42nd Annual Symposium / Le 42e symposium annuel

December 5 – 7, 2010
Delta Centre-Ville
777 University
Montreal, Quebec
H3C 3Z7
Tel. 514-879-1370

Linking Early Exposures to Late Effects: New Science, Tools and Modes of Action

Le lein entre les expositions précoces et les effets tardifs: science nouvelle, outils et modes d'action

Organised by / Organisé par
SOCIETY OF TOXICOLOGY OF CANADA
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Programme Committee / Comité du programme
Mike Wade, Health Canada, Chair
Douglas Bryant, Intrinsik Environmental Sciences, member
Sami Haddad, Université de Montréal, member

Sunday Dec 5 PM

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

7:00 Student mentoring session - Room 2810
Joanne Wan, Ashuren an Intertek Company
Douglas Bryant, Intrinsik Health Sciences

Linking Early Exposures to Late Effects: New Science, Tools and Modes of Action

Monday Dec 6 AM

7:30 Registration / Continental Breakfast - Foyer Régence AB

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

Session I: Toxicology and the Developmental Origins of Adult Health & Disease - Part I

Chairperson: Mike Wade, Health Canada

8:40 Introduction

8:45 **Jerry Heindel**, National Institute of Environmental Health Sciences
A good start lasts a lifetime: developmental basis of disease

9:30 **Alison Holloway**, McMaster University
In utero exposure to smoking cessation pharmacotherapies and postnatal health consequences

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

10:30 **Frederica Perera**, Columbia University, New York
Exposure to PAHs, epigenetic alterations, and health effects in children

11:15 **Deborah Cory-Slechta**, University of Rochester
The enduring consequences of developemental exposure to lead in conjunction with stress

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

Monday Dec 6 PM

Session II: Toxicology and the Developmental Origins of Adult Health & Disease - Part II

Chairperson: Douglas Bryant, Intrinsik Environmental Sciences

12:55 **Introduction**

1:00 **Steve Holladay**, University of Georgia, Athens
Developmental exposure to TCDD shifts the postnatal immune system toward an autoimmune phenotye in non autoimmune C57BL/6 mice and exacerbates autoimmunity in lupus-nephritis SNF1 mice

1:30 **Sue Fenton**, National Institute of Environmental Health Sciences
Low-dose developmental PFOA exposure in CD-1 mice affects multiple tissues resulting in adverse adult health outcomes

2:00 **2010 Henderson Award Lecture**
Jayadev Raju, Health Canada
Characterizing foods and food constituents as "safe for the colon" using morphological and molecular markers of carcinogenesis

2:30 **Coffee break and poster session**

3:00 **Advances in Toxicology and Environmental Health from the Posters**
Chairperson: Douglas Bryant, Intrinsik Environmental Sciences

Helen Renaud, University of Kansas Medical Center, Kansas City
Ontogeny of 78 cytochrome P450s during postnatal liver maturation in mice [Poster #16]

Errol Thomson, Health Canada
Contrasting biological potency of particulate matter collected at sites impacted by distinct sources [Poster #34]

Hongyan Dong, Health Canada
Thyroid hormone regulation of gene expression via microRNAs [Poster #2]

4:00 Session II adjournment

4:00 **Workshop:** MicroRNA technologies - Régence A
Coordinator: Sabina Halappanavar, Health Canada
Instructors:
Martin Angers, Agilent Technologies
Barbara Gould, Exiqon Life Sciences
Katia Nadeau, Qiagen Canada Inc.
Christoph Eicken, LC Sciences
Jeff Johnston, Life Technologies

4:00 **Annual Business Meeting** - St-Laurent

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

ToxQuiz **–** an animated challenge to your knowledge of Toxicology, risk assessment and posters at STC-2010
Host: Mike Wade

Tuesday Dec 7 AM

7:00 Continental Breakfast - Foyer Régence AB

Session III: MicroRNA in Toxicology

Chairperson: Sami Haddad, Université de Montréal

8:05 Introduction

8:15 **Thomas Duchaine**, McGill University
MicroRNAs: From decision-makers in development to effectors in genotoxic stress response

9:00 **Rajesh C. Miranda**, Health Science Center, Texas A&M University
MicroRNAs as epigenetic regulators of neural stem cell maturation: Evidence from teratogen exposure models of the developing fetal cerebral cortical neuroepithelium

9:45 **Coffee break and poster session**

10:15 **Olga Kovalchuk,** University of Lethbridge
Role of small RNAs in radiation responses and radiation-induced genome instability

11:00 **Sabina Halappanavar,** Health Canada
The role of MiR135b in particle-induced pulmonary inflammation

11:45 **Poster session and lunch**

Tuesday Dec 7 PM

Session IV: Novel Screening Methods in Developmental Toxicology
Chairperson: Louise Winn, Queen's University

1:00 Introduction

1:05 **Bob Chapin**, Pfizer
Embryonic stem cells: fountain of hope, window to awe and capitalist tool

1:50 **Tom Knudsen**, U.S. Environmental Protection Agency
Virtual embryo: systems modeling in developmental toxicity

2:35 **Cindy Zhang**, Bristol-Myers Squibb
Zebrafish development as a screening tool

3:15 **Roger Keefe**, Imperial Oil, President STC
Concluding remarks

3:25 Adjourn

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3:30 STC Board meeting - Bonsecours (1 hr or less)

Remerciements de la part de la Société de Toxicologie du Canada aux maisons qui ont, per leur appui financier, contribue à l'organisation et au succes de notre Symposium

Platinum

Vale

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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 6 AM

Session I:
Toxicology and the Developmental Origins of Adult Health & Disease I.

A Good Start Lasts a Lifetime: Developmental Basis of Disease

Monday, December 6, 8:45 AM - 9:30 AM

Jerrold J. Heindel, *Division of Extramural Research and Training, National Institute of Environmental Health Sciences, NIH/DHHS*

It is now clear that all complex diseases have both a genetic and an environmental component. It is also becoming clear that many if not all non- infectious complex diseases have their origins during development. Indeed stress, infections, drugs, nutrition and exposure to environmental chemicals during development can all lead to Increased susceptibility to disease later in life. There are now data showing that poor nutrition in utero or exposure to Environmental chemicals during development (*in utero* and the first few years of life) can result in increased susceptibility to all the major human diseases including, early puberty, infertility, ADHD, learning and cognitive disabilities, asthma, cardiovascular disease, immune diseases, neurodegenerative diseases and obesity and diabetes. The question then becomes how can poor nutrition or an environmental chemical cause effects long after the nutritional problem or environmental exposure is long gone? The answer at least in part is that these stresses during development can alter epigenetic programming. This talk will summarize the evidence linking poor nutrition or environmental toxicant exposures to epigenetic changes and show how these changes can lead to disease later in life. The data indicate that we may be able to actually prevent disease by improving perinatal nutrition and reducing environmental exposures during development.

Jerrold Heindel, has a PhD in Biochemistry from the University of Michigan. He was an assistant professor at the University of Texas Medical School at Houston and an associate professor at the University of Mississippi before joining the National Institute of Environmental Health Sciences (NIEHS). He started at NIEHS as head of the reproductive and developmental toxicology research group and then moved to his present position in the Division of Extramural Research and Training. He is currently the program director for the extramural endocrine disruptor and developmental basis of disease research programs.

In utero exposure to smoking cessation pharmacotherapies and postnatal health consequences

Monday, December 6, 9:30 AM - 10:15 AM

Alison Holloway, *Associate Professor, Department of Obstetrics and Gynecology, McMaster University, Hamilton, ON*

Cigarette smoking during pregnancy is associated with numerous obstetrical, fetal and developmental complications, as well as an increased risk of adverse health consequences

in the adult offspring. Nicotine replacement therapy (NRT) has been developed as a pharmacotherapy for smoking cessation, and is considered to be a safer alternative for women to smoking during pregnancy. The safety of NRT use during pregnancy has been evaluated in a limited number of short-term human trials, but there is currently no information on the long-term effects of developmental nicotine exposure in humans. However, animal studies suggest that nicotine alone may be a key chemical responsible for many of the long-term effects associated with maternal cigarette smoking on the offspring, such as impaired fertility, type 2 diabetes, obesity, hypertension, neurobehavioural defects and respiratory dysfunction. This presentation will review the long-term effects of fetal and neonatal nicotine exposure on postnatal metabolic outcomes and examine whether smoking cessation pharmacotherapies approved for use during pregnancy may have a better safety profile.

Dr Holloway received her BSc in Zoology from the University of Toronto in 1992. She then obtained her PhD in Zoology from the University of Guelph in 1996. Following her PhD, she worked as a postdoctoral fellow in the Department of Physiology at the University of Toronto. In 2003 she joined the faculty of the Department of Obstetrics and Gynecology at McMaster University where she is currently an Associate Professor.
Dr Holloway's current research studies how exposure to various chemicals during pregnancy can cause damage to the fetus while it is still in the womb. She is also interested in the long-term health consequences of these chemical exposures for the exposed children. The chemicals that are of interest to her laboratory include: chemicals we may intentionally expose ourselves to through lifestyle choices such as cigarette smoking or the use of over the counter natural health products; man-made chemicals present in the environment and naturally occurring chemicals in our diet (e.g. plant phytoestrogens). The majority of the work in her lab at this time focuses on the postnatal metabolic consequences of fetal and neonatal exposure to components in cigarette smoke and/or smoking cessation pharmacotherapies. Her research is currently funded by CIHR and NSERC.

Exposure to PAHs, epigenetic alterations, and health effects in children

Monday, Dec 6, 10:30 AM - 11:15 AM

Frederica P. Perera, Dr.P.H. *Professor of Environmental Health Sciences and Director, Columbia Center for Children's Environmental Health, Columbia University, N.Y., N.Y.*

There is increasing evidence of the fetal basis of adult disease. Environmental exposures, along with nutritional, behavioral and other factors, are thought to play a role. Here, we review the findings from research conducted by the Columbia Center for Children's Environmental Health. We have been following cohorts of pregnant women and their children from in utero to early adolescence and monitoring their exposure to a number of chemicals including polycyclic aromatic hydrocarbons (PAHs). These findings have implications for health and functioning over the life course.

Dr. Perera is a Professor at Columbia University's Mailman School of Public Health, where she serves as Director of the Columbia Center for Children's Environmental Health and of the DISCOVER Center. Dr. Perera pioneered the field of molecular epidemiology, beginning with studies of cancer and is now applying molecular techniques within studies of pregnant women and their children. Dr. Perera received her undergraduate degree from Radcliffe/Harvard University and her Masters and doctoral degrees in Public Health from Columbia University. Her areas of specialization include prevention of environmental risks to children, molecular epidemiology, cancer prevention, environment-susceptibility interactions in cancer,

developmental damage, asthma, and risk assessment. She is the author of over 200 publications and has received numerous honors, including: First Irving J. Selikoff Cancer Research Award, The Ramazzini Institute (1995); Newsweek, The Century Club Award (1997); First Children's Environmental Health Award, The Pew Center for Children's Health and the Environment (1999); Distinguished Lecturer, National Cancer Institute, Occupational and Environmental Cancer (2002); Doctoris Honoris Causa, Jagiellonian University, Krakow, Poland (2004); Children's Environmental Health Excellence Award, U.S. Environmental Protection Agency (2005); and CEHN (Children's Environmental Health Network) Award (2008).

The Enduring Consequences of Developmental Exposure to Lead in Conjunction With Stress

Monday, Dec 6, 11:15 AM - 12:00 PM

Deborah A. Cory-Slechta, Ph.D., *Professor of Environmental Medicine, University of Rochester School of Medicine & Dentistry, Rochester, N.Y.*

Background: Lead (Pb) exposure occurs in the context of other risk factors for different diseases and disorders. One such risk factor with which Pb co-occurs is stress, particularly in low socioeconomic status communities. Prenatal stress in particular is thought to have protracted adverse consequences. Pb and stress also share biological substrates and can lead to a variety of common adverse effects that include both cognitive and attention deficits. Thus, Pb and stress may act together to produce enhanced effects.
Objectives: These studies sought to determine the impact in rat and mouse models of combined exposures to elevated Pb and stress on cognitive behaviors, and potential underlying mechanisms including neurochemical and biochemical alterations in the central nervous system and in functioning of the hypothalamic-pituitary-adrenal (HPA) axis, the body's major stress response system. The studies have examined both maternal and continuous Pb exposure models in conjunction with prenatal stress (PS) or prenatal stress combined with stress directly to offspring (OS).
Methods: Exposure of dams to Pb in drinking water (0, 50 or 150 ppm) was initiated 2 mos prior to breeding and continued until offspring weaning (maternal Pb) or continued in offspring (continuous Pb). Dams were subjected to prenatal stress (restraint immobilization) 3x/day for 45 min each on gestational days (GD) 16-17 (rat) or from days 11-17 (mouse) and endpoints examined separately in male and female offspring. Corresponding steady state blood Pb levels have averaged <3, 5-11 and 25-40 µg/dl for 0, 50 and 150 ppm exposures, respectively.
Results: Both maternal only and continuous Pb exposure result in protracted if not permanent HPA axis dysfunction, with delays in glucocorticoid negative feedback pronounced in males. In female rats, both maternal Pb and continuous Pb exposures combined with PS or OS enhanced behavioral inefficiency on a Fixed Interval schedule of food reward, a behavioral baseline highly sensitive to Pb and considered a surrogate for impulsivity in children. Additionally, combined maternal Pb and stress increased levels of various brain catecholamines in both genders in the absence of effects of either Pb or PS alone. Combined exposures to continuous Pb and PS in female offspring impaired learning of specific sequences of responses that were not seen with Pb exposure alone. Statistical analyses that incorporate behavioral and neurochemical changes in females have suggested alterations in particular in frontal cortical norepinephrine in alterations in FI performance, as well as relationships among corticosterone levels, frontal cortex dopamine and nucleus

accumbens dopamine turnover with learning impairments.
Conclusions: Collectively, these studies show that Pb exposure effects can be unmasked or enhanced when co-occurring with stress; converse effects, e.g., mitigation of Pb effects could also conceivably occur. The findings have particular significance for public health protection, underscoring the need to advance cumulative risk assessment. Such efforts should include: 1) the evaluation of environmental chemical exposures such as Pb not in isolation, but rather in conjunction with other co-occurring risk factors with which they share biological substrates and common adverse effects, and 2) the corresponding need to develop animal models of toxicology that more realistically reflect human environmental conditions, and epidemiological studies with the capability to examine potential interactions among factors

Dr. Deborah Cory-Slechta became a faculty member at the University of Rochester Medical School (URMC) in 1982. She became Chair of its Department of Environmental Medicine and Director of the NIEHS Environmental Health Sciences Center in 1998, and served as Dean for Research from 2000-2002. She then became Director of the Environmental and Occupational Health Sciences Institute (EOHSI) and Chair of the Department of Environmental and Community Medicine at the UMDNJ-Robert Wood Johnson Medical School from 2003-2007, before returning to URMC as Professor in Environmental Medicine and Pediatrics. Dr. Cory-Slechta has served on national review and advisory panels of the National Institutes of Health, the National Institute of Environmental Health Sciences, the Food and Drug Administration, the National Center for Toxicological Research, the Environmental Protection Agency, the National Academy of Sciences, the Institute of Medicine, and the Agency for Toxic Substances and Disease Registry, Centers for Disease Control. She currently serves on the Science Advisory Board of the US EPA and on the Advisory Committee for Childhood Lead Poisoning Prevention of the CDC. In addition, Dr. Cory-Slechta has served on the editorial boards of the journals Neurotoxicology, Toxicology, Toxicological Sciences, Fundamental and Applied Toxicology, Neurotoxicology and Teratology, and American Journal of Mental Retardation. She has held the elected positions of President of the Neurotoxicology Specialty Section of the Society of Toxicology, President of the Behavioral Toxicology Society, and been named a Fellow of the American Psychological Association. Her research has focused largely on the relationships between brain neurotransmitter systems and behavior, and how such relationships are altered by exposures to environmental toxicants, particularly the role played by environmental neurotoxicant exposures in developmental disabilities and neurodegenerative diseases. These research efforts have resulted in over 120 papers and book chapters to date.

Monday Dec 6 PM

Session II:
Toxicology and the Developmental Origins of Adult Health & Disease II.

Developmental exposure to TCDD shifts the postnatal immune system toward an autoimmune phenotype in non autoimmune prone C57BL/6 mice and exacerbates autoimmunity in lupus-nephritis SNF1 mice

Monday, Dec 6, 1:00 PM - 1:30 PM

Steven D. Holladay, *Professor & Head, Anatomy and Radiology College of Veterinary Medicine, University of Georgia, Athens, GA* with A Mustafa and RM Gogal Jr

Inappropriate aryl hydrocarbon receptor (AhR) activation during development can have profound negative consequences in numerous organ systems. The classic environmental agent for studying AhR-related developmental toxicity is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), due to its high affinity and specificity for the AhR. We have found that a single mid-gestation dose of TCDD permanently skews the postnatal immune system toward an autoreactive phenotype in C57BL/6 mice, and both induces and exacerbates nephritis in autoimmune-predisposed SNF_1 mice. Male but not female C57BL/6 mice showed increased Vβ3+ and Vβ17a+ T cells in the spleen, and increased percentages of bone marrow CD24-B220$^+$ B cell progenitors. Activated splenocytes from female mice over-produced the cytokine IFNγ, while the males over-produced IL-10. Antibody titers to ssDNA, dsDNA and cardiolipin displayed increasing trends, reaching significance for anti-dsDNA in both sexes and for ssDNA in males. Immunofluorescent staining of IgG and C3 deposition in kidney glomeruli increased in both sexes, suggestive of early stages of autoimmune glomerulonephritis. In SNF1 mice, mid-gestation TCDD significantly changed B cell phenotypes in the adult bone marrow and spleen, increased autoantibodies to dsDNA and cardiolipin, and increased immune complex deposition in kidneys. These mice also had increased autoreactive CD4$^+$Vβ17a$^+$ peripheral T cells and increased IFNγ cytokine production by activated T cells. The prenatal TCDD also augmented hallmark indicators of SLE progression in the lupus-prone SNF_1 mice, including renal immune complex deposition, glomerulonephritis, and mesangial proliferation. These collective data show that developmental TCDD exposure results in persistent humoral and cell mediated immune dysregulation in mice, and induces an adult immune functional profile beyond the classic suppression response, to also include exacerbation of lupus-like autoimmune disease.

Dr. Holladay received his Ph.D. degree in toxicology from North Carolina State University in 1989. His post-doctoral studies were focused in developmental immunotoxicology at the National Institute of Environmental Health Sciences, with a joint appointment in the laboratories of Dr. Jerry Heindel (Developmental and Reproductive Toxicology) and Dr. Mike Luster (Immunotoxicology). He then held faculty appointments at North Carolina State University, Virginia Tech, and presently the University of Georgia, where he serves as Head of the Anatomy and Radiology Department in the College of Veterinary Medicine. Dr. Holladay served as editor for the textbook Developmental Immunotoxicology, published by CRC Press in 2005, and has published 130 peer-reviewed manuscripts. His research presently focuses on mechanisms by which developmental exposure to environmental contaminants may increase risk of immunologic disease later in life.

Low-dose developmental PFOA exposure in CD-1 mice affects multiple tissues resulting in adverse adult health outcomes

Monday, Dec 6, 1:30 PM - 2:00 PM

Suzanne Fenton, *National Institute of Environmental Health Sciences, NIH/DHHS* with Sally S. White, Madisa B. Macon, Jason P. Stanko

Perfluorooctanoic acid (PFOA) is an 8-carbon member of the perfluoroalkyl acid (PFAAs) family, which are synthetic chemicals that ubiquitously persist in the environment, in both humans and wildlife. PFOA is used primarily in the production of fluoropolymers and may accumulate following breakdown of flouro-telomer acids. Commercial uses of these

fluoropolymers include carpet and fabric coating, fire-fighting foam and grease-resistant food packaging. Primary exposure routes for humans and wildlife are not fully understood, but are thought to involve drinking water, consumption of contaminated food, and indoor contamination (house dust). Human health effects associated with PFAA exposure have been reported, and bio-monitoring and epidemiological studies are on-going to evaluate for long-term health effects. Toxicological findings have also been reported in laboratory animal models. Several sensitive adult health end points have been identified in developmentally PFOA-exposed mice. Our laboratory first indicated that prenatal exposure to 5 mg/kg/d PFOA delayed mammary gland development in exposed female offspring, as well as altered lactational performance or milk quality in exposed pregnant dams leading to decreased pup weight gain and increased mortality in the early postnatal period. More recent studies have utilized oral low-dose PFOA exposure (0.0, 0.01, 0.1, or 1.0 mg PFOA/kg body weight) during the latter half of gestation. Mammary gland development was abnormal in 21-day old treated animals, in a dose dependent manner ($p<0.05$), with notable changes in longitudinal and lateral epithelial growth, and fewer visible branch points and terminal end buds. Chronic exposure to PFOA in water (5 ppb) also affected developmental and adult mammary gland morphology. Full gestation-exposed female offspring at the lowest doses (0.01, 0.1) exhibited significantly elevated body weight after 10 weeks of age that was associated with increased serum insulin and leptin levels. Liver:body weight ratios in the 1.0 mg/kg dose group were elevated when compared to controls in animals exposed during late pregnancy, but was significantly elevated at 0.3 mg/kg following full gestation exposures. These increased ratios stemmed from increased hepatomegaly in both full and late gestation exposed pups. These data suggest that the lowest observable effect level of late gestational exposure to PFOA on liver:body weight ratio is 0.3 mg PFOA/kg, but is 0.01 mg PFOA/kg for mammary growth, excessive weight gain, and associated developmental effects. This abstract does not necessarily reflect NIEHS policy.

Dr. Suzanne “Sue” Fenton earned her M.S. and Ph.D. from the University of WI-Madison in the Endocrinology/Reproductive Physiology Program, working in the areas of artificial insemination and mammary gland biology. Her postdoctoral studies at the UNC-Chapel Hill Lineberger Cancer Center focused on the roles and gene regulation of epidermal growth factor receptor ligands in the mammary gland. Dr. Fenton was a Research Biologist at the US EPA’s Reproductive Toxicology Division from October 1998 to September 2009. She was recently recruited to the National Institute for Environmental Health Sciences where she is leading the Reproductive Endocrinology laboratory in the Cellular & Molecular Pathology Branch of the National Toxicology Program. Her current research involves identification of the effects of environmental components on early development, pubertal timing, lactational function, and tumor susceptibility of the mammary gland. Dr. Fenton has recently served on the Working Group for the NIEHS Breast Cancer and the Environment Research Centers, the Developmental and Reproductive Toxicology Executive Committee for ILSI HESI, and as a consultant to the California Breast Cancer Research Program Special Research Initiatives. She represents the National Toxicology Program at the Congressionally-mandated Inter-Agency Breast Cancer & Environment Research Coordinating Committee.

2010 Henderson Award Lecture

Monday, Dec 6, 2:00 PM - 2:30 PM

Characterizing foods and food constituents as "safe for the colon" using morphological and molecular markers of carcinogenesis

Jayadev Raju, Ph.D., *Toxicology Research Division, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, ON, Canada*

The identification and investigation of the carcinogenic potential of foods and food constituents are key factors in providing food safety advice by Canadian food regulators. Animal models of carcinogenesis such as chemically-induced rodent tumor models provide a unique opportunity to test hypotheses linking food constituents and their carcinogenic/anti-carcinogenic potential. The azoxymethane-induced rodent colon carcinogenesis model is ideal to study linkages between foods, nutrition and colon cancer. Aberrant crypt foci (ACF) are the earliest identifiable putative precancerous lesions of the colon in both humans and animal models. The rodent ACF system has been successfully adapted as a quantitative surrogate end-point biomarker to assess carcinogens/anti-carcinogens specific to the colon. The system has been vital in understanding the pathogenesis of colon cancer and marking vital cellular and molecular events in this multistep event. Many molecular candidates (e.g. transforming growth factor-β, estrogen receptor-β, cyclooxygenase-1/2, etc) are well implicated in colon carcinogenesis (early on in the process) and are being used as potential molecular markers to assess food carcinogens and anti-carcinogens. Similarly, enzymes involved in fatty acid metabolism are excellent molecular candidates in relating nutritional aspects that inhibit or exacerbate the effects of carcinogens/anti-carcinogens. *In vitro* studies are imperative in addressing specific molecular targets and their relationship to disease out comes. Our laboratory, in the federal food regulatory setting has since provided data with regards to the safety of foods and food components in terms of their potential carcinogenicity utilizing both *in vivo* and *in vitro* cancer models. We are currently investigating several food constituents categorized under the Government of Canada's Chemicals Management Plan to provide data on their potential health risks and molecular mechanisms so as to improve regulation and policy governing the use/occurrence of chemicals as/in foods.

Dr. Jayadev Raju is a Research Scientist (2007-present) in the Toxicology Research Division, Food Directorate, Health Products and Food Branch, Health Canada - the federal health authority for food safety regulation and policy in Canada. He received his Masters (1994) and Ph.D. (1999) from the Jawaharlal Nehru University, New Delhi, India and moved to Winnipeg as a Postdoctoral Fellow in the Department of Nutritional Sciences, University of Manitoba, Winnipeg, Canada. He served as a Research Associate at the American Health Foundation Cancer Centre (Institute for Cancer Prevention), Valhalla, NY, USA and at the University of Waterloo, Waterloo, Ontario, Canada. Dr. Raju is a recipient of the Graduate Research Fellowship (1994-1999) from the University Grants Commission, India; and a Visiting Scientist Fellowship (2004) from the German Cancer Research Centre (Deutsches Krebsforschungszentrum), Heidelberg, Germany. He is a recipient of research funding from the Federal Intramural Genomics Research and Development Fund, Government of Canada (2008-2011). He is published in peer-review journals from broad research areas ranging from foods & nutrition, biochemistry and cancer research.

Advances in Toxicology and Environmental Health from the Posters

Monday, Dec 6, 3:00 PM - 4:00 PM

Ontogeny of 78 Cytochrome P450s during Postnatal Liver Maturation in Mice [Poster #16]
Helen Renaud, *University of Kansas Medical Center, Kansas City*

Contrasting Biological Potency of Particulate Matter collected at sites Impacted by Distinct Sources [Poster #34]
Errol Thomson, *Health Canada*

Thyroid Hormone Regulation of Gene Expression via microRNAs [Poster #2]
Hongyan Dong, *Health Canada*

Workshop: MicroRNA Analysis

Monday, Dec 6, 4:00 p.m. - 6:00 p.m.

Coordinator: Sabina Halappanavar, *Health Canada*

This workshop will give participants an overview of the basic biology of non-coding microRNA as important mediators of cell function and then delve into the laboratory and bioinformatic tools for their analysis. Five instructors will describe different technical approaches and analytical platforms developed by their companies to measure and analyze cell and tissue levels of this important group of nucleic acids.

miRNA: Think BIG!!!
Martin Angers, Field Applications Scientist
Agilent Technologies - Life Science Research

Studying MicroRNA Biology -The Locked Nucleic Acid Advantage
Barbara R. Gould, Ph.D., Field Application Scientist
Exiqon Life Sciences

Providing Solutions for your Entire microRNA Workflow.
Katia Nadeau, PhD, Technical Sales Representative
Qiagen Canada Inc.
From extraction to analysis to functional studies, Qiagen offers beginner and experienced microRNA researchers alike a complete system for gene expression research.

Seq-Array - miRNA Discovery and Profiling Using a Customizable Workflow

Christoph Eicken, PhD, Head of Technical Services-Microarrays
LC Sciences, LLC

Current miRNA profiling methods rely on the limited sequence information available, hence the focus on a few model species. Seq-Array is a customized solution high-throughput genome-wide miRNA profiling to overcome these limitations. It combines and leverages three technologies: the latest deep sequencing technology, advanced bioinformatics, and µParaflo™ custom microarrays. This talk will present the workflow and a case study with 777 newly discovered miRNAs.

miRNA Functional Analysis Tools from Ambion

Jeff Johnston, PhD, Technology Sales Specialist
Gene Expression and Genetic Variation
Applied Biosystems - a part of Life Technologies

This presentation will be an overview of vectors for up-regulation, down-regulation and monitoring miRNA activity.

Tuesday Dec 7 AM

Session III: MicroRNA in Toxicology

MicroRNAs: from decision makers in development, to effectors in genotoxic stress response

Tuesday, Dec 7, 8:15 AM - 9:00 AM

Thomas Duchaine, *Assistant Professor, Goodman Cancer Research Center, Department of Biochemistry, McGill University*

MicroRNA-mediated gene silencing has emerged as a major layer in the gene regulation networks. According to well supported predictions, these small, non-coding miRNAs base-pair with, and negatively regulate more than 60% of the entire protein-coding genome. miRNAs are now known to be implicated in a wide range of functions during development, homeostasis, and disease. While they were first identified through forward genetic screens that revealed their wide role in animal developmental timing, in the last few years microRNAs have been implicated as key players in some of the most intensely investigated signaling cascades. I will review some of these fundamental functions, and outline the latest evidence now linking microRNAs to p53 genotoxic response, and additional cascades, in the induction of senescence and apoptosis in response to environmental cues and stresses. I will further discuss the implications of these findings for prospects to use microRNAs as biomarkers, or as therapeutic agents in radiation therapy.

Dr Thomas Duchaine has contributed to research in the field of gene regulation by RNA for more than 16 years. He was trained as a post-doctoral fellow in Massachusetts, USA, under the supervision of Dr Craig C. Mello (from 2001 to 2005), who received the Nobel Prize in Medicine with Andrew Fire for the discovery of RNAi in 2006. During a short second post-doctoral project, he was mentored by Dr Nahum Sonenberg, a leader in the field of translation regulation and one of Canada's most celebrated researchers. Since September 2006, Dr Duchaine has established his own research program as a member of the Department of Biochemistry, within the Goodman Cancer Research Centre. His research program aims to explore the biological implications of the RNAi phenomena, a series of genetic mechanisms that shut down gene expression, in embryonic development, but also in Cancer. For this, he exploits the experimental model organism C. elegans, just as well as human cultured cells. His work has been published in the most renown biomedical scientific journals such as Cell, Science and Molecular Cell.

MicroRNAs as epigenetic regulators of neural stem cell maturation: Evidence from teratogen exposure models of the developing fetal cerebral cortical neuroepithelium

Tuesday, Dec 7, 9:00 AM - 9:45 AM

Rajesh C. Miranda, PhD, *Texas A&M Health Science Ctr./College of Medicine, Dept. Neuroscience And Experimental Therapeutics, College Station, TX 77843-1114*

During the second trimester of development, the fetal brain undergoes a remarkable transformation, in which fetal neural stem and progenitor cells proliferate rapidly to

generate most of the neurons of the adult brain. In utero exposure to ethanol during this developmental period is likely to permanently alter the structure of the adult brain. We previously showed that neuroepithelial cells exposed to ethanol underwent increased cell proliferation and the elimination of cells expressing stem and progenitor cell markers. More recently, we identified specific microRNAs (miR9, 21, 153 and 335) that are altered following ethanol exposure. MiRNAs are short non-protein coding RNA fragments, which control gene expression by suppressing protein translation or destabilizing mRNA transcripts. Individual miRNAs control the expression of several hundred protein-coding genes to promote rapid changes in cell maturation and renewal. Therefore, as we recently showed (Sathyan et al., J Neuroscience, 2007) miRNAs are positioned to serve as important molecular transducers of the teratogenic effects of ethanol.
MiR335, a mammalian-specific miRNA is particularly interesting. It is located within the epigenetically-programmed MEST/Peg1 fetal growth-control locus. In humans, disruption of this locus is associated with fetal growth retardation and mild mental retardation. We will discuss data showing that miR335 is transiently expressed in the developing fetal brain and that its expression is regulated by genomic methylation and histone acetylation. We will also present data showing that the miR335 locus controls the maturation of fetal neural stem cells. These data collectively indicate that multiple ethanol-sensitive epigenetic components like miRNAs and chromatin remodeling effectively cooperate to shape the transcriptome, and consequently, neural stem cell responses to a teratogen.

Dr. Rajesh Miranda completed a MA in Clinical Psychology at Bombay University (Mumbai, India) in 1984, a Ph.D. in Neurobiology at the University of Rochester (Rochester NY) in 1989, and a post-doctoral fellowship at Columbia University, College of Physicians & Surgeons, Dept. Obstetrics & Gynecology (New York, NY) in 1995. In 1995, he joined the faculty of Texas A&M University System, HSC, and is now a tenured Professor in the Department of Neuroscience and Experimental Therapeutics. His research, funded by the NIH, has focused on teratology and neuro-degeneration with a specific focus on fetal brain development and neural stem cell maturation. More recently, his research has focused on the biology of microRNAs and their relevance to teratology. Rajesh Miranda has served as a reviewer in a variety of NIH grant review panels including as a chartered member in the Neurotoxicology and Alcoholism and AA-4 study sections.

Role of small RNAs in radiation responses and radiation-induced genome instability

Tuesday, Dec 7, 10:15 AM - 11:00 AM

Olga Kovalchuk, MD, PhD, *Professor, CIHR Chair in Gender and Health and Board of Governors' Research Chair, Biological Science Department, University of Lethbridge, Lethbridge Alberta, Canada*

While modern cancer radiation therapy has led to increased patient survival rates, the risk of radiation treatment-related complications is becoming a growing problem. Radiation poses a threat to the exposed individuals and their progeny. It causes genome instability that is linked to carcinogenesis. Radiation-induced genome instability manifests as elevated delayed and non-targeted mutation, chromosome aberration and gene expression changes. Its occurrence has been well-documented in the directly exposed cells and organisms. Radiation is also known to cause a wide variety of indirect effects. It can also cause genome instability in the unexposed progeny of the pre-conceptually exposed animals and humans. Yet, the mechanisms by which genome instability arises remain

obscure. We hypothesized that epigenetic changes and alterations in small RNA pools play leading roles in the molecular etiology of the radiation-induced genome instability. Amongst small RNAs, miRNAs and piRNAs are of special interest. MiRNAs are abundant, small, single-stranded noncoding RNAs that regulate gene expression at the post-transcriptional level. piRNAs are a novel class of small RNA molecules that have been linked to silencing of retrotransposons and other genetic elements.
In my talk I will present new and compelling evidence that epigenetic changes (DNA methylation and short RNA pool changes) are important for the molecular etiology of the radiation-induced genome instability.
The studies were supported by the CIHR, ACF and DOE grants.

Dr. Olga Kovalchuk is a Professor, Board of Governors' Research Chair in Radiation Biology and a CIHR Chair in Gender and Health in the Department of Biological Sciences at the University of Lethbridge, Alberta. Dr. Kovalchuk is a recipient of Canada's Top 40 Under 40 Award.
Research in the Kovalchuk's laboratory focuses on the somatic and germline effects of ionizing radiation exposure in animals and people. Dr. Kovalchuk's research interests include: epigenetics of carcinogenesis and cancer; radiation epigenetics and role of epigenetic changes in radiation-induced genome instability and carcinogenesis; epigenetic regulation of the cancer treatment responses; sex differences in radiation responses and carcinogenesis, and radiation-induced DNA damage, repair and recombination.

The role of microRNA-135b in particle-induced pulmonary inflammation.

Tuesday, Dec 7, 11:00 AM - 11:45 AM

Sabina Halappanavar, *Mechanistic Studies Division, Environmental Health Science & Research Division, Health Canada*

MicroRNAs (miRNA) are implicated in regulation of inflammation process. In this study pulmonary mRNA and miRNA expression profiles in mice exhibiting inflammatory phenotype following exposure to nanoparticles of titanium dioxide (nanoTiO2) were characterized. Female C57BL/6BomTac mice were exposed 1 hour every day to 42.4 ± 2.9 (SEM) mg surface coated nanoTiO2/m3 for 11 consecutive days by inhalation and were sacrificed 5 days following the last exposure. Pulmonary inflammation was quantified using differential cell counts in bronchalveolar lavage fluid (BALF). DNA microarrays were used to profile pulmonary gene and miRNA expression. The expression of 16 miRNAs was found significantly affected 5-days post exposure to nanoTiO2. Among the most induced were miR-1, miR-449a, and miR-135b with dramatic 60-fold induction of miR-135b. The total number of neutrophils was significantly increased in BALF of nanoTiO2-treated group compared to air-exposed controls. Furthermore, expression of a suit of genes in lung tissues associated with inflammation including acute phase response genes, several C-X-C and C-C motif chemokines (cxcl5, cxcl1, ccl2, ccl22, ccl7, ccr4) and cytokines was found upregulated following the exposure to nanoTiO2. Similar increase in pulmonary miR-135b levels was also observed in mice exhibiting inflammatory phenotype following exposure to particles of carbon black, suggesting a potential association between miR-135b expression levels and particle-elicited pulmonary inflammation process. To further clarify if the observed increase in miR-135b levels is specific to particle-induced inflammation, expression of miR-135b was profiled in vivo (mouse, pulmonary) and in vitro (cell culture) models of non-particle-induced inflammation. The presentation will

expand on details of the findings and put forward a hypothesis to explain the role of miR-135b in inflammation process.

Sabina Halappanavar, obtained her Ph.D. in Molecular and Cellular Biology from Université Laval, Quebec in 2003. She is currently working as a Research Scientist in the Mechanistic Studies Division, Environmental Health Scieince and Research Bureau of Health Canada. Her research at Health Canada focuses on epigenetics of cardiopulmonary effects of environmental toxicants. This includes effects of exposure to mainstream tobacco smoke, complex environmental matrices and engineered nano-sized particles. Another part of her research is directed towards validation of applicability of toxicogenomic tools in risk assessment.

Tuesday Dec 7 PM

Session IV: Novel Screening Methods in Developmental Toxicology

Embryonic Stem Cells: Fountain of Hope, Window to Awe, and Capitalist Tool

Tuesday, Dec 7, 1:05 PM - 1:50 PM

Robert Chapin, *Developmental and Reproductive Toxicology Center of Expertise, Pfizer, Inc. Groton, CT*

Stem cells present the latest unparalleled opportunity in medicine and science: that of using precursor cells to create differentiated cells either in situ or in vitro. Cells created for regenerative medicine hold the promise to treat and largely cure health problems that have been largely unaddressed by medicine so far. Each month brings new understandings of the biochemical and biophysical requirements for controlling the differentiation of these cells. While these understandings will be used to create effective and unprecedented treatments (we sure hope), it's also certain that everything we learn about the controlling biology will quickly be put to use in creating improved safety testing tools. One of the places of most immediate uptake will be in developmental toxicology. The cells in a conceptus undergo differentiation, multiplication, and migration. Differentiation and multiplication can be captured well enough in a stem cell culture, but until an environment or substrate is found which promotes predictable migration and interaction of multiple cell types, there will be a limit to how well stem cells can predict developmental toxicity. And it is for this reason that we turn to tiny whole-organism models to help test for effects on the migration aspect of development. This talk will review some of the current state of stem cell biology, and then reprise to trajectory of hope and frustration that our lab has experienced in developing predictive toxicity tools using these cells. Finally, to help remind us that change occurs logarithmically and not linearly, we will undertake a brief review of the rate of technologic change. If nothing else, stem cells and the promise they hold remind us that we live in one of the most exciting possible times.

Bob Chapin obtained his PhD in pharmacology from UNC-Chapel Hill, post-doc'd at CIIT, and then spent 18 years at the National Institute of Environmental Health Sciences in North Carolina, working mostly in male reproductive toxicology, but branching out occasionally to female and developmental tox. Bob has been at Pfizer for almost 9 years now, working with the most inspiring and wonderful group of people imaginable, trying to develop tools for predicting developmental tox, and using in vitro methods for mechanistic and

trouble-shooting investigations of various sorts. He's been lucky enough to publish >130 papers and book chapters, held various Society and committee positions, and always relishes the opportunity to interact with his friends to the North…. particularly in December.

Virtual Embryo: Systems Modeling in Developmental Toxicity

Tuesday, Dec 7, 1:50 PM - 2:35 PM

Thomas B. Knudsen, Ph.D., *National Center for Computational Toxicology, U.S. EPA, RTP, NC, USA 27711*

High-throughput and high-content screening (HTS-HCS) studies are providing a rich source of data that can be applied to in vitro profiling of chemical compounds for biological activity and potential toxicity. EPA's ToxCast™ project, and the broader Tox21 consortium, in addition to projects worldwide, are generating HTS-HCS data to construct in vitro cellular bioactivity profiles for thousands of chemical compounds in commerce or potentially entering the environment. EPA's ToxCast™ project generated HTS- HCS data on 309 environmental chemicals in more than 500 in vitro assays. Phase-I focused mostly on pesticidal and anti-microbial chemicals with rich in vivo animal testing data culled from the ToxRefDB database. The assays covered diverse biochemical activities, receptor binding activities, reporter gene activation and gene expression profiles, stress-response indicators, and perturbation in cell state and cellular function. Also included were assays to monitor effects in zebrafish embryos and pathways of differentiation in mouse embryonic stem cells. In vitro profiles (AC50 in uM) and in vivo endpoints (mg/kg/day dosage) are compared for each chemical in the ToxMiner™ database, with machine-learning algorithms used to identify patterns of biological activity and optimal feature selection for predictive modeling. Applying this approach to predictive modeling and mechanistic understanding of developmental toxicity faces several challenges: correlating in vitro concentration-response with internal dose-response kinetics; understanding how in vitro bioactivity profiles extrapolate from one cell-type or technology platform to another; and linking targets of in vitro bioactivity into pathways of developmental toxicity and mechanistic models. The latter would include in silico platforms that can be used to connect in vitro to in vivo effects with relevant knowledge about the developmental process, and computer simulations that run rules-based cellular behaviors to dissect complex multicellular responses at a systems-level. Addressing these challenges will require innovative computer models that simulate kinetics (ADME) and multicellular dynamics. EPAs virtual embryo project (v-Embryo™) is building a framework for incorporating knowledge gained from these projects into computational (in silico) models that execute morphogenetic programs to simulate developmental toxicity. [This abstract does not necessarily reflect US EPA policy].

Dr. Knudsen is a Developmental Systems Biologist at the US Environmental Protection Agency's National Center for Computational Toxicology (NCCT). He received his Ph.D. in Anatomy from Thomas Jefferson University in 1981 and postdoctoral training at the Children's Hospital Research Foundation in Cincinnati and Emory University. Dr. Knudsen was on the faculty of E Tennessee State University (1986-90), Jefferson Medical College (1990-2003), and U Louisville (2003-07). Before joining EPA he was Professor of Molecular, Cellular and Craniofacial Biology at the University of Louisville and a member of the Birth Defects Center and Center for Environmental Genomics and Integrative Biology. Dr. Knudsen was a member of the Human Embryology and Developmental Study Section at NIH, and served as President of the

Teratology Society (2007-08), and Adjunct Professor at the University of Louisville. His past research has focused on prenatal developmental toxicity, including mitochondrial mechanisms and genomics-systems biology. He has published over 80 scientific papers, book chapters, and edited one book. Dr. Knudsen has served as Editor in Chief of Reproductive Toxicology since 2003 and is on the editorial board of Birth Defects Research Part C: Embryo Today. Since joining NCCT, he is PI of EPA's 'Virtual Embryo Project' to build a framework and research program focusing on predictive models of developmental toxicity through the applications of ToxCast high-throughput screening (HTS) data, multicellular models and computational systems biology.

Zebrafish: A Model for Developmental Toxicology Screening

Tuesday, Dec 7, 2:35 PM - 3:15 PM

Cindy Zhang, *Bristol-Myers Squibb, Research and Discovery, Discovery Toxicology, Hopewell, N.J. 08534*
with Karen Augustine-Rauch

For many years, the zebrafish has shown great value in the developmental biology field as a model for gene discovery and functional characterization. As a research tool, the zebrafish embryo has many positive attributes including ease of culture, rapid organogenesis and cross-species conservation of many developmental pathways and embryological processes. There has been increasing interest in using the zebrafish as a model for assessing developmental toxicity of chemicals. To this end, a number of laboratories have independently reported on zebrafish in vitro developmental toxicology assay designs and their respective performance in correctly classifying known teratogens and non teratogens. Over the last 3 years, scientists engaged/interested in these efforts convened in two HESI workshops on in vitro developmental assays to identify gaps in the methodology and plan future steps for improving the assay. Acting upon this analysis, a consortium effort involving five pharmaceutical companies is underway to develop a harmonized zebrafish developmental toxicology assay that may be conducive to high throughput screening. This presentation will provide an overview of the zebrafish developmental toxicology assay, gaps identified from the workshops for improvement and consortium strategies that are currently underway to establish a harmonized assay for screening compounds for teratogenic potential.

Cindy Zhang received her BS in Wuhan, China and a M.S. in Asian Institute of Technology in Bangkok, Thailand. Both degrees were in the area of Aquatic Biology with focusing on fish genetics, disease, or nutrition. In 1995, she obtained M.S. in Immunology and Molecular Pathology from University of Florida, where she evaluated the protective effects of interleukin-1 on hematopoietic cells treated with the anti-oncologic agent, L-phenylalanine mustard. From 1995 to 1998, she worked in the Center for Cancer Treatment and Research in Richland Memorial Hospital affiliated with University of South Carolina. Cindy engaged in various cancer research projects, which included working with modified stem cells for long-term hematopoietic reconstitution. Cindy has subsequently worked in industry at GlaxoSmithKline and Bristol Myers Squibb Co. in the departments of Drug Safety Assessment and Discovery Toxicology, where her research projects have been mainly focused on assay development in hematotoxicity testing and teratogen screening using in vivo animal models, primary cells, cell lines, as well as in vitro rat or zebra fish embryo cultures.
Currently Cindy is engaged in evaluation and selection of predictive statistical models for supporting refined in vitro teratogenicity screen assays and generating guideline documents describing the criteria associated with the morphological score systems used in the rat and zebrafish embryo culture screening assays developed by BMS. At BMS, she has authored several publications, book chapters and reviews.

Session d'Affichage
Poster Session

IMMUNOHISTOCHEMICAL INVESTIGATION OF THE EFFECTS OF DIETARY POTASSIUM PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER

S.A.Aziz, G. Bondy, I. Curran, M. Barker, K. Kapal, P. Bellon-Gagnon, E. MacLellan, R. Mehta.
Toxicology Research Division, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario, Canada

Background: PFOS, an industrial chemical within the family of perfluoroalkylated compounds (PFCs), bioaccumulates in the food chain and human blood, suggesting widespread human exposure. Our recent studies in rats confirmed the liver is a primary target of PFOS, with altered fatty acid metabolism leading to hepatic peroxisome proliferation. Initial liver IHC showed an unusual cytoplasmic localization of the cell proliferation biomarker PCNA. PFCs may disrupt mitochondrial bioenergetics by induction of mitochondrial membrane permeability (MMP).

Objectives: The objective in this study was to employ IHC to investigate specifically, mitochondrial membrane disruption, and the interplay between cell proliferation (PCNA), tumor suppression (p53), cell cycle (p21) and apoptosis genes during PFOS induced liver toxicity.

Method: Formalin fixed livers from rats exposed to PFOS in feed (0 - 100 mg PFOS/kg diet) for 28 days were processed for biomarkers by IHC. Apoptosis was detected by TUNEL method. Biomarkers visualized as immuno-stained protein-antibody complexes, were quantified as numbers of positively stained cells per unit section area using microscopic image analysis.

Results: A predominance of cytoplasmic staining for PCNA with increasing dose of PFOS was observed. p53 expression was confined to the cytoplasm. p21 was expressed in both nucleus and cytoplasm, but with greater nuclear presence. Comparative analysis of PCNA and p21 revealed a pattern exhibiting a cellular ratio of 1:1. p21 expression usually depended on p53 presence, but occasionally, p21 was observed in p53 null cells.

Conclusions: IHC was found to be a useful tool for insight into protein expression of some hepatic genes affected by PFOS at an individual cell level. Future similar analyses of other peroxisome proliferator-type chemicals would aid in verifying if this same battery of genes would be applicable generally as a “biomarker” for health effects assessment of this class of chemical contaminants.

THYROID HORMONE REGULATION OF GENE EXPRESSION VIA MICRORNAs

Hongyan Dong[1], Martin Paquette[1], Andrew Williams[1], R. Thomas Zoeller[2], Mike Wade[1], Carole Yauk[1]
[1] Environmental Health Sciences and Research Bureau, Health Canada
[2] Molecular & Cellular Biology Program, University of Massachusetts

Background: MicroRNAs (miRNAs) are extensively involved in diverse biological processes. However, very little is known about the role of miRNAs in mediating the action of thyroid hormones (TH). Appropriate TH levels are known to be critically important for development, differentiation and maintenance of metabolic balance in mammals.

Objectives: To test the hypothesis that TH regulates specific miRNAs

Methods: We induced transient hypothyroidism in juvenile mice by short-term exposure to methimazole and perchlorate from post natal day (PND) 12 to 15. The expression of miRNAs in the liver was analyzed using Taqman Low Density Arrays (containing up to 600 rodent miRNAs). The gene expression profiles were examined with microarrays and were used to identify the target genes of miRNAs.

Results: We found the expression of 40 miRNAs was significantly altered in the livers of hypothyroid mice compared to euthyroid controls. Among the miRNAs, miRs-1, 206, 133a and 133b exhibited a massive increase in expression (50- to 500-fold). The regulation of TH on the expression of miRs-1, 206, 133a and 133b was confirmed in various mouse models including: chronic hypothyroid, short-term hyperthyroid and short-term hypothyroid followed by TH supplementation. TH regulation of these miRNAs was also confirmed in mouse hepatocyte AML 12 cells. The expression of precursors of miRs-1, 206, 133a and 133b were examined in AML 12 cells and shown to decrease after TH treatment, only pre-mir-206 and pre-mir-133b reached statistical significance. To identify the targets of these miRNAs, DNA microarrays were used to examine hepatic mRNA levels in the short-term hypothyroid mouse model relative to controls. We found transcripts from 92 known genes were significantly altered in these hypothyroid mice. Web-based target predication software (TargetScan and Microcosm) identified 14 of these transcripts as targets of miRs-1, 206, 133a and 133b. The vast majority of these mRNA targets were significantly down-regulated in hypothyroid mice, corresponding with the up-regulation of miRs-1, 206, 133a and 133b in hypothyroid mouse liver. To further investigate target genes, miR-206 was over-expressed in AML 12 cells. TH treatment of cells over-expressing miR-206 resulted in decreased miR-206 expression, and a significant increase in two predicted target genes, Mup1 and Gpd2.

Conclusion: The results suggest that TH regulation of gene expression in the liver may occur secondarily via miRNAs. These studies provide new insight into the role of miRNAs in mediating TH regulation of gene expression.

ACYCLOVIR ALDEHYDE MAY CAUSE ACYCLOVIR- INDUCED RENAL TUBULAR INJURY

Patrina Gunness[1,2], Katarina Aleksa[1,3] and Gideon Koren[1,2]
[1] Division of Clinical Pharmacology and Toxicology, The Hospital for Sick Children, Toronto, Ontario, Canada, [2]Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario, Canada, [3]Department of Pharmacy, University of Waterloo, Waterloo, Ontario, Canada

Background: Acyclovir is the first-line therapy for the treatment for several viral infections. Acyclovir is generally well tolerated, however, in some cases, nephrotoxicity has been observed. Crystalluria has been widely believed to be the mechanism of acyclovir – induced nephrotoxicity. However, clinical evidence of nephrotoxicity in the absence of crystalluria, suggests that acyclovir may induce direct renal tubular insult. Recently, we provided the first *in vitro* evidence which supports existing clinical evidence of direct renal tubular damage induced by acyclovir. We propose that acyclovir is metabolized to an intermediate acyclovir aldehyde metabolite that is subsequently metabolized to an end metabolite, 9-carboxymethoxymethylguanine (CMMG). It has been postulated that the alcohol dehydrogenase (ADH) enzyme may be responsible for the metabolism of acyclovir to acyclovir aldehyde and the aldehyde dehydrodenase (ALDH) enzyme may be accountable for the metabolism of acyclovir aldehyde to CMMG. Aldehydes are reactive chemical compounds that are often produced endogenously as intermediate drug metabolites and have been suggested to play an active role in the induction of several drug – induced organ toxicities, including hepatotoxicity, neurotoxicity and nephrotoxicity. The involvement of acyclovir aldehyde in acyclovir – induced nephrotoxicity has not been previously investigated. The ADH and ALDH enzymes are expressed in the human kidney and therefore, the kidney may have the ability to metabolize acyclovir to result in local production of its intermediate acyclovir aldehyde metabolite.

Objective: To determine whether acyclovir aldehyde plays a role in acyclovir – induced nephrotoxicity.

Methods: Human renal proximal tubular (HK-2) cell monolayers were exposed to acyclovir (500 – 2000 µg/mL) in the presence or absence of the ADH enzyme inhibitor, 4-methylpyrazole (200 or 500 µM) or the ALDH enzyme inhibitor, cyanamide (200 or 500 µM) for 24 hours. Cytotoxicity (assessed as a function of cell viability) was measured using the fluorometric alamarBlue® assay. Statistical analyses were performed using ANOVA followed by Tukey's HSD post hoc tests. Results were considered statistically significant if $p < 0.05$.

Results: Compared to cell monolayers exposed to acyclovir (500 – 2000 µg/mL), co-exposure to 4-methylpyrazole (200 or 500 µM) rendered significant protective effect ($p < 0.05$), probably due to inhibited production of the aldehyde.

Conclusions: Cytotoxicity results obtained from cell monolayers co-exposed to acyclovir and 4-methylpyrazole suggest that acyclovir aldehyde may be engaged in the direct renal tubular injury induced by acyclovir. Biologically plausible mechanisms of acyclovir aldehyde – induced cytotoxicity may include lipid peroxidation, DNA or protein damage. More studies are required to provide evidence of the potential mechanism(s) of acyclovir aldehyde – induced cytotoxicity.

ROLE OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN-INDUCIBLE POLY(ADP-RIBOSE) POLYMERASE IN DIOXIN-INDUCED ARYL HYDROCARBON RECEPTOR SIGNALLING

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Background: The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates the toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). AHR regulates the expression a hundreds of genes including TCDD-inducible poly(ADP-ribose) polymerase (TiPARP, PARP-7). TiPARP is a member of the PARP superfamily, which is an enzyme family that mediates poly(ADP-ribosyl)ation of protein targets. Poly(ADP-ribosyl)ation is post-translational modification associated with a number of biological functions including DNA repair, transcriptional regulation, apoptosis and cell division. TiPARP contains a C-terminal PARP catalytic domain, a conserved WWE (tryptophan-tryptophan-glutamic acid) domain and a single $CCCH_3$-type zinc-finger domain. However the biological role of TiPARP and whether TiPARP plays a functional role in AHR signal transduction is unknown.

Objective: The aim of the present study was to investigate modulation of TCDD-induced AHR activity by TiPARP.

Methods: We used RNA-interference to knockdown endogenous TiPARP in T-47D human breast carcinoma cells. TiPARP knockdown cells were treated with 10 nM TCDD for 24 h and cytochrome P450 1A1 (CYP1A1) and CYP1B1 gene expression and AHR protein expression were determined. AHR and ARNT (aryl hydrocarbon receptor nuclear translocator) recruitment in knockdown cells was determined by chromatin immunoprecipitation (ChIP) assays following 1 h TCDD treatment. Full-length TiPARP, TiPARP truncation and catalytic point mutants were overexpressed in HuH-7 human hepatoma cells and reporter gene assays were performed following 16 h TCDD treatment.

Results: RNAi-mediated knockdown of TiPARP significantly increased TCDD-induced CYP1A1 and CYP1B1 expression. Overexpression of exogenous TiPARP decreased TCDD-induced *CYP1A1*- and *CYP1B1*-regulated reporter activity in a dose-dependent manner. ChIP assays showed increased TCDD-dependent recruitment of AHR/ARNT to *CYP1A1* and *CYP1B1* enhancer regions following TiPARP knockdown. TiPARP knockdown also reduced TCDD-induced AHR protein degradation following 24 h treatment. Functional domain studies using TiPARP truncation mutants revealed TCDD-induced inhibition required the N-terminus (amino acid residues 1-234) and PARP catalytic domain. Single point mutations of two highly conserved residues of the PARP catalytic core motif abolished inhibition of TCDD-induced reporter gene activity.

Conclusions: Collectively, these results implicate TiPARP as a negative regulator of AHR signalling.

FOXA1 IS ESSENTIAL FOR THE AHR-DEPENDENT REGULATION OF CYCLIN G2

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Background: The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor known to mediate the toxic and carcinogenic effects of a wide variety of environmental contaminants such as dioxin (TCDD; 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin). AHR is thought to play a protective role in breast cancer development by blocking cell proliferation. This might occur through direct interaction with estrogen receptor alpha (ERα) or indirectly through modulation of ERα target genes. One such gene of interest is *cyclin G2* (*CCNG2*) which inhibits cell cycle progression by preventing cell cycle entry. This gene is negatively regulated by ERα, which might contribute to estrogen-dependent tumor growth. Our laboratory has shown that *CCNG2* is positively regulated by TCDD via AHR activation, suggesting that AHR might utilize this pathway to suppress tumor growth. However, the mechanism by which AHR regulates *CCNG2* is unknown.

Objective: This study was completed in order to fully understand how AHR regulates *CCNG2*. Elucidating its role could provide new mechanistic insight into the anti-proliferative function of AHR.

Methods: Using chromatin immunoprecipitation, gene expression, and siRNA technology on T-47D human breast cancer cells we investigated how AHR regulates *CCNG2*.

Results: We report that the prototypical AHR ligand TCDD (10nM) is able to recruit AHR and nuclear coactivator 3 (NCoA3) to the enhancer region of *CCNG2* (60% and 10% compared to a 5% total*)* leading to a 2-fold increase in gene expression after 6h of treatment with TCDD(10nM). Furthermore, our data reveal that the transcription factor Forkhead box A1 (FOXA1) is recruited to *CCNG2* in a TCDD-dependent manner. FOXA1 is known to be an important pioneer factor controlling the binding of ERα to its target genes. RNAi-mediated knockdown of FOXA1 abolished TCDD-dependent recruitment of AHR to *CCNG2* and reduced CCNG2 expression levels. Interestingly, knockdown of FOXA1 also caused a marked decrease in ERα, but not AHR protein levels. However, RNAi-mediated knockdown of ERα had no effect on TCDD-dependent AHR recruitment to or expression of *CCNG2*.

Conclusions: Our data show that FOXA1, but not ERα is essential for AHR-dependent regulation of *CCNG2*, assigning a role for FOXA1 in AHR action.

EFFECTS OF VALPROIC ACID AND VALPROMIDE ON LIMB MORPHOLOGY AND GENE EXPRESSION DURING DEVELOPMENT CORRELATE WITH THEIR HISTONE DEACETYLASE INHIBITION ACTIVITY

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Background: In utero exposure to valproic acid (VPA), an anticonvulsant and antidepressant, induces neural tube and skeletal malformations through a molecular mechanism of action that remains unsolved. A current hypothesis is that VPA may act through its activity as a histone deacetylase (HDAC) inhibitor. Indeed, Valpromide (VPD), an analog of VPA, has been reported to be less teratogenic and less potent as an HDAC inhibitor than VPA. HDACs are involved in chromatin remodeling and, consequently, their inhibition may affect the expression of certain genes. Two such genes are very important for limb development; Hif1a and Sox9 are key mediators of the cellular response to hypoxia and chondrocyte differentiation, respectively.

Objectives: The purpose of this study was to use an *in vitro* limb bud culture system to compare the effects of VPA and VPD on limb development, HDAC activity and the expression of Sox9 and Hif1a.

Methods: Timed-pregnant CD1 mice were euthanized on gestation day 12; the embryonic forelimbs were excised and cultured for 6 days in the absence or presence of VPA or VPD (0.6, 1.8 or 3.6 mM), stained with toluidine blue, and scored according to their morphology. Limbs were also cultured for 1, 3, 6 or 12h for the quantification of histone 4 acetylation by Western blot analysis and of Hif1a and Sox9 transcripts by qRT-PCR.

Results: VPA inhibited limb growth and differentiation and induced a significant decrease (70% at 3.6 mM) in limb score at all concentrations, whereas VPD caused a significant reduction in this score (29% at 3.6 mM) only in the group exposed to the highest concentration. The acetylation of histone 4 was increased at 3h in all VPA treatment groups (0.6 mM, 2.0 fold; 1.8 mM, 2.7 fold; 3.6 mM, 3.4 fold). In contrast, histone acetylation was not significantly affected in limbs exposed even to the highest concentration of VPD. While Sox9 and Hif1a transcripts were both downregulated (60 and 40%, respectively) 3h following exposure to the highest VPA concentration, no change in the expression of these genes was observed in the VPD treated limbs.

Conclusions: Together, these results suggest that the teratogenicity of VPA is correlated with its activity as an HDAC inhibitor and is associated with the disregulation of Sox9 and Hif1a during limb development. Further studies are needed to examine the mechanism linking decreased HDAC activity and the transcriptional regulation of Sox9 and Hif1a. These studies were supported by CIHR and FRSQ.

CHARACTERIZATION OF DIRECTLY-REGULATED THYROID HORMONE MEDIATED HEPATIC GENE EXPRESSION IN EARLY DEVELOPMENT

Martin Paquette[1,4], Hongyan Dong[2], Rémi Gagné[1], Andrew Williams[3], Morie Malowany[3], Mike Wade[2], Carole Yauk[1,4]
[1]Mechanistic Studies Division, [2]Hazard Identification Division, [3]Population Studies Division, Healthy Environments and Consumer Safety, Health Canada, [4]Department of Biology, Carleton University

Background: Toxicant induced disruption of thyroid hormone (TH) signalling during early life can alter growth, development and energy metabolism leading to persistent mental and physical deficits. THs exert their effects through interactions with thyroid response element (TRE)-bound TH receptors, thereby controlling target gene transcription. Despite the characterization of TH mode of action, only a few genes have been identified as being directly regulated by THs.

Objectives: To develop a better understanding of transcriptional regulation by THs during early development, the effects of short-term TH perturbation on hepatic mRNA transcription in juvenile mice have been evaluated. The objectives of this research are to further characterize the mechanisms of TH action during early liver development and to identify candidate biomarkers for the study of TH-disrupting chemicals. To develop a better understanding of TH signaling we are also identifying and characterizing novel DNA elements that bind to the TH receptor.

Methods: TH disruption was induced from postnatal day (PND) 12 -15 by adding goitrogens methimazole and sodium perchlorate to dams' drinking water (hypothyroid). A subgroup of TH-disrupted pups received intraperitoneal injections (*i.p.*) of replacement THs 4 hr prior to sacrifice (replacement). An additional group received only THs (*i.p.*) prior to sacrifice (hyperthyroid). Hepatic mRNA was extracted and hybridized to Agilent mouse microarrays. An *in silico* search of the genomic sequences of directly regulated genes for TREs was performed using a position weight matrix of known TREs coupled with a novel scoring system recently developed at Health Canada.

Results: Serum thyroxine levels in PND 15 pups confirmed that goitrogen and/or TH treatments generated the desired TH modulations. MAANOVA analysis identified approximately 400 significantly altered genes in male and/or female pups (false discovery rate-adjusted p-value < 0.05) in at least one treatment condition. Integrated systems biology analyses of microarray data indicated that several key pathways, including oxidative stress response (including *prdx1, txnrd1* and *herpud1*) and xenobiotic metabolism/signalling (including *cyp2c18, cyp3a4* and *fmo2*) were altered by thyroid hormone disruption. Several possible TREs were identified by bioinformatics search in the -8 to +2kb regulatory regions of a subset of genes (including *slc25a45, trim24* and *ihh*) immediately responsive to THs (i.e., directly-regulated).

Conclusion: These results provide insight into the TH-regulated hepatic transcriptome of juvenile mice and increase our understanding of the mechanism by which TH modulates liver development. By further understanding TH regulated gene expression we will be able to better understand and help predict deleterious adulthood effects resulting from TH signalling disruptions during early development. Further work will characterize TRE-containing genes as biomarkers of direct thyroid hormone action in the liver.

COMPARISON OF EFFECTS OF DIFFERENT LIGANDS AND Y322 ON ARYL HYDROCARBON RECEPTOR SIGNALLING

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Background: The aryl hydrocarbon receptor (AHR) mediates the toxic effects of halogenated aromatic hydrocarbons, but is also activated by a diverse range of compounds. Classic AHR ligands include 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) and 2,3,7,8-tetrachlorodibenzofuran (TCDF). Other compounds, such as omeprazole (Omp), are thought to activate AHR through phosphorylation of residue Y322. Mutations of the Y322 equivalent in rat AHR prevented activation by Omp but did not effect TCDD activation.

Objectives: In this study, we examined the ability of different compounds to activate AHR-dependent induction of cytochrome P450 1A1 (*CYP1A1)*, and hairy and enhancer of split 1 (*HES1*) expression in human breast cancer T-47D and hepatoma HuH7 cells, as well as the role of Y322 in AHR activation by these ligands.

Methods: T-47D and HuH7 cells were treated with DMSO, TCDD, PeCDF, TCDF or Omp and a time course of mRNA induction was analyzed by RT-PCR. AHR and coactivator (p300, nuclear receptor coactivator A1/A3) recruitment was determined by Chromatin Immunoprecipitation assay. AHR degradation was examined with Western blot of whole cell extract. To investigate the role of Y322 in AHR activation, AHR deficient MCF-7 AHR100 cells were transfected with hAHR, Y322F or Y322A mutants.

Results: Time course mRNA expression analysis showed that in cells treated with TCDD, PeCDF, TCDF or Omp *CYP1A1* mRNA induction increased throughout the 1.5 to 24h treatment, while *HES1* mRNA levels peaked after 1.5h treatment with no increase observed after 24h. Chromatin Immunoprecipitation assays revealed similar recruitment profiles for AHR and coactivator (p300, nuclear receptor coactivator A1/A3) to the regulatory regions of both genes; although some ligand differences were apparent. Proteolytic degradation of AHR was evident after treatment with TCDD, PeCDF, and TCDF, but not with Omp. To investigate the role of Y322 in AHR activation, AHR deficient MCF-7 AHR100 cells were transfected with hAHR, Y322F or Y322A mutants. Y322F resulted in 50% reduction of TCDD- and PeCDF-induced *CYP1A1* mRNA induction and AHR recruitment to *CYP1A1* relative to wt-hAHR, whereas this mutation ablated activation by TCDF or Omp. Y322A mutation failed to induce or recruit AHR to *CYP1A1* following exposure to all compounds.

Conclusions: Our findings show that (1) AHR regulates its target genes in temporally distinct manner; (2) different AHR activators recruit similar coactivators following AHR activation; and (3) Y322 is required for maximal ligand dependent activation of AHR.

A NOVEL GENOMIC-GUIDED PROTEOMIC STRATEGY TO IDENTIFY BIOMARKERS OF EXPOSURE TO DIESEL EXHAUST PARTICLES

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Background: Developing molecular approaches to identify biomarkers is essential for exposure assessment and improved prediction of toxicological hazard/risk. Here, we propose an approach integrating gene expression profiles and proteomic analyses to identify secreted biomarkers of exposures to/effect from extractable organics from diesel exhaust particles with mutagenic and carcinogenic hazard. Exposure to diesel exhaust particulate poses a significant risk to human health and environmental and occupational exposure to diesel exhaust particles is common.

Objectives: To illustrate that a genomic-guided proteomic strategy can be used *in vitro* with cell extracts and extracellular fluid to identify biomarkers of exposure to extractable organics from diesel exhaust particles (DEP).

Methods: Murine lung epithelial cells (FE1 cells) were exposed *in vitro* to organic DEP extracts for 6 hours. Cells were harvested for mRNA extraction and serum-free media was collected for purification of secreted proteins. The project is divided into three phases: (1) identification and validation of toxicogenomic profiles using high-density oligonucleotide microarrays (4x44K whole mouse genome), followed by RT-PCR validation; (2) linking toxicogenomic profiles to disease-related changes and toxicological pathways using bioinformatics tools (Ingenuity Pathway Analysis (IPA) software, DAVID bioinformatics) and identification of secretome elements; (3) targeted proteomic analysis (ELISA, Western blotting) to confirm the presence of the secreted protein biomarkers in extracellular fluid.

Results: Microarray gene expression profiling followed by a significance analysis identified 789 differentially expressed genes (1.5-fold; FDR adjusted $p < 0.05$). Selected genes were confirmed by RT-PCR. IPA software integrated the experimental findings into a cellular signalling context and highlighted the perturbed intracellular canonical pathways. The pathways affected included the NRF2-mediated oxidative stress response, xenobiotic metabolism, glutathione metabolism, TGF beta signalling, and biosynthesis of steroids. Several genes coding for secreted proteins were included in these pathways. Serpine1 and BMP4 have been identified as potential biomarkers of *in vitro* exposure to DEP extract.

Conclusions: The genomic-guided proteomic strategy, involving global gene expression profiling and targeted proteomic analysis, permits focussed biomarker discovery through linkage with toxicologically relevant pathways. The strategy will accelerate the deployment of “omics” technologies in applied toxicology, and ultimately assist in biomonitoring and risk management.

INVESTIGATING THE EFFECTS OF DIETARY FOLIC ACID ON SOMATIC DNA DAMAGE AND MUTATION IN MICE

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Background: Folate is essential for maintenance of DNA methylation, DNA synthesis, chromatin structure and appropriate gene expression. Folate deficiency is associated with increased risk for neural tube defects, cardiovascular disease and various cancers. Folate deficiency can induce DNA strand breaks leading to genome instability and micronuclei formation in somatic cells both *in vitro* and *in vivo*. However, folic acid supplementation can promote the progression of cancer when precancerous lesions are present in the colon. The present work examines the effects of diets enriched or deficient in folic acid on DNA damage and mutation in somatic cells of male mice. The PigA assay is a new *in vivo* technique for measuring DNA sequence mutation in reticulocytes and erythrocytes based on glycosylphosphatidylinositol (GPI) anchor deficiency. Micronuclei are pieces of damaged chromosomes that lag behind during anaphase, an indicator of chromosome damage. Mutation frequencies using the PigA assay and chromosome damage analyzed by the micronucleus assay were measured in erythrocytes from male mice.

Objectives: To examine the effect of dietary folic acid intake from weaning to maturity on DNA mutation rates and DNA damage in somatic DNA.

Methods: *Animal treatment:* Male BALB/c mice were weaned onto one of three diets on post-natal day 21containing supplemental folic acid (6 mg/kg), deficient folic acid (0 mg/kg) or control folic acid (2 mg/kg) for 15 weeks (n = 10/diet). Blood samples were collected and fixed for mutation analysis. Red blood cell folate levels were measured to determine folate status at sampling. *DNA mutation analysis:* PigA and micronucleus analyses were performed at Litron Laboratories. Micronuclei were measured in both reticulocytes (immature blood cells) and erythrocytes (mature blood cells), differentiated from one another using an antibody against the transferrin receptor, which is only expressed in reticulocytes. Propidium iodide was used to stain DNA and the sample was injected into a flow cytometer to measure the number of cells containing micronuclei. The PigA assay used an antibody against the GPI anchor (CD59) to sort cells containing a PigA mutation using a flow cytometer. Mutation frequency for the PigA assay was expressed as the number of GPI deficient cells per one million cells.

Results: Folic acid intake was not associated with changes in micronucleus frequency in reticulocytes or erythrocytes. Mean frequencies of micronuclei in reticulocytes were 0.42±0.05%, 0.47±0.07% and 0.47±0.06% per million cells for the 0, 2 and 6 mg/kg groups, respectively. Average micronucleus frequencies in erythrocytes were 0.31±0.03%, 0.25±0.03% and 0.25±0.03% per million cells for the deficient, control and supplemented groups, respectively. There was no effect of diet on mutation frequency using the PigA assay. The number of mutants in red blood cells was 6.8±6.45 18±37.4 and 5.1±3.9 per 4 million cells for the deficient, control and supplemented diets, respectively. The mutation frequencies in reticulocytes were 1.2±1.7, 1.7±2.7 and 1.1±1.8 per million cells for the 0, 2 and 6 mg/kg diets, respectively.

Conclusions: Differences in dietary folic acid intake over 15 weeks in the post-weaning period did not affect mutation frequency or DNA damage in red blood cells in male BALB/c mice.

INVESTIGATION OF HERITABLE GERMLINE MUTATION IN MICE EXPOSED TO PARTICULATE AIR POLLUTANTS *IN UTERO*

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Background: This project investigates induced inherited DNA sequence mutations arising in the germline (i.e., sperm or egg) of mice exposed *in utero* to diesel exhaust particles (DEP) via maternal inhalation compared to unexposed controls. Particulate Air Pollutants (PAPs) are widespread. Previous work has shown that PAPs from industrial environments cause DNA damage and mutation in the sperm of adult male mice. Effects on the female and male germline during critical stages of development (*in utero*) are unknown. In mice, expanded simple tandem repeat (ESTR) loci exhibit high rates of mutation and provide valuable tools for studying inherited mutation and genomic instability.

Objectives: The present study examines rates of inherited ESTR mutation in the descendants of mice exposed to DEP *in utero*.

Methods: Pregnant C57Bl mice were exposed by inhalation to 20mg/m^3 NIST 2975 for 1 hour daily from gestational day 7 until birth, alongside sham controls. Offspring were collected and mated with unexposed CBA mice. Parents and offspring were sacrificed at maturity and DNA extracted from somatic tissue. ESTR mutation frequencies were determined by comparing the length of the repeats in the offspring compared to parental alleles.

Results: Control mutation frequencies were approximately 6% for males at both alleles and approximately 3% at the C57Bl allele and 5% at the CBA allele in the females. There was no increase in mutation frequency in females exposed *in utero* to DEP. In contrast, a statistically significant increase in the mutation frequency of male mice exposed to DEP was observed (2-fold; $p < 0.05$). This indicates that maternal DEP exposure causes increased mutation in sperm during development.

Conclusions: The findings suggest that exposure *in utero* to 20mg/m^3 NIST 2975 (DEP) causes induced mutation mediated via the male germline. Additional work will be conducted to confirm this finding and examine the potential mechanism of action of DEP on the germline.

THE EFFECT OF CARBON NANOTUBES ON REPRODUCTION AND DEVELOPMENT IN *DROSOPHILA MELANOGASTER* AND CD-1 MICE

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Background: Carbon nanotubes (CNTs) are increasingly used in numerous applications, including electronics, water purification and food packaging. If functionalized by the attachment of various molecules, they have also been suggested to be useful for gene and drug delivery. After functionalization, cells readily take up CNTs, but the consequence of this uptake has been largely understudied.

Objectives: Since the impact of functionalized CNTs on reproduction and development in particular is almost entirely unaddressed, we have investigated the effect of ingested hydroxyl-functionalized CNTs on female reproduction and developmental processes in *Drosophila* and CD-1 mice.

Methods: Virgin female and male *Drosophila* were housed together and fed varying concentrations of CNTs (0.005% - 0.5%), a microparticle (MP) control or a vehicle control. Pregnant CD-1 mice (gestational day [GD] 9) were exposed to either 10 or 100 mg/kg CNTs, and on GD19 fetuses were removed and examined. Effects of CNTs on fecundity, fertility and development were assessed in *Drosophila* compared to the MP and vehicle control, and the impact of these CNTs on fetal development was measured in CD-1 mice.

Results: The CNTs had no impact on *Drosophila*, however they significantly altered offspring development in CD-1 mice. A single oral dose of CNTs (10 mg/kg) to pregnant dams significantly increased the number of resorptions, and resulted in fetuses with external morphological defects and skeletal abnormalities.

Conclusions: Differences between the responses of the two organisms likely reflect differences in reproductive strategies as well as differences in exposure. This research underscores the need to examine the effects of CNTs on reproductive health and development, particularly in mammals, before the utilization of CNTs continues to increase.

AN *EX VIVO* APPROACH TO THE DIFFERENTIAL PARENCHYMAL RESPONSES INDUCED BY CIGARETTE SMOKE AND ITS VAPOR PHASE

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Background: Terminal airway bronchioles and alveoli are the pathological sites of chronic diseases such as emphysema and lung fibrosis which are associated with cigarette smoke exposure. Owing to the lack of efficient experimental model, most of the studies focus on the later events of the pathogenesis. Consequently, the pulmonary responses to acute cigarette smoke exposure are rarely assessed. Establishing novel experimental model to assess the acute parenchymal responses to cigarette smoke at low concentration can help understanding the early events related to those diseases.

Objective: Using a rat lung slice model, compare the toxicity and stress responses induced by cigarette whole smoke (WS) to the ones induced by the vapor phase (VP) of the cigarette smoke.

Method: Freshly prepared lung slices were exposed to various dilutions of WS or VP for 30 minutes per day over 3 consecutive days. VP was separated from the WS by trapping the particulate phase using a Cambridge filter pad. Cytotoxicity was assessed by MTT assay and morphological examination. Expression of five stress related genes were examined by real time PCR analysis.

Results: The exposure of lung slices to 2, 5 and 10% dilutions of WS in air (v/v) resulted in 90, 42 and 16% relative survival in comparison to air exposure. Consistently, histological observations showed dose-related alveolar damages in the lung slices exposed to the same concentrations of WS. VP induced a similar toxicity profile as WS. Compared to VP, WS exposure lead to a 10-fold induction of the CYP1A1 gene and a 2-fold induction of the CYP1B1 gene, while a 4, 11 and 2-fold higher expression was observed for IL-1ß, B_1R and TNF-α genes, respectively.

Conclusion: These results highlight the preferential role of particulate phase in the substantial induction of IL-1ß, B_1R and CYP1A1 genes when compared to the effect of cigarette smoke VP. Both the particulate and vapor phases have equal contribution to the moderate induction of CYP1B1 and TNF-α genes. Finally, VP played a major role in cigarette smoke-induced toxicity to the lung parenchyma because there is no difference in toxicity between WS and VP. Overall, the observed results indicate that the *ex vivo* approach used to study the acute lung parenchymal responses induced by cigarette smoke can generate a useful model.

GLOBAL DIFFERENTIAL GENE EXPRESSION IN LUNG OF MICE EXPOSED TO CARBON BLACK NANOPARTICLES

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Background: Nanoparticles are hypothesized to mediate toxic outcomes via induction of inflammatory responses, DNA damage and oxidative stress. Carbon black nanoparticles are manufactured worldwide and used in a very broad range of products. As such, workplace exposure is a serious concern. In order to elucidate potential mechanisms of carbon black nanotoxicity, we investigated global differential gene expression in the lungs of exposed mice.

Objectives: This work employed DNA microarrays to identify genes that are perturbed by exposure to respirable carbon black nanoparticles in the lungs of mice. The research aims to increase understanding of dose response and persistence of nanoscale carbon black pulmonary toxicity.

Methods: Mice were exposed to a single dose of 0, 18, 54 or 162 μg Printex90 carbon black (i.e., 14nm diameter spherical particles) via intratracheal instillation and sacrificed on days 1, 3 or 28 for analysis. Differential gene expression was determined by the hybridization of extracted RNA to Agilent mouse microarrays (~41,000 genes). Data were LOWESS normalized and differential gene expression was identified using MAANOVA in R. Transcripts with ≥1.5 fold-changes relative to control mice and FDR adjusted p-values ≤0.05 were considered differentially expressed.

Results: Overall, 373 genes were differentially expressed in at least one of the exposure conditions. The most differentially expressed genes included acute-phase genes Saa1, Saa2 and Saa3 with fold changes reaching 65.3. Significant differential gene expression was shown to persist 28 days following the initial exposure. Primary biological pathways identified were involved in inflammatory and immune responses and included acute-phase response signalling, chemokine signalling, nuclear factor-kappa B signalling, interleukin-10 signalling and triggering receptor expressed on myeloid cells 1 signalling. Dendritic cell maturation signalling was strong at later time points.

Conclusions: Inflammatory responses were identified as the primary mechanism of carbon black nanotoxicity, although the exposure affected a broad range of biological pathways. Future work will be focused on confirming protein response and correlating gene expression data to biological effects of exposure.

THE ROLE OF MICRORNAS IN THE RESPONSE TO CHRONIC ORAL BENZO(a)PYRENE EXPOSURE

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Background: MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression. MiRNAs are initially transcribed as independent units in the nucleus, which then undergo a number of processing steps to produce mature miRNAs. Evidence is emerging to support a role for miRNAs in response to chemical exposures and may thus play a role in the resulting toxicity. MiRNAs are frequently altered in cancers. However, unlike mRNAs, loss of a single miRNA may lead to tumour promotion by enhancing the expression of a number of proteins that otherwise are suppressed, because a single miRNA can target hundreds of genes. Previous work in our laboratory found no changes of miRNA expression in mouse liver following 3 day exposures to benzo(a)pyrene (BaP). The results suggest a lack of miRNA transcriptional response to aryl hydrocarbon receptor agonists and/or DNA damage.

Objectives: In the present study we explore a larger dose range and a longer exposure period (28 days) to determine if chronic BaP exposure leads to hepatic miRNA perturbation.

Methods: Mature male mice (Muta™Mouse) were exposed by oral gavage to 25, 50, 75 mg/kg/day BaP alongside mice exposed to vehicle control (olive oil) for 28 days. Three days following the last exposure, mice were sacrificed and livers flash frozen. Agilent mouse 4 x 44 oligonucleotide microarrays were used to assess global gene expression, in parallel with miRNA hybridization on Agilent 8 x15 miRNA array slides. Pathway-focused gene expression profiling using Real Time- polymerase chain reaction (PCR) was performed for the p53 pathway. The expression of miR-34a was validated using the Qiagen miScript PCR system.

Results: MAANOVA identified 110 differentially expressed genes (adjusted $p < 0.05$) with fold change greater than 1.5. The genes most affected included those involved in xenobiotic metabolism, as well as the downstream targets of p53, indicative of DNA damage response and cell cycle arrest. P53 response was confirmed by pathway-specific Real Time-PCR. A significant increase, of 1.97-fold and 3.59-fold for 50 mg/kg/day and 75 mg/kg/day BaP respectively, in miR-34a expression was identified. This miRNA is known to be involved in p53 response.

Conclusions: Ingestion of BaP leads to widespread changes in gene expression in mouse liver, with enrichment of genes involved in cell cycle arrest, DNA damage response, and apoptosis via the p53 pathway. In contrast, miRNA expression was relatively unaffected. However, miR-34a was significantly up-regulated, and may therefore play a critical role in the post-transcriptional regulation of p53 and/or its downstream targets.

ONTOGENY OF 78 CYTOCHROME P450s DURING POSTNATAL LIVER MATURATION IN MICE

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Background: Once liberated from the uterine environment, the neonate is instantly exposed to a wide array of xenobiotics. The rapid and efficient biotransformation of these compounds by drug-metabolizing enzymes is an essential process if the infant is to avoid xenobiotic-induced cellular injury. Members of the first four families of the cytochrome P450s (Cyp1-4) metabolize numerous drugs and toxicants; however, little is known about how and when most of these Cyps become activated or suppressed during postnatal liver development, resulting in higher risks for adverse drug reactions in pediatric patients.

Objectives: Therefore, the purpose of the present study was to systematically determine the ontogeny of 78 Cyps in the Cyp1-4 families in mouse liver. In addition, we also determined the correlation between chromosomal location and coordinate gene expression of the 78 Cyps measured.

Methods: Livers were collected from C57BL/6 mice at gestational day (GD) 17.5 and various postnatal ages. The mRNAs of 78 Cyp isoforms (Cyp1-4) were quantified by a multiplex suspension array as well as RT-qPCR.

Results: The findings from this study revealed 4 ontogenic patterns of Cyps in liver: 36% of Cyp isoforms were expressed highest at day 30-45 (pattern 1), 27% were expressed highest at day 20 (pattern 2), 25% were highest from ages 10-15 days (pattern 3), and 12% were highest at neonatal ages GD 17.5 – postnatal day 5 (pattern 4). The majority of Cyps formed 8 genomic clusters, namely, Cyp1a on Chr 9 (cluster 1), Cyp2a-2b-2f-g-2t genes on chr 7 (cluster 2), Cyp2c genes on chr19 (cluster 3), Cyp2d genes on chr 15 (cluster 4), Cyp2j genes on chr4 (cluster 5), Cyp3a genes on chr 5 (cluster 6), Cyp4a-4x genes on chr 4 (cluster 7), and Cyp4f genes on chr 17 (cluster 8). Certain Cyps within a genomic cluster shared similar expression patterns, suggesting that Cyp mRNA expression is regulated by common pathways within gene clusters throughout liver development.

Conclusions: The successful completion of the Cyp ontogeny “blueprint” will lead to a better understanding of the development and regulation of the pediatric drug metabolism system, and will aid in improving therapeutic strategies as well as predicting toxicological responses in children. (Supported by NIH grants ES-07078, ES-09649, ES-09716, ES-013714, and ES-021940). Helen Renaud is the recipient of a CIHR Fellowship Award.

PRENATAL AND EARLY POSTNATAL EXPOSURE TO POLYCHLORINATED BIPHENYLS (PCBs) AND BEHAVIOUR IN INUIT PRESCHOOLERS

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Background: Polychlorinated biphenyls (PCBs) are developmental neurotoxicants routinely found in most samples of cord blood, breast milk and children blood worldwide. Prenatal exposure has been associated with several health effects including altered cognitive, psychomotor and behavioural functions in children. In a previous study on 11-month old Inuits, we showed that prenatal and postnatal exposures to PCB-153 were associated with decreased attention and reduced ability to control activity, respectively.

Objectives: In this study, we aimed to evaluate the persistence of PCB-induced behavioural effects in 5-year old children.

Methods: We assessed attention and activity in 112 children enrolled in a birth cohort by coding video recordings taken during the administration of psychomotor tasks. Prenatal exposure to PCB-153 was documented by measured cord blood levels. Postnatal exposure was estimated using a physiologically based pharmacokinetic (PBPK) model. Monthly areas under the blood concentration versus time curve (AUCs), proxies for internal exposure during each month, were abstracted from simulated profiles of blood PCB-153 for the first year of life. Children blood PCB-153 levels were also measured at the time of testing. Associations between PCB-153 exposures and behaviour were assessed through correlation analyses.

Results: Correlation analyses showed a pattern of prenatal PCB-induced decrements in children attention similar to those observed in 11-month old infants, although the associations were weaker and seemed to require additional exposure through breast-feeding as the strongest correlation (Pearson $r = 0.168$, $p=0.10$) was obtained with exposure during the second postnatal month. Similarly, concurrent blood PCB-153 were marginally associated with attention (Pearson $r = 0.160$, $p=0.12$). Activity was not associated to prenatal, postnatal or concurrent levels of PCB-153.

Conclusions: These findings suggest that the association between early PCB exposures and altered children attention, but not activity, is persistent throughout childhood. Whether PCB-induced decrements in children attention are predictors of later school achievement and behavioural deficits has yet to be elucidated.

AMERICAN EEL (*ANGUILLA ROSTRATA*) IS MORE SENSITIVE TO TCDD THAN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Background: Recruitment of juvenile American eel to L. Ontario has declined by an estimated 98% in the past three decades, leading to an almost four-fold reduction in commercial landings. For ecological and economic reasons, the American eel was designated a species of special concern in 2006. A potential cause of this decline is the maternal transfer of dioxin-like compounds (DLCs) accumulated over the female's life to her eggs during oogenesis, precluding proper embryonic and larval development. DLCs have previously been linked to the reproductive failure of lake trout (*Salvelinus namaycush*) in L. Ontario; due to similarities in trophic level and lifespan between the two species, a link may also exist between DLC accumulation by female American eels, transfer to oocytes, and the decline in juvenile recruitment. However, due to difficulties in obtaining and maintaining American eel embryos, the toxicity of DLCs is hard to assess directly. An indirect assessment of their toxicity in early life stages may be obtained by measuring potency for inducing cytochrome P4501A (CYP1A) at the juvenile stage.

Objectives: One, to compare CYP1A induction by TCDD between juvenile American eel, lake trout, and rainbow trout (a common reference species) to determine relative sensitivity to DLCs. Two, to compare basal CYP1A expression in adult L. Ontario eels to those of adults from other sites in Eastern Canada to determine the extent to which environmental exposure to DLCs varies with location.

Methods: Juvenile American eel, lake trout, and rainbow trout were exposed to IP-injected TCDD for 72 hours, at which point livers were excised. S9 fractions were prepared and CYP1A enzymatic activity assessed using the EROD assay. The excision, preparation, and enzymatic assessment was identical for adult eel samples.

Results: Based on ED50 values, juvenile eels were approximately ten times more sensitive to CYP1A induction than rainbow trout; lake trout data are currently being analyzed. Adults collected at two L. Ontario sites had significantly higher basal CYP1A activity (12.6 and 17.6 pmol/mg/min) than adults collected from two sites in Quebec (6.0 and 4.7 pmol/mg/min) and one in Nova Scotia (7.0 pmol/mg/min).

Conclusions: These data suggest that the early life stages of the American eel are highly sensitive to dioxin-like compounds, and that this stressor is of particular concern for females originating in L. Ontario. Collectively, the findings support the hypothesis that DLC-mediated reproductive failure may contribute to the recruitment decline of juvenile American eels to L. Ontario.

CHARACTERIZING THE ROLE OF OXIDATIVE STRESS IN VALPROIC ACID-INDUCED TERATOGENESIS

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Background: Exposure to the anticonvulsant drug valproic acid (VPA) *in utero* is associated with a 6-7% increase in the incidence of birth defects including neural tube defects (NTDs), skeletal malformations, and heart abnormalities. However, the molecular mechanisms by which VPA induces teratogenesis is unknown. Previous studies in our laboratory showed that VPA co-cultured with the antioxidative enzyme catalase significantly protected against NTDs in a murine whole embryo culture system, suggesting that reactive oxygen species (ROS) play a role in mediating VPA-induced teratogenesis. In addition to directly damaging cellular macromolecules, ROS can alter cellular signaling leading to alterations in cellular proliferation, differentiation, and cell death.

Objectives: The purpose of the current study is to characterize the role of oxidative stress in VPA-induced teratogenesis by measuring ROS formation in the developing embryo, measuring and localizing markers of oxidative damage, and assessing the embryo for apoptosis.

Methods: Whole embryos were dissected on gestational day 9.5 and cultured with vehicle, PEG-catalase (400 U/mL), VPA (0.60 mM), or PEG-catalase and VPA for 24 hours at 37°C after which membranes were removed and embryos collected for analysis. To measure ROS formation, whole mount fluorescent staining was performed with the ROS sensitive dye, CM-H_2DCFDA. Embryos were incubated with the dye for 1 hour, then washed with phosphate-buffered saline and examined by confocal microscopy. Western blotting and immunohistochemistry were used to assess 3-nitrotyrosine (3-NT) and 4-hydroxynonenal (4-HNE) adduct formation, as markers of protein nitration and lipid peroxidation respectively. Immunohistochemistry was used to measure 8-hydroxyguanosine (8-OHdG), a marker of DNA oxidation. To examine levels of apoptosis, Western blotting and immunohistochemistry were performed for cleaved caspase-3 and cleaved PARP.

Results: An increase in ROS formation was observed in the heads and somites of embryos exposed to VPA, which was attenuated by catalase co-culture. Western blotting and immunohistochemistry for 3-NT and 4-HNE did not show any differences among treatment groups, and 8-OHdG staining was not different among treatments. Expression of cleaved caspase-3 and cleaved PARP was significantly increased in VPA treated embryos and localized to the neuroepithelium and somites. Catalase supplementation attenuated these effects.

Conclusions: VPA induces an increase in ROS formation and apoptosis in the head and somites of embryos. As markers of oxidative damage were not increased, we now postulate that VPA is mediating teratogenic effects through alterations in redox-sensitive signaling pathways. (Support: CIHR).

THE USE OF VOLUNTARY EXERCISE TO MITIGATE COGNITIVE AND BEHAVIOURAL DEFICITS OF ETHANOL NEUROBEHAVIOURAL TERATOGENICITY IN THE GUINEA PIG

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Background: Ethanol consumption during pregnancy can produce a spectrum of teratogenic effects in offspring, which together are termed fetal alcohol spectrum disorders (FASD). The most debilitating consequence of prenatal ethanol exposure is neurobehavioural teratogenicity, which manifests as persistent impairment of learning, memory, attention, and motor function. Voluntary exercise can improve brain function by decreasing the risks of cognitive impairment, enhancing recovery from brain injury, increasing whole brain volume and enhancing synaptic plasticity. In rats that received chronic prenatal ethanol exposure (CPEE), voluntary exercise improved performance in spatial learning and memory tasks, and increased cell proliferation and neurogenesis in the hippocampus.

Objectives: Exercise appears to be therapeutic in CPEE offspring; however, the ability of voluntary exercise, via a dry-land maze, to attenuate neurobehavioural teratogenicity in both male and female offspring has not been studied. This study is designed to test the hypothesis that a structured voluntary exercise regimen will mitigate structural and functional deficits of ethanol neurobehavioural teratogenicity in both male and female postnatal offspring.

Methods: Pregnant Dunkin-Hartley-strain guinea pigs were randomly assigned to one of three experimental groups: ethanol (4 g ethanol/kg maternal body weight/day, 5 days/week throughout gestation); isocaloric-sucrose/pair-feeding; or no treatment. Following weaning on postnatal day (PD) 21, offspring were randomly assigned to one of two groups: a voluntary exercise (VE) regimen or no intervention. VE animals were placed in a dry-land maze in same-sex pairs for 30 minutes daily for 21 days starting on PD 24 to provide exercise training and socialization. Following the VE regimen (or no intervention), offspring were tested for spontaneous locomotor activity on PD 45 and rewarded alternation Y-maze task performance at PD 46-50. Structural MRI analysis will be conducted to determine the effects of voluntary exercise on whole brain volume, and immunocytochemical analysis will be conducted to measure neurogenesis in selected brain regions, including the hippocampus.

Results: CPEE offspring were growth restricted at birth and were hyperactive in spontaneous locomotor activity tests compared with the isocaloric-sucrose/pair fed nutritional control offspring. Preliminary data analysis indicated that offspring in each of the maternal ethanol and isocaloric-sucrose/pair-fed treatment groups that were exposed to a structured VE regimen manifested less spontaneous locomotor activity compared with animals that did not received the VE intervention.

Conclusions: Ongoing data analyses will determine whether cognitive and behavioural deficits of ethanol teratogenicity in the guinea pig are mitigated by voluntary exercise.
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MODULATION OF TRICHLOROETHYLENE METABOLISM BY DIFFERENT DRUGS IN RATS AND HUMANS

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Backgound: Trichloroethylene (TCE) is a volatile solvent to which humans are frequently exposed. We recently studied the impact of 15 drugs on the metabolism of TCE in suspended rat hepatocytes (acetaminophen, ibuprofen, lsalicylic acid, mefenamic acid, naproxen, diclofenac, sulphasalazine, cimetidine, ranitidine, valproic acid, carbamazepine, amoxicillin, erythromycin and gliclazide.) and identified that five altered the formation of two studied metabolites (trichloroethanol [TCOH] and trichloroacetic acid [TCA]).

Objectives: The objective of this study were i) to determine if the same interactions are observable in human hepatocytes, and ii) to characterize the most pronounced interactions using microsomal assays (oxidation and/or glucuronidation).

Methods: First, like we did in our previous study, the interacting drugs have been identified *in vitro* by measuring the metabolite formation rates in suspensions of human hepatocytes in the presence and absence of each of the drugs (10x therapeutic maximal blood levels) in closed vials. The concentrations of TCE and its metabolites, i.e. trichloroethanol (TCOH) and trichloroacetic acid (TCA), were measured by headspace gas chromatography coupled to mass spectrometry (GC-MS).

Results: Similar to observations in rats, salicylic acid and naproxen increased significantly TCA and TCOH levels. On the other hand, the decreased levels in both metabolites were not induced by the same drugs (rat: valproic acid, acetaminophen and gliclazide; and human carbamazepine and erythromycine). Drugs decreasing only TCOH levels were also different in both species: sulfasalazine and valproic acid in humans, and erythromycine in rat. Lowered levels of TCA only were observed with gliclazide in humans, whereas this was observed with cimetidine, diclofenac and amoxicillin in rats. Although metabolites levels in hepatocytes were shown to increase of in the presence of salicylic acid and to decrease with acetaminophen, no effect was detected using microsomes. We hypothesize that decreases in metabolite levels are explained by inhibition of cytochrome P450 activity while increases result from inhibition of glucuronidation. In rat liver microsomes, TCOH formation was inhibited by gliclazide with a K_i of 500 µM (partial noncompetitive inhibition). Valproic acid inhibited the hydroxylation of TCE to TCOH and TCA with K_i values of 452 µM and 785 µM, respectively (partial noncompetitive inhibition). Naproxen inhibited TCOH glucuronidation through partial competition (K_i = 295 µM).

Conclusions**:** Combined *in vivo* rat exposures of TCE with the identified stronger interacting drug are underway and *in vitro–in vivo* extrapolations of interactions will be validated with PBPK modeling to ultimately enable predictions of interactions in humans.

RAPID AND EFFICIENT METHODS OF EXTRACTION AND DETECTION OF MICROCYSTINS LR, RR AND YR FROM FISH TISSUES

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Background: Cyanobacteria are aquatic microorganisms which can be toxic to aquatic life and also to humans via food. In the recent years, the occurrence of cyanobacterial blooms has increased especially because of the eutrophication of lakes and rivers. Among the toxins they produce, there are the microcystins LR, RR and YR. These are hepatotoxic cyanotoxins very stable and persistent in the environment. Microcystins can thus accumulate in flesh and organs of fishes and potentially transfer along the food chain. These molecules are particularly harmful because they inhibit the activity of phosphatases which leads to destruction of hepatocytes.

Objectives: The objectives of this study were to develop a simple and efficient method for microcystin extraction from fish tissues and a rapid detection method for microcystins LR, RR and YR. In this study, we particularly focused on fish liver, muscles and blood. The liver is the target organ for microcystins, the muscles are usually the only part consumed by humans which makes them the principal vector of human exposure and blood allows the distribution of microcystins through the fish. We also verified whether it is adequate to lyophilize the tissue before extraction or not.

Methods: In this study, samples of liver, muscles and blood were incubated with agitation at 4°C during 4 to 12 hours in the presence of spiked standards of microcystins. For samples of liver and muscles, one half of the samples was lyophilized and the other half was frozen pending extraction. We chose a solid-liquid extraction with methanol 100% and 80%. The detection method developed is a high performance liquid chromatography (HPLC) coupled to tandem mass spectrometry (MS/MS) using multiple reaction monitoring (MRM).

Results: The extraction method developed allows a high recovery rate (minimum 80%) for microcystins YR, LR and RR extracted from non-freeze-dried liver and muscles and from the blood. In the case of extractions from freeze-dried tissues, the recovery is lower and varies between 43 and 67% for liver and between 26 and 39% for muscles. This method also allows a high and stable recovery rate for a large range of microcystin concentrations (25 to 240 $ug.L^{-1}$) for each microcystine and each tissue.

Conclusions: The extraction and detection methods we have developed are simple, fast and efficient compared to other previously published methods which require either the preparation of a specific internal standard and protein precipitation, or extraction in acidic water at high temperature in a filtration system based on sand. After validation in vivo, this method of analysis will be used to study the accumulation of microcystins in tissues of trout and perch. This will ultimately improve estimates of human exposure to microcystins through fish consumption.

EVALUATING BIOMARKERS OF EXPOSURE TO WOOD COMBUSTION PARTICULATE MATTER RESULTING FROM TRADITIONAL TEMAZCAL USE

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Background: Traditional temazcals (i.e., sweat lodges) are commonly used by native populations in Central America. Ninety percent of families surveyed in an earlier cohort study continue to use temazcals at least once or twice per week. Temazcals are heated via biomass combustion, and exposure to the resultant particulate emissions has been associated, in other studies, with a host of adverse health effects in humans (e.g., cancer, pulmonary injury, and asthma). Urinary (*Salmonella*) mutagenicity has previously been shown to be a useful biomarker of human exposure to combustion emissions, including wood smoke.

Objectives: The objective of this study is to evaluate urinary mutagenicity as a biomarker of exposure to biomass combustion emissions in Guatemalan families that regularly use traditional temazcals.

Methods: Study subjects included indigenous Guatemalan Mayan families (with children 5-10 years of age) who regularly use traditional temazcals, as well as control individuals that do not use temazcals. Urine samples were collected before and after temazcal exposure, and frozen at -80ºC until use. Thawed samples were enzymatically hydrolyzed overnight, and de-conjugated urinary metabolites were concentrated by solid-phase extraction on C_{18} silica. Mutagenic activity of concentrated extracts was assessed using the standard plate-incorporation version of the Ames/*Salmonella* mutation assay on strain YG1041 with exogenous S9 activation. All aspects of the study were approved by the Committee for Protection of Human Subjects (University of California) and the Health Canada Research Ethics Board.

Results: Urinary mutagenicity was modified by temazcal use. Six out of 9 individuals showed a significant increase in urinary mutagenicity following exposure (2 remained unchanged, and 1 showed a decrease). In most cases, mothers (who spend the longest time in temazcal) showed the greatest fold-change increase in the mutagenic potency of their urine after exposure, while children showed a lower (or no) increase.

Conclusions: In this pilot project we were able to successfully employ urinary mutagenicity as a biomarker of biomass combustion exposure associated with traditional temazcals. The use of temazcals appears to result in the production and urinary excretion of mutagenic metabolites. Moreover, the effect appears to be sustained over several days, and trends in urinary mutagenicity appear to be similar within families. Mutagenicity assessment of additional urine samples is currently underway to be compared with other biomarkers of oxidative stress in order to further characterize the health hazards associated with temazcal use.

PBPK MODELING OF THE MULTIROUTE EXPOSURES OF RATS TO TOLUENE, n-HEXANE, CYCLOHEXANE AND ISOOCTANE

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Background**:** Mixture risk assessments focusing on both the aggregate and cumulative exposures can be improved with an understanding of the change in internal dose. The modeling of the internal dose and pharmacokinetics of hydrocarbons following cumulative and aggregate exposures has not yet been undertaken.

Objective: The objective of this study was to develop a PBPK model to simulate the mixed exposure of rats to four hydrocarbons (toluene (T), n-hexane (H), cyclohexane (C) and isooctane (I); chosen to represent chemical classes in large volume petroleum products (e.g., gasoline, kerosene, gas oils)) by inhalation and oral routes.

Methods: The PBPK models of individual chemicals were initially developed, and then were interconnected using the metabolic interaction terms. The resulting model was first used to simulate the kinetics of each chemical following a single route of exposure, and then to simulate the data collected following multiroute exposure. The capacity of the PBPK model to adequately simulate the kinetics following aggregate and cumulative exposures was evaluated by comparing with the experimental data. These data were collected in groups of male Sprague-Dawley rats (n=5) exposed either to a single dose of each substance by inhalation (50 ppm of T and H; 300 ppm of C and I; 2-hr) or oral gavage (8.3, 5.5, 27.9, and 41.27 mg/kg, respectively, for T, H, C and I) or to multi-products given by both routes together at the same dose levels.

Results: The PBPK model could simulate the kinetic profiles in rats of all four marker substances from both, single route and multiroute exposures. The data-derived area under the blood concentration vs. time curve (AUC; mg/L x min) were 106.62, 12.97, 117.56 and 122.27 whereas the AUC values derived from the multiroute, multichemical PBPK model were: 114.04, 12.68, 58.99, and 123.42 mg/L x min, respectively, for T, H, C and I.

Conclusions: Overall, the PBPK model developed in this study is a useful tool for simulating kinetics of hydrocarbons, by accounting for not only the saturable metabolism but also for the interactive effects during aggregate and cumulative exposures.

CARACTÉRISATION DE LA DISTRIBUTION DE LA COMPOSITION TISSULAIRE DU RAT PAR ANALYSE BAYÉSIENNE

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Introduction: Les coefficients de partage tissu:air (Pta) sont des paramètres physicochimiques nécessaires au développement de modèles pharmacocinétiques à base physiologique (PBPK). Les algorithmes prédisant ces coefficients de partage à partir de la composition du tissu utilisent des estimés ponctuels comme paramètres entrant. La caractérisation des distributions des paramètres entrant dans l'algorithme, en particulier l'information sur la composition du tissu, n'a pas été étudiée à ce jour.

Objectif: Cette étude a pour objectif d'utiliser des données publiées de Pta de composés organiques volatils (COV) *in vitro* chez le rat dans des simulations Monte Carlo à chaîne de Markov (MCMC) pour analyser la variabilité des paramètres relatifs à la composition du muscle, du foie et du tissu adipeux (c. à d. pour la cellule : la fraction cellulaire du volume de tissu, C, la composition de la cellule en lipides neutres, LN, phospholipides neutres, PL et en eau, EC; et la composition en eau du liquide interstitiel, EI).

Methodes: La variabilité et l'incertitude associées aux paramètres de la composition du tissu ont été décrites par un modèle hiérarchique. Les valeurs *in vitro* de coefficient de partage huile:air et eau:air utilisées pour toutes les simulations ont été obtenues dans la littérature. L'analyse a été effectuée en deux étapes : i) En utilisant des données publiées de Pta pour 46 hydrocarbures halogénés, alcanes et composés aromatiques avec de l'information *a priori* sur la composition des tissus (distribution normale, moyenne obtenue de la littérature; 20 à 50 % de variabilité et d'incertitude); ii) En utilisant les distributions *a posteriori* obtenues après la première analyse conjointement à des données publiées de Pta pour 18 alcools, cétones, acétates et l'éther de diéthyle.

Resultats: Pour tous les tissus, les distributions obtenues après chaque analyse sont physiologiquement plausibles, avec des valeurs moyennes comparables aux distributions *a priori* et la variabilité décrue (entre 1 et 19 %) à l'exception des LN et PL du muscle et du foie dont la variabilité a augmenté (entre 30 et 44 %). Les deux analyses ont donné des distributions *a posteriori* similaires en ce qui concerne le muscle (C = 89 ± 0.5 %; EC = 68 ± 12 %; LN = 1.2 ± 0.5 %; PL = 1.1 ± 0.3 %; EI = 88 ± 3 %) et le tissu adipeux (C = 84 ± 14 %; EC = 3.4 ± 0.8 %; LN = 95 ± 0.5 %; PL = 0.2 ± 0.04 %; EI = 85 ± 2 %). En outre, pour le foie, les deux simulations ont mené à des distributions différentes pour LN et PL (1ère analyse : LN = 3.9 ± 1.7 %; PL = 3 ± 0.6 %; 2ème analyse : LN = 5.6 ± 1.7 %; PL = 4 ± 0.5 %); les autres distributions obtenues sont comparables entre les deux simulations (C = 82 ± 8 %; EC = 54 ± 17 %; EI = 83 ± 5 %).

Conclusion: Les résultats ont révélé que les données expérimentales contenaient assez d'information pour caractériser la variabilité associée avec la composition tissulaire du rat. Les distributions *a posteriori* résultant de l'analyse MCMC ci-dessus pourraient être utilisées avec les caractéristiques physicochimiques d'autres COV pour modéliser les distributions de leur Pta afin de développer des modèles PBPK probabilistes et des modèles à dosimétrie cellulaire
(financé par le CRSNG et l'AFSSET).

ARE SENSITIVE SUBPOPULATIONS BETTER PROTECTED BY DEFINING THE UNCERTAINTY FACTOR BASED ON THE FINITE SAMPLE SIZE MODEL OR THE SENSITIVE SUBPOPULATION MODEL?

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Background: The interindividual variability in risk assessment is addressed with the use of an uncertainty factor of 10. Recently, this factor has been subdivided into toxicokinetic and toxicodynamic components, each of a value of 3.16. When chemical-specific data are available for the human population, the kinetic component of the uncertainty factor can be estimated. This factor, referred to as human kinetic adjustment factor (HKAF), then aims to replace the default value of 3.16 to account for population variability in toxicokinetics, and is based upon two theoretical models, i.e. the finite sample size model and the sensitive subpopulation model.

Objective: To compare the two theoretical models for HKAF with regard to their ability to account adequately for the most sensitive subgroups in a population.

Methods: Monte-Carlo simulations were applied to a steady-state algorithm to generate distributions of blood concentration (C_{blood}) and rate of metabolism (RAM) for oral and inhalation exposure to chloroform (TCM) and styrene (STR) in a reconstructed population composed of different proportions of adults, elderly, children, neonates and pregnant women (PW). Subgroup-specific input parameters were obtained from the literature or P_3M software. Based on the finite sample size model, HKAF was computed as the ratio [99^{th} percentile/median] of dose metrics for the entire population. Based on the sensitive subpopulation model, it was calculated as the ratio of the 95^{th} percentile in each subpopulation on the median in adults.

Results: For oral exposure, C_{blood}-based HKAF based on the finite sample size model were 1.46 (TCM) and 1.66 (STR), whereas corresponding values based on RAM were 1.43 and 1.53. Comparable values were obtained for the inhalation route. Furthermore, the results suggest that C_{blood}-based HKAF would cover at least 95% of the individuals in all subgroups except for inhalation exposure of neonates to TCM (79%) and STR (74%), as well as PW (93% for both chemicals). Besides, RAM-based HKAF would protect 83% of PW for inhalation of both chemicals. Based on the sensitive subpopulation model, the highest HKAF for oral exposure was obtained in adults or PW (range: 1.38 – 1.40) while for inhalation exposure, highest HKAFs were obtained in neonates based on C_{blood} for TCM (1.64) and STR (2.34) and in PW based on RAM for TCM (1.53) and STR (1.79).

Conclusion: This study has for the first time compared the two theoretical models underlying the HKAF and showed that the sensitive subpopulation model provides better protection than the finite sample size model for the populations simulated in this study.

QUANTIFICATION OF ALTERNATIVELY SPLICED *Mbp* GENE TRANSCRIPTS IN RAT BRAIN: APPLICATION FOR NEUROTOXIC HAZARD IDENTIFICATION

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Background: Myelin sheaths are necessary for the proper insulation of axons and conduction of nerve impulses. Myelin basic protein (MBP) is one of the major protein constituents of myelin sheaths. Perturbation in *Mbp* gene expression was observed following exposure to various xenobiotics, which was often correlated with myelination defect and neurological abnormalities. In mammals, different *Mbp* isoforms are produced by alternative splicing of exons 2, 5 and/or 6.

Objectives: The objectives of this study were to develop a PCR-based strategy to quantify the expression of various *Mbp* isoforms and to assess the effects of methyl mercury (MeHg) exposure on *Mbp* gene expression in the developing rat brain.

Methods: Real-time quantitative PCR (qPCR) was performed in a Bio-Rad iQ5 cycler using SYBR Green dye. Primers spanning exon-exon boundaries were designed to identify specific *Mbp* isoforms. Developmental regulation of *Mbp* isoforms was assessed using RNA isolated from embryonic, juvenile and mature rat brains. The effect of MeHg, administered daily from the first day of gestation at 2 mg/Kg b.w, was assessed in rat cerebellum at postnatal days (PND) 14 and 21.

Results: The qPCR analyses performed using primers located within the constitutive *Mbp* exons 3 and 4 indicated an overall down-regulation of *Mbp* transcripts in PND 14 and 21 cerebellums following perinatal exposure to MeHg. Reverse transcriptase-PCR (RT-PCR) performed using primers spanning exon 5 boundaries revealed that this exon was prominently retained in the adult brain, but mostly spliced out in developing brain. This observation provided evidence regarding the existence of a novel *Mbp* isoform in rat lacking exon 5, which was confirmed by DNA sequencing.

Conclusions: A PCR-based strategy was developed to assess isoform-specific perturbations of *Mbp* gene expression following exposure to neurotoxicants. A novel *Mbp* isoforms that expresses predominantly at PND14 was identified. The impact of MeHg exposure on various *Mbp* isoforms during active phase of myelination is now being investigated.

ALTERATION OF VISUAL BRAIN RESPONSES IN 5-YEAR OLD CHILDREN FROM NUNAVIK EXPOSED TO POLYCHLORINATED BIPHENYLS (PCBs): EVIDENCE FOR AN EARLY POSTNATAL PERIOD OF VULNERABILITY.

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Background: Because of its traditional diet, the Inuit population of Northern Quebec (Nunavik) is highly exposed to several environmental contaminants including PCBs. In a study on visual evoked potentials (VEP) in 5-years old Inuits, we have reported that current blood concentration of PCBs was associated with alterations of visual processing. No association was found for prenatal exposure as estimated by cord blood measures. This finding was somewhat unexpected considering the abundant literature on child brain function impairments in association with *in utero* and perinatal exposure.

Objective: Using a validated physiologically-based pharmacokinetic (PBPK) model, we aimed at estimating the impact of PCB exposure during the first year of life, which is a crucial period for the development of the visual brain organization, on the child VEP responses to better understand the nature of our results observed at preschool age.

Methods: PBPK modeling of infant exposure was performed by using PCB-153 concentration in cord blood, physiological data from the mother and her child (height and weight) and breastfeeding duration in a sample of 78 children (mean age = 5.4 years). The model allowed generating estimates of exposure for each month from birth to 12 months of age. Monthly exposure data were then examined in relation to VEP amplitude (peak-to-peak amplitude of the N75 and P100 components), which was specifically associated with current PCB-153.

Results: Simple correlation analyses suggested that exposures from the 2nd to the 7th months were linked to decreased VEP child amplitude (*p* values ranging from 0.05 to 0.10). Further analyses revealed that breastfeeding duration was an important factor to account for these correlations. Using multiple regressions to take into account certain control variables (child sex, maternal marijuana exposure and breastfeeding duration), only exposures during the 2nd and 3th months were found to be significantly ($p < 0.05$) associated with child VEP amplitude. The strength of these associations (Standardized β= -0.248 and -0.263, respectively) were quite similar to the one observed with current levels (Standardized β= -0.276). Interestingly, PBPK modeling revealed that the most rapid increase in blood PCB levels in this population occured during the first months of life.

Conclusion: Although the VEP amplitude alteration observed at 5 years of age is likely dependent on bioaccumulation and chronic exposure to PCBs, the results of this study suggest that this visual processing impairment is largely explained by a critical time window of exposure occurring in the first trimester after birth.

COMPARATIVE ASSESSMENT OF NANO- AND MICRO-SIZED MINERAL PARTICLE-INDUCED CYTOTOXICITY

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Background: The use of manufactured nanoparticles in consumer products is on the rise. Currently, the majority of these materials represent metal- or mineral-based nanoparticles used in diverse applications such as cosmetics, food packaging or paints and coatings. Nanomaterials possess unique structural and chemical properties, on the basis of their nano-scale. Emerging studies show that the same properties may result in harmful alterations in cellular functions and thus pose a potential risk to human health and the environment. Therefore, comprehensive assessments of cellular responses to manufactured nanomaterials in relation to their physico-chemical and functional properties are needed to gain an understanding of the interaction of nanomaterials with biological systems.

Objective: The focus of this work was to assess the *in vitro* toxic potency of TiO_2 and SiO_2 nano- and micro-sized particles in a human lung epithelial and murine macrophage cell lines by utilizing a panel of cytotoxicity assays.

Methods: We have employed an automated *in vitro* bioassay that integrates the assessment of mitochondrial function (Alamar Blue reduction), membrane integrity (release of cytoplasmic LDH) and energy metabolism (steady state ATP levels) from the same cell culture well in a 96-well format. A549 human epithelial cells and J774A.1 murine macrophages were exposed to four nanomaterials (nano-anatase TiO2, nano-rutile TiO_2, mixture of nano-rutile and anatase TiO_2, nano SiO_2) and 2 NIST standard reference materials (SRM-1879a respirable crystalline silica, SRM-154b TiO_2) for 24 hours, at 0, 3, 30, 100 $\mu g/cm^2$.

Results: No interference of the particulate materials with the bioassay chemistry was observed. In general, the nano-sized TiO_2 particles had a higher toxic potency than micro-sized TiO_2 as well as micro- and nano-sized SiO_2 particles, as observed from the lower cellular ATP and increased release of LDH. Overall, the A549 epithelial cells and the J774A.1 macrophages responded to particles similarly.

Conclusion: Materials with distinct physico-chemical properties showed differences in their *in vitro* cytotoxic potency. The integrated bioassay represents a useful first-tier screening approach for the assessment of the cytotoxic potency of particulate materials and chemicals. Due to the diversity of the biochemical mechanisms underlying specific assays, the use of a panel of multiple assays is recommended.

EFFECTS OF SEASON AND SIZE ON THE CYTOTOXICITY OF AMBIENT URBAN PARTICULATE MATTER COLLECTED AT THREE DIFFERENT LOCATIONS ACROSS CANADA

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Background: Exposure to particulate air pollution has been linked to a number of health effects. It is conceivable that a number of factors such as local and distant sources of emissions, season and proximity to transportation source would impact the chemical composition of particles and therefore their biological potency. Understanding the relative differences in the toxic potency of particles derived from different locations and seasons and identification of underlying physicochemical determinants of toxicity will greatly benefit regulatory efforts.

Objectives: The objectives of this study were to assess whether ambient particulate matter collected from three different locations across Canada show differences in cytotoxic potency and to identify underlying determinants of toxicity including particle size and chemical composition.

Methods: PM10 and PM2.5 samples were collected from Downsview (Ontario), Saint John, (New Brunswick), and Pitt Meadows (British Columbia) during summer and winter seasons. Samples were analyzed for cytotoxicity in human lung epithelial (A549) and murine macrophage (J774) cell lines using an *in vitro* cytotoxicity assay (Alamar Blue). Cytotoxic potency (β) was calculated using dose-response data as Fold-effect = $(\text{Dose} + 1)^{\beta}$.

Results: The cytotoxic potency of the particles was determined by the geographical location of sample, season, and particle size. Three way ANOVA analyses of cytotoxic potency in A549 cell line with *location*, *season* and *particle size* as factors showed significant two way interaction between the *location* and *season* factors (*location* x *season*, p=0.026). Significant seasonal differences were noted in particles derived from Downsview and Saint John (Tukey's multiple comparison, *summer* < *winter* within Downsview, p=0.005; *summer* > *winter* within Saint John, p<0.001), but not in Pitt Meadows, a rural location. In addition, PM2.5 particles were generally more potent than PM10 across all locations (*size* main effect, p=0.043). The ranking of average potencies in J774 were generally consistent with rankings in A549.

Conclusions: Our results show that season can have a significant impact on potencies of particles derived from the same location. Size also impacted toxic potency. These findings suggest that identification of the underlying contributors of toxicity is critical for a more informed regulation of ambient PM. We are currently analyzing data on particle chemistry and wind patterns during sampling in order to identify determinants and point sources (local or distant) of toxicity.

BISPHENOL A POTENTIATES THE DIFFERENTIATION OF THE 3T3-L1MURINE PREADIPOCYTES AT NANOMOLAR CONCENTRATIONS

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Background: 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. Although sedentary life style and excess caloric intake cannot be denied as major contributing factors to the obesity epidemic, accumulating evidence indicates a significant contribution of adverse events, including exposures to bioactive environmental contaminants. Endocrine disrupters such as bisphenol A (BPA) are contaminants likely to play a role in the rise of obesity in the general population and in young children in particular. Several animal studies have shown that prenatal exposures to certain substances, BPA included, will increase the number of differentiated white adipose tissue cells (WATC) thereby predisposing exposed pups to increased weight gain.

Objectives: To assess the ability of low concentrations of BPA to affect the differentiation potential of preadipocytes to mature adipocytes and to investigate the molecular mechanisms that mediate the effect.

Methods: We used the murine 3T3 L1 preadipocyte model for the studies. The cells are held at confluency for two days when the cells are treated with 3-isobutyl-1-methyl xanthine (MIX) and dexamethasone (day 0). The media is then replenished every two days with fresh media containing insulin alone until day 8 when full differentiation is evident. At day 8 the cells are being 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. RNA was also extracted and upregulation of transcriptional regulators was investigated using quantitative real time PCR

Results: 3T3 L1 murine preadipocytes can be induced to differentiate into lipid-accumulating mature adipocytes, mimicking the hormonal signals required for adipocyte formation *in vivo*. We have found that nanomolar concentrations of BPA can partially replace and even increase the stimulatory action of glucocorticoids in inducing 3T3-L1 differentiation as indicated by the increased expression of the differentiation markers aP2 and adipsin. RT PCR analysis showed that BPA induced a modest increase in mRNA levels of important regulators of differentiation, and direct downstream effectors of GR, such as CCAAT enhancer binding proteins (C/EBPs). Estradiol, under the same conditions, did not induce 3T3-L1 differentiation suggesting that BPA is acting via a non-estrogen receptor mediated, novel mechanism of action. In efforts to delineate the mechanism of action we investigated the effect of BPA on the glucocorticoid receptor (GR) mediated transcription. We could not demonstrate a direct transcriptional transactivation of GR in response to BPA on several luciferase driven promoters and in several cell lines at a wide range of concentrations.

Conclusions: These studies suggest that very low doses of BPA can induce differentiation of preadipocyte. 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.

IDENTIFICATION OF TYROSINE ISOMERS AND 3-NITROTYROSINE AS OXIDATIVE STRESS BIOMARKERS IN HUMAN SALIVA SAMPLES USING HPLC-ELECTROCHEMICAL ARRAY TECHNIQUE

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Background: Oxidative stress is suggested to play a role in several pathologies including cancer. Free radicals such as reactive oxygen and nitrogen species can oxidize phenylalanine and tyrosine residues in proteins to form stable metabolites such as p-tyrosine, m-tyrosine, o-tyrosine, Cl-tyrosine and 3-nitrotyrosine that serve as biomarkers of oxidative stress. Several methods exist for the analysis of these biomarkers in plasma samples. However, the usefulness of these methods in biomonitoring studies could be limited due to the invasiveness of plasma sampling. Hence it will be advantageous to develop and validate new analytical methods for biomarkers identification in alternate biological fluids such as saliva that are obtained through non-invasive procedures.

Objectives: Our primary objective is to develop an analytical method for the simultaneous analysis of multiple oxidative stress biomarkers present at trace levels in human saliva samples using high performance liquid chromatography – electrochemical array detection (HPLC-ECD) technique. Presence of salivary proteins affects the proper identification and quantification of oxidative metabolites. Therefore we compared three different methods to remove proteins from saliva samples prior to HPLC-ECD analysis.

Methods: Nine 1 mL aliquots of human saliva samples were centrifuged at 5000g for 10 minutes and the supernatants were evaporated under nitrogen. One set of samples (n = 3) were precipitated by treatment with cold acid:acetone. Proteins were removed from the second set of saliva samples (n = 3) by passing through molecular weight cut-off filters (3 kDa). Proteins in the third set of saliva samples (n = 3) were removed by a combination of molecular weight cut-off filtration and by acid:acetone method. All samples were analyzed using HPLC-ECD technique.

Results: Peak area ratio of each analyte from saliva samples were calculated and compared to that of authentic standards. An area ratio variability of ± 10% (compared to the standard) was considered acceptable for the positive identification of the analytes. Using this criterion, p-tyrosine and 3-nitrotyrosine were positively identified in all saliva samples. Recovery of these analytes by all three methods was > 80%. However, m-tyrosine, o-tyrosine, Cl-tyrosine and n-tyrosine peaks did not pass the peak area ratio criterion suggesting possible contamination due to the presence of other matrix components. Among the methods tested, protein removal by molecular weight cut-off filtration proved to be simple.

Conclusion: Presence of proteins and other matrix components affects the analysis of oxidative metabolites in saliva samples. Although protein removal proved to be sufficient for the positive identification of p-tyrosine and 3-nitrotyrosine, further clean-up is required prior to HPLC analysis of m-tyrosine and Cl-tyrosine in saliva samples.

PREDICTIVE VALUE OF MATERNAL PLASMA BIOMARKERS FOR INFANT BIRTH WEIGHT

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Background: Environmental contaminants are known to affect reproductive health and child development. The project Maternal-Infant Research on Environmental Chemicals (MIREC) was initiated to define a national profile of in utero and lactational exposure to environmental contaminants and to investigate potential impacts on pregnancy outcomes.

Objectives: We hypothesized that exposure of mothers to heavy metals such as lead and mercury will result in oxidative stress and endothelial dysfunction, with potential alteration of utero-placental perfusion, and affect fetal development. Low birth weight is an indicator of the general health of newborns, and a key determinant of infant survival, health and development. Low birth weight infants are at a greater risk of dying during the first year of life, and of developing chronic health problems. We report here preliminary data on the relationship between infant birth weight and biomarkers of oxidative stress, endothelial dysfunction and inflammation in maternal plasma.

Methods: Plasma samples obtained at third trimester from pregnancies (n=64) in ten medical centres across Canada were stabilized at recovery and analyzed in our laboratory by high performance liquid chromatography-electrochemical array (HPLC-EC; o-tyrosine, m-tyrosine, p-tyrosine, and 3-nitrotyrosine as protein oxidation and nitration biomarkers), competitive enzyme immunoassay (EIA; 8-isoprostane as marker of lipid peroxidation), and protein array (Bioplex; cytokines and chemokines as markers of inflammation, and C-reactive protein as marker of endothelial dysfunction and inflammation).

Results: Backward stepwise regression applied to the series of analytes revealed a negative correlation ($p<0.05$) between maternal oxidative stress, indicated by plasma o-tyrosine and 8-isoprostane, and infant birth weight. C-reactive protein, a marker of inflammation and a predictor of endothelial dysfunction and adverse cardiovascular outcome, was elevated ($p<0.05$) in plasma of mothers with infants in the low 10^{th} percentile of birth weight, <2.7kg.

Conclusion: Our preliminary data support the view that oxidative stress and inflammation in pregnant mothers is associated with impacts on fetal development, resulting in lower birth weight. The relationship between oxidative stress and inflammation in mothers and their exposure to chemicals remains to be elucidated.

CONTRASTING BIOLOGICAL POTENCY OF PARTICULATE MATTER COLLECTED AT SITES IMPACTED BY DISTINCT SOURCES

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Background: The concentration of urban particulate matter is associated with cardiopulmonary morbidity and mortality. On the basis of this association, air quality standards in North America and Europe are expressed as mass concentrations for a given size range (i.e. PM_{10} and $PM_{2.5}$; particles with an aerodynamic diameter less than 10 µm and 2.5 µm respectively). It is clear, however, that biological responses to particulate matter are dependent not only on size, but also on other physicochemical factors such as elemental and organic composition, which are impacted by mode of generation, and therefore source. Although there is considerable data on health effects of ambient particles, specific determinants of potency and sources of toxic constituents remain poorly characterized.

Objectives: In the present work our objective was to compare the relative toxic potency of ambient particles from geographical areas with distinct pollution emission sources and investigate determinants of toxicity.

Methods: As part of an ongoing sampling campaign, size-fractionated particles ($PM_{0.1\text{-}2.5}$, $PM_{2.5\text{-}10}$, $PM_{>10}$) were collected on filters using a ChemVol High Volume Cascade Impactor at sites impacted predominantly by different sources. These were: a steel mill in Hamilton Beach, Ontario, petrochemical refineries in Sarnia, Ontario, and Montréal, Québec, a copper smelter in Montréal, Québec, and an aluminum refinery in Shawinigan, Québec. Particles were recovered from filters by aqueous extraction and sonication followed by vacuum-evaporation. The cytotoxic potency of particulate matter samples was determined in the human lung epithelial-like A549 and murine macrophage-like J774 cell lines using bioassays for metabolic activity (Alamar Blue) and membrane integrity (lactate dehydrogenase release). Potency (β) was determined from Fold-effect = $(\text{Dose}+1)^{\beta}$, and was regressed against the elemental composition of the particles.

Results: Comparison of sites by elemental composition revealed striking differences. For example, water-soluble metals in Hamilton Beach $PM_{0.1\text{-}2.5}$ were dominated by zinc, while Shawinigan $PM_{0.1\text{-}2.5}$ was dominated by aluminum. Particle potency was impacted both by size ($PM_{0.1\text{-}2.5} > PM_{2.5\text{-}10} > PM_{>10}$) and site. Interestingly, site-to-site differences in toxicity were most apparent in the fine $PM_{0.1\text{-}2.5}$ fraction. In contrast, few differences in particle potency were observed in the $PM_{>10}$ fraction.

Conclusions: This study confirms that sampling at sites impacted by distinct local emission sources produces samples with contrasting elemental profiles, which in turn affect particle potency. The data show that sources impact primarily the potency of respirable particles, and hence have the potential to impact human health. Attribution of toxic potency to specific particle constituents will provide critical data for identification of priority sources for regulatory action.

OZONE AND PARTICULATE MATTER ACTIVATE BIOLOGICAL PATHWAYS IN ORGANS THROUGHOUT THE BODY

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Background: There is a growing body of evidence supporting the contention that inhaled pollutants exert effects beyond the cardiopulmonary system. Studies have revealed translocation of particles from the lungs to other organs, and both particulate and gaseous pollutants can provoke responses in the lungs that have systemic consequences. However, little is known about the actual biological effects in potential target organs.

Objectives: Our objective was to screen for perturbation of toxicity pathways in a number of organs to gain insight into extrapulmonary impacts of inhaled particulate and gaseous pollutants.

Methods: Male Fisher-344 rats were exposed for 4 h by nose-only inhalation to particulate matter (0, 5, 50 mg/m^3) or ozone (0, 0.4, 0.8 ppm), and euthanized immediately or 24 h after exposure. To identify local and systemic impacts of inhaled pollutants, expression of a panel of genes representing a number of biological pathways was evaluated by real-time polymerase chain reaction in the lungs, heart, liver, kidney, and spleen. The panel included genes involved in xenobiotic metabolism, inflammation, oxidative stress, metal-response, endothelial dysfunction, and vasoregulation.

Results: Inflammatory responses appeared to be largely confined to the lungs, with ozone, but not particles, increasing expression of interleukin-6, cyclooxygenase-2, and monocyte chemotactic protein-1. In contrast, both particles and ozone independently activated xenobiotic metabolizing pathways (cytochrome P450 1A1 or 1B1) in the lungs and heart, with less pronounced effects in other organs. Both pollutants increased metal-response/oxidative stress endpoints (metallothioneins I, II) in the lungs, heart, and kidney, with pollutant-specific effects in the liver (particles) and spleen (ozone). Although particles and ozone increased endothelin expression (vasoregulation/endothelial dysfunction) in the lungs, ozone alone increased expression in the heart, kidney, and spleen, but not liver. Remarkably, both particles and ozone increased expression of hypoxia-inducible factor-3, member of a family of transcription factors sensitive to environmental stimuli, in all organs, suggesting that this factor may serve as a non-specific marker of systemic effects.

Conclusions: Our results indicate that both particulate and gaseous pollutants can activate transcriptional pathways in organs throughout the body, substantiating epidemiologic findings of impacts of inhaled pollutants outside the cardiopulmonary system. The distinct profiles of gene response observed for each organ suggest differential sensitivity to the exposures. Future work will be aimed at investigating the biological significance of observed extrapulmonary impacts of pollutant inhalation, and elucidating the signalling pathways involved.

ASSESSING EXPOSURE DOSE EQUIVALENT OF VINYL CHLORIDE IN DRINKING WATER FROM SHOWERING AND BATHING ACTIVITIES USING PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING

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Background: Historically, safety evaluation of chemicals in drinking water accounted only for exposures via the ingestion route. Recently, however, certain organizations performing chemical risk assessments for drinking water have begun to account for the inhaled and dermal routes of exposure in addition to daily exposure. Current assessment practices at Health Canada calculate daily intake or route equivalent dose using chemical data and average daily exposure assumptions. With more available information such as pharmacokinetic and toxicity data, the multiroute dose closely related to the target response can be estimated using a physiologically-based pharmacokinetic (PBPK) model.

Objective: In this study, the implications of using these methods in a proposed tiered analysis within a drinking water exposure assessment framework were investigated.

Methods: Exposure to high levels of vinyl chloride can cause liver toxicity and carcinogenic effects in rodents. A PBPK model was derived to determine the human dose equivalent of vinyl chloride in drinking water following a shower exposure scenario based on assumptions used in the derivation of Guidelines for Canadian Drinking Water Quality. For an average shower exposure, a dermal and inhaled point of departure in humans was extrapolated from a rodent PBPK model and target liver metabolism dose-response relationship.

Results: The resulting model dose estimates were transformed to an oral dose equivalent (L-eq) for vinyl chloride in drinking water, and compared with values calculated using the tiered approach. The modeled dose estimates were less conservative than the L-eq values that were generated using chemical exposure data.

Conclusion: A tiered analysis framework based on available data using the different computational techniques is a scientifically meaningful approach supporting risk assessment practices of chemicals and contaminants in drinking water.

MUTAGENIC ACTIVATION OF FURAN BY CHINESE HAMSTER V79 LUNG FIBROBLASTS EXPRESSING INFLAMMATION-RELATED GENES

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Background: Genes such as nitric oxide synthase (NOS) and prostaglandin H synthase 2 (PGHS2) are expressed during the inflammatory response and oxygen/nitrogen oxide species/radicals produced by the gene products are known to participate in chemical mutagenic activation. Inflamed tissues may thus act as secondary sites of chemical metabolism, increasing chemical exposure risk and effectively creating members of a temporary vulnerable population who would move in and out of higher risk throughout life according to inflammation status. Previously, mutagens were generated by preincubation of the food contaminant furan with either nitrous or peroxynitrous acids as shown with the Ames *Salmonella* test. Testing of this response in a mammalian system proved difficult because of reaction mixture toxicity. Therefore an alternative approach was needed to examine the effect of inflammation on furan mutagenicity.

Objectives: The effects of inflammation on mutagenic activation of furan was examined in Chinese hamster lung V79 cells transfected with commercially available vectors for NOS and PGHS2.

Method: Chinese hamster lung V79 cells were transfected with expression vectors for human NOS and PGHS2, either singly or in combination. The toxicity of furan was characterized using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay. The effects of NOS and PGHS2 on furan 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 cells transfected with an expression vector for cytochrome P450 2E1, the P450 isozyme implicated in furan metabolism/activation.

Results: Transfection of all genes reduced cell viability: untransfected>PGHS2>NOS>NOS/PGHS2. The presence of PGHS2 and NOS/PGHS2 increased furan toxicity at 24 hrs with NOS increasing toxicity at 48 hrs. Increased mutagenicity of furan was found with all constructs, approximately doubling at 1 ul/ml for PGHS, NOS/PGHS and 2E1 transfected cultures, while approximately tripling at 0.5 ul/ml for NOS.

Conclusions: The ability to metabolically activate furan to a mutagenic form using transfected, commercially available expression vectors for inflammation-related genes suggests a general method by which existing *in vitro* mutagenicity (or other toxicological endpoint) assays may be modified to examine the effects of inflammation or other processes of detoxification/metabolism/modification. The impact of pre-existing inflammatory conditions on chemical metabolism may be of relevance to how assessments of the potential health effects of this compound are undertaken.

EFFECT OF OZONE, TiO_2 NANOPARTICLES AND ETHANOL VAPORS ON LEUKOCYTES AND CYTOKINES IN A RAT MODEL OF ALLERGIC ASTHMA

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Background: Asthma, a chronic respiratory disease affecting 270 million individuals worldwide, is characterized by pulmonary inflammation. Epidemiological studies suggest that ozone, an important urban air contaminant, can increase asthmatic symptoms in exposed population. Nanoparticles and ethanol vapors are air contaminants of recent concern. We have shown that ethanol vapors do not increase pulmonary inflammation in a rat model of allergic asthma, while effects of nanoparticles in this model are unknown. The effect of combined exposure to these air contaminants on pulmonary inflammation has yet to be determined.

Objective: This study aimed to investigate the inflammatory pulmonary response, at the cellular and cytokine level, of inhaled TiO_2 nanoparticles, ozone, and mixtures with ethanol vapors in asthmatic rats.

Methods: Brown Norway rats were sensitized (s.c.) and challenged (15 min inhalation, 14 d later) with chicken egg ovalbumin (OVA); inflammation results from the OVA challenge. A concentration of 1.5% OVA and a time point of 48 h following the challenge to perform leucocytes counts (mostly eosinophils) in bronchoalveolar lavages (BAL) were found as optimal conditions since it caused approximately 30% of the maximum inflammatory response and it allowed monitoring of both upward and downward changes. Full body exposure to ozone (0.4 or 1 ppm), TiO_2 nanoparticles (9.35 mg/m^3) and ethanol vapors (3000 ppm) were done in a 500 L inhalation chamber. Using the Rat Cytokine Array Panel A (R&D Systems), the level of 29 cytokines was measured in pooled BAL supernatant.

Results: OVA challenge caused approximately a 4-fold increase in number of eosinophils in BAL compared to control rats. In OVA challenged rats, exposure to ozone (4 h/d, 3 consecutive days) showed a 3.3-fold decrease in eosinophils compared to controls (BAL were performed immediately following final exposure, i.e. 48 h post OVA challenge). Similarly, levels of cytokines know to be involved in asthmatic response, such as IL-13 and MIP-1α were decreased (6-fold and 4-fold, respectively). Co-exposure to ethanol vapors did not change the response caused by ozone alone. In OVA challenged rats, exposure to TiO_2 nanoparticles (6 h, single exposure) also decreased the eosinophil count compared to controls.

Conclusions: Both ozone and TiO_2 nanoparticles reduced the increase in leucocytes and cytokines levels caused by the OVA challenge. Thus, data obtained using a rat model of allergic pulmonary inflammation suggests a protection when the contaminants are administered subsequently to the induction of the inflammation. Ethanol vapors did not modify these responses. (Supported by AFSSET)

ALTERED METHANOL EMBRYOPATHIES IN EMBRYO CULTURE WITH MUTANT CATALASE-DEFICIENT MICE AND TRANSGENIC MICE EXPRESSING HUMAN CATALASE

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Background: The mechanisms underlying the teratogenicity of methanol (**MeOH**) in rodents, unlike its acute toxicity in humans, are unclear but may involve reactive oxygen species (**ROS**).

Objective: To determine whether embryonic catalase, although less than 10% of maternal activity, protects the embryo from MeOH embryopathies by detoxifying ROS.

Methods: We used whole embryo culture to remove confounding maternal factors, including metabolism of MeOH by maternal catalase. C57BL/6 (**C57**) mouse embryos expressing human catalase (**hCat**) or their wild-type (**C57 WT**) controls, and C3Ga.Cg-Catb/J acatalasemic (**aCat**) mouse embryos or their wild-type C3HeB/FeJ (**C3H WT**) controls, were explanted on gestational day (**GD**) 9 (plug = GD 1), exposed for 24 hr to 4 mg/mL MeOH or vehicle, and evaluated for functional and morphological changes.

Results: hCat and C57 WT vehicle-exposed embryos developed normally. MeOH was embryopathic in C57 WT embryos, evidenced by decreases in anterior neuropore closure, somites developed and turning, whereas hCat embryos were protected. Vehicle-exposed aCat mouse embryos had lower yolk sac diameters compared to C3H WT controls, suggesting endogenous ROS are embryopathic. MeOH was more embryopathic in aCat embryos than WT controls, with reduced anterior neuropore closure and head length only in catalase-deficient embryos.

Conclusions: These data suggest that ROS may be involved in the embryopathic mechanism of methanol, and that embryonic catalase activity may be a determinant of teratological risk.
(Support: Methanol Foundation and CIHR)

PRÉDICTIONS *IN SILICO* DES VALEURS LIMITES D'EXPOSITION PROFESSIONNELLE POUR LES SOLVANTS ORGANIQUES

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Contexte: En hygiène industrielle, le Vapour Hazard Ratio (VHR) est le rapport entre la concentration de vapeur saturante d'une substance volatile et sa valeur limite d'exposition (VLE). L'absence de VLE est une limite importante lorsqu'il est question de sélectionner un solvant de substitution sur la base du VHR.

Objectif: L'objectif de ce travail est de développer des modèles de relation quantitative propriété-propriété (QPPR) pour prédire des VLE et ainsi permettre le calcul du VHR.

Méthodes: Trois coefficients de partage lipide:air [n-octanol:air (K_{oa}), olive oil:air (K_{oila}) et fat:air (K_{fa})] ont été utilisés pour prédire les VLE des solvants avec une toxicité locale de type irritation. Pour les solvants avec une toxicité systémique, la prédiction des VLE considère les différences toxicocinétiques (facteur de variabilité toxicocinétique, FVT) et le potentiel toxicologique estimé à partir de la dose interne efficace (DIE). Les coefficients de partage n-octanol:water (K_{ow}), oil:water (K_{oilw}) et fat:water (K_{fw}) ont été utilisés pour évaluer la relation avec les DIE.

Résultats: Pour les solvants avec une toxicité locale, K_{oa} est le meilleur prédicteur des VLE [$VLE\ (ppm) = 10^{((-0,45 \times logKoa)+3,65)}$; $n=21$; $r^2=0,71$; $PRESS/SSY=0,36$; $F=45,5$ avec $p<0,001$] et la moyenne (±écart-type) (étendue) des rapports entre les VLE recommandées et prédites était de 1,04 ± 0,61 (0,2-2,5). Pour les solvants avec une toxicité systémique, FVT et DIE variaient de 0,73 à 41,4 µmol/L et de 1,20 to 848 µmol/L respectivement et K_{ow} est le meilleur prédicteur de DIE [$DIE\ (\mu mol/L) = 10^{((-1,16 \times logKow)+3,65)}$; $n=27$; $r^2=0,88$; $PRESS/SSY=0,12$; $F=181$ avec $p<0,001$]. Finalement, 61% et 87% des VHR prédits variaient d'un coefficient maximal de 2 et de 5 par rapport aux valeurs calculées.

Conclusions: Les modèles QPPR présentés offrent une nouvelle méthode de prédiction des VLE permettant d'estimer des indices VHR. Ces modèles sont les premiers à prédire des VLE en utilisant des paramètres physicochimiques et des constantes métaboliques (Vmax et Km). De plus, la considération de deux modèles distincts en fonction du mode d'action est aussi un élément d'originalité de la méthode.

PERINATAL EXPOSURE TO DIFFERENT MIXTURES OF ENVIRONMENTAL CONTAMINANTS CREATES DISTINCT SETS OF GENES DIFFERENTIALLY METHYLATED IN ADULTHOOD: DEVELOPMENT OF A DNA METHYLATION MICROARRAY ANALYSIS STRATEGY

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Background: Disadvantageous early life environment predisposes individuals to develop diseases during adulthood. A mechanism by which this could happen is changes in DNA methylation occurring early in development. DNA methylation is a persistent and stable epigenetic attribute involved in regulating gene expression. This work suggests that exposure to environmental contaminants during pregnancy and lactation induces differential methylation of genes in adulthood.

Objectives: (1) Develop a strategy for analysing DNA methylation microarray data. (2) Investigate DNA methylation changes in rat livers following developmental exposure to two mixtures of environmental contaminants.

Method: Two mixtures of contaminants identified as MM and AhR were tested. The mixtures were administered to the dams during pregnancy and lactation. Genes enriched in methylation in male offspring livers (10 control, 10 AhR, 8 MM), and in 5 aliquots of DNA from a cancer line, were revealed using NimbleGen DNA methylation microarrays (385,000 probes). Within each rat, genes enriched in methylation were identified using the Kolmogorov Smirnov test, followed by a scoring system to regroup significant probes into "peaks". Treatment effects were investigated by comparing the proportion of rats within each treatment group that possess genes with peaks of methylation.

Results: Among all 28 rats, 8,064 DNA segments displayed significant methylation enrichment. Of these, 6,787 were common to all three groups, and 1,277 were specific to the treatment groups. A test of the reproducibility of the methodology, assessed by analysing technical replicates, suggests that only 53% of the methylated DNA segments were detected by each of the five microarrays. This suggests the need for a careful examination and interpretation of the methylation status of the 1,277 DNA segments possibly differentially methylated by the treatments. The high level of methylation in the promoter of the gene Myelin Basic Protein measured by DNA methylation microarray (peak score = 15.05) was confirmed by pyrosequencing analysis revealing > 95% of methylated CpG.

Conclusions: These results suggest persistent mixture-specific effects on DNA methylation. These long term changes in methylation might reveal distinct mechanisms of action, valuable biomarkers of effects, and perhaps predispositions to diseases. However, definitive conclusions require ongoing validations by sodium bisulfite pyrosequencing and real time RT-PCR analysis.
(Funded by NCP, CMP, and Health Canada)

RAT DNA METHYLATION PYROSEQUENCING ASSAYS FOR GENES WHOSE ENVIRONMENTALLY ALTERED METHYLATION STATUS HAVE BEEN LINKED TO DISEASE STATES

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Background: Changes in the normal pattern of DNA methylation, which can modify gene expression, were suggested as a mechanism to explain how early events can predispose individuals to disease development later in life. DNA methylation involves the addition of a methyl group to the cytosine of the cytosine-guanine (CpG) dinucleotide. We obtained preliminary results suggesting that perinatal exposure to mixtures of environmental contaminants can: (1) create distinct sets of genes differentially methylated in adulthood (by DNA methylation microarray analysis), and (2) extend the duration of glucocorticoid hormone elevation induced by stress in adult offspring. The latter phenomenon is associated by others to changes in DNA methylation of the glucocorticoid receptor (GR) promoter.

Objectives: Develop and validate sodium bisulfite pyrosequencing DNA methylation assays for: (1) GR, and (2) for genes with contrasting DNA methylation levels [close to background (Cdkn2a, Clusterin), intermediate (H19/IGF2), or high (MBP)], as a set of control genes for other experiments in the rat.

Method: Two assays were designed for the rat GR promoter. The first assay targets GR exon1_7 (a predominant hippocampal exon) to measure methylation at seven consecutive CpG sites within the neuronal growth factor 1A binding domain. The second GR assay spans twenty CpG's within exon1_10, the most abundantly transcribed promoter in the liver. The assay for the tumor suppressor gene *cdkn2a* (p16INK4a) analyzes six CpG's within exon1. Other assays were validated for the gene of MBP, Clusterin, and Igf2/H19. Assays were optimized for PCR conditions, quantity of biotin-labelled amplicon, PCR annealing temperature, PCR bias, accuracy and reproducibility using dilutions of unmethylated and highly methylated genomic control DNA.

Results: Methylation measurements were highly correlated with the dilution of methylated and unmethylated DNA with r^2 values being >0.99 for most assays and most individual CpG positions. Within a DNA segment, the background level of methylation can significantly differ for each CpG position and ranged from <1% to 7%. The sensitivity of each assay, assessed above background readings for each CpG position, was always below 1.25%. In contrast to some literature, our preliminary analysis cannot support that changes in GR promoter methylation are associated with endocrine changes, GR mRNA or protein abundance. However, as expected from the literature, our other assays (MBP, Clusterin, Igf2/H19) have been measuring from 0 to 100% methylation depending on cell types.

Conclusions: These assays measure methylation accurately and reproducibly at individual CpG sites. In addition to generating key data for other ongoing studies, these assays provide us with a set of "control genes" that can be included in the validation steps of investigations of other genes with unknown methylation characteristics. (Funded by NCP, CMP, and Health Canada)

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