48th Annual Symposium / Le 48e symposium annuel

December 4th – 6th, 2016
The Westin Ottawa
11 Colonel By Drive
Ottawa, Ontario
http://www.thewestinottawa.com

HIGH-TECH TOXICOLOGY: NEW APPROACHES …. OLD PROBLEMS?

TOXICOLOGIE ET TECHNOLOGIE MODERNE: NOUVELLES APPROCHES…MÊMES DÉFIS

Organised by / Organisé par
SOCIETY OF TOXICOLOGY OF CANADA
LA SOCIÉTÉ DE TOXICOLOGIE DU CANADA

Programme Committee / Comité du programme
Sabina Halapannavar, Health Canada, Chair and Government Member
Leanne Bedard, Bedard ADME-Tox Solutions, Industry Member
Elaine Leslie, Academic Member

Contents

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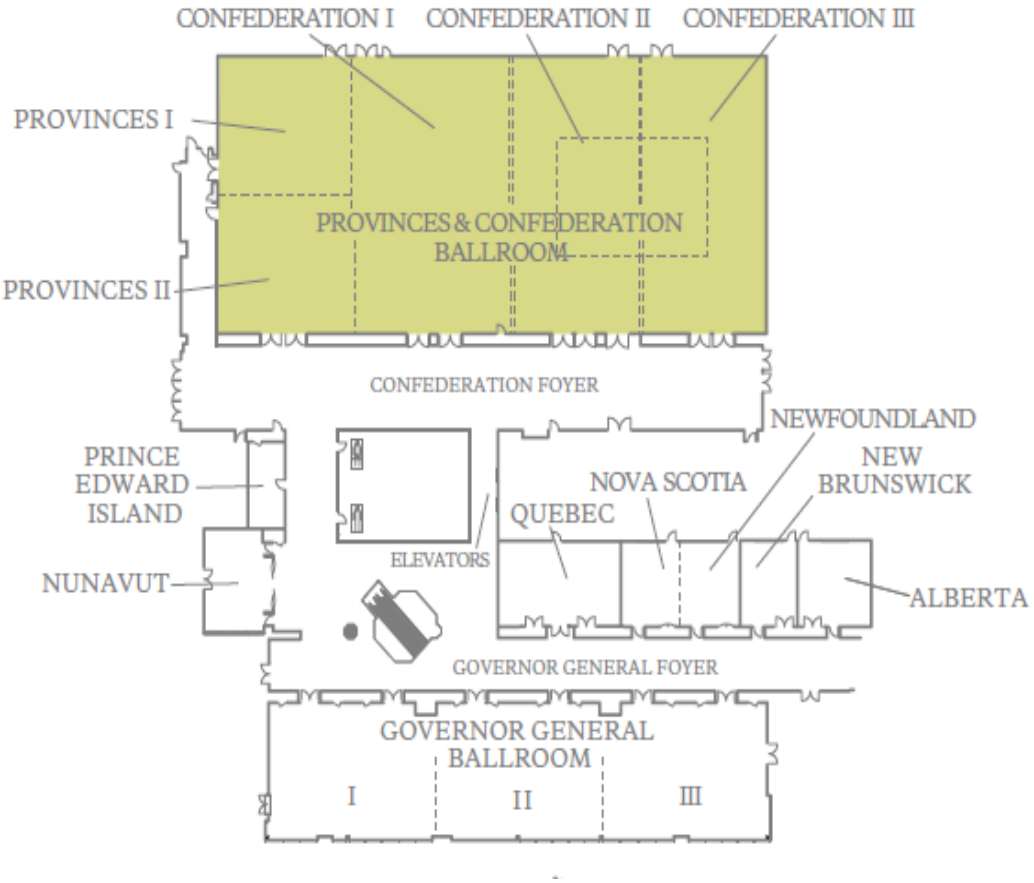
The Society of Toxicology of Canada is grateful to the above organizations for their valued contributions and financial support, which help make our Annual Symposium successful. Please Support these businesses.

Venue Information/ Plan Des Salles De Conférences

Westin Hotel, Ottawa - 4th Floor



Event Locations:

Symposium …… Governor General Ballroom III
Poster Viewing …… Governor General Ballroom II
Breaks/ Meals ……. GG Foyer
STC Annual Business Meeting ……Nova Scotia Room
Networking Workshop…… Governor General Ballroom III

Off Site Events:

Welcome Reception……The Pour House, 62 William St., Byward Marker
President's Reception…….Peter Devine's Pub, 67 Clarence St, Byward Marker

Reception Information / Activités de Réception et D'Accueil

Welcome Reception / Réception De Bienvenue

The Pour House, 62 William St., (William and George – One block south of Rideau)

Sunday, Dec 4th, starting at 7pm

Open to all registrants of the symposium. Trainees of all levels will be able to network with professionals working in toxicology from sectors including academia, industry and government to discuss possible career options and how to navigate the working world. A light dinner will be provided.

Ouverte à tous les participants du symposium. S'il vous plaît joindre à nous et les stagiaires de tous les niveaux qui auront l'occasion de faire du réseautage avec des professionnels qui travaillent en toxicologie, y compris à l'université, dans l'industrie et augouvernement, pour discuter des options de carrière et pour faire route en millieu professionnel. Un repas léger sera servi

Directions :

From the / Du Westin (5 min) https://goo.gl/maps/h2ZEuMqV1BT2

President's Reception /Réception du Président

Peter Devine's Pub, 67 Clarence ST (Byward Market), Ottawa

Monday, Dec 5th, Starting at 7 pm

This reception is open to all registrants of the symposium. Please join us for the presentation of awards, and test your knowledge of general toxicology and STC presentations at the **ToxTrivia Challenge**! A light dinner will be provided.

Ouverte à tous les participants du symposium. S'il vous plaît joindre à nous pour la remise des prix et pour participer au **Défi ToxTrivia** qui vous permettera d'évaluer vos connaissances sur la toxicologie générale et les présentations à la conférence ! Un repas léger sera servi

Directions :

From the / Du Westin (9 min) https://goo.gl/maps/CNTv8yNfntJ2

Symposium Program
High-tech toxicology: new approaches - old problems?

Sunday, Dec 4th PM

7:00 Welcome Reception – The Pour House, Upstairs at 62 William St., Byward Market

Monday, Dec 5th AM

Governor General Room (GG) III, Westin Hotel,

7:30 **Registration / Breakfast**

8:15 **Opening Ceremony** – Mr. Gordon Williams
Acknowledgement of the land

8:30 **Opening Remarks**
Mike Wade, STC President, Health Canada, Ottawa, Ontario

8:35 **SESSION I:**

Benefits and risks of nanomaterials – are they overstated?
Chair: Dr. Sabina Halappanavar
Introduction

8:40 **Warren Chan**, University of Toronto, Toronto, Ontario
Nanoparticle Toxicity: From Cells to Animals

9:10 **Suresh Neethirajan**, University of Guelph, Guelph, Ontario
Bionanotechnology - New Frontiers for Food, Agriculture and Animal Health Applications

9:40 **Jake Nikota,** Turnstone Biologics, McMaster University, Hamilton
New approaches in modeling carbon nanotube-induced toxicity

10:10 **Myriam Hill**, Health Canada, Ottawa, Ontario
Key Considerations in Human Health Risk Assessments of Nanomaterials – A Regulatory Perspective

10:40 **Coffee Break and Poster Session** (GG II, Westin; *Judging*)

11:20 **Gabriel Plaa Award lecture**
Genevieve Bondy, Health Canada, Ottawa, Ontario
Mycotoxins and everything else - 30 years of food toxicology

12:00 **Lunch and Poster Viewing** (GG II, *Judging, if required, during last 30 min*)

Monday, Dec 5th PM

Governor General Room (GG) III

12:55 **SESSION II: Toxicants - microbiome interaction**
Chair: Dr. Leanne Bedard
Introduction

1:00 **Jason Tetro**, Author of *The Germ Files,* Toronto, Ontario
Prevention Through Probiotics: The State of Knowledge and Where We May Go From Here

1:30 **Anita Kozyrkskyj**, University of Alberta, Edmonton, Alberta
Impact of Early Life Exposures on Infant Gut Microbiota: Cross-Sectional and Longitudinal Changes

2:00 **Rodney R. Dietert**, Cornell University, Ithaca, NY, USA
Protecting the Human Superorganism

2:30 **Coffee Break and Poster Viewing *(Judging if required)***

3:00 **SESSION III: TOXAM KEYNOTE SPEAKER**
Chair: Dr. Mike Wade
Prof. Martin Philbert, University of Michigan, School of Public Health, Michigan, USA
Nanotoxicology: From Simple Solutions to Complex Outcomes

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

4:30

The Science of Networking: Developing Your Personal Brand

Governor General Room (GG) II
Chair: Holly Campbell
Keynote: **Perry Monaco**, Head of Customer Success, LinkedIn

Panelists:
Pamela Shaver-Walker, ED Process Optimization, North American Safety Assessment at Charles River Laboratories
Jason Tetro, Author of "The Germ Files"

6:30

President's Reception and STC Awards

The "Heart and Crown", 67 Clarence Street (Byward Market), Ottawa
ToxTrivia – Challenge your knowledge of toxicology, and the STC symposium talks and posters 2016!
Chairs: Dr. Angela Hofstra and Dr. Leanne Bedard

Tuesday, Dec 6th AM

Governor General Room (GG) II

7:30 Breakfast

8:25 **SESSION IV: Emerging technology for functional toxicology**
Chair: Dr. Elaine Leslie
Introduction

8:30 **Mike Tyers,** Université de Montréal, Montréal, Québec
CRISPR/Cas9-based chemogenomic profiles for systematic assessment of toxin mechanism-of-action in human cells

9:00 **Dongeun Huh**, University of Pennsylvania, Philadelphia, PA, USA
Microengineered Physiological Biomimicry: Human Organ-on-Chips

9:30 **Simon Authier,** Deputy Chief Scientific Officer, Senior Director of Scientific Operations and Veterinary Science, CiToxLAB, Laval, Québec.
Trends with Non-Clinical and In Vitro Models for Proarrhythmia and Seizure Assessment

10:00 **Coffee Break and Poster Viewing** (GG II)

10:30 **SESSION V: Henderson Award Lecture**
Elaine Leslie, University of Alberta, Edmonton, Alberta.
The Role of Transport Proteins in Arsenic Detoxification.

11:10 **SHORT PRESENTATIONS**
Chair: Dr. Angela Hofstra
Introduction

11:15 **Nikki Philbrook**, Queens University, Kingston, Ontario
Investigating the Developmental Effects of the Flame Retardant, Triphynyl Phosphate, in C57BL/6 Mice. **(Poster #25)**

Dalibor Breznan, Health Canada, Ottawa, Ontario
Size and Surface Functionality of Mesoporous Silica Nanoparticles are associated with Cytotoxicity in Mammalian Cell Lines **(Poster # 8)**

William Willmore, Carleton University, Ottawa, Ontario
Role of the Antioxidant Response Element / Electrophile Response Element in the Response to Toxin Exposure. **(Poster # 35)**

11:55 **Lunch (and Poster Takedown)**

Tuesday, Dec 6th PM

Governor General Room (GG) II

12:55 **SESSION VI: Genetic toxicology: new twists on the double helix**
Chair: Dr. David Josephy
Introduction

1:00 **Steve Dertinger**, Litron Laboratories, Rochester, NY, USA
Multiplexed Flow Cytometric Assays Provide Information about Genotoxic Mode of Action

1:30 **Bruce Demple,** Stony Brook University Medical School, Stony Brook, NY, USA
Risky Repair: Oxidative DNA-Protein Crosslinks Driven by Mammalian Base Excision DNA Repair Mechanisms

2:00 **Robin Walker**, Independent Consultant, Mississauga, Ontario
Genotoxicity Evaluation of Impurities in Pharmaceuticals

2:30 **Closing Remarks**
Mike Wade, STC President, Health Canada, Ottawa, Ontario

Note: STC Board will meet briefly, after the Symposium

Speaker Abstracts and Biosketches

Keynote Speaker: Martin Philbert, PhD, FRSC

Affiliations: Dean, University of Michigan School of Public Health 1415 Washington Heights, Room # 1822 SPH I Ann Arbor, MI 48109-2029.

Title: Nanotoxicology: From Simple Solutions to Complex Outcomes

Biosketch: Dr. Philbert is professor of toxicology and dean of the University of Michigan School of Public Health. He earned his Bachelor of Science degree in 1984 from the College of Arts and Technology at Cambridge, and his doctorate in 1987 from the London University Royal Postgraduate Medical School. He was awarded a postdoctoral fellowship in the Neurotoxicology Laboratories at Rutgers University from 1988-90. Dr. Philbert served as a research assistant professor at Rutgers' Neurotoxicology Laboratories until 1995 when he joined the faculty at the University of Michigan School of Public Health as an assistant professor of toxicology. He was promoted to associate professor in 2000 and to professor in 2004. He served as associate chair for research and development in the department of environmental health sciences from 2000-03. In 2004, Dr. Philbert was appointed senior associate dean for research of the School of Public Health, a position he held through 2010 when he was appointed as Dean.

Dr. Philbert has maintained a continuously federally funded portfolio of basic research activities throughout his career. His research focuses on the development of flexible polymer nanoplatforms for optical sensing of ions and small molecules and the early detection and treatment of brain tumors. Other research interests include the mitochondrial mechanisms of chemically-induced neuropathic states. Most recently his work has been funded by the National Institutes of Health, the Department of Air Force and the National Cancer Institute. He is the author of more than 200 peer-reviewed scholarly manuscripts, abstracts and book chapters.

Dr. Philbert served as the inaugural chair of the US-EPA Chemical Assessment Advisory Committee that provides peer review of risk assessments produced under the auspices of the EPA's Integrated Risk Information System and was a standing member of the Agency's Science Advisory Board. He also served a four-year term on the National Advisory Environmental Health Sciences Council of the National Institute of Environmental Health Sciences, served as chair of the US-EPA Board of Scientific Counselors, chair of the US-FDA Science Advisory Board, and provides consultation to the federal agencies on a variety of issues surrounding emerging nanotechnologies, nanomedicine, health and safety. Dr. Philbert is an elected member of the Institute of Medicine of the National Academies of Science (USA), a fellow of the Royal Society of Chemistry (UK), a fellow of the Academy of Toxicological Sciences (USA), a member of the Division of Earth and Life Sciences of the National Academy of Science (USA), the Committee on Toxicology of the National Research Council (USA).

Gabriel Plaa Award lecture

Winner: Genevieve Bondy

Affiliations: Research Scientist, Regulatory Toxicology Research Division, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa ON

Title: Mycotoxins and everything else - 30 years of food toxicology

Abstract: History has been punctuated by recurring mycotoxicoses that have caused much suffering in humans, domestic animals and wildlife. Although the association of ergot consumption with illness dates back to the last millennium, recognition that consumption of toxins produced by moulds in food could lead to severe illness was not a consistently held opinion among food scientists. The study of mycotoxicology in recent decades dates back to the discovery of aflatoxins in the early 1960s. Since then there has been tremendous progress in identifying mycotoxins produced by a wide range of fungi, in detecting these toxins in raw commodities, foods and feeds, and in characterizing their hazards. In Canada, we regulate mycotoxins of concern based on whether Canadians are likely to be exposed to them and at what levels, and on the dose-response and nature of the hazard. Mycotoxin research in the Food Directorate, in my laboratory and those of my collaborators, is focussed on characterizing the health hazards associated with mycotoxins in foods consumed by Canadians as part of Health Canada's regulatory responsibilities under the Food and Drugs Act. We have conducted short and long term studies on carcinogenic fumonisin B1 that have contributed to international risk assessment by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Our chronic, alternative cancer bioassays for deoxynivalenol and ochratoxin A have, similarly, generated data in support of risk assessment at the national and international levels. Our current research is focussed on characterizing the reproductive and developmental toxicity of ochratoxin A. There are limited hazard data for juveniles, who are exposed to higher levels of ochratoxin A than adults. My experiences in the field of mycotoxicology provided the foundation for research on a wide range of regulatory food toxicology issues and knowledge gaps. For example, most key mycotoxins of concern globally are known to be immunosuppressive. Based on my early experiences in research on deoxynivalenol immunotoxicity, we have devised a streamlined approach to assessing chemical immunotoxicity in the context of regulatory toxicology studies. We are using this approach in collaborative studies on brominated flame retardants and other classes of chemical food contaminants. Our current research in the areas of chemical adjuvanticity, food allergy, and immunomodulation by food borne nanomaterials arose from our concern that, in addition to immunosuppression, inappropriate immune stimulation is also a potential adverse effect of chemical exposure via foods. The role of "traditional" regulatory toxicology studies in assessing genetically modified foods has also been a focus of critical review and research in our laboratories. In briefly highlighting my research in mycotoxicology and the broad spectrum of related research that has arisen from it, I will draw attention to the diverse and interesting field of regulatory food toxicology.

Henderson Award Lecture

Winner: Elaine Leslie

Affiliations: Departments of Physiology and Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB

Title: The Role of Transport Proteins in Arsenic Detoxification.

Abstract: Millions of people world-wide are chronically exposed to the environmental toxicant arsenic in drinking water. This has created a public health crisis because arsenic is a proven human carcinogen, and causes a myriad of other adverse health effects. Cellular transport pathways potentially have an important influence on regulating the cellular/tissue levels, epithelial passage, and ultimately elimination of arsenic. Thus, transport pathways are likely important regulators of the overall body burden of arsenic, and genetic differences in such pathways could influence arsenic-induced disease susceptibility. Arsenic is extensively methylated in human hepatocytes forming four major products monomethylarsonous acid (MMA^{III}), monomethylarsonic acid (MMA^{V}), dimethylarsinous acid (DMA^{III}), and dimethylarsinic acid (DMA^{V}). Arsenic elimination from the body occurs primarily through urinary excretion of dimethylated species. The ATP-binding cassette (ABC) transporter proteins, multidrug resistance protein 1 (MRP1/*ABCC1*), MRP2 (*ABCC2*) and MRP4 (*ABCC4*) are established transporters of various arsenic metabolites. Our *in vitro* studies have shown that human MRP1 transports the triglutathione conjugate of As^{III} [$As(GS)_3$] and the diglutathione conjugate of MMA^{III} [$MMA(GS)_2$], while human MRP2 is known to transport $As(GS)_3$, and the seleno-bis(*S*-glutathionyl) arsinium ion [$(GS)_2AsSe]^-$. We have shown that DMA^{V} and $MMA(GS)_2$ are transported with high affinity by MRP4. The localization of MRP4 at the basolateral surface of the hepatocyte and apical surface of the renal proximal tubule cell suggests it could be a critical pathway for arsenic elimination. We have recently found that single nucleotide polymorphic variants of MRP4 have differing abilities to transport arsenic through both altered function and membrane localization. Recent progress in the understanding of arsenic cellular efflux will be presented.

Biosketch: Elaine M. Leslie is currently an Associate Professor in the Departments of Physiology and Laboratory Medicine and Pathology at the University of Alberta, Edmonton, Alberta, Canada. She is an Alberta Innovates Health Solutions Scholar and was a Canadian Institutes of Health Research New Investigator. She received her BSc in Toxicology from the University of Guelph and her PhD in Pharmacology and Toxicology from Queen's University. Dr. Leslie completed postdoctoral training at the National Institutes of Environmental Health Sciences, Research Triangle Park, North Carolina, and at the University of North Carolina, Chapel Hill. Dr. Leslie has a long-standing interest in the involvement of ATP-binding cassette transporters and phase II biotransformation enzymes in detoxification. The current focus of the Leslie laboratory is understanding how multidrug resistance proteins (MRPs/ABCCs) and glutathione S-transferases are involved in the detoxification of arsenic.

Speaker: Warren C. W. Chan

Affiliations: Institute of Biomaterials & Biomedical Engineering (IBBME); Donnelly Centre for Cellular and Biomolecular Research (CCBR); Materials Science and Engineering
Chemical Engineering; Chemistry, University of Toronto

Title: Nanoparticle Toxicity: From Cells to Animals

ABSTRACT: Nanotechnology involves the engineering of structures, materials, and particle in the size range of 1 to 100 nm. These nanostructures have unique biological, optical, electronic and magnetic properties that are directly related to their size, shape, and surface chemistry. These structures are incorporated in a variety of health care devices for use inside and outside of the body for diagnosing and treating diseases. However, many nanoparticles contain heavy-metal components, can generate reactive oxygen species, and can be transported to different cellular and organ systems since their pharmacokinetic properties are related to their physicochemical properties. Many researchers have shown and suggested that nanoparticles may have unique toxicological profile because of these nanoparticle properties. Here, in this presentation, I will discuss the current state of nanotoxicology and nanomedicine. I will further discuss some of the current challenges, perceptions, and findings from this emerging field of research and how the developments in nanotoxicology research fits in with the greater objective of translating nanotechnology for patient care.

Biosketch:

Speaker: Suresh Neethirajan

Affiliations: Assistant Professor, School of Engineering, University of Guelph, Guelph, Canada

Title: Bionanotechnology - New Frontiers for Food, Agriculture and Animal Health Applications

ABSTRACT: Bionanotechnology provides novel tools for solving complex problems as we transform in to the 4th revolution in agriculture. Suresh will introduce discussion of the ways of testing biomarkers, proteins and contaminants using sensing techniques with the aid of hybrid nanomaterials. Microfluidic platforms and biosensors for single cell analysis paves the way for investigating the effects of drug testing catering to enhance in-vitro and in-vivo toxicology. Analytical and diagnostic hand-held tools that can emulate a lab in the field using microfluidic and nanotechnologies will be presented. These tools allows animals with health issues to be diagnosed on the spot without waiting hours or days for a test result. Suresh will address the session from the standpoint of agri-food nanotechnology that the government agencies, industries and academic communities should have on their radar.

Biosketch: Dr. Suresh Neethirajan is currently a registered Professional Engineer with the province of Ontario, and an Assistant Professor in the Biological & Biomedical Engineering program of the University of Guelph and serving as the Director of the BioNano Laboratory. He joined the University of Guelph in 2011 after working as a Research Engineer at the Oak Ridge National Laboratory of United States, and also as a Research Scientist at the National Food Research Institute of Japan. Dr. Suresh graduated from the University of Manitoba with both his PhD and master's degree in Biosystems Engineering. In 2010, he was recognised by NSERC Canada with the national Nano Innovation Platform award. Recently, he was awarded the 2014 Early Researcher Award by the Ontario Ministry of Research and Innovation, 2015 Young Engineer Achievement Award by Engineers Canada for demonstrated research excellence and academic contributions. Suresh is also the recipient of the Young Engineer of the Year award in 2015 by both the Canadian and American Society for Agricultural and Biological Engineers. The BioNano Laboratory headed by Suresh is focused on investigating pathogenic biofilms using nanotechnology approaches, and developing biosensors and bioinstrumentation tools for animal health diagnostics, human health and food safety applications. Suresh serves as the member of Executive Board of the Grand River Chapter and the Academic Requirements Committee of Professional Engineers of Ontario, and as the Vice-President (Technical) for the Canadian Society for Bioengineering. He is a member of Canada's National Nanotechnology Standards Committee, and the vice-chair of ASABE's IET-312 sub-committee of Emerging Information Systems. Suresh has been invited by the Parliament of Canada as an expert witness for enacting Bill S-11 regarding standards for nanotechnology in agriculture, food and biological systems. He has published over 120 technical papers including 60 peer reviewed international high impact journal papers, 5 book chapters, 5 United States Patent, and has supervised over 20 undergraduate students, 11 MSc students and 2 PhD students. He teaches Bio-Instrumentation Design, and Biological systems II, and Bio-nanotechnology courses at the University of Guelph.

Speaker: Jake Nikota

Affiliations: Industrial Postdoctoral Fellow, Turnstone Biologics, McMaster University, Hamilton

Title: New approaches in modeling carbon nanotube-induced toxicity

ABSTRACT: Carbon nanotubes (CNTs) are amongst the most widely produced and utilized nanomaterials worldwide. This diverse class of nanomaterial can possess unique physical properties allowing CNTs to be inhaled deep into the lungs and accumulate overtime due to ineffective interactions with pulmonary clearance mechanisms. The presence of CNTs within the lungs triggers inflammation, and over time, the development of fibrotic disease. The toxicity of different CNTs varies depending on their physical characteristics, necessitating a deep understanding of the mechanisms of CNT-induced lung pathology, and efficient experimental systems to screen newly developed CNTs. Traditional *in vitro* culture systems and *in vivo* mouse models have been used to address this issue, however our lab has expanded on this approach. We have proposed an adverse outcome pathway (AOP) to highlight the key biological events that occur after CNT inhalation which are important for the development of lung pathology. We have used this AOP as a roadmap to target genes in an *in vivo* mouse model system and have identified new mediators of CNT-induced lung disease. Additionally, we have developed an *ex vivo* precision-cut lung slice system that utilizes the strengths of *in vitro* and *in vivo* models that may be a useful tool for future screening strategies. Collectively, these approaches are expanding our understanding of CNT-induced toxicity.

Biosketch: Jake completed his PhD in Medical Science from McMaster University specializing in the field of Infection and Immunity. His thesis investigated the inflammatory mechanisms engaged in the lungs by cigarette smoke and bacterial infections. He recently finished a two year postdoctoral placement with Sabina Halappanavar at Health Canada in the Healthy Environments and Consumer Safety Branch studying mechanisms of nanomaterial-induced lung disease. Jake is currently pursuing an industrial postdoctoral placement with Turnstone Biologics investigating new viral based immunotherapies for the treatment of solid tumours.

Speaker: Myriam Hill

Affiliations: Section Head, Nanotechnology Section, New Substances Assessment & Control Bureau, Healthy Environments & Consumer Safety Branch, Health Canada.

Title: Key Considerations in Human Health Risk Assessments of Nanomaterials – A Regulatory Perspective

Abstract: Advances in nanotechnology have resulted in the commercialisation of many nano-enhanced consumer products, leading to the development of novel applications as well as providing improved performance and durability to existing products. In particular, nanomaterials are increasingly being used as additives in polymeric materials in food packaging, textiles, paints and coatings, cement, construction materials, sporting goods and personal care products. Although most jurisdictions agree that existing regulatory frameworks and statutes provide a firm foundation for the regulation and oversight of nanomaterials, the OECD Council Recommendation acknowledges that these should be adapted to take into account the specific properties of manufactured nanomaterials. In the absence of information on the toxicological effects specific to nanomaterials, identifying relevant exposure pathways during the life cycle of the products is crucial to mitigate potential health risks. In addition, consideration of the material's physico-chemical properties, use patterns, routes of exposure and any available hazard data may be used to develop criteria for grouping and read-across between nanomaterials with expected similar modes of action. The development of Adverse Outcome Pathways (AOP) relevant to nanomaterials may enhance the use and development of alternative testing strategies for use in regulatory risk assessments.

Biosketch: Myriam Hill received a B.Sc. degree in Chemistry from the National University of Colombia and a M.Sc. in Chemistry from the Weizmann Institute of Science in Israel. Ms. Hill is the manager of the Nanotechnology Section at the New Substances Assessment and Control Bureau at Health Canada, where she currently oversees the regulatory assessment of human health risks associated with the use of engineered nanomaterials. Her duties also include establishing priorities and research projects to identify and mitigate potential health risks to consumers resulting from both direct and environmental exposures to chemical substances, including nanomaterials. Ms. Hill has over 20 years of experience in the risk assessment and risk management of industrial chemicals, encompassing both exposure and hazard assessment of substances used in consumer products. In addition, Ms. Hill contributes to the development of risk assessment frameworks and policy principles for the regulation of products of nanotechnology in Canada. Ms. Hill has served as Health Canada's representative on a number of national and international nanotechnology committees including the Canada-US Regulatory Cooperation Council - (RCC) Nanotechnology initiative, the OECD Working Party on Manufactured Nanomaterials, and was the co-chair of the ILSI/NanoRelease-Consumer Products initiative.

Speaker: Jason Tetro

Affiliations: Visiting scientist at the University of Guelph; Author of The Germ Files

Title: Prevention Through Probiotics: The State of Knowledge and Where We May Go From Here

Abstract: Probiotics are defined as living organisms capable of providing a health benefit when ingested. Research over the last decade has revealed supplementation may be able to prevent several health ailments from infection to high cholesterol levels. The concept of using a probiotic to prevent toxin exposure has been explored and evidence does exist to suggest a possible benefit. This talk will discuss probiotics in general and explore the potential for reduction of harm from various environmental toxins.

Biosketch: Jason Tetro is a visiting scientist at the University of Guelph. He has studied health-related microbiology and immunology and has 25 years of experience in various fields including bloodborne, food and water pathogens; environmental microbiology; disinfection and antisepsis; and emerging pathogens such as Zika virus. Jason is better known in the public as The Germ Guy™. He regularly writes for The Huffington Post Canada, Popular Science, and The Globe and Mail. He also has a regular monthly column with CBC Radio. He has written two books, The Germ Code, which was shortlisted as Science Book of The Year (2014) and The Germ Files, which spent several weeks on the national bestseller list. He has also co-edited, The Human Microbiome Handbook, which provides an academic perspective on the impact of microbes in human health.

Speaker: Anita Kozyrkskyj

Affiliations: Professor, Dept Pediatrics, Dept Obstetrics & Gynecology, Faculty of Medicine & Dentistry; School of Public Health, University of Alberta, Edmonton

Title: Impact of early life exposures on infant gut microbiota: Cross-sectional & longitudinal changes

Abstract: With rising rates of cesarean section (CS) delivery and group B Streptococcus vaginal colonization during pregnancy, maternal intrapartum antibiotic prophylaxis (IAP) has become a routine part of the birthing process in North America. Longer-term these exposures have been associated with childhood obesity, asthma and allergy, conditions linked to gut microbial dysbiosis during early life. The presentation will draw on data from the gut microbiota profiles of 198 healthy term infants in the Canadian Healthy Infant Longitudinal Development (CHILD) pregnancy cohort study. IAP for group B Streptococcus prophylaxis was administered to 21% of mothers; another 23% received IAP for elective or emergency CS. Infant gut microbiota community structures differed significantly with all IAP & CS exposures, cross-sectionally at 3 months and longitudinally from 3 to 12 months.

Biosketch: Anita Kozyrskyj , Professor in Pediatrics, Faculty of Medicine & Dentistry, University of Alberta, leads the SyMBIOTA (Synergy in Microbiota Research) research program, involving a multidisciplinary research team across 5 Canadian universities. SyMBIOTA was established in 2010 through a 2.5 million microbiome team grant, awarded by the CIHR Microbiome Initiative to stimulate microbiome research in Canada. Based on data on 3,500 infants from the Canadian Healthy Infant Longitudinal Development (CHILD) pregnancy cohort study, SyMBIOTA investigates how interventions in early life, including birthing method, antibiotics and infant diet shape the gut microbiome throughout infancy, and how changes to microbial composition can lead to the development of overweight and allergic disease in childhood. SyMBIOTA's first infant gut microbiome paper on cesarean delivery received the 2014 Canadian Medical Association Journal Bruce Squires Award for the most influential publication.

Speaker: Rodney Dietert

Affiliation: Professor, Department of Microbiology and Immunology, Cornell University Ithaca, NY 14853

Title: Protecting the Human Superorganism

Abstract: Human safety evaluation and health protection are predicated on modeling, evaluating, estimating, and then responding to the likelihood of adverse outcomes from exposure to environmental chemicals, food, and drugs as well as physical and psychological factors. Fundamental to the process has been the assumption that the target organism being protected is the mammalian human. Until recently, little-to-no attention was directed toward the environmental vulnerability of thousands of non-mammalian, human-inhabiting species collectively known as the human microbiome. Yet, humans in their normal, healthiest state are by some measures a majority-microbial superorganism with a slight majority of microbial to mammalian cells and an even larger disparity among genes. Additionally, because the microbiome resides at the boundary between humans and their environment, it serves as a gatekeeper. Protection of the newly-defined human superorganism requires a re-thinking of what has been a largely mammalian-centric environmental health focus. This presentation considers how environmentally-induced changes in microbiome status drive human health risk and contribute to the ongoing epidemic of noncommunicable diseases.

Biosketch: **Rodney Dietert** is Professor of Immunotoxicology at Cornell University in Ithaca, New York having served on the Cornell faculty for 39 years. He received his PhD in immunogenetics from the University of Texas at Austin. At Cornell, Rodney previously directed Cornell's Graduate Field of Immunology, the Program on Breast Cancer and Environmental Risk Factors, and the Institute for Comparative and Environmental Toxicology, and served as a Senior Fellow in the Cornell Center for the Environment. In research, Rodney has more than 300 publications with most concerning the safety evaluation of drugs and environmental chemicals, the microbiome, the developing immune system, environmental health risks, and non-communicable diseases. Among his authored and edited scientific books are *Immunotoxicity Testing*, *Strategies for Protecting Your Child's Immune System*, *Immunotoxicity*, *Immune Dysfunction, and Chronic Disease* and the 2016 book from Dutton Penguin Random House titled: *The Human Superorganism*. Beyond Cornell, Rodney was President of the Immunotoxicology Specialty Section of SOT, served on grant, advisory, and document drafting panels for the NIH, WHO, EPA, USDA, NIH, and Department of Defense, consulted for industry on safety evaluation and currently is a toxicology book series editor for Springer. In 2014 he appeared in the award-winning documentary film, *Microbirth* and in 2015, Rodney received the James G. Wilson Best Paper of the Year Award from the Teratology Society for a paper on infant microbiome deficiencies as a new type of birth defect.

Speaker: Mike Tyers

Coauthors: Thierry Bertomeu, Jasmin Coulombe-Huntington, Corinne St-Denis, Karine Bourdages, Driss Boudeffa, Luisa Izzi

Affiliations: Professor, Institute for Research in Immunology and Cancer, Department of Medicine, Université de Montréal, Montreal, Canada

Title: CRISPR/Cas9-based chemogenomic profiles for systematic assessment of toxin mechanism-of-action in human cells

Abstract: The determination of precise mechanism-of-action for biologically active small molecules remains a general unsolved problem. This issue is central to the regulatory assessment of drugs, industrial chemicals and other environmental hazards. Recent policy documents and legislation calls for the replacement of traditional testing methods in animals with a rational tiered process that integrates information-rich genomics-based methods with targeted in vivo animal models. CRISPR/Cas9-based gene editing technology has recently been deployed on a genome-wide scale to enable functional knockout screens in human cell lines for the interrogation of gene function. We have generated a custom genome-wide CRISPR/Cas9 gene knockout library pool based on transduction of 278,754 different sgRNAs into human cells that conditionally express the Cas9 RNA-directed nuclease. Upon induction of Cas9, each sgRNA in the pool efficiency generates a site-specific insertion/deletion (indel) that causes loss of gene function. Growth of the pool in the presence of a specific drug or toxin allows the detection of chemical-genetic interactions by changes in sgRNA frequencies determined by next generation sequencing. The complete set of such changes forms a chemogenomic profile for the drug/toxin. These profiles reveal chemical mechanism-of-action and specificity, as well as novel aspects of gene function. In a proof-of-concept study, the NALM-6 human pre-B lymphocytic cell line was transduced with the sgRNA library and exposed to 19 different drugs or inhibitors to identify 21,452 significant chemical-gene interactions for 8,134 genes. Each chemogenomic profile provided direct evidence for compound mechanism-of-action and revealed many novel off-target effects for each particular agent tested. Progress on development of a large-scale chemogenomic matrix for 1000 bioactive agents of relevance to human health will be described.

Biosketch: Mike Tyers is a Principal Investigator at the Institute for Research in Immunology and Cancer and Professor in the Department of Medicine at the University of Montreal, where he holds a Canada Research Chair in Systems and Synthetic Biology. He is also a Visiting Professor at the University of Edinburgh and an Associate Member of the Lunenfeld-Tanenbaum Research Institute of Mount Sinai Hospital in Toronto. He was previously the C. H. Waddington Professor of Systems Biology at the University of Edinburgh and Director of the Scottish Universities Life Science Alliance. His contributions have been recognized by the Michael Smith Award, the McLaughlin Medal, a Howard Hughes Medical Institute International Scholar Award, and a Royal Society Wolfson Research Merit Award. He is a Fellow of the Royal Society of Canada, a Member of the European Molecular Biology Organization, and a Fellow of the Royal Society of Edinburgh.

Speaker: Dongeun Huh

Affiliations: Wilf Family Term Assistant Professor, Department of Bioengineering, University of Pennsylvania, USA

Title: Microengineered Physiological Biomimicry: Human Organ-on-Chips

Abstract: Human organs are complex living systems in which specialized cells and tissues are assembled in various patterns to carry out integrated functions essential to the survival of the entire organism. A paucity of predictive models that recapitulate the complexity of human organs and physiological systems poses major technical challenges in virtually all areas of life science and technology. This talk will present interdisciplinary research efforts to develop microengineered biomimetic models that reconstitute complex structure, dynamic microenvironment, and physiological function of living human organs. Specifically, I will talk about i) bioinspired microsystems that mimic the structural and functional complexity of the living human lung in health and disease, ii) an organ-on-chip microdevice that emulates the ocular surface of the human eye, and iii) microengineered physiological models of human reproductive organs.

Biosketch: Dan Huh is an Assistant Professor and Wilf Family Term Endowed Chair in the Department of Bioengineering at the University of Pennsylvania. He received a B.S. in Mechanical Engineering from Seoul National University, Master's degrees in Biomedical Engineering and Mechanical Engineering, and a Ph.D. in Biomedical Engineering from the University of Michigan. From 2007 to 2012, he was a postdoctoral fellow at Harvard Medical School and Harvard's Wyss Institute for Biologically Inspired Engineering. He is a pioneer of "organ-on-a-chip" technology, and his research group at Penn focuses on developing microengineered models of human anatomy and physiology for a wide variety of biomedical applications. Dr. Huh has won several honors and awards including the John J. Ryan Medal from the Royal College of Surgeons in Ireland, Design of the Year Award from London Design Museum, NIH Director's New Innovator Award, *Analytical Chemistry* Young Innovator Award, TEDx Fellow, NC3Rs Annual Award, SLAS Innovation Award from the Society for Lab Automation and Screening, Scientific Breakthrough of the Year from American Thoracic Society, Best Publication Award and Best Postdoctoral Award from the Society of Toxicology, Wyss Technology Development Fellowship from Harvard, Distinguished Achievement Award from Michigan, Widmer Award from microTAS, and Horace H. Rackham Predoctoral Fellowship.

Speaker: Simon Authier

Affiliations: Deputy Chief Scientific Officer, Senior Director of Scientific Operations and Veterinary Science, CiToxLAB

Title: Trends with non-clinical and in vitro models for proarrhythmia and seizure assessment
Abstract: Taken together cardiac and neurological adverse effects account for most of the drug failures observed during non-clinical drug safety testing. Early screening strategies and follow-up investigations have both evolved considerably over the last decade owing to emerging technologies and a better understanding of the underlying mechanisms involved with these adverse effects. As translational value remains the Holy Grail of *in vitro* and non-clinical assays, industry trends are solidifying with the influence from regulatory agencies and systematic evaluations of assay performance. New paradigms in cardiac and neurological drug safety testing will be discussed in the context of modern drug development.

Biosketch: Dr. Authier obtained a doctor in veterinary medicine degree from the University of Montreal and specialized in non-clinical studies after completing a Ph.D in preclinical pharmacology. He then completed a MBA in corporate finances and management. Over the past years, Dr. Authier investigated methodologies in non-clinical regulatory safety pharmacology studies with the objective of improving study designs for optimal sensitivity and decision making in this field. Dr. Authier overviews the work of a team of scientists and veterinarians specialized in non-clinical research for IND, BLA and NDA filing with a focus on pharmacology. He participated in face-to-face FDA pre-IND meetings and overviewed conduct of preclinical studies for a number of small and large molecules over the last 15 years working at CIToxLAB North America. He was an invited speaker at various scientific conferences including the Food and Drug Administration (FDA), the US National Institute of Allergies and Infectious Diseases, the Safety Pharmacology Society, the American College of Toxicologists, the Japanese Society of Safety Pharmacology and the American Association of Laboratory Animal Science to name a few. Dr. Authier is Associate Professor at University of Montreal, Canada where he is involved with clinical immunology and pharmacology and he has authored more than 70 peer reviewed articles and book chapters. To date, Dr. Authier has provided scientific overview for more than 800 preclinical studies.

Speaker: Stephen D. Dertinger
Other authors: Steven M. Bryce, Derek Bernacki, Jeffrey C. Bemis

Affiliations: Director of Research, Litron Laboratories, Rochester, New York, USA

Title: Multiplexed Flow Cytometric Assays Provide Information about Genotoxic Mode of Action

Abstract: We previously described a multiplexed *in vitro* genotoxicity assay based on flow cytometric analysis of detergent-liberated nuclei that are simultaneously stained with propidium iodide and labeled with fluorescent antibodies against p53, gH2AX, and phospho-histone H3. A known concentration of microspheres provides absolute nuclei counts. The assay is commercially known as MultiFlow™ DNA Damage Kit— p53, gH2AX, Phospho-histone H3. This presentation will describe the performance characteristics of the assay conducted with human lymphoblastoid TK6 cells treated with a diverse set of 85-well studied chemicals in 96-well plates. More recent lines of investigation will be briefly described, including an evaluation of inter-laboratory transferability, and the use of DNA repair inhibitors to provide additional insights into molecular targets.

Biosketch: Stephen graduated from the Rochester Institute of Technology with a B.S. in Biotechnology, and accomplished his post-graduate training in Toxicology at the University of Rochester. Since 2000, Stephen has been employed by Litron Laboratories where he serves as Director of Research. Over these years Stephen and his team have focused on the development high throughput, high information content genotoxicity assays, with a special emphasis on endpoints that can be applied across cell and animal models.

Speaker: Bruce Demple

Affiliations: Professor of Pharmacological Sciences, Department of Pharmacological Sciences
Stony Brook University School of Medicine

Title: Risky Repair: Oxidative DNA-protein Crosslinks Driven by Mammalian Base Excision DNA Repair Mechanisms

Abstract: Oxidative DNA damage is broadly implicated in a range of human diseases, including cancer, heart disease, and neurodegeneration. Free radical-mediated oxidative damage also contributes to the normal aging process. Beyond the endogenous sources, many environmental agents produce oxidative DNA damage either directly or indirectly to exert their biological effects. There is a complex array of oxidative DNA lesions, of which a substantial fraction, ~20% of the total, consists of several types of abasic sites and sugar fragments. Some lesions can be handled by familiar mechanisms, while others cause extra problems for DNA repair. Notable among these is the C1-oxidized 2-deoxyribonolactone (**dL**), which is formed at levels similar to 8-oxoguanine. Like other abasic lesions, dL interferes with DNA polymerases and lacks genetic information, potentiating mutagenesis. Unlike the others, dL can derail attempted repair: during "short-patch" base excision DNA repair, DNA polymerase β (**Pol**β) replaces the missing nucleotide and uses a separate lyase activity to excise the abasic residue, thus enabling ligation. With dL, attempted excision via the lyase leads to a dead-end product: Polβ covalently trapped on the lesion in a DNA-protein crosslink (**DPC**). Our recent work shows that Polβ-DPC are formed abundantly in mammalian cells treated with dL-forming oxidants, dependent on the Polβ lyase active site. Ubiquitylation and the proteasome act very rapidly to start clearing the DPC from the genome, and we have identified the Polβ residues required for this targeting and processing. We have now shown that several other repair proteins with abasic lyase activity also form DPC in cells treated to generate dL lesions in the genome.

Biosketch: Dr. Demple has extensive experience in the biochemistry, genetics and cell biology of oxidative stress responses and DNA repair. Starting in 1980, he mapped out cellular responses to oxidative stress and environmental mutagens, and investigated the genetic instability provoked by inadequate DNA repair. He has trained >60 postdoctoral fellows and 15 Ph.D. students, many of whom now direct their own laboratories and are tenured professors, while others direct research in government laboratories or work in environmental consulting or patent law. He has published over 200 publications. Dr. Demple obtained his PhD degree from University of California, Berkeley, CA in 1981.

Speaker: Robin M. Walker
Affiliations: Independent Consultant, Mississauga, Canada

Title: Genotoxicity Evaluation of Impurities in Pharmaceuticals.

Abstract: Evaluation of potential genotoxicity is an important aspect of the nonclinical safety assessment of drugs, but also of the impurities that are inevitably present in the drug substance, or active pharmaceutical ingredient (API), and in the formulated drug product, as well as degradation products that may form over time. The requirements for evaluation of the potential for genotoxicity of the API are stipulated in the International Conference on Harmonisation (ICH) Guideline S2(R1) and consist of conduct of *in vitro* and *in vivo* assays. The ICH requirements for evaluation of genotoxicty of impurities and degradants in drug substances and drug products were initially stipulated in ICH Guidelines Q3A and Q3B, respectively, finalized in 2005, and consist of bacterial and *in vitro* mammalian cell assays when impurity concentrations reach certain "qualification" (meaning safety assessment) thresholds that vary depending on the dose. However, it was recognized that the qualification thresholds were relatively high, particularly for high dose drugs where impurity doses of up to 3 mg/day or more might not be evaluated for potential genotoxicity and that there was a lack of clarity as to course of action in the event of positive findings. These and other issues were addressed in EMA and FDA guidance documents regarding genotoxic impurities in drugs and subsequently the ICH Guideline "Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Genotoxic Risk" (ICH M7), finalized in 2014. What is new in this process is the use of already existing bacterial mutagenicity and rodent carcinogenicity data to make predictions for unstudied impurities. Specifically, rodent carcinogenicity data for 730 chemicals were analyzed to arrive at a generic "Threshold of Toxicologic Concern" (TTC) dose of 1.5 μg/day, which is considered an acceptable daily intake even for most genotoxic impurities, since that dose is associated with a hypothetical low lifetime risk of carcinogenicity (<1:100,000). That conservatively calculated risk was based on linear extrapolations of the TD50 (dose associated with 50% tumor incidence) values in the carcinogenicity studies. Genotoxicity does not need to be evaluated for impurities at intakes below the TTC. Above the TTC, potential genotoxicity can be evaluated using *in silico* programs based on (Q)SAR, where the activity data are derived from bacterial mutagenicity assays. Negative results from 2 complementary *in silico* programs (rule-based and statistical) would classify an impurity as non-genotoxic. Further, negative findings in an appropriately conducted bacterial mutagenicity assay would supersede positive *in silico* alerts. Evaluation of genotoxic potential according to ICH Q3A/B Guidelines now only needs to be considered if an impurity intake exceeds 1 mg/day. The ICH M7 Guideline states that a 1.5 μg/day TTC would not be appropriate for particularly potent classes of carcinogens (specifically aflatoxins, N-nitroso, & azoxy structures), while higher acceptable intakes can be justified when a drug is indicated for "Less-Than-Lifetime" durations or the indication is for severe or life-threatening indications where treatment options are limited.

Biosketch: Dr. Robin Walker is an American Board of Toxicology certified toxicologist with over 30 years of experience in nonclinical aspects of drug development. After obtaining a PhD from the Department of Pathology in collaboration with the Department of Pharmacology and Toxicology at Queen's University, Dr. Walker worked for large pharmaceutical companies (Parke-Davis/Warner Lambert and Pfizer) for over 20 years and subsequently as a consultant to private companies and

regulatory agencies. Dr. Walker has extensive experience on international drug development teams with responsibilities for the design, overall direction, summarization, and issue resolution of nonclinical toxicology. He has been a member of highly successful development teams responsible for the development of marketed drugs (Neurontin and Cerebyx) and was responsible for the direction of exploratory and GLP-compliant laboratory operations that supported many other drug development programs. Dr. Walker has been active in the Society of Toxicology of Canada (STC) serving as a councilor on the Board of Directors, member and chair of the Program Committee, and member of the Organizing Committee and co-chair of the Program Committee for the International Congress of Toxicology when it was held in Montreal. He also has extensive experience as a study director and monitor of contracted GLP-compliant toxicology studies and has an extensive record of scientific publication. Consulting experience has been with respect to due diligence evaluations, development of preclinical development plans for small and medium sized pharmaceutical / biotechnology companies, preparation of CTA/IND/NDS/NDA/MAA and other regulatory documents for new chemical entities and medical devices, monitoring of toxicology studies at contract laboratories, and provision of toxicology advice to industry and Health Canada. The latter has included evaluation of the safety of impurities and degradation products in pharmaceuticals, particularly with respect to potential genotoxicity. Dr. Walker has also taught in the Regulatory Affairs Program at Humber College in Toronto and is an adjunct faculty member in the Department of Biomedical and Molecular Sciences at Queen's University in Kingston.

Posters and Abstracts

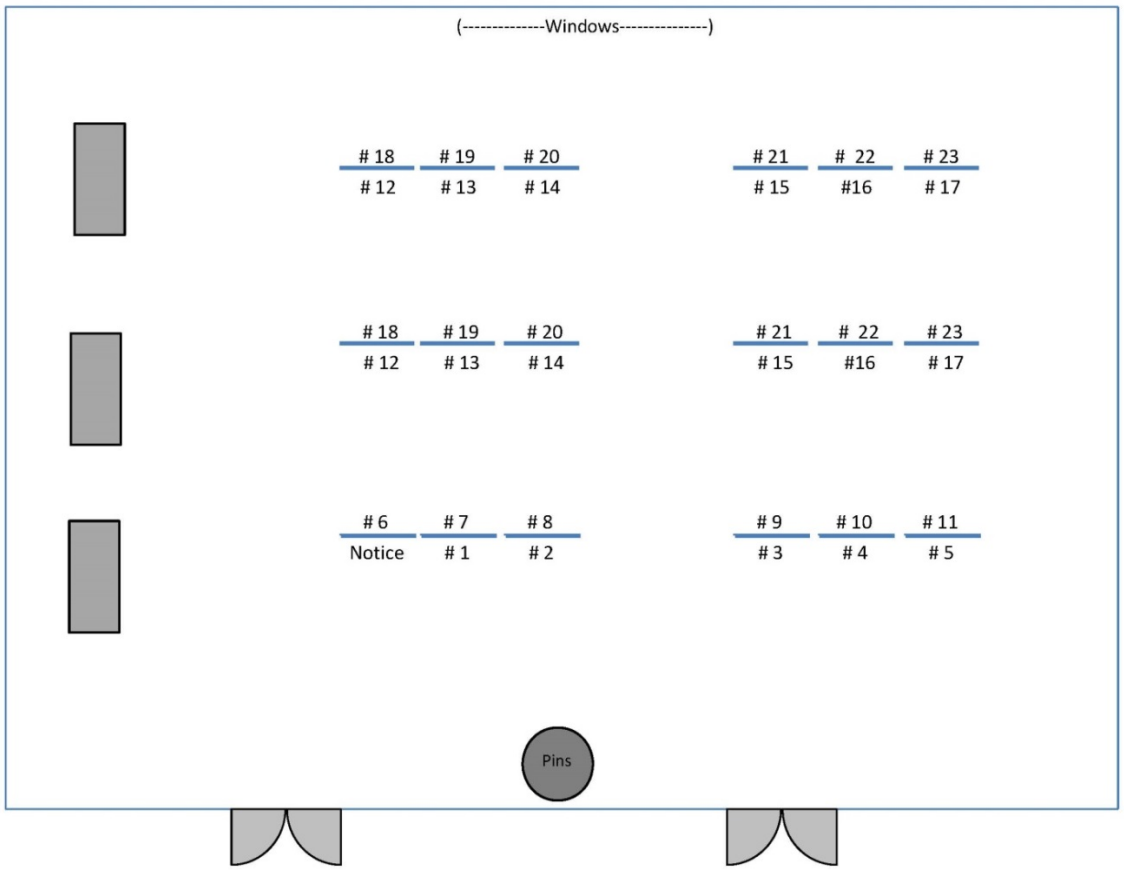
Poster Board Assignments:

Poster	Presenter	Title
1	Atlas, Ella	Adipogenic Effects and Gene Profiling of Firemaster® 550 Components in Human Primary Preadipocytes
2	Atlas, Ella	Bisphenol A and Bisphenol S disrupt breast epithelial cells organization in a 3D model.
3	Peshdary, Vian	Dechlorane Plus has obesogenic activity in 3T3-L1 preadipocytes
4	Aziz, Syed	28-day sub-acute dietary exposure to 2-monochloropropanediol (MCPD) in F344 rats causes skeletal muscle-related toxicity
5	Bahia, Simran	OXIDATIVE STRESS MECHANISMS IN X-RAY RADIATION EXPOSED HUMAN LENS EPITHELIAL CELLS
6	Bailey, Francis	Rodent Developmental Neurotoxicity (DNT) Study Paradigm: Establishing Additional Evaluation Guidance for Regulatory Reviewers.
7	Blais, Erica	Blood metal levels during pregnancy and changes in maternal plasma marker levels in understanding SGA/LGA birth outcomes in the MIREC Study
8	Breznan, Dalibor	SIZE AND SURFACE FUNCTIONALITY OF MESOPOROUS SILICA NANOPARTICLES ARE ASSOCIATED WITH CYTOTOXICITY IN MAMMALIAN CELL LINES
9	Cameron, Shana	EFFECT OF NANOSILVER ON CELLULAR OXIDATIVE STRESS RESPONSE AND DETOXIFICATION PATHWAYS
10	Cozzarin, Joseph	EFFECTS OF THE BENZENE METABOLITES, BENZOQUINONE AND HYDROQUINONE ON PU.1, AML-1 AND C/EBP-α TRANSCRIPTIONAL ACTIVITY
11	Cathy Cummings-Lorbetskie	Multiplex Measurement of Histone H3 Post-Translational Modifications following Exposure of Human Liver Cells to Copper and Zinc Organometallics.
12	Buick, Julie	THE TGx-28.65 GENOMIC BIOMARKER FOR DNA DAMAGING AGENTS WORKS EFFECTIVELY IN HUMAN HEPARG CELLS USING NEXT GENERATION SEQUENCING TECHNOLOGY
13	Gannon, Anne Marie	Gene and microRNA expression changes following a 28-day exposure to hexabromocyclododecane in a rat model
14	Greville, Lucas	TRICOLSAN ELEVATES ESTRADIOL LEVELS IN SERUM AND TISSUES OF FEMALE MICE
15	Hill, Katie	USE OF AN IN VITRO PROTEIN BINDING ASSAY TO CHARACTERIZE INTERACTIONS OF OP TRIESTERS AND METABOLITES WITH THYROXINE AND HUMAN TRANSTHYRETIN

Poster Number	Presenter	Title
16	Kaur, Gurnit	DIFFERENCES IN THE BILIARY EXCRETION OF ARSENIC BY RAT AND HUMAN SANDWICH-CULTURED HEPATOCYTES
17	Khan, Nasrin	EFFECTS OF CROSS-FOSTERING AND DEVELOPMENTAL EXPOSURE TO MIXTURES OF ENVIRONMENTAL CONTAMINANTS ON HEPATIC GENE EXPRESSION IN 21 DAY OLD AND ADULT LITTERMATES SPRAGUE DAWLEY RATS.
18	Kieskamp, Kyra	Is the Reference Dose for Perfluorooctanoic Acid (PFOA) Protective for Breastfed Infants? An Evaluation Using Pharmacokinetic Modeling
19	Kocmarek, Andrea	Toxicological and genomic responses in kidney, heart and liver tissues of male and female F344 rats following 28-days repeated dose sub-acute dietary exposure to 2-monochloro-1,3-propanediol (2-MCPD)
20	Lightbody, Elizabeth	Anticancer Activities of PPARγ in HER2+ Breast Cancer
21	Lofti, Laura	``Plasma Levels of Polybrominated Diphenyl Ethers (PBDEs) at 12, 24 and 36 Months of Age ``
22	Maciver, Rebecca	EFFECTS OF POST-NATAL ADRENERGIC RECEPTOR STIMULATION ON LATENT CONGENITAL HEART DEFECTS
23	Maurice, Clotilde	Integration of Germ Cell DNA Damage Assessment into OECD Guidelines for the Genotoxicity Testing of Chemicals
24	Nikota, Jake	Utilizing an adverse outcome pathway framework to investigate Interleukin-1 and STAT6 signaling in the pathology of carbon nanotubes in mice
25	Philbrook, Nikki	INVESTIGATING THE DEVELOPMENTAL EFFECTS OF THE FLAME RETARDANT, TRIPHENYL PHOSPHATE, IN C57BL/6 MICE
26	Pollock, Tyler	INTERACTION AMONG FIVE ENVIRONMENTAL CHEMICALS: ESTROGENIC POTENTIAL MEASURED VIA ELEVATIONS IN BISPHENOL A CONCENTRATIONS IN MICE
27	Rahman, Luna	In vivo pulmonary genotoxicity of multiwalled-carbon nanotubes
28	Ramsingh, Deborah	A RETROSPECTIVE ANALYSIS OF THE 1-YEAR DOG TOXICITY STUDY IN PESTICIDE HUMAN HEALTH RISK ASSESSMENTS
29	Ross, Bradley	Role of PPARg-dependent microRNA expression during breast tumour metastasis
30	Rubio, Carmen	Dietary exposure to Fluoride from wines
31	Rubio, Carmen	Could edible mushrooms be a dietary source of toxic metals?
32	Shi, Jia Yue	A Breast Tumour angiogenic role for PPARg Signaling

Poster Number	Presenter	Title
33	Thomson, Errol	ozone inhalation alters glucose metabolism and signalling in adipose tissue: role of glucocorticoids
34	Weaver, Rachel	A TALE OF THREE TOXICANTS: THE EFFECTS OF BUTYLPARABEN AND PROPYLPARABEN ON THE DISTRIBUTION OF BISPHENOL A
35	Willmore, William	ROLE OF THE ANTIOXIDANT RESPONSE ELEMENT/ELECTROPHILE RESPONSE ELEMENT IN THE RESPONSE TO TOXIN EXPOSURE

Poster Room Layout – Governor General II



Instructions:

Please mount your poster onto the designated board prior to the first coffee break on Mon., Dec 5. Poster pins will be provided

During the first break on Monday morning, presenters with posters on boards bearing even numbers should stand by their boards. For presenters with odd numbers, please stand by your posters during the lunch break, Monday from about 12:25 until the end.

Please take your posters down from the boards by the end of the lunch period on Tuesday.

Poster Abstracts

1. ADIPOGENIC EFFECTS AND GENE PROFILING OF FIREMASTER® 550 COMPONENTS IN HUMAN PRIMARY PREADIPOCYTES

Emily W.Y. Tung, Vian Peshdary, Remi Gagné, Andrea Rowan-Carroll, Carole Yauk, Adéle Boudreau and Ella Atlas

Environmental Health Science and Research Bureau, Health Canada, 50 Colombine Driveway, Ottawa, Ontario, Canada

Background: Exposure to flame retardants has been associated with negative health outcomes including metabolic effects. As polybrominated diphenyl ether flame retardants were pulled from commerce, human exposure to newer classes of flame retardants such as Firemaster® 550 (FM550) has increased. Although previous studies in murine systems have shown that FM550 and its main components increase adipogenesis at the expense of osteogenesis, the effects of the flame retardants in human models have not been elucidated.

Objectives: The objectives of this study were to investigate whether FM550 and its components were active in human preadipocytes, and to further investigate the mode of action of these chemicals.

Methods: Human primary preadipocytes were differentiated in the presence of FM550 and its components. Differentiation was assessed by lipid accumulation and expression of adipogenic markers. mRNA was collected for Poly-A RNA-sequencing and was used to identify differentially expressed genes (DEGs). Functional analysis of DEGs was undertaken in Ingenuity Pathway Analysis.

Results: FM550 and two of its components increased adipogenesis in human primary preadipocytes as assessed by lipid accumulation and mRNA expression of regulators of adipogenesis and adipogenic markers. Poly-A-RNA sequencing analysis revealed potential novel modes of action in the human cells.

Conclusions: This study is the first to show that FM550 and two of its components, triphenyl phosphate (TPP) and isopropylated triphenyl phosphates (IPTP), induced adipogenesis in human primary preadipocytes. Further, using global gene expression analysis, we show that while TPP likely exerts its effects through the PPARG receptor, IPTP may have distinct modes of action.

2. BISPHENOL A AND BISPHENOL S DISRUPT BREAST EPITHELIAL CELLS ORGANIZATION IN A 3D MODEL.

Valeria Dimitrova[1,2] and Ella Atlas[1,2]

[1]Environmental Health Science and Research Bureau, Health Canada, 50 Colombine Driveway, Ottawa, Ontario, Canada
[2]Department of Biochemistry Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada

Background: Exposure to environmental pollutants such as bisphenol-A (BPA) may lead to an increase in negative health outcomes including breast cancer. As increasing body of evidence suggests that BPA has endocrine disrupting effects it is being less used in consumer products and replaced with other plasticizers such as bisphenol S (BPS). Therefore, it is important to develop modes of screening for the replacement chemicals to investigate effects on the breast epithelium. It is widely accepted that the microenvironment of the mammary epithelium and the interactions with the extracellular matrix provide important signals that result in tissue polarization and proper organization of the cells into structures that resemble mammary ducts. Further, mammary epithelial cells that are grown in this microenvironment exhibit different sensitivities and outcomes in response to extracellular stimuli and insult. It is also established that normal breast epithelial cells grown in an extracellular matrix *in vitro* not only assume an organization similar to the mammary gland in situ but also assume the function of the mammary gland in response to hormonal stimulation. Using this model physiologically relevant model system we proceeded to study the effects of environmental pollutants with suspected endocrine disruptor activities on breast epithelial cells.

Objectives: The objective of the study was to compare the response of normal breast epithelial cells to BPA and bisphenol-S (BPS), in a 2D model as compared to a 3D model and to investigate the effects of the two chemicals on the organization of the cells in the 3D model.

Methods: Breast epithelial cell lines, MCF-12A expressing the estrogen receptor (ER) α and ERβ and MCF-10A (ERα–, ERβ+), were grown in Matrigel and treated with BPA and BPS. In these experiments morphological examination of the acini and protein expression of known molecular markers of breast cancer progression was performed using confocal microscopy. For 2D responses the proliferation rate of the cells treated with the chemicals was assessed.

Results: We found that MCF-12A and MCF-10A show distinct responses to BPA and BPS, as assessed by confocal microscopy and proliferation assays. More specifically, MCF-12A acini organization was disrupted by BPA and BPS, while MCF-10A acini formation was not. No effects on proliferation were observed in the 2D proliferation assays.

Conclusions: These results indicate that both BPA and BPS may affect mammary epithelium and perhaps increase breast cancer risk. This effect is likely mediated through the ERα. In addition, the 2D proliferation assay was not able to capture the effects.

3. DECHLORANE PLUS HAS OBESOGENIC ACTIVITY IN 3T3-L1 PREADIPOCYTES

Vian Peshdary, Gabriella Calzadilla and Ella Atlas

Environmental and Radiation Health Sciences Directorate, HECSB, Health Canada, Ottawa, Ontario, Canada K1A0H9

Background: PBDEs have been shown to be toxic and bioaccumulative and were phased out of commerce. Alternative FRs such as dechlorane plus (DP) have been introduced to replace PBDEs. DP is currently being produced in high volumes and it has been detected in ambient air, fish, and sediment samples from the Great Lakes region. In addition, DP was detected in human milk and in the serum of e-waste recycling workers. Although human exposure to DP is eminent, little is known about potential health effects as a consequence of this exposure. We and others have previously shown that some FRs are potential obesogens, defined as chemicals that promote the differentiation of preadipocytes into mature adipocytes a process that is known as adipogenesis. However, the effects of DP on adipogenesis have not been studied.

Objectives: The purpose of this study was to examine the ability of DP to induce adipogenesis.

Methods: Murine 3T3-L1 preadipocyte cells were differentiated in the presence of 0.0001-10 μM DP. Differentiation was assessed by lipid accumulation and mRNA of adipogenic markers and adipokines, as well as protein levels of select mature adipocyte markers.

Results: Our results show that treatment of 3T3-L1 preadipocytes with 10 μM DP increased the mRNA expression of mature adipocyte markers: adipocyte protein 2 (aP2), lipoprotein lipase (lpl), and perlipin; as well as adipokines: adipsin, and adiponectin. When 3T3-L1 preadipocyte were differentiated with 0.1 μM DP. However, the mRNA expression of adipogenic markers was reduced.

Conclusion: The current study shows that DP can induce adipogenesis at high concentrations. However, at lower concentrations DP may be impeding important signaling pathways relevant to adipocyte biology. The results will be used to determine whether exposure to DP has the potential to affect human health. In addition, these data will aid in the design of testing methods for the identification of chemicals that have effects on fat cell formation.

4. 28-DAY SUB-ACUTE DIETARY EXPOSURE TO 2-MONOCHLOROPROPANEDIOL (MCPD) IN F344 RATS CAUSES SKELETAL MUSCLE-RELATED TOXICITY

S.A. Aziz,[1] J. Abourgeili[4], K. Kapal[1], J. Roberts[1], D. Caldwell[2], D. Williams[3], R. Mehta[1], J. Raju[1].

[1]Regulatory Toxicology Research Division, [2]Scientific Services Division and [3]Chemical Health Hazard Assessment Division, Bureau of Chemical Safety, Food Directorate, HPFB, Health Canada; [4]Department of Chemistry and Bio-molecular Sciences, University of Ottawa; Ontario, Canada

Background: Chloropropanols (monochloropropanediol; MCPD) are food-borne contaminants resulting from high temperature processing of fat-containing matrices. To fill a regulatory gap in understanding the toxicity of 2-MCPD, we conducted a 28-day oral repeated-dose sub-acute exposure study of 2-MCPD in male and female F344 rats. 2-MCPD related gross and muscle pathological changes were observed in the heart.

Objective: The objective of this study was to address if dietary exposure of 2-MCPD caused any pathological changes in the skeletal (or striated) muscle.

Methods: The tibialis anterior muscle was dissected from F344 rats (male/female; n = 10 rats/group/sex) exposed *ad libitum* to diets containing 0, 382, or 764 mg 2-MCPD/kg diet to provide estimated daily doses of 0, 25, or 50 mg 2-MCPD/kg body weight; respectively. Fresh muscle biopsies were encased in dough consisting of tragacanth gum, before being snap-frozen in liquid N_2 and stored at -80°C. 10 µm histosections of the muscle biopsies were assessed for various markers of muscle pathology using histochemical/immunohistochemical techniques.

Results: Hematoxylin & eosin/Gomori trichome staining of muscle sections identified a significant increase ($p < 0.05$) in ragged red fibers, internal nuclei, clustered nuclei, nemaline rods and inflamed blood vessels in all MCPD groups compared to controls. Fiber size (Type-2A/2B) significantly decreased and fiber shrinkage significantly increased ($p < 0.05$) in all MCPD groups. The incidence of loss in both dystrophin expression and neuromuscular junctions was significantly increased ($p < 0.05$) in all MCPD groups compared to controls. NADH-TR staining assay revealed that the incidence of moth-eaten fibers and central cores were significantly higher ($p < 0.05$) in all MCPD groups.

Conclusions: The results of this study indicate that sub-acute dietary exposure to 2-MCPD in male and female F344 rats caused changes in several parameters indicating disruption of metabolic and neuromuscular processes in the skeletal muscle that may potentially lead to muscle weakness, myopathy and atrophy. To our knowledge, this is the first study that demonstrates muscle-related toxicity of 2-MCPD exposure in rats and provides data to support its hazard characterization.

5. OXIDATIVE STRESS MECHANISMS IN X-RAY RADIATION EXPOSED HUMAN LENS EPITHELIAL CELLS

Simran Bahia[1], Erica Blais[2], Vinita Chauhan[1], Prem Kumarathasan[2]

[1]Consumer and Clinical Radiation Protection Bureau, Healthy Environment and Consumer Safety Branch, Health Canada, Ottawa, Ontario Canada K1A 1C1
[2]Mechanistic Studies Division, Environmental Health Sciences and Research Bureau, Health Canada, Ottawa, Ontario, Canada K1A0K9,

Background: One of the responses caused by radiation includes the formation of reactive oxygen and nitrogen species which can alter cellular homeostasis. Currently there is limited information on dose-response relationships and toxicity mechanisms underlying radiation exposure-related adverse health effects and any differences in toxicity mechanisms after radiation exposures at low and high dose rates.

Objective: The aim of this work was to identify any changes in oxidative stress mechanisms in the Human Lens Epithelial cells exposed to radiation at a low and high dose rate, and to assess associated dose-response relationships.

Methods: Human Lens Epithelial cells were exposed at doses of 0, 0.01, 0.05, 0.25, 0.5, 2, and 5 Gy X-ray radiation at a low dose rate (1.62 cGy/min) or at a high dose rate (38.2 cGy/min). Cell culture supernatants were collected 20h post-exposure, stabilized, processed and were analysed for oxidative stress markers (metabolites of reactive oxygen species (ROS) and reactive nitrogen species (RNS)) using a HPLC-coulometric array detection methodology. Statistical analyses were conducted using 2-way ANOVA.

Results: Our findings on the metabolites of ROS (m-tyrosine and Cl-tyrosine) indicated that the 5 Gy was significantly increased ($p<0.05$) compared to the very low dose 0.05 Gy. At low dose rate exposures, RNS formation was favoured up to 2Gy compared to ROS generation based on dose-response curves, and above 2 Gy, ROS formation increased. In contrast, with high dose-rate exposures, the ROS pathway was favoured compared to RNS formation in these cells. Moreover, at 5 Gy exposures at both the dose-rates, the ROS and RNS formation were in the same order of magnitude.

Conclusion: The data show that the level and type of oxidative stress mechanisms are dependent on the dose as well as the dose rate of radiation exposures. The ROS and RNS reaction pathways appear to be two competing mechanisms depending on the type of radiation exposures suggesting different down-stream signalling pathways and associated biological effects.

6. RODENT DEVELOPMENTAL NEUROTOXICITY (DNT) STUDY PARADIGM: ESTABLISHING ADDITIONAL EVALUATION GUIDANCE FOR REGULATORY REVIEWERS.

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The rodent Developmental Neurotoxicity (DNT) study paradigm has evolved over time with the most recent test guidelines updated in 2007 with the introduction of Organizations for Economic Co-operation and Development (OECD) guideline 426. Over the past 5 years, a joint United States Environmental Protection Agency-Health Canada, Pest Management Regulatory Agency (US EPA-HC PMRA) intergovernmental group has been working to create a document to serve as internal guidance for regulatory reviewers in both countries.

Through 2011-2013, the PMRA engaged in an extensive consultative process with both governmental and non-governmental stakeholders to identify issues that presented challenges to both the study conduct and regulatory review. In 2013, a PMRA-USEPA intergovernmental technical group was formed to look at the issues identified in these consultations with an underlying objective for creating a document that would serve as internal guidance.

From a Health Canada perspective, the Department has undertaken this initiative to provide better context to key parameters necessary for the review of a DNT study, not only for the individual behavioural tests, but for their integration into the weight of evidence for the entire study and for the ultimate assessment of hazard and risk. Previous Health Canada Science Forum posters discussed the origin of the project and the consultative process, highlighting the key stakeholders which outlined the key outputs and provided examples of what the guidance would entail.

Over the past year, both Agencies have worked towards completing the remaining modules of the internal guidance document. This poster highlights the content of these modules and also highlights the application and use of the guidance going forward for both Health Canada reviewers as well as external stakeholders.

7. BLOOD METAL LEVELS DURING PREGNANCY AND CHANGES IN MATERNAL PLASMA MARKER LEVELS IN UNDERSTANDING SGA/LGA BIRTH OUTCOMES IN THE MIREC STUDY

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Background: Globally, adverse birth outcomes such as prematurity and low birth weight are determined to be major contributing factors to disability adjusted life years. Heavy metals are known to display endocrine disrupting properties. Prenatal exposures to metals are associated with preterm birth, low birth weight, fetal loss, neonatal deaths and neurotoxic effects. However, information on chemical exposure-related toxicity mechanisms that precipitate these adverse birth effects are unclear.

Objective: The objective of this study was to understand prenatal metal exposure-mediated changes in maternal pathways that may mediate small or large birth weights for gestational age by employing proteomic and metabolomic biomarker analyses.

Methods: The mother-infant cohort from the Canada-wide Maternal-Infant Research on Environmental Chemicals (MIREC) study was employed for this purpose. Maternal plasma samples were analysed for target proteomic/metabolomic markers (multiplex protein array, HPLC-Fluorescence, HPLC-Coularray and EIA). Statistical analyses were conducted to test associations among maternal blood metal (Cd, Hg, Pb, As, Mn) concentrations, plasma biomarkers, physiological changes and birth weight.

Results: Associations ($p<0.05$) were seen between maternal blood As, Hg, Pb concentrations and third trimester maternal plasma matrix metalloproteinases (MMPs), key enzymes in the process of pregnancy. Also, of the targeted biomarkers analysed in this study cohort, MMPs, vascular endothelial growth factor, cellular adhesion molecules, and chemokines relevant to inflammatory/vascular pathways were associated ($p<0.05$) with SGA or LGA births.

Conclusion: Our results suggested phenotypic heterogeneity based on targeted maternal plasma proteomic and metabolomic markers among the smaller and larger birth weight for gestational age groups that resembled potential infection, activated inflammation or vascular mechanisms. These findings suggest that future high-content proteomic, metabolomic and epigenomic/genomic studies will be useful in advancing our understanding of the environmental chemical exposure-mediated molecular mechanisms that may adversely affect infant birth weight and down-stream health effects later in life.

8. SIZE AND SURFACE FUNCTIONALITY OF MESOPOROUS SILICA NANOPARTICLES ARE ASSOCIATED WITH CYTOTOXICITY IN MAMMALIAN CELL LINES

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Background: Mesoporous silica nanoparticles (mSiNPs) are increasingly utilized in the development of pharmaceuticals, drug delivery, medical imaging, fuel cells and the catalytic oxidation of pollutants in wastewater among other applications. However, there are knowledge gaps in understanding their impacts on biological systems and the environment. One important aspect is the lack of knowledge of the potential adverse effects that these materials may exert in relation to their specific physicochemical properties.

Objective: The objective was to determine the effects of mSiNPs exposures in cells and to identify key physicochemical drivers of toxicity for these materials.

Methods: A size (25, 70, 100, 170, 600 nm) x surface functionalization (unmodified, C3-, C11-COOH) set of mSiNPs was previously synthesized. The nanoparticles, including reference particles were profiled for cytotoxicity (membrane integrity, metabolic activity, ATP content, cell proliferation) and induction of cytokines/chemokines in multiple cell lines (macrophages, epithelial cells) using an integrated in vitro bioassay. Also, their physicochemical characteristics were determined. A computational approach was applied to reduce the large dataset of cellular effects and to derive simple descriptors of biological potency of the mSiNPs. The biological potency and the nanoparticle properties were interrogated for associations using statistical tools.

Results: Overall, all mSiNPs were less potent than an amorphous SiNP-12nm reference particle. The cytotoxic potency of the mSiNPs was similar across the cell lines, while the cytokine/chemokine release profiles were cell line-specific. The mSiNPs were ranked differently for causing cytotoxicity and for eliciting cytokine/chemokine release. Overall, surface-modified mSiNPs had higher cytotoxic potency but lesser impact on cytokine/chemokine release compared to unmodified mSiNPs across nano-size (≤100nm). Cytotoxicity in macrophages but not epithelial cells was associated with nanoparticle surface area and DLS size/EM size ratio, a measure of the degree of nanoparticle agglomeration.

Conclusions: While mSiNPs induced effects in all cell lines, data suggest that the responses may occur via different biological pathways. Pathway analyses are underway to identify cellular functions impacted in each cell line. The results show that a combination of size and surface functionality and their interaction with the exposure media influence the cellular responses to this set of mSiNPs. The work provides data to aid in the evidence-based assessment and management of nanomaterials in the regulatory context.

9. EFFECT OF NANOSILVER ON CELLULAR OXIDATIVE STRESS RESPONSE AND DETOXIFICATION PATHWAYS

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Background: Nanosilver (1-100 nm silver particles) has antimicrobial properties and is widely used in many commercial, engineering, and biomedical applications. The potential effects of direct nanosilver exposure to human cells and other organisms through consumer products is largely unknown. Due to the increased use of nanosilver in these products, exposure to this nanomaterial is on the rise. Thus, it is crucial to understand the potential biological effects and mechanistic perturbations caused by nanosilver at the cellular level.

Objectives: The objective of this study was to examine the relative induction of the two primary detoxification pathways upon exposure to nanosilver. The first pathway is the aryl hydrocarbon receptor, which induces various cytochrome P450s through the xenobiotic response element (XRE). The second pathway is the nuclear factor-erythroid 2 like factors which induce various xenobiotic-metabolizing and antioxidant enzymes through the electrophile response element/antioxidant response element (EpRE/ARE).

Methods: Various mammalian cell lines were transfected with luciferase ARE or XRE reporter plasmids, treated with nanosilver, and the resulting luciferase induction measured. The production of transcription factors involved in the ARE pathway (nuclear factor erythroid 2 like 1 (Nrf1), and Nrf2) and in the XRE pathway (aryl hydrocarbon receptor, AhR) were examined by Western blotting. As well, flow cytometry was performed to examine cell cycle arrest.

Results: Our results indicate that nanosilver treatment induces activation of the ARE detoxification pathway through Nrf2, while suppressing the XRE detoxification pathway. Additionally, cell cycle arrest was observed, with an increased number of cells accumulating in the G1 and S phase for the nanosilver and $AgNO_3$ treated cells, respectively.

Conclusions: The results suggest that both ARE and XRE detoxification pathways are affected by nanosilver exposure. This research provides new information and insight into the mechanisms and pathways affected by nanosilver on the cellular level.

10. EFFECTS OF THE BENZENE METABOLITES, BENZOQUINONE AND HYDROQUINONE ON PU.1, AML-1 AND C/EBP-α TRANSCRIPTIONAL ACTIVITY

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Background: Benzene, a common chemical solvent and component of cigarette smoke, is a ubiquitous environmental pollutant that most Canadians are routinely exposed to by inhalation. Benzene is classified as a group 1A carcinogen by the IARC and exposure to benzene has been linked to myeloid leukemia and aplastic anemia in humans. Benzene is metabolized to toxic reactive metabolites by CYP 2E1 enzymes in the body and many studies have concluded that benzoquinone and hydroquinone are the most toxic of benzene metabolites. There are several transcription factors that play a significant role in hematopoietic differentiation. Of these transcription factors, PU.1, AML-1 and C/EBP-α are considered the most crucial to myeloid differentiation. PU.1, AML-1, and C/EBP-α are specific to cells in the hematopoietic system and play a critical role in hematopoietic differentiation as knockout mice deficient in any of these transcription factors have undifferentiated and impaired hematopoietic cells eventually resulting in death of the animal.

Objective: To test the hypothesis that exposure of HL-60 cells to hydroquinone and benzoquinone will result in decreased DNA binding activity and protein expression of the transcription factors PU.1, AML-1 and C/EBP-α.

Methods: HL-60 cells were plated at 1.5 x10^5 cells/ml in 10 ml plates and exposed to 0, 5, 10, 15, 25 μM equal parts hydroquinone and benzoquinone for 20 h. Following exposure, cells were harvested and nuclear fractions were isolated then applied to a filter plate assay to determine DNA binding to the corresponding transcription factor. Western blotting was also performed on nuclear extract samples in order to confirm protein presence.

Results: Filter plate assays revealed no significant differences in DNA binding amongst the 0, 5, 10, 15, 25 μM equal parts hydroquinone and benzoquinone exposure treatment groups when assaying for PU.1, AML-1 and C/EBP-α DNA binding. Western blotting performed on the nuclear extracts demonstrated the presence of PU.1, AML-1 and C/EBP-α with no observable difference in relative protein expression thus far for C/EBP-α transcription factor.

Conclusions: The toxicity observed following benzoquinone/hydroquinone exposure in HL60 cells does not appear to be mediated via altered DNA binding activity of PU.1, AML-1 and C/EBP-α.

11. MULTIPLEX MEASUREMENT OF HISTONE H3 POST-TRANSLATIONAL MODIFICATIONS FOLLOWING EXPOSURE OF HUMAN LIVER CELLS TO COPPER AND ZINC ORGANOMETALLICS

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Background: A number of zinc (Zn) and copper (Cu) based industrial chemicals, fungicides/pesticides, were found to be "data-poor" and their toxicity needs further investigation. There is a growing awareness that epigenetic alterations, including DNA methylation and histone post-translational modifications, are modes of action that contributes to metal toxicity.

Objective: As part of a larger *in vitro* project in which a list of Zn- and Cu-based chemicals are screened for their toxicity and DNA damaging potential, the current objective was to assess global changes in histone H3 post-translational modifications (H3-PTMs) through multiplex measurement of H3K9Ac, pan-acetyl H3, H3K4Me3, H3K27Me3, and H3K9Me3, for comparison with toxicity and DNA damage induction.

Methods: The HC-04 cell line was used as a metabolically and DNA repair competent human non-cancer liver cell line. Compounds found to be the most toxic [Cu(II) dimethyldithiocarbamate (CDMDC), Zn-diethyldithiocarbamate (ZDEDC)] were compared to soluble forms of Cu and Zn ($CuCl_2$; $ZnCl_2$) and to sodium-dimethyldithiocarbamate (NDMDC), to determine if the chemical potency can be attributed to the metal or to the organic portion of the molecules. Positive controls included known histone deacetylase inhibitor, Trichostatin A, and EZH2 histone methyltransferase inhibitor, GSK-126. After 24 h exposure in concentration-response experiments, acid lysates were prepared and normalised for protein concentration. The H3-PTMs were captured onto fluorescently labelled magnetic beads using the Active Motif Multiplex kit then measured with a Luminex 200 preformatted flow cytometry system.

Results: CDMDC changed the abundance of H3K9Ac and pan-acetyl H3 similarly across concentration groups ($p < 0.05$), with a small increase at 0.5 μM followed by a significant reduction at 2 μM. $CuCl_2$ significantly reduced ($p = 0.004$) H3K9 methylation with a lowest observable effect level (LOEL) of 1 mM; a non-cytotoxic concentration that exceeds levels of Cu found in human blood. No effects of ZDEDC and $ZnCl_2$ treatment on H3 methylation and acetylation were observed among the five epitopes investigated. The lowest observable adverse effect level (LOAEL) for CDMDC toxicity (0.6 μM) was similar to the above LOEL (2 μM) for epigenetic disturbance.

Conclusions: The results suggest that induction of changes in H3-PTMs is chemical specific and may become useful for chemical classification. The induction of DNA damage and epigenetic changes at similar concentrations of CDMDC raise concerns about possible predisposition to carcinogenic events in surviving cells. Multiplex measurement of H3-PTMs rapidly identified acetylation and methylation as epigenetic targets of CDMDC and $CuCl_2$ exposure, respectively, and is a method that facilitates the identification of epigenetic disturbance as a mode of action for risk assessment.

12. THE TGx-28.65 GENOMIC BIOMARKER FOR DNA DAMAGING AGENTS WORKS EFFECTIVELY IN HUMAN HEPARG CELLS, USING NEXT-GENERATION SEQUENCING

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Background: In vitro gene expression signatures to predict a specific toxicological response can expedite chemical screening for human health risk assessment purposes. Collaborative efforts through the Health and Environmental Sciences Institute's Genomics Committee developed and validated the TGx-28.65 genomic biomarker (a toxicogenomics (TGx) biomarker, developed using a 28 chemical training set, and comprising 65 genes). The biomarker is used in classifying compounds as genotoxic (DNA damage-inducing) and non-genotoxic in human lymphoblastoid TK6 cells in the presence/absence of S9 metabolic activation using Agilent gene expression microarrays.

Objectives: In this study, we tested the performance of the TGx-28.65 genomic biomarker using a different gene expression technology and in a metabolically competent human cell line (HepaRG™). Gene expression was measured using next generation sequencing technology on an Ion Proton sequencer to test the ability of the genomic biomarker to accurately classify chemicals across technical platforms. Furthermore, the performance of the TGx-28.65 biomarker was evaluated in metabolically competent human HepaRG™ cells to circumvent the need to add rat liver S9 to the cells in culture, which can be problematic for some compounds.

Methods: HepaRG™ cells were exposed to increasing concentrations of 10 test chemicals (5 genotoxic and 5 non-genotoxic) at 0, 24 and 48 h. Cells were collected 7 h and 96 h following the last exposure. Transcriptome profiles were generated at 7 h using the Ion AmpliSeq Transcriptome Human Gene Expression kit. Relative survival and micronucleus frequency were assessed by flow cytometry at 96 h. Statistical modeling and bioinformatics tools were utilized to classify chemicals as genotoxic or non-genotoxic using the TGx-28.65 biomarker genes.

Results: The TGx-28.65 genomic biomarker accurately classified the high concentrations of all 5 genotoxic compounds, in addition to all concentrations of the non-genotoxic chemicals using the Ion AmpliSeq Transcriptome approach in HepaRG™ cells. These results suggest that this genomic biomarker can be applied using a targeted sequencing approach in metabolically competent human cells. Moreover, new data mining is in progress to improve the gene expression predictors for genotoxicity.

Conclusions: The TGx-28.65 genomic biomarker has the potential to add significant value to existing approaches used to assess a chemical's genotoxic potential. This follow-up work demonstrates that the TGx-28.65 genomic biomarker accurately predicts genotoxicity using a different gene expression technology in metabolically competent human cells (HepaRG™). This genomic biomarker can be used as a follow up to the current regulatory genotoxicity testing battery to reduce reliance on animals and provide mechanistic insight into genotoxic effects. The TGx-28.65 gene expression signature is currently under formal evaluation by the US Food and Drug Administration and is set to become the first qualified and officially validated TGx biomarker. This approach is a first step in accomplishing a more integrated genotoxicity testing strategy to derive mechanistic information to better inform human health risk assessment.

13. GENE AND MICRORNA EXPRESSION CHANGES FOLLOWING A 28-DAY EXPOSURE TO HEXABROMOCYCLODODECANE IN A RAT MODEL

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Background: In recent decades, hexabromocyclododecane (HBCD), a brominated flame retardant, has been applied to consumer products to impair flame propagation of combustible materials. HBCD has been banned worldwide due to its environmental persistence and bioaccumulative properties, but continues to be a global health concern due to its persistence. HBCD exposure in experimental animals has been shown to alter thyroid function, increase liver weight, and cause changes in immune response; however, the mechanisms underlying its harmful effects are not clearly understood.

Objectives: To elucidate the molecular pathways involved in HBCD-induced toxicity via assessment of the potential exposure-related changes in expression of targeted genes and microRNA (miRNA) profiles.

Methods: A 28-day subacute toxicology study was conducted using male and female Fischer rats to assess the health hazards associated with exposure to HBCD. Liver, kidneys and serum were taken at necropsy and total RNA was isolated. Real-time PCR was conducted to identify genes and miRNAs with altered expression relative to controls.

Results: Genes involved in nephrotoxicity, stress and toxicity were surveyed. Genes from several biological pathways including cell cycle regulation, oxidative stress, immune response, steroidogenesis, renal damage and xenobiotic metabolism were found to be significantly altered following treatment. Many of these genes are downstream of CAR/PXR, receptors which regulate an important signalling cascade for xenobiotic-metabolizing genes. Several genes were found to be mediated by Nrf2, which is responsive to oxidative stress. Moreover, altered miRNA expression was observed in treatment groups which directly correlated with some of the genes investigated, and in many of the same pathways. Male rats demonstrated more genes with altered expression profiles at lower doses than did females. The significance of these findings indicates the interconnected response of genes and miRNAs in the transcriptome to chemical contaminants.

Conclusions: Our data suggest that HBCD exposure provokes responses from several cellular pathways associated with multiple target organs, a finding that is in keeping with effects seen with apical data. Taken together with previous findings, this study sheds light on possible mechanism of action of HBCD, and the continued concern for potential human health effects.

14. TRICLOSAN ELEVATES ESTRADIOL LEVELS IN SERUM AND TISSUES OF FEMALE MICE.

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Background: Triclosan is a synthetic antibacterial added to personal care products. It is absorbed through the skin and has been reliably detected in human urine samples. There are several studies demonstrating estrogenic effects of triclosan *in vivo*, despite weak evidence that triclosan directly binds the estrogen receptor.

Objectives: Our aim was to determine whether triclosan can modulate the concentrations of exogenous and endogenous estradiol in female mice.

Methods: Cycling and inseminated female mice were each injected with triclosan or vehicle prior to a 1 μCi injection of tritium-labeled estradiol (^{3}H-E_2). At 1 or 7 h after ^{3}H-E_2 injection, blood and tissues were collected and analyzed for radioactivity via liquid scintillation counting. In subsequent experiments, cycling and inseminated female mice were each injected with triclosan or vehicle and urine was collected between 2 and 12 h after injection. We measured unconjugated estradiol in urine via enzyme immunoassay.

Results: Triclosan increased radioactivity in the uterus of both cycling and inseminated females. Unconjugated urinary estradiol was significantly elevated in the urine of cycling and inseminated females.

Conclusions: Triclosan leads to an elevation of both exogenous and endogenous estradiol in cycling and inseminated female mice. As many personal care products contain triclosan, the elevation of estradiol by triclosan is potentially relevant to anti-reproductive and carcinogenic acions of excessive estradiol activity in human females.

15. USE OF AN IN VITRO PROTEIN BINDING ASSAY TO CHARACTERIZE INTERACTIONS OF OP TRIESTERS AND METABOLITES WITH THYROXINE AND HUMAN TRANSTHYRETIN

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Background: Organophosphate triester flame retardants (OPFRs) have been applied to plastics, foams and textiles for decades, and production of these additive flame retardants has increased substantially since the phase-out of polybrominated diphenyl ethers (PBDEs). Due to leaching, various OPFRs have been found in both indoor and outdoor environments leading to human and wildlife exposure. Recent studies have shown that the OPFR triphenyl phosphate (TPHP) is largely metabolized to hydroxylated metabolites, but also dealkylated to organophosphate diesters e.g. in vitro using human and herring gull liver microsomal assays as well as *in vitro* in chicken embryonic hepatocyte assay. Toxicological properties of OPFRs are not well understood to date, though increasing evidence suggests possible effects on the thyroid system. Perturbation of thyroid hormone transport is considered to be one mechanism of action that may affect thyroid function, and this is a toxicological concern with PBDEs and especially hydroxy-BDE metabolite compounds.

Objectives: The objectives of this study were to a) optimize an *in vitro* competitive protein binding assay that uses thyroxine (T4) as the natural ligand to thyroid hormone binding protein transthyretin (TTR, from human plasma), and b) apply this assay to investigate the abilities of select OPFRs identified in environmental biota, and diester and/or hydroxylated metabolites for each, to competitively displace T4 from TTR.

Methods: The present method employs size exclusion chromatography, using pre-packed polyacrylamide gel filters, to separate free and protein-bound radiolabeled T4 across a series of concentrations of each organophosphate competitor. Results are plotted as %T4-TTR binding compared to controls.

Results: The *in vitro* competitive binding assay was successfully optimized and validated with inter-day and inter-laboratory consistency of calibration results. Results indicate no competitive displacement of T4 from TTR for any of the compounds tested, but rather unexpectedly the T4-TTR binding increased in the presence of OP triester/diester (significantly different from controls at 64 to 512 nM, and 165 to 184% of controls at highest concentration of 2048 or 5000 nM).

Conclusion: For the first time, *in vitro* interactions between an OP diester or hydroxylated OP triester metabolite and T4-TTR binding were investigated. There was a consistent concentration-related increase in T4-TTR binding compared to controls for all compounds tested. One plausible explanation may be that allosteric interactions of OP triesters with TTR increase the ability/stability of T4-TTR binding, possibly allowing for both binding pockets to be occupied at once. While competitive displacement of T4 from TTR was not observed, these results could still indicate potential for OP triesters and metabolites to perturb thyroid homeostasis.

16. DIFFERENCES IN THE BILIARY EXCRETION OF ARSENIC BY RAT AND HUMAN SANDWICH-CULTURED HEPATOCYTES

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Background: Arsenic is a proven human carcinogen, causing cancers of the skin, lung, and bladder. More than two hundred million people worldwide are exposed to levels of arsenic above the World Health Organization guideline of 10 ppb. Yet, the cellular handling of arsenic remains inadequately understood. In the liver, the ATP-binding cassette transporter, multidrug resistance protein 2 (human MRP2/*ABCC2* and rat Mrp2/*Abcc2*) is localized to the apical surface of hepatocytes. In rats, Mrp2 is responsible for 99% of arsenic biliary excretion through the transport of arsenic triglutathione [$As(GS)_3$] and the diglutathione conjugate of monomethylarsonous acid [$MMA(GS)_2$]. Our studies using sandwich cultured primary human hepatocytes (SCHH) indicate that arsenic may undergo biliary excretion more extensively in rats than humans. We hypothesize that 1) rats more extensively excrete arsenic into bile than humans and 2) differences in the transport of arsenic by human and rat MRP2/Mrp2 are responsible for the different extents of biliary excretion.

Objectives: 1) To characterize the biliary excretion of arsenic using sandwich cultured primary rat hepatocytes (SCRH) for direct comparison with our published SCHH data. 2) To determine any differences in transport of $As(GS)_3$ and $MMA(GS)_2$ by rat and human Mrp2/MRP2-enriched membrane vesicles.

Methods: Biliary excretion of arsenic will be characterized in SCRH using B-CLEAR® technology. SCRH were treated with $^{73}As^{III}$ (1 μM) for 24 h, efflux of ^{73}As across basolateral and apical membranes measured, and the biliary excretion index (BEI) calculated. MRP2/Mrp2-enriched membrane vesicles were used to measure transport of $As(GS)_3$ and $MMA(GS)_2$. Arsenic vesicular transport was quantified using inductively coupled plasma mass spectrometry.

Results: Preliminary experiments with SCRH showed a higher arsenic BEI (range 44-76%) compared with SCHH (BEI range 0-31%). Using vesicular transport assays $MMA(GS)_2$ was found to be transported by both human and rat MRP2/Mrp2. Previous work from our lab has shown that $As(GS)_3$ is transported by human MRP2. Future work will characterize the kinetics of rat and human Mrp2/MRP2 transport of $As(GS)_3$ and $MMA(GS)_2$.

Conclusion: SCRH have a higher BEI for arsenic than SCHH, suggesting that rats excrete arsenic in bile to a greater extent than humans. $As(GS)_3$ and $MMA(GS)_2$ are transported by both rat and human Mrp2/MRP2 and determination of kinetic parameters will allow the comparison of transport efficiency. Understanding the rat and human Mrp2/MRP2 differences in arsenic transport will ultimately provide insight into the mechanism of action of MRP2, thereby leading us to better understand inter-individual differences in the handling of arsenic.

17. EFFECTS OF CROSS-FOSTERING AND DEVELOPMENTAL EXPOSURE TO MIXTURES OF ENVIRONMENTAL CONTAMINANTS ON HEPATIC GENE EXPRESSION IN 21 DAY OLD AND ADULT LITTERMATES SPRAGUE DAWLEY RATS.

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Background: Adverse health effects caused by exposure to chemical stressors such as environmental contaminants could potentially be amplified by the presence of non-chemical stressors. Our laboratory previously demonstrated that stress imposed on rat pups, by being adopted at birth by a nursing mother (cross-fostering), increased the adverse effects of exposure to environmental contaminants on hormonal stress-responses in the grown adults.

Objective: The current research is a follow up study to our previous work and investigates if cross-fostering as a source of stress, could modulate changes in gene expression profile of rat liver following developmental exposure to mixtures of environmental contaminants.

Methods: Sprague-Dawley rats were treated with low concentrations of mixtures of environmental contaminants, simulating the contaminant profile detected in the maternal blood of northern Canadians. Dams were fed cookies laced with corn oil (control) or a chemical Mixture (M) from pregnancy until 21 days after birth. At birth, some control (C) and M litters were cross-fostered and at 21 days of age and later during adulthood (78-86 days old) the livers were dissected and gene expression profiles were generated using Agilent SurePrint G3 Rat GE 8x60k microarrays. Bioinformatics analyses were performed to identify abnormal gene expressions leading to functions and pathways potentially affected by treatments.

Results: Our study revealed that exposure to the mixture of environmental contaminant modified the hepatic gene expression profile in rats. The altered expression of hepatic genes were mostly related to detoxification and energy metabolism (glucose and fat) in both age groups, but the genes affected at Day 21 differed from those affected at 78-86 days of age. Litters exposed to environmental contaminant that were also cross-fostered showed gene expression patterns that differed from those that were not cross-fostered. Moreover, a cross-fostering effect was detectable even in control groups not exposed to environmental contaminant. Top functions impacted by altered gene expression in cross-fostered control groups were energy production and lipid metabolism.

Conclusions: Abnormal gene expressions, along with the hormonal stress-responses illustrated by our previously published data, suggest that non-chemical stressors can modify the toxicity of environmental contaminants and represents an additional factor for consideration in chemical risk assessments. Sources and magnitude of stress that can modulate the effects of exposure to environmental contaminants should be investigated further

18. IS THE REFERENCE DOSE FOR PERFLUOROOCTANOIC ACID (PFOA) DERIVED BY THE U.S. ENVIRONMENTAL PROTECTION AGENCY PROTECTIVE FOR BREASTFED INFANTS? AN EVALUATION USING PHARMACOKINETIC MODELING

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Background: The U.S. Environmental Protection Agency (EPA) recently established a reference dose (RfD, 0.02 μg/kg/d) for PFOA. This dose was calculated based on an estimated average serum PFOA concentration in mouse dams exposed to 1 mg/kg/d from gestational day 1 to 17, a dose that led to reduced ossification and accelerated puberty in the offspring. We believe this approach overlooked two important factors: 1) PFOA concentration in the pup/infant is likely to be more toxicologically relevant than the concentration in the dam/mother and 2) lactational exposure in infants, which is a major route of PFOA exposure during infancy, was not considered.

Objective: We aimed to calculate an RfD for PFOA based on pup/infant concentrations estimated using physiologically based pharmacokinetic (PBPK) models.

Methods: Using a PBPK model of pregnant and lactating mice, we estimated the average serum PFOA concentration in the pups born to dams administered a 1 mg/kg/d dose from gestational day 1 to 17. We subsequently used a PBPK model of pregnancy and lactation in humans to estimate a maternal dose leading to an average infant serum concentration that matches the estimated serum concentration in pups. This maternal dose was then divided by the same uncertainty factors used by EPA (300) to derive an RfD.

Results: We estimated that pups born to dams exposed to 1 mg/kg/d had an average serum concentration of 3.5 mg/L during gestation and lactation. The model estimated maternal dose needed to reach an average serum concentration in human infants that matched the 3.5 mg/L serum concentration was 0.2 μg/kg/day. After dividing this value by uncertainty factors, we obtained an RfD of 0.0007 μg/kg/d, which is approximately 30 times lower than the RfD derived by the EPA.

Conclusion: Our results suggest that the PFOA reference dose calculated by the EPA may lead to an underestimation of the risks for developmental toxicity in breastfeeding infants.

19. TOXICOLOGICAL AND GENOMIC RESPONSES IN KIDNEY, HEART AND LIVER TISSUES OF MALE AND FEMALE F344 RATS FOLLOWING 28-DAY REPEATED DOSE SUB-ACUTE DIETARY EXPOSURE TO 2-MONOCHLORO-1,3-PROPANEDIOL (2-MCPD)

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Background: Chloropropanols (monochloropropanediol; MCPD) are known contaminants resulting from heat-induced processes in foods that contain vegetable oils. While there is sufficient data regarding the toxicology of 3-MCPD, no detailed toxicological studies of 2-MCPD are available to understand its health effects, creating a risk assessment gap.

Objective: The main objective of this study was to fill a regulatory gap in identifying the mode of action of dietary 2-monochloro-1,3-propanediol (2-MCPD) in kidneys, heart and liver tissues of F344 rats exposed for 28 days according to the *Organization of Economic Cooperation and Development (OECD) Test Guideline-407* using both apical and toxicogenomic endpoints.

Methods: Weanling male and female F344 rats (n = 10 rats/group/sex) were fed *ad libitum* AIN-93G diets containing 2-MCPD to provide estimated daily doses of 25, 50, 100 or 200 mg/kg body weight (BW). Within the first week of exposure, female rats in the 100 and 200 mg/kg BW dose groups of 2-MCPD became moribund and were euthanized. Male rats were spared from exposure to these high doses and these groups were excluded from the study. Remaining rats were killed 28 days after exposure, and their tissues (kidneys/heart/liver) were weighed and frozen in liquid N_2. RNA from target tissues was extracted using standard kits and MCPD-related changes in gene expression levels were assessed using PCR arrays for nephrotoxicity, cardiotoxicity, molecular toxicity, and oxidative stress. Genes that displayed significant ($p > 0.05$) fold changes ($\geq$ 1.5-fold) were validated using TaqMan Gene Expression assays (Life Technologies). Statistical analysis was performed using t-test (SigmaPlot 11).

Results: Non-cancerous lesions with minimal to moderate scores were observed specifically in the kidney (50 mg/kg BW in males), heart (50 mg/kg BW in females) and thyroid (25 and 50 mg/kg BW in males and 50 mg/kg BW in females). Weights of kidneys in both sexes were significantly higher in the 2-MCPD groups along with higher levels of creatine kinase and lower levels blood urea nitrogen. Heart weights were significantly higher in the 50 mg/kg BW groups in both sexes. Additionally, we observed significantly lower ALT and AST in males at both 25 and 50 mg/kg BW 2-MCPD, together with lower levels of high-density lipoproteins and cholesterol at 50 mg/kg BW 2-MCPD in both sexes. Genomic data indicates that in treated kidneys, 2-MCPD significantly increased *Hmox1* and *Ptgs2* genes both involved intrinsically in inflammation. Several pathways were targeted in the heart as a consequence of 2-MCPD exposure such as angiogenesis, metabolic regulation and cell migration. The liver tissue only showed limited changes in the battery of genes tested.

Conclusion: These results show that, for 2-MCPD, a no-observed-effect level (NOEL) was not reached in this study. This detailed sub-acute dietary exposure study provides toxicology and toxicogenomic data to support the hazard characterization of food-borne 2-MCPD for regulatory purposes and the lack of a NOEL provides impetus to further study 2-MCPD exposure.

20. ANTICANCER ACTIVITIES OF PPARγ IN HER2+ BREAST CANCER

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Background: Breast tumours overexpressing epidermal growth factor receptor 2 (HER2+) grow and spread faster than HER2-negative tumours, resulting in poor patient prognosis. Peroxisome proliferator-activated receptor (PPARγ) is a transcription factor that tightly regulates the expression of genes involved in sugar and fat metabolism. We previously showed PPARγ suppresses environmental carcinogen (DMBA)-mediated breast tumour progression *in vivo*. However, the role of PPARγ during HER2+ breast tumourigenesis and patient survival is unclear.

Objectives: We hypothesize PPARγ loss enhances HER2+ breast tumour progression.

Methods: We crossed a spontaneous HER2+ breast tumour mouse model, known as MMTV-Neu- IRES-Cre **(NIC)** with our unique PPARγ-floxed mice to create a novel mouse model **(NIC;PPARγKO)**, which have targeted PPARγ deletion in the same HER2+ transformed mammary epithelial cells that drive breast tumourigenesis. Western Blot and immunofluorescence (IF) assays were used to evaluate PPARγ and HER2 expression and localization in fresh and formalin fixed, paraffin-embedded (FFPE) tumours. A variety of HER2+ human breast cancer cell lines were stably transduced to create PPARγWT, PPARγKO, and PPARγmut cells to define the *in vitro* interactions between PPARγ and HER2 signaling. Mouse tumourigenic cell lines were also established in culture from a freshly isolated NIC;PPARγKO primary and lung metastatic tumours **(NIC;PPARγKO-lmets)**, and evaluated for angiogenic, invasive and migratory effects.

Results: Compared to NIC;PPARγWT mice, NIC;PPARγKOs have increased mammary tumour incidences and mammary tumour lung metastasis. Protein analysis of NIC;PPARγKO tumours hows PPARγ loss is inversely correlated with increased HER2 phosphorylation at tyrosine 877 (pY877HER2) in tumourigenic tissue. Immunofluorescent analysis also showed HER2 H-scores was significantly highest among tumours from NIC;PPARγKOs, but also correlated with targeted PPARγ loss in DMBA-induced primary and metastatic mammary tumours among PPARγWT and PPARγKO mice ($p<0.05$). To further investigate the role of PPARγ in the metastatic process, i*n vitro* analysis of several human HER2+ breast cancer cells lines and our NIC;PPARγKO-lmet cells shows migration, invasion and tumoursphere formation potential were significantly increased after epidermal growth factor (EGF, 20 ng/ml) treatment, and more interestingly, that co-treatment with a PPARγ activating drug (rosiglitazone, 10 μM) significantly abrogated these effects ($p<0.05$).

Conclusions: Together, these data provide the first evidence that PPARγ may be a useful prognostic/predictive biomarker for HER2+ breast tumours, and suggest the novel inclusion of PPARγ activating drugs may act to benefit a subset of HER2+ breast cancer patients.

21. PLASMA LEVELS OF POLYBROMINATED DIPHENYL ETHERS (PBDES) AT 12, 24 AND 36 MONTHS OF AGE

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Background: Studies have detected PBDEs in blood, adipose tissue and breast milk samples from the general population. Exposure to PBDEs is assumed to be greatest during infancy and childhood. Yet, few studies measured early-life children's plasma levels.

Objective: In this study, we aimed to document and compare levels of four PBDE congeners (BDE-47, BDE-99, BDE-100, BDE-153) in maternal plasma during pregnancy and children's plasma at the age of 12, 24 and 36 months.

Methods: Blood samples were collected from mothers and children participating to the Markers of Autism Risk in Babies (MARBLES) study in California. PBDE levels were measured by GC/EI-MS/MS and expressed on a lipid basis. To understand the relationship between maternal levels and children's levels, we calculated child:mother plasma level ratios by dividing children's levels (12, 24 and 36 months) by maternal levels measured during the third trimester of pregnancy.

Results: We found some of the highest children's plasma PBDEs levels ever reported, especially for BDE-47 with median (range) levels of 30 ng/g lipids (<LOD-216) at 12 months, 38 ng/g lipids (<LOD-417) at 24 months and 32 ng/g lipids (<LOD-501) at 36 months. Median child:mother plasma level ratios for the different congeners ranged from 2.6 to 3.8 at 12 months, 1.9 to 2.8 at 24 months and 2.1 to 3.5 at 36 months.

Conclusion: This study clearly showed that children's plasma levels can greatly exceed their mother's plasma levels during pregnancy. Given the extent of exposure and concurrent child development during infancy and childhood, studies are needed to evaluate the association between postnatal exposure to PBDEs and health effects.

22. EFFECTS OF POST-NATAL ADRENERGIC RECEPTOR STIMULATION ON LATENT CONGENITAL HEART DEFECTS

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Background: Congenital heart defects (CHD) are the most prevalent birth defect, occurring in approximately 1% of births. While 80% of structural CHD resolve spontaneously by one year of age, the long-term predisposition to adult onset heart pathologies is unknown. To understand the consequences of resolved CHD and to develop intervention strategies, we have developed an animal model in which pregnant rats are administered a potent heart teratogen. Although the offspring of these rats are born with about a 50% incidence of CHD, the CHD resolves spontaneously and cardiac performance in unstressed hearts is indistinguishable from controls when assessed using echocardiography. However, under the stress of pregnancy, rats with resolved CHD exhibit maladaptive responses, suggesting long-term latent heart vulnerabilities. It is unknown how male rats with resolved CHD respond to postnatal cardiac stressors.

Objectives: To use repeated adrenergic receptor stimulation as a cardiac stressor to test the hypothesis that hearts with resolved CHD are more vulnerable to stress-induced cardiac pathologies than control hearts using echocardiography to measure structural and functional cardiac changes longitudinally.

Methods: Time-mated, female Sprague-Dawley rats were dosed with 300 mg/kg dimethadione (or distilled water for pair-fed and ad libitum controls) via oral gavage every 12 h from gestational day 9-10. This dosing protocol has been previously used in our laboratory to produce offspring with a 50% incidence of CHD. Male offspring were monitored on post-natal days (PND) 4, 21, and 56 via ultrasound for structural and functional development of the heart. Adult rats were then treated with intermittent and increasing duration (from 1 and up to 7 days) of isonorepinephrine (INE), a β-adrenergic agonist at 0.01 mg/kg, s.c., with recovery periods (9 days) between dosing cycles. Echocardiography was performed at baseline, maximum cardiac hypertrophy (24 h after the last dose of INE), half-time to recovery of cardiac hypertrophy (4 days following last dose of INE) and at recovery from cardiac hypertrophy (7 days after last dose of INE) of every other dosing cycle.

Results: Significant structural and functional differences between dimethadione treated and control offspring were observed in the period leading up to INE exposure, including left ventricular mass, stroke volume, and cardiac output. With these differences accounted for, preliminary analysis of the INE exposure period suggests maladaptive responses to the stressor as observed by cardiac ultrasound. Further analysis will determine patterns in cardiac function, hypertrophy, and threshold for reversibility between control male rats and those with latent CHD.

Conclusion: While analysis is continuing, our initial findings underscore the growing body of evidence for long-term consequences of resolved CHD. More specifically, this study aims to determine if latent CHD predisposes an individual to an increased susceptibility to the pathophysiological effects of adrenergic hyperstimulation.

23. INTEGRATION OF GERM CELL DNA DAMAGE ASSESSMENT INTO OECD GUIDELINES FOR THE GENOTOXICITY TESTING OF CHEMICALS

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Background: Health Canada supports the development of test guidelines (TG), under the auspices of the Organisation for the Economic Co-operation and Development (OECD), for identifying chemicals that cause DNA damage. Recently, an OECD TG for detecting mutations in somatic cells and germ cells using transgenic rodents (TGR) was developed. However, guidelines for detecting DNA damage are only available for somatic cells. Current OECD test guidelines do not include assays for sperm DNA damage, resulting in a critical testing gap. Thus, developing a testing strategy that addresses this gap is a priority for risk assessment.

Objective: The aim of this project is to evaluate the performance of the Sperm Chromatin Structure Assay (SCSA), the Terminal Deoxynucleotidyl Transferase (TUNEL) and HT-COMET Comet assay to detect DNA damage in sperm, used in integrated testing with the transgenic rodent mutation assay (TG 488).

Methods: We simultaneously evaluated the genotoxic effects of triethylenemelamine (TEM), a multifunctional alkylating agent, in both somatic and germ cells of Muta^{TM}Mouse males. Animals received 0, 0.5, 1, or 2 mg/kg/day TEM for 28 days orally. Tissues were collected 2 (28+2; blood) and 3 (28+3; sperm and bone marrow) days later. LacZ mutant frequencies (MFs) were measured in bone marrow. Furthermore, the frequency of micronuclei in reticulocytes (% MN-RET) and normochromatic erythrocytes (% MN-NCE), the percentage of DNA fragmentation index (% DFI) and frequency of TUNEL positive cells in cauda sperm were analyzed by flow cytometry. The percentage of DNA in tail (%DNA tail) was also measured using HT-COMET, a novel automated high throughput version of the comet assay.

Results: Medium and high doses of TEM significantly increased MFs in bone marrow ($P < 0.05$) plus % MN-RET and % MN-NCE in a dose-dependent manner ($P < 0.05$). Both % DFI and % TUNEL positive cells demonstrated dose-related increases in sperm ($P < 0.05$), and the results of these two assays were strongly correlated ($R = 0.9298$). Although %DNA tail showed a significant increase in DNA damage at all TEM doses, there were no dose-related effects. Within the same animal, there was a good correlation between the %MN-RETs at 28+2 days and %DFI at 28+3 days ($R = 0.7189$).

Conclusions: Our results show that TEM induces DNA damage in both somatic and germ cells. All three sperm assays demonstrated significant induction of DNA damage at the low TEM dose. However, only the SCSA and TUNEL assay showed significantly more damage at the high TEM dose with respect to the two lower doses. This study demonstrates that sperm DNA damage can be easily integrated into standard OECD designs investigating genotoxicity in somatic cells. The results generated by these tests can provide key information on whether a chemical is genotoxic in germ cells and impact its risk assessment and subsequent regulatory decisions.

24. UTILIZING AN ADVERSE OUTCOME PATHWAY FRAMEWORK TO INVESTIGATE INTERLEUKIN-1 AND STAT6 SIGNALING IN THE PATHOLOGY OF CARBON NANOTUBES IN MICE

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Background: Carbon nanotubes (CNTs) are amongst the most widely produced and utilized nano-materials worldwide. Multi-walled carbon nanotubes (MWCNTs) are a subset of CNTs that can possess unique physical properties allowing for the material to penetrate deep into the lungs upon inhalation, while inhibiting clearance of the material from the lungs over time. Animal models have demonstrated that the accumulation of certain MWCNTs in the pulmonary environment can lead to inflammation and the development of disease similar to pulmonary fibrosis. Adverse Outcome Pathways (AOPs) are a new frame work for defining the key events that comprise the biological mechanism behind an undesirable event such as pulmonary fibrosis induced by MWCNTs. We hypothesize that AOPs can be utilized to guide more detailed mechanistic studies and identify specific genes and biological pathways crucial to disease development after exposure to a toxic substance.

Objectives: We targeted key events in an AOP that described MWCNT-induced fibrotic disease and assess the development of pathology. Specifically, we attempted to disrupt pulmonary inflammatory mechanisms by targeting IL-1R1, and disrupt the subsequent Th2/M2-associated healing response by targeting the transcription factor STAT6.

Methods: C57BL/6 and specific gene knock out (KO) mice were exposed to a high dose of a known disease-causing MWCNT by intratracheal administration. Two separated series of experiments were conducted, one utilized IL-1R1 KO mice and the other utilized STAT6 KO mice. Inflammation was assessed at 24 h and 28 days post MWCNT administration. The development of fibrotic disease was assessed 28 days post MWCNT administration, and whole-genome microarrays were performed on RNA isolated from lung tissue at all time points.

Results: Our data indicated that MWCNT-induced inflammation was ameliorated in IL-1R1 KO mice at the 24 h time point, but this suppressed inflammatory response was not observed 28 days post exposure. IL-1R1 KO did not significantly affect the development of fibrotic disease. STAT6 KO mice developed attenuated fibrotic disease in response to MWCNT administration. Unexpectedly, STAT6 KO mice also had reduced inflammation at early time points. Whole genome analysis identified a subset of differentially expressed genes associated with fibrosis.

Conclusion: This study has identified biological processes associated with STAT6 as being crucial to the development MWCNT-induced fibrotic disease. These results will be further investigated in a future study to identify STAT6 associated genes which can be used as effective biomarkers of MWCNT-induced pathology. The results also highlighted the robust nature of the inflammatory response associated with MWCNT exposure, as disrupting a key inflammatory mediatory, IL-1R1, was not sufficient to suppress inflammation beyond the initial inflammatory response. These data will prove useful in designing screening strategies that could be used by regulatory agencies to distinguish between MWCNTs of varying toxicity.

25. INVESTIGATING THE DEVELOPMENTAL EFFECTS OF THE FLAME RETARDANT, TRIPHENYL PHOSPHATE, IN C57BL/6 MICE

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Background: Triphenyl phosphate (TPP) is an organophosphorus flame retardant that has been added to numerous consumer products to serve as both a plasticizer and a flame retardant as a replacement for brominated flame retardants. TPP is not a structural teratogen. However, recent studies conducted in zebrafish have suggested that it may have metabolic and endocrine disrupting effects following developmental exposure.

Objective: Given its increased use in everyday products, the present study aimed to investigate the developmental effects of TPP in a murine model.

Methods: C57Bl/6 dams were exposed on gestational days 8, 10, 12, and 14 to 0, 5, 25, or 50 mg/kg TPP dissolved in corn oil via intraperitoneal injection. Dams were euthanized on gestational day 19, and maternal ovaries, thymus, kidney, spleen, and liver were excised and weighed. Subsequently, fetuses were removed, litter size and number of resorptions recorded, and placentas were weighed. Following fetal viability testing, fetuses were weighed, as well as crown-rump length and anogenital distance (AGD) were recorded. Fetal liver gene expression of insulin growth factor (*Igf*) 1 and 2, as well as peroxisome proliferator activated receptor alpha (*Ppar*α) was measured to determine preliminary metabolic effects of TPP.

Results: No significant differences between treatment groups were detected with respect to maternal organ weight or weight gained by dams during gestation. Similarly, no significant differences were detected between treatment groups with respect to litter size or number of resorptions per litter, or fetal weight or length. However, a significant increase in AGD and placenta size was found with increasing dose of TPP. Preliminary gene expression analysis demonstrated a significant increase in *Igf1*, *Igf2*, and *Ppar*α gene expression following exposure to 5 and 25 mg/kg TPP.

Conclusion: Taken together, these results support previous findings demonstrating that TPP does not cause overt structural developmental toxicity. However, the current results do support the potential endocrine and metabolic effect of TPP following exposure during development. Current analysis with tissue collected from this study is focusing on further investigation of the molecular evidence of endocrine and/or metabolic dysfunction in TPP-exposed fetal tissue.

26. INTERACTION AMONG FIVE ENVIRONMENTAL CHEMICALS: ESTROGENIC POTENTIAL MEASURED VIA ELEVATIONS IN BISPHENOL A CONCENTRATIONS IN MICE

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Background: Many toxicological studies examine the effects of a single chemical, whereas people are commonly exposed to multiple substances that may interact. We have recently demonstrated elevated 17β-estradiol (E2) and bisphenol A (BPA) concentrations in mice following treatment with one of five environmental chemicals. The lowest dose, given once per animal via subcutaneous injection, that significantly increased E2 and BPA concentrations varied by chemical: [1] triclosan (an antimicrobial agent; 0.6 mg), [2] tetrabromobisphenol A (a flame retardant; 1 mg), [3] butylparaben (a preservative; 1 mg), [4] diethylhexyl phthalate (a plasticizer; 3 mg), and [5] propylparaben (a preservative; 9 mg).

Objectives: We sought to determine whether concurrent administration of multiple chemicals would elevate BPA concentrations at lower doses than when given independently.

Methods: We subcutaneously injected female and male mice with 0.1 mg of each chemical, either alone or concurrently, then administered 50 :g/kg ^{14}C-BPA in a dietary supplement, and subsequently measured levels of radioactivity in serum and tissues.

Results: Whereas 0.1 mg of each chemical given alone was insufficient to modulate ^{14}C-BPA concentrations, 0.1 mg of all five chemicals given concurrently significantly elevated ^{14}C-BPA concentrations in serum, reproductive, and other tissues.

Conclusion: While BPA is an environmental estrogen with known endocrine disrupting effects and E2 is the most potent endogenous steroid hormone, the five chemicals studied here show very low or no affinity for estrogen receptors. Rather, all five chemicals potently bind and inhibit sulfotransferase, a major enzyme in steroid metabolism. These data are consistent with a mechanism whereby these chemicals compete with BPA for access to metabolic enzymes. Given that these chemicals are found in numerous consumer products, are readily absorbed into the body, and are widely detected in humans, these findings demonstrate the importance of considering studies of multiple toxicants when determining regulatory exposure limits.

27. IN VIVO PULMONARY GENOTOXICITY OF MULTIWALLED-CARBON NANOTUBES

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Background: Multiwalled carbon nanotubes (MWCNTs) are a type of nanomaterials that are extensively produced and widely used in several industrial applications. As a result, exposure to airborne MWCNTs in the environment or via consumer products is expected to increase significantly within the next decade. MWCNTs are shown to induce lung inflammation and fibrosis in experimental animals. Some studies suggest that MWCNTs are capable of inducing cancers. The International Agency for Research on Cancer (IARC) has classified MWCNTs as possible carcinogens in humans.

Objective: The objective of the present study was to investigate the mechanisms by which MWCNTs may induce cancers.

Methods: Adult male mice were exposed to 42.7 µg/mouse or 128 µg/mouse doses of two different types of MWCNTs (referred to as MWCNT-1 and MWCNT-2) once a week for four consecutive weeks via direct deposition of MWCNTs in lungs. Markers of cancer including DNA strand breaks, P53 expression and cell proliferation in lung tissue, lung inflammation, pathology, and changes in global gene expression levels were assessed on 60 days after the last exposure.

Results: The results showed that both MWCNT types persist in lungs 60 days post-exposure and induce lung inflammation and lung fibrosis to a similar extent. Increased cellular proliferation as measured by Ki67 expression (a marker of cell proliferation) was evident in both MWCNT groups; however, there was no evidence for DNA damage as indicated by the COMET assay which measures DNA strand breaks. Increased p53 expression was observed in the fibrotic foci of both MWCNT groups but the expression was higher in MWCNT-2 group compared to MWCNT-1. The analysis of gene expression data revealed perturbation of a large number of biological processes associated with cell death, irregular cell proliferation, free radical generation and other processes in both groups but the response was higher in MWCNT-2 group.

Conclusions: Despite the extensive tissue injury, the results at 60 days post-exposure revealed a massive effort by the organism to repair the injury and maintain homeostasis. Eventually, however, survival of injured cells resulting from imbalanced regulation of genes and biological processes involved in the tissue healing process may result in cancer. This study identified important markers of MWCNT-induced lung pathology, which can be used to screen other nanomaterials for their potential to induce similar lung pathology by Health Canada regulators.

28. A RETROSPECTIVE ANALYSIS OF THE 1-YEAR DOG TOXICITY STUDY IN PESTICIDE HUMAN HEALTH RISK ASSESSMENTS

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Background: The 1-year dog toxicity study is no longer required by certain pesticide regulatory jurisdictions, including the United States and Europe. As part of its commitment to the 3Rs (reduce, refine, replace the need for animal studies to the extent possible), Health Canada's Pest Management Regulatory Agency (PMRA) examined its current requirement for this study to determine if it could be refined or eliminated.

Objectives: A retrospective analysis was conducted to examine the impact of the 1-year dog study on human health risk assessment.

Methods: The Acceptable Daily Intake (ADI), a measure of the amount of a pesticide in food that can be ingested on a daily basis over a lifetime without an appreciable health risk, was the metric for this analysis. For 143 pesticides evaluated by the PMRA between 2008 and 2015, the supporting toxicology databases were examined to determine if other toxicology studies were protective of the findings in the 1-year dog study. When this criterion was not met, further investigation was undertaken to determine the potential impact of not having the 1-year dog study.

Results: For most of the pesticides, effect levels in the 1-year dog study were not substantially different from those in other toxicology studies, when considering factors such as dose-spacing and known experimental variability. For only one of the 143 pesticides investigated, the ADI was overestimated in the absence of the 1-year dog study. This would not have affected the registration status of this pesticide, however, when considering all information within the context of the overall risk assessment. The results of this analysis suggest that absence of the 1-year dog study would have minimal impact on the assessment of human health risk.

Conclusion: Therefore, Health Canada's PMRA has removed the routine requirement for the 1-year dog study from its pesticide data requirements. This scientifically–driven modification is in line with recent changes to requirements by other international regulatory partners and supports efforts towards the global harmonisation of data requirements that will result in tangible 3R benefits.

29. ROLE OF PPARγ-DEPENDENT MICRORNA EXPRESSION DURING BREAST TUMOUR METASTASIS

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Background: Metastasis is a complex, multistep process whereby cancer cells disseminate from their site of origin and establish secondary tumours in distant organs. In search of key regulators of this process, our lab focuses on peroxisome proliferator-activated receptor gamma (PPARγ) a ligand-activated transcription factor. We previously showed expression and activation of PPARγ decreases environmental chemical carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA)-mediated breast tumour progression. My research will extend these findings by determining whether PPARγ activation exerts these anti-metastatic effects in part by altering microRNA (miR) signaling.

Objectives: We hypothesize that activation of PPARγ upregulates anti-tumourigenic miRs that prevent breast tumour metastasis. To address this, we will use our established in vitro and in vivo models to define the PPARγ-dependent miR expression changes that are altered during DMBA-mediated breast tumourigenesis. Our goal is to determine whether activation of PPARγ upregulates miRs that prevent epithelial-to-mesenchymal transition (EMT) and/or invasion and migration of breast tumour cells. These studies may then provide novel information to reduce breast tumour metastasis.

Methods: Gene expression analysis will be conducted using highly metastatic human MDA-MB- 231 breast cancer cells transfected with either lentiviral empty vector controls or containing PPARγ expression plasmids for the wildtype ($PPAR\gamma^{WT}$) or non-ligand responding mutant ($PPAR\gamma^{mutant}$) protein. Transduced cells will be pre-treated for 24 hr with vehicle, or DMBA with and without the PPARγ activating drug rosiglitazone (ROSI). Treated cells will be evaluated for metastatic changes using an invasion assay (modified Boyden chamber). Total RNA will also be isolated from treated cells and screened for global miR expression changes. These results will be correlated to define PPARγ-dependent miR expression changes that predict metastatic potential. Putative miR candidates include pro-tumourigenic hsa-miR-9 and -21, and anti-tumourigenic hsa-miR-29b, -125b, and -205.

Results: Our preliminary results suggest parental 231 cells have significantly increased invasion potential when treated with DMBA, which is significantly abrogated in the presence of ROSI. Using the public miRanda algorithm database, we have also identified several pro-tumourigenic miRs that may target PPARγ for downregulation during breast tumour metastasis. Further cell line transductions and miR expression analyses are currently underway.

Conclusions: Preliminary data suggest DMBA enhances human breast cancer cell invasion, which may explain why breast tumours grow and spread in some but not all patients. The anti-metastatic effects of co-treating with the PPARγ activator ROSI also suggest that these drugs may be clinically useful as a novel way to treat breast cancer patients. With additional analyses, our studies will unveil PPARγ-dependent miR signaling patterns that may help predict which patients are at increased risk for developing metastatic breast cancer.

30 DIETARY EXPOSURE TO FLUORIDE FROM WINES

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Introduction: Fluoride (F) is an essential and toxic element for health. Values have been established regarding the Recommended Daily Intake (RDI) and Acceptable Daily Intake (ADI). The largest source of incorporation of F is water, but it can be found in other foods and beverages, such as wine. F can be found naturally occurring in wines at concentrations below 1 mg/L, the maximum limit recommended by the International Organization of Vines and Wine (OIV). The RDI values set for adults by the EFSA (European Food Safety Authority) for F are 3.4 and 2.9 mg/day for men and women, respectively. The ADI established for F by the Food and Nutrition Board of the Institute of Medicine is 10 mg/day. The data provided by the Spanish Agency of Consumer Affairs, Food Safety and Nutrition (AECOSAN) states that the average adult consumption of "table wines" is 111.72 mL/day.

Objectives: To establish the F concentration in organic and non-organic Spanish wines (mainland vs Canary Islands). To estimate F dietary intake derived from wine consumption and to evaluate the contribution of these dietary exposure levels to the RDI and ADI of moderate wine consumption.

Method: A total of 53 samples of red, white and rosé wines, both organic and non-organic, from different Spanish appellations of origin (mainland and Canary Islands) were analyzed by potentiometric determination with ion-selective electrode for F.

Results: None of the analyzed wines was found to have F concentrations exceeding 1 mg/L and were well below this value. The average concentration for mainland Spanish wines is 0.10 mg/L. The average concentration in non-organic Canary Islands wines is 0.13 mg/L. The white wines had a higher average F concentration. The average F concentration in organic wines is 0.20 mg/L. In general, the organic wines have higher F concentrations. Considering a 117.72 mL/day of wine consumption, the F concentrations observed (0.10; 0.13 and 0.20 mg/L for non organic Spanish mainland wines, non organic wines from the Canary Islands and organic Spanish wines, respectively) suggest a F estimated dietary intake (EDI) for adults of 0.011 mg/day for non organic Spanish mainland wines; 0.015 mg/day from non organic Canary Islands wines and 0.022 mg/day for Spanish organic wines. The percentages of wine consumption (117.72 mL/day) contribution to the F recommended dietary intake are: 0.32 and 0.38% for men and women after consuming non organic Spanish mainland wines; 0.44 and 0.52% for men and women after consuming non organic wines from the Canary Islands, and 0.65 and 0.76% for men and women after consuming organic Spanish wines. These results suggest that wine is a poor source dietary source of the recommended intakes of F. These percentages do not represent any toxicological risk to the health of adult consumers.

Conclusions: F intake from wine poses no risk to the health of adult wine moderate consumers.

31. COULD EDIBLE MUSHROOMS BE A DIETARY SOURCE OF TOXIC METALS?

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Background: Edible mushroom consumption, in terms of quantity and diversity (new species), is increasing. Due to their capacity to capture metals there is a toxicological interest about their metal content. Literature presents concentrations of metals in very few species (*Agaricus bisporus, Lentinula edodes and Lactarius deliciosus*) and no references concerning the type of packaging have been found, as the most frequent references only determined metals in fresh mushrooms. Pb and Cd levels in foods and dietary exposure to these metals are regulated by the European Food Safety Authority (EFSA). A TWI of 1 mg Al/kg bw/week, a PTWI of 2.5 µg Cd/kg bw/week and a reference value of 0.5 µg Pb/kg bw/day have been set.

Objectives: To determine 3 metals of toxicological interest (Al, Pb, Cd) in packed edible mushrooms sold in Spain by species (*Agaricus bisporus, Lactarius deliciosus, Lentinula edodes, Pholiota nameko and Pleurotus ostreatus*) and commercial presentation (glass vs metallic); to estimate the contributions of a daily mushroom consumption to the tolerable intake limits (TWI, PTWI and reference value) of these metals.

Methods: 96 samples of 5 edible mushrooms species sold in Spain either in glass or metallic containers were analyzed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) using Thermo Scientific spectrophotometer ICAP 6300. The dietary assessment exposure evaluation has been based on the adult consumption data on mushrooms (4.55 g/day).

Results: Al has the highest average value of 17.8 ± 6.09 mg/Kg (range: 3.21-36.2) followed by Pb with an average of 0.08 ± 0.06 mg / kg (range: 0.03-0.49) and Cd whose average is 0.005 ± 0.01mg/kg (range: ND-0.04). Al, Cd and Pb levels (mg/kg) were 18.2; 0.006 and 0.08 mg/kg for *Lactarius deliciosus*; 19.3; 0.002 and 0.08 for *Pholiota nameko*; 16.8; 0.009 and 0.09 for *Lentinula edodes*; 18.1; 0.004 and 0.1 for *Pleurotus ostreatus* and 16.8; 0.002 and 0.07 for *Agaricus Bisporus.* The average levels of both Pb and Cd are below the limits set by Regulation (EC) Nº1881/2006, which stipulates a maximum content of 0.30 and 0.20 mg/kg, respectively, for these metals. Significant differences in the concentration of Cd (0.009 ± 0.01 mg/kg) have been detected for *Lentinula edodes* with both its mean and its standard deviation being higher. This is consistent with previous results that suggest that *Lentinula edodes* is one of the main accumulator species of Cd. According to the commercial presentation, the mean metal contents are: glass container: Al (18.4 ±7.21); Pb (0.09±0.08); Cd (0.004±0.01) mg/kg; metallic container: Al (17.3±4.79); Pb (0.08±0.03); Cd (0.007 ±0.003) mg/kg. Statistically significant differences were only found in the Cd concentration. Overall contributions to the tolerable dietary intakes are insignificant, a fact conditioned to their low consumption, with Al being the greatest contribution the (average contribution of 0.85% of the TWI).

CONCLUSIONS: The studied edible mushrooms are a poor dietary source of toxic metals and therefore, can be considered as safe foods and pose no toxicological risk for consumers at the actual consumption rate. Nevertheless, monitoring the toxic metal content in mushrooms from contaminated areas or metal

intake in high consumers should not be disregarded. Maximum limits should be regulated for Al in mushrooms and other Al dietary sources. The species is a determining factor and exposure assessment studies should be designed considering the most consumed individual species, the different population groups and the dietary habits of the studied community. Consumers welcome scientific opinions that help choosing a type of container in terms of health protection.

32. A BREAST TUMOUR ANGIOGENIC ROLE FOR PPARγ SIGNALING

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Background: Angiogenesis is a key contributor to breast tumour metastasis, the main cause of patient deaths. We showed that peroxisome proliferator-activated receptor (PPAR) γ signaling in mammary stromal endothelial cells (ECs) suppresses 7,12-dimethylbenz[*a*]anthracene (DMBA)-mediated breast tumourigenesis *in vivo*. PPARγ activating drugs reportedly have anti-tumourigenic and anti-angiogenic effects, but the role of PPARγ signaling during breast tumour angiogenesis is unknown. We hypothesized that EC targeted loss of PPARγ would increase angiogenic signaling during breast tumour angiogenesis.

Objectives: Using our unique EC-targeted PPARγ-knockout (PPARγ-EC^{KO}) mice and their congenic controls (WT), we evaluated the angiogenic role of EC-specific PPARγ during *in vivo* breast tumourigenesis, and *in vitro* angiogenic stimulation of ECs from these mice.

Methods: Serum was collected from (n=5/group) 8-12 week old untreated mice, or (n=4/group) mice at necropsy post treatment with DMBA (1 mg/week p.o.) for 6 weeks and at continued at week 7 on normal chow diet (DMBA Only), or one supplemented with a PPARγ ligand (rosiglitazone, 4mg/kg/day; DMBA+ROSI) for 25 weeks. Expression changes were assessed using a Mouse Cytokine 23-plex serum assay kit. Formalin-fixed, paraffin-embedded breast tumours collected from mice treated as above were immunofluorescently labeled with a CD31 antibody to quantitate vascularity changes. Aortic rings isolated from untreated WT and PPARγ-EC^{KO} mice were analyzed for early angiogenic changes post treatment with VEGF ± ROSI using an aortic EC sprouting assay. Matrigel plugs with VEGF ± ROSI, or conditioned media from human MDA-MB-231 breast cancer cells treated for 24 h with vehicle or ROSI, were injected into mouse mammary fat pads of (n=3 mice/group), and assessed for vascularity changes after 7 days of implantation.

Results: PPARγ-EC^{KO} serum expression of several interleukins (5, 6, 10, and 17α), chemotaxins (eotaxin, CXCL1, MCP-1, and MIP-1β), and inflammatory factors (G-CSF, GM-CSF, and TNFα) were significantly lower versus WT mice ($p<0.05$). ROSI co-treatment significantly increased IL-1a and decreased IL-10 in PPARγ-EC^{KO} but not WT mice ($p<0.05$). VEGF-treated aortae from PPARγ-EC^{KO} mice showed a trend toward higher EC sprouting vs. WTs. ROSI co-treatment reduced EC sprouting in WTs but not PPARγ-EC^{KO} aortae. Vascular changes in breast tumours and matrigel plugs are currently being analyzed.

Conclusions: These data are the first evidence that loss of EC-targeted PPARγ alters the angiogenic environment during breast tumourigenesis, and support an early anti-angiogenic role for activating PPARγ signaling in breast cancer patients.

33. OZONE INHALATION ALTERS GLUCOSE METABOLISM AND SIGNALLING IN ADIPOSE TISSUE: ROLE OF GLUCOCORTICOIDS

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Background: Air pollution is associated with increased incidence of metabolic disorders including type 2 diabetes and obesity. We have recently shown that common air pollutants (airborne particulate matter, ozone) activate the hypothalamic-pituitary-adrenal axis, resulting in increased release of glucocorticoids (corticosterone in rodents, cortisol in humans) that exert profound effects on metabolic and inflammatory processes.

Objectives: In the present study we examined the role of glucocorticoids in mediating effects of ozone on clearance of glucose from the blood (an indicator of metabolic dysfunction) and on signaling in adipose tissues (important metabolic and endocrine organs).

Methods: Male Fischer-344 rats were exposed by inhalation to air or 0.8 ppm ozone for 4 h with or without metyrapone (0, 50, 150 mg/kg body weight), a drug that blocks production of corticosterone. A glucose tolerance test was used to measure clearance of administered glucose immediately after exposure. Transcript levels of genes implicated in glucose metabolism were assessed in epididymal and brown adipose tissues immediately or 24 h after exposure.

Results: Compared to air-exposed animals, ozone slowed the clearance of glucose from the blood, an effect that was exacerbated in animals administered metyrapone, but did not affect insulin levels. Ozone exposure decreased levels of genes involved in insulin signalling (e.g. insulin receptor substrate 1) and increased a sensor of low cellular glucose (hypoxia-inducible factor-3α) in adipose tissue, effects that were blocked by metyrapone.

Conclusion: Our results indicate that acute exposure to ozone affects glucose metabolism, including effects on biological pathways in adipose tissues relevant to insulin sensitivity and glucose metabolism that appear to be mediated, at least in part, by glucocorticoid action. Given the link between stress, glucocorticoids, and metabolic disorders, the results suggest that impacts on glucocorticoid production resulting from exposure to air pollutants could contribute to disease processes.

34. A TALE OF THREE TOXICANTS: THE EFFECTS OF BUTYLPARABEN AND PROPYLPARABEN ON THE DISTRIBUTION OF BISPHENOL A

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Background: Individuals in developed countries are exposed to multiple environmental chemicals, including bisphenol A (BPA), the monomer of polycarbonate plastics, as well as butylparaben and propylparaben, preservatives used in personal care products. All three of these chemicals are absorbed into circulation and reliably detected in human tissues and excretions. Although BPA is an established endocrine disruptor capable of binding estrogen receptors, evidence of endocrine disruption induced by butylparaben and propylparaben is limited.

Objectives: The objective of the present study was to determine whether butylparaben and/or propylparaben can modulate the distribution of BPA.

Methods: We subcutaneously injected female and male mice with 0, 1, 3, or 9 mg butylparaben or propylparaben, then administered 50 :g/kg ^{14}C-BPA in a dietary supplement, and subsequently measured levels of radioactivity in serum and tissues via liquid scintillation counting.

Results: In both females and males, butylparaben was a more effective modulator of BPA concentrations than was propylparaben. In females, doses as low as 1 mg butylparaben or 9 mg propylparaben were sufficient to elevate BPA concentrations in the uterus. In males, doses as low as 3 mg butylparaben were sufficient to elevate BPA concentrations in the epididymides, whereas propylparaben showed no effects.

Conclusions: These data show that BPA preferentially localizes to the uterus of females and the epididymides of males, consistent with the high expression of estrogen receptors in these tissues. They also indicate that butylparaben, and to a lesser extent propylparaben, can exacerbate the presence of BPA *in vivo*. This interaction is consistent with *in vitro* evidence that parabens inhibit enzymes responsible for the metabolism and excretion of BPA.

35. ROLE OF THE ANTIOXIDANT RESPONSE ELEMENT/ELECTROPHILE RESPONSE ELEMENT IN THE RESPONSE TO TOXIN EXPOSURE

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Background: Exposure to toxins often means exposure to more than one stress. This includes oxidative stress as many toxins are prooxidants. The toxin may also initiate endoplasmic reticulum (ER) stress (often referred to as the unfolded protein response) which leads to cell apoptosis. In response to oxidative stress, a key element involved in the regulation of antioxidant and detoxification genes is the antioxidant response element (ARE)/electrophile response element (EpRE) bound by nuclear factor erythroid 2-like/musculoaponeurotic fibrosarcoma oncogene (Nrf/Maf) heterodimers.

Objectives: This study aimed to answer the question "does Nrf1, a transcription factor bound to the ER and activating the antioxidant and ER stress responses, has a role in the stress response in mammalian cells exposed to sample toxins (polybrominated diphenyl ether (PDBE) flame retardants)". Activation of AREs/EpREs in response to penta-brominated PDBEs (penta-BDE or BDE-99) was assessed in mammalian cell lines. Protein (carbonyl groups) and lipid (8-isoprostanes) damage from oxidative stress, in response to penta-PBDEs was also assessed.

Methods: Human embryonic kidney (HEK293) cells were exposed to various concentrations of penta-PBDEs for various lengths of time. Cell viability was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Activation of AREs/EpREs was assessed by luciferase assays, with and without overexpression of the Nrf1 transcription factor. Nrf1 protein levels (and all of its truncated forms), in response to penta-PBDE treatment, were determined by Western blots. Protein carbonyl groups were measured using 2,4-dinitrophenylhydrazine (DNPH) conjugation. Lipid damage was measured using an 8-isoprostane ELISA kit. Senescence-associated β-galactosidase activity was measured by flow cytometry to determine if oxidative stress affected aging parameters in cell lines.

Results: The I.C.50 of penta-PDBEs was approximately 20 μM for 5.0×10^5 cells per well in a 6-well plate. Penta-PBDE treatment resulted in a) both increased Nrf1 and Nrf2 protein expression, b) an increase in ARE/EpRE transactivation activity, c) increased generation of protein carbonyl groups, d) an increase in 8-isoprostane production, and e) increased levels of β-galactosidase activity in flow cytometry.

Conclusion: This study shows an increased antioxidant response, stimulated by oxidative stress, with penta-PBDE treatment. This oxidative stress was also accompanied by an increase in cellular senescence parameters. It shows that the Nrf response is integral to the complete cellular response to toxins and that PDBEs induce oxidative stress in a cell culture model system. This study will present the importance and role(s) of the Nrf proteins in the response to toxins and how their function is essential for the activation of detoxification pathways. Future studies will focus on the activation of the ER stress response with exposure to penta-PBDEs through the Nrf proteins that are activated by the multivariate stresses which the toxins present.

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