50th Annual Symposium / 50e Symposium Annuel

December 10th – 12th, 2018
Courtyard by Marriott Toronto Downtown
475 Yonge St
Toronto, ON
M4Y 1X7

WHAT'S IN A NAM? (NOVEL APPROACH METHODOLOGIES) BENEFITS/ LIMITATIONS/TRANSLATION/COMMUNICATION

QU'EST CE QUE LES NAM? (NOUVELLES APPROCHES MÉTHODOLOGIQUES) AVANTAGES/LIMITES/INTERPRÉTRATION/ COMMUNICATION.

Organized by / Organisé par:

SOCIETY OF TOXICOLOGY OF CANADA
LA SOCIÉTÉ DE TOXICOLOGIE DU CANADA

Program Committee / Comité du programme:
Elaine Leslie, University of Alberta, Chair and Academic Member
Ella Atlas, Health Canada, Government Member
Joanne Wan, Intertek, Industry Member

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Eric Liberda, Ryerson University
David Riddick, University of Toronto

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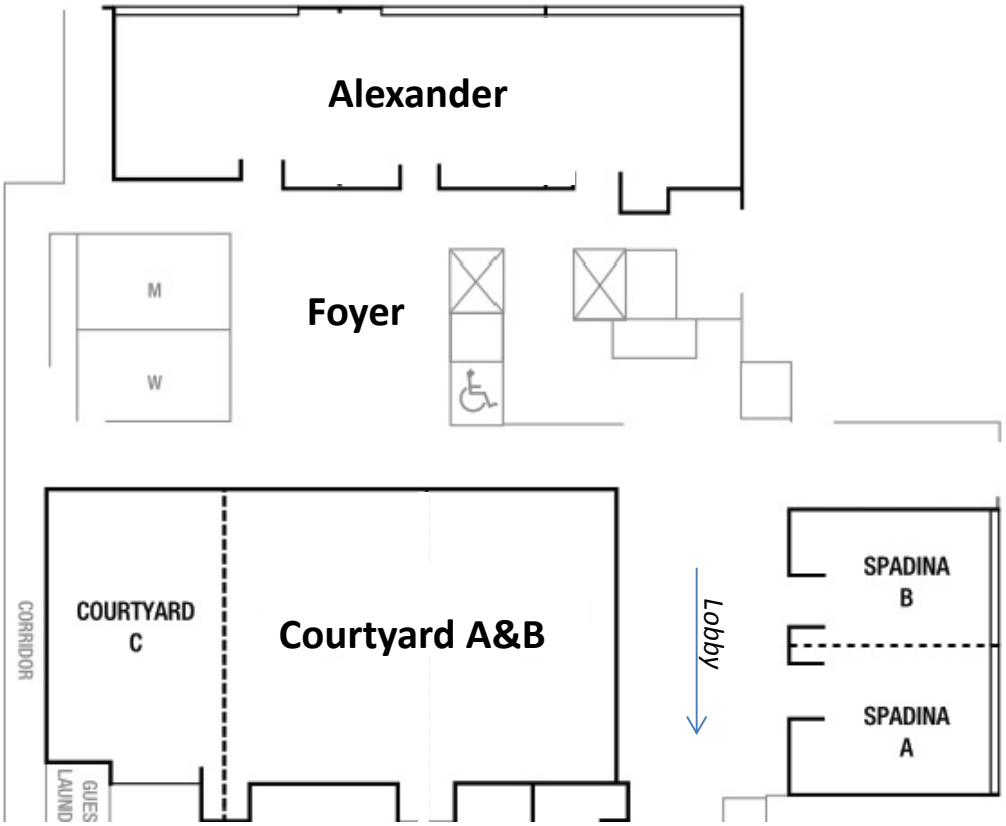
Contents

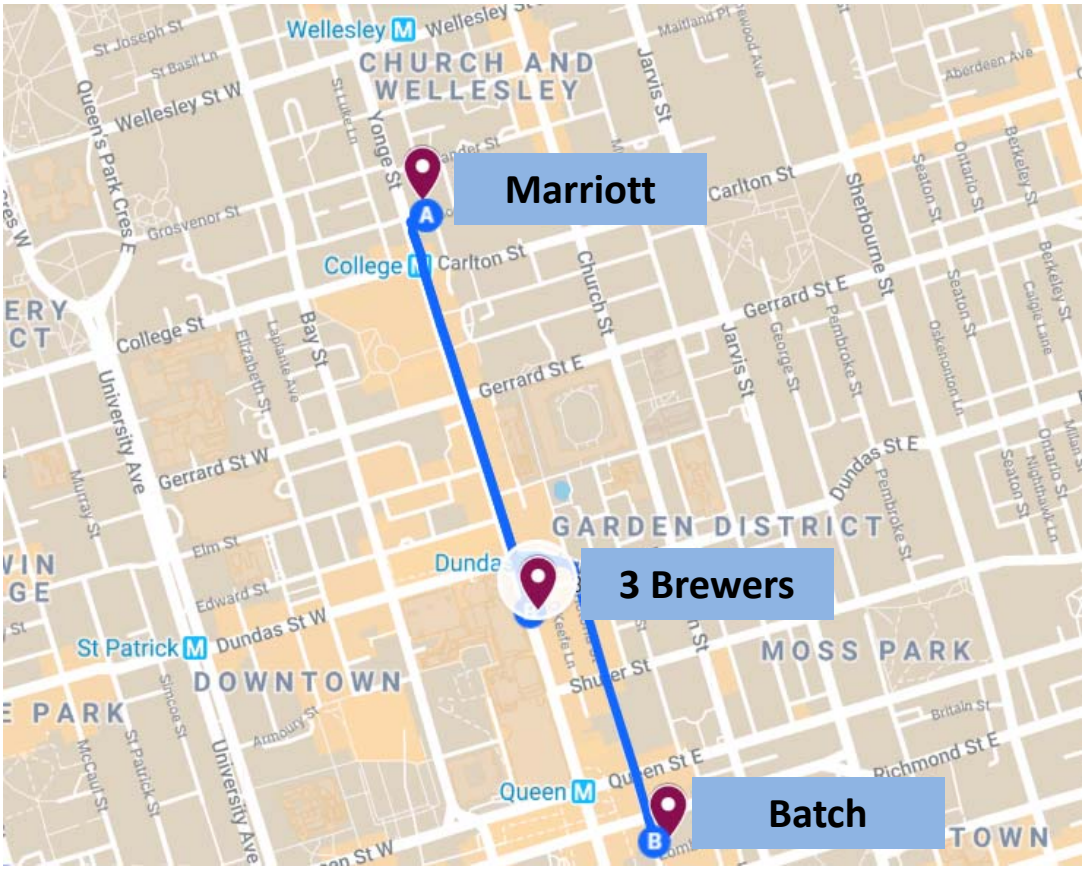
VENUE INFORMATION / PLAN DES SALLES DE CONFÉRENCES
Courtyard Marriott, Toronto
Ground Floor / Rez-de-Chaussée



Locations of On-Site Events / Localisation des différents événements

Event / Événement	Location / Salle
STC Board Meeting / Réunion du conseil d'administration	University A & B
Registration / Inscription (Dec.11, 12)	Courtyard Foyer
STC Symposium / Symposium STC	Courtyard Hall
Posters and Exhibits/Vendors / Présentations par affiche et exposants	Alexander A, B,& C
Coffee / Pauses café	Alexander A, B, & C
STC Annual Business Meeting / Réunion annuelle des membres	University A & B
Trainee Mentoring Session / Conférence pour les étudiants	Courtyard Hall
Breakfast, lunch Dec 11, 12 / Déjeuners, diners 11-12 déc.	Courtyard Foyer

LOCATION OF OFF-SITE EVENTS/ PLAN DES ACTIVITÉS HORS SITE
Downtown Toronto Centreville



Welcome Reception / Réception de Bienvenue
Monday, December 10, 7-9 pm / Lundi, 10 décembre 7-9h pm
3 Brewers Pub, 275 Yonge St

East side of Yonge St just S of Dundas (800 m South of the Hotel)
Rue Yonge (Coté est), au sud de Dundas (800 mètres au sud de l'hôtel)

President's Reception, 50th Reunion, and STC Awards / Réception du Président, 50ième réunion, et Prix STC
Tuesday, December 11, 6-9 pm / Mardi, 11 décembre, 6-9h pm
Batch, 75 Victoria St

Walk south on Yonge from Hotel, East at Dundas, South on Victoria to just S of Richmond (about 1.6 km S of hotel)
À partir de l'hôtel, marcher sur Yonge vers le sud, tourner vers l'est sur Dundas, puis prendre Victoria vers le sud (environ 1,6 km au sud de l'hôtel)

Symposium Program

Monday, December 10th PM

2:00 – 5:00 STC Board Meeting

7:00 – 9:00 **Welcome Reception**
Location: 3 Brewers, 275 Yonge Street, Toronto, ON M5B 1N8

Tuesday, December 11th AM

7:30 Registration / Breakfast
Location: Courtyard Foyer (ground level, Marriott)

8:45 Opening Remarks
Angela Hofstra, STC President, Syngenta Canada, Guelph, Ontario

8:50 **SESSION I: Alternative Approaches to Toxicity Testing**
Co-Chairs: Charu Chandrasekera, University of Windsor, Windsor, Ontario
Troy Seidle, Humane Society International, Toronto, Ontario

08:50-9:00 **Charu Chandrasekera**, University of Windsor, Windsor, Ontario
"Overview of the New Canadian Centre for Alternatives to Animal Methods"

09:00-9:30 **Anna Lowit**, Senior Science Advisor, Office of Pesticide Programs, Environmental Protection Agency, Washington, DC
"Recent Progress on Implementing New Approach Methodologies at USEPA"
Sponsored By: Canadian Centre for Alternatives to Animal Models, University of Windsor

9:30-10:00 **Milica Radisic**, Professor and Canada Research Chair (Tier 2), Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario
"Engineering Atrial and Ventricular Cardiac Tissues for Drug Testing."

10:00-10:30 **Michael-Rock (Rocky) Goldsmith,** Computational Discovery Chemistry Lead, Bayer U.S., Chesterfield, Missouri
"Molecular De-risking by Design: Modeling and Simulation to Advance Safer Chemistries"

10:30-11:15 **Coffee Break and Poster Viewing**

11:15-11:45 **Carole Yauk,** Health Canada, Ottawa, Ontario
"Dose-response Modelling of Transcriptomic Data: Progress and Potential for Risk Assessment Applications."

11:45 to 12:15 **Andy Nong,** Health Canada, Ottawa, Ontario
"Good and New Computational Practices for 21st Century Risk Assessment."

12:15-1:30 **Lunch and Poster viewing** *(judging)*

SESSION II: Alternative Approaches to Toxicity Testing Continued
Chair: Elaine M. Leslie, University of Alberta, Edmonton, Alberta

1:30-2:00 **Yadvinder Bhuller**, Health Canada, Ottawa, Ontario
"New Approach Methodologies – Is there another way to address the underlying regulatory question?"

2:00-2:45 **KEYNOTE TALK: George Daston**, Victor Mills Society Research Fellow at The Procter & Gamble Company, Cincinnati, Ohio
"New Approaches for Predicting Toxicity"

2:45-3:15 **Break/Poster viewing** *(Judging)*

SESSION III

3:15-4:00 **Gabriel Plaa Award Lecture**
*Chair***:** Angela Hofstra, Syngenta Canada, Guelph, Ontario

Peter G. Wells, University of Toronto, Toronto, Ontario
"A Novel Developmental Role for 'Tumor' Suppressor Genes in Protecting the Fetus From Reactive Oxidative Species."

4:00 **MEMBERS - STC Annual Business Meeting**

4:00 **Student/Post-doctoral Fellow Mentoring Session**
Alternatives to Academic Careers
Location: Courtyard Hall
Co-chairs: Shama Bhatia, PhD candidate, University of Toronto, Toronto, Ontario
Marc Bossou, PhD candidate, Université de Montréal, Montreal, Quebec
Speakers/Participants:
Margaret Magdesian, Ananda Devices, Montreal, Quebec
Carole Yauk, Health Canada, Ottawa, Ontario
Anna Lowit, Environmental Protection Agency, Washington, D.C.
David Clarke, Pfizer Inc, Pearl River, New York
Rocky Goldsmith, Bayer U.S., Chesterfield, Missouri
Tania Onica, Ontario MECP, Toronto, Ontario

6:00 **President's Reception, 50th Reunion, and STC Awards**
Location: Batch, 75 Victoria St, Toronto, M5C 2B1
ToxTrivia – Challenge your knowledge of toxicology, STC history, and the 2018 STC symposium talks and posters!

Wednesday, December 12th AM

7:30 Breakfast

8:25 **SESSION IV: Translational Toxicology**
Chair: Ella Atlas, Health Canada, Ottawa, Ontario

8:30-9:00 **Jack Uetrecht**, Professor of Pharmacy and Medicine, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario
"Finally, a Valid Animal Model of Idiosyncratic Adverse Drug Reactions"

9:00-9:30 **David Clarke**, Drug Safety Research and Development, Pfizer Inc, Pearl River, New York
"Challenges in the Preclinical Assessment of Efficacy and Safety for Immune Oncology Products."

9:30-10:00 **Eric Liberda**, School of Occupational and Public Health, Ryerson University, Toronto, Ontario
"Indigenous Knowledge Holders and the "Two-Eyed Seeing" Approach to Environmental Health Investigations."

10:00-10:25 **Coffee Break and Poster Viewing**

10:25 **SESSION V: INVITED TRAINEE PLATFORM PRESENTATIONS**
Chair: Christopher Nicol, Queen's University, Kingston, Ontario

10:30 **Amy Hoff**, MSc Candidate, University of Guelph, Guelph, Ontario
Abstract #26: "Searching for Binding Partners of Human Glutathione Transferase Theta1- a Moonlighting Protein?"

10:45 **Shama Bhatia**, PhD Candidate, University of Toronto, Toronto, Ontario
Abstract #4: "Oxoguanine Glycosylase 1 Knockout Mice Exhibit Increased Sex-dependent Cerebellar and Hippocampal DNA Strand Breaks and Postnatal Brain Function Disorders."

11:00 **Rita-Josiane Gouesse,** PhD Candidate, INRS, Laval, Quebec
Abstract #23: "Brominated Flame Retardants: Perinatal Exposure to an Environmentally-Relevant Mixture Disrupted Cell-Cell Interactions and Thyroid Homeostasis in Rat Mammary Glands at Puberty"

11:15	**Camila Gonçalves Athanasio**, Postdoctoral Fellow, McGill University, Montreal, Quebec Abstract #21: "Effects of Early-life Exposure to AHR Ligands in Chicken Embryos: Epigenetic Regulation and Interindividual Variation."
11:30	**SESSION VI: Risk Communication and Assessment** *Chair:* Joanne Wan, Intertek, Mississauga, Ontario
11:30-12:10	**Ron Brecher and Trevor Smith Diggins,** RiskPartners.ca, Guelph, Ontario "Risk Communication: Make the Science Make Sense"
12:10-1:10	**Lunch (and Poster Takedown)**
1:10	**Lois Haighton**, Director, Toxicology & Regulatory Affairs, Food and Nutrition, Intertek, Mississauga, Ontario "Risk Assessment of Cannabis, Not Just Another Food Ingredient"
1:40	**Andrea Amendola and Ruwan Jayasinghe,** Golder, Mississauga, Ontario "Applications of Toxicology in Support of Human Health Risk Assessments".
2:15	**Miriam Diamond**, Professor, Department of Earth Sciences, University of Toronto, Toronto, Ontario. "The Gulf Between Stakeholders Illustrated by Flame Retardants in Electronic Products."
2:45-3:00	Closing

Speaker Abstracts and Biographical Sketches

Speaker: Anna B. Lowit

Recent Progress on Implementing New Approach Methodologies at USEPA

Anna B. Lowit[1] and Louis Scarano[1]

[1]USEPA OCSPP, Washington, DC.

EPA's Office of Chemical Safety and Pollution Prevention (OCSPP)'s mission is to protect public health and the environment from potential risks due to pesticides (through the Office of Pesticide Programs, or OPP) and commercial chemicals (through the Office of Pollution Prevention and Toxics, or OPPT). EPA OPP and EPA OPPT are working together towards advancing and adopting new approach methodologies (NAMs). EPA has worked with the Interagency Coordinating Committee on the Validation of Methods (ICCVAM) and NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to develop the new "Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States." ICCVAM is composed of representatives from 16 U.S. federal regulatory and research agencies. Each of these regulatory and research agencies require, use, generate, or disseminate toxicological and safety testing information. In addition, OPPT has recently published its "Strategic Plan to Promote the Development and Implementation of Alternative Test Methods Within the TSCA Program." This presentation will discuss these documents and illustrate their application using recent NAM implementation activities in the areas of skin sensitization and inhalation. In 2018, EPA released a draft, interim science policy on the use of alternative approaches for skin sensitization as a replacement for laboratory animal testing. In addition, EPA is convening a meeting of the FIFRA Scientific Advisory Panel (SAP) in December, 2018 on a proposed approach to refine the inhalation risk assessment for point of contact toxicity using *in vitro* methods coupled with dosimetry modeling in lieu of *in vivo* testing.

Biosketch: Dr. Anna B. Lowit received her Ph.D. in Environmental Toxicology from the University of Tennessee in 1998 where she was a Graduate Fellow in Sustainable Waste Management. Dr. Lowit began her career with EPA in 1998 with the Office of Pesticide Programs, where she remains today. Dr. Lowit is currently the Senior Science Advisor at the EPA's Office of Pesticide Programs where she advices senior managers and leads multidisciplinary teams on a variety of cross-cutting topics. She is currently one of the Co-Chairs of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). ICCVAM is composed of representatives from 15 U.S. Federal regulatory and research agencies that require, use, generate or disseminate toxicological and safety testing information and whose purpose is to promote and facilitate the 3Rs of toxicity testing (reduce, refine, replace) in regulatory toxicity testing.

Speaker: Milica Radisic

Engineering Atrial and Ventricular Cardiac Tissues for Drug Testing

Milica Radisic[1,2,3]

[1] Department of Chemical Engineering and Applied Chemistry; University of Toronto; Toronto; Ontario, M5S 3E5; Canada. [2] Institute of Biomaterials and Biomedical Engineering; University of Toronto; Toronto; Ontario, M5S 3G9; Canada. [3]Toronto General Research Institute, Toronto; Ontario, M5G 2C4; Canada.

Profound physiological differences among species, in action potentials, ion current profiles, and contractile rates, motivate the development of human cardiac tissues for evaluation of the structural and functional changes induced by pharmacological compounds and disease modelling. An ideal platform should be able to yield distinct atrial and ventricular tissues of high biological fidelity; yet cardiac tissue engineering starting from human pluripotent stem cells has focused on reproducing ventricular myocardium and assessing adverse ventricular events. Our goal here was to develop a versatile resource for the community, a platform that enables creation of electrophysiologically distinct atrial and ventricular tissues, and that is capable of providing months long biophysical stimulation of 3D tissues to model a polygenic disease. This platform, termed Biowire II, enables growth of thin, cylindrical tissues, similar to human trabeculae, suspended between two parallel polymer wires whose deflection can be used to conveniently quantify passive and active forces simultaneously with Ca^{2+} transients. With appropriate choices of directed differentiation protocols and optimized electrical conditioning, atrial vs. ventricular specification was robustly achieved. We demonstrated chamber specific drug responses, specifically that a low concentrations of 4-AP (<50μM) prolonged AP duration in atrial but not ventricular tissues; whereas the acetylcholine analogue, carbachol, shortened atrial APDs only. We also observed higher expression levels of KCNJ2 responsible for Kir2.1 protein production and the corresponding I_{K1} current in ventricular tissues compared to atrial tissues.

Biosketch: Dr. Milica Radisic is a Professor at the University of Toronto, Canada Research Chair (Tier 2) in Functional Cardiovascular Tissue Engineering and a Senior Scientist at the Toronto General Research Institute. She is also the Associate Chair-Research for the Department of Chemical Engineering and Applied Chemistry at the University of Toronto and Director of the NSERC CREATE Training Program in Organ-on-a-Chip Engineering and Entrepreneurship. She obtained B.Eng. from McMaster University, and Ph.D. form the Massachusetts Institute of Technology. She is a Fellow of the Royal Society of Canada-Academy of Science, Canadian Academy of Engineering and the American Institute for Medical and Biological Engineering. She received numerous awards and fellowships, including MIT Technology Review Top 35 Innovators under 35. She was a recipient of the Professional Engineers Ontario-Young Engineer Medal in 2011, Engineers Canada Young Engineer Achievement Award in 2012, Queen Elizabeth II Diamond Jubilee Medal in 2013 and NSERC E.W.R Steacie Fellowship in 2014. The long term objective of Dr. Radisic's research is to enable cardiovascular regeneration through tissue engineering and development of new biomaterials. Her research interests also include microfluidic cell separation and development of in vitro models for drug testing. Currently, she holds research funding from CIHR, NSERC, CFI, ORF, NIH, and the Heart and Stroke Foundation. She is an Associate Editor for ACS Biomaterials Science & Engineering, a member of the Editorial Board of Tissue Engineering, Advanced Drug Delivery Reviews and Regenerative Biomaterials. She serves on review panels for Canadian

Institutes of Health Research and the National Institutes of Health. She is actively involved with BMES (Cardiovascular Track Chair in 2013 and 2104) and TERMIS-AM (Council member, Chair of the Membership Committee). She was a co-organizer of a 2017 Keystone Symposium, "Engineered Cells and Tissues as Platforms for Discovery and Therapy". Her research findings were presented in over 160 research papers, reviews and book chapters with h-index of 51 and over 9400 citations. She is a co-founder of a New York-based company TARA Biosystems, that uses human engineered heart tissues in drug development and safety testing for major pharmaceutical companies. She serves on the Board of Directors for Ontario Society of Professional Engineers and TARA Biosystems.

http://www.labs.chem-eng.utoronto.ca/radisic/

Twitter: @milicaruoft

Speaker: Michael-Rock (Rocky) Goldsmith

Molecular De-risking by Design: Modeling and Simulation to Advance Safer Chemistries

Michael-Rock Goldsmith[1]

[1]Bayer U.S. Crop Sciences, Chesterfield MO 63017

Molecular design approaches have traditionally been driven by functional purpose (new additives, new surfactants, new inhibitors, new pharmaceuticals, new agrochemicals) and are highly target and use-class centric. As one identifies leads for a functional purpose and optimizes iteratively to make a better molecular "product" it is critical to take stock of emerging in silico technologies and advancements in computational toxicological and molecular modeling approaches to provide feedback to the molecular design and lead selection process. Holistically capturing the safety landscape requires considering the likely or intended chemical exposure routes, the entire chemical life-cycle and hazard flags for both target and off-target species. The process to identify model needs, modeling environments and how to scope the molecular design landscape early on can pave the way that enable molecular design optimized for safer, more effective chemistries. We describe these approaches and showcase some functional tools that fit into molecule de-risking workflows that can guide in compound selection.

Biosketch: Michael-Rock ("Rocky") Goldsmith is the Computational Discovery Chemistry Lead for the Emerging Technology platform of Small Molecules Research at Bayer Crop Sciences in Chesterfield MO. Prior to this position he was at Chemical Computing Group Inc., a Montreal-based life-science informatics and modeling software company that produces the Molecular operating Environment

(MOE); a fully integrated molecular-discovery platform. He also worked for nearly a decade as a principal investigator at the US EPA, where he performed post-doctoral research at the National Center for Computational Toxicology in the field of in silico chemical genomics methods and virtual high-throughput screening, and later started much of the computational exposure sciences with a focus on Consumer Product chemical exposures. During his undergraduate studies at Concordia University in Montreal he worked in pharmaceutical, tobacco and explosives industries in both R&D and product development and subsequently completed his Ph.D. in theoretical chemistry and molecular biophysics at Duke University (Durham, North Carolina) working on theoretical optical activity of molecular assemblies and aggregates, theory-assisted determination of absolute stereochemistry of chiral natural products, and elucidating the molecular mechanisms of biological sequestration and in vivo distribution of the food contaminant Ochratoxin.

Speaker: Carole Yauk

Dose-response Modelling of Transcriptomic Data: Progress and Potential for Risk Assesment Applications

Carole Yauk[1]

[1]Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada; Phone: 613-941-7376; E-mail: carole.yauk@canada.ca

Despite widespread recognition of the value of global gene expression profiling in toxicological testing (i.e., toxicogenomics), its applications in human health risk assessment has not been fully realized. A significant roadblock to application has been the amount of time and expertise required to derive meaningful data from these extremely large data sets. Two particularly useful approaches include the use of transcriptional signatures that predict specific modes of actions or toxicities, and benchmark-dose modeling of global gene expression data to derive toxicogenomic point of departures (PODs). This presentation will review lessons learned from using *in vitro* and *in vivo* toxicogenomic data in chemical evaluations at Health Canada, and our resulting proposed paradigms for application. Case studies will be described for exposures to prototype chemicals to demonstrate the use of transcriptomic signatures and BMD modeling for: (1) tiered testing or prioritization; (2) potency assessment; (3) mode of action analysis; and (4) derivation of point of departure (POD) and margin of exposure. Overall, our work shows that hazards and PODs identified from transcriptional data are consistent with those derived from analysis of conventional endpoints. The work demonstrates practical approaches for the use of toxicogenomics in chemical prioritization and preliminary evaluation of hazards, to enhance our knowledge of the mode of action, and identify PODs to support and improve human health risk assessment.

Biosketch: Carole Yauk obtained her Ph.D. in biology from McMaster University in Hamilton, Ontario, Canada, where she studied the effects of urban air pollution on heritable mutations. In her post-doctoral

research at the University of Leicester, England, she developed novel single-molecule PCR techniques to study induced mutations in sperm. She went on to become a research associate in the Dept. of Genetics at the University of Leicester, where she studied meiotic recombination hotspots in the mouse genome. She returned to Canada to join the Healthy Environments and Consumer Safety Branch as a Research Scientist in 2002. She is currently lead of the Genomics Laboratory in the Healthy Environments and Consumer Safety Branch at Health Canada, and an adjunct professor of Biology at Carleton University. Her current research is focused on the development of genomic approaches for chemical risk assessment and on improving regulatory assessment of heritable effects. She has over 150 publications in these research fields.

Speaker: Andy Nong

Good and New Computational Practices for 21st Century Risk Assessment

Andy Nong[1]

[1]Environmental Health Sciences and Research Bureau, Health Canada, Ottawa, ON

Health Canada Chemical Management Plan has been supporting research on the use of new approach methodologies to advance risk assessment and management of environmental chemicals. The rapid pace of research technology and techniques has led to an increasing amount of information for environmental chemicals. The wealth of data being produced to researchers and risk assessors will be increasingly dependent on computational and data science tools for storage, management, analysis, and visualization of data. Eventually, harmonizing the use of computational tools will be important for international risk assessment uptake and applications. With the onset of informatics developed for new approach methodologies, this presentation will focus the evolution of computing practices for the risk assessment of environmental chemicals. We will observe perspectives from Toxicologists, Computer Modelers, and Risk Assessors on the application of toxicokinetics for environmental contaminants. A case study on the use of in vitro to in vivo extrapolation (IVIVE) toxicokinetics modeling to scale in vitro experimental data for human based values will be used to learn about key components on addressing uncertainties and practicalities for risk assessment. Lastly, we will look at international efforts for the advancement of informatics to uptake of new approach methodologies to predict toxicity and safety. Computational research will play a central role at helping identify future research need and emerging health concerns. Eventually, the outcome from the research will generate computer tools that will help with the prioritization, assessment, decision making of managing environmental chemicals at Health Canada.

Biosketch: Dr. Andy Nong is a Research Scientist at Health Canada and leads the Computational Toxicology Laboratory at the Environmental Health Sciences and Research Bureau. Dr Nong works has been recognized for toxicokinetic and biological modeling approaches in risk assessment. His current research program explores the use of computer biological models to advance alternative approaches for chemical evaluation. It consists of developing computational tools to assess exposure of emerging chemicals such as kinetics or dynamic models, dose-response models or even chemical structure activity

models. He has also helped developed Biomonitoring Equivalents to interpret biomonitoring data with the Canadian Health Measures Survey and more recently is working at evaluating computer tools for the in vitro to in vivo extrapolation of high throughput screening content such as the US EPA ToxCast for Health Canada Chemical Management Plan. In addition to his research activities, Dr Nong has been involved in many expert committees such as the National Academies of Sciences (NAS), World Health Organisation International Programme on Chemical Safety (WHO/IPCS), Organisation for Economic Co-operation and Development (OECD), and European Union Reference Laboratory for alternatives to animal testing (EURL-ECVAM). He received his PhD in Public Health from University of Montreal and was formerly a Research Investigator and Postdoctoral Fellow at The Hamner Institutes for Health Sciences. Dr. Nong is author/co-author of several publications including peer-reviewed articles and book chapters, and once served on the Board of Editors of the Journal of Applied Toxicology.

Speaker: Yadvinder Bhuller

New Approach Methodologies – Is There Another Way to Address the Underlying Regulatory Question?

Yadvinder Bhuller[1]

[1]Director, Health Effects Division I, Health Evaluation Directorate, Health Canada's Pest Management Regulatory Agency, 2720 Riverside Drive, Ottawa, Ontario, K1A 0K9

Animal and *in vitro* studies along with published literature are relied upon to determine the acceptability of human health risks from potential exposure to a pest control product. This has resulted in a data rich environment for many pesticide active ingredients. Over the years and as a means to: reduce, refine and/or replace existing animal studies, non-animal based alternative approaches have and continue to be developed. The Canadian regulatory framework for pest control products has sufficient flexibility to allow for incorporation of validated alternative approaches such as: *in silico* methods ([quantitative] structure activity relationship ([Q]SAR) models), integrated approaches to testing and assessment (IATA), adverse outcome pathways (AOPs), and Tox21 and RISK21 approaches. Given the importance of human safety and protection, when considering alternative approaches, robust scientific scrutiny of these methods is necessary. The global acceptance of such approaches highlights the importance of having internationally recognized technical guidelines, such as those developed by the OECD. More recently, consideration of new approach methodologies (NAMs) has come to the forefront and there has been a recent emergence of potential machine and deep learning methods. This has reignited discussions on the current pace of incorporation of alternative methods and has pushed the need towards a strong science and design-based mindset. For the latter, this incorporates a framework from the digital world, referred to as 'digital thinking', where a user experience-based approach is determined by experimentation and incorporation of the knowledge learnt from failed experiments as a means to improve the desired outcome.

Disclaimer: The views expressed in this abstract are those of the author and do not necessarily represent the views or policies of Health Canada's Pest Management Regulatory Agency.

Biosketch: Prior to joining Health Canada's Pest Management Regulatory Agency in 2011, Yadvinder (Yad) Bhuller was responsible for managing the regulatory approval of submissions for human clinical trials conducted in Canada with investigational pharmaceuticals. In this role, Yad was key in advancing the development and publication of clinical trial practice guidelines along with the clinical trials e-manual. In his current role, as the Director of the Health Effects Division 1, Yad continues to incorporate his clinical trials related experience along with his background in Pharmaceutical Sciences and Toxicology. This includes considering regulatory mechanisms on how to incorporate alternative approaches in both the toxicology and exposure assessments of pesticides.

PLENARY SPEAKER: George Daston

New Approaches for Predicting Toxicity

George Daston[1]

[1]The Procter & Gamble Company, Cincinnati, Ohio USA

New approach methodologies are starting to be applied for chemical safety assessment. This talk will focus on the use of cheminformatics and toxicogenomics as a means of predicting toxicity. The field of toxicology began by describing the adverse effects produced by chemicals, but research on the underlying modes of action quickly followed. Advances in biotechnology have provided the tools to query modes of action rapidly and with high throughput; however, the steps of pathogenesis between initial molecular interaction and adverse outcome are less well characterized. Therefore, the initial use of these data is in support of read-across, where the toxicity of a new chemical is predicted based on existing toxicity data from an analogous chemical. The use of large databases to identify analogs will be described, as well as projects to catalog all known modes of action based on chemistry information. Read-across is based on an assumption of similar biological activity among analogs; we use of toxicogenomics to test this assumption. As more information linking gene expression with specific modes of action becomes available, it will be possible to rely solely on this information to connect chemicals with similar biological activity. Approaching toxicity prediction at a mode of action level also facilitates the elucidation of multi-factorial causes of disease, as it is at the mode of action level that genetics, exogenous chemical exposure and nutritional and other host factors interact.

Biosketch: George Daston is Victor Mills Society Research Fellow at the Procter & Gamble Company, the highest scientific rank achievable. He has published over 100 articles and book chapters and edited five books in toxicology and risk assessment. His most cited work is on the topics of mechanistic approaches to characterize dose-response and risk from estrogenic chemicals, the role of toxicity-induced micronutrient deficiencies in developmental toxicity, and identifying that the triazines melamine and cyanuric acid were responsible for an epidemic of acute renal failure in dogs and cats in North America, and later in babies in China. His current research efforts are in the areas of cheminformatics, toxicogenomics and predictive and mechanistic toxicology, particularly in addressing how findings in these fields can improve risk assessment for chemicals and the development of non-animal alternatives. Dr. Daston has served as President of the Teratology Society, Vice President-Elect, Councilor and Treasurer of the Society of Toxicology, on the USEPA's Science Advisory Board, Board of Scientific Counselors and Endocrine Disrupter Screening and Testing Advisory Committee, National Toxicology Program Board of Scientific Counselors, National Research Council's Board of Environmental Studies and Toxicology, and National Children's Study Advisory Committee. In his advisory role at EPA, he oversaw the chartering and first five years of EPA's acclaimed Computational Toxicology Program. He was the founding editor of *Birth Defects Research: Developmental and Reproductive Toxicology*. With scientists at the Humane Society of the US, Dr. Daston manages the AltTox website, which is devoted to the exchange of scientific information leading to the development of non-animal replacements for toxicity assessments. Dr. Daston has been awarded the Josef Warkany Lectureship and the Distinguished Service Award by the Teratology Society, the George H. Scott Award by the Toxicology Forum, the Society of Toxicology's Best Paper of the Year Award, and is an elected Fellow of AAAS. Dr. Daston is an adjunct Professor of Pediatrics at University of Cincinnati.

GABRIEL PLAA AWARD RECIPIENT: Peter G. Wells

A Novel Developmental Role for 'Tumor' Suppressor Genes in Protecting the Fetus from Reactive Oxygen Species

Peter G. Wells[1,2], Danielle M. Drake[1] and Shama Bhatia[1]

[1]Division of Biomolecular Sciences and Centre for Pharmaceutical Oncology, Faculty of Pharmacy, University of Toronto, Toronto, Ontario [2]Dept. of Pharmacology & Toxicology, Faculty of Medicine, University of Toronto, Toronto, Ontario

Reactive oxygen species (**ROS**) like hydrogen peroxide, superoxide and hydroxyl radicals are essential components of many physiological cellular processes, yet they also can cause disease by adversely altering the structure and function of cellular macromolecules (lipids, proteins, DNA, RNA). ROS-initiated diseases can arise in biochemically predisposed individuals who have enhanced pathways for ROS formation, and/or deficiencies in one or more of the many antioxidants and antioxidative enzymes that detoxify ROS, or that repair ROS-initiated DNA damage. Superimposed upon this complex balance is the potential enhancement of ROS formation and ROS-initiated diseases by numerous drugs (e.g. phenytoin, thalidomide, ethanol, methamphetamine) and environmental chemicals (e.g. benzo[a]pyrene), collectively termed xenobiotics. ROS-initiated developmental disorders likely occur at least in part via non-mutagenic mechanisms involving epigenetic changes caused by oxidative lesions in DNA and RNA. "Tumor" suppressor genes, including those that repair DNA damage, are widely known for their role in suppressing cancer. However, throughout most of evolution, animals including humans died before they could develop most cancers, so the evolutionary pressure for development of DNA repair genes likely arose from the developmental necessity to produce healthy progeny. In support of this hypothesis, recent studies in knockout mice provide evidence that deficiencies in various DNA repair proteins like p53, ataxia telangiectasia mutated (**ATM**), oxoguanine glycosylase 1 (**OGG1**) and breast cancer 1 (**BRCA1**) result in enhanced ROS-initiated embryonic oxidative DNA damage and disorders in developmental morphology and/or postnatal neurodevelopment when compared to wild-type (DNA repair-normal) progeny from the same litter. These mechanisms may contribute to many developmental disorders including autism. Moreover, these and additional morphological and postnatal neurodevelopmental disorders in progeny deficient in the above DNA repair proteins or Cockayne Syndrome B (**CSB**) are caused by *in utero* exposure to ROS-initiating xenobiotics including phenytoin and alcohol (ethanol), which may contribute respectively to the mechanism of disorders like fetal hydantoin syndrome (**FHS**) and fetal alcohol spectrum disorders (**FASD**). In addition to genetic predisposition, susceptibility also can be enhanced by environmental factors, evidenced by a substantial reduction in embryonic BRCA1 caused by *in utero* ethanol exposure. Growing evidence suggests a primary role for DNA repair proteins in protecting the developing embryo and fetus from ROS-initiated developmental disorders. (Support: CIHR; University of Toronto Faculty of Pharmacy)

Biosketch: Dr. Peter G. Wells is a professor at the Univ. of Toronto in the Division of Biomolecular Sciences in the Faculty of Pharmacy, and in the Dept. of Pharmacology & Toxicology in the Faculty of Medicine. His research interests are in the toxicology of reactive intermediates, particularly involving oxidative stress and DNA damage and repair in teratogenesis, neurodegeneration and carcinogenesis. He was a keynote speaker for the International Union of Toxicology (**IUTOX**) 11th International Congress of Toxicology in Montreal (2007), and the Brain Awareness Week, Univ. of Saskatchewan (2007). Distinctions include the Chappel Memorial Lecturer, Univ. of Guelph (2015); Career Research Award,

Assoc. of Faculties of Pharmacy of Canada (2011); Grass Traveling Scientist Award, Saskatchewan Neuroscience Network (2007); and, Deichmann Nomination, Society of Toxicology of Canada (**STC**) (2005). Dr. Wells was president of the Reproductive & Developmental Toxicology Specialty Section of the Society of Toxicology (**SOT**) (2001-2002). His laboratory's research papers have been highlighted in Cancer (2009), Chemical Research in Toxicology (2013), The FASEB Journal (2011), Nature Medicine (1999) and Toxicological Sciences (2004, 2011). Dr. Wells has given over 125 invited presentations, including symposium talks at annual meetings of SOT, American Society for Pharmacology & Experimental Therapeutics, Teratology Society (USA), Int. Society for the Study of Xenobiotics, STC, Int. Union for Pharmacology and IUTOX (2007, 2019). He has supervised 24 Ph.D. and 14 M.Sc. students, 7 postdoctoral fellows and over 115 undergraduate research students, with over 140 peer-reviewed publications. He has received research grant support from the Canadian Institutes of Health Research (**CIHR**), the National Institute of Environmental Health Sciences (NIH) and the National Cancer Institute of Canada. Dr. Wells has served on grant review committees for CIHR (Pharmaceutical Sciences, Pharmacology & Toxicology, and University-Industry Panels), the National Cancer Institute (NIH) and the U.S. Environmental Protection Agency.

Speaker: Jack Uetrecht

Finally, a Valid Animal Model of Idiosyncratic Adverse Drug Reactions

Jack Uetrecht[1]

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Idiosyncratic drug reactions (IDRs) represent a significant problem for patients and drug development. Little is known with certainty about the mechanisms of IDRs. Attempts have been made to reproduce IDRs in animals by giving very high doses of a drug or using other interventions such as glutathione depletion. However, these models all have characteristics very different from IDRs in patients. In particular, they are acute; in contrast IDRs are delayed in onset. For idiosyncratic drug-induced liver injury (IDILI) the delay is usually more than a month. There is evidence to suggest that most IDRs are immune mediated. The dominant immune response in the liver is immune tolerance. A recent development in the treatment of cancer is the use of immune checkpoint inhibitors that prevent immune tolerance and promote an immune response that sometimes destroys the cancer. One immune checkpoint is PD-1, and PD-$1^{-/-}$ mice have increased delayed onset liver injury when treated with amodiaquine, but it resolves despite continued treatment. Another checkpoint is CTLA-4, and addition of anti-CTLA-4 antibodies further increases the liver injury, and the histology looks the same as the histology of IDILI in humans. Depletion of CD8 T cells is protective, which indicates the injury is mediated by cytotoxic T cells. The same model unmasks the ability of other drugs to cause IDILI, although the injury is not as severe. This model will allow us to study the sequence of events leading up to the immune response that can lead to IDILI. Funded by CIHR.

Biosketch: Dr. Uetrecht is Professor of Pharmacy and Medicine and held the Canada Research Chair in Adverse Drug Reactions from 2001 to 2015. He received his Ph.D. in organic chemistry at Cornell University in 1972, M.D. at Ohio State University in 1975 and did his medical residency at the University of Kansas Medical Center from 1975-1978. He completed his clinical pharmacology fellowship in 1981 at Vanderbilt University and then joined the faculty as an assistant professor. He moved to the University of Toronto in 1985 as an associate professor and was the associate dean of pharmacy from 1994 to 1998. He is a Fellow of the Canadian Academy of Health Sciences. He chaired the Gordon Conference on Drug Metabolism in 2002 and initiated and chaired a new Gordon Research Conference on Adverse Drug Reactions, the first of which was held in 2005. He was the chair of the organizing committee for the 2018 North American ISSX meeting. He received the Janssen-Ortho Research award in 2001, the Student's Administrative Council Undergraduate Teaching Award in 2005, the McEwan Lectureship in 2007, and was voted Teacher of the Year by the 3rd year class in both 2007 and 2008. He received the Vos Award for Lifetime Career Achievement in Immunotoxicology from the Society of Toxicology in 2018. He has over 170 research publications, 35 book chapters, and has published a book with Bill Trager on drug metabolism. His research is focused on the mechanisms of idiosyncratic drug reactions with an emphasis on reactive metabolites and immune mechanisms, and he consults extensively for the pharmaceutical industry on problems with idiosyncratic drug reactions.

Speaker: David Clarke

Challenges in the Preclinical Assessment of Efficacy and Safety for Immune Oncology Products.

David Clarke[1]

[1]Drug Safety Research and Development, Pfizer Inc, Pearl River, New York

Therapies for the treatment of cancer are undergoing a transformation. Historically cancers have been treated with medicines that that were specific to biochemical properties of the cancer cell in a somewhat nonspecific manner. While more specific modalities have been utilized, there is growing interest in the importance of the immune system in treating cancer, and the immune system is being specifically engaged as a means to treat cancer. However, the engagement of the immune system presents some of the greatest challenges with both evaluating the therapies nonclinically and translation of the nonclinical findings into a clinical setting. I hope to present some of the nonclinical strategies being employed, but also some of the limitations and considerations in evaluating immune therapies in the nonclinical setting.

Biosketch: David W Clarke, PhD, DABT is the Drug Safety R&D Therapeutic Area Lead for Vaccines, located in Pearl River, NY, supporting projects within the Vaccines Research & Early Development and Cancer Vaccines and Immunotherapeutics units. Previously I was a Regulatory Strategy Lead within DSRD supporting primarily oncology and vaccines programs, and Head of Regulatory Toxicology, Discovery Interface, and Therapeutic Area Head for Oncology within the Wyeth organization. I've held previous positions with Nycomed Pharma in Linz Austria and the Parke-Davis Research Institute, Sheridan Park, Canada. I have a PhD in Pharmacology and Toxicology from Queen's University, Kingston, Canada, am a diplomat of the American Board of Toxicology, and have over 30 years of experience working within toxicology in the pharmaceutical industry.

Speaker: Eric Liberda

Indigenous Knowledge Holders and the "Two-Eyed Seeing" Approach to Environmental Health Investigations

Eric N. Liberda[1,*], Meaghan Wilton[2], Roger Davey[3], and Leonard J.S. Tsuji[2]

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The Two-Eyed Seeing approach to conducting research with Indigenous communities utilizes "western" science and Indigenous knowledge complementarily. The principle of utilizing multiple perspectives, particularly Indigenous knowledge, to address complex environment-and-health related issues has been used by our research team for approximately three decades, even though the two-eyed seeing term was only more recently coined. Applying the strengths of both Indigenous knowledge and western science results in robust research questions that are driven by both academic researchers and Indigenous community members. Herein, we illustrate how the Two-Eyed Seeing approach has been utilized by our research group and highlight how original research questions evolved, and how Indigenous knowledge and western science inform each other to provide a better understanding of complex environmental health issues that work towards a solution. For several examples, we highlight how research questions evolved to ultimately be one of environmental contamination. We also examine other projects involving traditional foods such as wild game and fish. In many of our examples Indigenous knowledge holders inform analytical findings by providing historical context which would have otherwise not developed a full picture of contamination sources. When working with Indigenous communities, it quickly becomes evident that all knowledge constructs should be utilized to inform each other to get a better understanding of complex environmental issues.

Biosketch: Dr. Liberda began his studies as an undergraduate and then a Master's student at the University of Waterloo (Environmental Health). This was followed by a second Master's degree in toxicology (RMIT, Australia), and ultimately a doctorate at New York University in toxicology and exposure sciences (School of Medicine, Environmental Medicine). His focus on science-based policy and regulatory matters is informed by his research in toxicology and human health risk assessment. Dr. Liberda's studies have resulted in publications and grants that work towards protecting Canadians from the adverse health effects of toxicants such as pesticides and toxic metals while providing insight into regulating novel compounds such as nanoparticles. The majority of his toxicology and risk assessment work has been with remote First Nations communities in Canada.

Speakers: Ron Brecher and Trevor Smith Diggins

Making the Science Make Sense - A Risk Communication Framework

Ronald W. Brecher[1], Trevor Smith Diggins[1]

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Toxicologists and other scientists are frequently called upon to provide opinions on issues that may have impacts on human health or the environment. Understanding the principles of risk communication can help technical experts prepare to respond to complex questions in high-profile conversations about risk. This presentation introduces a framework for risk communication planning, and tools to help communicate more effectively with decision-makers and stakeholders, building credibility in the process.

Biosketch: Dr. Ronald W. Brecher is recognized in Canada and internationally for his expertise in toxicology, risk assessment and risk communication. He obtained a B.Sc. (Hon.) in biochemistry from Carleton University and a Ph.D. in Medicinal Biochemistry from Sussex University. He is a Diplomate of the American Board of Toxicology (DABT), and a Chartered Chemist (C.Chem.)in Ontario. Dr. Brecher has more than 30 years of experience as a consultant in toxicology, with an emphasis on assessing and communicating about the human health impacts of chemicals in air, food, water, soil and consumer products. In addition to his technical consulting, Dr. Brecher provides training in toxicology, risk assessment and risk communication for clients at all levels of government and within the private sector. He is regularly called upon to provide his expertise as an expert witness in legal proceedings. Ron has taught at York, Guelph and Waterloo universities.

Biosketch: Trevor Smith Diggins has been a communication strategist for over 30 years, Trevor's work in risk and crisis response has involved high-profile assignments throughout North America and around the globe. He has delivered risk communication training for Health Canada, Environment Canada, Defence Canada, Indigenous and Northern Affairs Canada, the Ontario Ministry of Environment, the Sudbury Soils Study, the U.S. Army, the U.S. Navy Bureau of Medicine, NASA, Nova Scotia Health, BC Health, and BC Environment, among others. He provided risk communication training to first responders at US Navy hospitals around the world, including San Diego, Camp Pendleton, Pearl Harbor and Okinawa. Trevor was invited to the Pentagon by the Under Secretary of Defense to recognize the success of an installation restoration project at The Memphis Depot, where he managed the communication strategy and stakeholder engagement programs. Trevor currently serves as an advisor to FloodSmart Canada.

Speaker: Lois Haighton

Risk Assessment of Cannabis, Not Just Another Food Ingredient

Lois A. Haighton[1], Dayna Lozon[1], Rebecca Rogerson[1], Joanne Wan[1]

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Recreational use of dried/fresh cannabis and cannabis oil is currently legal in Canada with restrictions (Bill C-45; *Cannabis Act*); however, marketing recreational cannabis edibles remains illegal, with regulations allowing their use expected late 2019. Several challenges exist in fitting such products within the existing regulatory frameworks for foods (Part B; *Food and Drug Regulations*) and Natural Health Products (NHPs). Food ingredients must meet standards of consistency and purity and be safe for all consumers. As cannabis is a complex herbal matrix rather than a single entity, specifications characterizing it to ensure consistency are needed. Viable candidates for standardizing composition might be delta-9-Tetrahydrocannabinol (THC) and phytocannabinoids. Some ingredients have pharmacological activities, such as the stimulant effects of caffeine, however, the neuropsychoactivity of cannabis is not characteristic of food ingredients. Also, there are data to suggest that THC adversely affects the developing brain, which would normally prohibit its use in foods, and, as such, edibles would not be suitable for children or teens. It is also necessary to establish a safe level of use that will not cause extreme psychoactive effects or general toxicity. Safety and dosing data should be based on oral studies, currently lacking. To establish the safety of edibles, oral studies can either be conducted or inhalation safety data will need to be extrapolated for oral use, which is not ideal. For this presentation, an overview of published safety information on cannabis/THC to-date, including potential effects on fertility, will be presented, and limitations/gaps in the dataset, for supporting oral safety, highlighted.

Biosketch: Lois Haighton is a Director in the Food & Nutrition Group at Intertek Scientific & Regulatory Consultancy where she is responsible for providing companies with regulatory and safety support for various products including food ingredients, dietary supplements, pharmaceuticals, cosmetics and consumer products. Ms. Haighton graduated from Queen's University in 1989 with an honours B.Sc. in Life Sciences. She has been a Diplomate of the American Board of Toxicology since 2002 and a European Registered Toxicologist since 2007. She joined the Society of Toxicology (SOT) as an associate member in 2001 and upgraded to a full member in 2011. She is also a member of the Society of Toxicology Canada (STC) and is currently serving as President of the Lake Ontario Regional Chapter of SOT. Ms. Haighton has over 25 years of experience in safety evaluation and risk assessment. She plays an integral role as a project manager and scientific resource person on a wide variety of health related projects. In this capacity, she prepares numerous technical reports for both government and industry on issues related to chemical contamination, pesticide use, new food ingredients, pharmaceuticals, and medical devices. She also has co-authored several publications in peer-reviewed journals, and she has presented and co-authored numerous posters at the Society of Toxicology annual meetings since 2004, as well as being Chair of a Workshop Session on the regulation and safety of probiotics at the 2017 SOT meeting in Baltimore.

Speakers: Andrea Amendola and Ruwan Jayasinghe

APPLICATIONS OF TOXICOLOGY IN SUPPORT OF HUMAN HEALTH RISK ASSESSMENTS

Andrea Amendola[1], Ruwan Jayasinghe[1], and Tessa Roselli[2]

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Human health risk assessment (HHRA) often relies upon assumptions from regulatory guidance documents or the scientific literature that are intended for broad application to a variety of sites for a range of land uses. These guidance-based assumptions in many cases are not representative of site-specific exposure scenarios. These assumptions can introduce unnecessary conservatism into an HHRA and result in overestimates of potential exposure and risk. Where possible, site-specific knowledge can be incorporated into HHRA as in Environmental Assessments through the collection of traditional knowledge and land use information. Two examples of the use of site-specific information are presented. The first example uses applied toxicology to characterize the bioaccessibility of metals in soil to better define the proportion of metal contamination that is absorbed. Site-specific in vitro bioaccessibility testing was applied in three HHRAs for federally-owned metallic lead-contaminated sites evaluated under Health Canada's Detailed Quantitative Risk Assessment guidance. All three sites were assessed for lead, and two sites for arsenic using the U.S. EPA validated bioaccessibility methods. The second example uses a dietary study and hair samples from an exposed human population to define actual exposures to mercury from fish consumption. Both the baseline dietary study and hair biomonitoring are key supporting studies to the overall baseline HHRA effort that will provide area-specific information regarding potential and actual human exposure to mercury/methylmercury.

Biosketch: Andrea Amendola is an environmental risk assessor and toxicologist with an honours B.Sc. in biomedical toxicology from the University of Guelph. Since joining Golder in 2003, Andrea has provided technical direction, review, and support to risk assessments and other toxicological evaluations, conducting and interpreting laboratory studies in support of risk assessments or toxicological evaluations, and project management. Andrea is a Qualified Person for Risk Assessment (QPRA) under Ontario Regulation 153/04, and is a practitioner with the Ontario Ministry of the Environment, Conservation and Park's TRV Expert Working Group. Beyond O. Reg. 153/04, Andrea's 15 years of experience have included providing technical expertise to environmental assessments under the Canadian Environmental Assessment Act (CEAA) and for the Canadian Nuclear Safety Commission (CNSC), federal contaminated site risk assessments including Preliminary Quantitative Risk Assessments (PQRAs) and Detailed Quantitative Risk Assessments (DQRAs), risk assessments in the United States under the Resource Conservation and Recovery Act (RCRA), and risk assessments in support of projects in South America, Africa, Europe, Australia, New Zealand and China. In addition to risk assessment, Andrea has led bioaccessibility testing and research & development at Golder Associates' Mississauga office and has been a guest lecturer at the University of Toronto for a graduate-level course entitled Technical Aspects of Environmental Regulations.

Biosketch: Ruwan Jayasinghe is a Senior Risk Assessor and a Diplomate of the American Board of Toxicology (DABT)-certified Toxicologist with Golder Associates Ltd. in Mississauga, Ontario, Canada. With over 18 years of experience in environmental risk assessment and toxicology across Canada, Ruwan has provided his expertise and supervision to risk assessments in support of Environmental Assessments, Site-specific Risk Assessments, and Brownfield Risk Assessments for variety of clients in the Mining, Oil & Gas, Commercial, Real Estate, and Power & Energy Sectors, in addition to risk

assessments in support of governmental agencies at the various levels, and globally in Indonesia, Australia, China and the United States. In Ontario, Ruwan is recognized by the Ministry of the Environment, Conservation and Parks (MECP) as a Qualified Person for Risk Assessment (QPRA) and is one of the MECP's Vendor of Record Peer Reviewers of consultant risk assessments. Ruwan is also well versed in providing expert support in relation to fate & transport modelling of contaminants in the environment, public health and public consultation aspects related to human health and toxicology. In addition to his expertise in consulting, Ruwan is an Adjunct Lecturer and member of the Graduate Faculty at the University of Toronto. At the University of Toronto, he teaches a course in Environmental Risk Assessment that introduces the principles of environmental risk assessment and toxicology to students of the Masters of Environmental Science Program. The course presents the quantitative methods used to assess the human and ecological health risks associated with exposure to toxicants and includes an overview of Canadian regulations and policies and their impact on practitioners, policy makers and stakeholders. Furthermore, Ruwan's research has been published in various international peer-reviewed journals related to environmental risk assessment, health and toxicology.

Speaker: Miriam Diamond

The Gulf Between Stakeholders Illustrated by Flame Retardants in Electronic Products

Miriam Diamond[1]

[1]Department of Earth Sciences, University of Toronto

The perception of risks posed by industrial chemicals varies widely among stakeholders. The differing perceptions come from interpreting the same data on chemical use, exposure and toxicity, if such data are available. These differences are being played out in debates about flame retardants of FRs (those that are semi-volatile organic compounds) which are widely used in electrical and electronic equipment (EEE) and digital devices. Use of digital devices has reached up to 50% of the world's population and nearly 100% of North Americans. The number and concentrations of FRs in EEE is not known. Our recent study found 79 FRs in air and dust samples from an Ontario e-waste facility, with at least 60 compounds having at least 50% detection frequency. Many of these chemicals are listed on the Domestic Substance List compiled up to 1986 and as such, have been "grandfathered" in based on minimal data on chemical properties and toxicity. Other FRs are not on this list or the non-domestic substance list. Risk assessments are being developed under the Canadian Chemical Management Plan (CMP) for those FRs on the DSL that have been prioritized through initial screening. To date, few FRs have been found to be "toxic" under the Canadian Environmental Protection Act based on industry-supplied data on chemical usage which does not necessarily include those chemicals imported into Canada within a final finished product, such as EEE. The CMP assessments use best-available-data, and QSARs and read-across to fill data gaps. There is heavy reliance on in vivo test data, although efforts are turning towards the use of New Approach Methodologies (NAMs). The FR polybrominated diphenyl ethers, which are now controlled for new uses in Canada, are commonly found in Canadian residential air and dust, as well as surface wipes of current-use EEE. These data indicate widespread, on-going exposure to the Canadian population. The National Academies of Sciences (US) systematic review for evaluating low-dose toxicity

concluded that estimates of human exposure to PBDEs are “several orders of magnitude” lower than benchmark doses obtained from the meta-analysis of in vivo test data but that the benchmark dose may not “accurately predict exposures at which humans are affected.” This paper will discuss how and why these interpretations of the knowledge and knowledge gaps surrounding FRs vary widely depending on the stakeholder.

Biosketch: Miriam Diamond is a professor in the Department of Earth Sciences with cross-appointments to the Department of Chemical Engineering and Applied Chemistry, the Dalla Lana School of Public Health, School of the Environment, Department of Geography and Program in Planning, and the Physical and Environmental Sciences Program at Scarborough College. She received her B.Sc. in Biology from the University of Toronto (1976), M.Sc. from the University of Alberta in Zoology (1980), M.Sc.Eng. from Queen’s University (Kingston, Ontario) in Mining Engineering (1984), and her Ph.D. from the Department of Chemical Engineering and Applied Chemistry from University of Toronto (1990).
The goal of Prof. Diamond’s multidisciplinary research program is to improve our understanding of chemical contaminants from emission, through to transport indoors and outdoors, and ultimately to human and ecological exposure. This research has been published in over 150 articles and chapters in addition to receiving media attention. Prof. Diamond is an Associate Editor of the journal *Environmental Science and Technology* and sits on the Editorial Review board of *Journal of Exposure Science and Environmental Epidemiology.* Prof. Diamond is the co-chair of the Canadian Chemical Management Plan Science Committee, and is involved in several national and international organizations. She is a Fellow of the Canadian Geographical Society and the Society of Environmental Toxicology and Chemistry. In 2007 she was named Canadian Environmental Scientist of the Year and is a finalist for 2018 the Nature Inspiration Award from the Canadian Museum of Nature. She was Co-chair of the Ontario Ministry of the Environment’s Toxic Reduction Scientific Expert Panel that helped usher in Ontario’s Toxic Reduction Act.

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Poster Abstracts

1 Hazard identification and potency prioritization of 13 data-poor compounds using integrated in silico and in vitro genotoxicity testing

Yen K. Tran[1,2], Julie K. Buick[1], Jennifer L.A. Keir[3], Andrew Williams[1], Carol D. Swartz[4], Carole Yauk[1,2], Paul A. White[1,3]

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Traditional toxicological testing that involves the use of experimental animals is expensive and laborious. Thus, integrated testing employs both *in silico* and *in vitro* assessment tools to increase efficiency, and has become a core component of contemporary chemical toxicity assessment. Consequently, modern genotoxicity assessment strategies incorporate *in silico* predictions to direct priority candidates for *in vitro* testing, and then quantitative approaches establish potencies for further prioritization. At present, over a thousand chemicals on the Canadian Domestic Substances list (DSL) have not been tested for genotoxicity. Thus, there is a need to efficiently assess the genotoxicity of these data-poor substances.

In the present study, *in silico* screening and *in vitro* testing were integrated for genotoxicity assessment of 13 data-poor chemicals on the DSL. First, chemicals were flagged for potential genotoxicity based on quantitative structure-activity relationships (QSAR) in Leadscope® and OASIS-TIMES' expert and statistical systems; this information was used to prioritize chemicals for *in vitro* testing. Mutagenicity was assessed using the Ames test with Salmonella strain YG1041. Mammalian chromosome damage was assessed in human TK6 cells via the flow-cytometric micronucleus test. The Benchmark Dose approach was used determine genotoxic potency; bacterial mutagenicity and mammalian chromosome damage potencies were compared to the potency of benzo[*a*]pyrene.

Six of the investigated chemicals were flagged as bacterial mutagens by QSAR, and mutagenicity was confirmed in the Ames test. The three most potent mutagens were 1-anilino-4-hydroxyanthraquinone, Pigment Red 177, and benz[*de*]isoquinoline. Six were flagged as potential clastogens by QSAR; eight were positive in the micronucleus test, and three were equipotent with benzo[*a*]pyrene: 1-anilino-4-hydroxyanthraquinone, benz[*de*]isoquinoline, and 1,4 diaminoanthraquinone. Overall, the results revealed that 12 of the 13 DSL compounds investigated elicited a positive response in at least one of the tests.

Given the potencies exhibited by three chemicals equipotent to BaP, further testing and careful analysis of exposure may be required. Overall, our study demonstrates the effective use of integrated genotoxicity testing and quantitative dose-response assessment, through which we have identified the genotoxic hazard of 13-data poor substances on Canada's DSL. This information can be used to determine the need for any follow-up investigations to the prioritized chemicals.

2 Characterizing the role of the Akt-p300 axis in valproic acid induced exencephaly in CD-1 mouse embryos

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Background: Valproic acid (VPA), is a widely used antiepileptic drug and an effective treatment of a variety of seizures, bipolar and migraine disorders. The incidence of neural tube defects (NTDs) is 1-2% following VPA exposure during pregnancy. VPA is a known histone deacetylase (HDAC) inhibitor and *in vitro* exposure to VPA downregulates p300 histone acetyltransferase [HAT] protein. Akt, a family of serine-threonine kinases, phosphorylates p300 enhancing its HAT activity and plays an important role in cell survival and proliferation. Three isoforms of Akt including Akt1, 2 and 3 have been identified in mammals with approximately 80% amino acid homology and primary substrate specificity. Here it was hypothesized that VPA downregulates Akt and p300 following *in utero* exposure in GD9/GD10 CD1 mouse embryos. *Methods*: Pregnant CD-1 dams were exposed to a single dose of 400 mg/kg VPA or the vehicle control via subcutaneous injection on GD9 and embryos were harvested at 0, 1, 3, 6 and 24 hrs post-exposure. GD10 embryos were grouped depending on whether they had as open or closed neural tube. Fetuses harvested on GD 13 were grouped as control, non-affected or exencephalic. Protein expression of p300, Akt1/3 and Akt2 was determined by western blot using whole embryos for GD9/GD10 and heads only for GD13. *Results*: In CD-1 mouse embryos, Akt1/3 and Akt2 were detected in GD9 embryos while in GD10 and GD13 tissues only Akt2 bands were present. There was a decreasing trend of p300 and Akt2 in the 24 hr open neural tube (n=3) embryos. GD13 fetuses (n=3 where three heads were used as independent samples from each litter, control=9, non-affected = 8-9 and exencephalic=8 heads) showed no significant difference of either Akt1/3 or Akt2 protein expression. Statistical analysis done by one-way ANOVA ($p<0.05$). *Conclusion*: Akt isoforms show differential expression in GD9, GD10 and GD13 embryos. The Akt-p300 axis does not seem to play a significant role in VPA-induced open neural tube defects and exencephaly.

3 Peroxisome proliferator-activated receptor gamma (PPARγ) agonists reverse EcoHIV-1 induced inflammation in primary cultures of mouse glial cells.

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Purpose: HIV-associated neurological disorders (HAND) is the spectrum of neurological, behavioural and motor deficits observed in HIV-1 infected individuals. Evidence suggests that HAND is associated with neurodegeneration due to the release of neurotoxins and proinflammatory cytokines/chemokines by microglia and astrocytes in the central nervous system. Currently, there is no treatment available for HAND with patients relying on combined antiretroviral therapy as the primary approach of delaying or preventing the onset of cognitive impairment. PPARγ is a nuclear receptor that is involved with glucose metabolism and fatty acid storage. Several groups have demonstrated that treatment with PPARγ agonists is neuroprotective in various models of neurological impairment. The aim of this study was to investigate the anti-inflammatory potential of PPARγ agonist (i.e., rosiglitazone or pioglitazone) treatment *in vitro,* in primary cultures of mouse glial cells exposed to a chimeric strain of HIV-1, EcoHIV. EcoHIV has been engineered to specifically infect mouse cells through replacement of HIV-1 viral envelope glycoprotein 120 with murine leukemia virus glycoprotein 80, which facilitates entry into mouse cells.

Methods: Primary cultures of mouse glial cells (astrocytes and microglia) were treated with either rosiglitazone (25μM), pioglitazone (50μM) or vehicle for 1h prior to EcoHIV (17, 500pg of p24) exposure for 24h. Inflammatory cytokine/chemokine markers (IL-1β, TNFα, iNOS, CCL2, CCL3 and CXCL10) were then measured using qPCR. To further determine if anti-inflammatory effects were specific to PPARγ activation, cultures were co-treated with the specific PPARγ antagonist GW9662 (10μM) and respective agonists (pioglitazone or rosiglitazone).

Results and Conclusions: Exposure of EcoHIV to primary cultures of glial cells resulted in a significant elevation of IL-1β, TNFα, iNOS, CCL2, CCL3 and CXCL10 which was attenuated with either rosiglitazone or pioglitazone treatment. Co-treatment with the specific PPARγ antagonist reversed the effects of pioglitazone and rosiglitazone. Herein, we show that rosiglitazone and pioglitazone are effective in reducing inflammation in primary glial cultures. This data suggests that targeting PPARγ may provide a molecular target for preventing/treating HIV-associated brain inflammation. Future studies will examine the anti-inflammatory potential of these compounds in an *in vivo* mouse model of EcoHIV.

4 Oxoguanine glycosylase 1 knockout mice exhibit increased sex-dependent cerebellar and hippocampal DNA strand breaks and postnatal brain function disorders

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Oxoguanine glycosylase 1 (**OGG1**) primarily repairs the reactive oxygen species (**ROS**)-initiated DNA lesion 8-oxo-2'-deoxyguanosine (**8-oxoG**), although global 8-oxoG brain levels in *Ogg1* knockout (**KO**) mice are not increased until old age. OGG1 polymorphisms in humans have been associated with an increase in risk for cancer, diabetes, cataracts, etc.; however, their role in brain development has not been extensively studied. Herein, we investigated in *Ogg1* KO mice the levels of single and double DNA strand breaks (**SSBs**, **DSBs**) in brain, and brain function disorders, at 2-3 months of age. Strand breaks were measured via single cell gel electrophoresis (comet assay), and behavioural tests included measurements for repetitive behaviour (nesting material shredding, marble burying), object recognition and spatial memory (novel location and object recognition tests), and startle response (pre-pulse inhibition). Cerebellar SSBs were increased in male but not female *Ogg1* -/- mice compared to *Ogg1* wild-type controls (+/+) ($p<0.0001$), with no differences in hippocampal levels for either sex. However, DSBs were increased in both the cerebellum and hippocampus of male but not female *Ogg1* -/- mice ($p<0.0001$). Across all groups, SSBs were approximately 2-fold higher in the hippocampus compared to the cerebellum, with a lesser increase in DSBs. Behavioural results showed sex- and gene-dependent differences. *Ogg1* -/- males showed decreased nesting material shredding behaviour compared to *Ogg1* +/+ males, with opposite results in females ($p<0.05$). In contrast, *Ogg1* -/- males exhibited increased marble burying behaviour compared to *Ogg1* +/+ males ($p<0.05$), with opposite results in females ($p<0.0001$). In novel location recognition tests, at a short retention time (90 min), *Ogg1* +/+ males and females ($p<0.0001$, $p<0.05$ respectively) but not -/- mice showed the expected preference for objects placed in novel locations. No *Ogg1*-dependent preference for original vs. novel location was seen at 24 h (long retention time) for either sex. For the novel object recognition test at 24 h (but not 90 min), *Ogg1* -/- females but not males showed impaired long-term retention with no preference for the novel object, whereas *Ogg1* +/+ females showed the expected preference for the novel object ($p<0.01$). For startle response, male but not female *Ogg1* -/- mice showed decreased pre-pulse inhibition ($p<0.05$). The increased single and double strand breaks primarily in *Ogg1* KO males and sex-dependent behavioural differences in *Ogg1* KO mice suggest roles for DNA damage and OGG1 in brain disorders, possibly including autism. (Support: CIHR; Univ. of Toronto Faculty of Pharmacy).

5 Tackling ToxTracker data: the development of a data analysis pipeline to facilitate genotoxicity screening

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To minimize the chance of adverse human health effects, chemical safety assessments require toxicity screening. Toxicity screening must include assessment of genotoxicity, which has been linked to heritable genetic diseases and cancer. To permit efficient genotoxicity screening, a Dutch company Toxys B.V. recently developed a New Approach Methodology (i.e., NAM) that comprises a suite of assessment tools collectively called the ToxTracker assay; it detects genotoxicity in mouse embryonic stem cells by simultaneously monitoring fluorescent biomarkers linked to six reporter genes: *Rtkn*, *Ddit3*, *Bscl2*, *Btg2*, *Srxn1* and *Blvrb*. Those genes are indicative of DNA double strand breaks, the unfolded protein response, DNA replication stress, P53 activation and the antioxidant responses of NRF2 and HMOX1, respectively. More specifically, the reporter genes are fused to fluorescent biomarkers that permit measurement of a DNA damage response via flow cytometry. The response profile is then used to assess the genotoxic hazard of the tested compound. The ToxTracker assay generates large amounts of complex, multivariate dose-response data, creating a need to devise appropriate data processing techniques that facilitate rapid and effective interpretation of assay results.

This study involved the use of novel strategies to develop a data analysis and interpretation pipeline to efficiently scrutinise large amounts of ToxTracker dose-response data. More specifically, the work is (i) examining the variability of control responses, (ii) defining a threshold for identification of a significant positive response, (iii) defining endpoint-specific critical effect sizes by using the 95th percentile of the log-normal distribution in control responses; and (iv) using the benchmark dose covariate approach to effectively rank validation compounds by genotoxic potency.

The results obtained define thresholds of 1.5 and 1.7-fold change for identification of a weak and true positive response, respectively. The weak positive response value was consistent with the one advocated by Toxys B.V. However, the 1.7-fold change differs from the 2-fold change advocated by the company. A pipeline for potency ranking for rapid interpretation of data for new test articles was also developed. It readily permits potency evaluations for responses of each of the six reporters.

Next steps include an investigation of functional and statistical relationships between the six ToxTracker genetic toxicity reporters.

This study contributed to the continuing development of robust data analysis pipelines to facilitate effective use of NAM dose-response data for chemical classification and regulatory decision-making. More specifically, the study allows for a robust and effective interpretation of ToxTracker dose-response data for routine regulatory screening.

6 Prenatal paternal exposure to Arctic pollutants: Folic acid supplementation, a strategy that is not without consequences

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Introduction

Traditional diets of Inuit people result in low folate intake and high body burdens of Persistent Organic Pollutants (POPs), known to have negative health effects. Indeed, Inuit have more adverse pregnancy outcomes and shorter life expectancies than non-Inuit Canadians. Recent studies have shown that the father's lifestyle influences his offspring health. Therefore, we hypothesized that folic acid (FA) supplementation attenuates pathologies associated with prenatal paternal exposure to POPs over multiple generations.

Methods: We used a 4 generation Sprague-Dawley rat model, where founder females (F0) were divided into 4 treatments and gavaged with corn oil or an environmentally-relevant level of an Arctic POPs mixture composed of >15 POPs, including PCBs, Chlordane, DDT and DDE (Anas *et al*. Biol Reprod 2005) before mating and until parturition.

The diet of the F0 females contained either a basal level of FA (2 mg/kg), representing intakes from Canadian fortified foods, or supplemented level of FA (6 mg/kg), corresponding to periconceptional recommendations for women.

The four treatment groups obtained are: 1) FA-basal, without POPs 2) FA-basal, with POPs 3) FA-supplemented, without POPs 4) FA-supplemented, with POPs. The resulting F1 males were mated to untreated females to produce F2 rats and so on until F4.

At each generation, placentas, fetuses (19.5 dpc) and adult males (PND 150) were assessed for congenital anomalies by necropsies and histopathology.

Results: The fetal:placental weight (FW:PW) was reduced by POPs*FA interaction in F1, suggesting an inadequate placental efficiency resulting in smaller fetuses ($p<0.0001$). F1 placentas might adapt to enhanced fetal needs in the presence of POPs, because the labyrinth zone area was larger whereas the basal zone area was smaller ($p=0.03$). Surprisingly, the FA-supplemented lineages exceeded the expected incidence of fetal malformations in F1, F2 and F4 generations.

In F1, both POPs and FA increased kidney weight ($p=0.03$), whereas in F2, it was reduced in the FA-supplemented lineage ($p=0.02$). Histology of F1 kidneys revealed a lower score for progressive chronic nephropathy in FA-supplemented rats ($p=0.02$), suggesting a protective effect of FA on age-associated renal degeneration.

Conclusion: Intergenerational transmission of the paternal environment is apparent and may occur via the sperm epigenome or placental disruption. Although this study partially supports our hypothesis, FA supplementation may not represent an ideal solution to counteract the consequences of POPs. Achieving our objectives will broaden current understanding of the toxicological impacts of the environment on human health and the developmental origins of disease.

7 Ethanol-enhanced fetal proteasomal degradation of BRCA1 protein and macromolecular damage in the brains of Brca1 knockout mice

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Alcohol (ethanol, **EtOH**) is a Group 1 carcinogen (World Health Organization), and *in utero* exposure causes Fetal Alcohol Spectrum Disorders (**FASD**). The breast cancer 1 (**BRCA1**) susceptibility protein, commonly known for its tumor suppression role in breast and ovarian cancers, also facilitates DNA repair and protects the developing embryo from EtOH-initiated DNA damage and developmental abnormalities (Shapiro *et al*., Redox Biology 7: 30-38, 2016), including neurodevelopmental disorders (Drake *et al*., Birth Defects Research 110: 755 [No. 3], 2018). We hypothesize that deficient BRCA1-dependent DNA repair enhances FASD. Herein, +/- *Brca1* knockout (**KO**) mice were mated, and pregnant dams were treated once on gestational day 17 with either EtOH (4 g/kg i.p.), which enhances reactive oxygen species (**ROS**) formation in fetal brains, or its saline vehicle, with or without pretreatment with the free radical trap α-phenyl-N-tert-butyl nitrone (**PBN**). Fetal brains were extracted 6 hr post treatment and assessed for BRCA1 protein and expression levels, macromolecular damage including DNA double strand breaks (**DSBs**) and protein oxidation, as well as proteasomal activity, which is enhanced by low levels of oxidative stress. BRCA1 protein levels were about 2-fold higher in fetal vs. maternal *Brca1* +/- brains. EtOH reduced BRCA1 protein levels by 44% and 53% respectively in wild-type (**+/+**) and +/- *Brca1* fetal brains ($p<0.0001$), regardless of sex, without decreasing mRNA. This EtOH-initiated BRCA1 reduction may result from proteasomal degradation, as its activity was enhanced by 73% and 50% respectively in +/+ and +/- fetal brains exposed to EtOH ($p<0.001$). DSBs were about 60% higher in saline-exposed +/- vs. +/+ fetal brains. Compared to saline controls, EtOH enhanced DSBs by about 60% in the brains of both sexes of *Brca1* +/-, and to a lesser extent +/+ progeny ($p<0.0001$). The increases in DSBs in saline-exposed +/- vs. +/+ brains, and in EtOH-exposed +/+ and +/- brains, were blocked by PBN, suggesting a ROS-dependent mechanism. Interestingly, protein oxidation was similarly enhanced by EtOH in the brains of both *Brca1* genotypes, but only in males ($p<0.01$), and was blocked by PBN. In addition to increasing ROS formation, we propose a secondary risk mechanism whereby EtOH enhances oxidative DNA damage by increasing BRCA1 degradation, thereby reducing DNA repair. This proteasomal effect of EtOH may further enhance the risk for FASD as well as organ damage and cancer caused by EtOH use in adults. (Support: CIHR; University of Toronto Faculty of Pharmacy; CPO)

8 Public Health Ontario's Environmental Burden of Disease Project

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Public Health Ontario (PHO), a Crown corporation that provides decision-makers with scientific and technical advice to protect and promote the health of Ontarians, is working to estimate the environmental burden of disease for the 14 million residents of the province. Due to methodological and operational considerations, we separated diseases into cancer and "non-cancer" categories.

The *Environmental Burden of Cancer in Ontario* report, published in 2016, was a joint effort by PHO and Cancer Care Ontario. In it, we assessed the cancer risk from population-level exposure to 23 carcinogens found in indoor and outdoor air, food, drinking water, and indoor dust using predominantly a risk assessment model. We identified slope factors from Health Canada, U.S. Environmental Protection Agency (EPA), and the California EPA. We characterized current exposures using the best available information and applied appropriate exposure factors. We summed the excess cancer risk across ingestion and inhalation routes of exposure using a Monte Carlo approach. The environmental burden of cancer from exposure to the identified carcinogens was approximately 4,800 annual cancer cases (plausible range: 3,540 to 6,510 cases). The top contributors were exposure to sunlight, radon, and air pollution. Our findings, particularly the relative burden rankings of carcinogens, appear to have been recognized in the Ontario Ministry of Health and Long-Term Care's *Healthy Environment and Climate Change Guideline* (2018).

Currently, PHO is focused on estimating the environmental burden of non-cancer diseases. We selected 11 environmental hazards based on their inclusion in previous environmental burden of disease studies (e.g., WHO's (2016) global assessment of the burden of disease from environmental risks). The hazards are air pollution, foodborne and waterborne pathogens, lead, aeroallergens, mold/dampness, poisons, temperature, noise, UV radiation, and second-hand smoke exposure. We are conducting a probabilistic burden of disease analysis using exposure, mortality, and health administrative data to estimate these physician office visits, emergency department visits, hospitalizations, and deaths attributable to the 11 hazards. Our preliminary results attribute approximately 2,000 hospitalizations and 6,000 premature deaths to exposure to air pollution, with 3.5-fold more hospitalizations and 60-fold less premature deaths attributable to exposure to foodborne pathogens. We plan to compare the burden across all environmental hazards, including the 23 carcinogens. These results will be useful to decision-makers, with the ultimate aim of reducing population-level exposure to leading environmental hazards in Ontario.

9 Effects of organophosphate esters (OPEs) on a human granulosa cell line

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The use of the polybrominated diphenyl ether (PBDE) flame retardants has been regulated due to their adverse health effects. The PBDEs have been replaced by organophosphate esters (OPEs) that are now found ubiquitously in the environment. Despite their widespread presence, little information is available on the safety of OPEs. Previous studies have suggested that exposure to OPEs may be detrimental to female fertility in humans. Here, we focused on the effects of OPEs on granulosa cells, endocrine cells surrounding the oocyte that play a key role in ovarian function. To test the hypothesis that OPEs alter the cellular characteristics of granulosa cells to a lesser extent than PBDEs, we compared the effects of a major PBDE, 2,2',4,4' tetrabromodiphenyl ether (BDE-47), to those of three OPEs, tris(methylphenyl) phosphate (TMPP), triphenyl phosphate (TPHP) and isopropylated triphenyl phosphate (IPPP). KGN immortalized human granulosa cells were exposed to BDE-47 or an OPE (0.001 – 100 μM) for 48h. Effects on cell counts, lysosomes, and lipid droplets were determined using fluorescent dyes and high content imaging. All of the chemicals tested caused concentration-dependent decreases in cell survival, with IC50 values for IPPP < TMPP < BDE-47 < TPHP. Exposure to BDE-47, TMPP or IPPP decreased the number of lysosomes per cell whereas TPHP had no effect. BDE-47 significantly increased the total area of lipid droplets in the cells at concentrations ≥ 50 μM; all three OPEs tested affected the area of lipid droplets to a greater extent than BDE-47 (~ 2 to 4 fold) at lower concentrations. Significant effects of IPPP were observed at concentrations ≥ 3.2 μM, of TMPP at ≥ 5 μM, and of TPHP at ≥ 10 μM. Thus, we observed prominent effects of the OPEs tested on KGN cells at lower concentrations than BDE-47, suggesting that these alternatives may be more toxic than the PBDE flame retardants that they have replaced.

Supported by CIHR.

10 New proofs of endocrine disrupting properties for DEHP and mEHP: Increased proliferation of breast cancer cells through progesterone receptor activation

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The phthalates family are plasticizers add in plastic products, such as food containers, medical devices and toys, to increase their flexibility. Because they are not forming covalent bonds with the plastic, phthalates are gradually released from the plastic, resulting in a chronic exposure for humans. While these plasticizers, particularly the diethylhexylphthalate (DEHP), are characterised as endocrine disruptor compound, the mechanisms implicated in their toxicity are poorly understood. To fill these gaps, this study aimed to determine the effects of an exposure to DEHP and one of its major metabolite, the monoethylhexylphthalate (mEHP), on breast cancer cells by analysing markers involved in breast carcinogenesis. T-47D cells were exposed to environmentally relevant and higher doses of DEHP and mEHP (0.1 to 10 000 nM) daily for 4 days. Our results showed a significant increase of T-47D cell proliferation for the 10 000 nM dose of DEHP and the lowest concentration of mEHP (0.1 nM). We also observed an increase of the protein levels of the progesterone receptor (PR) at 10 000nM of DEHP and at the lowest concentrations of mEHP, although the latest was not significant. Since PR activation has been linked to hormone dependent breast cancer, we then determine its localization in cells exposed to DEHP and mEHP. Our results showed that nuclear localisation of PR was increased for DEHP and mEHP exposure, but significantly only for 10 000nM of DEHP. To confirm the implication of PR in the phthalates-induced proliferation, we treated the T-47D cells with 10nM of Mifepristone, an antagonist of PR. The proliferation induced by both phthalates was inhibited by Mifepristone, as well as PR activation, as demonstrated by its localization. These results suggest that an exposure to DEHP or mEHP increase cell proliferation by activating PR signaling, which could potentially increase the risks of breast cancer. The mechanism of activation of the progesterone pathway by those phthalates and the long-term consequences of this activation remained to be elucidated. Funded by NSERC, SRC, FRQS, QBCF, FAF.

11 The effects of in utero benzene exposure on fetal NF-KB cell signalling in CD-1 mice.

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In utero exposure to benzene, a known carcinogenic environmental toxicant, is associated with the development of childhood leukemia. We have previously demonstrated that *in utero* exposure to benzene in CD-1 mice can alter the expression of the transcription factor NF-kB, which regulates genes involved in cell proliferation and programmed cell death, however, a full characterization of benzene's effects on fetal NF-kB is still needed. Since NF-kB regulates genes involved in cell proliferation, inappropriate activation can result in the proliferation of damaged cells potentially leading to the development of childhood leukemia. We hypothesize that benzene metabolism in the maternal liver leads to fetal changes in NF-kB signalling. To test this hypothesis, pregnant CD-1 mice were exposed to 200 mg/kg benzene or its vehicle control on gestational days 8, 10, 12, and 14. Dams were sacrificed at 2, 4, and 24 hours after the last benzene dose. Fetal livers were collected, and immunoblotting was done to assess changes in protein levels of phospho-p65 (Ser276), phospho-p38-MAPK (Thr 180/Tyr 182), and inhibitor of NF-kB alpha (IkB-α). Preliminary results show that after *in utero* benzene exposure, protein levels of phospho-p65 (Ser276) and phospho-p38-MAPK (Thr 180/Tyr 182) did not change significantly after benzene treatment but protein levels of IkB-α significantly increased 6 hours after the last benzene dose. Future steps will assess mRNA expression levels of IkB-α as well as DNA binding activity of p65.

12 DNA damage and perturbed topoisomerase IIa as a target of 1,4-benzoquinone toxicity in murine fetal liver cells

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Recent studies suggest that maternal exposure to benzene during fetal development may lead to leukemia (blood cancer) in offspring. While the etiology of fetal benzene-induced leukemia is unknown, benzene is known to affect the critical DNA repair enzyme topoisomerase IIα (TOP2a), which is known to be associated with childhood leukemias. Here we describe the first study to examine the effects of the benzene metabolite benzoquinone (BQ) on fetal TOP2a in cultured fetal liver cells taken from gestational day CD1 mice. Cultured fetal liver cells were exposed to BQ at a concentration of 12.5 µM, as this was shown to be non-cytotoxic. Fetal TOP2a activity was measured at 2, 12 and 24 hours following BQ exposure and results demonstrated significantly decreased TOP2a activity at 24 hours. DNA-TOP2a covalent complexes were detected at 24 hours following 12.5 µM exposure and 30 minutes exposure following 50 µM exposure, indicating that BQ is a TOP2a poison in cultured fetal liver cells. Additionally, double-stranded DNA breaks were significantly higher after 24 hours of exposure, implying that TOP2a is involved in DNA damage following BQ exposure. Lastly, elevated γH2AX, a marker of DNA damage, in BQ-treated cells suggests an association between ROS and TOP2a, a previously contested hypothesis. Further *in vivo* complementary experiments will confirm and characterize the role of fetal TOP2a in transplacental benzene carcinogenesis. Support: CIHR

13 Endothelial-targeted loss of PPARγ increases susceptibility to DMBA-mediated tumourigenesis.

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Peroxisome proliferator-activated receptor (PPAR) γ plays a role in tumourigenesis. PPARγ is expressed in many mammary associated cell types. Previously, we showed PPARγ suppresses 7,12-dimethylbenz[a]anthracene (DMBA)-induced breast tumour progression in a cell type specific manner. Angiogenesis is key in the progression of many cancers. Based on reports suggesting PPARγ ligands block angiogenesis *in vitro*, we hypothesized PPARγ expression and signaling within endothelial cells (ECs) inhibits breast tumour angiogenesis *in vivo*. Female EC-targeted *PPARγ* knockout (*PPARγ*$^{E\text{-}KO}$) and congenic wildtype (WT) mice were treated with DMBA, and then maintained on a normal chow diet or one supplemented with the PPARγ activating ligand rosiglitazone (ROSI) and monitored weekly for up to 25 weeks. Here we show DMBA-only treated *PPARγ*$^{E\text{-}KO}$ mice have significantly worse *in vivo* tumourigenic outcomes compared to respectively treated WT controls. More importantly, co-treatment with ROSI significantly improved tumourigenic outcomes among controls, but not *PPARγ*$^{E\text{-}KO}$ mice. Western analysis revealed that signaling changes differ between *PPARγ*$^{E\text{-}KO}$ and WT mice irrespective of treatment. Interestingly, *PPARγ*$^{E\text{-}KOs}$ had a significantly higher DMBA-induced thymic tumour incidence compared to WT controls, which was not altered by ROSI co-treatment. Our *in vitro* studies also show PPARγ expression or absence did not significantly alter the inherent ability of ECs to sprout, migrate or invade. In contrast, early *in vitro* angiogenic events were significantly decreased by ROSI among aortic rings isolated from WT but not *PPARγ*$^{E\text{-}KO}$ mice. Together, our data provide the first direct *in vivo* evidence that EC-targeted loss of PPARγ increases DMBA-mediated breast and thymic tumourigenesis, and that ROSI activates PPARγ-dependent anti-cancer effects in EC that may be critical during early angiogenic events. Our data also support a novel breast cancer therapeutic role for PPARγ activating drugs and suggest stromal PPARγ expression may be a novel predictive biomarker for improved clinical outcomes among a subset of breast cancer patients.

14 No correlation between blood manganese and cognitive and motor impairment at age 6-7 in Canadian cohort GESTE

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Background: Manganese (Mn) is an essential element for growth and Mn deficiency has never been described in humans. Overexposure to Mn has been reported among workers, who show signs of intoxication known as "manganism", a disease with symptoms similar to Parkinson's disease. Increasing evidence suggest that high exposure to Mn could impair brain development. In Canada, particularly in Easter Townships, there are several environmental sources of Mn including drinking groundwater. Our objective was to examine the relationship between blood Mn and psychomotor skills in 212 school aged children in Eastern Townships, Qc, Canada.

Methods: Participant's mothers were recruited between 2008 and 2010 during pregnancy (n = 800) to participate in a prospective cohort GESTE. At 6-7 years, a total of 358 children completed a series of neuropsychological tests. Blood Mn was analysed by ICP-MS. A battery of selected tests from Wechsler Intelligence Scale for Children - Fourth Edition (WISC-IV) and NEPSY-II was administered to children aged 6-7, those tests allow us to assess working memory, long-term memory, visuospatial precision and motor skills. Caregivers were also asked to answer questionnaires to investigate for potential confounding factors. Multivariate statistic modelling was used to consider potential confounding factors. We first try to assess linear relationship between psychomotor outcomes and blood Mn. Considering a potential U-shape relationship with Mn, a quadratic non-linear dose-response models were also tested.

Results: The median blood Mn was 9.9 µg/L (range 4.7-21.4 µg/L). There was no association between blood Mn and both cognitive and motor outcomes.

Conclusion: Our study suggests that there is no association between psychomotor skills and blood Mn in school aged children in our population.

15 Detection of Ochratoxin A Using a Fluorescent Internal Linker Modified Aptamer

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Ochratoxin A (OTA) is a toxic metabolite produced by *Aspergillus* and *Penicillium* fungi. OTA accumulates as a secondary metabolite and is known to be acutely and sub-chronically nephrotoxic leading to kidney failure. It contaminates a wide range of human foodstuffs and has been classified as a possible human carcinogen by the International Agency for Research on Cancer. Therefore, it is important to have a quick and facile detection platform to test for its presence in foodstuffs. OTA detection has been a focus in the field of small molecule detection, prompting several research groups to select DNA aptamers for OTA. Aptamers are functional nucleic acid oligonucleotides that bind targets with high affinity and specificity and have been employed for the detection of various biologically relevant targets. Moreover, chemical modification within an aptamer can create a fluorescent nucleobase analog with the potential to provide a sensitive emission. Previous work has demonstrated modifications of natural bases where the synthesis involves many time-consuming steps. Therefore, we have designed a practical linker-style internal fluorescent probe which undergoes excitation at 530 nm and emission at 590 nm. Presented herein is the utility of our internal probe for determining OTA binding within the antiparallel quinine quadruplex structure of the OTA aptamer.

16 Synthesis of Fluorescent Aromatic Amines and the Incorporation of C8 Aryl dG Adducts into DNA

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Aromatic amines have gained notoriety over the past decades as significant and prominent environmental carcinogens. Many aromatic amines are converted into reactive intermediates *via* metabolism and form addition products (adducts) with DNA bases. More specifically, bioactivated amines are known to readily alkylate to the C8 position of deoxyguanosine (dG) which can cause structural alterations and promote atypical base-pairing, introducing mutations that can ultimately lead to carcinogenesis. Mutational propensity following introduction of dG adducts has been researched extensively in short DNA sequences that are known to contain "hotspots" for frameshift mutations. To complement the data derived biological assays, we have exploited the utility of fluorescence spectroscopy. Turn-on fluorescent probes are sensitive to their microenvironment and structural data can be translated through qualitative and quantitative analysis of fluorescence emission spectra following the probe's incorporation into DNA. This prompted our lab to synthesize a variety of fluorescent aromatic amines to serve as adduct analogs. This poster will discuss the optical properties of these amines as well as their mutagenic potential. In addition, adduct synthesis and incorporation into DNA will be reviewed.

17 Investigation of the mechanism of lamotrigine-induced skin rash

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Circumstantial evidence suggests that most idiosyncratic drug reactions (IDRs) are caused by a chemically reactive metabolite of the drug that covalently binds to proteins, which can induce an immune response. A common IDR is skin rash. There are few drug metabolizing enzymes in the skin, but an exception is sulfotransferase. We previously developed an animal model of a drug-induced skin rash in which nevirapine is oxidized in the liver to a benzylic alcohol and this is converted to a reactive sulfate conjugate in the skin. A topical sulfotransferase inhibitor prevented covalent binding in the skin and also the skin rash.

Other drugs may also cause skin rash by formation of a reactive sulfate metabolite in the skin. Lamotrigine (LTG), an antiepileptic drug, causes a relatively high incidence of skin rash. LTG is oxidized to an N-oxide, and our collaborator (Ming Liu, University of Toledo) found that LTG-N-oxide is a good substrate for several SULTs. We also know that LTG-N-oxide sulfate reacts with N-acetylserine. The next step was to make a LTG antiserum to study its covalent binding.

A reductive amination reaction of lamotrigine and glyoxylic acid using sodium cyanoborohydride was carried out to form a carboxylic acid intermediate, which was further reacted with EDC/NHS to form an activated LTG ester. This activated ester was reacted with BSA to form a LTG-modified BSA conjugated that was used to immunized rabbits to produce LTG antisera. The hapten density on the protein was determined by MALDI/MS and the specificity of the antisera produced was determined by ELSIA.

Female Brown Norway rats have been treated with LTG (100 mg/kg/day for 4 days). Animals were sacrificed and covalent binding of LTG to liver and skin proteins will be determined. Bioactivation of LTG to the N-oxide metabolite is a minor pathway, although it can probably be reduced back to LTG; therefore, the total amount produced is unknown. We synthesized the N-oxide by reaction of LTG with meta-chloroperoxybenzonic acid; this will be used to treat animals as well. The blood levels of both the LTG and LTG-N-oxide will be determined in both treatment groups.

18 The Development Of A CRISPR/Cas-Mediated PD-1 Knockout Rat Model To Study Idiosyncratic Drug Reactions Including Nevirapine-Induced Liver Injury

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Idiosyncratic drug reactions (IDRs) are rare but serious adverse drug reactions that are specific to an individual and can lead to patient morbidity. Animal models are important tools for mechanistic studies of IDRs, but they must display the same clinical picture as those seen in patients. We have developed the first valid idiosyncratic drug-induced liver injury (IDILI) model using PD-1 deficient mice in conjunction with anti-CTLA-4 to block immune checkpoints. We hypothesize that most IDILI is immune mediated and immune tolerance, the dominant immune response in the liver, prevents most patients for getting serious IDILI. By impairing immune tolerance, we are able to unmask the potential of this mouse model and allowing us to vigorously test hypotheses of IDILI. However, there are species differences and a spectrum of sensitivity to drugs; thus, PD-1 deficient mice are not a good model for all IDRs. Using a CRISPR-Cas9 approach, we have generated a PD-1 mutant rat analogous to the mouse model to investigate the drugs that cannot be studied in mice. We hypothesize the PD-1 mutant rats will be more sensitive to drugs, such as nevirapine, compared to wild type animals in terms of incidence and severity of the skin rash and/or liver injury. Male/female Sprague-Dawley wild type and PD-1 mutant rats were treated with 0.2% w/w concentration of nevirapine in rodent meal. None of the rats developed a skin rash; however, nevirapine treatment led to delayed onset marked increases in ALT activity. Histopathological samples of the nevirapine-treated PD-1 mutant rat liver exhibited diffuse hepatitis with marked depletion of hepatocytes and active necrosis/apoptosis with the infiltration of mononuclear leukocytes. Interestingly, immunophenotyping of lymphocytes from the liver of female PD-1 mutant rats exhibit increases in CD3+CD8+ T-cells, suggesting the liver injury is mediated by cytotoxic T-cells. The finding of what appears to be liver failure in the CRISPR-mediated PD-1 mutant rats treated with nevirapine is exciting as we have the versatility to use this newly generated model to investigate the mechanism of IDILI. Future directions will determine if other drugs that cause IDILI in humans also cause more severe liver injury in the PD-1 mutant rat than in the P-D-1- deficient mouse model. This research was supported by grants from the Canadian Institutes of Health Research (CIHR).

19 The effects of two new generation flame retardants on markers of oxidative stress and cell cycle regulation during endochondral ossification in mouse limb buds

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Flame retardants (FRs) are applied to products to slow burning. The use of "new generation" organophosphate esters (OPEs) as FRs has surged in the last 15 years, but information on their safety remains limited. Previously, we showed that several OPEs, including triphenyl phosphate (TPHP) and *tert*-butylphenyl diphenyl phosphate (BPDP), suppressed endochondral ossification in the *ex vivo* mouse limb bud culture model. One of the effects observed was a reduction in the differentiation of proliferating chondrocytes into hypertrophic chondrocytes. The literature suggests that both a tightly controlled increase in reactive oxygen species (ROS) and an upregulation in the expression of cyclin-dependent kinase inhibitors (CKIs) play an important role in directing the exit of chondrocytes from the cell cycle and subsequently permitting their terminal differentiation. These changes must occur at the appropriate time and to the appropriate degree in order for proper bone formation to take place. We tested the hypothesis that OPE exposure alters the oxidative stress response and cell cycle regulation by assessing the expression of *Hmox1*, an oxidative stress marker, as well as *Cdkn1a* and *Cdkn1c*, two CKIs, using qRT-PCR. Compared to control, 10 µM TPHP-treated limbs initially expressed lower levels of *Hmox1* and *Cdkn1a*, followed by a sustained upregulation. *Cdkn1c*, on the other hand, was downregulated. In contrast, BPDP exposure had smaller effects on *Hmox1* and *Cdkn1a* while not affecting *Cdkn1c* at all. Thus, although TPHP and BPDP similarly suppress chondrocyte differentiation at 10 µM, there are some differences in their effects on markers of oxidative stress and cell cycle regulation.

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20 Comparing National Biomonitoring Data from Multiple Countries: Bisphenol A as a Case Study

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National biomonitoring data serve as an important indicator of population exposures to environmental chemicals. As the capacity and longevity of biomonitoring programs continue to expand, these data are becoming increasingly useful to quantify the impacts of regulatory risk management decisions. Here we use bisphenol A (BPA) as a case study for comparing national biomonitoring data from three countries spanning several years. We report urinary BPA concentrations and estimated BPA intakes in the Canadian population using data from the Canadian Health Measures Survey (2009–2015), in the US population using data from the National Health and Nutrition Examination Survey (2009–2014), and in the Korean population using data from the Korean National Environmental Health Survey (2009–2014). We found that median urinary BPA concentrations did not substantially differ among countries in the most recent years examined for each survey. Over similar time periods, we showed that urinary BPA concentrations were consistently low (1.1–1.2 ng/ml) in the Canadian population, while they decreased (from 1.9 to 1.3 ng/ml) in the US population and increased (from 0.7 to 1.1 ng/ml) in the Korean population. Estimated BPA intakes at the 95th percentile are over an order of magnitude below current health-based guidance values. We discuss methodological and physiological factors that could influence these data, such as the impacts of different analytical methods and respondent fasting times. We also explore the generalizability of these factors to other chemicals measured as part of biomonitoring programs.

21 Effects of early-life exposure to AHR ligands in chicken embryos: epigenetic regulation and interindividual variation

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Dioxin-like compounds (DLCs) and polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants that induce expression of xenobiotic metabolizing enzymes through the aryl hydrocarbon receptor (AHR) response pathway. Both PAHs and DLCs are lipophilic and can be found in the eggs of wild birds. We are interested in how this early life exposure to contaminants affects sensitivity to subsequent exposures later in life, and whether sensitivity to re-exposure is regulated by epigenetic mechanisms. In the current study, tetrachlorodibenzo-*p*-dioxin (TCDD), benzo[*k*]fluoranthene (BkF), or vehicle control (DMSO) was injected into fertilized chicken eggs prior to incubation. After 19 days of incubation livers were harvested, sliced and cultured. *In vitro* exposures were conducted using graded concentrations of TCDD or BkF and the vehicle control for 24h. Levels of expression of genes involved in the AHR pathway were assessed in each group. We observed a 50-fold induction of CYP1A mRNA expression in tissues cultured from TCDD- but not BkF-treated embryos, consistent with our previous finding that PAHs are metabolized by the embryo prior to embryonic day 19. Re-exposure to graded concentrations of TCDD or BkF resulted in dose-dependent increases in CYP1A expression for both test chemicals. We observed high inter-individual variability in CYP1A induction after chemical re-exposures, and a sensitization to CYP1A induction in liver slices from pre-exposed birds. Low levels of DNA methylation of the CYP1A promoter suggests that DNA methylation does not play a significant role in this phenotype. We are currently investigating the contribution of histone modifications on the organismal response to re-exposure to xenobiotics. Epigenetic mechanisms involved in xenobiotic metabolism may be useful as biomarkers describing an association between early life exposures to environmental contaminants and sensitivities to subsequent exposures later in life. The same mechanisms can also be a source of the observed high interindividual variability in the response to an AHR-activator.

22 GARD® – assessing skin and respiratory sensitizers in vitro using a genomics-based platform

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Chemical sensitizers are chemicals that induce sensitization in exposed and immunologically susceptible individuals. Many chemicals in consumer products are tested for sensitizing properties in order to prevent adverse effects in the general population or in occupational environments.

Genomic Allergen Rapid Detection (GARD) is a state-of-the-art non-animal-based platform for the testing and identification of sensitizing chemicals. Classifications are based on differentially expressed, disease-associated genomic biomarker signatures measured in human myeloid dendritic cell-like cells. To date, the GARD platform has generated three readily available assays for the prediction and classification of skin sensitizers (GARDskin and GARDpotency) and respiratory sensitizers (GARDair).

The GARDskin assay classifies chemicals as either skin sensitizers or non-sensitizers. A formal validation procedure for GARDskin was recently performed (OECD TGP 4.106) in which the assay demonstrated an outstanding predictive performance with an accuracy of 94%, sensitivity of 93%, and specificity of 96%. An additional application, GARDpotency, based on a complementary set of biomarkers further subcategorizes chemicals according to their relative skin sensitizing potency in alignment with the CLP (Cat 1A, Cat 1B, No Cat) with an accuracy of 82%.

Lastly, GARDair is the most recent extension of the GARD platform; a first of-its-kind assay for assessment and identification of respiratory sensitizing chemicals. Performance data on GARDair has thus far demonstrated an accuracy of 89%, well balanced between sensitivity and specificity and with 100% reproducibility between experiments. GARDair is unique in that it is capable of accurately identifying respiratory sensitizers and distinguishing them from both true non-sensitizers and skin sensitizers.

The performance demonstrated with all three of the GARD assays shows that the GARD platform is a robust method for assessing the sensitization properties of chemicals.

23 Brominated Flame Retardants: Perinatal Exposure to an Environmentally-Relevant Mixture Disrupted Cell-Cell Interactions and Thyroid Homeostasis in Rat Mammary Glands at Puberty

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Mammary gland development requires precise hormonal regulation during puberty, pregnancy and lactation. Exposure to endocrine disruptors, such as brominated flame retardants (BFRs), is ubiquitous and may alter mammary development and function. BFRs are chemical compounds added to consumer products to satisfy flammability standards. We have previously shown that gestational and lactational exposure to BFRs disrupted proteins of the adherens junctions in the mammary glands of rat dams, likely in a PKA-dependant manner. *Here, we hypothesized that perinatal exposure to BFRs also disrupts junctional proteins and signaling pathways that control mammary gland development in pups.* Female Sprague-Dawley rats were exposed through diet to an environmentally relevant mixture of BFRs, based on levels of these observed in house dust, designed to deliver doses of BFRs of 0 (control), 0.06, 20 or 60 mg/kg/day. Dams were exposed before mating, and during pregnancy and lactation, resulting in perinatal exposure of the pups. Female offspring were euthanized on post-natal day 46 (Post-pubertal) and mammary glands were collected. Exposure to BFRs (0.06 mg/kg/day) significantly down-regulated the levels of adherens junction proteins E-cadherin and β-catenin, and of the gap junction protein p-Cx43. No effects were observed on the protein levels of estrogen and progesterone receptors. However, exposure to this low dose of BFRs, but not higher doses, down-regulated thyroid hormone receptor alpha (TRα) protein levels in the mammary glands of the offspring. Together, our results suggest that perinatal exposure to an environmentally relevant mixture of BFRs disrupts cell-cell interactions and thyroid hormone homeostasis during puberty, a critical period of breast development. Supported by FRQS, QBCF, NSERC (IP), by CIHR (ML, BFH, BR), by MERSCI, RQR-CIRD (RJG) and by FAF (ED)

24 Highly Mutagenic Cyanonitroanilines: Structure-activity Relationships

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Azo dyes are the most important class of synthetic dyes, as they are globally used to colour textiles, paints, papers, etc. There is evidence that these dyes can be reduced to aromatic amines upon ingestion, releasing potentially mutagenic compounds. For many Disperse Blue dyes, the reduction products are cyano-substituted nitroanilines (NA). Over 30 years ago, 4-nitroaniline (4NA) was identified as a weak mutagen in the Ames test. Remarkably, 2-amino-5-nitrobenzonitrile (2A5NBN) is over 10,000-fold more mutagenic than the parent compound 4NA (Environ. Mol. Mutagen. 57: 10-16, 2016). Mutagenicity of 2A5NBN is strongly dependent on the bacterial N-acetyltransferase (NAT) and nitroreductase (NR) enzymes, as evidenced by much greater mutagenicity in NAT-overproducing strain YG1024 and much weaker mutagenicity in NR-deficient strain TA98NR, compared to TA98.

We have synthesized and tested 5-amino-2-nitrobenzonitrile (5A2NBN), an isomer of 2A5NBN, to examine the structure-activity relationship of these potent mutagens. The mutagenicity of 5A2NBN in strain YG1024 is much less than that of 2A5NBN, but still much greater than 4NA.

We hypothesize that the addition of cyano substituents increases the rate of reduction by bacterial nitroreductases, a critical bioactivation step. Examination of the structure of a putative cyanonitroaniline-dG adducts leads to the hypothesis that cyclization could occur between the carbon atom of the cyano group and purine N7, forming a polycyclic DNA adduct.

25 Proteomic Profiling of Liver Toxicity Biomarkers in Rat Serum: A Rapid and Predictive in vivo Hepatotoxicity Assay for Preclinical Toxicity Safety Assessment

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Introduction: Pre-clinical assessment of compounds for pharmaceutical use for potential liver toxicity is a pre-requisite for regulatory submission. Although this requirement is currently satisfied using classical toxicological analysis, there is opportunity to use 'omics technologies to provide quicker, sensitive, and temporal readouts for the prediction of a compound's potential for liver toxicity during *in vivo* pre-clinical safety assessment. The purpose of this study was to develop a LC/MS/MS method for the quantification of biomarkers in the serum of rats to predict potential liver toxicity and monitor clinical safety. A panel of protein biomarkers was selected based on a thorough review of scientific publications.

Methods: A LC/MS/MS method for the quantification of biomarkers was developed for quantification of over 140 biomarkers for liver toxicity in rat plasma were identified through literature review and network biology analysis using publically available gene expression datasets. Rats were treated either with a variety of control vehicles or a single high dose of a variety of different hepatotoxic compounds. Protein biomarker data were collected from rat serum by LC-MS/MS using SRM methods. Protein biomarker data were assessed using multivariate statistical analysis to categorize the treatments.

Results: The biomarker assay was capable of discriminating hepatotoxicants from non-toxic control compounds and pre-dose samples as evaluated by partial least squares discriminant analysis (PLS-DA) and hierarchical clustering. The biomarkers that had the highest predictive capacity were a combination of proteins normally secreted by the liver together with intrinsic liver proteins that are presumably released during pathogenesis in a similar manner as classical liver toxicity enzymes. Many biomarkers – such as Alpha-1-antitrypsin, C-Reactive Protein, Retinol- Binding Protein, Afamin Protein, Serpin protein, Haptoglobin – overlapped in terms of 'importance' across most treatment groups with hepatotoxicant drugs.

Conclusions: This study has demonstrated the capability of the proteomic profiling LC-MS/MS biomarker assay of reasonable prediction of drug candidates for liver toxicity and the high throughput protein biomarker assay is highly suited for detection of hepatotoxicity in rats. Although work is required to connect the magnitude of each biomarker measurement to liver toxicity mechanisms, the assay can be used presently as a rapid predictive *in vivo* hepatotoxicity bioassay for early-phase drug discovery.

26 Searching for binding partners of human glutathione transferase Theta 1 – a moonlighting protein?

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Human glutathione transferase Theta 1 (GSTT1) is a member of the GST superfamily. GST enzymes are found in almost all organisms; they protect cells from damage due to electrophilic (mutagenic/carcinogenic) compounds, catalyzing their conjugation with the non-protein thiol, glutathione.

Several properties of human GSTT1 are unusual. The enzyme has relatively low catalytic activity. A homozygous null *GSTT1* genotype is present in about one-third of the population. Although GSTs are regarded as enzymes of xenobiotic detoxication, GSTT1 expression is reported to be higher in thyroid and prostate than in liver or kidney.

We hypothesize that GSTT1 has a "moonlighting" function independent of its catalytic activity. Recent studies have shown that several other GSTs, notably GST Pi, have binding interactions with signaling proteins (e.g., TRAF4, JNK, ASK1). We are identifying possible binding partners (protein-protein interactions) of GSTT1, which could provide clues to a role in signaling or other processes.

Purified recombinant his-tagged GSTT1 was used as bait in "pull-down" experiments with cell lysates prepared from cultured human prostate cells. Following trypsin digestion of eluted proteins, proteomic mass spectrometry was performed to identify binding partners. Preliminary results have identified potential binding partners, including the peroxiredoxins PRDX1, PRDX3, PRDX4, and PRDX6; SPAG9; and MCRIP2.

27 Accumulation and Sequestration of Arsenite and Selenite in Human Red Blood Cells

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Over 200 million people worldwide are exposed to the proven human carcinogen arsenic, due to contaminated drinking water. Animal studies have shown that arsenic and the essential trace element selenium can undergo mutual detoxification through the formation of the seleno-bis(S-glutathionyl) arsinium ion $[(GS)_2AsSe]^-$ which undergoes biliary excretion, resulting in fecal elimination of both compounds. $[(GS)_2AsSe]^-$ has been shown to form in animal red blood cells (RBCs), resulting in the sequestration of arsenic and selenium. This sequestration slows the distribution of both metalloids to the liver and other organs susceptible to toxic effects. In human RBCs (hRBCs), the influence of arsenic on selenium accumulation, and vice versa, is largely unknown. The aim of this study was to determine if selenite (Se^{IV}) and arsenite (As^{III}) increase the accumulation of the other in hRBCs and ultimately to determine if this is through the formation and sequestration of $[(GS)_2AsSe]^-$. In rat RBCs, Se^{IV} uptake is inhibited by 4,4'-diisothiocyanatodihydrostilbene-2,2'-disulfonic acid (H_2DIDS), suggesting uptake is mediated by the erythrocyte anion-exchanger 1 (eAE1, or Band 3). This led us to hypothesize that the presence of As^{III} would increase radioactive $^{75}Se^{IV}$ accumulation in hRBCs by means of Band 3 and $[(GS)_2AsSe]^-$ formation. $^{75}Se^{IV}$ accumulation assays were performed ± As^{III} ± H_2DIDS ± BSA. As^{III} was able to increase $^{75}Se^{IV}$ accumulation by approximately two-fold after 10 minutes in the presence of BSA (35 mg/mL). Radioactive $^{73}As^{III}$ accumulation assays ± Se^{IV} showed increased accumulation of $^{73}As^{III}$ + Se^{IV} over time. X-ray absorption near-edge structure (XANES) was used to determine the species of As and Se in hRBCs exposed *in vivo* to both compounds. Upon exposure to 30 µM As^{III}+Se^{IV} for 45 min, the presence of $[(GS)_2AsSe]^-$ was observed. Thus, under physiological conditions, As^{III} and Se^{IV} accumulation is mutually increased in hRBCs, with Se^{IV} uptake being mediated by the Band 3 protein. The presence of Se^{IV} in hRBCs can facilitate the formation of $[(GS)_2AsSe]^-$ and allow for the sequestration of As^{III}. Thus, Se could potentially reduce As-induced disease by slowing As distribution to susceptible tissues.

28 BISPHENOL A REPLACEMENTS EFFECTS ON ADIPOGENESIS IN MURINE 3T3LI PREADIPOCYTES

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Background: The use of bisphenol A (BPA) in consumer goods and food packaging has been associated with negative health outcomes due to its endocrine-disrupting capabilities. This prompted its removal from many products and replacement with other bisphenol analogs such as, bisphenol-S (BPS), bisphenol-F (BPF), bisphenol-AP (BPAP), Tetramethyl Bisphenol F (TM-BPF), Fluorene-9-bisphenol and 2,2'-Isopropylidenediphenol (2,2'-BPA) and others. Despite the structure similarities to BPA the obesogenic ability of these chemicals was not yet determined.

Objectives: The objective of our study was to determine if the BPA analogues function similarly to BPA as an endocrine-disrupting chemical by inducing the adipogenic differentiation of murine 3T3L1 preadipocytes.

Methods: Murine 3T3L1 preadipocytes were used to determine the adipogenic potential of these chemicals. Cells were treated with 0.01-20□M and we determined lipid accumulation and mRNA expression of adipogenic markers.

Results: Our results indicate that treatment of 3T3L1 cells with some of the BPA analogues induces increased mRNA expression of key adipogenic markers similar to the effects seen with BPA. We show that TM-BPF, BPF and BPS can upregulate lipoprotein lipase (Lpl), fatty acid binding protein-4 (Fabp4), perlipin (Plin) and others.

Conclusions: Some replacement BPA analogues are as potent if not more potent than BPA at inducing adipogenesis in the 3T3-L1 preadipocyte model.

29 Assessing Air Contaminants in Ontario: Permitting vs Ambient Air Quality Assessments

Onica, T., Attard, B., Jugloff, D. and Schroeder, J.

Ministry of the Environment, Conservation and Parks

The Human Toxicology and Air Standards Section (HTAS) of the Ministry of the Environment, Conservation and Parks (MECP) assesses health risk associated with contaminants in air. These assessments are carried out to support a number of programs, including setting benchmarks, permitting of industrial releases, and evaluations of ambient air quality. These assessments can be used to inform risk management decisions within a community.

In permitting, contaminants emitted to the environment must be assessed for their potential to cause adverse effects. To achieve this, maximum concentrations of contaminants emitted from the facility are estimated using air dispersion models, which inform conservative assumptions of exposure. These modelled concentrations are either assessed against MECP benchmarks below which an adverse effect is not expected to occur, or undergo a toxicological assessment to determine the potential of an adverse effect occurring. Often, the chemicals assessed through this process are data-poor, which presents a challenge to HTAS toxicologists.

HTAS can also be asked to interpret risk monitored concentrations of contaminants in air. These requests can be in association with environmental assessments, special studies investigating an incident (e.g. spill to air), and evaluations of general air quality in a community. For these types of assessments, conservative exposure scenarios are developed using the monitoring data provided and considering the toxicity profile of the contaminant. Often, technical issues such as the type of monitors used (stationary vs mobile), sampling times, and detection limits are also considered.

Case studies for evaluating emissions and general air quality will be presented to demonstrate how toxicological analyses inform environmental protection programs.

30 A Consultative Approach for Review and Selection of Toxicity Reference Values

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Human Toxicology and Air Standards Section, Technology Assessment and Standards Development Branch (TASDB), Ontario Ministry of the Environment, Conservation and Parks (MECP)

The Ontario Ministry of the Environment, Conservation and Parks (the ministry) relies on human Toxicity Reference Values (TRVs) which are used in risk assessments for contaminated sites and in other program applications. Selection of TRVs involves a review of the rationales from multiple agencies.

Recently the ministry introduced a process to enhance the transparency and efficiency of TRV selection. The process involves a review of TRV derivations based on established criteria and the application of a weight of evidence approach. Mode of action (MOA) analysis is applied to support the selection of the cancer-based TRVs. For example, for ethylbenzene, the ministry concluded that the MOA underlying the development of renal neoplasia does not involve direct interaction with DNA, and therefore did not select an oral cancer slope factor or inhalation unit risk value.

Each TRV is evaluated and then classified according to its supportability. A final TRV is selected from the most supportable TRVs and a confidence rating is assigned. In rare cases, the ministry suggests minor modifications (mainly with respect to the uncertainty factors (UF) applied) based on ministry's weight-of-evidence analysis. For example, for cyanide, the ministry suggested reducing the database deficiency UF of 10 applied by the US EPA IRIS in its inhalation reference concentration. The ministry's draft evaluations and recommendations are then provided to a multi-stakeholder group of external practitioners with expertise in toxicology and risk assessment practice. Their input is considered in the final selection by the ministry. When no single preferred TRV can be identified, the ministry notes the acceptability of all supportable TRVs. The updated TRV selection process facilitates the selection of the most scientifically supportable TRVs and enhances the transparency and efficiency of TRV selection process. Details of the process and criteria used to critically evaluate the derivations will be presented with case studies.

31 A Method for Developing Recreational Sediment Guidelines for Human Health

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In response to concerns regarding risks to humans from exposures to sediment contaminants occurring from recreational use of a water body in Ontario, preliminary site-specific recreational sediment guidelines were developed to inform and guide risk management of the shoreline.

To determine the contaminants needing the most attention, sediment concentrations were screened against health-based soil guidelines and background concentrations, identifying lead, mercury, and benzo(a)pyrene [B(a)P]. A conceptual site model was developed. For *high*- and *low-exposure activity* areas, recreational exposure scenarios were described and potential exposures to recreational users were expressed in the form of equations. Appropriate receptor parameters values were selected and rationales were provided. For each of the contaminants, toxicity reference values and relative absorption values were identified. Target risk values were assigned a hazard quotient (HQ) of 0.2 or 1 and incremental lifetime cancer risk (ILCR) of 10^{-6} or 10^{-5}.

Sediment concentrations of lead, mercury, and B[a]P corresponding to lower target risk levels (HQ of 0.2, ILCR) of 10^{-6}) and higher target risk levels (HQ of 1, ILCR of 10^{-5}) were calculated for both the *high*- and *low-exposure activity* areas.

Three risk management action levels were assigned based on corresponding target values of HQ and ILCR:

- No action: HQ <0.2 or ILCR level $<10^{-6}$
- Risk Management Action Level 1: HQ from 0.2 to 1 or ILCR from 10^{-6} to 10^{-5}
- Risk Management Action Level 2: HQ >1 or ILCR $>10^{-5}$

Results of the calculations for each contaminant were organized into a matrix of sediment guidelines for three risk management action levels based on contaminant concentrations and the type of recreational area (high- or low-exposure activity area). Sample calculations, key inputs, and a preliminary sediment guideline matrix will be presented in further detail.

32 Significance of the Fish Consumption Pathway in Human Health Risk Assessment

Sara Tavakoli and Jim Gilmore

Ministry of the Environment, Conservation and Parks

Water bodies and sediment can be contaminated with many bio-accumulative pollutants including polychlorinated biphenyls (PCBs), polychlorinated dibenzo dioxins and furans (PCDD/Fs), polybrominated diphenyl ethers (PBDEs), methylmercury, and chlorinated pesticides such as dichlorodiphenyltrichloroethane (DDT) and its metabolites (to name a few). For the majority of the general population, exposure to persistent and bio-accumulating contaminants occurs mainly through the ingestion of contaminated foods. A particular focus is placed on fish consumption because fish constitute a major route of exposure to bio-accumulating and persistent contaminants. In order to facilitate the evaluation of the fish consumption pathway for risk assessments, the Ministry of Environment, Conservation and Parks (MECP) is providing a streamlined equation for calculating exposure estimates to persistent organic pollutants (POPs) in fish. This equation, and the exposure parameters that feed into it, highlight the complexity of this exposure pathway. Each exposure parameter is presented to illustrate the site-specific nature of the fish consumption pathway as well as the overall variability and uncertainty with this quantitative approach. For example, the estimation of exposure to POPs in fish requires information about the nature of the exposed receptor population (i.e., general population, recreational fishermen, and subsistence fishers), type of fish consumed, intake rates, and if the catch is "diluted" by fish from other water bodies. Other considerations include preparation methods (skin-on or skin-off fillet, or whole fish) and cooking methods (e.g. baked, fried). These considerations are important as contaminants do not bio-accumulate to the same degree across different fish species and are not distributed uniformly in fish tissues, as some contaminants bind primarily to lipids and others to proteins. Fatty and/or larger fish also often contain higher POP concentrations than leaner, smaller fish. A case study for methylmercury is presented to illustrate the practical application of this information, in the form of a quantitative analysis of the fish consumption pathway in human health risk assessment.

33 Immunological protein concentrations in milk from Canadian mothers participating in the Maternal–Infant Research on Environmental Chemicals (MIREC) study

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Proteins in maternal milk support the health, growth and development of the infant. Studies in large cohorts are required to identify maternal factors that impact milk immunological protein composition, including health and socioeconomic variables, and factors related to milk production and infant feeding. Likewise, cohort studies provide a means of identifying correlations between milk immunological protein concentrations and infant health. To study these relationships, milk from 795 participants from the MIREC study was analysed for total and immunological protein content, and the data were correlated with maternal and infant health data. Milk samples were collected within 10 weeks postpartum. Maternal and infant data were derived from questionnaires administered to study participants. Milk was processed to mitigate fat interference with immunoassays. Total protein was analysed using a bicinchoninic acid-based colorimetric assay. Immunoglobulin A (IgA), lactoferrin, lysozyme, and prolactin were analysed using immunoassays. MIREC study participants were predominantly well-educated, socioeconomically advantaged, low-parity mothers, over 30 years in age. Total protein, lactoferrin and prolactin concentrations in milk from study participants declined with lactation time, while lysozyme increased. IgA did not significantly change over time. Milk lysozyme concentrations correlated significantly with lower maternal education and income, higher maternal weight during pregnancy, and body mass index at lactation. Milk prolactin concentrations correlated with factors related to baby growth, such as lower baby weight at birth and lactation, and lower baby length at birth. Trends in milk immunological protein over time were consistent with established knowledge of human milk composition during the lactation period. Overall, the relative lack of associations between maternal variables and milk proteins may reflect the homogeneity of MIREC study participants. Significant associations between milk lysozyme and maternal socioeconomic status are consistent with some reports in the literature, but causality is unknown at present. Relationships between prolactin and infant size suggest a role for milk prolactin in regulating neonatal growth and development. This research provides new Canadian information on factors affecting milk protein composition and contributes to a goal of the MIREC study, which is to obtain provide more knowledge of beneficial constituents and harmful contaminants in milk, and their associations with maternal and infant health.

34 Naphthenic acids and metabolic health: a focus on PPARα-mediated pathways

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Introduction: The Alberta oil sands have the third largest oil reserves in the world containing an estimated 2.5 trillion barrels of recoverable bitumen. Bitumen extraction generates residual tailings water known as oil sands process-affected water (OSPW). Although OSPW contains several major classes of contaminants, the toxicity of OSPW has been primarily attributed to naphthenic acids (NA). Studies in non-mammalian vertebrates have shown that exposure to both OSPW and NA can adversely impact hepatic metabolic processes, although the molecular pathways underlying these observations are not fully understood. A recent study has identified that chemicals in OPSW can act as activators of the peroxisome proliferator-activated receptor γ (PPARγ). The PPARs are ligand activated nuclear receptors that regulate cellular homoeostasis and metabolism. PPARs control the expression of genes involved in fatty-acid and lipid metabolism, oxidative stress and inflammation. Importantly, PPARγ and PPARα, the main isoform in liver, regulate many of the same genes however the impact of chemicals in OSPW on PPARα-regulated gene networks is unknown. Therefore the goal of this study was to determine the effects of NA exposure on hepatic PPARα-regulated gene targets.

Methods: McA-RH7777 cells, a rat hepatoma cell line, were exposed to 1.25 and 125 mg/L of a commercial technical NA mixture (SigmaAldrich) for 24h; these concentrations are within the range of NA concentrations reported in OSPW. We assessed mRNA expression of 5 confirmed direct targets of PPARα (Peroxisomal acyl-coenzyme A oxidase 1 [*Acox1*]; Angiopoietin-like 4 [*Angptl4*]; Carnitine palmitoyltransferase 1a [*Cpt1a*]; Carnitine/acylcarnitine translocase (*Slc25a20*] and cytochrome P450 1A1 [*Cyp1a1*]) by real-time PCR.

Results and Discussion: In general the expression of all PPARα target genes was increased by NA treatment, however this did not reach significance for *Acox1* (p=0.059) or *Slc25a20* (p=0.062). There was a significant increase in *Cyp1a1* expression with NA treatment which likely does not reflect solely PPARα activity as NAs have been shown induce *Cyp1a1* expression via AhR-mediated pathways as well. *Angptl4* and *Cpt1a* were significantly increased by NA treatment. Interestingly, overexpression of *Angptl4* has been shown to cause hyperlipidemia and hepatic steatosis in mammals, whereas overexpression of *Cpt1a* and *Acox1* (the first enzyme of the fatty acid beta-oxidation pathway) is related to increased fatty acid oxidation which, in some studies has been shown to be associated with obesity.

Conclusion: Although it is well established that the commercial NA mixtures do not wholly represent those found in OSPW, these results provide proof of concept that NA's have the potential to act as a PPARα activator and perturb PPARα regulated pathways. Since PPARα plays a key role in glucose and lipid homeostasis these findings suggest that it is biologically plausible that exposure to NAs in mammals may result in metabolic perturbations.

35 Treatment of the murine model of Lymphoma L5178Y with silver nanoparticles and its genotoxic effect.

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Introduction: Lymphoblastic lymphoma is a cancer responsible for 40% lymphomas in childhood. Silver nanoparticles (AgNP) have gained interest in cancer treatment for its cytotoxic effects by oxidative stress[1]. Inconsistencies have been reported genotoxicity since it varies according to the physio-chemical properties of the studied AgNP[2]. The micronuclei (MN) are biological markers for determinate genotoxicity. The objective was to analyse the genotoxic effect in the treatment of lymphoma L5178Y with AgNP.

Methods: Experimental study in which 42 male DBA/2 mic, six weeks old were used. Seven groups were studied including a control group without treatment, treated with cisplatin and AgNP, Dyed with acridine orange for fluorescence microscopy quantification of, polychromatic (PC), micronucleated (MN) and micronucleated polychromatic cells (PCMN). The statistical analysis was performed with the SPSS v20 software. The data was analyzed with Shapiro-Wilk test. The nonparametric tests of Kruskal Wallis and U of Mann Whitney were used. A value of $p < 0.05$ was considered significant. The study was conducted according to the NOM-062-ZOO-1999.

Results: a higher frequency of PC was observed in the control group versus the group treated with cisplatin. The group treated with AgNP showed a PC frequency of 212.5 ± 90.7 that was higher than observed in cisplatin group. There was observed a significant difference between the MN frequency of the control group versus the group treated with cisplatin; The group treated with AgNP showed a decrease of MN, this decrease was less than the observed in cisplatin group.

Conclusion: In this study, a myelosuppression effect was observed after the administration of cisplatin. Our findings suggest that AgNP could have a cytotoxic effect observed by the decreasing of bone marrow cells and simultaneously could have a protective effect against myelosuppression generated by cisplatin. A longitudinal analysis is required to determine the genotoxic effect of AgNP.

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36 Genotoxicity and Cytotoxicity of Topical Hydrocortisone and Fluocinolone in a Murine Model *in vivo* by Micronucleated Erythrocytes.

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Introduction: Fluocinolone and hydrocortisone are topical corticosteroids indicated in inflammatory dermal conditions, it is unknown whether they have a cytotoxic effect in bone marrow, or if it causes any damage to the genetic material (Bali, et al., 1990).

Objective: To evaluate the genotoxicity and cytotoxicity of topical fluocinolone and hydrocortisone in a murine model *in vivo* using the micronuclei test in peripheral blood.

Material and methods: Twenty-four male Balb-C mice (5 weeks and 25 gr) were used for this research, under the ethical criteria of the General Health Law. It was administered topically every 24 hours / 5 days to 8 groups of 6 murines with; 1) Negative control petrolatum (300 / mg), 2) 5-Fluorouracil 5%, positive control (37.5 mg), 3-5) fluocinolone and 6-8) hydrocortisone, at doses of 75, 150 and 300 mg. Sample taking and analysis: Two smears per peripheral blood sample every 24 hours / 9 days, before the application of any compound, air dried, set (80% ethanol), stained (acridine orange) and analyzed with microscope (100X). We identified the frequency of micronucleated erythrocytes (MNE) in 10,000 total erythrocytes, polychromatic erythrocytes (PCE) in 1000 erythrocytes and micronucleated polychromatic erythrocytes (MNPCE) in 1000 PCE (Zúñiga-González GM, 2003).

Results: Hydrocortisone showed genotoxicity 24 hours after the three doses, and cytotoxicity 96 hours after; fluoxinolone showed no genotoxic or cytotoxic effect.

***Discussion*:** Topical corticosteroids are widely used and it is important to identify whether they penetrate the bone marrow and induce genotoxic and / or cytotoxic damage, or not. This study showed that hydrocortisone does have these effects, as opposed to fluocinolone.

Conclusion: The experiment demonstrated that the use of fluocionlone is safer compared with hydrocortisone, due to its nonexistent cytotoxic or genotoxic effect.

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37 Blood Arsenic Levels and the Risk of Familial Breast Cancer in Poland

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Abstract

Arsenic is an established carcinogen; however, the relationship between arsenic and breast cancer risk is inconclusive. Thus, we conducted a prospective study of circulating arsenic and breast cancer risk among 1,702 women with a family history of the disease but no inherited *BRCA1* mutation. We included women aged 40 and above who had received genetic counselling and genetic testing at the Pomeranian Medical University between September 2010 and April 2017. Subjects completed a detailed research questionnaire and total blood arsenic concentration was measured by inductively coupled plasma mass spectroscopy. Subjects were classified into quartiles according to the blood arsenic level. Over an average of 4.5 years (range 0.7 to 7.3) of follow-up and 7,731 person-years, there were 110 incident cases of cancer diagnosed in the cohort. Based on the 68 breast cancer cases diagnosed, the annual breast cancer incidence rate is 914/100,000 per year and was 4.9 times greater than the expected risk based on Polish statistics. Women in the highest quartile of arsenic had a highly significant 12-fold increased risk of developing breast cancer compared to women in the lowest quartile (HR = 12.72; 95%CI 3.89-41.57). After five years of follow-up, the cumulative incidence was 0.7% for quartile 1, 3.8% for quartile 2, 4.2% for quartile 3 and 9.5% for quartile 4. Results were similar in the analysis including all incident cancers (HR quartile 4 vs. quartile 1 = 13.14; 95%CI 4.72-36.51). These findings suggest that in Poland blood arsenic status (even at low concentrations) is a strong predictor of cancer, including breast cancer.

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