the Genetic Toxicology Association.

Save the date:

GTA meeting 2023

3-5 May 2023

Clayton Hall, University of Delaware,

Newark, DE

Letter from the Chair

Dear fellow GTA members and 2022 Annual Meeting attendees,

The 2022 annual scientific meeting of the Genetic Toxicology Association was held virtually in May and proved to be highly interactive and informative. This year's Scientific Program Committee included Kevin Cross, Ji [Zane] Zhiying and Sheroy Minocherhomji. Together with the session co-chairs, we were able to provide a comprehensive program focused on important and emerging issues and advances in the field of genetic toxicology. The 2022 GTA annual meeting was a virtual meeting and was made possible by the cumulative dedication of numerous current and past members of the GTA BOD, volunteers, and sponsors. Attendance at the GTA meeting represented a global audience.

This year's speakers as was the case for our 2021 meeting deserve a special recognition for their flexibility and steadfastness in delivering an exciting set of talks and workshops which were followed on by Q&A sessions with attendees, virtually. The 2022 GTA keynote address was given by Prof. David Pellman (Harvard Medical School) on Mechanisms Driving Rapid Evolution of Genomes which was very well received and invigorated robust scientific discussion. For the 2022 meeting we had two workshops and six symposiums.

The case study workshops were chaired by Maria Engel and Zhanna Sobol and included talks on "Ames positive follow ups" and "Applied Genetox". The six symposiums discussed the current state-of-the-art in the field of genetic toxicology, including Symposium 1 (co-chaired by Penny Leavitt and Laura Custer on "Genetox related updates"), Symposium 2 (co-chaired by Silvana Libertini & Mick Fellows on "Assays to measure off-target mutagenicity and insertional mutagenesis of gene therapies"), Symposium 3 (co-chaired by Zhiying [Zane] Ji and Stephen Dertinger on "In vivo micronucleus test in alternative tissues"), Symposiums 4 and 5 (co-chaired by Catrin Hasselgren & Kevin Cross on "Nitrosamine risk assessment/updates Parts 1 and 2"), Symposium 5 (co-chaired by Stefan Pfuhler & Ashley Allemang on "Applied Genotoxicity Testing") and Symposium 6 (co-chaired by Melisa Masuda-Herrera & Vincent Reynolds on "Pig-a assay regulatory acceptance"). The program was rounded out by 3 poster sessions and breakout rooms for social networking. Presentations from this year's meeting are accessible in the members area of the GTA website.

The Excellence in Science Award was presented to Dr. Stephen Dertinger who took us on a trip down the genetox memory lane describing work that he engaged in over his career in the field, including reflecting on historical controls distributions and how to use them to determine biological relevance in genetox assays. The GTA student outreach committee granted 6 poster awards to students and early-stage investigators.

We welcomed new GTA BOD members (Zhiying [Zane] Ji and Wen Sun), chair-elect Penny Leavitt and Ashley Allemang as the GTA Secretary. I and the BOD would especially like to thank and recognize our deepest appreciation of the generous support from our sustaining corporate members, meeting sponsors, and vendors and exhibitors. As the GTA is a non-profit, volunteer run organization, both members and non-members are encouraged to actively participate in the GTA. Please reach out to me or a member of the GTA BOD if you are interested in becoming an active volunteer or a member interested in running for the board in the future.

The next scientific meeting of the GTA is planned for May 3-5th, 2023, and is anticipated to take place as an in-person meeting at Clayton Hall, University of Delaware, Newark, DE. The 2023 GTA Scientific Program Committee co-chairs (Laura Markley, Wen Sun and Yi Yang) are planning a scientifically stimulating and robust program and will be leveraging valuable feedback collected from the 2022 post meeting survey. Finally, we will continue to work through the challenges of organizing a scientific meeting and top-notch scientific content for our meeting attendees. I look forward to you joining us at the 2023 GTA meeting, in person at Clayton Hall, University of Delaware, Newark, DE. Until then, may you all continue to thrive and stay healthy.

Best wishes,

Sheroy Minocherhomji PhD

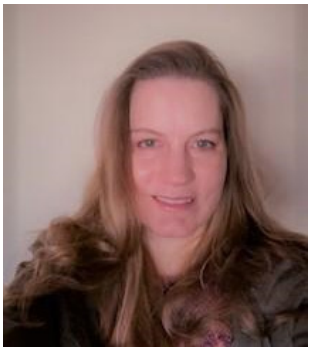
Chair, Genetic Toxicology Association (GTA)

GTA Board of Directors 2022-2023

Chair (2023): Sheroy Minocherhomji

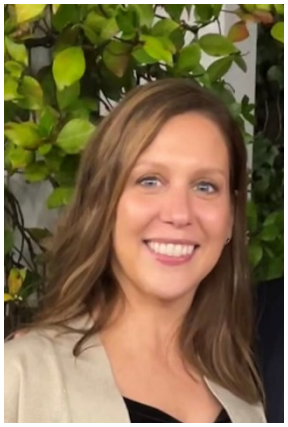
Dr. Sheroy Minocherhomji is currently Senior Director within the Toxicology and Pathology department at Eli Lilly and Company. He has >10 years of industry and academic experience in regulatory and discovery toxicology, impurity qualification of drugs, genomics, precision oncology, DNA repair, and safety assessment of multi-modality therapeutics, from discovery through to marketing and in oncology, cardiometabolic disorders and neuroscience therapeutic areas. He was previously a Principal Scientist/Toxicologist and Head of the Genetic Toxicology Unit within the Translational Safety department at Amgen and an Assistant Professor at the University of Copenhagen, Denmark. He received his MSc. and DIC degrees in Human Molecular Genetics from Imperial College London, UK and his PhD as a Marie Curie Fellow from the University of Copenhagen, Denmark in Health & Medical Sciences. He has been invited to speak at numerous global conferences and has authored more than 20 peer-reviewed publications including 10 as first/co-first and 4 as senior last author in high-impact journals including *Nature*, *Nature Cell Biology*, *PNAS, Molecular Cell* and *Regulatory Toxicology & Pharmacology*. He has been the recipient of numerous awards and grants and most recently the Early-Stage Investigator and Emerging Scientist Awards by the Genetic Toxicology Association (GTA) and the Environmental Mutagenesis & Genomics Society (EMGS). He is also a steering committee member of HESI's Genetic Toxicology Technical Committee (HESI-GTTC) and a past co-chair of the HESI-GTTC ecNGS working group.

Chair-Elect (2023): Penny Leavitt



Penny Leavitt has been dedicated to Genetic Toxicology efforts since joining Bristol Myers Squibb (BMS) in 2011. Current responsibilities are heavily weighted in mutagenic impurities risk assessment with a cross-functional role in Chemical Process Development and CMC Regulatory. Efforts support IND and NDA dossier submissions as well as responding to Health Authority requests as they arise. External efforts include data sharing projects with commercial in silico providers for genotoxic endpoints and contributing expert knowledge in external working groups. In addition, Penny is responsible for ensuring effective external partnerships with CROs and monitoring of Genetox studies. Prior to tenure at BMS, Penny's career experience spanned multiple areas of drug discovery utilizing a breadth of techniques in both industry and academia, with a particular aptitude for microbiology, biochemistry, and chemistry SAR. Most recently, Penny has expanded horizons in general and regulatory Toxicology, with culmination of earning status as a Diplomate of American Board of Toxicology, achieving position of Nonclinical Safety

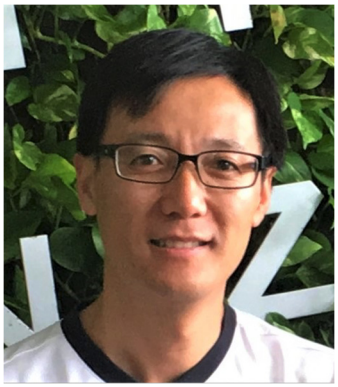
Project Representative, and responsibilities in review/authoring internal exposure monographs deriving chemical exposure limits to support chemical development and related quality events. Penny has been a member of GTA since 2011. For the past few years she has volunteered to support efforts for the GTA annual meeting, in particular co-chairing the GTA student outreach, aligning with her demonstrated advocacy for mentoring young scientists throughout her 20+ career.



Maria Engel is a Principal Scientist in the Global Portfolio Regulatory & Strategy group at Pfizer and is currently responsible for providing nonclinical toxicology support for submissions to regulatory agencies worldwide. She previously worked in genetic toxicology gaining over 20 years of practical experience in the application of genetic toxicology testing in support of pharmaceutical development. Maria contributes to genetic toxicology mutagenicity assessments for marketed products. She is a long time member and volunteer of the Genetic Toxicology Association and served as the Newsletter Editor (2008-2010), Secretary (2011-2015), Student Outreach Chair (2010-2019), previous member of the Board of Directors (2012-2014) and currently serving a second term. Maria holds a BS in Diagnostic Genetic Sciences (cytogenetics) from the University of Connecticut and a MS in Pharmaceutical Regulatory Affairs from Temple University.

Ashley Allemang 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.

Melisa Masuda-Herrera is a Senior Associate Scientist in the Environmental and Occupational Toxicology group within Nonclinical Safety & Pathobiology at Gilead. Her responsibilities include authoring health-based risk assessments to support product quality and occupational toxicology programs, monitoring and reviewing worker safety studies and environmental risk assessments and providing toxicology support to the Environmental Health and Safety group for global hazard communication programs. Before joining Gilead, Melisa worked as a Scientific Researcher at Genentech in the Product Quality and Occupational Toxicology group. While at Genentech, her primary focus was on authoring and reviewing documents to support Genentech product quality and occupational toxicology programs and conducting and interpreting *in silico* assessments of impurities for potential genotoxic activity using quantitative structure–activity relationship programs. Prior to graduate school, she worked as a Laboratory Assistant/Field Technician at the California Department of Public Health where she assumed the lead role in validating new methods, and assisting in standard methods, of chemical analysis of marine toxins for biotoxin monitoring. Melisa received her Bachelor of Science degree at UC Berkeley in Molecular Toxicology and her Master of Science degree at UC Santa Cruz in Environmental toxicology. She is currently a member of the Society of Toxicology, American College of Toxicology, ELSIE Consortium, and Occupational Toxicology Roundtable.



Dr. Zhiying (Zane) 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; 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 over 20 peer-reviewed publications. He is an active member of GTA, EMGS and SOT.

Dr. Wen Sun is a Senior 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.

GTA Volunteers 2021-2023

The following individuals are not board-elected but volunteer their time and talents to both enrich our organization and keep it running smoothly.

Treasurer: Leon Stankowski

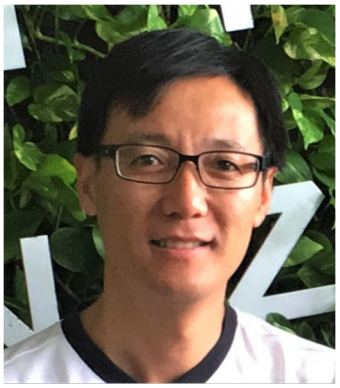
Assistant Treasurer: Sara Hurtado

Secretary, Web Liaison: Ashley Allemang

Account Administrator, Communications Co-chair, ESA Chair: Dan Roberts

Communications co-chair: Teresa Wegesser

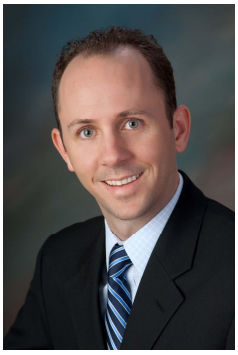
Student Outreach: Zhiying Ji



GTA Volunteers 2021-2023 (*continued)*

Student Outreach: Penny Leavitt

Financial Auditor: Chris Farabaugh



GTA Photographer: Robert Preston

Newsletter: Paula van Rossum and Jennifer Sasaki

GTA Historian: Volunteer needed!

Corporate Sustaining Members

We thank the following companies for their support in 2022. If you would like to renew your sponsorship or are interested in information on how your company can support the GTA as a corporate sustaining member and/or a meeting sponsor please click here.

(alphabetical order)

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2022 GTA Meeting – recap

All the chairs of the workshops and symposia have been kind enough to write a short recap of what was discussed during the event to refresh our memories. If you are a GTA member you can also access the slides that have been presented in the members area of the GTA the website.

Keynote: Mechanisms Driving the Rapid Evolution of Genomes
By: Prof. David Pellman (Harvard Medical School)
Chairs: Sheroy Minocherhomji & Maria Engel

On Wednesday, May 18th, 2022, Prof. David Pellman (Harvard Medical School) gave this year's GTA Keynote Address on the "Mechanisms driving the rapid evolution of genomes". Professor David Pellman is the Margaret M. Dyson Professor of Pediatric Oncology at the Dana-Farber Cancer Institute, a Professor of Cell Biology at Harvard Medical School, an Investigator of the Howard Hughes Medical Institute, and the Associate Director for Basic Science at the Dana-Farber/Harvard Cancer Center.

In his keynote address he discussed research from his lab showing how cellular genomes can evolve through episodic bursts of mutagenesis. His talk focused on describing the mechanisms of catastrophic mutational processes, including the process of chromothripsis, which involves massive rearrangement(s) of one or a few chromosomes in one cell division cycle. Finally, he described a mechanism explaining why chromosomes from micronuclei are fragmented to generate chromothripsis.

Workshop 1: Ames Positive Follow-up Case Studies
Chairs: Maria Engel & Zhanna Sobol

The first speaker was Dr. Tim Robison, a Pharm Tox Supervisor at the U.S. FDA in the Division of Pharmacology-Toxicology for Immunology & Inflammation, who described the appropriate follow-up assays to an in vitro bacterial reverse mutation (Ames) test positive investigational drug candidate (active pharmaceutical ingredient). Dr. Robison discussed the use of the in vivo TGR mutational assays and the Pig-a assay as appropriate follow ups to an Ames positive finding because these assays can evaluate the same types of genetic damage detected by the Ames test.

Dr. Patricia Escobar, Executive Director at Merck & Co. Inc., presented a case study of Molnupiravir (MOV), a therapeutic that has been authorized for emergency use as a five-day oral treatment regimen in patients with COVID-19. Dr. Escobar presented from a sponsor's perspective and described the use of the Big Blue® (*cII* Locus) transgenic rodent (TGR) assay as a follow up to a positive bacterial reverse mutation (Ames) assay to assess the in vivo relevance of the in vitro mutagenicity finding.

Dr. Robert Heflich, Director at the Division of Genetic and Molecular Toxicology at the U.S. FDA/NCTR, presented an assessment of available genotoxicity data for MOV from

a regulatory perspective. Dr. Heflich also presented new data from FDA/NCTR labs showing induction of mutations in bacterial and mammalian cells in vitro. The TGR assay was deemed an appropriate follow up to both bacterial and mammalian cell mutagenicity findings in vitro, in accordance with ICH S2(R1) guideline.

The final case study was presented by Dr. Sheroy Minocherhomji, Principal Scientist and Head of Genetic Toxicology at Amgen, who described and characterized a false positive mutagenicity finding that was caused by a contaminant. Dr. Minocherhomji demonstrated how QSAR modeling and root cause analyses were utilized to identify and characterize known mutagenic contaminants that were responsible for a positive Ames response when two non-mutagenic impurities were tested.

During the question-and-answer session the speakers addressed questions regarding the sensitivity of TGR assays for detecting in vivo mutagens and provided additional details about data analysis and testing strategy for each of the case studies.

Symposium I: Genetox Related Updates
Chairs: Penny Leavitt & Laura Custer

Symposium 1 consisted of 3 diverse topics with presentations for Genetic Toxicology Updates with global impact.

Dr. Stefan Pfuhler, Research Fellow, Global Expert in Genetic Toxicology at Procter & Gamble, provided an overview of the global effort to determine the genotoxicity potential of Titanium Dioxide. Dr. Pfuhler is one of a panel of experts, comprised of experts in genetic toxicology, general toxicology, bioavailability, carcinogenicity, and nanoparticle characterization that has convened to perform review of the reported genotoxicity tests on TiO2 reviewed by EFSA, and additional publications and unpublished reports conducted by industry. He described the robust weight of evidence approach the panel used to review the datasets and moreover the conclusions the panel have reached.

Dr. Giel Hendriks, CEO of Toxys, discussed the ongoing efforts of the OECD Test Guideline 34 compliant- international inter-laboratory validation of the ToxTracker assay, a stem-cell based (geno) toxicity screening platform giving insight into mode of action of genotoxic compounds. He provided an overview of the ToxTracker assay and the interlaboratory pre-validation efforts performed to determine transferability and reproducibility that preceded the SPSF approval in 2017 towards OECD Guideline for ToxTracker and concluded with summarizing the current results, next steps and expected timelines to OECD acceptance.

Dr. Susanne Stalford, Principal Scientist at Lhasa Limited, introduced the ongoing efforts of an industry led working group involved in evaluating SAR of N-nitrosamines to address reliance on Global Regulatory default acceptable intake (AI) limits in pharmaceuticals. She discussed the efforts specifically related to carcinogenicity SAR of N-nitrosamines and the framework which was developed to review the available rodent carcinogenicity data to assess its usability to derive compound-specific acceptable intakes and moreover to improve SAR knowledge intended to support carcinogenicity and AI assessment of N-nitrosamines with unknown carcinogenic potential for regulatory submission.

Symposium II: Assays to Measure Off-target mutagenicity and Insertional Mutagenesis of Gene Therapies
Chairs: Silvana Libertini & Mick Fellows

Dr. Irene Gil-Farina, Senior Director at ProtaGene CGT GmbH, provided an overview of the technical landscape available around vector integration analyses, which comprises Linear Amplification mediated-PCR (LAM-PCR), Ligation Mediated-PCR (LM-PCR) and Target Enrichment Sequencing (TES), amongst others. She also explored current strategies for insertional mutagenesis detection in preclinical and clinical studies as well as open questions to address a gene therapy vector's genotoxic potential.

Dr. Tony Cathomen, Professor for gene and cell therapy at the University of Freiburg, discussed the genotoxicity associated with designer nucleases, such as off-target activity and chromosomal translocations. He introduced CAST-Seq, a diagnostic assay that detects CRISPR-Cas and TALEN-induced chromosomal aberrations in a genome-wide manner and proposed the use of designer nickases and base editors as mitigation strategy with reduced genotoxic side effects.

Dr. Myriam Lemmens, Senior Scientist at Novartis, introduced two in vitro transformation assays, the soft agar colony-forming assay (SACF) and the growth in low attachment assay (GILA), for the tumorigenicity assessment of CRISPR/Cas9 edited cells. She described the identification of appropriate controls, the sensitivity of both methods, and how these assays could be used for the preclinical safety assessment of gene therapy products.

Symposium III: In vivo micronucleus test in alternative tissues
Chairs: Zhiying (Zane) Ji & Stephen Dertinger

Development of repeated-dose liver micronucleus assay and integration into general toxicity study by Shuichi Hamada
In Dr. Hamada' talk "Development of repeated-dose liver micronucleus assay and integration into general toxicity study", Dr. Hamada talked about the significance and development process of performing a liver MN assay by 28-day repeated dosing. In addition, he presented a high correlation between MN induction and liver carcinogenesis and reported that the liver carcinogenicity of a test substance can be detected with high precision by a repeated dose study of around four weeks in combination with histopathological examination. Finally, a liver MN assay using the formalin fixation method would not only enables to easily integrate genotoxic indices into a general toxicity study but also makes it possible to perform retrospective evaluation of liver MN induction using liver specimens obtained in the general toxicity studies conducted in the past.

Flow cytometric method for the assessment of chromosome damage in rodent liver hepatocytes by Jeff Bemis. Dr. Bemis delivered a presentation entitled "Flow cytometric method for the assessment of chromosome damage in rodent liver hepatocytes". The talk included an introduction to the flow cytometric technique along with data that established the working parameters of the methodology. A summary of additional work

included results from a 13-compound study that incorporated several in vivo genotoxicity endpoints as well as a review of an optimized experimental design that focused on 3Rs considerations.

Simultaneous Evaluation of Liver Micronucleus and Other Genotoxicity Endpoints in a Single Study – A Proof of Concept Study with Clofibrate by Zhiying Ji
Dr. Zhiying Ji presented a proof-of-concept study of simultaneous evaluation of liver micronucleus and other genotoxicity endpoints in a single repeated dose rat study using clofibrate as the model chemical. Clofibrate was administered by oral gavage at doses of 0 (vehicle control), 125, 250, and 500 mg/kg/day to young adult (6.4 weeks old at first dose) male Han/Wistar rats for 29 consecutive days. All surviving rats were euthanized on day 29 and liver and blood samples were collected for liver micronucleus test, liver comet assay, blood micronucleus test, and blood Pig-a mutation assay. Additional groups treated with diethylnitrosamine (DEN), ethylnitrosourea (ENU), cyclophosphamide monohydrate (CP), and/or ethyl methanesulphonate (EMS) served as positive controls. The findings support that liver micronucleus test and other commonly used in vivo genotoxicity assays (i.e., liver Comet assay, blood MN test, and blood Pig-a assay) can be simultaneously evaluated in a single repeated dose study. This approach provides data from same animals for comprehensive genotoxicity evaluation and greatly promotes the 3Rs principle.

Symposium IV: Nitrosamine risk assessment/updates – Part I & II
Chairs: Catrin Hasselgren & Kevin Cross

Five speakers were featured in the session: Kevin Cross from Instem, Joel Bercu from Gilead, Andrew Teasdale from AZ, Robert Heflich from FDA/NCTR and Bevin Engelward from MIT

In the talk “Advancing prediction of nitrosamine potency”, Dr. Cross presented an update on the various collaborative groups studying risk assessment of nitrosamines. He then presented current statistics on the sensitivity of the Ames test for detecting rodent carcinogenicity where retesting results of all known Ames false negatives, (identified predominantly as isopropyl N-nitrosamines), were now all positive when using hamster S9. This was particularly important for testing the nitrosated “olol” drug class which include the isopropyl substituent.

In the talk “Setting Acceptable Intakes for Nitrosamine Impurities with Limited Carcinogenicity Data”, Dr. Bercu discussed methods for setting AI levels using existing biological data, proposed mechanisms, QSAR and quantum mechanics. Several toxicity data categories were defined with various levels of robustness for use within ICH M7 and establishing precision limits in AI assessments, including: robust, limited, insufficient information with SAR, and no information or SAR. When applying a weight-of-evidence approach, a case study assessing the AI of DIPNA resulted in it not being a Cohort of Concern.

In the talk, HCTZ – a Nitrosamine concern? or another twist in the tail”, Dr. Teasdale presented while it is known that HCTZ forms nitrosamines, the positive Ames test resulted in an unanticipated strain profile with a positive result without using S9 and with TA1535 testing negative. Nitrosated HCTZ was found to be unstable and rapidly

degrade at physiological pH using LC (and under Ames test conditions). No diazonium ion was formed in the degradation pathway to a final thiatriazine and aromatic amine impurity. However, degradation resulted in the equimolar formation of formaldehyde which had a similar Ames strain profile to the tested nitrosated NCTZ. Consequently, none of the degradation products are in the Cohort of Concern and they can be controlled at TTC levels for impurities.

In the talk, “Optimizing the Ames Test to Enhance Assessment of the Mutagenic Potential of Nitrosamine Drug Impurities and Other Genotoxicity Studies Conducted at FDA/NCTR”, Dr. Heflich discussed how the mutagenicity of nitrosamines in the Ames test can be affected by the choice of protocol conditions, including tester stain, pre-incubation vs plate incorporation, length of preincubation, pH of the medium, animal species used to prepare liver S9, concentration of the S9, and the solvent/vehicle used for the assay. Results also were presented from studies on the genotoxicity of nitrosamines in human cells transduced with human CYPs. Consideration of possibly using the in vivo Pig-a assay for mutagenicity assessment generated much discussion, particularly around demonstrating metabolic competence of nitrosamine metabolites in the bone marrow.

In the talk, “Excision of mutagenic replication-blocking lesions suppresses cancer but promotes cytotoxicity and lethality in nitrosamine-exposed mice”, Dr. Engelward studied NDMA in Aag-/- and Aag-overexpressing mice that harbor increased levels of either replication-blocking lesions (3-methyladenine, or 3MeA) or strand breaks (BER intermediates). After integrating a suite of molecular, cellular and physiological analyses, it was found that unrepaired 3MeA is somewhat toxic but highly mutagenic (promoting cancer), whereas excess strand breaks are poorly mutagenic and highly toxic (suppressing cancer and promoting lethality). Levels of a single DNA repair protein (namely, Aag) tips the balance between blocks and breaks, and thus dictates the disease consequences.

Workshop 2: Case Studies of Applied Genetox
Chairs: Zhanna Sobol, Maria Engel

Three speakers were featured in the session: Dr. Sucheta Mukherjee from Aligos Therapeutics, Abby Myhre from Corteva Agriscience, and Anne-Marie Fortin from University of Ottawa.

Dr. Mukherjee reviewed the risk management strategy of in vitro micronucleus findings of Compound X in development for a non-oncology indication in a talk entitled “De-Risking of a Positive In Vitro Micronucleus Test Result: A Case Study of a Small Molecule in Early Development”. Concentration dependent increases in micronucleus frequency across all assay conditions was accompanied by a non-genotoxic effect at the lowest concentration, indicating a threshold mechanism. Mode of action (MOA) investigation classified compound X as a clastogen via a non-DNA reactive mechanism. Results from two in vivo studies (micronucleus and comet) did not show any evidence of clastogenicity or DNA damage. These results supported a weight of evidence approach that compound X presented a low risk of causing genotoxicity. Questions addressing the approach to conduct parallel in vitro MOA and in vivo studies, testing related to ROS

toxicity and bioaccumulation, and dose selection and safety margin over projected clinical efficacious exposures of the in vivo studies.

Abby Myhre's presentation "Effects of Hemolysis on the HPBL In Vitro Micronucleus Test and How it Correlates to In Vivo Results" described the investigation of the potential impact of extreme hemolysis induced in vitro by an agricultural formulation. Human peripheral blood lymphocytes treated with an agricultural formulation as well as the blank formulation (without active ingredient) resulted in micronucleus induction accompanied by pronounced hemolysis in vitro. In both cases, hemolysis confounded the toxicity calculation based on the cytokinesis-block proliferation index (CPBI). The active ingredient alone did not cause micronucleus induction in both in vitro and in vivo studies. The same agricultural formulation (blank and with active) did not result in positive micronucleus findings in vivo. In addition, no excessive hemolysis or proliferative effects were observed in vivo regardless of treatment. It was concluded that in vitro hemolysis and micronucleus induction did not translate in vivo. During the question-and-answer period, Abby addressed exploring alternate cell types to investigate if the hemolysis effects would still be observed and whether the in vitro micronucleus induction would be reproducible without the hemolysis effect.

Anne-Marie Fortin described “Application of a New Approach Methodology (NAM)-based Strategy for Genotoxicity Assessment of Data-poor Substances”. To illustrate this, 10 reference compounds identified by in silico tools as being likely genotoxic were selected for testing. The in vitro MicroFlow® assay and TGx-DDI transcriptomic biomarker assay were used to classify compounds as DNA damage-inducing (DDI) or non-DDI. Benchmark concentration modeling of transcriptomic data and corresponding MicroFlow® revealed comparable potency ranking results. This study demonstrated good concordance between the assays and how pairing the two assays provides robust hazard identification supporting an integrated approach for the testing and assessment of data poor compounds. Questions regarding potency rankings, methods related to the conduct of the TGx-DDI assay, and oxidative stress detection in this platform were addressed.

Symposium V: 3Rs in Applied Genotoxicity Testing
Chairs: Stefan Pfuhler & Ashley Allemang

Three speakers were featured in the session: Yax Thakkar (RIFM), Inger Brandsma (Toxys) and Ashley Allemang.

In his talk “Utility of animal alternative models (3DRSM and HET-MN) to address misleading positive in traditional in vitro micronucleus assay (OECD 487)”, Y. Thakkar presented data generated with substances present in fragrances as well as flavor additives and showed that both the 3DRSMN and HET-MN assays were in 100% agreement with the in vivo micronucleus assay results. These data support the importance of these assays as direct replacement of animal studies and that they can help minimize misleading positive outcomes from standard in vitro genotoxicity assays. Dr. Brandsma presented on “Examples of the use of ToxTracker data in in vitro testing approaches to reduce animal testing”. These included the use of the ToxTracker to read-across and grouping of cobalt substances, and as a tool to efficiently look at the genotoxicity of mixtures. In the case of the cobalt substances, she showed that

ToxTracker data could be used to group substances together, while for mixtures of pesticides the ToxTracker was used to distinguish the response of individual components within a mixture, both leading to estimated reduction in animal use.
The last featured speaker, A. Allemang, presented an “In in vitro-only approach to the safety assessment of DNA reactive botanical contaminates”. The chemicals featured in her talk were pyrrolizidine alkaloids (PAs) which are DNA reactive plant compounds commonly found as botanical contaminates in products such as tea or honey. The in vitro-only approach to the safety assessment of PAs she presented based on the understanding of toxicokinetics, metabolic activation and genotoxicity potency and lends support to the rank ordering of PA toxicity to aid risk assessment.

Taken together, the session aimed at demonstrating three different ways to reduce/avoid animal testing: direct replacement of in vivo assays via use of higher tier in vitro models (1), support of grouping/read across and aid prioritization of chemicals with an aim of reducing animal studies (2), and how in vitro genotoxicity data, in conjunction with additional ADME elements generated in vitro, can aid in vitro to in vivo extrapolation (IVIVE).

Symposium VI: Pig-a assay regulatory acceptance
Chairs: Melisa Masuda-Herrera & Vincent Reynolds

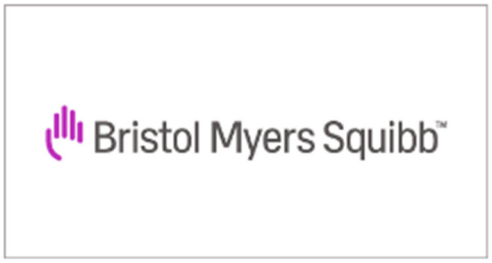
Two speakers were featured in the session: Javed Bhalli from Frontage Laboratories and Vasily Dobrovolsky from FDA.

In his talk “Strengths and Weaknesses: Technical Aspects of the Pig-a Assay”, Dr. Bhalli provided and overview of the Pig-a assay, highlighted the advantages of the assay, and addressed areas of improvement.

In his talk “*Pig-a* OECD Test Guideline (an update)”, Dr. Dobrovolsky provided an overview of the process to get the Pig-a OECD guideline approved. He also provided an overview of the assay itself and uses of the assay.

During the question-and-answer session the speakers addressed questions regarding the guideline approval process and future directions/applications of the assay.

2022 Meeting Sponsors and Exhibitors



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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.

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

Or send this completed application and appropriate funds (checks made payable to the Genetic Toxicology Association) by regular postal mail to:

Leon Stankowski, Treasurer
Genetic Toxicology Association
c/o 1712 DaVinci Lane
Clarks Summit, PA 18411

2022 GTA Award Recipients

Poster award – Student category

Genetic Toxicology Association

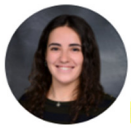
GTA Poster Awards
Students

Mariam Habil
PhD candidate, University of Louisville
Laboratory of Dr. David Hein

Aromatic Amine Induced Genotoxicity
Poster Session 1
Wednesday, May 18th

Jamie Hsing-Ming Chang
PhD Student, Stony Brook University
Laboratory of Dr. Bruce Demple

The Genotoxic and Cytotoxic Impact of Lunar Dust Simulants on A549 Human Lung Cells
Poster Session 3
Friday, May 20th



Idoia Meaza Isusi
M.S, M.S, PhD candidate, University of Louisville
Laboratory of Dr. John Pierce Wise

Particulate Hexavalent Chromium Causes DNA Double Strand Breaks and RAD51 Inhibition, Leading to Increased Chromosome Instability in Human Bronchial Epithelial Cells
Poster Session 3
Friday, May 20th

Poster award – Early Stage Investigator Category

Genetic Toxicology Association

GTA Poster Awards
Early-Stage Investigators

Elliot Corless
Postdoctoral Associate, MIT

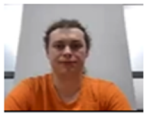
Laboratory of Dr. Bevin Engelward

Exploiting AI To Improve DNA Damage Detection and Quantification
Poster Session 1
Wednesday, May 18th

Lee Pribyl
Postdoctoral Fellow, Massachusetts Institute of Technology

Laboratory of Dr. Bevin Engelward

Impact of Mgmt-Mediated DNA Repair on Mutation Susceptibility and Cancer in Mice
Poster Session 2
Thursday, May 19th



Robert Thomas
Senior Data Scientist, Lhasa Limited

Impact of Dose-group Allocation on TD50 Reliability
Poster Session 2
Thursday, May 19th

Environmental Mutagenesis and Genomics Society (EMGS) Emerging Scientist Award

Environmental Mutagenesis and Genomics Society (EMGS) Emerging Scientist Award

The 2022 GTA EMGS Emerging Scientist Award is presented to:

Lee Pribyl

Postdoctoral Fellow,
Massachusetts Institute of Technology

Laboratory of Dr. Bevin Engelward

Impact of Mgmt-Mediated DNA Repair on Mutation Susceptibility and Cancer in Mice

Poster Session 2
Thursday, May 19th

GTA Excellence in Science Award

The GTA was pleased to award **Stephen Dertinger, Ph.D.** with the 2022 GTA Excellence in Science Award. His reflections are featured on Page 22.

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.** The awardee is selected 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 (May 3-5, 2023). Nominations should be sent to Dan Roberts, Chair of the ESA selection committee.

Nominations for the 2023 award are due by December 1, 2022.
Please email your nominations to: d.roberts@toxys.com.

2022 GTA Excellence in Science Award

Excerpts from **Stephen Dertinger's** GTA ESA Acceptance Speech

It's an honor receiving the GTA's 2022 Excellence in Science Award. Past awardees have been a great source of inspiration, so it's incredibly flattering to count myself among that group of individuals. Clearly no one that receives this award does so without a lot of help. So, I'd like to take this opportunity to shine a light on some of the individuals that have been especially instrumental to my scientific career.

In my early years working at Litron Laboratories, Andrew and Carol Tometsko, Dorothea Torous, and Nikki Hall helped me understand what good experiments look like, and how to go about executing them. Some of Dr. T's sayings from this period continue to guide me to this day: "if it's real it's reproducible," and "one experiment is worth a thousand opinions."

After several years at Litron I enrolled in the University of Rochester's Toxicology Program. At the U of R I had the great fortune of working under Tom Gasiewicz. It was Tom that taught me the importance of orthogonal evidence.

It's hard to adequately express the amount of help Jim MacGregor has provided over the years. Early on, Jim encouraged me to think of micronuclei as a translational biomarker that should be utilized across species of toxicological interest. He also taught me to stop thinking of genetic toxicology as separate and distinct from toxicology. The same principles apply, and it behooves us to stop trying to excuse ourselves from that paradigm.

I've benefitted from fantastic collaborators at several government agencies. In particular, I'd like to thank Bob Heflich and colleagues at NCTR for all the incredible work they put into validating the Pig-a assay, and for spearheading the OECD Test Guideline. Paul White and several Health Canada scientists have greatly inspired our work through the years, most recently with their point-of-departure analyses. Our colleagues at NIH, especially Kristine Witt, Stephanie Smith-Roe, and Dan Shaughnessy, were early supporters of our human blood-based analysis efforts, and NIEHS supported several rounds of grant funding that helped us reduce the methods to practice.

I've been super fortunate to have collaborated with many of the top industry and academic scientists in the field of genetic toxicology. This is not an exhaustive list, but I'd like to highlight some colleagues that Litron has done our most important work with: Leon Stankowski & Dan Roberts; Maik Schuler, Krista Dobo, Ron Fiedler, Michelle Kenyon, Randy Spellman, Maria Engel & Stephanie Coffing; Les Recio, Cheryl Hobbs & Carol Swartz; Andreas Zeller; Azeddine Elhajouji; Anthony Lynch & Julia Kenny; Elisabeth Lorge; Veronique Thybaud; Matt Tate; Laura Custer; Rob Smith; Bas-jan Van Der Leede, Freddy Van Goethem & Sandy Weiner; Sheila Galloway, Patricia Escobar & Zhanna Sobol; Stefan Pfuhler & Ashley Allemang; John Nicolette; George Johnson, Bevin Engleward & Jenny Kay. Thank you all!

One last thought about inter-lab work before I leave this subject. Our best outcomes seem to occur when colleagues feel comfortable telling us difficult truths.

“Honesty is more than not lying. It is truth telling.” J.E. Faust. I have many anecdotes I could share, but the first that came to mind has to do with our Pig-a ring trial. Work was well underway when Anthony Lynch stopped me at a meeting to say not nearly enough reticulocytes were being evaluated. That was a difficult thing to hear, but it made all the difference. We paused the trial and puzzled out an immunomagnetic separation technique that provided an order of magnitude more reticulocytes for analysis. Thank you, Anthony, and all our past and current collaborators for helping ensure we’re doing the best possible work we can.

I touched on the early years at Litron, and now want to acknowledge several contemporary co-workers. Jeff Bemis wears many hats at our small company. In doing so, Jeff has a way of making everything we do better. Steve Bryce has advanced every aspect of our research in profound ways, and I really don’t know what we’d do without him. MultiFlow kits and services is just one recent example. We give Svetlana Avlasevich our most difficult benchtop problems. It should no longer surprise me, but I’m always amazed to see how efficiently she cuts through all the issues, one after another. Thank you, Jeff, Steve and Svetlana!

Moving on to my personal life, I need to thank my wife and kids for all their support through the years. Science is decidedly not a 9 to 5 job, and their understanding and support has made all the difference. Thank you, Molly, Samantha, Ben, Trevor, and Claire!

Thank you very much for selecting me for the 2022 ESA. It provided me with a nice opportunity to shine a light on many of the people that shaped my scientific career, and that have contributed to the successes we’ve had a Litron. I hope to see you all, in person, at the 2023 GTA meeting!

Announcements

INVITATION TO EXHIBITORS

Exhibition space will be available at the in-person 2023 meeting to allow interested vendors the opportunity to showcase their products or services to the conference attendees. Exhibitor spaces are limited, so please inquire as soon as possible

GTA CORPORATE SPONSORSHIP

The Board of Directors and members of the Genetic Toxicology Association (GTA) are inviting organizations to support the GTA's efforts to foster sustained excellence in genetic toxicology. The GTA is a tax-exempt educational and scientific organization and any contributions will be used to help the GTA continue to support its activities to promote scientific excellence in this field. Several contribution mechanisms are available: **Sustaining Membership** (Gold: $500 or Silver: $250) for general, non-designated use by the GTA; *additional* **Targeted Contribution** towards a specific event or function (e.g., meeting breaks or lunch, offsetting general meeting expenses, student travel award, etc.); and/or *additional* **Session Sponsorship** ($1000), on a first come first serve basis.

Advertising Space

We are offering an opportunity for companies to purchase advertising space on our home page and meeting program website. If interested or for additional information please email the GTA Treasurer, **Leon Stankowski**, at leon.stankowski@crl.com for information and prices.

Thank you

For their contributions to the GTA:
We would like to thank the board members and volunteers. Their dedication and contributions are invaluable to our organization and we thank you!

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The Genetic Toxicology Association would like to thank the following Exhibitors and Sponsors for generously supporting our 2022 Meeting

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