43nd Annual Symposium / Le 43e symposium annuel

December 4 – 6, 2011
Delta Centre-Ville
777 University
Montreal, Quebec
H3C 3Z7
Tel. 514-879-1370

Low Dose Effects and Their Use in Risk Assessment: When is an Effect Adverse?

Les effets de faibles doses et le recours à ceux-ci dans l'évaluation des risques: Dans quels cas un effet est-il nocif?

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

Programme Committee / Comité du programme
Douglas Bryant, Intrinsik Science Inc., chair
Sami Haddad, Université de Montréal
Jayadev Raju, Health Canada

Sunday Dec 4 PM

1:00 – 5:00 STC Board meeting - Room 532

7:00 – 9:00 Student mentoring session - Room 2810

Low Dose Effects and Their Use in Risk Assessment: When is an Effect Adverse?

Monday Dec 5 AM

7:30 Registration / Continental Breakfast - Foyer Régence AB

8:30 **Roger Keefe**, Imperial Oil, President STC
Opening remarks and Introduction

Session I: Low Dose Risk Assessment, Theory and Practice

Chairperson: Douglas Bryant, Intrinsik Science Inc

8:40 Introduction

8:45 **Dan Krewski**, McLaughlin Centre for Population Health Risk Assessment, University of Ottawa
New Directions in Toxicity Testing: Understanding Key Events in Toxicity Pathways at Low Doses

9:30 **James Bus,** Dow Chemical Company
Low Dose Risk Assessment: Experimental Verification of Extrapolation from High Doses

10:15 **Coffee break and poster session**

10:45 **Edward Calabrese**, University of Massachusetts
Hormesis: Its Significance for Toxicology, Pharmacology and Risk Assessment

11:30 **Alexander Suvorov,** Boston University
New Pipeline to Integrate Low-Dose Gene Expression Microarrays Studies into Risk Assessment [Poster #21]

Laetiscia Lavoie, Université de Sherbrooke
Polybrominated Diphenyl Ethers (PBDE) Exposure During Early Pregnancy and Maternal and Fetal Thyroid Function [Poster #9]

Monday Dec 5 PM

12:00 **Lunch**
Poster Session + Cantox Award Judging - Régence B

Session II: Adverse Effects – Biological Perturbations at Low Exposure

Chairperson: Douglas Bryant, Intrinsik Environmental Sciences

1:10 **Introduction**

1:15 **Carole Yauk**, Health Canada
Effects of Air Pollutants on Male Germ Cells at Environmentally Relevant Exposure Levels

2:00 **Craig Parfett**, Health Canada
Assessment of Subclinical Changes in Hepatic Gene Expression Profiles after Low Dose, Short Term Exposures

2:45 **Coffee break and poster session**

3:15 **Catherine Klein,** New York University Langone Medical Center
Low Dose Considerations and Epigenetic Effects of Environmental Agents

4:00 **Hamzeh Mahsa,** National Research Council Canada
A Study of the Mechanism of *in vitro* Cytotoxicity and Genotoxicity of Titanium Dioxide (TiO_2) Nanoparticles: Impact of Physico-Chemical Characteristics [Poster #16]

Pavine L.C. Lefèvre, McGill University
An Environmentally-relevant Exposure to Brominated Flame Retardants (BFRs) *in utero* Induces Fetal Limb Malformations in Sprague-Dawley Rats [Poster #10]

4:30 **Workshop** Toxicogenomics : Experimental Design & Data Analysis of Microarray Studies – Régence A
Instructors: Craig Parfett and Andrew Williams, Health Canada

4:30 **Annual STC Business Meeting** – Régence C

6:00 **President's reception & STC awards** – La Terrasse

ToxQuiz – an animated challenge to your knowledge of Toxicology, risk assessment and posters at STC 2011
H*ost*: Sami Haddad

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Tuesday Dec 6 AM

7:00 Continental Breakfast - Foyer Régence AB

Session III: Carcinogenesis and Low Exposure Toxicology

Chairperson: Sami Haddad, Université de Montréal

8:00 Introduction

8:05 **Henderson Award Lecture**
Jason Matthews, University of Toronto
TiPARP is a Negative Regulator of Aryl Hydrocarbon Receptor Transactivation

8:50 **Julian Preston**, NHERL, US EPA, Research Triangle Park, NC
Risks at Low Doses of Radiation: Implicatons for Diagnostic Radiology

9:35 **Coffee break and poster session**

10:05 **James Swenberg,** University of North Carolina
Endogenous *vs.* Exogenous DNA Adducts: Their Role in Carcinogenesis, Epidemiology and Risk Assessment

10:50 **Carlos Sonnenschein,** Tufts University
Tissue Organization Field Theory – a Replacement for the Somatic Mutation Theory of Cancer

11:35 **Poster session (please remove posters by 1:00)**

12:00 **Lunch**

Tuesday Dec 6 PM

Session IV: Low Dose Toxicity in Humans

Chairperson: Jayadev Raju, Health Canada

1:00 Introduction

1:05 **Robert Schnatter**, ExxonMobil Biomedical Sciences Inc
New Benzene Findings in Occupational Epidemiologic Studies and Implications for Benzene Risk Assessment

1:50 **D. H. Garabrant**, University of Michigan
Dioxin Exposure Pathways in the Population of Midland Michigan

2:35 **Summary and Closing Remarks**
Roger Keefe, Imperial Oil, President STC

3:00 **Adjourn**

La Société de Toxicologie du Canada remercie les entreprises qui ont contribué par leur appui financier à l'organisation et au succès de notre Symposium

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The Society of Toxicology of Canada is grateful to the above organizations for their valued interest and support of our Annual Symposium

Speaker abstracts and biographies

Monday Dec 5 AM

Session I:
Low Dose Risk Assessment – Theory and Practice

New Directions in Toxicity Testing: Understanding Key Events in Toxicity Pathways at Low Doses

Monday, December 5, 8:45 – 9:30 AM

Daniel Krewski, *McLaughlin Centre for Population Health Risk Assessment, University of Ottawa*

In 2007, the U.S. National Research Council released a report entitled *Toxicity Testing in the 21st Century: A Vision and a Strategy*, which proposes a paradigm shift in the way toxicity testing of environmental agents is conducted. The vision, commonly referred to as TT21C, is based on the notion that exposure to environmental agents can lead to adverse health outcomes through the perturbation of human toxicity pathways. Significant progress in implementing TT21C has been made since that time, and international support for the vision has been increasing. Implementation of the vision will require a concomitant paradigm shift in exposure assessment. New directions in exposure assessment include consideration of exposures at all levels of biological organization, including those directly linked to toxicity pathway perturbations. Increased reliance on molecular and genetic epidemiology will entail greater use of biomarkers of both exposure and biological change. With the anticipated widespread reliance on high throughput *in vitro* screening assays to identify pathway perturbations, there will be need for the development and application of new methods for *in vitro* to *in vivo* dosimetric extrapolation, including those based on reverse toxicokinetics. As this paradigm shift in exposure assessment takes place, human exposure guidelines may be based on biomonitoring equivalents defined in terms of serum concentrations of relevant exposure biomarkers rather than exogeneous exposure levels. The role of exposure assessment in chemical prioritization for risk assessment purposes is explored. The application of these new approaches to exposure assessment is illustrated using prototype risk assessments involving in vitro biomarkers of biological effect and reverse toxicokinetics.

Dr. Daniel Krewski is currently Scientific Director of the McLaughlin Centre for Population Health Risk Assessment at the University of Ottawa and holds a Natural Sciences and Engineering Research Council (NSERC) of Canada Industrial Research Chair in Risk Science. Since 2008, Dr Krewski has also served as Associate Scientific Director for PrioNet Canada, a Network of Centres of Excellence involving 15 Canadian universities, investigating the health risks of prion diseases such as bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob disease (vCJD).

Dr Krewski is also President and CEO of Risk Sciences International Inc. since 2007; Director of the Network for Environmental Risk Assessment and Management (NERAM-Ottawa node) since 2000; Director of the Graduate Certificate in Population Health Risk Assessment and Management, Institute of Population Health since 2004; Adjunct Research Professor of Statistics in the Department of Mathematics and Statistics at Carleton University since 1984. While with Health Canada, he also served as Acting Director of the Bureau of Chemical Hazards and as Chief of the Biostatistics Division in the Environmental Health Directorate. Dr Krewski is also the principal investigator of a multi-centre project involving 5 universities in

the National Population Health Study of Neurological Conditions funded by the Public Health Agency of Canada (PHAC).

Dr. Krewski obtained his Ph.D. in statistics from Carleton University and subsequently completed his M.H.A. at the University of Ottawa. He is internationally known for his research on environmental determinants of health, has contributed to 160 peer-reviewed scientific papers, served as principal or co-investigator on 59 externally funded grants/contracts, and mentored 31 trainees (25 pre-doctoral and 6 post-doctoral) in the last five years. Dr. Krewski has published over 600 articles in scientific and technical reviews; he has also authored or directed the publication of six books to date.

New Tools of Toxicology and Exposure Science: Informing the Shape of Dose-Response under Conditions of Low-Dose Exposure

Monday, December 5, 9:30 – 10:15 AM

James Bus, *Dow Chemical Company, Midland, Michigan*

The advent of a variety of exciting new technologies such as toxicogenomics, bioinformatics, and high-throughput mechanism-based screening tests affords an opportunity to dramatically reframe the existing toxicity testing and risk assessment paradigm that is inarguably burdened with concerns of excessive costs, speed of delivery, use of animals, ability to inform risk, among others. Nonetheless, implementation of these promising technologies must be done thoughtfully and with great caution. In particular, in order to improve estimation of human risks occurring under conditions of real-world exposures to environmental chemicals, future toxicity testing must direct close attention to better understanding dosimetry relationships from findings of toxicity studies, both *in vivo* and *in vitro*, to anticipated or known human exposures. Recent rapid advances in analytical sciences often permits sensitive and rapid quantitation of chemical parent and/or key metabolite(s) both in whole animal toxicity studies and in human biomonitoring programs. Thus the opportunity now exists to better position doses of test materials and/or their metabolites examined in whole animal toxicity test systems to appropriate internal, target organ dosimetry contexts generated not only in conventional animal tests but also in humans under conditions of real-world exposures. Equally important, appropriate matching of *in vitro* dosimetry to *in vivo* dosimetry will greatly facilitate the potential value of high-throughput assay outputs to understanding potential hazard and risk. Thus, developing better fundamental understanding of dose response relationships under conditions of real-world, low-dose exposures will significantly improve understanding of potential human health risks associated with those exposures.

James S. Bus is Director of External Technology, Toxicology and Environmental Research and Consulting at The Dow Chemical Company (1989-present). He previously held positions as Associate Director of Toxicology and Director of Drug Metabolism at The Upjohn Company (1986-1989), Senior Scientist at the Chemical Industry Institute of Toxicology (CIIT, 1977-1986), and Assistant Professor of Toxicology, University of Cincinnati (1975-1977). Dr. Bus currently serves on the Board of Directors of The Hamner Institutes (formerly CIIT). He has also has served as Chair of the American Chemistry Council and International Council of Chemical Associations Long-Range Research Initiatives; the USEPA Office of Research and Development Board of Scientific Counselors (1997-2003) and Chartered Science Advisory Board (2003-2009); the National Toxicology Program Board of Scientific Counselors (1997-2000); the FDA National Center for Toxicological Research Science Advisory Board (2004-2010); and the National Academy of Sciences/National Research Council Board on Environmental Studies and Toxicology (BEST; 2005-2011). He serves as an Associate Editor of Toxicology and Applied Pharmacology,and on the Editorial

Boards of Environmental Health Perspectives and Dose Response. Dr. Bus is a member of the Society of Toxicology (serving as President in 1996-97), the American Society for Pharmacology and Experimental Therapeutics, the American Conference of Governmental and Industrial Hygienists, and the Teratology Society.

He is a Diplomate and Past-President of the American Board of Toxicology and a Fellow of the Academy of Toxicological Sciences (member of Board of Directors, 2008-present; Vice-President and President, 2010-2011). Dr. Bus received the Society of Toxicology Achievement Award (1987) for outstanding contributions to the science of toxicology; the Society of Toxicology Founders Award (2010) for leadership fostering the role of toxicology in improving safety decisions; Rutgers University Robert A. Scala Award (1999) for exceptional work as a toxicologist in an industry laboratory; and the K.E. Moore Outstanding Alumus Award (Michigan State University, Dept. Pharmacol. and Toxicol.).

He received his B.S. in Medicinal Chemistry from the University of Michigan (1971) and Ph.D in pharmacology from Michigan State University (1975) and currently is an Adjunct Professor in the Dept. Pharmacology and Toxicology at that institution. His research interests include mechanisms of oxidant toxicity, defense mechanisms to chemical toxicity, relationships of pharmacokinetics to expression of chemical toxicity, and general pesticide and industrial chemical toxicology. He has authored/co-authored over 100 publications, books, and scientific reviews.

Hormesis: Its Significance for Toxicology, Pharmacology, and Risk Assessment

Monday, December 5, 10:45 – 11:30 AM

Edward Calabrese, *University of Massachusetts, Amherst, Massachusetts*

This presentation provides an assessment of hormesis, a dose-response concept that is characterized by a low-dose stimulation and a high-dose inhibition. It will trace the historical foundations of hormesis, its quantitative features and mechanistic foundations, and its risk assessment implications. It will be argued that the hormetic dose response is the most fundamental dose response, significantly outcompeting other leading dose-response models in large-scale, head-to-head evaluations used by regulatory agencies such as the EPA and FDA. The hormetic dose response is highly generalizable, being independent of biological model, endpoint measured, chemical class, physical agent (e.g., radiation) and interindividual variability. Hormesis also provides a framework for the study and assessment of chemical mixtures, incorporating the concept of additivity and synergism. Because the hormetic biphasic dose response represents a general pattern of biological responsiveness, it is expected that it will become progressively more significant within toxicological evaluation and risk assessment practices as well as having numerous biomedical applications, some of which will be emphasized in this presentation.

Edward J. Calabrese is a Professor of Toxicology at the University of Massachusetts, School of Public Health and Health Sciences, Amherst. Dr. Calabrese has researched extensively in the area of host factors affecting susceptibility to pollutants, and is the author of over 600 papers in scholarly journals, as well as more than 10 books, including Principles of Animal Extrapolation; Nutrition and Environmental Health, Vols. I and II; Ecogenetics; Multiple Chemical Interaction; Air Toxics and Risk Assessment; and Biological Effects of Low Level Exposures to Chemical and Radiation. Along with Mark Mattson (NIH) he is a co-editor of the recently published book entitled Hormesis: A Revolution in Biology, Toxicology and Medicine. He has been a member of the U.S. National Academy of Sciences and NATO Countries Safe Drinking Water committees, and on the Board of Scientific Counselors for the Agency for Toxic Substances and Disease Registry (ATSDR). Dr. Calabrese also serves as Chairman of the Biological Effects of Low Level Exposures

(BELLE) and as Director of the Northeast Regional Environmental Public Health Center at the University of Massachusetts. Dr. Calabrese was awarded the 2009 Marie Curie Prize for his body of work on hormesis. He was recently announced as the recipient of the International Society for Cell Communication and Signaling-Springer award for 2010. The award will be officially given at the annual conference in Ireland in October.

Over the past 20 years Professor Calabrese has redirected his research to understanding the nature of the dose response in the low dose zone and underlying adaptive explanatory mechanisms. Of particular note is that this research has led to important discoveries which indicate that the most fundamental dose response in toxicology and pharmacology is the hormetic-biphasic dose response relationship. These observations are leading to a major transformation in improving drug discovery, development, and in the efficiency of the clinical trial, as well as the scientific foundations for risk assessment and environmental regulation for radiation and chemicals.

Selected Highlights from the Posters

Monday, December 5, 11:30 – 12:00 AM

New Pipeline to Integrate Low-Dose Gene Expression Microarrays Studies into Risk Assessment [Poster #21]
Alexander Suvorov, *Boston University*

Polybrominated Diphenyl Ethers (PBDE) Exposure During Early Pregnancy and Maternal and Fetal Thyroid Function [Poster #9]
Laetiscia Lavoie, *Université de Sherbrooke*

Monday Dec 5 PM

Session II:
Adverse Effects – Biological Perturbations at Low Exposure

Effects of Air Pollutants on Male Germ Cells at Environmentally Relevant Exposure Levels

Monday, December 5, 1:15 – 2:00 PM

Carol Yauk, *Health Canada*

Particulate air pollution contains compounds that are genotoxic and carcinogenic. Air particles are associated with polycyclic aromatic compounds and metals which can be directly or indirectly mutagenic through the creation of DNA adducts and oxidative stress. Air quality standards around the world are primarily based on the increased cardiopulmonary events that are observed following exposure to particulate air pollution. These events are highly relevant to overall public health and well-being. Data from our lab demonstrate that male germ cells are also highly sensitive to environmental levels of particulate air pollution. Our work investigates the genetic consequences of parental exposure to particulate air pollutants on their gametes and their unexposed descendants.

We have established that exposure to various sources of particulate air pollutants causes mutations at repetitive DNA elements in rodent sperm. For example, exposure of male mice to ambient particulate air pollution in Hamilton, Ontario exhibit increased DNA strand breaks, DNA mutations and altered DNA methylation in sperm relative to animals breathing HEPA-filtered air. Levels of oxidative stress are higher in testes than in brain, heart or liver of these particle-exposed mice relative to mice breathing HEPA-filtered air. Mice exposed to both mainstream and sidestream tobacco smoke at environmentally relevant levels show similar increases in mutation frequency, above those doses leading to induction of micronuclei in circulating red blood cells. Various sperm endpoints (sperm motility and DNA damage) measured in these mice are consistent with observations in human smokers. The data demonstrate that particles derived from combustion cause DNA damage and mutation in gametes at environmentally-relevant exposure levels, and that germ cells may be a particularly responsive cell type relative to other somatic cells. The mechanisms for gamete susceptibility to particles remain elusive but are the subject of research in our laboratory. DNA damage in germ cells can lead to heritable mutations that may result in a wide variety of detrimental outcomes, from embryonic lethality to genetic disease in the offspring. Thus, hazards to germ cells are critically important to evaluate in the context of the health of future generations.

Dr. Carole Yauk obtained a Ph.D. in biology from McMaster University in Hamilton, Ontario and won the Natural Sciences and Engineering Research Council (NSERC) doctoral prize for her dissertation on germline mutation in herring gulls inhabiting contaminated urban/industrial locations. She obtained a NSERC post-doctoral fellowship to travel to the United Kingdom for two years to work with Professor Sir Alec Jeffreys and Dr. Yuri Dubrova at the University of Leicester. There, Dr. Yauk developed novel approaches to study mutation and recombination directly in sperm. She remained at the University of Leicester for an additional two years as a Wellcome Trust research associate. In 2002, Dr. Yauk returned to Canada to establish her own research program in germline toxicology and toxicogenomics. Dr. Yauk is currently a research scientist at Health Canada and an adjunct professor at Carleton University. In 2006, Dr. Yauk won the Deputy Minister's Award of Excellence in Science for Most Promising Scientist (Health Canada). She is on the editorial board of *Mutation Research Fundamental and Molecular Mutagenesis* and is the associate editor of *Environmental and Molecular Mutagenesis*.

Assessment of Subclinical Changes in Hepatic Gene Expression Profiles after Low Dose, Short Term Exposures

Monday, December 5, 2:00 – 2:45 PM

Craig Parfett, *Health Canada*

Gene expression profiling that examines critical, toxicologically-relevant gene and signal-response pathways promises to improve risk assessment and safety evaluation of low-dose chemical exposures. As an approach to achieving this goal, mechanistic interpretations based upon gene expression changes that are determinants of adverse toxicological outcomes were applied to the analysis of low-dose gene expression profiles. RNA for expression profiling was obtained from mice given short-term gavage exposures to diminishing doses of four toxicants: 3,3',4,4',5-pentachlorobiphenyl (PCB126), phenobarbital (PB), isoproterenol (IPR), and lead acetate (PbAc). Lowest doses were below the no-observable effects levels established using standard clinical toxicology

parameters. Hepatic gene expression profiles were analyzed using a custom, focused oligonucleotide DNA microarray, the HC ToxArray™, containing toxin-responsive and toxicologically-determinant genes. Expression data were compared to changes in conventional clinical chemistry parameters and drug metabolism activities. PCB126 and PB demonstrated a dose-dependent correlation between minimal changes in biochemical markers, hepatic metabolism and induction of gene expression profiles. PbAc exposure gave a small adaptive profile at the highest dose. IPR- and PCB126-induced changes were detected at doses below those required to alter the traditional biochemical endpoints and included genes with causal roles in hepatic toxicity, insulin resistance, atherosclerosis, angiogenesis and hypertension. Likely adverse phenotypic consequences resulting from expression changes lead to assignments of "Lowest Observed Adverse Transcriptional Expression Levels" (LOATEL) for each agent. These results support the suggestion that altered expression profiles of genes contributing to toxicologically-relevant pathways provide useful tools for reducing uncertainty in establishing no-effect levels and for designing longer-term toxicity studies.

Since 1989, Dr. Parfett has been a research scientist within Health Canada and is currently in the Mechanistic Studies Division, Environmental Health Science and Research Bureau, Environmental Health Directorate. Tunney's Pasture, Ottawa, Ontario. Prior appointments were as Assistant professor in the Dept. of Pharmacology and Toxicology, and the Dept. of Biochemistry, University of Western Ontario, which followed his MRC Postdoctoral Fellowship in the Cancer Research Laboratory at UWO.

Dr. Parfett's major interests and efforts since joining Health Canada have centred on molecular aspects of tumour promotion and development of cell transformation assays for application in regulatory testing, especially in the detection of non-genotoxic carcinogens. He also has been developing rapid assays for genetic instability induced indirectly by chemical exposures through changes in the expression of genes involved in DNA replication, DNA repair and DNA metabolism as a mode of action. Toxicogenomic approaches provide important insights in these endeavours. Some of his work has also been directed toward refinement of the methodologies and data analysis involved in array-based gene expression profiling with a view to the data requirements for decision-making within regulatory agencies.

Low Dose Considerations and Epigenetic Effects of Environmental Agents

Monday, December 5, 3:15 – 4:00 PM

Catherine Klein, *New York University Langone Medical Center, Tuxedo Park, New York*

Epigenetic toxicology is an emerging field that interrogates various epigenetic endpoints to ascertain potentially heritable effects of genotoxic and nongenotoxic carcinogens and other environmental agents. Many of the same principles that apply to traditional genetic toxicology studies, including dose response, low dose effects, duration of exposure, persistence of effect upon withdrawal of the agent, and response mitigators can also be applied to epigenetic toxicology studies. Using examples from ongoing research on carcinogenic metals, arsenic and anti-mutagenic compounds, these principals will be demonstrated. Goals for future epigenetic toxicology studies will be discussed.

Catherine B. Klein, Ph.D., is an Assistant Professor of Environmental Medicine at NYU Langone Medical Center. She received her Ph.D. in Environmental Health Sciences from NYU. Her research and expertise in mutagenesis and epigenetic effects has been focused around the effects of carcinogenic metals, nickel,

chromium and arsenic. She has co-authored several papers demonstrating the first evidence of epigenetic gene silencing by nickel and chromium. She currently serves on the editorial boards of *Mutagenesis*, *Mutation Research Reviews* and *Environmental and Molecular Mutagenesis*. Dr. Klein is the incoming (2011) President of the Environmental Mutagen Society (EMS) and served in October as Program Chair for the 2011 annual meeting of the EMS, the theme of which was "Environmental Impacts on the Genome and Epigenome: Mechanisms and Risks".

Selected Highlights from the Posters

Monday, December 5, 4:00 – 4:30 PM

A Study of the Mechanism of *in vitro* Cytotoxicity and Genotoxicity of Titanium Dioxide (TiO_2) Nanoparticles: Impact of Physico-Chemical Characteristics [Poster #16]
Hamzeh Mahsa, *National Research Council Canada*

An Environmentally-Relevant Exposure to Brominated Flame Retardants (BFRs) *in utero* Induces Fetal Limb Malformations in Sprague-Dawley Rats [Poster #10]
Pavine L.C. Lefèvre, *McGill University*

Workshop

Toxicogenomics: Experimental Design and Data Analysis of Microarray Studies

Monday, Dec 5, 4:30 – 6:00 PM

Instructors: Craig Parfett and Andrew Williams, *Health Canada*

The adoption of appropriate experimental design may be the greatest change for toxicogenomics. Many recent studies which are available in public repositories such as the National Center for Biotechnology Information (Gene Expression Omnibus) or European Bioinformatics Institute (ArrayExpress) exhibit inadequate consideration of experimental design issues. Such as the lack appropriate matched controls and/or confound the biology with known microarray batch effects which may compromise the analysis and interpretation of the data. This workshop will cover various experimental designs for microarrays and discuss various batch effects that often appear in microarray data. The importance of having technical and biological variability and matched controls will also be discussed. Quality control and different statistical analyses such as tools for reducing the dimensionality through gene sets will be demonstrated using publicly available data.

Outline

1. Introduction
2. Comparison of microarray platforms

   Briefly discuss the various microarray platforms and the initial investigations (years 2000–2003) that found discordance in the gene expression measures produced by different microarray technologies. Many of these studies were flawed with regards to the experimental design and/or poor annotation of the probes on the microarrays.
3. Experimental design

   We will examine experimental design issues that occur in microarrays such as sources of variation. We will review some of the commonly used designs such as the reference design, loop based designs, direct comparisons and randomized block designs. We will discuss dye-swaps and other technical sources of variation that are often more pronounced than the biology. An emphasis on blocking, randomization and ensuring that effects of interest are not confounded with secondary effects.
4. Data processing, filtering and quality control

   Improved data normalization and quality control has led to much higher levels of concordance between platforms among more recent publications (2004 to present).
5. Data analysis

   Here we will look a number of available tools for per gene analysis such as SAM and MAANOVA as well as a number of gene set enrichment methods such as GSEA, Global and the Rank Test. We will also look at methods and examples for class discovery and class prediction for example assigning an exposure to its phenotype group.
6. Microarray Confirmation

   Briefly we will discuss various methods for validating a microarray study using techniques such as RT-PCR and discuss discrepancies that may occur in the confirmation process.
7. Data repositories and publically available databases

   For most microarray publications the data is publicly available. Here we will discuss how these data may be used in study design for example in power calculations. Will also discuss how these data and publicly available databases can add support to an existing project.

Andrew Williams received a MSc in biostatistics from Carleton University, Ottawa, Ontario. He joined the Biostatistics Unit in the Environmental Health Sciences and Research Bureau at Health Canada in 2001, where he provided statistical support to research within the Bureau. In 2002 the focus of his work shifted to development of methodologies to analyze and interpret global gene expression data, with an emphasis on DNA microarrays. His research contributions include technological validation exercises, and methods development for data handling and pathway analysis. Additional research is aimed at emerging genomics endpoints including analysis of changes in DNA methylation, transcription factor binding (i.e., ChIP-chip and ChIP-SEQ) and global microRNA analyses. His primary focus is on developing statistical tools to help biologist interpret their microarray data through data visualization techniques and gene set statistics. Mr. Williams has over 30 publications in this field of research.

Tuesday Dec 6 AM

** 2011 Henderson Award Lecture **

TiPARP is a Negative Regulator of Aryl Hydrocarbon Receptor Transactivation

Tuesday, December 6, 8:05 – 8:50 AM

Jason Matthews, *Department of Pharmacology and Toxicology, University of Toronto*

The aryl hydrocarbon receptor (AHR) was initially identified as a receptor that mediates the toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other environmental toxicants; however, AHR also has significant biological roles and is being recognized as an important therapeutic target for a number of human diseases including breast cancer. Although microarray and ChIP-chip experiments have identified numerous AHR target genes, our understanding of mechanisms that regulate AHR transactivation remains incomplete. Moreover, the roles of many AHR target genes in AHR-mediated toxicity or AHR-dependent signaling pathways have not been fully investigated. For example, TCDD-inducible poly(ADP-ribose) polymerase (TiPARP) is an AHR target gene that is induced following TCDD treatment in almost all cell and animal model examined, but very little is known about its function. To this end we investigated the effect of loss and overexpression of TiPARP on AHR-dependent regulation of cytochrome P450 1A1 (CYP1A1) and CYP1B1. RNAi-mediated knockdown of TiPARP in T47D human breast cancer and other cell lines significantly increased TCDD-induced CYP1A1 and CYP1B1 expression. TiPARP overexpression decreased TCDD-induced *CYP1A1*- and *CYP1B1*-regulated reporter activity in a dose-dependent manner. This was observed for both mouse and human, but not chicken TiPARP. The inhibition was rescued with AHR but not ARNT overexpression. TiPARP truncations and point mutants revealed TCDD-induced inhibition required the zinc-finger and catalytic function. Co-immunoprecipitation and co-localization studies showed that TiPARP and AHR are part of the same protein complex and interact. We also observed that TiPARP possesses mono-ADP-ribosyltransferase (mART) activity rather than poly(ADP-ribosyl)ation activity, suggesting that TiPARP belongs to the growing family of PARP-like mART proteins. Collectively, these findings suggest that TiPARP is a mART and acts as a negative regulator of AHR transactivation.

Jason Matthews received his B.Sc. Degree in Toxicology and Environmental Science from the University of Western Ontario. He completed his Ph.D. degree in 2001 in Biochemistry and Environmental Toxicology at Michigan State University under the supervision of Professor Tim Zacharewski. He then moved to Sweden to train as a postdoctoral fellow in Professor Jan-Åke Gustafsson's laboratory at the Karolinska Institute. In 2006 Jason joined the faculty at the University of Toronto where he is currently an Associate Professor in the Department of Pharmacology and Toxicology. Research in his laboratory is focused on understanding the mechanisms of transcription factor dependent gene activation/repression by aryl hydrocarbon and estrogen receptors. In addition, his laboratory is interested in understanding the molecular mechanisms by which environmental chemicals modulate receptor signaling pathways, potentially increasing the risk of developing hormone dependent cancers.

Session III: Carcinogenesis and Low Exposure Toxicology

Risks at Low Doses of Radiation: Implications for Diagnostic Radiology

December 6, 8:50 – 9:35 AM

Julian Preston, *US EPA, Research Triangle Park, North Carolina*

Cancer risk estimates are used in the setting of radiation protection standards by international and national organizations, and for this purpose need to be developed for low doses of radiation. The approach has involved extrapolation from human cancer mortality and incidence values at higher doses to predict low dose estimates. Such an extrapolation has generally involved the use of the linear non-threshold (LNT) theory. Recent reports from the National Research Council (BEIR VII), the International Commission on Radiological Protection (ICRP), and the Environmental Protection Agency (EPA) reached the overall conclusion that current scientific evidence remains consistent with the LNT hypothesis, while appreciating that this might not rule out the possibility that other extrapolation models might well be valid but require further evaluation and additional research to establish their validity. There are data from radiobiology studies that show that non-targeted effects (e.g., bystander, genomic instability) increase the response from linearity at low doses, particularly in vitro. Also, it has been proposed that observed adaptive responses or hormetic responses could lower cancer risks at very low doses. However, at this time there is insufficient evidence on the impact of these effects on cancer frequency at low doses to include them in the cancer risk assessment process.
The current cancer risk estimation process as utilized by ICRP (ICRP 2007) and BEIR VII (NRC 2006) will be used in the presentation to assess the potential risks from annual whole-body CT screens using information and an approach published by Brenner and Ellington. The major conclusion is that potential radiation risks need to be considered along with the pros and cons of the detection limits of the procedure and the comparative benefits and risks. This serves to highlight the importance of using radiation protection practices in modern radiological imaging.

R. Julian Preston, PhD is currently Associate Director for Health for the National Health and Environmental Effects Research Laboratory of the U.S. EPA in Research Triangle Park, NC. He served as Director of the Environmental Carcinogenesis Division at the EPA from 1999 until August 2005. He served as the Senior Science Advisor at the Chemical Industry Institute of Toxicology (now The Hamner Institute) in Research Triangle Park, NC from 1991-1999. He was employed at the Biology Division of the Oak Ridge National Laboratory in Oak Ridge, Tennessee from 1970-1991. He also served as Associate Director for the Oak Ridge – University of Tennessee Graduate School for Biomedical Sciences. Dr. Preston received his BA and MA from Peterhouse, Cambridge University, England in genetics and his Ph.D. from Reading University, England in radiation genetics.

Currently, Dr. Preston is Chair of Committee 1 of the International Commission on Radiological Protection (ICRP), a member of the US Delegation to the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), Chair of a Committee on Uncertainty in Risk Estimation for the National Council on Radiation Protection (NCRP) and a member of the HESI DNA Adducts and Risk Assessment Project and Risk 21Committee. He has served on several NAS Committees including as chair on the Committee to review The Radiation Exposure Screening and Education Program and currently as chair of the Committee to Review the NASA Space Radiation Cancer Risk Projections and Uncertainties. He is an Associate Editor for

Environmental and Molecular Mutagenesis, Mutation Research, and Chemico-Biological Interactions. Dr. Preston's research and current activities have focused on the mechanisms of radiation and chemical carcinogenesis and the approaches for incorporating these types of data into cancer risk assessments. Dr Preston has published over 200 peer-reviewed papers and chapters.

Formaldehyde Carcinogenicity: Use of Molecular Toxicology to Evaluate Biological Plausibility and Low Dose Risk Assessment

Tuesday, December 6, 10:05 – 10:50 AM

James A. Swenberg, *Department of Environmental Sciences and Engineering, University of North Carolina*

Formaldehyde is classified as a known animal and human carcinogen, causing nasal cancer. It also has epidemiology studies that suggest a causal role in the induction of leukemia in humans. What has gotten less attention is that formaldehyde is also an essential chemical in all living cells. This endogenous presence of formaldehyde raises interesting issues related to low dose risk assessment. We have developed very sensitive LC-MS/MS methods to quantitate formaldehyde DNA adducts and have combined this approach with the use of stable isotope-labeled formaldehyde inhalation exposures so that a better understanding of the relationships of inhaled formaldehyde to endogenous formaldehyde is known. Studies have been conducted in rats for 1-5 days of exposure at concentrations ranging from 0.7 – 15 ppm, and in monkeys exposed to 2 or 6 ppm [$^{13}CD_2$]-formaldehyde. DNA adduct studies have demonstrated that inhaled formaldehyde only reaches sites of direct contact and that sites distant to these do not contain [$^{13}CD_2$]-labeled DNA adducts, but always have non-labeled DNA adducts. The ratio of exogenous/ endogenous DNA adducts is highly non-linear in nasal mucosa. The data support both a cytotoxic and a genotoxic Mode of Action for the induction of cancer in the upper respiratory system, but do not support a causal role for the induction of leukemia.

Formaldehyde joins several other known human and animal carcinogens that have identical endogenous DNA adducts, including vinyl chloride, urethane, ethylene oxide and acetaldehyde. For these agents, clear evidence exists for mutagenic endpoints related to Hazard Identification. However, little data have been developed related to low dose mutagenicity. In contrast to exogenous DNA adducts, mutations do not extrapolate down to zero. Rather, they always have a background rate of mutations. We propose that this background rate is the result of endogenous DNA damage, some of which are identical to chemically-induced damage and others that have a variety of sources. Since mutations drive the induction of cancer, this constant endogenous DNA damage is likely to drive the biology of low dose mutagenesis and needs to be considered when extrapolating risks down to extremely low exposures. Such information should greatly contribute to science-based risk assessments. *Co-authors*: Kun Lu, Benjamin Moeller and Tom Starr.

James Swenberg is a Kenan Distinguished Professor of Environmental Sciences and Engineering and Professor of Nutrition, and Pathology and Laboratory Medicine at the University of North Carolina at Chapel Hill. He also serves as the Director of Center for Environmental Health and Susceptibility and is a member of the Lineberger Comprehensive Cancer Center at the University of North Carolina at Chapel Hill. Jim served as Director of the Curriculum in Toxicology at UNC from 1992-2010. Before joining the University of North Carolina, he was a Department Head at the Chemical Industry Institute of Toxicology from 1978-89,

Research Scientist and Section Head, Pathology & Toxicology Research Unit, The Upjohn Company from 1972-78, and Assistant and Associate Professor of Veterinary Pathology at the Ohio State University.

Dr. Swenberg earned his D.V.M. degree from the University of Minnesota and his Ph.D. degree in Veterinary Pathology from the Ohio State University. He is a Diplomate of the American College of Veterinary Pathologists and a member of the American Association for Cancer Research, American Association of Neuropathologists, American Society for Investigative Pathology, Society of Toxicologic Pathologists, and the Society of Toxicology. He has served on the Board of Scientific Counselors, Division of Cancer Etiology, NCI, Board of Scientific Counselors, National Toxicology Program, NIEHS, and Board of Scientific Counselors, NIEHS, as well as a member of the FIFRA Scientific Advisory Panel, EPA, and was elected to the Society of Toxicology Council. He was awarded the George Scott Award from the Toxicology Forum, the John Barnes Prize Lectureship from the British Toxicology Society, the Distinguished Alumnus Award from The Ohio State University College of Veterinary Medicine, and the Distinguished Research Alumnus Award from the University of Minnesota, College of Veterinary Medicine, and the Society of Toxicology Merit Award for 2007. His research group won the Best Publication Award for 2010 from *Toxicological Sciences*.

Dr. Swenberg has published over 370 scientific papers and has served on the editorial boards of *Cancer Epidemiology, Biomarkers and Prevention*; *Cancer Research; Carcinogenesis; Chemical-Biological Interactions; Chemical Research in Toxicology; Environmental Health Perspectives; Food and Chemical Toxicology; Fundamental and Applied Toxicology; Neuro-Oncology;* and *Toxicologic Pathology*. His research focuses on mechanisms of carcinogenesis and toxicology, with emphasis on the roles of DNA damage and repair and cell proliferation. He has published extensively on the use of mass spectrometry for DNA and protein adducts, including those arising from environmental and endogenous chemicals. Most recently, he has been investigating direct and indirect DNA damage arising from oxidative stress and environmental chemicals, including vinyl chloride, formaldehyde and PCBs. In addition, he has been actively involved in research related to differences in the dose response for DNA adducts and mutations and the implications of these differences for cancer risk assessment.

In his spare time, Jim is an avid fly fisher and enjoys gardening.

Tissue Organization Field Theory - a Replacement for the Somatic Mutation Theory of Cancer

Tuesday, December 6, 10:50 – 11:35 AM

Carlos Sonnenschein, *Tufts University, Boston, Massachusetts*

The somatic mutation theory of carcinogenesis has been and remains so far the prevalent theory aimed at explaining how do tumors arise and progress. Briefly, this theory proposes that cancer is a cell-based disease and implicitly assumes that quiescence is the default state of cells in metazoa. Curiously, despite its popularity the published record indicates that this theory has never been rigorously tested despite the long-standing controversy that questioned the theory's heuristic and pragmatic values. In my presentation, I will propose to implement a protocol that might answer the usual criticisms generated by this theory. At the same time, I will also offer evidence that an alternative theory of carcinogenesis, the tissue organization field theory, was tested and proven to be compatible with the proposition that cancer is a tissue-based disease and that proliferation and motility are the default state of all cells. Adoption of these alternative premises to design experimental approaches and interpret the data collected should change perceptions of basic developmental events, as well as of therapeutic and toxicological approaches.

Dr. Carlos Sonnenschein is a professor in the Department of Anatomy and Cellular Biology at Tufts University School of Medicine, in Boston, MA. For over three decades, Dr Sonnenschein's research interests have centered on a) the control of cell proliferation by estrogens and androgens, b) the impact of endocrine disruptors on organogenesis and the reproductive function and c) carcinogenesis during adult life and, specifically, on the role of stroma/epithelial interactions on rat and human mammary carcinogenesis. The current experimental tools he uses include 3D tissue culture and Systems Biology.

In collaboration with Professor Ana M. Soto, they co-authored a book entitled THE SOCIETY OF CELLS (Bios-Springer-Verlag, 1999) in which they critically evaluated the status of research in the fields of control of cell proliferation and carcinogenesis. The major contributions discussed in the book were that a) the default state of all cells in multicellular organisms (and of course, including those in animals) is *proliferation,* and that b) the so-called sporadic cancers (95% of all clinical cases) represent diseases specifically anchored at the tissue level of biological organization. These postulates are at the core of their proposition of the *tissue organization field theory of carcinogenesis* (TOFT). Ever since, Dr Sonnenschein and Soto have published a number of research communications and reviews buttressing the values of their theory. The English version of THE SOCIETY OF CELLS has been translated into French in 2006 (LA SOCIETE DE CELLULES, 2006 Ed. Sylepse, Paris, France) and an Addendum was added to this translation incorporating new data and commentaries published since 1999. Again, in collaboration with Dr Soto they have authored an invited review in NATURE REVIEWS ENDOCRINOLOGY that appeared in the July 2010 issue in print. He is frequently invited to talk at national and international meetings on subjects referred to above.

Toward the end of the 1980s, Professors Soto and Sonnenschein developed two *in vitro* bioassays capable of reliably identify xenoestrogens and androgens agonists and antagonists (E-SCREEN and A-SCREEN, respectively) that are being used worldwide to detect the presence of natural and man-made estrogenic compounds. Dr. Sonnenschein was a member of the SACATM, a National Institute of Environmental Health Sciences (NIEHS) advisory panel that deals with the approval of bioassays in the field of environmental toxicology. He has also been a consultant for the European Commission in the fields of contamination of foods and of water resources by environmental endocrine disruptors, and the EPA on *in vitro* bioassays for cell toxicity.

Dr Sonnenschein was invited to deliver lectures at the ANNUAL e-HORMONE SYMPOSIUM in New Orleans, LO, and at the "BIOPHYSICAL ASPECTS OF COMPLEXITY IN HEALTH AND DISEASE" at Milan, Italy. He also delivered seminars in Rome (Universita La Sapienza), Siena (Universita di Siena), Universita de Urbino, Universite de Paris VI, Ecole Normal Superioure in Paris, and at the Harvard School of Public Health, in Boston, MA.

Tuesday Dec 6 PM

Session IV: Low Dose Toxicity in Humans

New Benzene Findings in Epidemiologic Studies and Implication for Risk Assessment

Tuesday, December 6, 1:05 – 1:50 PM

A Robert Schnatter, *ExxonMobil Biomedical Sciences Inc, Clinton, New Jersey*

Benzene is a known to cause acute myeloid leukemia (AML) and is also hematotoxic, but basic questions remain for risk assessment. Two important questions are whether other lymphohaematopoietic (LH) cancer subtypes can be caused by benzene, and if so, at what exposure levels? These questions are difficult to address, because there is no accepted

animal model for benzene-induced AML, thus studies have relied on observational epidemiologic research. This presentation will review some recent epidemiologic findings and present some new focuses for future risk assessment.

There is general agreement that (a) benzene must be metabolized to produce bone marrow effects, (b) benzene causes decreases in blood cell counts, (c) benzene causes some forms of genotoxicity, and (d) benzene causes acute myeloid leukemia. However, linking these effects to suggest a mode of action based on data has been elusive. Thus, most risk assessments take a conservative approach and assume a no-threshold, linear-at-low-dose exposure/response curve. The US EPA uses this approach to arrive at a concentration associated with a one-in-a-million excess of leukemia of about 40 parts per trillion, which is two orders of magnitude lower than existing ambient concentrations.

Recent studies of benzene have needed to use large population sizes due to the ever-decreasing exposure levels and the rarity of the diseases under investigation. I will summarize two recent studies: one in a population of Shanghai workers exposed from low (sub-ppm) to high (>200 ppm) levels of benzene, and one in a pooled population of petroleum distribution workers exposed to lower levels (approximately 0.02 – 6 ppm).

The Shanghai studies suggest that: (a) exposures of 7-8 ppm are needed to produce decrements in blood cell counts, and (b) non-Hodgkin lymphoma is not associated with benzene, although both AML and myelodysplastic syndrome (MDS) are. In addition, a mode of action associated with bone marrow inflammation and autoimmunity is suggested. The distribution worker pooled analysis suggests that: AML, chronic lymphoid leukemia (CLL), chronic myeloid leukemia (CML), and myeloproliferative disease (MPD) are not associated with lower level benzene exposure, but MDS is. Potential risk assessment implications of finding MDS associated with lower benzene exposures will be discussed.

Rob is a Distinguished Scientific Advisor at ExxonMobil Biomedical Sciences, Inc. in Clinton, NJ. Rob earned a bachelor's degree at Rutgers University in Quantitative Biology, a master's in Biostatistics and Genetics at the University of Pittsburgh, and his doctorate degree in Epidemiology at Columbia University. Rob has served on numerous scientific advisory panels and trade associations in the areas of benzene exposure and health effects, petroleum worker studies, asbestos exposure and disease, and various other substances. He has served as adjunct faculty at Western Connecticut State University and Florida Institute of Technology. Rob formerly worked for Union Carbide Corporation and where he initiated worker health surveillance systems. He then moved to ExxonMobil Biomedical Sciences, where he also put a vital status registry in place, which has served as the basis for several subsequent publications in ExxonMobil workers. He later became Section Head of Epidemiology, while studying and publishing on health effects in petrochemical and laboratory workers and benzene exposure in petroleum workers. He previously chaired the American Industrial Health Council's Epidemiology Subcommittee, and served on WHO, EPA and ATSDR scientific advisory panels for epidemiologic data in risk assessment as well as health-based exposure standards. Currently, Rob is an advisor to the U.S. Public Health Service. In his role as Distinguished Scientific Advisor at EMBSI he is co-investigator (with the University of Colorado and Fudan University) of a large study on benzene exposure in Shanghai, China; and also a co-PI for an international collaborative pooled study on benzene exposure with Australia and UK investigators.

Dioxin Exposure Pathways in the Population of Midland, Michigan

Tuesday, December 6, 1:50 – 2:35 PM

D.H. Garabrant, *University of Michigan School of Public Health, Ann Arbor, Michigan*

The University of Michigan Dioxin Exposure Study (UMDES) was undertaken to address concerns that dioxins (polychlorinated dibenzodioxins [PCDDs] and polychlorinated dibenzofurans [PCDFs]) released from the Dow Chemical Company over the past 100 years have led to increased body burdens of these compounds in residents of Midland and Saginaw Counties, Michigan USA. Soils in Midland downwind of the Dow facilities are contaminated with dioxins having a congener profile that is rich in PCDDs, while soils in the floodplain of the Tittabawassee River downstream of the Dow facilities are contaminated with dioxins having a congener profile that is rich in PCDFs. In both geographic areas there is concern among the general public that living on contaminated soils and river sediments contributes to the body burden of these compounds. In addition, PCBs are present in the sediments of the Saginaw River due to sources other than the Dow Chemical Company. The goal of the UMDES is to identify and quantify exposure pathways from the environment in Midland/Saginaw Counties to the serum of the population in the region. Large numbers of residents were potentially exposed to very high concentrations of specific congeners in soil.

Demographic factors (including age, gender, BMI, weight loss, breast feeding, and smoking) were the greatest factors in determining the dioxin TEQ concentration in the serum. The number of years lived in Midland/Saginaw between 1960 and 1979 was an explanatory variable for many PCDD and PCDF compounds except OCDD and was the second greatest explanatory variable for 2,3,7,8-TCDD, suggesting historical exposure. Neither soil nor household dust dioxin concentrations were significant predictors of serum TEQ, 2,3,7,8-TCDD, OCDD or 2,3,4,7,8-PeCDF. There were some small contributions from soil concentrations to the variance in serum concentrations for some dioxin-like PCBs; however, this may not reflect a direct exposure pathway from soil and dust. Instead, this may be indicative of direct aerial exposure to ongoing sources of PCBs. Consumption of fish and game from the contaminated areas did not contribute to serum PCDD or PCDF levels. Consumption of cattle raised on highly contaminated soil was associated with elevated serum 2,3,4,7,8-PeCDF in a small number of residents who were regularly exposed to this source. This study does not support a link between serum dioxin levels and living on contaminated soil other than through the consumption of animal products raised on contaminated soil.

Professor Garabrant is Emeritus Professor of Occupational Medicine and Epidemiology at the University of Michigan. He has conducted research in occupational and environmental epidemiology for the past 30 years. He is board certified in internal medicine, occupational medicine, and preventive medicine. Since joining the faculty at the University of Michigan in 1988, he has served as Director of the Occupational Medicine Program, Director of the Occupational Health Program, Director of the Center for Occupational Health and Safety Engineering, and Director of the Cancer Prevention Training Program, and Founding Director of the Risk Science Center. His educational background includes: BS, Chemical Engineering, Tufts University; 1972, MD, Tufts University, 1976; MPH, Harvard University, 1979; and MS, Physiology, Harvard University, 1980. He has served on and chaired the SOH study section and numerous other review sections for the NIH, and has served on the board of editors of the Journal of Occupational Medicine and the Journal of Exposure and Public Health. He is a member of the Society for Epidemiologic Research, the International Epidemiology Association, and the American College of Occupational and Environmental Medicine. He was the recipient of the 2006 Excellence in Research Award from the University of Michigan School of Public Health and the 2007 Research Excellence Award from the Risk Science Center at the University of Michigan. He is the author of over 200 peer-reviewed publications.

Session d'Affichage
Poster Session

CYTOTOXICITY OF THE LYOPHILIZED AQUEOUS EXTRACT OF SEAWEEDS *HALIMEDA MONILE* AND *HALIMEDA OPUNTIA*.

Ana E. Batista-Gonzalez[1], Alexis Vidal Novoa[2], Adyary Fallarero[1] and Pia Vuorela[1]
[1] Department of Bioscience. Pharmaceutical Sciences. Åbo Akademi University. Finland,
[2] Department of Biochemistry. Faculty of Biology. University of Havana. Cuba

Background: The biological activity of seaweeds of *Halimeda* genus has been exhaustively study. Antioxidant activity of *Halimeda monile* and *Halimeda opuntia* has been evaluated in terms of neuroprotection and hepatoprotection, but the toxicological profile of these seaweeds has not been yet investigated.

Objectives: The aim of this work was to evaluate the cytotoxicity of the lyophilized aqueous extract of *Halimeda monile* and *Halimeda opuntia* in a panel of different cell lines.

Methods: Seaweeds *Halimeda monile* and *Halimeda opuntia* were collected and dried at room temperature. An extraction in water was made and after centrifugation the supernatant was lyophilized. Two cell lines were used in order to assess the cytotoxicity of the seaweeds. GT1-7 (immortalized hypothalamic mouse cell line) and HepG2 (human hepatocarcinoma cell line) were exposed to different concentrations (0.05-10 mg/mL) of the lyophilized aqueous extract of both seaweeds and after 24 h the cellular viability was measured using the resazurin reduction assay.

Results: The lyophilized aqueous extract of *Halimeda monile* and *Halimeda opuntia* showed a dose dependent cytotoxicity in HepG2 cells. Both seaweeds showed the same cytotoxicity profile and they started to be toxic above 7.5 mg/mL, with a 79 percentage of cell viability at that concentration. When the seaweeds were assessed in GT1-7 cells they didn't show any cytotoxicity at any concentration. These results suggest that the extract could be metabolized by the hepatic cells producing cytotoxic metabolites which are not produced in neuronal cells. This effect is only significant in terms of cytotoxicity at higher concentrations of the extracts.

Conclusions: The lyophilized aqueous extract of *Halimeda monile* and *Halimeda opuntia* were more toxic in hepatic cells than in neuronal cells, but they were not toxic at the concentrations that they showed biological activity before. These seaweeds can be further investigated for other biological properties in order to use them in a future to improve the human health.

METHAMPHETAMINE EMBRYOPATHIES IN BRCA1 CONDITIONAL KNOCKOUT MICE IN EMBRYO CULTURE

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[2] Department of Pharmacology and Toxicology, University of Toronto, Toronto

Background: Mutations in Breast Cancer 1 (*Brca1*), a DNA repair gene, increase the incidence of certain breast and ovarian cancers. Knocking out *Brca1* in mice causes embryolethality before gestational day (GD) 7.5 with gross genomic and morphological abnormalities, suggesting an important role for this gene in early embryonic development. We have shown that embryonic DNA repair can protect progeny from structural and functional anomalies caused by teratogens like methamphetamine (METH) that initiate reactive oxygen species (ROS) formation.

Objectives: Determine if embryonic deficiencies in BRCA1 expression will increase oxidatively damaged DNA and embryopathies caused by the ROS-initiating teratogen METH.

Methods: Early embryolethality observed in traditional *Brca1* knockouts was avoided by the use of conditional knockouts. *Brca1* conditional knockouts were produced by mating floxed *Brca1* mice with Cre-expressing transgenic mice on the Sox2 promoter, an embryo-specific marker that appears on GD 6.5. The resulting wild-type and *Brca1* knockout embryos were explanted on GD 8 and exposed in culture for 24 hours to subembryopathic and embryopathic concentrations of METH. Oxidatively damaged DNA was measured as 8-oxoguanine using high-performance liquid chromatography with tandem mass spectrometry, and embryos were assessed for developmental anomalies.

Results: Preliminary data suggest that embryos are susceptible to METH-initiated embryopathies, which are further exacerbated by a lack of functional *Brca1*. Studies are ongoing to confirm these trends and quantify oxidatively damaged DNA.

Conclusions: The prevalence of *Brca1* mutations is high in certain populations but is currently considered a risk factor only for breast and ovarian cancers. Our preliminary results if confirmed suggest a novel pathogenic implication for the developing embryo, possibly warranting prenatal genetic screening in susceptible populations. BRCA1 protection against ROS-initiating teratogens also suggests novel mechanisms in the embryopathic effects of oxidative stress. [Support: Canadian Institutes of Health Research]

INCREASED NUCLEOTIDE EXCISION REPAIR ACTIVITY OF AFLATOXIN-B_1-N^7-GUANINE ADDUCTS BUT NOT AFLATOXIN-B_1-FORMAMIDOPYRIMIDINE ADDUCTS IN LIVERS OF MICE EXPOSED CHRONICALLY TO AFB_1

Jeanne E. Mulder[1], Rekha Mehta[2], Genevieve S. Bondy[2] and Thomas E. Massey[1]
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[2] Toxicology Research Division, 2202D, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario, K1A 0K9.

Background: Aflatoxin B_1 (AFB_1) is produced by species of *Aspergillus*, moulds that can grow on grains, oilseeds and spices. Following inhalation or ingestion, AFB_1 is biotransformed into the highly reactive AFB_1-*exo*-epoxide, which binds preferentially to the N^7 position of guanine residues in DNA and can then hydrolyze spontaneously to a formamidopyrimidine adduct (AFB_1-FAPY). These adducts, which are normally excised by nucleotide excision repair (NER), damage target cellular DNA and may induce cancer. Previous work in our laboratory showed that acute exposure of CD-1 mice to a single tumourigenic dose of AFB_1 caused a 3.5 fold increase in hepatic repair activity of AFB_1-N^7-Gua.

Objectives: To assess effects of low-dose chronic dietary AFB_1 exposure on hepatic repair of AFB_1-N^7-Gua and AFB_1-FAPY adducts.

Methods: Male C57Bl/6J mice were exposed to 0, 0.2 or 1.0 ppm AFB_1 in AIN 93M semi-purified diet for 26 weeks. DNA repair synthesis activity of liver nuclear protein extracts was assessed with an *in vitro* assay that reproduces the NER reaction, specifically global genome repair, using adducted plasmid DNA as a substrate.

Results: Activity for repair of AFB_1-N^7-Gua was three-fold greater in extracts from livers of mice exposed to 0.2 ppm AFB_1 in diet compared to extracts from livers of control mice ($p<0.05$). In contrast, 0.2 ppm AFB_1 did not alter repair of AFB_1-FAPY, and no difference in repair of either AFB_1-N^7-Gua or AFB_1-FAPY was found in liver extracts between control and 1.0 ppm AFB_1 treated mice.

Conclusions: These results indicate an increase in hepatic NER activity that is both dose and substrate specific, following chronic AFB_1 exposure. (Supported by CIHR Grant No. MOP-89698 and GRDI)

EFFECTS OF ENVIRONMENTAL CONTAMINANTS ON EXPRESSION OF DRUG METABOLISM PHASE I ENZYMES, AND DNA METHYLATION OF DNA REPEATED ELEMENTS IN TWO HUMAN LIVER CELL LINES

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EHSRB, HECSB, Health Canada, Ottawa ON

Background: DNA methylation is a normal chemical modification of our genetic material. It occurs in various regions of the DNA, including promoters (regions regulating our genes) and various DNA segments that are repeated numerous times throughout the DNA. Abnormal methylation in these DNA repeated elements and in promoters is associated with numerous diseases, including cancers.

Objectives: In the context of short term 72h cell culture experiments that can be used in strategies to screen chemicals for prioritization for further testing, or for chemical classification based on mechanism of action, our objective was to investigate if changes in DNA methylation of various DNA repeated elements are sensitive indicators of chemical exposure.

Method: The HC04 and HepG2 liver cell lines were characterized based on differences in DNA methylation relative to a normal human liver biopsy, and based on dose-response effects of chemicals. Vanadium, nickel, and three mixtures of polybrominated diphenyl ethers (PBDE-71, -79, and 83) were tested, in addition to activators of the AhR and CAR nuclear receptor pathways (polychlorinated biphenyls126 and 153, respectively), and a demethylating standard, 5-aza-2'-deoxycytidine (5aCdR). DNA methylation of five DNA repeated elements (Line-1, AluYb8, NBL, Sat-alpha and D4Z4) was measured using pyrosequencing assays. These endpoints were compared to changes in metabolic activity (AlamarBlue assay as surrogate for toxicity) and expression of 84 drug metabolism phase-I enzymes.

Results: The methylation level differs between the biopsy DNA, the HC04 and HepG2 cells (e.g., 81%, 64% and 63% methylation in Line-1, respectively). Both cell lines responded to the demethylating agent 5aCdR, with decreases in DNA methylation but the effects differed between cell lines and among the repeated elements. Effects of the other chemicals on DNA methylation were minor despite significant inductions of numerous phase-I enzyme genes and decreases in metabolic activity. Vanadium was more toxic than nickel and PBDE 71 was the most toxic PBDE.

Conclusion: DNA methylation of the repeated elements differed among the cell types, but these endpoints were not sensitive indicators of exposure to environmental chemicals. The cell lines responded differently and therefore using both cell lines, instead of one, provided better coverage of potential effects. Methylation of gene promoters is being investigated.

ETHANOL METABOLIC TERATOGENICITY IN THE GUINEA PIG AND EFFECTS OF A VOLUNTARY EXERCISE INTERVENTION.

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Background: Maternal ethanol consumption during pregnancy can lead to teratogenic effects in offspring, collectively termed fetal alcohol spectrum disorders (FASD). Ethanol can produce metabolic teratogenicity, including insulin resistance and impaired glucose metabolism. Voluntary exercise (VE) is a promising intervention to mitigate ethanol metabolic teratogenicity, and has been shown to reverse insulin resistance and improve glycemic control. This study tested the hypothesis that VE mitigates ethanol metabolic teratogenicity in the guinea pig.

Objectives: To determine whether chronic maternal ethanol administration causes metabolic teratogenicity in the guinea pig and to determine whether voluntary exercise mitigates this ethanol metabolic teratogenicity.

Methods: Pregnant Dunkin-Hartley-strain guinea pigs received ethanol (4 g/kg maternal body weight/day throughout gestation) or isocaloric-sucrose/pair-feeding (nutritional control). On postnatal day (PD) 21, offspring were randomly assigned to VE or no intervention. VE animals were placed in a dry-land maze for 30 min daily for 21 days (PD 24-44). Weight gain was recorded from birth until euthanasia. Fasting blood glucose concentration was measured prior to euthanasia (PD 150-200), when liver and pancreas were collected.

Results: CPEE offspring had decreased birth weight compared with offspring of the nutritional control group. Shortly after birth, female CPEE offspring showed accelerated weight gain which continued into puberty, leading to increased body weight compared to female nutritional control offspring. This weight gain was mitigated by VE. CPEE offspring demonstrated dysregulated fasting blood glucose concentrations and increased liver weight at PD 150-200. CPEE animals had increased intralobular fat and structural abnormalities in pancreatic islets. These effects were not mitigated by VE.

Conclusions: The data demonstrate that this chronic maternal ethanol regimen produces metabolic teratogenicity in the guinea pig, and that VE mitigates CPEE-induced weight gain in female offspring. (Supported by CIHR grants MOP84553 and ELA80227).

MITIGATING CHRONIC ARSENIC TOXICITY THROUGH SELENIUM - CONTROLLED DIETS

Shweta Sah and Judit Smits
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Background: Arsenic (As) toxicity causes serious health problems in humans world-wide that are concentrated on Indo-Gangetic Plains. Selenium (Se), an important micronutrient and antioxidant, is recognized as an antagonist of arsenic. Therefore, Se levels in food might be a potential treatment for populations in areas with high arsenic exposure.

Objectives: The objective of this research was to evaluate the potential effects of Se-fortified diets in mitigating symptoms of chronic arsenic (As) toxicity in mammals. Here we focused on the usefulness of Se to reduce indicators of arsenic poisoning, such as liver damage and impaired immune response.

Methods: We performed an 18 week study using 36 male Wistar rats weighing 150-170 g at the start of the study. Rats were exposed to arsenic (40 ppm and 80 ppm) in drinking water while receiving dietary selenium at deficient (<0.01 ppm), adequate (0.15 ppm) or fortified (0.6 ppm) levels . Clinical variables were assessed daily. To assess As-induced liver damage and oxidative stress, malondialdehyde (MDA)lipid peroxidation assays and glutathione (GSH) assays were performed on liver and whole blood respectively. Kidney and liver samples were analyzed for total As and Se concentrations. Immune function was evaluated by testing the secondary antibody response to keyhole limpet hemocyanin (KLH), a nonpathogenic antigen.

Results: In the low As - exposed groups; liver As showed a positive correlation with the lipid peroxidation ($r = 0.45$). Lipid peroxidation (MDA) in liver decreased in the Se supplemented groups as compared to Se deficient group ($p = 0.02$) . Also, there was lower secondary antibody response (IgG) in Se deficient rats ($p = 0.01$) compared with Se adequate and Se fortified groups. These results demonstrated a beneficial effect of Se on As-triggered liver damage and immune suppression. In addition, lower kidney As and elevated glutathione levels (GSH Assay) observed in the Se fortified group ($p = 0.003$) indicated a protective effect of Se fortification.

Conclusions: These findings support that arsenic toxicity may be decreased through dietary Se supplementation. Our assays indicate that increased dietary Se decreases liver damage, one of the important target organs for As toxicity. Se fortified diet effectively decreased As levels in the kidney. Moreover, Se supplementation has a potential to reverse the arsenic-induced immunosuppression. More work will be required to establish the optimal doses of selenium in the diet to ensure maximal health benefits, and to determine the validity of extrapolation between laboratory rodents and humans.

THE FREE RADICAL SPIN TRAPPING AGENT PHENYLBUTYLNITRONE REDUCES POSTNATAL COGNITIVE DEFICITS CAUSED BY *IN UTERO* ETHANOL EXPOSURE IN CD-1 MICE

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Background: Fetal ethanol (EtOH) exposure during pregnancy can result in a spectrum of morphological, cognitive and behavioral anomalies, collectively termed Fetal Alcohol Spectrum Disorders (FASD). Reactive oxygen species (ROS) have been implicated in the underlying mechanism of postnatal cognitive deficits in both clinical studies and animal models of various teratogens. However, this mechanism has been implicated in the morphological but not the behavioral anomalies resulting from *in utero* exposure to EtOH.

Objectives: To determine the role of ROS in postnatal neurodevelopmental deficits due to *in utero* EtOH exposure using a free radical spin trapping agent.

Methods: A passive avoidance learning task was assessed postnatally at 6, 9, 12 and 16 weeks in the progeny of CD-1 mice treated on gestational day (GD) 17 (plug = GD1) with 4 g/kg of EtOH or its saline vehicle via intraperitoneal (i.p.) injection, with or without pretreatment with *alpha*-phenyl-N-tert-butylnitrone (PBN) (40 mg/kg i.p.), a free radical spin trapping agent previously shown to trap EtOH-initiated free radicals, protect against oxidative damage initiated *in utero* by other ROS-initiating teratogens such as phenytoin and thalidomide, and mitigate ROS-mediated neurotoxicity.

Results: Saline-exposed progeny met the criteria of the task by 12 weeks of age indicating an age-dependent learning curve. PBN alone had no significant effect on learning compared to saline-treated progeny. EtOH-exposed progeny performed worse than saline- and PBN-exposed controls at 12 weeks of age, suggesting a window of deficient cognition ($p<0.001$), while this deficit continued to 16 weeks in male animals ($p<0.01$), possibly reflecting a gender predisposition. PBN pretreatment improved learning in EtOH-exposed animals at 12 and 16 weeks of age ($p<0.01$).

Conclusions: These data show that *in utero* EtOH exposure enhances postnatal cognitive deficits in 12 and 16-week old CD-1 mice, and provides the first evidence that ROS may be involved in the mechanism of EtOH-initiated neurodevelopmental deficits. (Support: CIHR)

HEPATOTOXICITY THROUGH VEGFR-2-PATHWAY INHIBITION IN SPRAGUE DAWLEY RATS.

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Background: The liver is a complex structurally and functionally heterogeneous organ. Its lobar-pattern may influence responses to drugs due to lobe-dependent portal blood supply and drug delivery. Alanine transferase (ALT) is presently the most reliable drug-induced liver injury (DILI) biomarker. A proprietary vascular endothelial growth factor receptor-2 (VEGFR-2) inhibitor is among several drugs that failed preclinical testing. This target-molecule therapy increases ALT activity without morphology evidence of necrosis. Additionally, this drug causes lobe-specific changes in ALT levels with implications for biopsy procedures used for drug liabilities assessment.

Objectives: To show that VEGFR-2 Inhibitor, given orally, has an ALT and mitochondria phenotypic lobe anchoring.

Methods: Sprague-Dawley rats (24:12 controls and 12 treated), were kept on a 12h: 12h light-dark cycle and orally given 400mg/kg/day VEGFR-2 inhibitor (treated) or 0.5% CMC (controls). Blood and liver tissue were collected on days 7 and 21. Morphological analysis by light and transmission electron microscopy (LM and TEM), immunohistochemistry (TUNEL and Caspase-3) and mitochondria function tests were performed on liver tissue sections and mitochondria freshly isolated from caudate, left lateral and right median lobes.

Results: Body and liver weights decreased in treated rats. Plasma alanine transferase (ALT) and aspartate transaminase (AST) and red blood cells (RBC) count increased while total plasma proteins decreased in treated rats. Liver ALT response was biphasic, being elevated on day 7 and considerably reduced on day 21. No histological evidence of necrosis was found on day 7 or 21. Masson trichrome staining revealed increase in connective tissue around portal triads. TEM showed signs of fibrosis in the Space of Disse and decreased vascular area fraction in the left lateral lobe of treated rats. TUNEL revealed lobe- and time-dependent increase in DNA fragmentation. Elevated caspase-3 immuno-reactivity corresponding with increased apoptosis was observed in treated rats. Mitochondria function analysis showed reduced O_2 consumption in mitochondria isolated from all the three lobes of treated rats with the caudate and left lateral lobes being most sensitive to VEGFR-2 inhibitor.

Conclusion: We demonstrate VEGFR-2 inhibitor induced liver injury. It appears that VEGF-suppression causes apoptosis through caspase-3 activation leading to fibro genesis without signs of necrosis. The mitochondrial dysfunction observed suggests that disruption of cellular energy homeostasis plays a vital role in VEGFR-2 inhibitor-induced hepatotoxicity.

POLYBROMINATED DIPHENYL ETHERS (PBDE) EXPOSURE DURING EARLY PREGNANCY AND MATERNAL AND FETAL THYROID FUNCTION

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Background: One critical role of thyroid hormones is growth and differentiation of many organs, especially the brain. The foetal thyroid begins functioning at the beginning of the second trimester. Prior to this, the embryo is entirely dependent on maternal thyroid hormones. Polybrominated Diphenyl Ethers (PBDE) are endocrine disruptors that interact with the thyroid pathway, in part by competing for thyroid hormone transporters, and may disturb neurodevelopment.

Objectives: To investigate the relationship between maternal blood PBDE measured in early pregnancy and serum thyroid function in pregnancy and the fetus.

Methods: The following were measured in 380 pregnant women in the first trimester: low-brominated PBDE, three PCB, total (TT4, TT3) and free thyroid hormones (FT4, FT3), thyroid-stimulating hormone (TSH), thyroperoxidase antibodies (TPO atb), urinary iodine, selenium, and total blood mercury. Thyroid hormones were also assessed at delivery and in cord blood (n=260).

Results: In samples taken between 3 and 20 weeks of pregnancy**,** we observed a negative relationship between maternal PBDE and total thyroid hormones and a positive relation with free thyroid hormones, and TPO atb (an autoimmune biomarker). At delivery, maternal TT4 and FT3 as well as fetal TSH, TT4 and FT4 decreased in relation to PBDE. Fetal TSH decreased in relation to BDE-47, BDE-100 and the sum of all PBDE. The above relationships were independent of PCB levels and selenium.

Conclusions: Our results show that the presence of PBDE in early pregnancy can interfere with thyroid status. Those findings raise a high concern for foetal neurodevelopment.

AN ENVIRONMENTALLY-RELEVANT EXPOSURE TO BROMINATED FLAME RETARDANTS (BFRs) *IN UTERO* INDUCES FETAL LIMB MALFORMATIONS IN SPRAGUE-DAWLEY RATS

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Background: BFRs are incorporated into diverse consumer products to reduce flammability. High concentrations of BFRs have been measured in mid-gestation human fetal liver and placenta. Since elevated levels of BFRs in breast milk have been positively correlated with abnormal fetal development, *in utero* development may represent a critical window for exposure to BFRs.

Objectives: The *in utero* effects of exposures to single BFR congeners or technical mixtures have been investigated in animal models, but the dose, route of administration and composition of the mixtures have not mimicked human environmental exposures to BFRs. Thus, the aim of this study is to mimic human BFR exposure in an animal model to test the hypothesis that BFRs affect pregnancy outcome and fetal development.

Methods: Adult female Sprague-Dawley rats (n=35-38 per group) were exposed to 0, 0.06, 20 and 60 mg/kg/day of a mixture of BFRs formulated to mimic the relative congener levels in house dust, the major source of human exposure. This mixture was composed of three commercial brominated diphenyl ethers (BDEs) (52.1% DE-71, 0.4% DE-79, 44.2% decaBDE-209) and hexabromocyclododecane (all 3 isomers, 3.3%) and incorporated in the diet. Treatment started two weeks before mating, and lasted until females were euthanized on gestational day (GD) 20 (the females were sperm positive on GD0). Body weights were measured once a week before mating and at GD0, GD3, GD8, GD13, GD16 and GD20. At GD20, litter size and resorption sites were counted. Fetal development was analyzed by measuring fetal weights, crown rump lengths, and anogenital distances and evaluating external malformations.

Results: Increases in body weight before mating and during gestation in females from the control and BFR-treated groups were comparable. Parameters of pregnancy outcome such as mating, fertility and fecundity indices and litter size were not significantly affected by exposure to the BFR mixture. No effects on postimplantation loss, fetal viability, sex ratio, fetal weight or crown rump lengths were detected in litters collected from treated groups in comparison to controls. However, fetal external examinations revealed limb malformations such as clinodactyly and fused digits in all groups. The numbers of malformed fetuses and litters with malformed fetuses were significantly higher after exposure to the highest dose of BFRs compared to the controls (8.8 % vs 2.6 % with $P<0.05$, and 44.5% vs 25.0 %, with $P<0.001$, respectively).

Conclusions: Our results suggest that an environmentally-relevant exposure to BFR during pregnancy resulted in fetal malformations in the absence of maternal toxicity. Therefore, this study supports the hypothesis that *in utero* exposure to BFRs can have adverse effects on fetal development. Supported by grant RHF100625 from the Institute for Human Development, Child and Youth Health, (CIHR).

THE ACTIVATION AND INTRACELLULAR DISTRIBUTION OF P38 MITOGEN-ACTIVATED PROTEIN KINASE (P38 MAPK) IN EMBRYOS EXPOSED TO THE DEVELOPMENTAL TOXICANT HYDROXYUREA

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Background: The administration of hydroxyurea (HU) to timed-pregnant CD1 mice causes birth defects, including severe and characteristic damage to the fetal brain (exencephaly), appendages (clubbed feet), paws (ectro- and polydactyl), and tail (short and kinked). At embryotoxic doses, HU induces oxidative stress and activates p38 MAPK. Moreover, the inhibition of p38 MAPK increases fetal death in HU exposed embryos, demonstrating that this pathway plays an essential role in the survival of the conceptus after insult. Together with its upstream MAP2K, MEK3/6, activation of p38 MAPK can mediate cell death, cell differentiation and cell cycle checkpoints. While MEK3/6 is only expressed in the cytosol, p38 MAPK can accumulate in the cytosol or translocate into the nucleus, thereby influencing different downstream targets. Despite the number of studies showing activation of this pathway induced by stress, the mechanism by which it is activated and regulation of the the intracellular distribution of p38 MAPK remain unclear.

Objective: The goal of this study was to investigate the effects of HU on the activation and localization of MEK3/6 and p38 MAPK and on the subcellular distribution of p38 MAPK in the organogenesis stage mouse embryo.

Methods: Timed-pregnant CD1 mice were treated with vehicle (saline) or HU (low dose: 400mg/kg; high dose: 600 mg/kg) by i.p injection on gestation day 9 (GD9). Dams were euthanized at 0.5, 3, or 6h post-treatment and embryos explanted. Total proteins were isolated and protein expression was quantified by Western blot analysis. The localization of activated MEK3/6 and p38 MAPK was analyzed in embryo tissue sections using immunofluorescence. Subcellular distribution of activated p38 MAPK was assessed by confocal microscopy and 3D imaging analysis.

Results: HU exposure induced the activation of MEK3/6 and p38 MAPK as detected in Western blots by an increase in phospho-MEK3/6 and phospho-p38 at 0.5h, 3h and 6h. Activated MEK3/6 and p38 MAPK immunoreactivities were widespread in embryos, including in the neural tube, somite, heart, caudal and rostral neuroepithelium. In HU treated embryos, the nuclear expression of phospho-p38 MAPK was increased in all structures in the embryo.

Conclusion: HU increased activation of MEK3/6 and p38 MAPK and promoted the nuclear accumulation of p38 MAPK. These data suggest that p38 MAPK may phosphorylate nuclear targets which play a role in coordinating the response of the embryo to insult. [Supported by CIHR. SB is the recipient of awards from REDIH and FRSQ]

EFFECTS OF CHRONIC EXPOSURE TO AN ENVIRONMENTALLY RELEVANT MIXTURE OF BROMINATED FLAME RETARDANTS (BFRs) ON THE REPRODUCTIVE SYSTEM AND THYROID IN ADULT MALE RATS

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Background: Brominated flame retardants (BFRs) are added to consumer items to reduce their flammability. BFRs are readily released into home and work environments because they are not covalently linked with the polymer matrices; dust is the major source of exposure. Since BFRs are implicated as endocrine disruptors, it is of concern that human body burdens of BFRs have risen in North America over the past decade.

Objective: Animal studies have generally characterized the outcome of acute or chronic exposure to a single BFR technical mixture or congener but not the impact of environmentally relevant BFR mixtures. The goal of this study was to test the hypothesis that chronic exposure to BFRs found in house dust has adverse impacts on the adult male rat reproductive system and thyroid function.

Methods: Adult male Sprague-Dawley rats were exposed to a complex BFR mixture composed of three commercial brominated diphenyl ethers (52.1% DE-71, 0.4% DE-79, 44.2% decaBDE-209) and hexabromocyclododecane (all 3 isomers, 3.3%), formulated to mimic the relative congener levels in house dust. BFRs were delivered in the diet at doses of 0, 0.02, 0.2, 2 or 20 mg/kg/day for 70 days. Hepatic P450 drug-metabolizing enzymes (DME) activities were quantified by alkylresorufin-O-dealkylase assay (EROD, MROD, PROD and BROD) and Cyp1a1 mRNA expression by qRT-PCR as indicators of xenobiotic response. Testosterone (T), thyroxine (T4) and thyroid-stimulating hormone (TSH) were measured in serum. Reproductive health measures, such as testis morphology, the expression of steroidogenesis genes and sperm motion parameters, were assessed. Data were analyzed by ANOVA followed by multiple comparison tests.

Results: There were no differences among the treatment groups with respect to body weights or the weights of the gonads or sex accessory tissues. BFR exposures also did not significantly affect reproductive health or serum T or TSH levels. Compared to controls, males exposed to the highest dose of BFRs (20 mg/kg/day) displayed a significant increase in the weights (mean ± SEM, in grams) of the liver (31.9±1.0 vs 38.9±1.0, $P<0.0001$) and kidneys (5.4±0.2 vs 6.2±0.2, $P<0.05$). All four hepatic DME activities assayed and Cyp1a1 expression were significantly increased (0.009±0.002 vs 0.550±0.141, $P<0.05$), and serum T4 levels were significantly decreased (5.6±0.3 vs 4.1±0.1 μg/dl, $P<0.0001$).

Conclusions: Toxicity assessment across 3 orders of magnitude of dose revealed effects on liver and thyroid physiology but not on reproductive parameters, suggesting that this environmentally relevant BFR mixture does not affect reproductive function in male rats.

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THE ARYL HYDROCARBON RECEPTOR (AhR) EXERTS POST-TRANSCRIPTIONAL CONTROL OVER CIGARETTE SMOKE-INDUCED CYCLOOXYGENASE-2 (COX-2) PROTEIN EXPRESSION

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Background: The aryl hydrocarbon receptor (AhR) is well-known to mediate toxic effects of man-made environmental contaminants, such as dioxin. Activation of the AhR by dioxin can increase inflammatory mediator production, including cyclooxygenase-2 (Cox-2), an immediate/early gene responsible for the production of prostaglandins (PG) and thromboxanes. Cox-2 protein expression is increased in chronic obstructive pulmonary disease (COPD), a lung disease caused by cigarette smoke. We have previously published that the AhR, whose physiological function is still unknown, suppresses cigarette smoke-induced Cox-2 protein, but not mRNA, expression. The ability of the AhR to prevent Cox-2 protein expression may involve RNA-binding proteins (RBP) such as HuR, which increases mRNA stability and thus promotes protein translation. Therefore, we hypothesize that AhR modulates cigarette smoke-induced Cox-2 protein expression by altering Cox-2 mRNA stability via the RBP HuR.

Objectives: The objectives of this study are (1) to determine if the AhR suppression of cigarette smoke-induced Cox-2 protein is due to Cox-2 mRNA destabilization and (2) assess the mechanism by which the AhR stops Cox-2 protein expression.

Methods: $AhR^{-/-}$ and $AhR^{+/+}$ mouse lung fibroblasts were exposed to cigarette smoke extract (CSE) for 3 hours followed by treatment with Actinomycin D (ActD; an inhibitor of RNA synthesis) for 30 minutes, 1 or 3 hours. Following treatments, whole cell lysates and total RNA were harvested for the determination of Cox-2 protein and mRNA by Western Blot and real-time PCR (qPCR), respectively. HuR was assessed by western blot and immunofluorescence (IF).

Results: Steady-state Cox-2 mRNA levels significantly increased upon exposure to CSE for 3 hours only in the $AhR^{+/+}$ lung fibroblasts. Treatment with ActD revealed that the AhR destabilized Cox-2 mRNA. Cigarette smoke-induced Cox-2 mRNA levels in $AhR^{+/+}$ lung fibroblasts decayed, whereas there was little change in steady-state Cox-2 mRNA levels in $AhR^{-/-}$ lung fibroblasts. HuR is expressed in primary lung fibroblasts, and its expression remained constant upon exposure to CSE. HuR was predominantly nuclear in media-only $AhR^{-/-}$ and $AhR^{+/+}$ lung fibroblasts. Exposure to CSE resulted in a dramatic increase in cytoplasmic HuR only in $AhR^{-/-}$ lung fibroblasts.

Conclusions: These results suggest that AhR prevents Cox-2 protein expression by mRNA destabilization. The increased Cox-2 protein associated with AhR deficiency may be the result of HuR translocation to the cytoplasm in response to cigarette smoke. The ability of the AhR to prevent inflammatory Cox-2 protein expression via a post-transcriptional mechanism is a novel finding that furthers our understanding of inflammatory events associated with the development of chronic pulmonary diseases caused by cigarette smoke, such as COPD and lung cancer.

CIGARETTE SMOKE PROMOTES MMP1 AND INHIBITS FOLLISTATIN GENE EXPRESSION IN HUMAN LUNG FIBROBLASTS ACTIVATED WITH TGF-ß1

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Background: Interstitial lung fibrosis and emphysema are two distinct pathological outcomes associated with cigarette smoke. The former is characterized by excess deposition of alveolar extracellular matrix (ECM) while the latter by alveolar destruction. Increasing evidences have shown that both pathological features can exist within the same lung. Recent study further revealed that deposition of parenchymal type I collagen was followed by emphysematous alterations in the lungs of mice subjected to subchronic cigarette smoke exposure. It is not known how cigarette smoke is implicated in the process of such controversial and complex pathogenesis which involves unbalanced ECM homeostasis. Fibroblast is the major cell type accountable for ECM metabolism in the lungs and TGF-ß1 activates fibroblasts in terms of trans-differentiation and ECM synthesis. This study tests the hypothesis that cigarette smoke plays dual roles in promoting both emphysematous and fibrous effects in TGF-ß1 activated human lung fibroblasts (HLF).

Objective: To examine the expression of the genes involved in the ECM homeostasis in TGF-ß1 activated HLF following treatment with the total particulate matter (TPM) of cigarette smoke. Specific genes examined were matrix metalloproteinase -1 (MMP1), collagen 1a1 (Col1a1), α-smooth muscle actin (α-SMA) and follistatin (FST, a fibrosis regulatory mediator).

Method: Human lung fibroblasts were grown for 5 days to reach confluence. TGF-ß1 was administered for 3 days to activate HLF in a serum free medium. TPM, at concentrations of 2.5, 5 or 10 µg/ml, was then added for 24 hours to either a serum free or a 10% fetal calf serum (FCS) containing medium. At the end of theTPM treatment, total RNA was harvested for cDNA synthesis and real-time PCR analysis. A dual luciferase assay was used to verify the MMP1 promoter activation following TPM treatment.

Results: Activation of HLF with TGF-ß1 treatment was confirmed by a significant increased expression of α-SMA, Col1a1 and FST genes. TGF-ß1 mediated inhibition of MMP1 was noted but was not statistically significant. A 24-h TPM treatment in serum free condition, did not alter α-SMA expression in TGF-ß activated cells except at the concentration of 10 µg/ml, neither did it change the expression of Col1a1. Slight but not significant induction of MMP1 expression was observed. Interestingly, FST gene expression was inhibited by TPM at all concentrations. The same observations were further confirmed with the TPM treatment (5 µg/ml) in the 10% FCS containing medium. Dual luciferase reporter analysis was performed to verify whether TPM could activate MMP1 promoter. The results showed that TPM at 5 µg/ml significantly induced HLF promoter activity.

Conclusion: TPM treatment enhanced MMP1 and inhibited follistatin gene expression in TGF-ß1 activated human lung fibroblasts. MMP1 is a potent ECM protease promoting parenchyma destruction and follistatin is known to moderate fibrous progression by binding to activin. These results show that TPM, a complex chemical mixture, could antagonize the effects of TGF-ß1 to lung fibroblasts with regard to ECM homeostasis and further suggest a possible pathogenic mechanism of cigarette smoke-related fibrosis and emphysema.

RESOURCES TO EVALUATE TOXICOGENOMIC DATA

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Background: Gene expression analysis using real-time quantitative PCR (qPCR) technique is now widely used in toxicological research because of its high dynamic range and sensitivity. However, interpretation of the relevance of gene expression data in risk assessment is often hampered by incomplete or confusing methodological description. Furthermore, numerous qPCR experiment protocols and data acquisition methods exist, depending on qPCR machine design. Although, there is a qPCR publication guideline describing the minimum requirements for qPCR data -"Minimum information for the Publication of Quantitative Real-Time PCR experiments (MIQE)", it is not followed widely.

Objectives: We propose that while evaluating qPCR data for risk assessment, it is essential to verify the sequences used in gene expression analysis by accessing genomic databases through web-based bioinformatics tools. This is extremely important, because genomic resources are growing at a fast pace and our knowledge on intra-specific genetic variability, that might impact toxicogenomic response, are evolving with time.

Method and Results: Rat is the most widely used model in toxicology and pharmaceutical research. We will illustrate, i) how to use available rat genomic resources to verify sequence identity and its location within a gene, ii) how the presence of gene splice variants could confound gene expression study, and iii) the importance of considering Single Nucleotide Polymorphism (SNP) in various strains of rat to interpret intra-specific variability in gene expression data.

Conclusion: Better understanding of genomic resources and their utilisation for the verification of gene sequence data will assist the regulators/evaluators in the integration of qPCR data in risk assessments.

A STUDY OF THE MECHANISM OF *IN VITRO* CYTOTOXICITY AND GENOTOXICITY OF TITANIUM DIOXIDE (TiO_2) NANOPARTICLES: IMPACT OF PHYSICO-CHEMICAL CHARACTERISITICS

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Background: There are increasing safety concerns about the development and abundant use of nanoparticles because of their wide range of applications. The unique physical and chemical characteristics of titanium dioxide (TiO_2) nanoparticles results in generating different chemical and biological activities when compared to their larger (micron-sized) counterparts. Earlier studies in *in vitro* and *in vivo* have shown the potential toxicity of nano-TiO_2 in the environment and human health. The physico-chemical characteristics including dispersibility, size, surface area, surface charge, crystal phase, composition, and surface modification can play an important role in influencing toxicity. Among several routes of nano-TiO_2 exposure, inhalation is apparently more prevalent relative to others such as ingestion, and dermal penetration. As such, nano-TiO_2 can induce lung cancer in rats, and toxicity in human bronchial cells.

Objective: Therefore, our objective is to investigate the cytotoxicity and genotoxicity of TiO_2 nanoparticles with different physical and chemical characteristics, as well as the role of surface coating of these nanoparticles.

Methods: We treated Chinese hamster lung fibroblast cell V79 cells with several forms of TiO_2 particles (non-coated nano- and micron-sized TiO_2, and coated nano-TiO_2) at different concentrations and durations of exposure. We then determined the effect of such particles on the cell viability, apoptosis, ROS generation, as well as DNA damage using the comet assay.

Results and Conclusions: Results indicated that all types of TiO_2 could affect cell viability in a concentration-dependent manner, and that nano-sized particles were more cytotoxic compared to the micron-size control. In addition, coated nano-TiO_2 could be cytotoxic but only at a higher concentration (100 mg/L). ROS was generated in cells exposed to all types of TiO_2, but to a lesser extent compared to the positive control (100 μM hydrogen peroxide). Although all types of TiO_2 induced more cell death and more apoptosis (early and late stages) compared to the negative control group, non-coated nano-TiO_2 induced more apoptosis and dead cells compared to either the micron-size or coated nano-TiO_2. A similar pattern of response was observed for DNA damage. Together, these results describe the first comprehensive study of TiO_2 nanoparticles-induced toxicity with respect to the different physico-chemical properties, including particle surface modification. Given that nano-TiO_2 particles are more toxic compared to their larger counterparts, surface modification could change these results. In view of the growing use of TiO_2 nanoparticles, our findings may help to improve the development of new nanoparticles by reducing their potential adverse effects.

THE EFFECT OF PRENATAL ETHANOL EXPOSURE ON EPIGENTIC FACTORS AND CRITCAL SIGNALLING PATHWAYS WITHIN THE DEVELOPING FOREBRAIN

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Background: Prenatal ethanol exposure has been linked to a wide range of cognitive and neurobehavioural abnormalities referred to as fetal alcohol spectrum disorders (FASD). Ethanol-related disruptions in CNS development constitute the most devastating consequences, and maternal ethanol consumption during the first trimester and throughout pregnancy are the most common patterns of exposure. Ethanol may induce changes in DNA methylation that could alter signaling pathways critical in forebrain development, causing impairments in learning, memory, attention, and motor function. TGFβ, Hedgehog and Wnt signaling pathways have been identified as high probability candidate pathways associated with FASD.

Objectives: The purpose of this study is to determine whether prenatal ethanol exposure during the first trimester and throughout entire pregnancy induces DNA methylation modifications within the embryonic/fetal forebrain, and whether DNA methylation modifications leads to changes in gene expression within the TGFβ, Hedgehog and Wnt pathways.

Methods: Pregnant Dunkin-Hartley-strain guinea pigs were assigned to one of three groups: ethanol, isocaloric-sucrose/pair-feeding, or no treatment. Embryonic/fetal brains were collected at the end of the first trimester (GD 23) and third trimester (GD 65). Forebrains from GD 23 embryos were microdissected using laser capture microscopy, and global DNA methylation was quantified. qRT-PCR was utilized to examine TGFβ1, SHH and Wnt3a gene expression in GD 23 forebrain and GD 65 hippocampus.

Results: GD 23 ethanol-exposed embryos were growth restricted in terms of head length and crown-rump length compared with controls. Data analysis suggests an increase in global DNA methylation in GD 23 ethanol-exposed embryos. Preliminary data analysis suggests a decrease in TGFβ1 expression in GD 23 ethanol-exposed embryos, and a decrease in Wnt3a expression in sucrose-treated embryos suggesting a nutritional effect. An increase in TGFβ1, SHH and Wnt3a gene expressions were observed in GD 65 ethanol-exposed fetuses.

Conclusions: Prenatal ethanol exposure results in global DNA hypermethylation in embryonic forebrain, as well as embryonic growth restriction. Maternal ethanol consumption during the first trimester and throughout pregnancy led to changes in gene expression within pathways critical for normal forebrain development, including the TGFβ, Hedgehog, and/or Wnt pathways. These results suggest that ethanol consumption during pregnancy may affect embryonic/fetal forebrain development through changes in DNA methylation and subsequent changes within the TGFβ, Hedgehog and Wnt pathways.

TRACKING PARTICULATE POLLUTANT-INDUCED PROTEOMIC CHANGES IN MACROPHAGES BY MS-BASED ANALYSES

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Background: There is mounting evidence associating environmental particle exposures to a wide range human health effects, predominantly cardiovascular and pulmonary health outcomes. Screening a wide range of particles to gain knowledge on their relative toxicity mainly involves high throughput *in vitro* exposure studies. Typically, classical toxicity assays are used for this purpose, but are limited in terms of their information output. Mass spectrometry (MS)-based proteomic analyses have been shown to generate high content data and can be useful in understanding relative toxicity of particles.

Objectives: The purpose of this work is to apply MS-based proteomics to grade particles based on their relative potencies, compare with cytotoxicity results and to associate with their physico-chemical properties.

Methods: Particles of varying, but well characterized physico-chemical properties (EHC-6802, SRM 1650, CB, SiO2, TiO2, SWCNT-P, SWCNT-O, MWCNT-P and MWCNT-O) were used in this study. J774 murine macrophage cells were exposed to these particles at 0-100 μg/cm2 (96-well plates) in serum-free medium for 24h. Classical cytotoxicity assays (Alamar blue, MTS, ATP and BrdU) were carried out in either cell supernatant or lysate. Shotgun proteomic analyses of tryptic digests of fractionated cell lysates were performed by direct MALDI-TOF-TOF-MS after sample clean-up to remove interferences and experimental artifacts. Saturated alpha-cyano-4-hydroxycinnamic acid served as MALDI matrix. MS data in the m/z region up to 6kDa were mined using ClinPro Tools software (Bruker Daltonics) to identify candidate biomarkers. MS/MS analyses were performed to characterize these biomarkers.

Results: Our results clearly indicated that J774 cells exposed to particles exhibited characteristic m/z profiles. Particle exposures resulted in up or down regulation of a number of proteins. Extent of changes in these candidate biomarkers were different based on the type of particle exposure. Of them, endothelin-1 a marker of inflammatory status of macrophages exhibited a dose-related elevation with particle exposure. Meanwhile, lactate dehydrogenase an indicator of membrane integrity was down regulated upon particle exposures. Also, biomarkers of cytoskeletal changes and antigen processing were identified after particle exposures.

Conclusion: Our results indicated that tracking of proteomic changes due to particle exposures using this mass spectrometry-based analysis method can yield valuable high-content information. Mining of MS data allows categorization of particle exposures based on their toxicity characteristics and also permits identification of biomarkers that can provide new insights into mechanisms of particle toxicity assisting exploration of associations with their physico-chemical properties.

CONSIDERATIONS FOR THE TOXICOLOGICAL ASSESSMENT OF CARBON NANOTUBES *IN VITRO*

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Background: Carbon nanotubes (CNTs) are allotropes of carbon with a cylindrical nanostructure and very high aspect (length-to-diameter) ratios. Their unique structural, electrical and thermal properties have generated a great interest for their application in a wide range of material sciences including nanotechnology, electronics, optics and architecture, as well as medical research, for drug delivery and biosensing. However, evidence is emerging for their potential to cause toxicity, thereby CNTs need to be assessed for possible health and environmental impacts. Many efforts have been dedicated worldwide to address the safety of nanomaterials including CNTs. However, due to their unique physico-chemical properties, difficulties have arisen in the employment of current scientific methodologies in the safety assessment of nanomaterials.

Objective: Evaluate the applicability of an *in vitro* integrated bioassay platform for the screening toxicity of CNTs.

Methods: We have employed our integrated bioassay to screen CNTs including unmodified and surface-modified single-walled and multi-walled CNTs for cytotoxicity. A549 lung epithelial cells and J774A.1 macrophages were exposed to multiple doses of CNTs, in 96-well plates for 24h. The integrated bioassay consists of four assays which determine different cellular pathways, while utilizing distinct chemistries and detection/quantification methods. The assays include the determination of cell metabolic capacity (Cell-Titer Blue), cell proliferation (BrdU incorporation), energy metabolism (ATP levels) and cell membrane damage (LDH release).

Results: We have assessed the integrated bioassay for potential of chemical and physical interactions with the CNTs. CNTs were found to interact with the Cell-Titer Blue assay, confounding the fluorescence-based quantification of cytotoxicity. The non-biological interference was physical, with artifact increasing with increase in particle mass in the well. The assay procedure was modified to assess clarified aliquots taken from the wells, rather than the wells themselves, prior to fluorescence reading. The remaining endpoints of the bioassay did not appear to be affected by CNTs.

Conclusion: Our results suggest the need for rigorous assessment of test systems utilized for toxicological screening of as-received and modified nanomaterials, to circumvent potential interactions with the assays employed. Toxicological testing of nanomaterials including CNT's can be successfully conducted, when extensive characterizations of the assays and modifications of manufacturer's standard protocols are performed.

EPITHELIAL EXPRESSION OF THE ARYL HYDROCARBON RECEPTOR (AHR) PREVENTS CIGARETTE SMOKE INDUCED APOPTOSIS AND MITOCHONDRIAL DYSFUNTION

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Background*:* Cigarette smoke is a major cause of oxidative stress, apoptosis and inflammation, pathological processes involved in chronic lung diseases such as chronic obstructive pulmonary disease (COPD). Pulmonary epithelial cells are a direct target for smoke-induced damage, and undergo apoptosis after cigarette smoke exposure. The AhR is a ligand-activated transcription that responds to man-made toxicants. Although the physiological function of the AhR is unknown, evidence suggests that expression of the AhR may prevent oxidative stress and apoptosis. We therefore hypothesized that AhR expression would attenuate cigarette smoke-induced apoptosis in lung epithelial cells.

Objectives*:* To investigate if the AhR suppresses cigarette smoke-induced pulmonary epithelial cell death and mitochondrial dysfunction.

Methods*:* Mouse lung epithelial cells (MLE-12) (ATCC) were transiently transfected with 60 nM of AhR siRNA or control siRNA (Santa Cruz) and exposed to cigarette smoke extract (CSE-made according to established protocols) or control media. Verification of target knock-down was done by western blot 48 hours after transfection. Mitochondrial function was assessed by MitoTracker®Red immunofluorescence and apoptosis by morphology (Hematoxylin & Eosin staining), chromatin condensation (Hoechst fluorescence) and cleaved PARP levels (western blot).

Results*:* Transfection of MLE-12 cells with AhR siRNA significantly reduced AhR expression (to approximately 42% compared to control siRNA). Knockdown of the AhR in the absence of CSE increased morphological parameters of apoptosis, including nuclear condensation. Following exposure to 2% CSE, there was a further increase in apoptotic markers. AhR-knockdown lung epithelial cells also exhibited mitochondrial dysfunction upon CSE exposure, as evidenced by diffuse cytoplasmic fluorescence of MitoTracker® Red. In contrast, control siRNA epithelial cells exhibited punctuate cytoplasmic staining. Finally, reducing AhR levels in mouse lung epithelial cells resulted in a significant increase in the levels of cleaved PARP when exposed to CSE.

Conclusions*:* Expression of the AhR attenuates cigarette smoke-induced apoptosis in lung epithelial cells. The mode of apoptosis caused by AhR deficiency involves the intrinsic pathway, as evidenced by heightened mitochondrial dysfunction. These results, in concert with our recently published work, support the hypothesis that the AhR is a novel and central regulator of pathogenic processes implicated in COPD etiology and progression.

NEW PIPELINE TO INTEGRATE LOW-DOSE GENE EXPRESSION MICROARRAYS STUDIES INTO RISK ASSESMENT

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Background: Analysis of all genome gene expression by microarrays is becoming a routine practice in toxicological studies. Integration of these studies into risk assessment procedures is lagging. The typical pipeline of analysis of microarray data consist of (1) normalisation, (2) determination of the list of significantly differentially expressed genes by application of arbitrary thresholds of fold change and statistical significance, (3) enrichment analysis of molecular pathways and ontology terms. Limitation of this pipeline for a risk assessment purposes include: (1) arbitrary selection of a list of significantly differentially expressed genes; (2) results of enrichment analysis do not correspond often to any phenotypic effect, especially in low-dose experiments, and may hardly be interpreted from "adversity" point of view; (3) luck of clear health outcome hinders transfer of results obtained in animal experiments to assessment of risk for humans.

Objectives: To develop a pipeline for gene expression micoarrays analysis which produce results "friendly" for risk assessment.

Methods: We used our previously published data of rat liver and brain frontal lobes transcriptome response to low dose developmental exposure to brominated flame retardant 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) to develop a pipeline producing data corresponding to the following criteria: minimizing arbitrary settings in analysis, relevance to adverse health effects, easy integration of results into assessment of risk for humans.

Results: The developed pipeline consists of the following steps: (1) normalisation, (2) conversion of the list of genes used in microarray into the list of human orthologs, (3) ranking of the list in accordance with expression ratio, (4) gene-set enrichment analysis against gene-sets relevant to human disease.

Conclusions: Pipeline described here may facilitate integration into risk assessment of results of numerous experiments using gene expression analysis by microarrays in response to low dose exposure to toxins.

EVALUATING THE POTENTIAL FOR MATERNAL PRETREATMENT WITH SULFORAPHANE TO ACTIVATE VARIOUS XENOBIOTIC METABOLIZING PATHWAYS IN MATERNAL AND FETAL CD-1 MICE

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Background: Sulforaphane (SFN) is a phytochemical derived from cruciferous vegetables that has been shown to increase the activity of key drug-metabolizing enzymes and alter levels of signaling molecules involved in carcinogenesis. While the protective effects of SFN have been demonstrated in cell lines and adult animal models, determining whether treatment with SFN can prevent the development of cancers initiated *in utero* have yet to be addressed. Given that cancers are a major cause of death in children, and that *in utero* exposure to certain toxicants has been linked to childhood cancer, assessing potential preventative agents in *in utero* models of carcinogenesis is warranted. Therefore, the aim of this research is to establish a dose of SFN, which when administered to pregnant CD-1 dams increases the activity of drug-metabolizing enzymes and proteins involved in anti-cancer pathways in maternal and/or fetal tissue.

Methods: Pregnant CD-1 mice were administered SFN (0, 1, 10, or 50 mg/kg) via I.P. injection on gestational days (GDs) 7, 9, 11, and 13, or 0 or 50 mg/kg daily from GDs 7 - 13. On GD 14, dams were sacrificed and maternal heart (positive control for NQO1 activity assay), liver, and fetal livers were removed, and resorption rate noted. Differences in NADPH dehydrogenase, quinone 1 (NQO1) activity between treatment groups were measured by comparing the reduction of 2,6-dichlorophenol-indophenol by NQO1 in the presence or absence of dicoumarol using a spectrophotometer. Additionally, levels of NFkB were measured by Western blot analysis.

Results: NQO1 activity levels did not differ between treatment groups (0, 1, 10, or 50 mg/kg SFN on GDs 7, 9, 11, 13) and the vehicle control in either maternal or fetal tissue. While exposure of pregnant dams to 50 mg/kg SFN daily (GDs 7-13) increased activity of NQO1 in maternal and fetal livers, this increase was not significant. Little difference in the levels of NFkB between any of the treatment groups has been detected. Although dams treated with a daily dose of 50 mg/kg SFN (GDs 7-13) had a higher incidence of resorptions, this difference was also not statistically significant.

Conclusions: SFN has been implicated for use as a potential chemopreventative agent, and many studies to-date have illustrated its benefits. Although previous studies in various models have indicated that SFN increases NQO1 activity and levels of NFkB, this was not seen in the present study, likely due to lower doses and/or shorter treatment periods. Doses were chosen modestly in attempt to detect a low dose that could be useful in activating maternal and fetal NQO1 activity and NFkB levels without causing toxicity to the embryo. Future studies will focus on exposing mice to similar concentrations of SFN over the entire gestational period prior to examination.

BENZO[a]PYRENE INCREASES HOMOLOGOUS RECOMBINATION FREQUENCY *IN VITRO*: A POSSIBLE MECHANISM OF CARCINOGENESIS

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Background: Benzo[a]pyrene (BP) is a polycyclic aromatic hydrocarbon and carcinogen that is released into the environment through natural and man-made sources. BP toxicity is dependent on its metabolism by cytochrome P450s to the reactive metabolite benzo[a]pyrene diol epoxide (BPDE). BPDE is associated with DNA adduct formation and increased frequency of mutations. Furthermore, BP can be metabolized to benzo[a]pyrene quinones that can undergo redox cycling and induce oxidative stress. DNA damage by reactive oxygen species can result in DNA double strand breaks, which can be repaired by homologous recombination or non-homologous end joining. As these processes are not error-free, aberrant DNA repair may be contributing to the toxicity of BP.

Objective: The purpose of this study was to examine if BP exposure induces homologous recombination in an *in vitro* model.

Methods: The Chinese hamster ovary 3-6 cell line was used to measure homologous recombination frequency. This cell line contains a neomycin direct repeat recombination substrate. Cells were exposed to 0, 0.25, 1, 5, or 10 µM BP for 4 or 24 hours and evaluated for viability and recombination frequency.

Results: All of the doses tested were not cytotoxic at both time points assessed. A significant increase in homologous recombination was observed at 10 µM BP after 24 hours of exposure.

Conclusion: BP increases homologous recombination frequency *in vitro*, which may be contributing to its mechanism of carcinogenesis. Ongoing studies are now evaluating the transplacental effects of BP exposure in the pkZ1 mouse strain, which allows measurement of non-homologous end joining frequency *in vivo*.

BEHAVIOURAL ASSESSMENT OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD)-DEFICIENT MICE WITH AGING AND TREATMENT WITH METHYLENEDIOXYAMPHETAMINE (MDA)

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Background: Glucose-6-phosphate dehydrogenase (G6PD) is important for regenerating the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). During cellular oxidative stress, NADPH maintains reduced levels of glutathione, which is essential for the detoxification of reactive oxygen species (ROS), including free radicals and hydroperoxides. NADPH also maintains the catalytic activity of catalase, which detoxifies hydrogen peroxide. G6PD deficiencies constitute the most common human enzymopathy, affecting over 400 million people and up to 60% of some populations. It is generally believed that G6PD deficiencies constitute a problem only for mature red blood cells; however, we have shown an embryoprotective role for G6PD against ROS-initiated teratogenesis. We also have found enhanced pathological changes in the brains of aged G6PD-deficient mice, suggesting a protective role for G6PD in the aging brain.

Objective: Using mutant G6PD-deficient mice, we evaluated several behavioural tests of motor coordination and learning and memory to assess the protective role of G6PD in aged mice, and in young and aged G6PD-deficient mice treated with an amphetamine derivative for which ROS have been implicated in its neurodegenerative effects.

Methods: Motor coordination, taste aversion learning (TAL) and passive avoidance learning (PAL) were measured in young and aged G6PD-normal and G6PD-deficient mice that were either untreated or treated with the major active metabolite of ecstasy, methylenedioxyamphetamine (MDA).

Results: Motor coordination was significantly reduced in aged G6PD-normal female mice but not in the G6PD-deficient females nor in any of the male groups. Motor coordination was not affected in MDA-treated mice in any of the genders and genotypes. TAL was not affected in aged mice, but in young animals, MDA caused a significant deficit in the female animals, with no differences among G6PD genotypes. G6PD-normal and deficient aged mice did not differ in PAL, nor was this type of learning affected by MDA.

Conclusions: The behavioural results for the tests employed do not suggest a protective role for G6PD in these outcomes, which conflicts with the apparent protective role of this enzyme in reducing adverse cellular changes in the brain with advanced age. Future studies will evaluate other behavioural tests reflecting additional brain areas and cell types. The gender-dependent deficit in taste aversion learning caused by a single dose of MDA is consistent with a ROS-dependent mechanism in decreased learning. This differed from the MDA learning deficit measured in the passive avoidance test, which showed no G6PD effect, suggesting complexity in the sensitivity and specificity of behavioural tests for CNS effects of oxidative stress. (Support: CIHR)

MICROARRAY ANALYSIS OF MOLECULAR CHANGES ELICITED IN MICE BY DIETS HIGH IN FAT, SUGAR, OR CHOLESTEROL, AND DIETS DEFICIENT IN CALORIES OR ESSENTIAL FATTY ACIDS

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Background: Nutrition is a focal point of medical research as excess caloric intake is correlated with detrimental health outcomes, such as the metabolic syndrome; however in contrast, low-calorie diets and diets enriched in omega-3 essential fatty acids (EFAs) have beneficial health effects, such as lower cancer rates. However, our understanding of the causal relationship between diet and health remains largely elusive.

Objective: We studied the molecular changes elicited by diets high in fat, sugar, or cholesterol, as well as diets deficient in calories or omega-3 and -6 fatty acids in C57BL/6 male mice.

Methods: Microarrays were conducted on liver samples from 3 mice/diet and detected 20,449 unique genes of which 3,561 were responsive to diet.

Results: Correlational analysis found that diet restriction was the most unique diet because it correlated the least with, and affected more genes than the other diets. The majority of the diets affected several canonical pathways including the citric acid cycle, FXR/RXR activation, LPS/IL-1 mediated inhibition of RXR function, short chain fatty acid metabolism, and NRF2-mediated oxidative stress response. Of the 498 transcription factors (www.TFCat.ca), 87 were responsive to the diets with the majority belonging to the Zipper-Type (38%) and Zinc-Coordinating (31%) classes of transcription factors. Two-way hierarchical clustering of the nine diets identified three major patterns of transcription-factor expression: 1) most highly expressed in the diet-restriction group; 2) most lowly expressed in the diet-restriction group; and 3) most lowly expressed in the high-fructose and EFA-deficient diets.

Conclusions: This study provides considerable insight into the molecular changes incurred by different diets and furthers our understanding of the causal relationships between diet and health. [Support: NIH grants DK-081461, ES-019487, and ES-009649; CIHR]

OXIDATIVE STRESS IN THE DEVELOPMENTAL TOXICITY OF METHANOL IN MICE

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Background: Reactive oxygen species (ROS) are implicated in the mechanism of methanol (MeOH) teratogenesis in rodents. Of particular interest is the role of catalase, which in rodents both detoxifies ROS and metabolizes MeOH and its formic acid (FA) metabolite.

Objectives: We evaluated the *in vivo* role of ROS in MeOH developmental toxicity in preliminary dose optimization studies employing two pharmacological probes that respectively enhance catalase activity or trap free radicals.

Methods: Pregnant mice expressing either high catalase activity (transgenic mice expressing human catalase, hCat) or their respective C57BL/6J wild-type (WT) controls, with either MeOH (two doses of 2 g/kg i.p., 4 h apart, 4 g/kg total dose) or saline vehicle on gestational day (GD) 8 (plug date = GD 1). Additionally, in order to ensure that catalase activity was increased sufficiently in order to see an effect, pregnant hCat mice were pretreated with polyethylene glycol (PEG)-conjugated catalase (50 KU/kg) 8 h before either MeOH or saline vehicle on GD 8. The respective C57BL/6J wild-type (WT) controls were pretreated with a free radical spin trapping agent, alpha-phenyl-N-t-butylnitrone (PBN) in order to elucidate the role of ROS in teratogenesis. PBN (15, 30 and 40 mg/kg ip) was given either 30 min before the first dose only, 30 min before both doses, or 30 min before the first dose of MeOH/Saline and once more, 4 h after the second dose of MeOH/saline. Fetuses were assessed for gross morphological anomalies on GD 19.

Results: General litter parameters (resorptions, fetal body weight) were unaffected by MeOH alone or by either probe. Neither probe alone increased the incidence of malformations. hCat fetuses and their WT controls were similarly susceptible to MeOH-initiated ophthalmic abnormalities, with increased incidences in ophthalmic abnormalities in both strains ($p<0.05$), and increased incidences of cleft palate in the WT ($p<0.05$) and hCat ($p>0.05$) mice. PBN did not appear to protect against MeOH teratogenicity, and was associated with novel anomalies not seen with MeOH alone. Similarly, PEG-catalase pretreatment provided no protection against teratological outcomes of *in utero* MeOH exposure, and appeared to increase the incidence of MeOH-initiated ophthalmic abnormalities and cleft palate ($p<0.05$), as well as the aforementioned novel anomalies.

Conclusions: These results revealed that neither probe protected against MeOH-initiated birth defects *in vivo*, although other studies found both were protective in embryo culture. The role of ROS in MeOH developmental toxicity as tested using these probes may be obscured in an *in vivo* system, possibly due in part to the potentially confounding role of maternal catalase in MeOH and formic acid metabolism, as distinct from its antioxidative role. In light of the slow elimination of MeOH, an expanded exposure period for PBN and PEG-catalase are warranted to corroborate these results. (Support: Methanol Fdn., CIHR)

DEVELOPMENT OF A HIGH-THROUGHPUT SCREEN FOR CHANGES IN DNA METHYLATION USING HIGH RESOLUTION MELT-CURVE ANALYSIS

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Background: Epigenetic markers such as DNA methylation play a critical role in human health and disease through their regulation of gene expression. Environmental contaminants can alter DNA methylation, making it an important endpoint for toxicological study. Establishing a method for high-throughput screening of changes in DNA methylation would be of considerable use for hazard identification. High-resolution melt-curve analysis (HRM), which can be performed using high-throughput PCR systems, is one approach that may serve this purpose.

Objectives: Methylation detection by high resolution melt-curve analysis may result in complex melt profiles, hindering true quantification. In order to use HRM for high-throughput detection of changes in DNA methylation, the informative limits of the approach must be determined. Our objective was to use combinations of synthetic oligonucleotide sequences with varying numbers of methylated sites to determine how changes to the composition of a sample can affect the final melt profile and categorization of samples of differing methylation level.

Methods: Serial dilutions and equimolar combinations of synthetic oligonucleotides with known sequences were used to precisely control methylation profiles for assay validation. Primers were designed to amplify bisulfite-converted DNA, regardless of methylation status. Adjustments to the primer annealing temperature were used to optimize the range and resolution of the assay. Following PCR-amplification, DNA was assessed by HRM, and fluorescence normalization was performed for all melt curves. The area under the curve was calculated and used as an index of methylation. To assess the utility of the HRM analysis in an exposure model, human alveolar epithelial cells (A549) were treated with the hypomethylating drug 5-aza-2'deoxycytidine (DAC; 0, 0.1, 5mM) for 24 and 72h, and examined for changes in DNA methylation.

Results: Altering primer annealing temperature allowed detection of 1% methylation in a population of unmethylated sequences. As little as one differentially methylated site was sufficient for discrimination between homogeneous populations. Combining sets of synthetic oligonucleotides generated melt curve profiles corresponding to mixtures of homogeneously and heterogeneously methylated template DNA, serving as a model enabling the detection and discrimination of complex methylation signatures derived from HRM analysis. Analysis of melt profiles following HRM allowed the detection of a significant decrease in methylation of the long interspersed element (LINE-1) in A549 cells treated with 0.1mM DAC, with no change observed at higher dose (2-way ANOVA, Dose x Time interaction, $p<0.001$), consistent with published pyrosequencing data. Methylation status of specific gene promoters did not necessarily follow the trend observed for LINE-1, suggesting changes in methylation may be pathway dependent.

Conclusions: Insight gained from the controlled synthetic oligonucleotide model enabled sensitive detection and categorization of changes in DNA methylation in exposed A549 cells. Future work will focus on determining the endpoints most relevant to environmental pollutant exposures and using this approach to complement gene expression data for high-throughput toxicity screening.

IN VITRO METHODS TO EXAMINE INFLAMMATON-RELATED MUTAGENIC/GENOTOXIC ACTIVATION OF BISPHENOL A

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Background: Bisphenol A (BPA) is a component of polycarbonate plastics and epoxy resins. Exposure to BPA has been linked to possible estrogenic endocrine disruptor activity, adverse developmental effects and carcinogenesis. Previously, the laboratory has shown nitrous acid-mediated mutagenic activation of BPA, leading to the possibility of similar reactions occurring during an inflammatory response. Inflammation has been associated with increased risk of carcinogenesis and involves increased expression for genes coding for prostaglandin H synthase 2 (PGHS2), nitric oxide synthase (iNOS), and myeloperoxidase (MPO). The resulting enzymes produce a variety of free radical species which can participate in chemical mutagenic activation through corresponding oxidation, nitrosation and chlorination reactions.

Objectives: The possible mutagenic activation of BPA was tested using the mouse RAW 264.7 macrophage cell line which can be induced to express inflammation-related gene products. In addition, DNA transfection technology was used to examine the effect of BPA on p53 gene expression in human MCF-7 breast cancer cells as a relatively fast indication of DNA damage induction.

Methods: Mouse RAW 264.7 macrophage cells were stimulated with lipopolysaccharide and interferon γ. The toxicity of BPA was characterized using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay. The effect of inflammation on BPA mutagenicity was then examined at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) gene locus by assessing formation of thioguanine resistant mutant colonies. Results were compared with unstimulated cells. MCF-7 cells were co-transfected with an iNOS expression vector as well as a p53 promoter-firefly luciferase reporter plasmid and constitutive *Renilla* luciferase expression vector to control for transfection efficiency. Luciferase expression was measured 24 hours after BPA addition.

Results: The toxicity of BPA, as measured by the MTT assay, was comparable in both unstimulated and stimulated RAW 264.7 cell cultures with an LD_{50}=55 μM found for unstimulated cells and LD_{50}=43 μM found for stimulated cultures. When examined for mutagenicity at the HGPRT gene locus, little evidence for BPA mutagenicity could be found in unstimulated cultures when tested to 10 μM, but BPA was found to be mutagenic in stimulated cultures, with a two-fold increase in mutagenicity found at 100 pM BPA, increasing to 6-fold increase at 1 μM. The presence of iNOS in MCF-7 cells resulted in increased p53 promoter dependent expression of firefly luciferase, first evident at 100pM BPA.

Conclusions: Inflammation-mediated mutagenic activation of BPA was found in RAW 264.7 cells and iNOS expression resulted in increased p53 expression in MCF-7 cells. Both approaches provide commercially available platforms to examine the effects of inflammation induction on endpoints related to carcinogenicity. Increased attention may be needed to examine the impact of inflammation on chemical metabolism and health effects.

EXPRESSION OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTORS-α, -δ/β, AND – γ IN PERFLUOROOCTANCE SULFONATE (PFOS) TREATED RAT LIVERS : AN IMMUNOHISTOCHEMICAL INVESTIGATION

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Background: Determining the mechanism(s) of toxicity of chemical food contaminants is essential to characterize, manage and mitigate the health risks that may be posed by their occurrence in foods for Canadians. PFOS, an industrial chemical, bioaccumulates in the food chain and human blood, suggesting widespread human exposure. Our recent rat studies suggested perturbations in lipid metabolism as a major contributor to liver toxicity of PFOS. Such changes in lipid homeostasis may be attributed to liver peroxisome proliferation (PP) via PFOS interaction with peroxisome proliferator-activated receptors (PPARs). To date, three PPAR subtypes (PPAR- α, -δ/β, and – γ) with a distinct, tissue-specific expression pattern and role(s) have been identified in many species including humans. In this study, we have determined the relative expression of these three forms of PPARs in untreated and PFOS-exposed rat livers in order to relate their potential role in PFOS hepatotoxicity.

Objectives: To study the expression of PPAR sub-types and their potential contribution to rat hepatotoxicity induced by PFOS exposure.

Method: Formalin fixed livers from rats exposed to PFOS in feed (0 - 100 mg /kg diet) for 28 days were processed and stained for PPARs using the immunohistochemical (IHC) method. PPARs, visualized as immuno-stained protein-antibody cellular complexes, were quantified as numbers of positively stained cells per unit area of liver section using microscopic image analysis.

Results: IHC indicated that compared to untreated livers, PFOS treatment significantly increased PPAR-α ($p=0.019$) and PPAR-γ (($p=0.022$) protein expression in both male and female livers. PPAR- δ/β protein expression increased in a dose dependent fashion in both male & female livers, but with no significant differences between PFOS exposed and untreated rats ($p=0.516$).

Conclusions: The increase in the liver-specific PP-induced PPAR-α in PFOS exposed rat livers correlates with our previously observed altered fatty acid profiles and elevated acyl-coenzymeA oxidase 1 in livers from the same study, thus confirming a PP-type mode of action for PFOS. PPAR- γ, shown by others to be expressed more highly than PPAR-α in human tissues and predominantly in adipose tissues, was also significantly increased in PFOS treated rat livers. Current evidence suggests that in general, humans are refractory towards PPAR-α -dependent hepatic peroxisome proliferation, and therefore, perhaps to PFOS hepatotoxicity. However, future studies are necessary to assess the toxicological and human health significance of the observed PFOS induced PPAR- γ activation in the rat model in relation to PFOS levels detected in human blood.

EFFECTS OF THYROID HORMONE DISRUPTION ON MOUSE BRAIN DEVELOPMENT IN EARLY GESTATION

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Objective: The long range objective is to understand the adverse effects of thyroid hormone (TH) disrupting chemicals on brain development, and identify biomarkers of TH disruption. In this experiment, we identify genes regulated by TH in the late fetal neurocortex.

Methods: Timed-pregnant C57BL/6 mice were made transiently hypo- or hyperthyroid by treatment with antithyroid drugs for 3 days (starting from gestation day 13 (G13); "hypo") or injection with TH (on G15, 12 hrs prior to sacrifice; "hyper"), respectively. A third group (hypo+) received both treatments while the control group received the vehicle only. The left cerebral cortex was collected to measure T4 levels, while the right one was used for RNA extraction. Global gene expression was analysed with Affymetrix microarrays, and miRNA analysis applied Agilent miRNA arrays. Statistical analysis was conducted in R software.

Results: Cerebral TH levels were significantly decreased in hypo, and increased in hypo+ and hyper, relative to control. Cluster analysis indicated that gene expression patterns were similar between control and hypo, while hypo+ and hyper clustered separately. There were 29 genes that were significantly altered in common in hypo+ and hyper, relative to control. We confirmed 7 out of 8 of these genes with RT-PCR (88% confirmation of microarray results). miRNA expression was analyzed in the control and hyper groups. TH treatment increased the expression of the most miRNAs. The largest increases were observed for miR-693/30/302.

Conclusions: The genes affected by altered TH levels are involved in pathways critical to neurodevelopment, including thyroid hormone receptor activation, long term potentiation, neurotrophin and reelin signaling. Altered miRNAs play a role in the release of neurotransmitters and neurobehaviour. These findings provide insight into impaired brain development induced by TH disrupting chemicals. Further investigation of these genes and miRNAs will identify potential biomarkers of TH disruption in the developing brain.

PRO-ATHEROGENIC ARSENIC EXPOSURE ALTERS MACROPHAGE POLARIZATION

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Background: Environmental arsenic exposure is linked epidemiologically to increased atherosclerosis. Moreover, we showed that arsenic exposure altered plaque composition. However, the mechanisms by which arsenic enhances atherosclerosis are still unknown. Monocytes and macrophages are key players in atherosclerosis. Different macrophage phenotypes (M1, M2 or Mox) with different biological functions are present within atherosclerotic plaques. M1 are classical macrophages with inflammatory characteristics, M2 are reparative macrophages and Mox respond to oxidative stress in an nrf2-dependant manner (nuclear erythroid related factor-2).

Objective: As arsenic activate nrf2, we hypothesize that it increases atherosclerosis by skewing macrophage polarization toward a Mox phenotype through nrf2 activation.

Methods: Therefore, we investigated the effects of arsenic on murine bone marrow derived macrophages by first culturing these cells in M-CSF into resting macrophages and then polarizing these into M1 with IFNγ or into M2 with IL-4. Macrophages or polarized macrophages were then exposed to arsenic (1.33 μM) and gene expression, phagocytosis and cholesterol transport were evaluated.

Results: Arsenic-exposed naïve macrophages showed increased mRNA marker of Mox, including heme oxygenase-1. Furthermore, arsenic increased Mox markers in M1 and M2 macrophages while decreasing markers of M1 and M2 (iNOS and arginase1, respectively), regardless of their primary polarization. Although these data suggest that arsenic skews macrophage differentiation toward Mox, characterized by a decrease in phagocytosis, which was not seen after arsenic exposure. In addition, arsenic-exposed macrophages showed decreased pro-atherogenic cholesterol efflux. To better characterize the arsenic-enhanced plaque, we exposed $ApoE^{-/-}$ mice, a well-describe model of atherosclerosis, to arsenic (200 ppb) for 13 weeks and characterized the macrophage composition within the plaque.

Conclusions: Our observations may lead to a better understanding of the role of macrophages in arsenic-induced atherosclerosis.

MECHANISMS OF CHEMICALLY INDUCED OBESITY, FOCUSSING ON DEXAMETHASONE AND BPA

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Introduction: The metabolic syndrome which affects a large proportion of the Western population world wide is associated in large with an increase in type two diabetes and cardiovascular disease. Accumulating evidence indicates a possibly significant contribution of adverse events, including exposures to bioactive environmental contaminants such as endocrine disrupter chemicals including bisphenol A (BPA). These contaminants are likely to play a role in the rise of obesity in the general population and in young children in particular. Therefore, understanding the mechanism of action of chemicals leading to the differentiation of cells into adipocytes is of great importance.

Objectives: To investigate the molecular mechanisms that mediates the differentiation process effect in response to chemicals such as Dex and BPA, focusing on early transcriptional events.

Methods: We used the murine 3T3 L1 preadipocyte model for the studies. We have established defined, serum free conditions for the investigation of the chemically induced effects. Under these conditions we have established the lowest effective dose of dexamethasone necessary for the induction of differentiation. In order to initiate adipose differentiation the cells were held at confluency for two days prior to treatment with 3-isobutyl-1-methyl xanthine (MIX) and dexamethasone (day 0). RNA was extracted at several early time points following the initiation of treatment and potential target genes were identified using microarrays. Changes in gene expression were further confirmed using quantitative real time PCR. In addition, differentiation was carried to day 8 when the cells were assessed for differentiation using Oil-Red O which stains lipid droplets and Western Blot for the expression of terminal differentiation markers such as aP2 and adipsin. The ability of chemicals to act as potential obesogens was also examined using BPA as the model chemical. For this purpose the cells were treated with BPA in the absence or presence of the previously identified low dose of dexamethasone.

Results: Several potential early genes upregulated in this process were identified using this methodology. The changes in the expression of these target genes were further confirmed using real time PCR. Additional experiments investigating the involvement of these genes in the regulation of adipogenesis are currently conducted. We have also found that nanomolar concentrations of BPA affected the expression of some target genes in a persistent yet inconsistent fashion. In efforts to delineate the mechanism of action of BPA we will increase the doses of the chemical to accomplish a consistent effect. Using these high concentrations of BPA we hope to delineate the molecular targets of this elusive chemical.

Conclusions: These studies have identified several target genes that could potentially be used as markers for the induction of fat cell formation. In addition these studies reinforce the notion that chemicals such as BPA need to be investigated in conjunction with other environmental factors and in the context of appropriate hormonal environment to understand the full potential hazard. As WATC play a significant physiological etiological role in the metabolic syndrome our results further support the hypothesis that the obesity epidemic of the western world is at least partly chemically-induced.

REPEATED DOSES INTRAMUSCULAR INJECTION OF THE CIMAVAX-EGF VACCINE IN SPRAGUE DAWLEY RATS INDUCES LOCAL AND SYSTEMIC TOXICITY

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Background: CIMAvax-EGF consists of human recombinant Epidermal Growth Factor (EGF), coupled to P64k, recombinant carrier protein from *N. meningitis*, and Montanide ISA 51 as adjuvant. The vaccine immunization induces specific antibody production, inhibiting the EGF/EGF-R interaction through EGF deprivation.

Objective: The objective of this study was to assess the CIMAvax-EGF toxicity in Sprague Dawley rats after intramuscular administration of repeated doses (6 months) and at the same time to determine if rat is a relevant species for studying of CIMAvax-EGF vaccine.

Methods: Rats were randomly distributed into four groups: Control, Montanide ISA 51, Treated with 1X and 15X of human total dose of the antigen. Animals were immunized weekly during 9 weeks, plus 9 immunizations every 14 days. Rats were inspected daily for clinical signs. Body weight, food consumption, and rectal temperature were measured during the administration of doses. Blood samples were collected for hematological, serum biochemical determinations and EGF titles at the beginning, three months and at the end of experimentation. Gross necropsy and histological examination of tissues were performed on animals at the end of the assay.

Results: Vaccine provoked the apparition of antibodies against EGF in the rats, demonstrating rat species relevance in these studies. Neither body weight gain nor food and water consumption were affected. CIMAvax-EGF and Montanide ISA 51 produced local damaged at the administration site, showing multiple cysts and granulomas. Both vaccine-treated groups showed neutrophil elevation, besides AST increase probably related with the damage at the administration site. Rectal temperature was found to be significantly higher in 15X Treated group after immunizations, probably induced by the inflammatory process at the injection site. In summary, the clinical pathology findings together with the body temperature results, appears to be caused by the inflammatory reaction at the administration site of the vaccine, mainly mediated by the oil-based adjuvant Montanide ISA 51, probably enhanced by the immunological properties of the antigen.

Conclusions: This study showed evidences that intramuscular administration during 26 weeks of CIMAvax-EGF at doses up to 15x human total dose is well tolerated in rats and it has a clinical importance since this long lasting study in relevant species allows to treat cancer patients with tumors during long periods with relative weight safety margin.

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