47th Annual Symposium / Le 47e Symposium Annuel

8th – 10th December 2015
The Westin Ottawa
11 Colonel By Drive
Ottawa, Ontario
http://www.thewestinottawa.com

MODERN TOXICOLOGY ESSENTIALS: MODELS, TOXICOGENOMICS, POPULATIONS & SAFETY (WHAT HAVE WE LEARNED IN 40 YEARS?)

ESSENTIELS DE TOXICOLOGIE MODERNE: MODÈLES, TOXICOGENOMIQUE, POPULATIONS ET SÉCURITÉ (QU'AVONS NOUS APPRIS EN 40 ANS?)

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

Programme Committee / Comité du programme
Christopher Nicol, Queen's University, Chair and Academic Member
Leanne Bedard, Bedard ADME-Tox Solutions, Industry Member
Sabina Halapannavar, Health Canada, Government Member

Table of Contents • Table des Matières

La Société de Toxicologie du Canada tient à remercier les organisations suivantes pour leurs précieuses contributions et le soutien financier qui appuient la réussite de notre Symposium Annuel.

GOLD • OR

Charles River Laboratories

SILVER • ARGENT

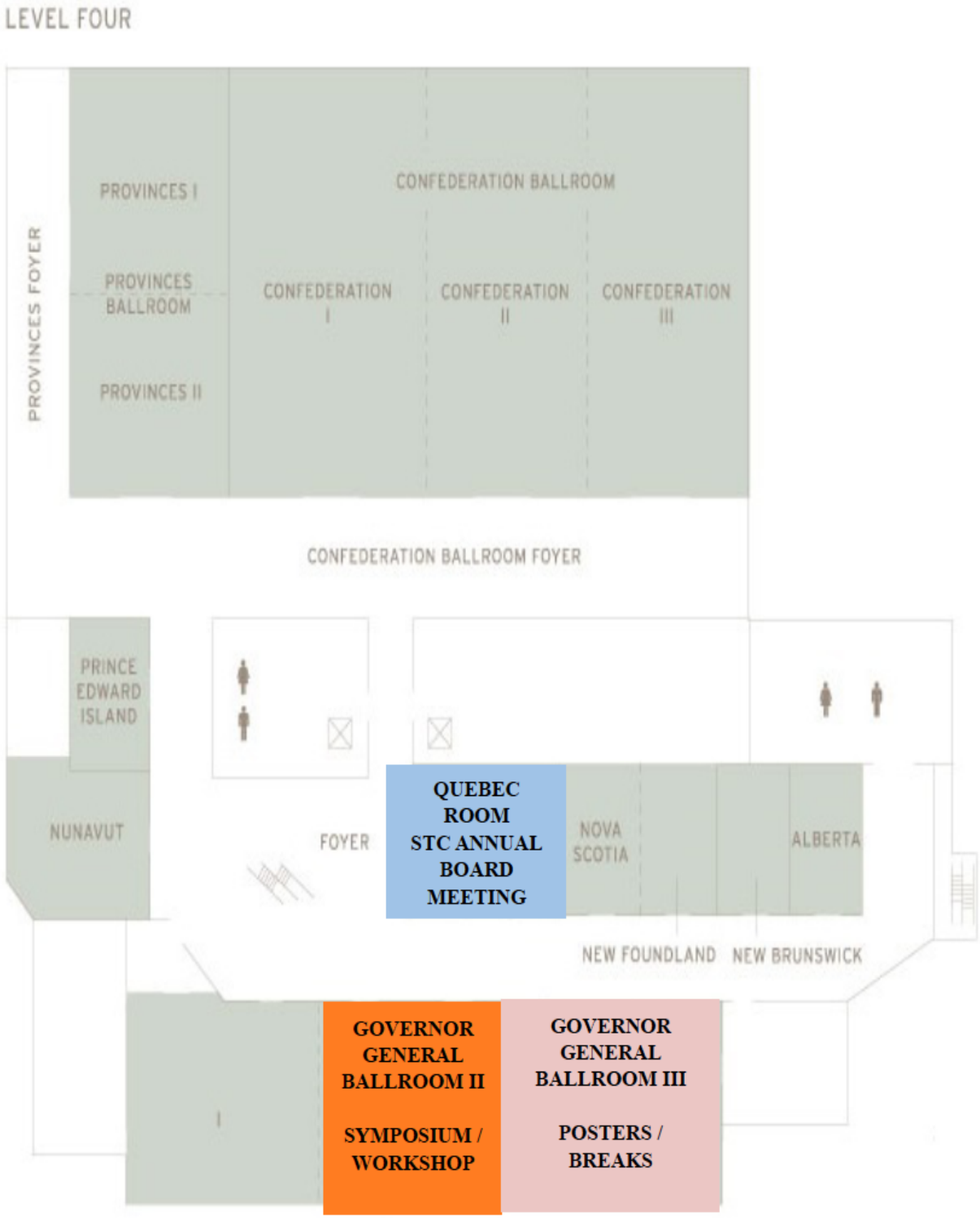
Intertek Canada
Syngenta Crop Protection Canada
CiToxLAB
Canadian Science Publishing/FACETS
Algorithme Pharma, An Altasciences Company

BRONZE

David Josephy Teaching & Consulting Services
Bedard ADME-Tox Solutions
EY

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.

VENUE INFORMATION / PLAN DES SALLES DE CONFÉRENCES
Westin Hotel, Ottawa



RECEPTION INFORMATION / ACTIVITÉS DE RÉCEPTION ET D'ACCUEIL

Welcome Reception / Réception De Bienvenue

Café Nostalgica, 601 Cumberland Street, on the University of Ottawa Campus. http://cafenostalgica.ca/
Tuesday 8th December 2015, starting at 7pm

Open to all registrants of the symposium. Trainees at 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 in 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 au gouvernement, pour discuter des options de carrière et pour faire route en milieu professionnel.. Un repas léger sera servi.

Directions:

- From the Westin: https://goo.gl/maps/494NK4k9NQF2
  ~ 11 min walk from the Westin

President's Reception / Réception du Président

Heart & Crown Pub, 67 Clarence Street (Byward Market), Ottawa
Wednesday 9th December 2015, starting at 6pm

This reception is open to all registrants of the symposium. Please join us for the presentations of awards, and test your knowledge of general toxicology, STC talks and posters 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, des conférences et des affiches STC! Un repas léger sera servi.

Directions:

- From the Westin: https://goo.gl/maps/8qEUe2tQaA52
- ~8 min walk from the Westin

Tuesday, 8 Dec. / Mardi, 8 Déc. PM

2:00 – 5:00 STC Board Meeting (Board Members Only), Tunney's Pasture, Ottawa, Ont.

7:00 Welcome Reception / Réception De Bienvenue – (All Registrants) Café Nostalgica, 601 Cumberland St., U. Ottawa Campus, Ottawa, Ontario

Wednesday, 9 Dec. / Mecredi, 9 Déc. AM

7:30 Registration / Breakfast – *Sponsored by Syngenta Canada*

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

8:30 *Opening Remarks*
David Josephy, STC President, University of Guelph, Guelph, Ontario

8:35 **SESSION I: Emerging Safety Assessment Concepts**
Chair: **Leanne Bedard**, Bedard ADME-Tox Solutions, Montréal, Québec
Introduction

8:40 **Thomas A. Baillie**, University of Washington, Seattle, Washington
Reactive Drug Metabolites and Targeted Covalent Inhibitors: A Risk-Benefit Perspective

9:10 **Tara Barton-Maclaren**, Health Canada, Ottawa, Ontario
Integrating New Approaches to Support Risk Assessment under Canada's Chemicals Management Plan

9:40 **INVITED STUDENT PLATFORM PRESENTATIONS**
9:45 **Julie Buick**, *Poster #7*

9:55 **Tanya Lalvani**, *Poster #21*

10:10 **Coffee Break and Poster Session (*Judging*)**

10:55 **SESSION II: Mallé Jurima-Romet Memorial Symposium**
Chair: **Lena King**
Introduction

11:00 **Denis Grant**, University of Toronto, Toronto, Ontario
Pharmacogenetics and Drug-induced Hepatotoxicity

11:30 **J. Fred Pritchard**, Celerion, Ebony, Virginia
Assessing Safety Risk in Early Clinical Studies: Science or Art?

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

Wednesday, 9 Dec. / Mecredi, 9 Déc. PM

12:55 **SESSION III: Clinical and Population Exposure Study Insights**
Chair: **Sabina Halapannavar**, Health Canada, Ottawa, Ontario
Introduction

1:00 **Tye Arbuckle**, Health Canada, Ottawa
The Maternal-Infant Research on Environmental Chemicals (MIREC) Research Platform: The Early Life Exposome

1:30 **Graham Wood**, Algorithme Pharma, Laval, Québec
Moving from Preclinical to Clinical Studies – The Right Dose

2:00 **Dr. Rebecca Auer**, Ottawa Hospital Research Institute, Ottawa, Ontario
Therapeutic Use of Oncolytic Viruses in Perioperative Period for Cancer Patients

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

3:00 **SESSION IV: KEYNOTE SPEAKER**
Chair: **David Josephy,** University of Guelph, Guelph, Ontario
Introduction

Moshe Szyf, McGill University, Montréal, Québec
Implications of New Discoveries in Epigenetics for Toxicology; Life-Long and Transgenerational Implications of Transient Exposures

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

4:00 **TRAINEES – Mentoring/Networking Workshop,**
Chairs: **Holly Campbell**, Health Canada, Ottawa, Ontario
Katherine Crewe, Women in Biotech, Montréal, Québec, and
Laurie Chan, University of Ottawa, Ottawa, Ontario

6:00 **President's Reception / Réception du Président**
Heart and Crown Pub, 67 Clarence Street, Ottawa, Ontario
STC Awards and ToxTrivia

Thursday, 10 Dec. / Jeudi, 10 Déc. AM

7:30 Breakfast – *Sponsored by Bedard ADME-Tox Solutions and David Josephy Teaching & Consulting Services*

8:25 **SESSION V: Progress in Developmental Toxicity Models**
Chair: **Angela Hofstra**, Syngenta, Guelph, Ontario
Introduction

8:30 **Elise Lewis**, Charles River, Pennsylvania
Alternative Approaches to Reproductive, Developmental and Juvenile Toxicity Testing

9:00 **Timothy J. Shafer**, US EPA, Research Triangle Park, North Carolina
Using Neural Networks Grown on Microelectrode Arrays ("Brains on a Chip") to Screen Chemicals for Potential Developmental Neurotoxicity

9:30 **Leena Hilakivi-Clarke**, Georgetown University, Washington, DC
Maternal Exposure to Estrogen-like Compounds During Pregnancy Alters Offspring Breast Cancer Risk

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

10:30 **SESSION VI: Gabriel Plaa Award Lecture**
Chairs: **Robin Walker,** CanBioPharma Consulting Inc, Mississauga, Ontario
Leanne Bedard, Bedard ADME-Tox Solutions, Montréal, Québec
Introduction

Thomas E. Massey, Queen's University, Kingston, Ontario
Pulmonary Toxicity of Cardiac Drugs—and Some Other Thoughts

11:10 **INVITED STUDENT PLATFORM PRESENTATIONS**
Chair: **Christopher Nicol**, Queen's University, Kingston, Ontario
Introduction

11:15 **Shaimaa Ahmed**, *Poster #1*

11:25 **Élyse Caron-Beaudoin**, *Poster #9*

11:35 **Rebecca Maciver**, *Poster #25*

11:45 **Shinjini Pilon**, *Poster #30*

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

Thursday, 10 Dec. / Jeudi, 10 Déc. PM

12:55 **SESSION VII: Advances in the Use of Toxicogenomics**
Chair: **Christopher Nicol**, Queen's University, Kingston, Ontario
Introduction

1:00 **Aurore Varela**, Charles River Laboratories, Montréal, Québec
Assessment of Bone Tissue in Preclinical Toxicology Studies

1:30 **Robert Turesky**, University of Minnesota, Minneapolis, Minnesota
Mass Spectrometry-Based Methods to Biomonitor DNA Adducts of Carcinogens

2:00 **Darrell Boverhof**, Dow Chemical Company, Midland, Michigan
Toxicogenomics Applied To Risk Assessment- Learning From The Past To Guide The Future.

2:30 *Closing Remarks*
David Josephy, STC President, University of Guelph, Guelph, Ontario

2:35 *Closing Ceremony*
Mr. Gordon Williams, Ottawa, Ontario

2:40 *Meeting Adjourned*

BRIEF BIOGRAPHIES OF PLENARY SPEAKERS (in order of presentations)

COURTES BIOGRAPHIES DES CONFÉRENCIERS PLÉNIERS (dans l'ordre des présentations)

Session I – Emerging Safety Assessment Concepts (Wed AM)

Thomas A. Baillie, Ph.D.

Dr. Thomas A. Baillie is Dean Emeritus of the School of Pharmacy at the University of Washington in Seattle, WA, where he also served as Vice Provost for Strategic Initiatives. He was born in Scotland and educated at the University of Glasgow, where he earned B.Sc. (Hons) and Ph.D. degrees in Chemistry in 1970 and 1973, respectively. He also holds an M.Sc. degree in Biochemistry from the University of London (1978) and was awarded the degree of D.Sc. in Chemistry from the University of Glasgow in 1992.

Following postdoctoral research at the Karolinska Institute in Stockholm, Sweden (1973-75), Dr. Baillie held successive faculty positions at the University of London (1975-78), University of California San Francisco (1978-81), and University of Washington (1981-94). He then joined Merck Research Laboratories in West Point, PA, where he was Global Vice President of Drug Metabolism & Pharmacokinetics until 2008, at which point he returned to the University of Washington to assume his teaching and administrative positions.

Dr. Baillie's research interests center on the application of mass spectrometry and allied techniques to mechanistic studies on the metabolism of foreign compounds, with particular emphasis on the generation of chemically-reactive, potentially toxic products of biotransformation. He has co-authored over 200 peer-reviewed publications, serves on the Advisory Boards of a number of journals and academic research centers, and acts as a consultant to several companies in the pharmaceutical and biotechnology industries.

Dr. Baillie was awarded a Fogarty Senior International Fellowship from the NIH in 1988, was the recipient of the James R. Gillette Award from the American Society for Pharmacology & Experimental Therapeutics (2001), and received the Lifetime Achievement Award from the International Isotope Society (2009). In 2010, he was elected as a Fellow of the Royal Society of Chemistry and a Fellow of the Japanese Society for the Study of Xenobiotics. In 2011, Dr. Baillie became a Fellow of the American Chemical Society and, in 2012, he received the Founder's Award from the ACS Division of Chemical Toxicology. Most recently, he was the 2014 recipient of the North American Scientific Achievement Award from the International Society for the Study of Xenobiotics.

Session I – Emerging Safety Assessment Concepts (Wed AM)

Tara Barton-Maclaren, Ph.D.

Dr. Tara Barton-Maclaren is Manager of the Hazard Methodology Division in the Existing Substances Risk Assessment Bureau, Healthy Environments and Consumer Safety Branch of Health Canada since September 2012. As the focal point for hazard assessment expertise in the Bureau, the division follows advancements in toxicology and risk assessment and works toward the development of strategies for the integration of emerging data and novel methodologies for the assessment of chemicals existing in the Canadian marketplace. In support of the global transition to 21st Century Toxicology, she participates in initiatives under the Organization for Economic Cooperation and Development (OECD) and engages in various scientific collaborations both nationally and internationally in the areas of QSAR, Adverse Outcome Pathways (AOPs), Integrated Approaches to Testing and Assessment and new approaches to support regulatory decision-making.

Dr. Barton-Maclaren joined Health Canada in the Existing Substances Risk Assessment Bureau in 2007 and has led various risk assessment files as well as hazard methodology initiatives. She obtained her BSc Honours from the University of Guelph with a specialization in Biomedical Science in 2000 and her PhD in Reproductive Toxicology from McGill University, Montreal, Quebec in 2007.

Session II – Mallé Jurima-Romet Memorial Symposium (Wed AM)

Denis Grant, Ph.D.

Dr. Denis Grant received his B.Sc. in Biochemistry from McMaster University and his Ph.D. in Pharmacology from the University of Toronto, under the supervision of Werner Kalow. Following a postdoctoral fellowship in molecular pharmacogenetics with Urs Meyer at the Biozentrum of the University of Basel, he was appointed as a Scientist in the Research Institute of the Hospital for Sick Children, and as an Assistant Professor in the Departments of Pharmacology, Pediatrics and Pharmaceutical Sciences at the University of Toronto. In 1999 he took a position as Senior Director of Pharmacogenetics at Orchid Biosciences in Princeton, New Jersey, and in 2002 he returned to the University of Toronto to become Chair of the Department of Pharmacology & Toxicology from 2002 to 2012. He is currently a Professor in Pharmacology & Toxicology and Pharmaceutical Sciences at the University of Toronto.

Dr. Grant's research career has focused on the study of interindividual variation in drug and chemical response and toxicity, including the first studies on the pharmacogenetics of caffeine metabolism and subsequent work to elucidate the molecular genetics and toxic consequences of human acetylation polymorphisms in human populations. He has over 100 peer-reviewed articles, books and book chapters to his credit, and has been an invited speaker at almost 100 international and national conferences and venues. His current research utilizes genetically modified mouse models and cell culture systems to investigate the mechanisms of cellular toxicity associated with exposure to the aromatic amine and nitrosamine classes of chemicals.

Session II – Mallé Jurima-Romet Memorial Symposium (Wed AM)

J. Fred Pritchard, Ph.D.

As Vice President of Drug Development Services at Celerion, Dr. J. Fred Pritchard leads a global team of drug development and regulatory affairs experts, project management professionals and alliance managers that work actively with clients to bring their drug products efficiently through early clinical testing.

Dr. Pritchard brings 35 years of drug development experience to his work, including pre-registration work on five currently marketed pharmaceuticals and several that are currently in clinical development. Leveraging his experience in leading international teams of scientists at large pharmaceutical companies, Fred founded and led a group of consultants and program directors within a full service CRO that collectively worked on nearly 40 integrated drug development programs, bringing several of these through IND into phase 1 and 2 clinical trials. Dr. Pritchard has given numerous invited lectures at scientific meetings and academic institutions and is an author on over 130 scientific publications and abstracts. He was the 2000 Alumni Fellow of the Penn State College of Medicine, and named one of nine "Notable People in Pharmaceutical R&D" by R&D Directions magazine in 2009. He currently serves on the Therapy Acceleration Program Committee for the Leukemia and Lymphoma Society (LLS), the LLS North Carolina Chapter Board of Trustees and the Executive Committee of the Research Triangle Park Drug Metabolism Discussion Group where he has been past Chair and Treasurer.

Prior to his current role with Celerion, Dr. Pritchard held positions at MDS Pharma Services, GlaxoSmithKline, GlaxoWellcome, Glaxo, and McNeil Pharmaceutical. He was trained in Life Sciences and Pharmacology (B.Sc (Hons), M.Sc., Queen's University, Canada) and Clinical Pharmacology (Ph.D, The Pennsylvania State University College of Medicine).

Session III – Clinical and Population Exposure Study Insights (Wed PM)

Tye E. Arbuckle, Ph.D.

Dr. Tye Arbuckle received her PhD in Epidemiology from the University of North Carolina and is currently a senior epidemiologist and research scientist with the Population Studies Division, Healthy Environments and Consumer Safety Branch of Health Canada. She is an adjunct professor with the School of Epidemiology, Public Health and Preventive Medicine at the University of Ottawa and the Department of Public Health Sciences at Queen's University. Dr. Arbuckle is an associate editor with the Journal of Exposure Science and Environmental Epidemiology. Her current research interests involve assessing exposure to environmental chemicals and potential health effects during pregnancy and early life.

Session III – Clinical and Population Exposure Study Insights (Wed PM)

Graham Wood, Ph.D.

Dr. Graham Wood is currently the Executive Vice President of Phase I Clinical Development at Algorithme Pharma, an Altasciences Company. Since finishing graduate school, Dr. Wood has focused on clinical pharmacology and has had the chance to work on three new molecular entities from toxicology to Phase IIa, over twenty compounds from first-in-human to Phase IIa and well over 200 clinical pharmacology studies. Dr. Wood has worked at a number of the leading CROs where he consulted with pharmaceutical and biotechnology companies on speeding up the clinical development process, in particular in going from first-in-man to proof of concept. He has published and presented these techniques at a number of conferences. He received his Hon BSc from McMaster University in biochemistry and his PhD from McGill University in neurology, as well as completing a Fellowship at the National Institute for Mental Health in Washington.

Session III – Clinical and Population Exposure Study Insights (Wed PM)

Rebecca Ann C. Auer, B.Sc., M.D., M.Sc., FRCSC, FACS

Dr. Rebecca Auer is a Surgical Oncologist specializing in Colorectal Surgery and Abdominal Sarcomas at The Ottawa Hospital. General Campus. She is an Assistant Professor in the Department of Surgery at the University of Ottawa and a Scientist in the Center for Cancer Therapeutics at the Ottawa Hospital Research Institute.

Dr. Auer's translational research program focuses on understanding the promotion of metastatic disease in the perioperative period, following surgical stress. She runs a research laboratory that studies these therapies in pre-clinical models and is the principle investigator on related clinical trials of perioperative cancer therapies.

Session IV – Keynote Speaker (Wed PM)

Moshe Szyf, Ph.D., FRSC.

Dr. Moshe Szyf received his Ph.D from the Hebrew University Medical School in 1985 and did his postdoctoral fellowship in Genetics at Harvard Medical School. He joined the department of Pharmacology and Therapeutics at McGill University in Montreal in 1989 as an assistant professor and currently holds a James McGill Professorship and GlaxoSmithKline-CIHR Chair in Pharmacology at McGill University and is a fellow of the Royal Society of Canada.

Dr. Szyf is the founding co-director of the Sackler Institute for Epigenetics and Psychobiology at McGill. Dr. Szyf has been the founder of the first "Pharma" to develop epigenetic pharmacology "Methylgene Inc." and the first journal in epigenetics "Epigenetics". The Szyf lab proposed three decades ago that DNA methylation is a prime therapeutic target in cancer and other diseases, and postulated and provided the first set of evidence that the "social environment" early in life can alter DNA methylation launching the emerging field of "social epigenetics". The Szyf lab is interested in understanding basic epigenetic mechanisms and their broad implications in human behavior, health and disease.

Session V – Progress in Developmental Toxicity Models (Thurs AM)

Elise M. Lewis, Ph.D.

Dr. Elise Lewis is the Director, Reproductive and Neurobehavioral Toxicology at Charles River in Horsham, Pennsylvania. She is responsible for recruiting and supervising study directors and principle research scientists, and has an integral role in implementing and overseeing the science and systems to ensure that Charles River maintains its' leadership position in the field of Reproductive, Developmental and Juvenile Toxicology.

Dr. Lewis received her doctorate in Developmental Toxicology from the University of Alabama in Tuscaloosa. She has authored and co-authored multiple publications in various areas of reproductive, developmental, and juvenile toxicology, and has presented at several industry symposia. She also co-edited the first pediatric nonclinical drug testing book entitled "Pediatric Non-Clinical Drug Testing: Principles, Requirements, and Practice". Dr. Lewis is a member of the Teratology Society, Society of Toxicology, European Teratology Society, the American College of Toxicology, and the Mid-Atlantic Reproductive Toxicology Association.

Session V – Progress in Developmental Toxicity Models (Thurs AM)

Timothy Shafer, Ph.D.

Dr. Timothy Shafer received his B.S. from Hope College in 1986 and Ph.D. in Pharmacology and Toxicology from Michigan State University in 1991. He was a postdoctoral fellow in the Neurotoxicology Division of the U. S. EPA from 1991 -1994 and in 1995 became a Principal Investigator. Currently, he is a Principal Investigator in the Systems Biology Branch of the Integrated Systems Toxicology Division of the U.S. EPA. The focus of Dr. Shafer's research career has been to examine mechanisms of neurotoxicant effects mediated through disruption of ion channels and cellular neurophysiology. This has included examination of the actions of heavy metals, PCBs, herbicides and pesticides on neuronal function using a variety of approaches including patch-clamp recordings, oocyte recordings and imaging experiments using ion- or voltage-sensitive dyes. Currently, Dr Shafer's research is focused on using microelectrode arrays to develop medium throughput screens for neurotoxicity and developmental neurotoxicity.

Dr. Shafer has also served as the Acting Assistant Laboratory Director for Human Health Research at the EPA, was on the ALTOX3/NAL study section at NIH and on the Society of Toxicology Program Committee. He currently is an Associate Editor for the journal *NeuroToxicology* and on the Editorial Board of *Toxicology and Applied Pharmacology*.

Session V – Progress in Developmental Toxicity Models (Thurs AM)

Leena Hilakivi-Clarke, Ph.D.

Dr. Leena Hilakivi-Clarke is a professor of Oncology at Georgetown University. She also is a director of Tumor Biology MS program, co-director of Animal Model Shared Resource and chair of the Committee of Appointments and Promotions at the Medical Center. Dr. Hilakivi-Clarke received her PhD in Experimental Psychology in 1987 from University of Helsinki, Finland, where she studied the role of maternal exposures during pregnancy in affecting offspring's later susceptibility to depression and alcohol abuse in preclinical models. Next, during a Fogarty postdoctoral fellowship (1987-1990) at the National Institute of Alcohol Abuse and Alcoholism in Bethesda, Maryland, she studied the effects of alcohol and social stress on aggressive behaviors in mice. In 1991, she joined the Lombardi Comprehensive Cancer Center and was appointed as an Associate Professor of Psychiatry at Georgetown University, Washington DC. Her research focus shifted from behavioral neurosciences towards studying maternal exposures during pregnancy and breast cancer risk in mothers and daughters. In addition, she began to investigate the impact of childhood exposures on breast cancer risk. Exposures include estradiol and synthetic ethinyl estradiol, plant derived estrogenic compounds, dietary fats and obesity. Most of these studies have been done using animal models, but over the past 15 years she has been collaborating with epidemiologists to study if maternal and childhood exposures also affect breast cancer risk in women.

Recently, Dr. Hilakivi-Clarke has discovered that early life exposures to synthetic estrogens and maternal obesity affect response of offspring's mammary tumors to antiestrogen treatment, and she is pursuing studies to identify the mechanisms involved, focusing on epigenetics and unfolded protein response, autophagy and tumor immune responses. Her publication record consists of over 140 journal articles. She is a recipient of multiple research grants through her research career, including being a program director for NCI funded U54 program project entitled "*Timing of dietary exposures and breast cancer risk*" to investigate nutritional modulation of genetic pathways leading to cancer. She currently is a Principal Investigator of RO1 and AICR grants, co-investigator of UO1 grant and project PI of U54 program on systems biology and breast cancer.

Session VI – Gabriel Plaa Award Lecture (Thurs AM)

Thomas E. Massey, Ph.D.

Dr. Thomas Massey received his B.Sc. in Physiology & Pharmacology from the University of Western Ontario and his Ph.D. in Pharmacology & Toxicology from Queen's University. He then pursued postdoctoral studies at the National Institute of Environmental Health Sciences in North Carolina. In 1985, he received a Medical Research Council of Canada Development Grant and returned to the Queen's Pharmacology & Toxicology Department, where he worked his way through the ranks, being promoted to Professor in 1997.

In 2003, Dr. Massey was appointed Head of Pharmacology & Toxicology at Queen's, a position he held until a merger occurred of basic medical science departments in 2011. While Department Head, Dr. Massey led an initiative that resulted in the establishment of a stream in Drug Development and Human Toxicology in the Queen's undergraduate Life Sciences program.

Dr. Massey's research program focusses on biochemical and molecular mechanisms of toxicity, particularly in the lung. His research has been funded continually by tricouncil operating grants since 1985. Dr. Massey has supervised numerous graduate students and post-doctoral fellows. He has received the Basmajian Award for Excellence in Research from the Faculty of Medicine at Queen's, the Merck Frosst Award from the Pharmacological Society of Canada, and the Velyien Henderson Award from the Society of Toxicology of Canada. In 2008, he received the Queen's Life Sciences Students' Choice Teaching Award, and in 2009, he was the sole recipient of the Queen's Faculty of Health Sciences Education Award.

Dr. Massey has served on several provincial and national grant review panels as well as on a number of journal editorial boards, including *Toxicological Sciences, Toxicology,* and *The Canadian Journal of Physiology and Pharmacology*. He has served the STC in a number of capacities, and was Secretary of the Society from 1995 to 2001, during which he established the Society's first website.

Session VII – Advances in the Use of Toxicogenomics (Thurs PM)

Aurore Varela, DVM, MSc, DABT

Aurore Varela is a Scientific Director of Nonclinical Biomedical Imaging at Charles River in Montreal, with more than 13 years of experience in preclinical radiology, imaging, bone biomarkers, biomechanics, study design and conduct in musculoskeletal research and toxicology, and plays a key role in designing and executing studies, and interpreting data relevant to musculoskeletal research, musculoskeletal and metabolic toxicology, and imaging. She has co-authored several research papers in peer-reviewed scientific journals and contributed to chapters in several books related to Musculoskeletal Research and Bone Pathology. She is also a Diplomate of the American Board of Toxicology.

Session VII – Advances in the Use of Toxicogenomics (Thurs PM)

Robert Turesky, Ph.D.

Dr. Robert Turesky is a Professor in the Department of Medicinal Chemistry, and Director of the Masonic Cancer Center's Analytical Biochemistry shared resource, a mass spectrometry facility devoted to the cancer and chemoprevention programs at the University of Minnesota. Dr. Turesky received his B.Sc. in biochemistry from the University of Massachusetts, Amherst, and PhD in nutrition and food science from M.I.T. Prior to this position, Dr. Turesky was Group Leader of the Biomarkers Unit, Nestlé Research Center, Lausanne, Switzerland (1986 – 2000); Division Director of Chemistry, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR, (2000 – 2004); and Principal Investigator, Wadsworth Center, New York State Department of Health (2004 – 2013).

Dr. Turesky's research is focused on the biochemical toxicology of dietary and environmental genotoxicants. Biomarkers of these genotoxicants, such as urinary metabolites, protein- and DNA adducts are established and state-of-the-art mass spectrometric methods are applied to measure these biomarkers in collaborative molecular epidemiology studies that seek to understand the role of chemical exposures in the origin of cancer. Novel techniques are being developed to identify DNA adducts of carcinogens, by MS-based technologies, in formalin fixed paraffin embedded tissues and exfoliated urinary cells, two largely underutilized biospecimens in cancer biomarker research. These bioanalytical mass spectrometric-based approaches will significantly advance our knowledge about chemicals that damage DNA and may be contributing factors to the etiology of cancer.

Session VII – Advances in the Use of Toxicogenomics (Thurs PM)

Darrell R. Boverhof, Ph.D.

Dr. Darrell Boverhof is currently the Director of Product Sustainability Consulting within Dow's Toxicology and Environmental Research and Consulting (TERC) organization. In this role, Darrell has responsibility for all Mammalian and Environmental Toxicology Consulting and Risk Assessment, Consulting Support Services, and Global Safety Data Sheet Support for the company.

Dr. Boverhof joined Dow in 2006 as a Toxicologist within TERC. He has held several technical and leadership roles in TERC including as Leader of the Cellular and Molecular Toxicology group were he was responsible for the initial implementation of Dow AgroSciences' Predictive Toxicology program. In addition, he served as a toxicology consultant for the Epoxy and Epoxy Systems businesses and as the Dow Nanotechnology EH&S focal point. Prior to his current role he was the Global EH&S Product Sustainability Leader for Dow Building & Construction, Dow Energy Materials and Dow Corporate Ventures. In this role he was responsible for facilitating business success through global leadership in product stewardship including prevention of adverse impacts to people and the environment from Dow products and ensuring compliance with regulations and the product aspects of Responsible Care®.

During his career, he has represented Dow externally on the American Chemistry Council's Nanotechnology Panel and the Center for the Polyurethanes Industry, as well as the ILSI-HESI Immunotoxicology Technical Committee. He also serves on the Medical Committee for the Elsa U. Pardee Foundation, the Editorial Board for Toxicological Sciences, and was formerly a Councilor for the Michigan Society of Toxicology. He has published over 35 manuscripts/book chapters and has co-edited a book on toxicogenomics in safety evaluation and risk assessment.

Dr. Boverhof holds a PhD in Biochemistry/Toxicology from Michigan State University and a BSc in Biomedical Toxicology from the University of Guelph, Canada. Prior to his graduate work, he was employed as a Scientific Evaluator with Health Canada where he was involved the categorization and evaluation of chemicals on the Canadian Domestic Substances List.

POSTER ABSTRACTS
(in alphabetical order of presenting authors)

ABSTRAITS DES AFFICHES
(dans l'ordre alphabétique des auteurs)

BISPHENOL S-INDUCED ADIPOGENIC DIFFERENTIATION OF MURINE 3T3LI PREADIPOCYTES REQUIRES PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPAR γ).

Shaimaa Ahmed[1] and Ella Atlas[1].
[1] Environmental Health Science and Research Bureau, Health Canada, 50 Colombine Driveway, Ottawa, Ontario, K1A 0K9, Canada.

Background: The use of bisphenol A (BPA) in consumer goods and food packaging has been associated with negative health outcomes due to its endocrine-disrupting capabilities. This prompted its removal from many products and replacement with other bisphenol analogs. Bisphenol S is one such replacement analogue, but its ability to function as an endocrine-disrupting chemical has not been determined.

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

Methods: Murine 3T3L1 preadipocytes were used to determine the adipogenic potential of BPS. Cells were treated with BPS (0.01-50 μM) and we determined lipid accumulation, mRNA and protein expression of adipogenic markers, and its ability to activate peroxisome proliferator-activated receptor (PPARγ), a key transcription factor involved in adipogenesis.

Results: Our results indicate that treatment of 3T3L1 cells with BPS induces significant lipid accumulation (10-50 μM), increased mRNA and protein expression of key adipogenic markers (1-50 mM) similar to the effects seen with BPA. We show that BPS can upregulate lipoprotein lipase (Lpl), adipocyte protein 2 (aP2), peroxisome proliferator-activated receptor (PPARγ), perlipin (Plin), adipsin, and CCAAT/enhancer-binding protein alpha (Cebpα) expression levels in a dose-dependent manner. Furthermore, using transcriptional assays, we show that BPS functions as a partial agonist for PPARγ in a PPRE (PPARγ response element)-dependent luciferase. Co-treatment of cells with the selective PPARγ antagonist GW9662 inhibits BPS-dependent adipogenic differentiation suggesting the PPARγ is required for mediating adipogenesis caused by BPS treatment.

Conclusions: This is the first study to suggest that BPS can directly activate PPARγ to induce adipogenesis of murine 3T3L1.

EXPOSURE TO A TOXICANT MIXTURE DURING PREGNANCY AND POST-PARTUM: EFFECTS ON PREGNANCY AND CEREBRAL GLIAL CELL COUNTS

Ramez Antoun[1] and Anne T.M. Konkle[1,2].
[1] Interdisciplinary School of Health Sciences, University of Ottawa, Ontario, Canada;
[2] School of Psychology, University of Ottawa, Ontario, Canada.

Background: Environmental toxicants abound. Their neurotoxic effects are often assessed in vulnerable populations such as the fetus and newborn. Many, such as PCBs, methyl mercury and organochlorine pesticides have shown deleterious effects in these populations, when administered individually. Animal models also show effects on bone development, thyroid function and protein changes in the hippocampus and cerebellum when exposed *in utero* to a mixture of these three classes of toxicants, which is a more realistic scenario. However, the effects of these toxicants, or the mixture, have never been assessed on the pregnant dam.

Objectives: Given the importance of maternal care on development of the newborn, the overarching aim of this work is to assess whether pregnancy and maternal care are affected by exposure to this mixture of toxicants during pregnancy and the nursing period. Our more specific objective is to assess the health of the dams during pregnancy and to begin to explore potential changes in the post-partum brain.

Methods: Individually caged female Sprague-Dawley rats (Charles River) were placed with male rats and checked for pregnancy daily. As soon as a positive sperm plug was detected, females were administered daily oral doses (via a cookie) of one of the following: Vehicle (corn oil), low or high (0.01; 1.0 mg/kg) dose or methyl mercury, low or high (0.04; 4.0 mg/kg) dose of the mixture (methyl mercury, PCB, organochlorine pesticides). Dosing occurred until post-partum day 21 at which time the rats were euthanized and organs and brain collected. Body weight was chronicled daily through the dosing period. Pregnancy measures were also assessed at birth or the time of sacrifice. Brains were fixed (4% paraformaldehyde) then sectioned at 30 μm using a cryostat. Free floating immunohistochemistry using a glial fibrillary acetic acid (GFAP) antibody was performed in order to identify mature glial cells in key brain regions involved in maternal behaviour. Cells were counted using ImageJ and the counts compared across groups.

Results: The number of uterine implantation sites and number of live births appeared to be unaffected by any treatment. The distribution of males and females was also not influenced by the treatments. While overall body weights during pregnancy and post-partum appeared unaffected, a closer look reveals that dams having received the high dose of methyl mercury (1.0 mg/kg) had significantly lower body weight versus vehicle dams. GFAP cell counts are currently being assessed in the hippocampus and medial preoptic area, two key areas important in maternal behaviour.

Conclusions: This work is the first to look at the effects of exposure to a mixture of environmental toxicants on pregnancy and post-partum measures, including changes in the brain. A better understanding of brain plasticity during this period will help shed some light on the potential impact of drugs and environmental toxicants on the brain and related behaviours; of particular interest is maternal behaviour, as differences in this behaviour are well documented to differentially influence offspring development both molecularly and behaviourally.

SCREENING OF IN VITRO POTENCIES RELATED TO SOURCE EMISSION PARTICULATE MATTER: TOXICOPROTEOMICS OF J774 MACROPHAGES

Marianne B. Ariganello[1,2], Dharani D. Das[2], Dalibor Breznan[1], Errol Thomson[1], Ngoc Vuong[1], Agnieszka Bielecki[2], Christine MacKinnon-Roy[1], Fred Elisma[2], Renaud Vincent[1] and Premkumari Kumarathasan[2].

[1] Inhalation Toxicology Laboratory, Health Canada, Ottawa, ON, Canada, K1A 0K9;

[2] Analytical Biochemistry and Proteomics Laboratory, Health Canada, Ottawa, ON, Canada, K1A 0K9.

Background: Particulate matter (PM) is a heterogeneous mixture of inorganics and organics found in air pollution. There is clear evidence that ambient PM is associated with adverse health effects; however the relative importance of constituents derived from distinct sources remains uncertain. Evaluating the toxicity of PM is complex because the composition varies based on physicochemical characteristics influenced by source emissions. Particles produced from different sources may elicit different biological responses and a systematic approach is required to screen biological effects related to these source emission particles.

Objective: Our objective was to identify differences in the molecular signature of cells exposed to particles collected from different emission sources across Canada, and correlate these differences to toxic responses in cells. This comprehensive approach will allow discrimination between emission sources and provide insight into processes underlying differences in particle potency.

Methods: Cells were exposed for 24 h to particles with an aerodynamic diameter of 0.1-2.5 μm ($PM_{0.1\text{-}2.5}$) collected from five emission sources across Canada: Hamilton (HB, steel-mill), Sarnia (SR, petrochemical industry), Shawinigan (SW, aluminum smelter), and two sites in Montreal (MA: petrochemical industry; MC: copper refinery); as well as an Ottawa urban air particle (EHC6802) and two reference mineral particles (TiO_2, SiO_2). Cellular cytotoxicity endpoints including resazurin reduction and lactate dehydrogenase (LDH) were measured and cell lysates were collected for proteomic analysis. The complex mixture was simplified using molecular weight fractionation and the 50-100 kDa fraction was digested overnight (trypsin and lys C). The tryptic-digests were analyzed by shot-gun MALDI-TOF-TOF-MS and peptides exhibiting differential responses were subjected to MS/MS analyses for protein identification.

Results: Analyses revealed distinct patterns of protein changes in response to particles from different sources. In general, the most cytotoxic particles (HB, SW, MA) elicited the largest alterations in proteomes, in both number of proteins affected and magnitude of changes. There were major differences between the responses to Montreal particles MA and MC (which share the same geographical vicinity). We identified 30 candidate biomarkers out of 300 identified proteins that will be used to cluster particles from different sources.

Conclusions: Our results demonstrate that proteomic profiling of in vitro cellular responses can discriminate among particles from distinct emission sources within the same air-shed. On-going analyses are aimed at elucidating molecular pathways related to toxicity and associations with chemical composition.

DEVELOPMENT OF AN INTEGRATED APPROACH FOR COMPARISON OF IN VITRO AND IN VIVO RESPONSES TO PARTICULATE MATTER

Dalibor Breznan[1], Subramanian Karthikeyan[1], Marcelle Phaneuf[1], Prem Kumarathasan[2], Sabit Cakmak[3], Michael S. Denison[4], Jeffrey R. Brook[5] and Renaud Vincent[1].
[1]InhalationToxicology Laboratory, Health Canada, Ottawa, ON, Canada, K1A 0K9;
[2]Analytical Biochemistry and Proteomics Laboratory, Health Canada, Ottawa, ON, Canada, K1A 0K9;
[3]Biostatistics and Epidemiology Division, Health Canada, Ottawa, ON, Canada, K1A 0K9;
[4]Department of Environmental Toxicology, University of California, Davis, CA, USA;
[5]Air Quality Processes Research Section, Environment Canada, Toronto, ON, Canada, M3H 5T4

Background: Association of particulate matter with adverse health effects has been established in epidemiological studies and animal experiments. Epidemiological studies are difficult to undertake while animal studies are impractical for high-throughput toxicity testing. The ease and rapidity of in vitro tests emphasizes their potential for use in risk assessment of chemicals and particles.

Objective: Examine the association between in vitro and in vivo responses to ambient particles, to determine the potential of cell-based assays as standalone toxicity screening tools using a novel approach that integrates large sets of toxicity data from cellular bioassays and animal tests.

Methods: Assays of cytotoxicity and key inflammatory mediators were applied to determine the in vitro biological potency of a panel of urban and mineral particles in J774A.1 macrophages and A549 lung epithelial cells. The particles were also screened for the presence of AhR agonists using the Ah receptor-dependent gene induction assay and for endotoxin using the Limulus amebocyte lysate assay. A subset of the particles with a contrasting in vitro toxicity profile was delivered intratracheally in BALB/c mice to assess their in vivo biological potency. Results from various bioassays were combined within the in vitro and in vivo models. The combined potency measures were examined for associations.

Results: Overall, J774A.1 cells were more sensitive to particle effects than A549 cells. Whereas the combined cytotoxicity estimates were highly correlated between the two cell lines, the combined in vitro inflammatory potency estimates were not, emphasizing functional differences of the cell types. Secretion of inflammatory markers by J774A.1 cells was correlated with AhR ligand binding profile and endotoxin levels of particles. Particle instillation led to an acute toxicity response in BALB/c mice, with neutrophilia and release of inflammatory mediators. While the combined toxicity estimates were not correlated between in vitro and in vivo models, the combined inflammatory and integrated potency estimates (toxicity and inflammation) showed a marginal trend towards significance in a correlation within both in vitro and in vivo models, with a ranking of fine particle (DWR1), minerals (TiO_2, CRI) and coarse particles (SRM-, EHC-type) from low to high potency.

Conclusions**:** Integration of in vitro endpoints shows promise in determining adverse outcomes of particle exposures in vivo. The devised approach may prove useful in developing quantitative models for predicting hazard potential of particles however distinct models may be needed for particles of different type (*e.g.*, urban versus mineral particles).

THE EFFECTS OF VALPROIC ACID EXPOSURE ON P300, EGR1 AND STAT3 PROTEIN EXPRESSION IN P19 EMBRYONAL CARCINOMA CELLS

Jordan K. Bricker[1] and Louise M. Winn[1,2].
[1] Department of Biomedical and Molecular Sciences, Graduate Program in Reproductive and Developmental Sciences, Queen's University, Kingston, Ontario, Canada;
[2] School of Environmental Studies, Queen's University, Kingston, Ontario, Canada.

Background: Valproic acid (VPA), a commonly used anticonvulsant drug, is associated with an increased risk of fetal malformations, including neural tube defects (NTDs). Previous *in vivo* results have found that VPA-exposed embryos with a NTD have altered expression of several proteins which are regulated by p300, a histone acetyltransferase (HAT) protein. Egr1 and Stat3, which both function as transcription factors, are regulated by p300 through its HAT activity. Additionally, Egr1, which has been found to play a role in the pluripotent differentiation of P19 embryonal carcinoma (EC) cells, has also been found to regulate p300 expression, leading to crossregulation between these two proteins. Meanwhile, there is also evidence to suggest that Stat3, which has been found to play a role in cellular proliferation, acts upstream of both Egr1 and p300 in other pathways which may help to regulate cellular proliferation. In summary, these three proteins appear to have a complex relationship with many potential implications in cellular proliferation and differentiation, and consequently may be involved in VPA-mediated teratogenicity.

Objective: The objective of our study was to evaluate the *in vitro* effects of VPA exposure on the protein expression of p300, Egr1, and Stat3 in P19 EC cells.

Methods: P19 cells were plated and allowed to adhere for 24 h, and then exposed to various concentrations of VPA (0-5 mM) for 24 h. Western blotting was used to quantify the protein expression of p300, Egr1 and Stat3, with β-actin used as a loading control.

Results: Our study demonstrated that p300 protein expression is significantly decreased as a result of VPA exposure in P19 cells *in vitro*. Additionally, results demonstrated a significant increase in Stat3 protein expression in cells exposed to 5 mM VPA *in vitro*, while the impact of VPA exposure on Egr1 protein expression in P19 cells *in vitro* is ongoing. Additionally, other timepoints (4, 12 and 48 h) are being investigated in order to better understand the temporality with respect to these changes, as we expect this information to further our understanding of the relationship between these proteins as a result of VPA exposure *in vitro*.

Conclusion: We expect that these results will allow us to better understand how these three proteins interact in response to VPA exposure *in vitro*. As a result, the results obtained in this study will help to elucidate the role of p300, Egr1 and Stat3 in VPA-mediated teratogenicity.

Support: The Canadian Institute for Health Research MOP115188

ASSESSING THE IMPACT OF ADULT PHYSIOLOGICAL VARIABILITY ON THE MAGNITUDE OF CHEMICAL-CHEMICAL METABOLIC INTERACTIONS.

Michel Bteich[1] and Sami Haddad[1].
[1]Department of Environmental and Occupational Health, IRSPUM, Université de Montréal, Montreal, Quebec, Canada, H3C 3J7.

Background: A variation in commonly known reactions of the population may become more significant due to the existence of interindividual variability.

Objectives: The purpose of this study was to assess the impact of adult interindividual variability in physiological and biochemical characteristics on the magnitude of chemical-chemical competitive inhibition at the hepatic clearance level.

Methods: In this study, theoretical chemicals were considered bound or unbound to albumin and distinctively metabolized by CYP2E1 (CV= 4.08%), CYP3A4 (CV= 33%) and CYP1A2 (CV= 54.76%), which are the most solicited enzymes for the biotransformation of chemicals in the human liver. Thus, three models of hepatic clearance (CLh) were selected; the Well-Stirred (W.S.), the Parallel-Tube (P.T.) and the recently-adapted model developed by Poulin *et al.* (2012) for the *in vitro-in vivo* extrapolation of clearance (IVIVE-POULIN). Statistical data of pertinent parameters served as inputs in the PBTK mathematical equations to run Monte Carlo iterations (n= 1000; confidence interval of 95%).

Results: The theoretical graphics obtained from iterated simulations, showed a decrease of CLh (L/min) and a narrowing of variability bands, all depending on the increase of the inhibitor's concentration ([I]/ki) values. The change of CLh became statistically significant at different values of [I]/Ki according to the type of the quantitative model, the enzyme (CYP450) and the intrinsic clearance (CLint). As for the IVIVE-POULIN model, the variability of the unbound fraction in incubation also had an impact on the predicted hepatic clearance.

Conclusions: This PBTK modeling approach succeeded in evaluating the impact caused by the physiological variability on the magnitude of the competitive inhibition for the chosen adult subpopulation. This novel approach may be useful, for different subpopulations, when incorporated in environmental and health risk assessment of ingested toxicants that interact in the human body.

APPLICATION OF THE TGx-28.65 GENOMIC BIOMARKER TO ASSESS THE GENOTOXICITY OF AN ORANGE AZO DYE AND 1,2,4-BENZENETRIOL IN HUMAN TK6 CELLS

Julie K. Buick[1], Andrew Williams[1], C.D. Swartz[2], L. Recio[2], and Carole L. Yauk[1].
[1] Environmental Health Science and Research Bureau, Environmental and Radiation Health Sciences Directorate, HECSB, Health Canada, Ottawa, Ontario, Canada;
[2] Integrated Laboratory Systems (ILS), Research Triangle Park, North Carolina, USA.

Background: In vitro toxicogenomics signatures to predict mode of action can facilitate chemical screening. We recently demonstrated the use of the TGx-28.65 genomic biomarker (representing: toxicogenomics (TGx), developed using a 28 chemical training set, and comprising 65 genes) in classifying agents as genotoxic (DNA damaging) and non-genotoxic in human lymphoblastoid TK6 cells.

Objectives: We previously developed and validated an in vitro genomic biomarker that accurately predicts whether a chemical is genotoxic or non-genotoxic in the presence of S9 metabolic activation. In this study, we applied the TGx-28.65 genotoxicity signature to determine whether two compounds of regulatory interest, which have limited and unclear genotoxicity data, are classified as genotoxic or non-genotoxic using the signature.

Methods: TK6 cells were exposed to increasing concentrations of an orange azo dye and 1,2,4-benzenetriol in the presence of rat liver microsomal S9 fraction for 4 h. Cells were collected 4 h and 20 h post-exposure (8 h and 24 h time points, respectively). Relative survival and micronucleus frequency were assessed by flow cytometry at 24 h. Transcriptome profiles were generated using Agilent human 8x60K microarrays. Statistical modeling and bioinformatics tools were utilized to classify both chemicals as genotoxic or non-genotoxic using the gene expression signature.

Results: The TGx-28.65 gene expression signature clearly classified both chemicals as genotoxic. The strength of the induction of the signature was consistent with measures of micronucleus induction and cell survival. The weight of evidence from this experiment strongly suggests that the orange azo dye and 1,2,4-benzenetriol are genotoxic.

Conclusions: Our previously published data and this proof of concept study suggest that the TGx-28.65 genomic biomarker has the potential to add significant value to existing approaches used to assess a chemical's genotoxic potential. The positive responses for genotoxicity of the orange azo dye and 1,2,4-benzenetriol strongly suggest that follow-up work is required to assess whether these chemicals are also genotoxic in vivo. This genomic biomarker can be used in conjunction with the current genotoxicity testing battery to allow for more effective regulation of genotoxic chemicals. The TGx-28.65 gene expression signature is currently under formal evaluation by the US Food and Drug Administration as a first step in accomplishing a more integrated genotoxicity testing strategy to better inform human health risk assessment.

NANOSILVER INDUCTION OF DETOXICATION PATHWAYS IN MAMMALIAN CELL LINES AND THE EFFECTS OF HYPOXIA

Shana Cameron[1], Owen Hovey[2] and William Willmore[1,2].
[1] Department of Chemistry, Carleton University, Ottawa, Ontario, Canada;
[2] Department of Biology, Carleton University, Ottawa, Ontario, Canada

Background: Nanosilver (1-100 nm silver particles) has antimicrobial properties and is widely used in many commercial, engineering, and biomedical applications. Due to its increased use in industrial products, the average person's exposure to this nanomaterial is also increasing, and thus it is critical to understand the biological effects and mechanistic perturbations caused by nanosilver at the cellular level.

Objectives: The objective of this study was to determine the induction levels of the two primary detoxification pathways upon exposure to nanosilver, and the effects of hypoxia. The first pathway is the aryl hydrocarbon receptor which induces various cytochromes P450 through the xenobiotic response element (XRE), and the second is the nuclear factor-erythroid 2 p45 subunit-related 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 under hypoxic or normoxic conditions, and the luciferase induction measured. As well, flow cytometry was performed to determine cell viability, amount of Reactive Oxygen Species (ROS) present, and cell aging.

Results: Our results show that nanosilver induces activation of the ARE and XRE detoxification pathways, and that hypoxia treatment inhibits this activation.

Conclusions: This research provides new insight into the mechanisms by which nanosilver contributes to the generation of ROS, cell death, and aging.

NEONICOTINOIDS INDUCE A PROMOTER-SWITCH IN *CYP19* EXPRESSION IN BREAST-CANCER CELLS AND ALTER AROMATASE ACTIVITY AND HORMONE PRODUCTION IN A FETOPLACENTAL CO-CULTURE MODEL

Élyse Caron-Beaudoin[1], Rachel Viau[1], Pascal Chhay[1], Michael Denison[2] and Thomas Sanderson[1].
[1] INRS - Institut Armand-Frappier, Université du Québec, Laval, QC, Canada;
[2] Department of Environmental Toxicology, University of California, Davis, CA USA.

Background: In hormone-dependent breast cancers, *CYP19* expression is increased via activation of several normally inactive promoters (PII, I.3, I.7) and by the inhibition of the normal I.4 promoter. CYP19 biosynthesizes estrogens, which stimulate proliferation of hormone-dependent breast cancer cells. Estrogens are also responsible for healthy development of the placenta, where promoter I.1 regulates *CYP19* expression. Exposures to certain pesticides, such as the herbicide atrazine, are associated with increased expression of *CYP19*. However, little is known about the endocrine disrupting potential of neonicotinoid insecticides.

Objectives: To develop cell-based assays to identify chemicals that alter the promoter-specific expression of *CYP19*, and to determine the effects of neonicotinoids in these cell assays, including a co-culture model of steroidogenesis in the fetoplacental unit.

Methods: Human H295R adrenocortical carcinoma, Hs578t breast cancer and BeWo trophoblast cells were exposed to atrazine or the neonicotinoids imidacloprid, thiacloprid and thiamethoxam (0.1-30 μM) for 24h. Tissue/promoter-specific *CYP19* expression was measured by quantitative RT-qPCR using two reference genes and aromatase activity was measured by tritiated water-release method. A co-culture of H295R and BeWo cells (feto-placental model) underwent the same treatments. Estradiol, estrone, DHEA and β-HCG production was determined by *ELISA*.

Results: In H295R cells, atrazine concentration-dependently increased PII- and I.3-mediated *CYP19* gene expression (7-fold) and aromatase activity (2-fold). Thiacloprid and thiamethoxam (0.1, 0.3 and 10 μM) significantly induced PII and I.3-mediated *CYP19* expression (14-fold) and aromatase activity. In Hs578t cells, thiacloprid and imidacloprid inhibited I.4- and induced PII promoter-mediated *CYP19* expression (up to 50-fold), which resulted in induced aromatase activity. In BeWo cells, all three neonicotinoids concentration-dependently inhibited I.1-mediated CYP19 expression. In the co-culture model, all three neonicotinoids increased estrogen production.

Conclusions: Atrazine and neonicotinoids alter *CYP19* gene expression, aromatase activity and estrogen biosynthesis in a promoter-specific manner. Therefore, the endocrine disrupting effects of these pesticides on estrogen biosynthesis in humans would be of a tissue-specific nature. We are the first to provide a mechanistic basis for the potential of neonicotinoids to alter the biosynthesis of estrogens in placenta during pregnancy or in hormone-dependent breast cancer via the tissue-specific promoters of CYP19. The novel mechanistic application of our cell-based screening tools will be helpful in assessing the risk certain chemicals may pose by altering tissue-specific estrogen biosynthesis in exposed women.

EFFECTS OF THE BENZENE METABOLITES BENZOQUINONE AND HYDROQUINONE ON HL-60 HUMAN LEUKEMIA CELL DIFFERENTIATION AND IMPACT ON PU.1 TRANSCRIPTIONAL ACTIVITY

Joseph P. Cozzarin[1] and Louise M. Winn[1,2].
[1] Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada;
[2] School of Environmental Studies, Queen's University, Kingston, Ontario, Canada.

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 benzene metabolites. PU.1 is a transcription factor that is specific to cells in the hematopoietic system and plays a critical role in hematopoietic differentiation as PU.1 knockout mice have undifferentiated and impaired immune cells which results in the death of the animal. In addition, PU.1 hyperphosphorylation has been demonstrated to be concomitant with immature myeloid cell phenotypes. PU.1 activity is increased following phosphorylation and has been previously shown to become hyperphosphorylated in the presence of hydroquinone; suggesting that hydroquinone can disrupt PU.1 activity.

Objective: We hypothesize that exposure of HL-60 cells to hydroquinone and benzoquinone will result in a hyperphosphorylation of PU.1 resulting in increased activity and subsequently alterations in the expression of genes regulated by PU.1 ultimately leading to a decrease in terminal differentiation.

Methods: HL-60 cells were plated at 1.5×10^5 cells/ml and exposed to 25 μM benzoquinone and hydroquinone for 2, 4, 8 or 24 h. Following exposure, cells were induced to differentiate with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (3.0×10^{-8} M). Undifferentiated and differentiated cells were evaluated for CD 11b surface markers using flow cytometry in order to determine the percent differentiation.

Results: Flow cytometry studies to date have demonstrated that cells exposed to TPA matured to macrophages as indicated by 65% of cells in the treated sample possessing CD11b surface markers. Future studies will isolate differentiated cells from non-differentiated cells by flow cytometry, assess the effects of benzoquinone/hydroquinone exposure on the level of cell differentiation and assess PU.1 transcription factor activity via immunoprecipitation of PU.1 protein followed by western blot using a phospho-serine antibody.

Conclusions: These results will contribute to the existing knowledge of benzene induced leukemia as the mechanism of benzene toxicity is poorly understood.

OXIDATIVE STRESS AND CELLULAR AGING IN RESPONSE TO POLYBROMINATED DIPHENYL ETHER FLAME RETARDANTS

Mary Daniel[1] and William Willmore[2].
[1]Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Canada K1S 5B6;
[2]Institute of Biochemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Canada K1S 5B6

Background: Individuals are exposed to thousands of toxic chemicals on a daily basis. Many toxic chemicals produce reactive oxygen species (ROS), which can greatly contribute to protein modification and the accumulation of protein carbonyl groups; often used as an indicator of cellular aging. The antioxidant response to toxic chemicals involves activation of the antioxidant response elements (AREs)/Electrophile Response Elements (EpREs), located in the promoter regions of the genes of antioxidant and phase two enzymes, and regulated by the nuclear factor-erythroid 2 subunit-related transcription factors (Nrf1, Nrf2, and Nrf3).

Objective: Activation of the AREs/EpREs, in particular by Nrf1, in response to polybrominated diphenyl ether (PBDE) flame retardants was investigated.

Methods: Protein damage as a result of PBDE treatment, as assessed by carbonyl assays, and cellular aging, as assessed by β-galactosidase assays, were examined.

Results: We found that PDBE-induced oxidative stress causes activation of AREs/EpREs. We also found that PDBE-induced oxidative stress further promotes accumulation of carbonyl groups on proteins and general cellular aging. The results provide insight into how the antioxidant response is activated by PDBEs, how they damage proteins through ROS and how damage by these compounds is related to advanced cellular aging.

Conclusions: As a significant proportion of our population will reach their senior years in the very near future, an understanding of how toxic chemicals influence the aging process is required.

DEVELOPMENT OF A HIGH-THROUGHPUT SCREENING TOOL FOR CHEMICALS DISRUPTING THYROID HORMONE UPTAKE VIA TRANSPORTER MCT8

Hongyan Dong[1] and Mike Wade[1].
[1] Hazard Identification Division, Health Canada, Ottawa, Ontario, Canada.

Background: Thyroid hormones (THs) play important roles in various biological processes. THs are actively taken up into target cell via mainly transporter MCT8. MCT8 defect have consequence for the severe neuromuscular phenotype. Some chemicals that humans are constantly exposed to are suspected to be TH disrupting chemicals (TDCs), especially affect TH uptake via MCT8. However, current there is no approach available that could be used to screening this kind of chemicals.

Objectives: To develop a high-throughput assay that is fast, cost-effective and avoiding the use of radioisotopes, as well as apply the system to screen TDCs which disrupt TH uptake via MCT8.

Methods: MDCK_MCT8, a canine kidney cell line over expressed MCT8, was used in the screening system. TH uptake was examined by detecting the amount of iodide moieties in the system using Sandel-Kolthoff assay which depending on the oxidation-reduction process between As3+ and Ce4+ in the presence of iodide. The iodide content of the reaction mixture is reflected by the destaining kinetics of Ce4+. Microtiter plate (96-well plate) format was used in High-through put screening system. Assay performance, such as reliability, uniformity and variability, was evaluated. The system was applied to screen TDCs of seven pharmaceutical chemicals.

Results: We examined time course and dose-response of T3 uptake in the system of MDCK-MCT8 cells. T3 uptake increased with incubation time and T3 concentration. The known MCT8 inhibitor bromsulphthalein (BSP) was used as a positive control to exam the ability of the system for detecting the inhibition effect of chemicals. We found the IC50 of BSP is about 100 μM. The performance of high-throughput system was evaluated by calculating the CV, Signal window and Z score. All indexes meet the acceptable criteria. No significant drift and edge effect was observed. Using this system, we found tyrosine kinase inhibitors such as sunitinib, imatinib, dasatinib and bosutinib disrupting MCT8 regulated TH uptake, while no significant disrupting was found for chemicals such as phenytoin, indocyanine green and meclofenamic acid.

Conclusions: The high throughput screening assay developed here is a powerful tool to identify and characterize substances to which Canadians are exposed and present hazards to health due to thyroid hormone disruption. Data from this tool will support risk assessment and risk management activities.

ACTIVATION OF THE P53 SIGNALING PATHWAY IN RESPONSE TO HYDROXYUREA EMBRYOTOXICITY DURING ORGANOGENESIS.

Nazem El Husseini[1] and Barbara F. Hales[1].
[1] Pharmacology & Therapeutics, McGill University, Montreal, QC, Canada.

Background: Hydroxyurea (HU), an anticancer agent and potent teratogen, is used as a model drug to study the embryonic stress response during organogenesis. Previously, we demonstrated that HU activates a DNA damage response (DDR) pathway in the gestation day (GD) 9 mouse embryo. The p53 tumor suppressor protein is a possible downstream effector of this pathway. P53 plays an important role in embryonic development; however, its function in response to teratogen-induced stress is not well understood.

Objectives: We hypothesize that HU exposure at the organogenesis stage activates p53 and that p53 mediates downstream events leading to cell cycle arrest and cell death in the regions where malformations are observed

Methods: CD-1 embryos at GD9 were exposed to saline (Control-CO) or two doses of HU (HU400= 400 mg/kg; HU600= 600 mg/kg) *in vivo*; 3 hours later the embryos were extracted and samples prepared for the analysis of gene and protein expression.

Results: Microarray analysis of whole-genome expression showed that the expression of 1346 genes significantly changes in embryos exposed to HU vs. control and that they are significantly associated with the p53 signaling pathway. Prediction analysis suggested that p53, along with the family proteins p63 and p73, is significantly activated in the embryo in response to HU. While qRT-PCR analysis revealed that *Trp53* mRNA levels were not changed by HU treatment Western blot analysis demonstrated that HU induced a significant, dose-dependent increase in p53 protein concentrations [HU400: 2.8 ±0.4; HU600: 4.0 ± 0.5, N=4-5, fold change ±SEM] and in phospho-p53 (S15) [HU400: 3.1 ±1.5; HU600: 5.5 ±1.2, N=5]. Using confocal microscopy, we determined that there is a significant increase in nuclear translocation of phospho-p53 with HU exposure, but that there are no region-specific translocation differences within the embryo. The expression of p53 target genes was determined to assess whether the transcriptional activity of p53 was increased by HU treatment. The expression of *Cdkn1a,* (aka *p21*), a cell cycle inhibiting factor [HU400: 12.02 ±1.90; HU600: 26.11 ±7.95 N=5], *Fas,* the cell surface receptor related to apoptosis [HU400: 2.83 ±0.54, HU600: 5.6 ±1.59; N=4-5] and *tp53inp1,* a proapoptotic protein that is also involved in autophagy [HU400: 7.77 ±0.93, HU600: 7.52 ±0.44; N=5] were all significantly upregulated. P53inp1 protein expression is also significantly increased in response to HU [HU400: 9.95 ±0.75; HU600: 10.78 ±2.29, N=4-5] suggesting that p53 mediates the activation of downstream cell death factors in response to HU embryotoxicity.

Conclusions: Together, these data show that p53 signaling is activated in almost all regions in the embryo in response to HU embryotoxicity during organogenesis and leads to an increase the transcription and translation of cell cycle arrest and cell death factors.

Funded by CIHR MOP-57867. NELH is the recipient of an award from CIHR-REDIH.

EXPOSURE TO ORGANOPHOSPHATE PESTICIDES DURING PREGNANCY: DEVELOPMENT OF A PREDICTIVE MODEL TO IMPROVE EXPOSURE ASSESSMENT

Karl Forest-Bérard[1,2], Maryse Bouchard[2] and Marc-André Verner[2,3].
[1] Department of Pharmacology, Université de Montréal (UM), Montreal, Quebec (QC), Canada;
[2] Department of Environmental and Occupational Health, UM, Montreal, QC; Canada;
[3] Université de Montréal Public Health Research Institute (IRSPUM), UM, Montreal, QC, Canada.

Background: Uses of organophosphate (OP) pesticides are widespread across Canada, and residues can be detected in urine samples of the general population. Ongoing embryological processes essential to the normal development of the nervous system during pregnancy are especially vulnerable to the neurotoxic effects of such exposure. Epidemiological studies on prenatal exposure to environmental chemicals with a short biological half-life like OP pesticides usually rely on urinary metabolite levels in one or a few spot samples from pregnant women to estimate exposure. However, recent studies have shown that intra-individual variability in measured levels is often greater than inter-individual variability. Failure to adequately characterize inter-individual variability in exposure is a major obstacle to the evaluation of exposure-outcome associations.

Objectives: The main objective of the present project was to develop a predictive model, which would refine exposure assessment for OP pesticides in pregnant women.

Methods: We recruited 44 pregnant women in their 1st trimester, who provided 10 spot urine samples over 10 consecutive days. Dialkylphosphate (DAP) metabolites of OP pesticides were measured in the 1st urine sample and in the 10-sample pool. Our model attempted to predict the DAP concentration of the 10-sample pool (dependent variable, seizing a larger share of temporal variation) using the 1st urinary metabolites measurement, combined with data gathered from a questionnaire (independent variables). Variables associated with the DAP concentration in the 10-sample pool at a $p<0.3$ (ANOVA or Spearman's correlation) were selected to be used in the predictive regression model. We restricted our multiple regression model to the 7 variables that were the most strongly associated with the dependent variable to avoid overfitting.

Results: All samples tested positive for traces of DAP metabolites. In a univariate regression model, DAP levels in the 1st urine sample alone explained 16% of the variability of the 10-sample-pool DAP levels. When adding other independent variables in a multiple regression model (i.e., body weight and body mass index, average sampling time, number of beans, potatoes, raspberries and cherry tomatoes consumed in the last month), the explained variability in the 10-sample-pool DAP levels rose to 43%.

Conclusions: Our results suggest that it is possible to build predictive models using this approach, and that it can improve the resolution of exposure estimates. This sets the stage for the development of other predictive models using more advanced techniques (e.g., machine-learning algorithms). Epidemiological studies on exposure to chemicals with short biological half-life should consider conducting panel studies to develop predictive models, subsequently using them to refine estimates in all study participants, improving overall representativity.

ASSOCIATION OF MATERNAL POLYBROMINATED DIPHENYL ETHER (PBDE) EXPOSURE WITH CRYPTORCHIDISM

Cynthia G Goodyer[1,2], Shirley Poon[8], Katarina Aleksa[8,9], Laura Hou[6], Veronica Atehortua[1], Amanda Carnevale[8], Roman Jednak[3], Sherif Emil[4], Darius Bagli[7], Barbara F Hales[5] and Jonathan Chevrier[6].

[1] The Research Institute of McGill University Health Centre, Montreal, QC, Canada;
[2] Departments of Pediatrics, [3] Pediatric Urology, [4] Pediatric General and Thoracic Surgery, [5] Pharmacology and Therapeutics, [6] Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, QC, Canada;
[7] Department of Pediatric Urology, Hospital for Sick Children, Toronto, ON, Canada;
[8] Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, Canada;
[9] Leslie Dan School of Pharmacy, University of Toronto, Toronto, ON, Canada.

Background: Polybrominated diphenyl ethers (PBDEs) are flame retardants used throughout North America during the past four decades. They have been added to many household products, including appliances, electronics, foam upholstery and building materials. PBDEs leach out as these products age and are found in dust of the home and workplace; PBDEs have also migrated into the environment and entered the food chain. Thus, individuals are exposed through both dust and diet. Clinical studies suggest that PBDEs can disrupt endocrine systems, leading to alterations in thyroid function, growth and development, including of the reproductive system. In the present study, we examined whether there is a link between maternal exposure to PBDEs and the risk of having a male child with undescended testes (cryptorchidism).

Methods: Control (n=158) and case (n=137) participants were recruited through pediatric urology and surgery clinics in Montreal and Toronto; inclusion criteria were a full-term normal birth, child age (3-18 mo), surgically-defined cryptorchidism in case infants and absence of genitourinary abnormalities in controls. Seven BDEs (BDE-28, -47, -99, -100, -153, -154,-209) were measured by GC-MS in maternal and child hair samples; mothers completed a demographics/lifestyle questionnaire.

Results: The geometric mean of ∑PBDEs for maternal hair was 45.35 pg/mg for controls and 50.27 for cases; for infant hair, it was 65.37 for controls and 67.95 for cases. Logistic regression analysis revealed that every 10-fold increase in maternal hair BDE-99 (OR=2.41; 95% CI=1.25, 4.65; $p<0.01$) and BDE-100 (OR=2.32; 95% CI=1.24, 4.34; $p<0.01$) was associated with more than a doubling in the risk of having a male infant with cryptorchidism; confounders taken into account included maternal age, ethnicity, income, paternal and familial history of cryptorchidism amongst others. Paired maternal-infant hair samples showed significant correlations for each of the PBDEs ($r= 0.34$-0.71; $p<0.001$).

Conclusions: Our results suggest that maternal exposure to BDE-99 and BDE-100 may be associated with abnormal migration of testes in the male fetus. This may be due to the PBDEs' anti-androgenic properties. (*Supported by grant RHF100625 from the IHDCYH/CIHR*)

THE POTENT MUTAGENICITY OF 2-CYANO-4-NITROANILINE AND 2,6-DICYANO-4-NITROANILINE, COMPONENTS OF DISPERSE DYES.

P. David Josephy*[1], Muhammad Zahid[2], Joban Dhanoa[1], Hilary Groom[1], Meghan Lambie[1] and Giovanna Brondino Duarte de Souza[3].
[1] Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada;
[2] Department of Chemistry, University of Agriculture Faisalabad, Pakistan;
[3] State of Sao Paulo University "Julio de Mesquita Filho" (UNESP), Araraquara, Sao Paulo State, Brazil.

Background: Many synthetic dyes (including acid, basic, disperse, reactive, and solvent dyes) are aromatic azo compounds. Regulatory agencies have conducted risk-assessment reviews of azo dyes and their aromatic amine precursors, *e.g.*, the recent "Aromatic Azo and Benzidine-based Substance Grouping" component of the "Substance Groupings Initiative", Environment Canada/ Health Canada. The chemical structures of many of these dyes include "structural alerts" for mutagenicity, such as nitroaromatic and arylamine functional groups. Nevertheless, there are many knowledge gaps in the toxicology/ genotoxicology data for azo dyes and their components. Several disperse dyes (used for colouring polyester and other synthetic fabrics) are based on cyano-substituted anilines, *e.g.*, Disperse Violet 33 and Disperse Red 73 (component: 2-cyano-4-nitroaniline, CNNA); Disperse Blue 165 and Disperse Blue 337 (component: 2,6-dicyano-4-nitroaniline; CNCNNA. No genotoxicology data is available on these cyanobenzene (benzonitrile) derivatives.

Objectives: To study the genotoxicity of CNNA, CNCNNA, and some structurally related compounds in the *Salmonella* mutagenicity assay (Ames test).

Methods: The Ames test was carried out according to standard protocols; mammalian metabolic activation was not used. The tester strains used were TA98, TA100, TA98NR, and YG1024. TA98 and TA100 are the standard strains used for detection of frameshift and base-substitution mutations, respectively. TA98NR is a derivative of TA98 which lacks the "classical" *Salmonella* nitroreductase activity. YG1024 carries a plasmid for high expression of acetyl CoA: arylamine N-acetyltransferase (NAT).

Results: CNCNNA and CNNA are exceptionally potent direct-acting frameshift mutagens. Indeed, the mutagenic potency of CNCNNA is higher than that of nitro-polycyclic aromatic hydrocarbons, such as 1-nitropyrene. The mutagenic potencies of CNNA and CNCNNA are orders of magnitude greater than those of structural analogues in which chlorine substituents replace the cyano groups. As expected, mutagenicities were much lower in strain TA98 than in YG1024, and were further reduced in TA98NR.

Conclusions: Cyano-substituted nitroanilines are extraordinarily potent mutagens. These mutagens could be released by reductive cleavage of the azo bonds of disperse dyes (a reaction which may be catalyzed, for example, by enzymes in the gut flora), and their high mutagenicity is a previously-unrecognized genotoxic hazard.

Supported by NSERC Canada.

ELIMINATION OF ARSENIC SPECIES BY SINGLE NUCLEOTIDE POLYMORPHIC VARIANTS OF THE HUMAN MULTIDRUG RESISTANCE PROTEIN 2 (MRP2/*ABCC2)*

Gurnit Kaur[1] and Elaine M. Leslie[1,2].
[1] Division of Analytical and Environmental Toxicology, Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada;
[2] Department of Physiology, University of Alberta, Edmonton, Alberta, Canada.

Background: Arsenic and selenium are toxic compounds, however in vivo exposures to arsenite and selenite result in mutual detoxification. The molecular basis of this can be explained by the biliary excretion of the seleno-bis(*S*-glutathionyl) arsinium ion $[(GS)_2AsSe]^-$ by the ATP-binding cassette (ABC) transporter, multidrug resistance protein 2 (MRP2/*ABCC2*). The *ABCC2* gene is highly variable; >50 single nucleotide polymorphisms (SNPs) have been identified. Several SNPs have been shown to alter the toxicokinetics of important therapeutic agents.

Objectives: The objective of this study was to determine whether *ABCC2* SNPs that result in the amino acid changes, R412G, V417I, S789F, R1150H, R1181L, N1244K, P1291L, V1188E, A1450T, T1477M, C1515Y and C1515Y/V1188E, displayed altered $[(GS)_2AsSe]^-$ transport activity in comparison to wild-type (WT) MRP2.

Methods: *ABCC2* SNPs were generated using site-directed mutagenesis and expressed in HEK293T cells. Plasma membrane-enriched vesicles were isolated and relative MRP2 levels were determined by western blotting. Cell-surface biotinylation experiments were done to confirm plasma membrane localization of selected variants. Transport activities of WT and variant MRP2 were compared using $[(GS)_2AsSe]^-$.

Results: All mutants were detected in whole cell lysates except for T1477M. S789F and A1450T were not detected in plasma membrane enriched vesicles. R412G and R1150H displayed lower $[(GS)_2AsSe]^-$ transport activity compared to WT.

Conclusions: The differences in cellular localization of S789F, A1450T and T1477M suggest that these amino acids may contribute to correct folding and trafficking of MRP2. Arsenic exposed individuals with MRP2 SNPs that display reduced transport activity and mislocalization may not benefit from selenium supplementation.

IN VITRO INHALATION EXPOSURE STUDY FOR ASSESSING TOXICITY OF AIRBORNE METAL NANOPARTICLES

Jong Sung Kim[1,2], Eileen Burns[1], Jae-Hong Park[3] and Jacqueline Yakobi-Hancock[1].
[1] Department of Community Health and Epidemiology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada;
[2] Department of Microbiology and Immunology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada;
[3] Department of Occupational and Environmental Health, College of Public Health, University of Iowa, Iowa City, Iowa, USA.

Background: Airborne metal nanoparticles are of particular concern over human exposure, as they can readily move in ambient air and enter the body through inhalation. Current *in vitro* models for airborne nanoparticle toxicity assessment have been criticized as being not pertinent to real human exposure. There is great interest in developing rapid screening methods that predict *in vivo* toxicity of nanoparticles. However, adequate testing models for assessing nanoparticle toxicity remain largely underdeveloped.

Objectives: The objective of the present study was to develop and validate the *in vitro* nanoparticle exposure system capable of providing a more robust and authentic replication of inhalation exposures to nanoparticles.

Methods: We used a spark discharge system capable of generating and delivering airborne metal nanoparticles directly onto lung cells at an air-liquid interface. The generated nanoparticles were characterized by using a scanning mobility particle sizer, inductively coupled plasma-mass spectroscope and electron microscopes. To better model *in vivo* repeated-low dose protocols we sequentially exposed lung cells to nanoparticles *in vitro* (4 h exposure-2 h rest in an incubator-4 h exposure) and cell viability was determined by Alamar Blue assay at 4 h post-exposure.

Results: Our nanoparticle exposure system produced stable metal nanoparticle aerosols for 4 h (Cu, 8 x 10^6 particles/cm^3). Particle size distribution indicated the geometric mean diameter of the generated particles to be average 5 nm with a geometric standard deviation of 1.5. SEM and TEM results confirmed the deposition of nano-sized particles on cell-free membranes. The cellular concentration of Cu nanoparticles was 5.9 μg Cu/transwell (4.7 cm^2) and a substantial amount of Cu was released to the basolateral medium (0.3 μg) during air-delivery of Cu nanoparticles in 4 h. Viability for cells exposed to Cu nanoparticles was significantly reduced to 79% (p<0.05) at 4 h post-exposure compared to cells maintained in an incubator.

Conclusions: Our results demonstrated that this *in vitro* model can be useful for generating metal nanoparticles and simulating an *in vivo* pulmonary exposure to airborne metal nanoparticles. This system will ultimately allow better understanding of the toxicological mechanisms by which nanoparticles produce biological responses.

POTENTIAL INVOLVEMENT OF MATRIX METALLOPROTEINASES (MMPS) IN METAL EXPOSURE-MEDIATED BIRTH OUTCOMES IN THE MIREC STUDY COHORT

Premkumari Kumarathasan[1], Renaud Vincent[1], Agnieszka Bielecki[1], Erica Blais[1], F. Au[2], Dharani D. Das[1], A. Filiatreault[1], Sabit Cakmak[1], M. Fisher[1], J. Gomes[2], A. Basak[2], Tye E. Arbuckle[1] and W. D. Fraser[3].

[1] Environmental Health Science and Research Bureau, HECSB, Health Canada, Ottawa, ON, Canada;
[2] University of Ottawa, Ottawa, ON, Canada;
[3] Centre hospitalier universitaire de Sherbrooke, Sherbrooke, QC, Canada.

Background: There is growing evidence suggesting that environmental chemical exposures (*e.g.* heavy metals, air pollutants) during pregnancy can lead to increased risk of low birth weight and preterm birth. Yet, mechanistic pathways underlying such associations need to be identified in order to gain insight into how chemical exposures mediate maternal and infant health effects.

Objective: This work was conducted to employ a spectrum of target biomarkers in the third trimester plasma of mothers from the Maternal Infant Research on Environmental Chemicals (MIREC) study cohort, to identify metal exposure-related perturbations in biochemical mechanisms that can potentially translate into altered maternal physiology, low birth weight and preterm birth.

Methods: Third trimester maternal blood plasma samples were analysed for proteomic and metabolite changes by affinity-based multiplex protein array (e.g MMPs, cytokines), HPLC-Fluorescence (*e.g.* endothelins), HPLC-Coularray (metabolites) and EIA (8-isoPGF2alpha) methods. Bioinformatic and biostatistical analyses were conducted to test the associations between maternal blood metal (Cd, Hg, Pb, As, Mn) levels (1st and 3rd trimesters), plasma biomarkers (3rd trimester), physiological changes and pregnancy outcomes.

Results: Our results revealed statistically significant associations ($p<0.05$) between maternal plasma MMPs and maternal blood metal levels, as well as with infant birth weight and gestational age. Changes in MMPs were associated with circulating endothelin levels. Also, protein expression and interaction patterns were suggestive of metal-specific and dose-related responses.

Conclusions: Our findings imply that targeted proteomic and metabolomic markers can add value to the understanding of metal exposure (As, Cd, Hg, Pb, Mn)-mediated impacts on maternal mechanistic pathways. Notably, maternal metal levels appeared to influence MMP-related inflammatory pathways and vascular performance that may adversely affect pregnancy outcomes.

BMDEXPRESS DATA VIEWER - A VISUALIZATION TOOL TO ANALYZE BMDEXPRESS DATASETS

Byron Kuo[1], Anna Francina Webster[1], Russell S. Thomas[2] and Carole L. Yauk[1].
[1] Mechanistic Studies Division, Environmental Health Science and Research Bureau, Health Canada, 50 Colombine Driveway, Tunney`s Pasture, Ottawa, Canada, K1A 0K9;
[2] United States Environmental Protection Agency, Office of Research and Development, National Center for Computational Toxicology, Research Triangle Park, NC, USA 27711

Background: Benchmark Dose (BMD) modelling is a mathematical approach used to determine where a dose-response change begins to take place relative to controls following chemical exposure. BMDs are being increasingly applied in regulatory toxicology to estimate acceptable exposure levels in humans. BMDExpress is an open-source software that establishes transcriptomic BMDs following chemical exposure, and is gaining popularity in evaluating genomics datasets.

Objectives: Using BMDExpress, thousands of genes can be modelled simultaneously and analyzed individually or as part of pathways, molecular functions or biological processes. However, BMDExpress output files can contain thousands of rows and over 60 columns. Analyzing these data can be time-consuming and difficult. An automated solution to organize and prepare the datasets in graphical formats will facilitate the effectiveness of the interpretation and evaluation of BMDExpress data.

Methods: We developed a web-based application, *BMDExpress Data Viewer,* as a complementary visualization component to facilitate analysis, interpretation and graphical presentation of BMDExpress data.

Results: We conducted case studies to demonstrate this tool can be used to perform five major functions: 1) Summarize and visually present complex datasets (to assess quality and statistical metrics); 2) Estimate and predict point-of-departure (POD) BMDs; 3) Identify the most sensitive biological processes and BMD values following chemical exposure; 4) Compare multiple pathways to identify dose changes within confidence intervals; and 5) Compare multiple datasets to reveal important trends (*e.g.* how toxicity of a chemical changes over time or across tissues). Moreover, important biological processes can be selected and easily compared across experiments using built-in statistical and graphical tools.

Conclusions: We believe that *BMDExpress Data Viewer* will greatly facilitate the use and interpretation of BMD modeled gene expression data, which is critical for advancing the application of genomics data in risk assessment. BMDExpress Data Viewer is available as a browser-based application, and is freely available at:
http://apps.sciome.com:8082/BMDX_Viewer/ (MIT license).

Disclaimer: This abstract does not reflect EPA policy.

THE APPLICATION OF TOXICITY REFERENCE VALUES FOR DEVELOPING PUBLIC HEALTH ADVICE: TRICHLOROETHYLENE AS A CASE STUDY

Tanya Lalvani[1], Nicole Somers[1] and Ray Copes[1].
[1] Environmental and Occupational Health, Public Health Ontario, Toronto, Ontario, Canada.

Background: Toxicity Reference Values (TRVs) are derived by various regulatory agencies across the world to derive chemical-specific environmental media standards or criteria that are considered to be protective of health. Trichloroethylene (TCE) is a volatile organic compound commonly used as a solvent. It has been confirmed to cause kidney cancer, and there is evidence that it may also be linked to non-Hodgkin lymphoma and liver cancer. TCE has been found in soil and groundwater at various communities across Ontario. Consequently, public health staff need to interpret the results of indoor air, soil and groundwater testing to provide advice to the public and where necessary to identify actions that may be effective in reducing personal exposure to TCE.

Objectives: To determine the usefulness of TCE TRVs for assessing the likelihood of adverse health outcomes from elevated TCE concentrations in soil, drinking water, and indoor air and as a basis for developing information about TCE risks for the public; and to identify actions that may be effective in reducing exposure.

Methods: A jurisdictional scan of TCE TRVs from the United States Environmental Protection Agency, Health Canada, California Environmental Protection Agency, and Agency for Toxic Substances and Disease Registry was conducted. In addition, the derivation of current Ontario standards and criteria for TCE in environmental media was reviewed.

Results: In Ontario, TRVs for TCE have been used to derive soil and groundwater standards that are enforceable by the MOECC; however, when public health units are dealing with TCE contamination at a local level, these standards may not be useful for assessing and communicating site specific risks to local residents for the following reasons: (i) Regulatory standards and criteria incorporate policy decisions including target/acceptable risk levels (e.g., a cancer risk of one-in-one million). At a local level, risks of this magnitude are not expected to result in a measurable increase in number of cancers attributable to TCE over a lifetime. (ii) Currently, the selected non-carcinogenic TRVs in Ontario are set on the basis of cardiac teratogenicity and therefore may not be a relevant endpoint for all members of the affected population. (iii) Individual variability, sensitive populations, multiple or co-exposures have not been accounted for in these selected values.

Conclusions: TRVs and standards for environmental media are useful as screening tools, and can be employed in conjunction with other information when developing public health advice. Other information that needs to be considered includes: (i) whether measured concentrations accurately reflect exposure to the public and comparison to background exposures, (ii) the presence of sensitive individuals (e.g., women of child-bearing age) exposed to elevated concentrations, (iii) the anticipated duration of exposure, and (iv) what exposure reduction options are available. These considerations are now being utilized by several public health units including Halton, London-Middlesex and Simcoe-Muskoka to interpret and communicate the results of indoor air, soil and groundwater testing at local communities that are affected by TCE contamination.

POTENTIAL ADIPOGENIC PROPERTIES OF SHORT-CHAIN CHLORINATED PARAFFINS AND PENTACHLOROPHENOL IN 3T3-L1 PREADIPOCYTES

Enoch Lam[1] and Laurie HM. Chan[1].
[1] Department of Biology, Graduate program in Chemical and Environmental Toxicology, University of Ottawa, Ottawa, Ontario, Canada.

Background: Persistent organic pollutants (POPs) are compounds with environmental and human health concerns associated to their bioaccumulative and hydrophobic properties. Previously studied POPs including poly-chlorinated biphenyls (PCBs) have been shown to alter the development of adipocytes in human and murine models. We are studying the effects of pentachlorophenol (PCP) and short-chain chlorinated paraffins (SCCPs) in adipocytes. PCP is used as a wood preservative, while SCCPs are a class of commercial chemicals that have applications as flame retardants and plasticizers. Both PCP and SCCPs are present in significant concentrations in the environment and have the ability to bioaccumulate in regions of the human body including adipose tissue with unknown health effects.

Objective: The objective of this study was to evaluate the adipogenic effects of PCP and SCCPs in the 3T3-L1 preadipocyte murine cell model at environmental concentrations.

Methods: Cultured 3T3-L1 cells were individually exposed during the induction of differentiation through the use of isobutylmethylxanthine, dexamethasone and insulin with PCP (0.442 – 4420 nM), the SCCP mixture tetrachlorodecane (0.357 - 3570 nM), and monomer 1,2,5,6,9,0-hexachlorodecane (0.287 - 2870 nM) in DMSO. Lipid accumulation was evaluated through the use of oil red o staining. QPCR was also performed to verify the differentiation of each exposure concentration and determine the effect of exposure on lipid metabolism.

Results: Significant increases in lipid accumulation were identified for PCP at 4420 nM, at 35.7 and 3570 nM for tetrachlorodecane, and at 28.7, 287 and 2870 nM for 1,2,5,6,9,0-hexachlorodecane. PCP exposure resulted in a 75% increase in lipids when compared to differentiated controls and 25-50% increases were observed for tetrachlorodecane and 1,2,5,6,9,0-hexachlorodecane exposures.

Conclusion: This study shows preliminary data that indicate pentachlorophenol and short-chain chlorinated paraffins may potentially alter the adipogenesis of adipocytes.

INVESTIGATING VALPROIC ACID-INDUCED ALTERATIONS TO NF-κB IN P19 EMBRYONAL CARCINOMA CELLS

Christina L. Lamparter[1] and Louise M. Winn[1,2].
[1] Department of Biomedical and Molecular Sciences, Graduate Program in Pharmacology and Toxicology, Queen's University, Kingston, Ontario. Canada;
[2] School of Environmental Studies, Queen's University, Kingston, Ontario, Canada.

Background: The transcription factor NF-κB is an important regulator of gene expression and a protective factor against the apoptotic cascade. Reduced NF-κB DNA-binding and increased apoptosis have been observed in susceptible tissues following exposure to several developmental toxicants including cyclophosphamide and thalidomide. We have previously demonstrated that exposure to the widely prescribed anticonvulsant valproic acid (VPA) decreases the protein expression of NFκB in mouse embryos with a neural tube defect (NTD) but is unaltered in embryos where the neural tube is closed.

Objective: This study aims to evaluate the contribution of NF-κB-mediated anti-apoptotic signaling to the teratogenicity of VPA and investigate the basis for its differential expression in embryos with/without a NTD.

Methods: P19 embryonal carcinoma cells plated at 1×10^5 cells/mL were exposed to 5 mM VPA or PBS control for 24 h with NF-κB protein expression quantified by Western blotting using antibodies targeting the N- or C-terminus. NF-κB stability was also evaluated following 16 h VPA exposure by switching cells to media containing 30 μg/mL cycloheximide (an inhibitor of new protein synthesis) and harvesting cells for up to 4 h.

Results: NF-κB protein expression was decreased following VPA exposure and was accompanied by the appearance of a prominent smaller molecular weight band (~55 kDa) detected only with the N-terminal targeting antibody. VPA exposure also decreased the stability of NF-κB assessed by cycloheximide exposure and again resulted in an additional small molecular weight band detected only with the N-terminal antibody. Ongoing studies are evaluating this lower band as a potential cleavage product and evaluating its DNA binding ability which is essential for cell survival.

Conclusions: Our preliminary results indicate that VPA exposure alters the stability of NF-κB which could be mediated by its cleavage. Understanding the impact of NF-κB stability on DNA binding and cell survival following VPA exposure will hopefully provide insight into the mechanism and susceptibility to NTD formation associated with VPA teratogenesis.

Support: The Canadian Institutes for Health Research MOP115188

PPARγ LOSS INCREASES METASTASIS OF HER2+ BREAST TUMOURS

Elizabeth D. Lightbody[1], Kathleen M.J. O'Connell[2], Rachel E. Rubino[2], Anthony J. Apostoli[2], Mark M. Schneider[1], Sandip K. SenGupta[1] and Christopher J.B. Nicol[1-3].
[1] Dept. of Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada;
[2] Cancer Biology and Genetics Division, Cancer Research Institute, Kingston, ON, Canada;
[3] Dept. of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada.

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: To test the hypothesis that 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;PPARγ^{KO}), which have targeted PPARγ deletion in the same HER2+ transformed mammary epithelial cells that drive breast tumourigenesis. Mice were then monitored weekly for breast tumour formation, volume and location. Western Blot and immunofluorescence (IF) assays were used to evaluate PPARγ and HER2 expression and localization in formalin fixed, paraffin-embedded (FFPE) tumours. Mouse tumourigenic cell lines were also established in culture from a freshly isolated NIC;PPARγ^{KO} lung metastatic tumours (NIC;PPARγ$^{KO\text{-}lmets}$), to define the *in vitro* interactions between PPARγ and HER2 signaling, and metastatic potential.

Results: NIC;PPARγ^{KO} females (n=17) have high levels of mammary tumour incidences and multiplicity, and more interestingly, enhanced lung metastases compared to studies of the parental NIC strain. Expression analysis of NIC;PPARγ^{KO} tumours shows PPARγ protein levels decrease as the mammary gland progresses from normal to tumourigenic tissue. Loss of PPARγ expression was also inversely matched to increased HER2 phosphorylation at tyrosine 877 (pY877HER2). IF analysis on sections of FFPE samples assessed HER2 expression levels in the NIC;PPARγ^{KO} tumours compared to controls including DMBA-induced breast tumours from congenic wildtype controls and mammary epithelial HER2^{Low};PPARγ^{KO} mice. HER2 expression was significantly increased in primary and metastatic tumours versus controls ($p<0.05$). Scratch wound and boyden chamber assays using NIC;PPARγ$^{KO\text{-}lmet}$ cells showed migration and invasion is significantly enhanced after epidermal growth factor (EGF, 20 ng/ml) treatment; whereas, *in vitro* co-treatment of NIC;PPARγ$^{KO\text{-}lmets}$ with a PPARγ activating drug (rosiglitazone, 10μM) decreased EGF-mediated cell migration and invasion potential ($p<0.05$).

Conclusions: Together, these data provide the first evidence that PPARγ is a useful prognostic/predictive biomarker for HER2+ breast tumours, and suggest the use of PPARγ ligands may benefit some HER2+ breast cancer patients.

LATENT CONGENITAL HEART DEFECTS AND PREDISPOSITION TO CARDIOVASCULAR DISEASE FOLLOWING POSTNATAL HYPERSTIMULATION OF ADRENERGIC RECEPTORS AS A MODEL OF POST-TRAUMATIC STRESS DISORDER

Rebecca D. Maciver[1], Michael A. Adams[1], Louise M. Winn[1] and Terence R. S. Ozolinš[1].
[1] Department of Biomedical and Molecular Sciences, Graduate Program in Pharmacology and Toxicology, Queen's University, Kingston, ON, Canada.

Background: Congenital heart defects (CHD) are the most prevalent birth defect with an incidence of approximately 1%. While 80% of structural CHD resolve spontaneously, the long-term predispositions to disease in later life are unknown. In the active military, a healthy population by standard measures, there is a paradoxical two-fold increased risk of cardiovascular disease CVD and a two- to four-fold increased risk of post-traumatic stress disorder (PTSD) compared to the general population. This PTSD-associated CVD has a distinct pathology, independent of Framingham risk factors such as obesity, physical inactivity and smoking. It has been hypothesized, although not formally tested, that adrenergic (AR) hyperstimulation (associated with PTSD) may be an aetiologic agent. Moreover, it has been suggested that the increased rate of CVD may be the result of latent CHD undetected during recruitment. Taken together, we hypothesize that rats with resolved CHD are at increased risk of the pathophysiological consequences of AR hyperstimulation.

Objectives: Repeated, long-term episodes of AR hyperstimulation were induced pharmacologically as a surrogate for PTSD. The structural and functional changes of the heart induced by the AR response are being assessed longitudinally using high-resolution ultrasound (HRUS) and compared between control rats and those with resolved CHD.

Methods: Time-mated 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 (GD) 9-10 to produce offspring with a 50% incidence of CHD, of which 80% resolve spontaneously by weaning (postnatal day (PND) 21). Offspring were monitored on postnatal d (PND) 4, 21 and 56 via HRUS for structural and functional endpoints. To mimic PTSD-like stress, rats were treated with intermittently and with increasing duration (from 1 and up to 7 d) of isonorepinephrine (INE), a β-AR agonist at 0.01 mg/kg, s.c., with recovery periods (9 d) between cycles. HRUS was performed at baseline, peak cardiac hypertrophy (24 h), half-time to recovery of cardiac hypertrophy (4 d) and at recovery from cardiac hypertrophy (7 d) of each dosing cycle.

Results: Preliminary findings show differences between groups with regards to maternal body weight gain during pregnancy and postnatal viability and body weight gain of offspring. Analysis of HRUS will longitudinally measure cardiac function, hypertrophic effects and threshold for reversibility during periods of INE treatment.

Conclusions: While these studies are ongoing, if our hypothesis is correct, rats with resolved CHD (DMO treated) will exhibit an increased susceptibility to the pathophysiological effects of INE, our pharmacological model of PTSD. This would add to a growing body of evidence demonstrating the potential for long-term consequences of resolved CHD.

UTILITY OF FLUORESCENT GUANINE MIMICS FOR APTAMER BINDING TO OCHRATOXIN A

Kaila L. Fadock[1] and Richard A. Manderville[1].
[1] Department of Chemistry, University of Guelph, Guelph, Ontario, Canada.

Background: Ochratoxin A (OTA) is a carcinogenic mycotoxin commonly found in grain, pork, grapes and feedstock. OTA is regulated for consumption with a tolerable daily intake (TDI) of 17 ng/kg/day set by the European Food Safety Authority; Health Canada has proposed a TDI of only 4 ng/kg/day. DNA aptamers are single-strand oligonucleotides which have been developed to bind a target molecule with high affinity and specificity. The OTA aptamer has the ability to bind to OTA, but lacks any signaling response to act as a detection platform for the toxin.

Objectives: The objective of this study is to incorporate fluorescent 8-aryl-guanine bases within the OTA aptamer to develop a simple detection platform for the presence of OTA at government regulation concentrations.

Methods: The OTA aptamer was characterized to form an anti-parallel guanine quadruplex (GQ) through the use of circular dichroism (CD). Anti-parallel GQs contain at least two G-tetrads, which consist of four Gs in alternating *anti*- and *syn*-conformations that are stabilized by certain metal ions, notably K^+, and through Hoogsteen H-bonding interactions. The 8-thiophene-deoxyguanosine (thio-dG) probe is well-suited for insertion into anti-parallel GQs as it exhibits a conformational preference for *syn*-Gs. Thio-dG was systematically inserted into each G location within the 31-base OTA aptamer to identify the ideal probe location for GQ stability and aptamer binding affinity to OTA. GQ formation by the thio-dG-modified aptamers was characterized using thermal melting experiments, CD studies, and fluorescence response. Binding affinity titrations were then performed on thio-dG-modified aptamer samples with decreasing OTA concentrations of 3 μM, 500 nM, and 100 nM.

Results: When inserted into a *syn*-G within the G-tetrad, the thio-dG probe stabilized the GQ by 3.0-9.2 °C, but destabilized the GQ by -0.5 to -8.2 °C when placed at an *anti*-G. The thio-dG at the 11th position in the aptamer was determined to be the most suitable position for GQ stability and aptamer binding to 100 nM OTA.

Conclusions: This is the first time that fluorescent G mimics have been inserted into the OTA aptamer and have been used as a detection platform for nM concentrations of the toxin. The results of this study will provide a guideline for G probe incorporation into the OTA aptamer. The goal of this project is to increase fluorescence response and provide a visible emissive signal for development of a handheld platform for OTA detection.

N-HYDROXYMETHYLACRYLAMIDE DOES NOT INDUCE GENE MUTATIONS IN SOMATIC TISSUES AND GERM CELLS OF MICE

Francesco Marchetti[1], John Gingerich[1], Lynda Soper[1], George R. Douglas[1] and Carole Yauk[1].
[1] Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada.

Background: A general assumption in risk assessment is that evaluations based on somatic cell mutation are sufficient to identify agents that are likely to enhance the risk of germ cell mutations. However, emerging evidence suggests that germ cell mutations arise at doses of chemicals below those that cause increased somatic cell mutation. For example, genotoxicity following exposure to acrylamide occurs at lower doses in germ cells than in somatic cells and n-hydroxymethylacrylamide (NHMA) induces a strong dominant lethal response without affecting the frequencies of micronuclei in blood of exposed mice.

Objectives: The objective of the present study is to use the Muta^{TM}Mouse transgenic rodent model to evaluate the mutagenicity of NHMA in both somatic tissues and germ cells.

Methods: Muta^{TM}Mouse males (8 per group) received 0, 180, 360 or 720 ppm NHMA by oral gavage for 28 d. Bone marrow, germ cells from the seminiferous tubules and sperm were collected at 3, 42 and 72 d after the last daily exposure and the *lacZ* assay was conducted to establish mutant frequencies (MF). This approach enabled the evaluation of the mutagenic effects of NHMA on postmeiotic germ cells, dividing spermatogonia and stem cell spermatogonia, respectively. Additionally, peripheral blood was collected from a subset of animals 2 d after the last exposure and used to detect micronuclei (MN) in red blood cells by flow-cytometry.

Results: The results of the *lacZ* assay showed that there was no dose- or time-related increases in MFs in any of the tissue samples analyzed. NHMA did produce a statistically significant ~1.5-fold increase in MN frequencies in circulating reticulocytes and normochromatic erithrocytes at the two highest doses tested.

Conclusions: The present results show that NHMA does not cause gene mutations in bone marrow and germ cells of mice and that it is a weak clastogen in bone marrow. These findings suggest that the strong response observed in the dominant lethal test is likely due to the fixation of NHMA-induced sperm DNA lesions into chromosomal aberrations following fertilization. These data suggest that current approaches for germ cell testing should consider post-fertilization events when characterizing germ cell genotoxicity.

REFINING CURRENT GUIDELINES FOR SOMATIC AND GERM-CELL GENOTOXICITY TESTING METHODS

Clotilde Maurice[1], Marianela Rosales[1], Carole Yauk[1] and Francesco Marchetti[1].
[1] Environmental Health Science and Research Bureau, Health Canada, Ottawa, Ontario, Canada.

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 using transgenic rodents (TGR) was developed. However, the recommended experimental design in the TG for assessing mutations in somatic tissues is not optimal for germ cells. It encompasses a period of spermatogenesis that does not include DNA synthesis nor DNA repair, events that are necessary to fix mutation in the transgenic reporter system.

Objectives: The long-term objective of our work is to identify an experimental design that will enable the evaluation of mutations in somatic and germ cells with comparable sensitivity. The short-term goal is to study the sensitivity of somatic tissues and sperm to the induction of mutations by triethylemetamelanine (TEM), a chemical that is known to break chromosomes but that has not been assessed for the ability to induce mutations.

Methods: Muta^{TM}Mouse males received 0, 0.5, 1 or 2 mg/kg TEM for 28 d orally and were euthanized 3, 28, 42 and 70 d after the last exposure. These timepoints were selected to evaluate effects on postmeiotic germ cells, meiotic germ cells, dividing spermatogonia and stem cell spermatogonia, respectively. At each timepoint, several somatic tissues, as well as mature and developing sperm were collected. DNA extracted from each tissue was used to evaluate mutant frequencies (MFs) in the *lacZ* transgene. Furthermore, the frequency of micronuclei in circulating red blood cells was analyzed by flow cytometry.

Results: Preliminary results show that MFs increased in bone marrow and testicular somatic cells 3 d after the last TEM exposure but not in germ cells from the seminiferous tubules. MFs appear to decline in bone marrow and testicular somatic cells collected 28 d after exposure. The percentage of MN reticulocytes was significantly increased at the medium and high doses ($P < 0.05$), while a significant dose response was observed in normochromatic erythrocytes ($P < 0.05$).

Conclusions: These data confirm that TEM is genotoxic to somatic cells and demonstrate that similar mutational endpoints can be simultaneously analyzed and compared between somatic tissues and germ cells. Analyses are ongoing to determine whether TEM induces mutations in spermatogonia.

ASSOCIATION BETWEEN PERFLUOROOCTANOIC ACID (PFOA) AND ENDOMETRIOSIS: POTENTIAL INFLUENCE OF ORAL CONTRACEPTIVES USE

Gerard Ngueta[1,2], Miyoung Yoon[3], Harvey Clewell[3,4], Melvin E. Andersen[3,4], Matthew P. Longnecker[4] and Marc-André Verner[1,2].
[1] Department of Occupational and Environmental Health, Université de Montreal, Canada;
[2] Universite de Montreal Public Health Research Institute (IRSPUM), Université de Montreal, Canada;
[3] Hamner Institutes for Health Sciences, Research Triangle Park, NC, USA;
[4] Ramboll Environ, Research Triangle Park, NC, USA

Background: A recent epidemiological study reported an association between endometriosis and plasma PFOA levels. Women with endometriosis experience more pain during menses (dysmenorrhea) than controls and are therefore more likely to use oral contraceptives (OCs) as a treatment for pain. Reduction of menstrual blood loss by OCs (70%) could decrease PFOA elimination and confound the association between PFOA and endometriosis.

Objective: We estimated the influence of OC use for dysmenorrhea on the association between plasma PFOA and endometriosis.

Methods: We used a one-compartment pharmacokinetic model of plasma PFOA and a Monte Carlo process to simulate 20,000 women like those in the epidemiological study. The probability of disease (case/control) and dysmenorrhea (yes/no) for each woman was determined based on published distributions. In women with dysmenorrhea, we evaluated different probabilities of taking OCs to treat pain (0%-100%). Duration of OC treatment for pain was randomly sampled (0.5-10 years before diagnosis). The relation between endometriosis and simulated plasma PFOA levels (ng/ml) was evaluated using logistic regression.

Results: We observed greater odds ratios with increasing probabilities of women using OCs to treat dysmenorrhea. When assuming that 100% of women with dysmenorrhea used OCs, the odds ratio was 1.08 (95% CI=0.98-1.18). In comparison, the odds ratio in the epidemiological study, which was not adjusted for OC use, was 1.62 (95% CI=0.99-2.66). Future plans include consideration of different probabilities of OC use for dysmenorrhea, depending on disease status.

Conclusions: Under the assumptions made, our results suggest that confounding by OC use may explain some of the epidemiological association between endometriosis and plasma PFOA levels.

ROLE OF THE STRESS AXIS IN REGULATING METABOLIC AND INFLAMMATORY EFFECTS OF OZONE

Shinjini Pilon[1], Josée Guénette[1], Michael G. Wade[1], Ella Atlas[1], Alison C. Holloway[2], Andrew Williams[1], Renaud Vincent[1] and Errol M Thomson[1].
[1] Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, ON, Canada;
[2] Department of Obstetrics and Gynecology, McMaster University, Hamilton, ON, Canada.

Background: Adverse health effects of air pollution have been shown to extend beyond respiratory and cardiovascular disease. Recent epidemiological and experimental studies suggest that exposure to air pollutants can produce systemic inflammatory responses and oxidative stress, and increase the incidence of metabolic diseases such as type 2 diabetes, and neurological disorders such as depression. We have shown that exposing rats to common air pollutants activates the hypothalamic-pituitary-adrenal (HPA) stress axis, resulting in increased plasma levels of the glucocorticoid corticosterone (the rodent equivalent of cortisol). Chronic elevation of glucocorticoids can initiate inflammatory dysregulation, and is thought to play an important role in the pathogenesis of metabolic disorders.

Objectives: Our objective was to determine whether glucocorticoids mediate effects of ozone on metabolic and inflammatory pathways.

Methods: To assess involvement of the stress response, male Fischer-344 rats were administered vehicle or metyrapone, a drug that blocks synthesis of corticosterone, at two doses (50 and 150 mg/kg). Rats were then exposed to either air or ozone for 4 h. Circulating levels of metabolic and inflammatory biomarkers were measured, and expression of relevant genes was assessed in target tissues.

Results: Ozone inhalation altered plasma levels of insulin and glucagon and the expression of genes involved in glucose metabolism such as FGF21 and PEPCK in the liver. Some effects of ozone were blocked in metyrapone-treated rats, including the ozone-induced increase in serum corticosterone, demonstrating involvement of the stress response. Metyrapone-treated rats showed an exaggerated inflammatory response to ozone, consistent with a role for glucocorticoids in limiting inflammation.

Conclusions: Our data are consistent with the hypothesis that certain metabolic effects and inflammatory responses resulting from short-term ozone exposure were mediated by glucocorticoids. This project provides evidence supporting a biologically plausible mechanism for metabolic impacts of air pollutants. Such data should assist interpretation of population studies and inform future research aimed at assessing the risk posed by air pollutants.

DEVELOPMENT AND REFINEMENT OF A NEW METHOD TO DETECT MUTATIONS IN MOUSE SPERM FOR THE QUANTIFICATION OF POTENTIAL HERITABLE HAZARDS

Andrea Rowan-Carroll[1], M. A. Beal[1], A. Williams[1], Francesco Marchetti[1] and Carole L. Yauk[1].
[1]Mechanistic Studies Division, Environmental Health Science and Research Bureau, Health Canada, Ottawa, Ontario, K1A 0K9

Background: Understanding the heritable consequences of chemical exposure is of the utmost importance to population health, as mutations in the germline are passed onto subsequent generations. Previously, we have shown that germline mutations may be detected in unstable Expanded Simple Tandem Repeat (ESTR) sequences using the single-molecule PCR (SM-PCR) technique. However, mutation detection with ESTR sequences is laborious and time consuming. In our current research, we explore the use of the SM-PCR technique with microsatellite tandem repeats. This offers several advantages: unlike ESTRs, microsatellites are found in most organisms; they are much easier to work with experimentally due to their small size; and, they can be sequenced by capillary electrophoresis to precisely identify repeat length changes.

Objectives: In this study, we apply SM-PCR of microsatellites to determine the sensitivity of the method in identifying genetic hazards to sperm of mice exposed to the known mutagenic chemical, and common environmental pollutant, benzo[*a*]pyrene (BaP).

Methods: Mice were exposed to 0, 50 or 100 mg/kg BaP for 28 d and sperm sampled 42 d after the final exposure to assess the induction of microsatellite mutations in dividing spermatogonia. Samples were diluted to a single DNA molecule per PCR amplification. Microsatellites Mm2.2.1 and Mm19.2.3 were then amplified and repeat length of the microsatellite allele was assessed to determine whether exposure to BaP had an effect on the mutation frequency at these loci.

Results: Mice exposed to the 100 mg/kg BaP show over a 2-fold significant increase in mutations relative to the controls at both loci. Likewise, mice exposed to 50 mg/kg BaP show significant increases in mutations relative to controls. Multiplexing SM-PCR experiments, in which both Mm2.2.1 and the Mm19.2.3 loci were amplified in the same reaction, revealed similar increases in mutation frequencies.

Conclusions: The results from our current work indicate that mutation frequencies in the germline may be detected using two hypervariable microsatellite loci. Furthermore, using multiple microsatellite loci increases our ability to detect these changes by increasing statistical significance and thus the sensitivity of the assay. With the increased refinement and sensitivity that the microsatellite/ SM-PCR technique shows promise as a useful tool for regulators in their assessment of chemicals mutagens which are present at low and environmentally relevant levels.

NEUROTOXIC EFFECT OF THE ALKYL POLYCYCLIC AROMATIC HYDROCARBONS IN HUMAN NEUROBLASTOMA CELLS

Sailendra N. Sarma[1], Jules M. Blais[1] and Hing M. Chan[1].
[1] University of Ottawa, Department of Biology, Center for Advance Research in Environment Genomics, Ottawa, Ontario, Canada.

Background: Alkylated polycyclic aromatic hydrocarbons (alkyl-PAH) are the major constitute of total PAH in crude oil and originated from incomplete combustion process. The potential differential toxicity and related mechanism are not known.

Objective: The objective of the present study was to elucidate the mechanism of two abundant alkyl-PAHs (dibenzothiophene and retene) and a well-studied parent PAH (benzo[*a*]pyrene) induced neurotoxicity using human neuroblastoma cells.

Methods: The SK-N-SH neuroblastoma cells were differentiated into neuronal cells and exposed with the different concentration for 24 h to obtain the cytotoxic concentrations. The 10 % and 20% lethal concentration (LC_{10} & LC_{20}) were found at around 10 μM and 40 μM. Differentiated neuronal cells were exposed with the PAHs treatments to determine the biological end points.

Results: 24 h treatment showed significant dose-dependent increase levels of reactive oxygen species, superoxide dismutase activity and the amount of lipid hydroperoxide. Alkyl-PAHs showed to be more toxic than the parent PAH and more potent in inducing all the end point measured. The exposure of alkyl-PAHs with sub-lethal concentration (500 nM) also increased the reactive oxygen species level and superoxide dismutase activity.

Conclusions: Our present data showed that benzo[*a*]pyrene, dibenzothiophene and retene may induce the neurotoxicity by the redox signaling pathway. The western blot analysis and gene expression profiles will be analyzed to elucidate the pathway of neurotoxicity.

THE ROLE OF PPARγ DURING BREAST TUMOUR ANGIOGENESIS

Jia Yue (Amelia) Shi[1], Anthony J. Apostoli[2], Rachel E. Rubino[3] and Christopher J.B. Nicol[1-3].
[1] Department of Biomedical & Molecular Sciences, Queen's University, Kingston, ON, Canada;
[2] Department of Pathology & Molecular Medicine, Queen's University, Kingston, ON, Canada;
[3] Division of Cancer Biology & Genetics, Cancer Research Institute; Queen's University, Kingston, ON, Canada.

Background: Breast cancer-related deaths result from tumour growth and spread, to which angiogenesis is a key contributor. Previously, 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 loss of PPARγ would alter angiogenic signaling during breast tumour angiogenesis.

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

Methods: Serum was collected from (n=5/group) untreated 8-12 week old WT and PPARγ-EC^{KO} mice, or (n=4/group) mice at necropsy post treatment with DMBA (1 mg/week p.o.) for 6 weeks and either continued on normal chow diet at week 7 (DMBA Only), or a diet 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. Aortic rings from untreated WT and PPARγ-EC^{KO} mice were analyzed post-treatment with VEGF ± ROSI using an aortic EC sprouting assay.

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 ($p<0.05$). In contrast, DMBA significantly decreased IL-5 and increased IL-10 and CXCL1 in PPARγ-EC^{KO} but not WT mice ($p<0.05$). DMBA+ROSI 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. VEGF+ROSI treatment reduced EC sprouting in WTs but not PPARγ-EC^{KO} aortae.

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

EVALUATING THE IMPACT OF MULTIPLE EXPOSURES ON THE MEASURE OF HUMAN VARIABILITY IN TOXICOKINETICS

Mathieu Valcke[1], Honesty Tohon[2] and Sami Haddad[2].
[1] INSPQ, Montreal, QC, Canada;
[2] IRSPUM, Université de Montréal, Montreal, QC, Canada

Background: Vinyl chloride (VC), tri- and tetrachloroethylene (TCE and PERC) may appear together in the environment, but their risk assessment seldom considers their simultaneous occurrence.

Objective: The objective of this study was to assess the impact of concomitant exposures on the magnitude of interindividual variability (IV) in internal dose metrics (IDM), using TCE and PERC as proofs-of-concept.

Methods: PBPK (Physiologically based pharmacokinetic) models for adults (AD, 30 yrs) and infants (INF, 2-6 mo) were used to simulate inhalation exposure to "low" (ACGIH's TLV) or "high" (US EPA's AEGL1) concentrations of TCE or PERC alone, together, and in binary or ternary mixture with VC. Distributions of subpopulation-specific parameters were taken from the literature and Monte Carlo simulations allowed generating distributions of area under the curve of either parent compound (AUC) or circulating metabolite (AUCTCA), as well as the maximum arterial blood concentration (Cmax) and the amount of parent compound metabolized per liter of liver (AMET). Coefficients of variation (CV) of IDM were computed as proxy of intra-group variability. Also, IV of a given IDM was assessed as the ratio of the 95th percentile value in INF over AD's median.

Results: For "low" exposure to single substances, AUC-based IV ratios were 1.6 and 1.3 for TCE and PERC respectively. Corresponding AMET-based numbers were 0.74 and 1.53 whereas those based on AUCTCA where 0.96 and 1.96. These numbers barely changed in mixtures. For "high" exposure, IV ratios were 1.57 and 1.33 for TCE and PERC alone, respectively, and slightly decreased to 1.48 and 1.27 in ternary mixtures. Conversely, AMET-based IV ratios increased, from 0.77 to 0.83 for TCE and from 1.57 to 1.76 for PERC. In all cases, CV was $\leq$ 20% but AMET and AUCTCA from PERC exhibit CV values >40% in both AD and INF. Cmax-based trends followed AUC's.

Conclusion: This study analyzed the effect of multiple exposures on interindividual variability in toxicokinetics. Our results suggest that the effect depends on the chemicals' concentrations and biochemical properties as well as the IDM considered.

TRIPHENYL PHOSPHATE AND ISOPROPYLATED TRIPHENYL PHOSPHATES INDUCE AP2 EXPRESSION AND DIFFERENTIATION OF MURINE 3T3-L1 PREADIPOCYTES VIA PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR GAMMA (PPARγ)

Emily W.Y. Tung[1], Shaimaa Ahmed[1] and Ella Atlas[1].
[1]Hazard Identification Division, Health Canada, Ottawa, Ontario

Background: Firemaster 550 is a proprietary mixture of brominated and non-halogenated compounds currently used as additive flame retardants in commercial products following the phase out of polybrominated diphenyl ethers in 2004. This mixture is composed of 4 principal chemicals: 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB), bis(2-ethylhexyl) tetrabromo-phthalate (TBPH), triphenyl phosphate (TPP) and isopropylated triphenyl phosphates (IPPP). A recent study showed that Firemaster 550 can induce adipogenesis by diverting differentiation from the osteogenic pathway in multipotent mesenchymal stromal cell models. Subsequent studies identified PPARγ as a likely target for TPP and IPPP.

Objectives: The purpose of this study was to study the potential for these compounds to induce adipocyte differentiation in a murine cell model and to further elucidate the downstream mechanisms involved in the differentiation process induced by Firemaster 550 components.

Methods: 3T3-L1 preadipocytes were exposed to a differentiation cocktail containing Firemaster 550, TPP, TBPH, TPP, or IPPP (0-200 μM) in the presence or absence of the PPARγ inhibitor GW9662 for 9 d. The extent of adipocyte differentiation was assessed by Western blotting for terminal differentiation markers [fatty acid binding protein 4 (aP2), perilipin and lipoprotein lipase], as well as fluorescent staining of lipids with Nile red. RT-PCR was used to measure expression of the transcription factors PPARγ and CEBPα throughout the differentiation process. PPARγ activation was assessed by measuring activity of the aP2 enhancer region via luciferase. PPARγ binding to aP2 promoter regions was assessed by Chromatin immunoprecipitation.

Results: TPP (20 μM), IPPP (10 μM), and Firemaster 550 (100 μM), increased adipocyte differentiation in 3T3-L1s as indicated by increased protein expression of aP2, perilipin, and lipoprotein lipase, as well as increased lipid accumulation assessed by Nile red staining. Preliminary data suggest that differentiation was inhibited with GW9662. RT-PCR revealed PPARγ and CEBPα expression were increased starting at day 4 for TPP (20 μM), IPPP (10 μM), and Firemaster 550 (100 μM), with peaks occurring at day 6. Furthermore, TPP (20 μM), IPPP (10 μM), and Firemaster 550 (100 μM) increased PPARγ activity as measured by luciferase activity of the aP2 enhancer. In addition, chromatin immunoprecipitation assays revealed increased binding of PPARγ to the aP2 promoter following treatment with TPP (20 μM) and IPPP (10 μM).

Conclusions: We established that Firemaster 550, TPP, and IPPP induce the differentiation of preadipocytes to mature adipocytes in 3T3-L1s through PPARγ activation.

USING HUMAN RECOMBINANT ENZYME AMES ASSAYS FOR ENHANCED DETECTION OF AIR BORNE CARCINOGENS.

Aaron A. Witham[1], Will Lush[1] and David P. Josephy[2].
[1] Environmental Bio-detection Products Incorporated (EBPI), Mississauga, Ontario, Canada;
[2] Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada.

Background: Air pollution remains a prominent cause of adverse health effects despite increasing monitoring strategies and government legislation. Chronic diseases like cardiopulmonary disease and lung cancers are attributed to air borne carcinogens and fine particulate matter ($PM_{10\text{-}2.5}$) which can penetrate into lower airways and be absorbed by the alveoli. Identifying and quantifying human exposure to toxic compounds in air pollution is critical to assess public health risk, but the diversity of toxicants in air pollution and their potential interactions means that accurate human risk assessments cannot be adequately addressed using chemical analysis alone. Biological effect measurements, like mutagenicity testing, improve risk assessments by measuring sub-chronic effects from unidentified substances, and accounting for synergistic or antagonistic effects from pollutant mixtures.

Objectives: The main objective of this work was to compare the efficacy of recently developed bacterial strains from EBPI that express human metabolic enzymes to traditional strategies for the detection of mutagenic effects from air pollutant extracts. The design of a filtration and extraction system that was compatible with the test methods was also highly desired to potentially implement this technology for commercial analysis. The study present compared bacterial strains expressing CYP1A2 enzymes to traditional Ames strains including S9 activation for their ability to detect mutagenic responses for filtered air samples from a variety of sources.

Methods: Ames assays were conducted on 4 dilutions of concentrated extracts filtered from cigarette smoke and incense using traditional Ames strains, traditional strains with S9 addition and new Ames-Express™ bacteria with human CYP1A2 enzyme expression. A modified ISO procedure was used (EBPI), based on the traditional fluctuation assay method (ISO 11350). All samples were run in triplicate and a student's *t*-test was used to evaluate significance.

Results: Upon testing the PM extracts, statistically positive responses ($p < 0.01$) were observed for most concentrations tested using the Ames-Express™ P450 1A2 strains. These positive responses were not observed using traditional TA 98 and TA 100 bacteria which was suggestive of selective bioactivation of pro-carcinogens in the extracts by the P450 1A2-expressing strain. The levels of mutagenicity from the P450 1A2-expressing strain were lower than traditional strains with S9 addition however, the rates of mutagenicity were comparable between these two systems. Some PM components appear to me bioactivated with enzymes other than CYP1A2.

Conclusions: The Ames-Express™ P450 1A2 bacteria demonstrated significant improvements in sensitivity over traditional Ames test strains to detect mutagenic responses from PM extracts. The comparable mutagenicity to S9 addition is significant, as liver homogenate is expensive to produce, more difficult to use, and could be reliably replaced with the CYP1A2 strains. We believe that this technology has great potential to provide sensitive and facile analysis of genotoxic effects in air samples collected from industrial and residential sources.

POSTER INDEX / INDEX DES AFFICHES

La Société de Toxicologie du Canada tient à remercier les organisations suivantes pour leurs précieuses contributions et le soutien financier qui appuient la réussite de notre Symposium Annuel.

GOLD • OR

charles river | every step of the way.

SILVER • ARGENT

Intertek
Valued Quality. Delivered.

syngenta

CiToxLAB
Safety and Health Research Laboratories

FACETS
a multidisciplinary open access science journal

Altasciences
CLINICAL RESEARCH
ALGORITHME PHARMA

BRONZE

Bedard ADME-Tox Solutions

David Josephy, Ph.D.
Teaching & Consulting
Biochemistry & Toxicology

EY

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.