40th Annual Symposium/ Le 40e symposium annuel

December 1 and 2, 2008
Delta Centre-Ville
777 rue University Street
Montreal, Quebec
H3C 3Z7
Tel. 514-879-1370

Toxicity testing tomorrow: What does the future hold?
Que nous réservent les essais de toxicité de demain?

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

<u>Program Committee/ Comité du programme</u>
Lynne LeSauteur (Chairperson), Charles River Laboratories-CTBR
Kannan Krishnan, Université de Montréal
Michael Wade, Health Canada

PROGRAM

Sunday 30 November 2008

PM

2:00-5:00 **STC Executive Committee meeting**

7:00 **Evening Session:** Careers in Toxicology-Perspectives from Government, Industry and Academia.

Invited guest: Ed Short, Director of Human Resources, Charles River Laboratories

Topic: Toxicity testing - the HR perspective.

Monday 1 December 2008

AM

8:00 **Registration**

8:45 Opening remarks and introduction
Genevieve Bondy, President, Society of Toxicology of Canada

Session I. Toxicology testing of tomorrow: A regulatory perspective

Chairperson: Lynne LeSauteur

8:50 Introduction

9:00 Toxicity testing in the twenty-first century: A vision and a strategy
Daniel Krewski, University of Ottawa/ **Melvin Andersen**, The Hamner Institute of Health Sciences

9:45 Biotechnology and toxicity testing: Regulatory toxicology and risk assessment
Kenneth L. Hastings, Sanofi Aventis

10:30 Coffee break and poster session

11:00 An EPA perspective on testing for developmental neurotoxicity: How do we go from where we are now to where we need to be?
Kevin Crofton, US EPA

11:45 Panel discussion and Chairperson's concluding remarks

12:00 Lunch and poster session

Monday 1 December 2008
PM

Session II. Toxicology testing tomorrow: Compound characterization and *in vitro* toxicity testing.

Chairperson: Lynne Lesauteur

1:25	Introduction
1:30	The US EPA's ToxCast Program for predicting hazard, characterizing toxicity pathways and prioritizing the toxicity testing of environmental chemicals **David Dix**, US EPA
2:00	*In vitro* surrogates, genomics, and *in vivo* biomarkers: How do they translate to safety in drug development? **Holly Smith**, Eli Lilly
2:30	Coffee break
3:00	Accelerated cytotoxic mechanism screening for hepatotoxic drugs **Peter O'Brian**, University of Toronto
3:30	Student speaker: **Katherine Schoeman**, University of Western Ontario
3:45	Student speaker: **Helen Badham**, Queen's University
4:00	Discussion and Chairperson's concluding remarks
4:15	Annual Business Meeting
4:15	Student Workshop: Drug toxicity testing today. **Anna Adamou**, Scientific Director, IAF Preclinical Services
6:00	President's Reception and STC Awards
8:00	STC Dinner

Tuesday 2 December 2008

AM

Session III. Toxicology of the future: In vivo toxicity testing, dose-response and extrapolation modeling

Chairperson: Kannan Krishnan

8:50	Introduction
9:00	Consideration of alternative approaches for the purpose of reducing animal numbers in the preclinical development of biotherapeutic products **Laura Andrews**, Genzyme
9:45	Zebrafish test systems as tools for early safety evaluation in pharmaceutical R&D: Issues and opportunities **Ernie Bush**, Cambridge Healthtech Associates
10:30	Coffee break and poster session
11:00	Dose response modeling and toxicology of the future **Kannan Krishnan**, Université de Montréal
11:45	Discussion and Chairperson's concluding remarks
12:00	Lunch

Tuesday 2 December 2008

PM

Session IV. Toxicity testing strategies: Population-based and human biomonitoring data

Chairperson: Mike Wade

1:25	Introduction
1:30	Biomonitoring Equivalents (BEs) as screening tools for interpretation of human biomonitoring data. **Lesa L. Aylward**, Summit Toxicology
2:00	MIREC (Maternal Infant Research in Environmental Chemicals) – A cohort study. **William Fraser**, Hopital St-Justine
2:30	Biomonitoring of environmental chemicals in the Canadian Health Measures Survey **Doug Haines**, Health Canada
3:00	Discussion and Chairperson's concluding remarks
3:15	Conclusion: Genevieve Bondy, President, STC

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

CANTOX Health Sciences International

Charles River Laboratories Canada

GlobalTox International Consultants Inc.

Health Canada

Réseau de recherche en santé environmentale/ Environmental Health Research Network

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*

(Alphabetically)

*Not all abstracts available at press.

Anna Adamou

Workshop on drug toxicity testing today: An overview of the drug development process.
Topics will include regulatory agencies/regulations, types of testing, phases of drug development, pre-clinical plans, non-clinical studies required for an IND/NDA submission and special considerations.

Biography: Adamou, Anna
Completed MSc at the University of Montreal option General Toxicology. Started at PCS-MTL in 1997 in the pathology group and subsequently transferred to the Quality Assurance group as an Inspector and ultimately to the Toxicology group as a Study Director in June 1998. Currently Scientific Director of PCS-MTL satellite facility in Laval.

Dr. Laura Andrews

Consideration of alternative approaches for the purpose of reducing animal numbers in the preclinical development of biotherapeutic products.
As the development of biotherapeutics becomes a more advanced science based challenge, the selection of relevant animal models, utility of traditional species and alternatives to traditional safety approaches are becoming more accepted and in fact, necessary.

The last ten years has seen a significant advancement of our knowledge and development of biotechnology-derived products for the treatment of debilitating, life threatening diseases. As the therapies being developed are more sophisticated and generally more specific the need to establish safety in relevant models has become more and more of a challenge.

Alternatives to the traditional safety approach include the use of homologous proteins, transgenic animals, animal models of disease as well as state of the art non-invasive, non-terminal technologies such as high resolution imaging and scanning methods. In addition, a science based approach to rationale study design has allowed for a better use of animals through the development process. Study design considerations must be addressed in order to most effectively utilize animals and wherever possible reduce the need for large numbers and multiple studies. The opportunities and challenges for these approaches as well as the approach to implementing these areas to help reduce animal use and advance the science of biotechnology drugs will be discussed.

Biography: Andrews, Laura
Laura Andrews is Vice President of Pharmacology and Toxicology at Genzyme where she directs the nonclinical development programs for biotherapeutics. She is responsible for the nonclinical development programs for therapeutic biologics, gene therapy products, and cell based therapies. Laura oversees the design, implementation and interpretation of the in vivo GLP studies and the in vitro assays to support product development. Laura has authored the pharmacology and toxicology section for several Genzyme INDs and marketing applications in several different territories. Dr. Andrews received a BS (1983) in Biology and Chemistry from Dickinson College, and a Ph.D. (1987) in Pathology and Cell Biology from Thomas Jefferson University and Medical College in Philadelphia, PA. She was Board Certified in General Toxicology in1998. She holds memberships in the Society of Toxicology (SOT), Society for Toxicologic

Pathology (STP) and the American College for Toxicology (ACT). She is currently on the Board of Directors for the American Board of Toxicology.

Dr. Lesa Aylward

Biomonitoring Equivalents (BEs) as screening tools for interpretation of human biomonitoring data.

Increasingly sensitive analytical data on concentrations of chemicals in human biologic media such as blood or urine in persons from the general population are being generated by biomonitoring programs conducted by the U.S. Centers for Disease Control (CDC) and other researchers, and more such programs are planned, including the Canadian Monitoring Programme. However, there are currently few screening tools available for interpretation of such data in a health risk assessment context. This presentation describes the concept and implementation of Biomonitoring Equivalents (BEs), estimates of the concentration of a chemical or metabolite in a biological medium that is consistent with an existing exposure guidance value such as a tolerable daily intake (TDI) or reference dose (RfD). Key concepts regarding the derivation and communication of BE values resulting from an expert workshop held in 2007 are summarized. BE derivations for four case study chemicals (toluene, 2,4-dichlorophenoxyacetic acid, cadmium, and acrylamide) are presented, and the interpretation of available biomonitoring data for these chemicals is presented using the BE values. These case studies demonstrate that a range of pharmacokinetic data and approaches can be used to derive BE values; fully-developed physiologically based pharmacokinetic (PBPK) models, while useful, are not required. The resulting screening level evaluation can be used to classify these compounds into relative categories of low, medium, and high priority for risk assessment follow-up. Application of BE values as tools in risk assessment and risk management are discussed.

Biography: Aylward, Lesa

Lesa Aylward is a Principal at Summit Toxicology and received Bachelor's and Master's degrees in engineering from M.I.T. Ms. Aylward has published extensively in the areas of pharmacokinetic modeling, chemical risk assessment, and methods for interpreting biomonitoring data in a health risk context. Ms. Aylward has published extensively on pharmacokinetics and biomonitoring for persistent organochlorines including dioxins and furans and related PCB compounds. With collaborating scientists, she has developed and published novel approaches to assessing the biologically relevant exposures to TCDD and related compounds, and analyzed the impact of these methods on conclusions drawn from conventional risk assessment methods.

Dr. Ernie Bush

Zebrafish test systems as tools for early safety evaluation in pharmaceutical R&D: Issues and opportinities

Cambridge Healthtech Associates has worked closely with several vendors of Zebrafish test systems to evaluate their optimal use and value to the pharmacuetical R&D community. As part of this process, an industry wide survey was administered to get feedback and concerns from a broad range of users, consumers and managers in the pharma space. This presentation will review the overall state of the zebrafish situation especially focusing on the current perceptions of the optimal value proposition, where the tools need

better validation, and what capabilities are missing from the current tool set. Finally, the presentation will summarize the optimal test system and validation strategy that has evolved from this collaborative peer to peer evaluation.

Biography: Bush, Ernest D., Ph.D.

VP & Scientific Director
Cambridge Healthtech Associates

Dr. Bush has spent over 23 years working in the field of biomedical R&D most of which working in preclinical safety departments for major pharmaceutical companies. From 1992 to 2005 he worked at Hoffmann-La Roche, Inc. and for the last 5 years of that time he led their Non-Clinical Drug Safety Department. This department was comprised of: Drug Metabolism, Non-Clinical Pharmacokinetics, Bioanalytical, Toxicology (including Safety Pharmacology and Teratology), and Pathology. The scope of their responsibilities spanned from early Discovery support through to NDA with particular emphasis on providing an integrated safety assessment to project teams and senior management.

Although Dr. Bush's graduate training and early career focused on bioanalytics and drug metabolism, he has always had a special interest in how pharma safety data was collected, managed and interpreted; this interest has evolved into a major job function due in part to the exploding volume of safety data generated in today's R&D environment. Consequently, Dr. Bush has been asked to lead several global initiatives on safety information management practices, including a global safety LIMS system at Roche and a global information repository for toxicogenomics data for the International Life Sciences Institute (ILSI).

Since joining CHA in 2005, Dr. Bush has been the guiding force behind collaborative technology evaluations in the pharmaceutical safety space. These projects bring together experts from multiple companies to improve the economics and effectiveness of how the industry conducts preclinical safety evaluations.

Therefore, his in-depth knowledge and broad experience in both the informatics and safety assessment fields has made him a unique leader in today's complex drug development landscape.

Dr. Kevin Crofton

An EPA Perspective on Testing for Developmental Neurotoxicity: How Do Go from Where We Are Now to Where We Need to Be

A number of recent reports have highlighted the need new methods and models to assess the hazards of the large number of untested environmental chemicals. In response to this need, the US Environmental Protection Agency has launched new research to develop rapid and efficient tools to detect hazard. One example is an interdisciplinary effort to develop models to screen for developmental neurotoxicity.

Adverse effects on the nervous system following exposure to environmental contaminants during development are well documented (e.g., lead, methyl mercury). Unfortunately, current regulatory guidelines for developmental neurotoxicity (EPA, ORDC) are expensive in terms of both scientific resources and time, and do not represent a viable approach to screen large number of chemicals. The Office of Research and Development has begun a variety of efforts including improved data collection and interpretation from current methods, as well as developing targeted in vivo testing based on available mode-of-action information. These efforts will allow better use and interpretation of existing technologies. The challenge for developmental neurotoxicologists, regulatory agencies, and the regulated community is to develop true first-tier high-throughput screening technologies. We are currently developing alternative methods that are capable of higher throughput for much lower costs. This includes high-throughput assays that use clonal animal and human cell lines, human progenitor cells, as well as use of small vertebrate species (e.g., zebrafish).

These methods will allow prioritization of further testing based on toxic potential, leading to more efficient testing. Major scientific issues that need to be resolved include validation of new test methods, development of tiered decision logic, and regulatory acceptance. (This abstract does not represent USEPA policy)

Biography: Crofton, Kevin

Dr. Kevin M. Crofton is a developmental neurotoxicologist in the National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina. Dr. Crofton received a B.A. and M.S. in Zoology from Miami University, Oxford, Oh, and Ph.D. in Toxicology from the University of North Carolina, Chapel Hill. He has been a toxicologist at EPA since 1988. His research interests include developmental neurotoxicity, the cumulative risk of thyroid disruptors and pesticides, and development of high-throughput testing methods. He is an Adjunct Assistant Professor in the Curriculum in Toxicology, University of North Carolina at Chapel Hill, and in the Department of Environmental and Molecular Toxicology at North Carolina State University. Dr. Crofton's professional activities include membership in numerous scientific societies and participation many professional review boards. He is currently on the editorial boards of several scientific journals, including Toxicological Sciences, NeuroToxicology, and the Journal of Environmental Neuroscience and Biomedicine. He has presented invited lectures for a variety of government agencies in Europe, Canada, and the U.S., and for numerous professional societies and universities. In addition, he has authored or coauthored over 115 additional peer reviewed publications.

Douglas Haines

Biomonitoring of environmental chemicals in the Canadian Health Measures Survey

Currently, there are limited nationally-representative biomonitoring data for the general Canadian population. In order to fill this data gap, Health Canada is collaborating with Statistics Canada on the Canadian Health Measures Survey (CHMS). The CHMS is a national 'direct measures' health survey that will provide benchmark data representative of 97% of the Canadian population on indicators of chronic diseases, infectious diseases, fitness, nutritional status, environmental exposures, as well as risk factors and protective characteristics related to these health indicators.

The CHMS, launched in March 2007, is being conducted over a period of 24 months. Approximately 5000 Canadians, roughly 500 males and 500 females in each of the following age groups are being sampled: 6 to 11, 12 to19, 20 to 39, 40 to 59, and 60 to 79 years. Health questionnaires are being administered in participants' homes followed by physical health exams conducted in mobile clinics at 15 collection sites across the country.

The CHMS is the first survey to include a comprehensive biomonitoring component measuring concentrations of environmental chemicals in biological specimens of Canadians. A total of 91 chemicals or their metabolites are currently being measured in blood and urine samples. Classes of chemicals being measured include: metals (including lead, cadmium and mercury), phthalates, polychlorinated biphenyls (PCBs), organochlorine pesticides, brominated flame retardants, organophosphate pesticide metabolites, phenoxy herbicides, pyrethroid pesticides, cotinine, perfluorinated compounds, and Bisphenol-A.

One of the most important contributions from the CHMS will be the development of baseline data in the Canadian population that will allow for the assessment of national distributions of health measures. This will permit the establishment of current population levels for a broad range of environmental chemicals and provide representative data to enable both tracking of trends and comparisons of data within Canada and with other countries.

Results from this survey will also allow future research efforts to explore relationships between environmental chemicals, other physical measures, and self-reported information, as well as provide information to governments to guide action.

This presentation will provide an overview of the population-based biomonitoring component currently underway in the Canadian Health Measures Survey.

Biography: Haines, Douglas

Douglas Haines is with Health Canada's Chemicals Management Directorate where he is managing the implementation of national biomonitoring initiatives to track Canadians' exposures to environmental chemicals. Mr. Haines previously managed Health Canada's Great Lakes Health Effects Program and the health component of the Canada-Quebec St. Lawrence Vision 2000 Program which focused on assessing and managing health risks posed by environmental pollution in these two ecosystems. On assignment with Statistics Canada, Mr. Haines led the development of the proposal for the Canadian Health Measures Survey, launched in March 2007. He is currently a member of the Federal-Provincial-Territorial Health and Environment Tracking/Surveillance Task Group.

Kannan Krishnan, Thomas Peyret and Mathieu Valcke

Dose response modelling and toxicology of the future

The vision of the U.S. National Academy of Sciences for toxicity testing in the 21st century is a logical one that shifts the attention from adverse effects seen at high doses towards perturbations of key toxicity pathways. For the successful implementation of this paradigm shift, it is essential to develop data, tools and models based on systems biology. The quantitative models would facilitate the integration of dosimetric information as well as perturbations of toxicity pathways determined *in vitro*. In this regard, physiologically-based pharmacokinetic (PBPK) models play a critical role because they facilitate the simulation of human exposure conditions (dose, scenario, etc.) corresponding to intracellular concentrations associated with perturbations of toxicity pathways. The PBPK models are quantitative descriptions of the absorption, distribution, metabolism and excretion of chemicals based on interrelationships among the critical determinants of these processes. These models can be particularly useful to predict microlevel and macrolevel kinetics in intact animals and humans (including sensitive subpopulations) on the basis of *in vitro* data on metabolism rates and partition coefficients. Similarly, *in silico* approaches may also be used for generating initial estimates of chemical-specific parameters for constructing PBPK models to simulate the time-course of blood or tissue concentrations of a chemical and its metabolite. When PBPK models are integrated with biologically-based pharmacodynamic models, they would allow not only the time-course of internal dose in exposed animals but also the toxicological responses based on *in vitro* data on perturbations of pathways or the mode(s) of action. Recent research advances in PBPK modeling demonstrate the feasibility of accounting for lifestage differences in physiology and enzyme polymorphism to facilitate the prediction of population distribution of tissue dose. It would appear that integrated molecular-level, cellular-level and population-level PBPK models will lead the way of sophisticated dose-response modeling in the future. These approaches will not only improve our understanding of the exposure-dose-response relationships but also facilitate the use of a systems approach to solve complex problems in toxicology, thus ensuring the successful shift in the toxicity testing paradigm.

Further Reading:

Andersen ME, Krewski D and Mantus E and Zeise L. (2008). Toxicity testing in the 21st century. http://www.alttox.org

Collins FS, Gray GM, Bucher JR. (2008). Transforming environmental health protection. Science. 319(5865):906-7.

Krishnan K and Andersen ME (2007) Physiologically-based pharmacokinetic and toxicokinetic modeling. In: **Principles and Methods in Toxicology**. A Wallace Hayes (ed.), Taylor and Francis Inc., New York pp232-291

NAS/NRC (2007). Toxicity testing in the 21st century. A vision strategy. The National Academy Press, Washington, D.C.

Biography: Krishnan, Kannan

Dr. Kannan Krishnan is Professor of Occupational and Environmental Health at Université de Montréal and Director of the Inter-University Toxicology Research Center (CIRTOX), Montréal. He obtained his Ph.D. in Public Health from Université de Montréal (1990) and completed his post-doctoral training at The Hamner Institute (formerly CIIT), Research Triangle Park, NC (1992). He has maintained an active research program that focuses on computational toxicology and health risk assessment methods. Kannan received the *Veylian Henderson Award* of STC in year 2000, and was co-recipient of SOT's *Best paper award in Toxicological Sciences* (2003) for a land-mark publication on the modeling and health risk assessment of chemical mixtures. He has served in various capacities at the national and international levels including: U.S. National Academy of Sciences' Sub-committee on Acute Exposure Guideline Levels (2001-'04); Mixtures, Modeling and Risk Assessment Specialty Section Councils of the Society of Toxicology (2004-todate); International Programme on Chemical Safety of World Health Organization (2003-todate); Human Studies Review Board of U.S. EPA (2006-'08); and Workgroup of the International Agency for Research on Cancer (IARC/WHO; 2007). He has also served on the editorial boards of *Toxicological Sciences, International Journal of Toxicology, Journal of Applied Toxicology, Journal of Child Health* and the open-access *Journal of Toxicology*. Listed in *Canadian Who's Who*, Kannan is currently a *Fellow* of the Academy of Toxicological Sciences and *Diplomate* of the American Board of Toxicology.

Professor Peter O'Brien

Accelerated cytotoxic mechanism screening for hepatotoxic drugs

Hepatotoxicity is the commonest cause of drug failure and hepatocytes are the gold standard for in vitro metabolism studies or toxicity testing. Hepatocytes freshly isolated from rats have similar phase 1 & 2 drug metabolizing enzyme activities and GSH levels for several hours as are found in vivo . Most cells lose some of these activities on culturing. Recently we have shown that screening 10 halobenzene analogues to find the relative concentration required to cause 50% cytotoxicity (EC50) using hepatocytes isolated from CYP2B induced rats was similar to the relative dose of each analogue required to cause in vivo hepatoxicity in CYP2B induced rats at 24hrs. Furthermore the QSAR derived halobenzene EC50s equation for normal uninduced hepatocytes was dependent on log P and E_{homo} suggesting cytotoxicity was dependent on the ease of halobenzene oxidation whereas the EC50s for hepatocytes isolated from CYP2B induced rats was dependent on log P and μ (dipole moment) indicating the rate limiting step toxic factor was now the asymmetric charge distribution of the halogen substituents (OBrien PJ 2007 CBI 165,165-174). The low P=0.001 of the QSAR mathematical equations indicates they could be used to predict the in vivo toxicity of other untested halobenzenes and would be a useful alternative laboratory test that could reduce animal testing of toxins. However even more useful information can be obtained by using "Accelerated Cytotoxic Mechanism Screening

(ACMS)" techniques. This involves using cell death as an endpoint which can be used to determine the time point at which the toxic pathway becomes irreversible and the cells cannot be rescued. This varies enormously depending on the toxin and the toxin concentration. Useful rescue agents include traps of toxins or toxin reactive metabolites or reactive oxygen species traps. The role of metabolizing enzymes in activating or detoxifying cytotoxins can also be readily found using the effect of inhibitors of these metabolizing enzymes on cell death (O'Brien 2005 Current Drug Metabolism 6,101-111). Finally redox agents can be used to normalise the cellular redox potential changed by the toxin. Cellular reductive versus oxidative stress can be obtained by determining the ratios for GSH/GSSG, lactate/ pyruvate and β-hydroxybutyrate /acetoacetate. Oxidative stress could be determined from ROS, Fox assays and protein carbonylation. Bioenergetic stress can be measured from mitochondrial membrane potential and ATP/AMP ratios. Liver inflammation that activated Kupffer cells & caused neutrophil infiltration markedly increased hepatocyte susceptibility to drugs by releasing cytokine, myeloperoxidase (MPO) and ROS . A marked increase (x 20-60 fold) in idiosyncratic drug susceptibility was also found when hepatocytes were exposed to non cytotoxic H_2O_2 levels or TNF. ACMS studies showed mitochondrial oxidative stress was the molecular cytotoxic mechanism for hepatocyte death induced by the following drugs:- troglitazone, amodiaquine, hydralazine, isoniazid. Other drug cytotoxicities e.g. ibuprofen,aspirin were not affected by H_2O_2 with or without MPO .This H_2O_2 inflammation model should prove to be a more robust screen for the hepatotoxicity potential of drug candidates.

Biography: O'Brien, Peter John

POSITION TITLE
Professor emeritus (Toxicology), Graduate Department of Pharmaceutical Sciences, Faculty of Pharmacy and Department of Pharmacology, Faculty of Medicine, University of Toronto (1986-).
EDUCATION AND TRAINING
B.Sc. Honours, Biochemistry, Department of Biochemistry, University College London, UK
M.Sc. Radiobiology, Department of Physics, Birmingham University, UK
Ph.D. Biochemistry, (Advisor, Prof. G. Hubscher) Department of Medical Biochemistry, Birmingham University, UK
Post Doc fellowship with Prof., Alistair Fraser, Department of Medical Biochemistry, Birmingham University, UK

ACADEMIC APPOINTMENTS
Senior Research Associate, Department of Medical Biochemistry, Birmingham University, UK (1965-7)
Associate Professor of Biochemistry, Department of Biochemistry, Memorial University of Newfoundland, St. Johnís Nfld. (1974-86)
Professor, Graduate Department of Pharmaceutical Sciences, Faculty of Pharmacy and Department of Pharmacology, Faculty of Medicine, University of Toronto, (1986-)
Graduate Chairman (1987-1999) and Associate Dean of Research (1988-91), Faculty of Pharmacy, University of Toronto. Graduate Coordinator (2003-4)

EDITORIAL BOARDS
Associate Editor, Can. J. of Biochem. (1987-1999), Xenobiotica (1982-1995), Drug Metab. and Drug Interacns. (1988-), Chem. Biol. Interacns. (1992-1998 and 2001-), Journal of Toxicology (2007-)

HONOURS AND AWARDS
Recipient McNeil (Janssen-Ortho) Award for Pharmaceutical Sciences Research (1996)
Recipient of Medical Research Council Development Grant (1986-1996)
Recipient of National Science and Engineering Research Council Operating Grants (1968-2013)

PUBLICATIONS
Senior author of over 340 scientific papers and 15 book chapters

GRADUATE STUDENT SUPERVISION
20 Ph.D. completed and 2 in progress
30 M.Sc. completed and 2 in progress
21 Post Doc fellows and 1 in progress

MAJOR RESEARCH INTERESTS
Molecular Toxicology Mechanisms, Oxidative Stress, Molecular Mechanisms of Cell Death, Screening of drug candidates for safety, QSARs for predicting xenobiotic safety.

Holly Smith

In vitro surrogates, genomics, and in vivo biomarkers: How do they translate to safety in drug development?

The future of efficient drug development includes the need for earlier attrition of candidate compounds, and a large percentage of the attrition rate of compounds is unexpected toxicity. Therefore new techniques and models for toxicity assessment are being developed for implementation earlier in the drug development process. *In vitro* surrogate cell-based assays are developed for high-throughput screening, toxicogenomic evaluations are performed on cell surrogate and *in vivo* samples, and biomarkers are measured in early *in vivo* pharmacology and efficacy studies. How well do these techniques and models translate across one another and how well do they predict the safety of the compounds? We present here preclinical case studies where many of these approaches were applied and the outcomes were evaluated to determine the success of the predictions.

Biography: Smith, Holly

Holly Smith is currently a Senior Research Scientist with Eli Lilly and Company in the Investigative Toxicology Division. She received her BA degree in Microbiology at Miami University (Oxford, Ohio) and has over 20 years of experience in immunoassay development supporting nonclinical toxicology. Her recent investigations include the identification and development of biomarkers for monitoring preclinical drug-induced target organ toxicities. In addition to her biomarker efforts, Ms. Smith has extensive expertise in the assessment of the potential immunogenicity of biotherapeutics and she is co-author on several immunogenicity guidance white papers.

Session d'Affichage
Poster Session

GENOMIC MAPPING OF TCDD ACTIVATED ARYL HYDROCARBON RECEPTOR AND ESTROGEN RECEPTOR ALPHA REVEALS SHARED GENOMIC REGULATION

[*]Shaimaa Ahmed[1], [*]Eivind Valen[2], [*]Albin Sandelin[2] & Jason Matthews[1]

Department of Pharmacology & Toxicology, University of Toronto, Toronto, Ontario, CANADA M5S 2A8[1] The Bioinformatics Centre, Department of Molecular Biology & Biotech Research and Innovation Centre University of Copenhagen, Denmark[2]

In this study, chromatin immunoprecipitation followed by microarray technology (ChIP-chip) was used to compare the direct genomic targets of the aryl hydrocarbon receptor (AHR) and estrogen receptor alpha (ERα). Understanding their genomic regulation will help elucidate the biological importance of AHR and ERα cross-talk. Experiments were completed on chromatin isolated from asynchronous T-47D human breast cancer cells treated with 10 nM of the potent AHR ligand: 2,3,7,8- tetrachlorodibenzo-ρ-dioxin (TCDD) for 1 h. Protein:chromatin complexes were isolated using specific antibodies against AHR and ERα. Immunoprecipitated DNA was linearly amplified and hybridized to Affymetrix® human promoter tiling arrays that contain 25, 500 promoter regions. Data was analyzed using CisGenome® set at a 1% false detection rate. Of the 905 ERα identified regions 214 overlapped (>50 % sequence identity) with the 1191 AHR identified regions following TCDD treatment. Experimental technique was validated using conventional ChIPs and target gene expression levels were measured. Selected regions confirmed the recruitment of AHR and ERα but varied in relative levels and gene expression activation. Furthermore, to investigate whether AHR and ERα were recruited together or separately to the shared regions sequential ChIPs were performed. Results indicate that both AHR and ERα are recruited at the same time in the subset of regions tested. Furthermore, computational analysis of the shared regions revealed that there was an over-representation of AHREs and not ERE suggesting that AHR drives the recruitment of ERα. Knockdown of AHR confirmed that it is required for the recruitment of ERα to the shared regions. However, knockdown of ERα did not affect the recruitment of AHR to the subset of shared regions tested. Overall, AHR modulates the genomic binding patterns of ERα following the addition of the AHR selective agonist TCDD. Our findings confirm the strong functional overlap between ERα and AHR signaling pathways.

Significance of the Observed Cytoplasmic Immuno-localisation of Proliferating Cell Nuclear Antigen (PCNA) in Studies to Evaluate the Safety of A Chemical Food Contaminant.

Syed A. Aziz, Ivan Curran, Genevieve Bondy, Kamla Kapal, Eric Lok, Rekha Mehta.

Toxicology Research Division, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario, CANADA

Summary: Emerging chemical food contaminants must be assessed for their toxicity and potential health effects in order to regulate, make policy decisions and minimize health risks to Canadians. Various validated short-term tests are also applied for determining the potential for carcinogenicity without resorting to conducting long-term cancer bioassays. One such test is the cell proliferation index, which is routinely determined in target organs of the exposed test animal using PCNA as an

immunohistochemical indicator. PCNA is normally expressed in the nuclei of cells in S-phase of the cell cycle. Our focus in this study was to understand the preferential localization of PCNA in the cytoplasm when rats were exposed to the food contaminant, perfluorooctane sulphonate (PFOS). **Objectives/Background/Issue(s):** To maximize food safety for Canadians, an emerging chemical food contaminant, PFOS, was recently assessed in our laboratory for its toxicity, including its potential to cause cancer. When evaluating the cell proliferation index, PCNA staining was predominantly localized to the cytoplasm instead of the nucleus where PCNA is normally expressed. Other toxicity data from the same study showed that PFOS affects lipid metabolism in rat liver, and behaves like a weak peroxisome proliferator (PP). The significance of cytoplasmic expression of PCNA in relation to the PP-like activity of PFOS has, therefore, been investigated by comparing the cellular localization of PCNA in livers of rats treated with ciprofibrate (CPF), a known strong PP and a non-genotoxic carcinogen. **Design/Method/Description:** Rats were exposed to either PFOS in feed (0 - 100 mg PFOS/kg diet) for 28 consecutive days, or to CPF in the diet, according to a tumour inducing regimen for 20 weeks. At necropsy, livers were fixed in formalin, and then processed for PCNA immunohistochemistry. **Outputs/Results:** In control diet rats, nuclear PCNA labeling remained at a base-line steady state level. In contrast, in hepatocytes from both PFOS and CPF treated livers, PCNA-specific staining was primarily localized to the cytoplasm.

Impacts/Outcomes/Conclusions/Implications/Next Steps: Our data show that both CPF and PFOS induce cytoplasmic localization of PCNA, suggesting this as a common phenomenon for PP type of chemical contaminants. PFOS, CPF and other PP are known to interact with the peroxisome proliferator receptor, contributing to dysregulation of lipid metabolism, and leading to altered membrane permeability in the nucleus, mitochondria or the cell. Thus, cytoplasmic location of PCNA may be one consequence of these cellular events. Further delineation of the significance of our observation may facilitate the application of *cytoplasmic* PCNA as a biomarker that is specific for PP-type chemical contaminants and/or non-genotoxic carcinogens.

IN UTERO EXPOSURE TO BENZENE CAUSES REACTIVE OXYGEN SPECIES-MEDIATED ALTERATIONS IN HEMATOPOIETIC PROGENITOR CELL GROWTH.

H.J. Badham[1], L.M. Winn[1,2]

[1]Department of Pharmacology and Toxicology and [2]School of Environmental Studies, Queen's University, Kingston, Ontario, Canada, K7L 3N6

Leukemia is the most prevalent childhood cancer and its incidence has increased by 20% within the last two decades. It is hypothesized that the primary cause of this increase in leukemia is *in utero* exposure to environmental pollutants such as benzene. Epidemiological studies have correlated *in utero* benzene exposure with an increased incidence of childhood leukemia. In addition, studies have shown that *in utero* exposure to benzene in mice increases the number of erythroid and myeloid progenitor cells in fetal tissue. The molecular mechanisms behind benzene-initiated leukemia after *in utero* exposure are unknown. One proposed mechanism is that the bioactivation of benzene causes an increased production of reactive oxygen species (ROS), which leads to deregulation of the cell cycle. Our first objective was to determine if *in utero* exposure to benzene causes an increase in ROS in fetal liver tissue, which is the primary site of hematopoiesis. Our second objective was to determine if deregulation of blood cell development after *in utero* benzene exposure is abrogated by pretreatment with the antioxidative enzyme catalase. Pregnant C57Bl/6N mice were injected i.p. on gestational days (GD) 8, 10, 12, and 14 with either vehicle (corn oil) or 200 mg/kg benzene. In addition, these mice were also injected i.p. with either PBS or

25 KU/kg PEG-catalase 16 hours prior to each oil or benzene injection. To measure ROS, a single cell suspension of GD 16 fetal liver tissue was incubated with the ROS sensitive dye dichlorofluorescein diacetate and fluorescence intensity was measured using flow cytometry. In addition, GD 16 fetal liver cells were plated in semi-solid media and incubated for 3-12 days after which colony-forming units (CFU) and blast-forming units (BFU) of the erythroid lineage (CFU-E and BFU-E), and of the myeloid lineage (CFU-granulocyte, CFU-monocyte, and CFU-granulocyte/monocyte) were counted using a light microscope. Our results show that in utero exposure to benzene causes a significant increase in ROS in the fetal liver and this effect is abrogated by pre-treatment with catalase. In addition, catalase protects against benzene-induced alterations in CFU-E, BFU-E, CFU-M, CFU-G, and CFU-GM colony numbers. These results suggest that ROS play a key role in the development of *in utero*-initiated benzene toxicity. The growing incidence of childhood leukemia and the prevalence of benzene contamination in our environment emphasizes the need for further research in this area. (Support: CIHR)

CONTRIBUTION OF TOXICOKINETIC MODELING FOR THE STUDY OF THE IMPACT OF PHYSICAL EXERTION ON THE ASSESSMENT OF OCCUPTIONAL EXPOSURE TO ACETONE

A. Bérubé[1], G. Charest-Tardif[1], I. Sari-Minodier[2], R. Tardif[1]
1 GRIS, Université de Montréal, Montréal, Québec, H3T 1A8, 2 Laboratoire de biogénotoxicologie et mutagenèse environnementale, Aix-Marseille Université, France.

This study aims at assessing the impact of workload on urinary levels of acetone (ACE) and its potential influence in the previous determination of the corresponding biological exposure indices (BEI). A physiologically based pharmacokinetic (PBPK) model was developed and validated in order to simulate weekly occupational exposure (8h/day, 5 days) to ACE at the threshold limit value (TLV) according to the American Conference of Governmental Industrial Hygienists (500 ppm). Simulations were then conducted under workload levels corresponding to rest (12.5 W), 25 W and 50 W and the impact on the urinary level of ACE at the end of the last shift was studied in a typical worker. Other values of ACE excretion such as blood level and expired air concentration for different workloads were also examined. The predicted values were compared to the results of both experimental and field studies which supported the adoption of the current BEI (50 mg/L) for ACE. For an exposure to TLV, the end-of-shift values for workloads of 25 W and 50 W showed 1.6-fold and 3.5-fold increases compared to the value at rest (27.9 mg/L), respectively. These results show that not taking into account the workload level leads to an underestimation of the actual exposure for workers whose physical activity is as great as 50 W. Also, there was a slight accumulation of ACE among the week (5-days), with a prior-to-shift value of 6.9 mg/L at 50 W on the last day, which is about 4.5 higher than the given endogenous level (1.5 mg/L). The PBPK model described well the magnitude of the significant impact of workload on ACE BEI and internal exposure. The predicted values revealed that workers with heavy tasks may be at greater risk. Overall, these results showed that workload should be taken into account when interpreting biomonitoring exposure indicators for ACE for an optimal protection of workers. (Supported by Afsset, France and IRSST, Canada)

INVESTIGATING APOPTOTIC AND NECROTIC PATHWAYS INDUCED BY AMIODARONE AND ITS MAJOR METABOLITE DESETHYLAMIODARONE.

*JE Black, JF Brien, WJ Racz and TE Massey. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, K7L 3N6.

Amiodarone (AM) is a potent antidysrhythmic agent associated with several adverse effects, one of which is potentially life-threatening pulmonary fibrosis. The mechanisms involved in amiodarone-induced pulmonary toxicity (AIPT) have yet to be fully elucidated. Desethylamiodarone (DEA) is a primary metabolite of AM and may contribute to the pathogenesis of AIPT. The objective of this study was to characterize the cytotoxic pathways activated by AM and DEA using the HPL1A human peripheral lung epithelial cell line as a model of target cells in AIPT. Determination of apoptotic cell death was achieved by annexin-V-FITC staining with flow cytometric analysis and detection of activated caspase-3 was measured by immunoblotting. Necrotic cell death was determined by propidium iodide staining, followed by flow cytometric analysis. Treatment with 20 μM AM for 24 h increased the percentage of necrotic cells from 10.8% (control) to 81.4%, but decreased the percentage of apoptotic cells from 9.1% (control) to 1.9% ($p<0.05$). In contrast, treatment with 3.5 μM DEA for 24 h increased the percentage of cells exhibiting necrosis from 10.8% (control) to 20.5%, and increased the percentage of apoptotic cells from 9.1% (control) to 21.5% ($p<0.05$). Treatment with DEA, but not AM, increased intracellular levels of activated caspase-3. In addition, DEA induced cell death at an earlier timepoint and at lower concentrations than AM, confirming that DEA is a more potent cytotoxicant than AM. Hence, despite a difference of only one ethyl group in chemical structure, AM activates primarily necrotic pathways, whereas DEA activates both necrotic and apoptotic pathways. In view of these results, therapeutic treatments targeting AIPT will have to address cell death pathways initiated by both AM and DEA. (Funded by CIHR Grant No. MOP-13257). Keywords: amiodarone, desethylamiodarone, cytotoxicity.

Polychlorinated Biphenyls and Dioxin-Like Activity in n-3 Polyunsaturated Fatty Acid Supplements

Julie A. Bourdon[1,2], Tania M. Bazinet[2], Trevor T. Arnason[2], Lynda E. Kimpe[2], Jules M. Blais[2], Paul A. White[1]*

[1]Mechanistic Studies Division, Safe Environments Directorate, Healthy Environments and Consumer Safety Branch, Health Canada.
[2]Center for Advanced Research in Environmental Genomics, Department of Biology, University of Ottawa.

Abstract

This research investigated total polychlorinated biphenyls (PCBs) and dioxin-like activity in 17 omega-3 fatty acid supplements (e.g., fish, mammal and plant derived oils). Total PCBs were measured using gas chromatography-electron capture detection and dioxin-like activity was measured using the DR-CALUX assay. Results for total PCBs ranged from 0.1 to 792.9 ng g-1 oil, with salmon and seal yielding the highest values. Values for dioxin-like activity ranged from 1.3 to 72.1 pg TEQ g-1 oil, with salmon and tuna yielding the highest values. Considering the average daily dietary intake of the contaminants, an average adult body weight of 70 kg and manufacturer dosing recommendations, it was determined that dietary supplementation with five of the products can cause exceedance of the upper limit of the World Health Organization tolerable daily intake (TDI) (4 pg kg-1 body weight day^{-1}) for dioxins. Only one product can cause

exceedance of the TDI for total PCBs (20 ng kg^{-1} body weight day^{-1}). The beneficial properties of fish oil and the results of this study suggest that it is prudent to consume fish supplements derived from small, short lived fatty fish. Nonetheless, quality control measures implemented by the manufacturer will be important determinants of supplement contamination.

TOXICOLOGY OF URBAN PARTICULATE MATTER: *IN VITRO* AND *IN VIVO* BIOASSAYS

Dalibor Breznan[1,2,3], PhD; Marcelle Phaneuf[1], MSc; Yunus Siddiqui[1], MSc; and Renaud Vincent[1,2], PhD

[1]*Inhalation Toxicology and Aerobiology Section, HECS, Health Canada, Tunney's Pasture, Ottawa, ON, K1A 0K9*

[2]*University of Ottawa, Faculty of Medicine, Department of Biochemistry, Microbiology and Immunology, 451 Smyth Road, Ottawa, ON, K1H 8M5*

Current Address: [3]*In Commerce Substances Unit, Chemicals Management Directorate, HECS, Health Canada, 123 Slater Ave, Ottawa, ON, K1A 0K9; Contact:* (**dalibor_breznan@hc-sc.gc.ca**)

Exposure to air particulate matter (PM) is epidemiologically associated with increased incidence of respiratory and cardiovascular disease in humans. Although inflammation, oxidative stress and toxicity have been implicated in the pathophysiological response to air pollution, the physicochemical properties of the particles responsible for the adverse effects are yet to be elucidated. *In vitro* assays are widely used to study the toxicity of particles, however, they have not been comprehensively validated *in vivo*. This work aimed to evaluate *in vitro* cytotoxicity and inflammatory cytokine assays as screening tools for relative toxicity of PM, validate their capacity to predict toxicity of PM in BALB/*c* mice, develop a cell-interaction model for the study of the biological effects of PM, and evaluate its performance in relation to PM effects in animals. Results show that *in vitro* assays conducted in macrophage (J774A.1) and alveolar epithelial (A549) cell lines can discriminate between PM, based on their cytotoxic and inflammatory potency. SRM- and EHC-type particles were more potent than DWR1 ($PM_{2.5}$) and mineral particles both *in vitro* and *in vivo*, although PM rankings varied considerably between assays. PM with different compositions had different toxic potencies. Combinations of 2 to 4 *in vitro* assays showed a predictive potential for PM-induced effects *in vivo*, represented by bronchoalveolar lavage (BAL) neutrophilia in mice. In a co-culture, epithelial (A549) and macrophage (THP-1) cells synergistically modulate their production of pro-inflammatory cytokines (IL-1β, IL-6, IL-8, MCP-1 and TNF-α), ICAM-1 and VEGF in response to PM exposure. Cellular mediators from PM-exposed co-cultures can activate endothelial cells (HPAE) to produce cytokines (IL-6, IL-8, GM-CSF, MCP-1) and adhesion factors (ICAM-1, VCAM-1 and E-selectin). Exposure of mice to EHC-6802, DWR1 and SRM-1650 particles led to BAL neutrophilia and elevated cytokines (IL-6, KC, MIP-1α, TNF-α), with a stronger inflammatory response at 2 hrs then 24 hrs. Statistical comparison of the cell interaction model responses with PM-induced BAL neutrophilia *in vivo* shows that the co-culture model correlates well with biological effects of particles in mice. Along with its ability to simulate physiologically relevant cell-cell interactions, it is a good model for studying mechanisms of PM toxicity, and for relative toxicity screening of particles.

TEMPERATURE INSENSITIVITY OF NON-EXPRESSING TM16 MUTANTS OF MRP1 (ABCC1)

Marina Chan*, Gwenaëlle Conseil* and Susan P.C. Cole.

Department of Pharmacology & Toxicology and Division of Cancer Biology & Genetics, Cancer Research Institute, Queen 's University, Kingston, ON, K7L 3N6

MRP1 (ABCC1) plays an important role in cancer drug resistance and the body's defense against xenobiotics such as aflatoxin B. MRP1 transports a wide range of compounds including conjugated exogenous and endogenous organic anions, some of which are involved in immune responses (e.g. leukotriene C4). Previous studies have identified MRP1 mutant proteins R1202D and E1204K as being poorly expressed in mammalian cells although their mRNA levels were comparable to cells expressing wild-type MRP 1 [1]. These mutations, which involve residues predicted to be in TM1 6, appear to disrupt the folding of MRP1 such that the transporter cannot acquire or maintain its native three-dimensional structure and so is targeted for degradation. The goal of the present study was to determine whether expression of these two mutants (R1202D and E1204K) could be rescued by incubation of cells expressing these proteins at reduced temperatures. Thus HEK293T cells were transiently transfected with wild-type and mutant pcDNA3.1 expression vectors and 24 hr later, cells were incubated either at 37 ºC or 30 ºC for 48 hr. Whole cell lysates were prepared and MRP1 levels determined by immunoblotting. Cells were also examined by confocal microscopy. In cells transfected with wild-type or mutant vectors, a mixture of fully glycosylated (190 kDa) and underglycosylated immature (170 kDa) MRP1 proteins was observed when the incubation temperature was lowered from 37 ºC to 30 ºC. However, even at the lower temperature, the levels of the MRP1 mutants remained just 30 –40% of wild-type MRP1 levels at 37 ºC. As the temperature was lowered from 30 ºC to 27 ºC, there was a greater proportion of both wild-type MRP1 and mutants being expressed as the immature form. Confocal microscopy showed that at these temperatures, both mutants displayed membrane localization patterns comparable to wild-type MRP1 suggesting these mutations disrupt folding of MRP1 at a post-translational stage leading to early degradation of the mutant proteins even though they can still traffic to and insert into the plasma membrane. We conclude that the relative temperature insensitivity of the mutant proteins together with their similar pattern of localization as wild-type MRP1 at reduced temperatures indicates that the mutations disrupt the kinetic and/or thermodynamic stability of the nascent MRP1 polypeptide, which cannot be compensated for by lower temperatures.

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VALIDATION OF EXTENDED STABILITY AND OF A SINGLE PLATFORM METHOD FOR IMMUNOPHENOTYPING AND ABSOLUTE LYMPHOCYTE COUNTS IN CYNOMOLGUS MONKEY WHOLE BLOOD SAMPLES BY FLOW CYTOMETRY.

S. Corneau[1], H. Khalil[1], C. Dumont[1], A. Jawad[1], M. Kakkar[1], J. Huang[1] and L. LeSauteur[1]

[1]*Immunology, Charles River Laboratories, Preclinical Services Montreal Inc., Montreal, Canada*

Email: *Hayssam.khalil@crl.com*

Immunophenotyping is a tool often used for determination of the immunotoxicity potential of compounds in preclinical research. Growing demand for this type of assay has made the analysis of freshly collected blood samples a challenge. The purpose of this study was thus to validate an extended stability period for Cynomolgus monkey whole blood samples collected in Cyto-Chex BCT® tubes. BCT® tubes contain stabilizing agents which allow for preservation of blood samples prior to staining. Single platform method for absolute lymphocyte counts using Trucount™ tubes was also validated. Samples were analyzed by flow cytometry to determine the relative percentages of the lymphocyte populations of interest (T [total, helper and cytotoxic], B and NK). Results obtained in BCT® tubes were compared to those obtained in EDTA tubes with regards to sample stability, reactivity and precision. Extended stability was achieved when using BCT® tubes. Now, samples can be stored for up to 5 days after collection, processed and stored for another 24h prior to analysis. Antibody reactivity results obtained were similar and all markers under study met the acceptance criteria for the assay precision, regardless of the type of tube used. The absolute lymphocyte counts obtained by flow cytometry or from an automated counter were similar. The single platform method is thus suitable for its intended use. This extended stability will now allow shipping and improve the processing capacity of Cynomolgus monkey whole blood samples.

Methylmercury Altered Cellular Distribution and Protein Expression of Paraoxonase-2 (PON2), Thioredoxin Reductase-1 (TrxR1), Glutathione Peroxidase-1 (GPx1) in Human Coronary Artery Endothelial Cells (HCAEC)

Melanie Coughlan, Nikia Kearns, Jessica Skof, Yan Jin, Xiaolei Jin

Toxicology Research Division, Food Directorate, Health Canada

Hypothesis: Epidemiological studies revealed that high MeHg body burdens were associated with increased risk of cardiovascular diseases. However, the underlying mechanisms remain unclear. We hypothesize that MeHg may contribute to the pathogenesis of cardiovascular disease by attacking antioxidant enzymes leading to endothelial dysfunction. This study examined the effects of MeHg on cell viability and morphology, and distribution and expression of PON2, TrxR1, and GPx1 in cultured HCAEC.

Methods: HCAEC were treated with 0-250 µM MeHg for 24 h. Cell viability was determined by MTT assay. Cellular distributions of PON2, TrxR1, and GPx1 were examined in cells treated with 7.5 µM

MeHg (LD_50) for 3 or 6 hours using immunofluorescent staining. Protein expressions were determined by Western blots.

Results: MeHg decreased cell viability in a dose-dependent fashion. MeHg at $_{LD50}$ altered cell morphology even at 3 h after dosing, with loss of cell connection and attachment, and transformation from protruding to more round shapes. In the control cells, PON2 was visualized as microtubule-like filaments covering the whole cytoplasmic region, but diffused in the nuclei. TrxR1 was diffused in both the cytoplasm and nuclei, while GPx1 was associated with lysosome-like granular structures in the cytoplasm. MeHg increased the intensity and granularity of PON2 staining in the nuclei, and disrupted filamentous staining in the cytoplasm. MeHg induced a decreased and punctuated cytoplasmic staining and an increased nuclear staining of TrxR1, and decreased or abolished cytoplasmic staining of GPx1. These effects of MeHg intensified with duration of exposure. MeHg markedly increased PON2 protein expression in a time-dependent fashion, slightly increased GPx1 protein expression only at 3 h after treatment, but had not effects on TrxR1 protein expression.
Conclusions: These results suggest that cytoskeleton proteins are primary targets of MeHg, and GPx1 may be more sensitive than PON2 and TrxR1 to MeHg-induced structural damage. The compensatory increase in PON2 protein expression along with the nuclear translocation of TrxR1 in MeHg-treated cells implies an important role of these antioxidant enzymes in regulating stress response, protecting structural integrity and function, and repairing oxidative damage in HCAEC.

IDENTIFICATION OF THYROID HORMONE RECEPOTR BINDING SITES AND TARGET GENES USING ChIP-on-chip IN DEVELOPING MOUSE CEREBELLUM

Hongyan Dong[1*], Carole L. Yauk[2], Andrea Rowan-Carroll[2*], Seo-Hee You[3*], R. Thomas Zoeller[3*], Iain Lambert[4*], Michael G. Wade[1]
1 Hazard Identification Division; 2 Mechanistic Study Division, EHSRB, Health Canada, 50 Columbine Driveway, Ottawa, Ontario, Canada, K1A 0K9; 3 Molecular & Cellular Biology Program, University of Massachusetts, Amherst, MA, USA, 01003; 4 Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, Canada, K1S 5B6

Thyroid hormone (TH) is critical to normal brain development, but the mechanisms operating in this process are poorly understood. We used chromatin immunoprecipitation to enrich regions of DNA bound to thyroid receptor beta (TRβ) of mouse cerebellum sampled on post natal day 15. Enriched target was hybridized to promoter microarrays (ChIP-on-chip) spanning -8 kb to +2 kb of the transcription start site (TSS) of 5000 genes. We identified 91 genes with TR binding sites. Roughly half of the sites were located in introns, while 30% were located within 1 kb upstream (5') of the TSS. Of these genes, 83 with known function included genes involved in apoptosis, neurodevelopment, metabolism and signal transduction. Two genes, MBP and CD44, are known to contain TREs, providing validation of the system. This is the first report of TR binding for 81 of these genes. ChIP-on-chip results were confirmed for 10 of the 13 binding fragments using ChIP-PCR. The expression of 4 novel TH target genes was found to be correlated with TH levels in hyper/hypothyroid animals providing further support for TR binding. A TRβ binding site upstream of the coding region of myelin associated glycoprotein was demonstrated to be TH-responsive using a luciferase expression system. Motif searches did not identify any classic binding elements, indicating that not all TR binding sites conform to variations of the classic form. These findings provide mechanistic insight into impaired neurodevelopment resulting from TH deficiency and a rich bioinformatics resource for developing a better understanding of TR binding.

ASSESSMENT OF HAIR AND BONE AS POTENTIAL BIOINDICATORS OF BERYLLIUM EXPOSURE

Katherine Drolet-Vives*, Joseph Zayed, Sébastien Sauvé*
Université de Montréal, Montréal, Québec, H3C 3J7

The objective of this research is to assess hair and bone as biological indicators of Beryllium (Be) exposure. Two groups of mice, C3H/HEJ, were exposed to two different particles sizes Be metal (< 2.5 μm, called here fine Be or Be-F and < 10 μm, called here inhalable Be or Be-I) during three weeks of nose-only inhalation exposure at a target concentration of 250 μg m^{-3}. Another group exposed to HEPA filtered air was used as a control group. Mice were sacrificed either 1 or 3 weeks (this last duration is only related to a sub-group of Be-F) after the end of exposure. Mice were shaved and the bones were extracted. Hair and bones were wet digested in open vessel with nitric acid and 30% (v/v) hydrogen peroxide, and with nitric acid only respectively. These solutions were filtered and the volume was adjusted with MilliQ distilled water. The Be content in hair and bones was determined using an inductively coupled plasma - atomic emission spectrometer (ICP-AES). For washed hair, results of mice sacrificed one week after the end of exposure 8.3 ± 1.4 μg kg^{-1} for the control group, 114 ± 42 μg kg^{-1} for Be-I, and 159 ± 65 μg kg^{-1} for Be-F. These results are different with those related to unwashed samples since the mean Be concentrations for Be-I and Be-F were 654 ± 808 μg kg^{-1}, and 754 ± 386 μg kg^{-1} respectively. Results of mice from Be-F sacrificed three weeks after the end of exposure showed an average Be concentration in washed hair of 419 ± 100 μg kg^{-1}. It is than possible to hypothesis that excretion of Be in hair increases with time. Be concentration in bones were 6 ± 3 μg kg^{-1} for the control group, compared to 24 ± 7 μg kg^{-1} for Be-I and 34 ± 6 μg kg^{-1} or 43 ± 8 μg kg^{-1} for Be-F sacrificed one or three weeks after the end of exposure respectively. These results demonstrate the potential of using hair and bone as bioindicators of Be exposure.

HEAVY METAL CONCENTRATIONS IN MUSSELS FROM THE BAIE DES CHALEURS, NEW BRUNSWICK

Marc Fraser*[1] <<SPON: Cathy Vaillancourt>>, Céline Surette*[1]<<SPON.: Cathy Vaillancourt>>, Cathy Vaillancourt[2]
[1]Université de Moncton, Moncton, Nouveau-Brunswick, E1A 3E9
[2]INRS – Institut Armand-Frappier, Laval, Québec, H7V 1B7

The Baie des Chaleurs ecosystem is subject to pressure from industrial activities, such as thermal plants and a lead smelter, that can cause heavy metal contamination. This contamination can lead to bioaccumulation in the aquatic food web and thus can contaminate the fishery resources. Important lead and cadmium contamination in the Belledune area and mercury contamination in the Dalhousie area has been reported in the last 30 years. Heavy metals, even at low concentrations, can have negative impact on the health and the wellbeing of the population. Local inhabitants have expressed concerns and are anxious for the potential consequences of environmental contamination on their health. These concerns are stimulated by reported higher rates of death, cancer and hospitalization in this area than in the rest of the province of New Brunswick. Thus, our global objective is to better understand the links between environmental contaminants found in fish and seafood and human health at a community level, especially in communities relying heavily on the fishery resources. To achieve this goal, our first step is to establish a spatio-temporal distribution of heavy metals in the aquatic biota present in the Baie des Chaleurs. Our hypothesis is that

sites closer to industries will be more contaminated and that the further sites are from the industries, the lower heavy metal concentrations in biota will be. Furthermore, since anti-pollution mechanisms have been implemented, levels of heavy metals in the environment and in biota should decrease year after year. In 2006, we sampled blue mussels in 6 different sites (25 mussels per site). In 2008, we sampled the same 6 sites and added 3 new sites (30 mussels per site). We chose to sample blue mussels as they are particularly good bioindicators due to their reduced mobility, their ubiquity, and their feeding habits. Heavy metal concentrations in blue mussels (Mytilus edulis) were determined. Our preliminary results reveal mercury levels between 0.117 and 1.04 µg/g (ww) and the lead levels between 6.82 and 89.6 µg/g (ww) and cadmium rates between 1.24 and 7.71 µg/g (ww). In Canada, the allowable lead and mercury content in fish and seafood that are sold commercially are established at 0.5 µg/g (ww). Our results show that these values can be reached in mussels from contaminated area of the Baie des Chaleurs. In fact, fishing restrictions are in place in industrial areas of Belledune and Dalhousie.

EFFET DE L'EXPOSITION MULTIVOIE SUR LA DOSE INTERNE DU N-HEXANE ET CYCLOHEXANE CHEZ LE RAT

Michelle Gagné[1]*; Ginette Charest-Tardif[1*]; Kannan Krishnan[1]; Robert Tardif[1]

[1]GRIS – Santé environnementale et santé au travail, Université de Montréal, Montréal, QC, Canada, H3T 1A8.

La contribution de l'inhalation et de la voie percutanée à l'exposition totale de composés organiques volatils (COVs) présents dans l'eau potable est une problématique qui suscite un intérêt grandissant en santé publique et au niveau réglementaire. Jusqu'à récemment, seule l'ingestion était considérée dans l'évaluation du risque des contaminants de l'eau. L'objectif de ce projet était de caractériser l'effet de l'exposition multivoie à deux COVs, le n-hexane (HEX) et le cyclohexane (CYCLO), sur leur dose interne chez le rat à partir d'un modèle expérimental animal. Des études préliminaires ont d'abord montré que la voie percutanée n'était pas significative dans le cas de ces deux composés. Dans un premier temps des groupes de 5 rats Sprague-Dawley ont reçu une dose unique d'HEX (5,5 or 22,3 mg/kg) par voie orale ou ont été exposés à 50 ou 200 ppm (2h) par inhalation. Pour le CYCLO, les doses orales étaient respectivement de 27,9 et 108,9 mg/kg, alors que les concentrations d'inhalation étaient de 300 ou 1200 ppm (2h). Dans un deuxième temps, des groupes supplémentaires ont été exposés simultanément par les deux voies (orale et inhalation) aux hautes doses pour l'HEX, et aux faibles et fortes doses pour le CYCLO. À partir de prélèvements sanguins (25-200µl) nous avons, dans chaque cas, caractérisé la cinétique du HEX ou du CYCLO durant la période post-administrations. En général, les niveaux sanguins obtenus lors de l'exposition multivoie aux hautes doses d'HEX étaient plus élevés que la somme des niveaux associés aux expositions par voie unique (45 à 70 %) contrairement au CYCLO pour lequel les niveaux étaient similaires. Ces résultats suggèrent que la dose interne résultant d'une exposition multivoie ne peut pas toujours être prédite correctement (ex, HEX) en additionnant simplement les doses internes mesurées après exposition par voie unique. Ce phénomène serait explicable par les caractéristiques propres aux voies d'absorption ainsi que par le degré de saturation du métabolisme pouvant résulter d'une exposition multivoie à des doses suffisamment élevées. Projet financé par ExxonMobil et CRSNG (CRD-335163)

THE ADAPTIVE RESPONSE TO 3-METHYLCHOLANTHRENE IS ALTERED IN THE LIVER OF ADRENALECTOMIZED RATS

Anne K. Mullen **Grey** and David S. Riddick
Department of Pharmacology & Toxicology, University of Toronto, Toronto, Ontario M5S 1A8

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates most effects of aromatic hydrocarbons, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 3-methylcholanthrene (MC); a prototypical response is induction of cytochrome P450 1A1 (*CYP1A1*). The *in vivo* regulation of hepatic AHR and whether changes in AHR levels affect aromatic hydrocarbon responsiveness are not fully understood. We previously found that the level of hepatic AHR protein was depleted by 50-60% in male adrenalectomized (ADX) rats four days after surgery. In order to study the consequences of AHR protein depletion following ADX, we treated ADX and SHAM-operated rats with MC (20 mg/kg by gavage) and measured expression of aromatic hydrocarbon-responsive genes in the liver at 6 and 54 h post-MC. At four days after surgery, hepatic AHR protein was depleted by 75% in ADX rats relative to SHAM. At 6 h post- MC, livers from SHAM and ADX rats had no detectable AHR protein in cytosol or homogenate. At six days after surgery and 54 h post-MC, hepatic AHR protein level was increased by 70% by MC in ADX but not SHAM rats; a difference in the level of hepatic AHR protein between vehicle-treated SHAM and ADX rats was no longer apparent at this later time-point. Hepatic CYP1A1 mRNA was induced ~1200- and ~600-fold by MC at 6 h in SHAM and ADX rats, respectively. At 54 h post-MC, CYP1A1 mRNA was induced ~4- and ~70-fold in SHAM and ADX rats, respectively. While CYP1A1 protein was not detected in liver homogenate from vehicle-treated rats, the level of CYP1A1 protein in MC-treated ADX rats was 60% less than SHAM at 6 h and 3-fold greater than SHAM at 54 h post-MC. Hepatic NADPH-cytochrome P450 reductase (CYPRED) mRNA was induced by 50% by MC in SHAM rats at 6 h, with no induction by MC in ADX rats. However, CYPRED mRNA was induced by 80% by MC in ADX rats at 54 h, with no induction by MC in SHAM rats at this later time-point. Auto-regulation of the AHR by its own ligands has been reported. In our study, AHR mRNA induction by MC followed a similar pattern to that seen for CYPRED mRNA. Hepatic AHR mRNA was elevated 4-fold by MC in SHAM rats at 6 h, with no induction by MC in ADX rats at 6h. MC induced hepatic CYP1A2 mRNA to a similar extent in SHAM and ADX rats at 6 and 54 h. The results observed with CYP1A1 mRNA/protein and CYPRED and AHR mRNA suggest that the adaptive response to MC, an AHR ligand, at 6 h is impaired in the liver of ADX rats relative to SHAM in a gene-dependent manner. This diminished hepatic response to MC corresponds to the decrease in AHR protein seen at four days post-surgery. However, the effect of MC on some, but not all, responsive genes is sustained and enhanced at the later time-point in ADX rats. We are studying whether genes down-regulated by MC respond in a similar manner to the inducible genes reported here. We plan to compare the pharmacokinetics of MC in SHAM and ADX rats to determine if reduced clearance of MC is responsible for the sustained effect of MC in ADX rats.

This study contributes to our understanding of how AHR levels affect the response to aromatic hydrocarbons *in vivo* and the physiological factors that contribute to this altered response.

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THE EFFECTS OF PATERNAL EXPOSURE TO CYCLOPHOSPHAMIDE ON THE RATE OF SPERMATOZOAL DECONDENSATION AND THE PATTERNING OF CHROMATIN REMODELING IN EARLY POST-FERTILIZATION ZYGOTES

L. Grenier[1], B. F. Hales[1], B. Robaire[1,2]

Departments of Pharmacology and Therapeutics[1], and Obstetrics and Gynecology[2],
McGill University, Montréal Québec H3G 1Y6

Following fertilization, the entry of the mature spermatozoon triggers the mature oocyte to complete its second meiotic division; oocyte proteins are activated to induce sperm decondensation. Sperm decondensation is divided into four distincts stages: condensed, type *a* (partially decondensed), type *b* (totally decondensed) and type *c* (recondensed) nuclei. Once the spermatozoon has started decondensing, chromatin remodeling is initiated, during which protein exchange occurs and sperm specific proteins are replaced with maternal histones. Cyclophosphamide (CPA) is an anticancer and immunosuppressive agent, causing DNA crosslinks and strand breaks; it is used as a model male mediated developmental toxicant. Paternal exposure to CPA results in time specific and dose dependent adverse effects on progeny outcome. The treatment of male Sprague Dawley rats with a chronic low dose of CPA, 6mg/kg/day for 4 weeks, targets highly sensitive post meiotic male germ cells undergoing chromatin remodeling. The proper packaging of the male genome is essential for delivery to the oocyte and the early embryonic development. We hypothesize that paternal exposure to CPA during spermiogenesis leads to improper packaging of the male genome, disturbing chromatin decondensation and remodelling in early zygotes. Our objectives were to determine when, during sperm decondensation, post-translational modified histones are present on the paternal chromatin in control group and then assess whether CPA alters the time required for sperm decondensation or the patterning of chromatin remodeling. Control and CPA treated male Sprague Dawley rats were mated overnight to naturally cycling females; early zygotes were collected the following morning at 9am and prepared for analysis by immunofluorescence and confocal microscopy. The classification of sperm decondensation stages was determined by the chromatin compaction level of spermatozoon stained with propidium iodide. Sperm decondensation occurred first at the base, then the tip and finally in the center region of the sperm head. Our results suggest that the progression of zygotes over the sperm decondensation stages is accelerated significantly in type *c* sperm nuclei in zygotes sired by CPA males compared to their respective controls. The pattern and distribution of modified histones during chromatin remodeling was assessed with H4ack12 and H3S10ph. We propose two distinct patterns of chromatin remodeling. H4ack12, a marker for open chromatin structure, had a homogenous staining, whereas H3S10ph, a marker for condensed chromatin structure, displayed a ring-like staining around the sperm head. Chromatin remodeling varied significantly along the longitudinal section of spermatozoa within and between sperm decondensation stages but was not affected by paternal exposure to CPA. We propose that paternal exposure to CPA disturbs the compaction of chromatin structure of male germ cells, affecting the rate of sperm decondensation, without altering the distribution pattern of modified histones in early post-fertilized zygotes. Supported by CIHR

CHEMICAL ASSESSMENT OF A WHOLE SMOKE EXPOSURE SYSTEM

N. Kaur*[1-2], M. Lacasse*[2], K.C. Waldron*[1], A. Fürtös*[1], J-L. Cabral*[2], A. Morin*[2]
"(SPON: Michel Charbonneau)"

1 *Department of Chemistry, Université de Montréal, Montréal, Canada, H3C 3J7*
2 *Imperial Tobacco Canada Ltd, Montréal, Canada, H4C 3P6*

Email: *nkaur@itl.ca*

Most of the research relating cigarette smoke to its biological activity has been based on either exposing cells to cigarette smoke condensate (C SC) or direct *in vivo* studies. Unfortunately, both methods are limited in terms of their exposure techniques. Some studies indicate that the vapor phase may be responsible for the majority of the toxicological response. Thus, exposure of cells to more than just the CSC should be more biologically relevant. The Borgwaldt RM-20S™ smoke delivery instrument in combination with Transwell™ technology is a relatively recent *in vitro* technique that allows for direct whole cigarette smoke exposure to cells at the air-liquid interface. Unfortunately, the dosing method of the RM-20S system has not been chemically characterized. The objective was to assess the reliability of the smoke delivery of the instrument, by evaluating markers of both of the particulate and vapor phases; and secondly, to determine the biological effects of each phase (particulate and vapor) on a specific biological system. For particulate phase measurements, reliability of the smoke dilution, syringe and run order were assessed over three days by measuring solanesol, a tobacco marker, using liquid chromatography with ultraviolet detection (LC/UV). Using a Cambridge filter pad (in place of the exposure chamber) to collect the particulate phase delivered, the precision was found to range between 5.7 and 14%. The cigarette tips were analyzed for solanesol content to evaluate the puffing precision of the instrument. The precision (%RSD) associated with the latter was approximately 12%. The precision of vapor phase delivery was estimated with a methane gas standard used for the instrument calibration and analyzed with a hydrocarbon analyzer equipped with a flame ionization detector (FID). The precision was determined to be between 0.4 and 4.7%. The amount of particulate and vapor phase generated by the instrument was considered to be reproducible when taking into account the error due to the puffing precision of the instrument and the analytical measurement itself. Finally, the neutral red uptake (NRU) assay was employed to compare the exposure of human alveolar basal epithelial cells (A549 cells) to whole smoke versus the vapor phase only. The results confirm that the vapor phase plays a key role in the toxicological response of the exposure in this particular system. In summary, this instrument offers a convenient and realistic system allowing for the exposure of cells directly to whole tobacco smoke.

Direct Analysis of Cell Lysates by MALDI-TOF-TOF-MS for Particle-Induced Biological Changes

P. Kumarathasan, D. Das, S. Mohottalage, Y. Siddiqui, K. Subramanian and R. Vincent.
Safe Environments Programme, Health Canada, Tunney's Pasture, Ottawa, Ontario, Canada, K1A 0K9.

Urban air pollutants are associated with adverse cardiorespiratory and central nervous system effects. The mechanisms of adverse acute health impacts of particles at low exposure levels remain obscure. Particulate air pollutants exhibit complex chemical compositions based on source emissions, atmospheric reactions and size characteristics. We have used a proteomic approach to compare the effects of 10 types of particles

in the J774 murine macrophage cell line: TiO2, SiO2, urban dust samples, diesel exhaust particles (DEP), carbon black and a $_{PM2.5}$ sample. Cells were exposed to the particles at 0-100 $\mu g/cm^2$ (96-well plates) in serum-free medium for 24h. Biological changes were followed by conducting a shot-gun peptide/protein profiling of cell lysates by direct MALDI-TOF-TOF-MS. Saturated alpha-cyano-4-hydroxycinnamic acid served as the matrix. The mass spectral profiles were interrogated in the region <6kDa using k-nearest neighbours clustering algorithm. Shotgun proteomic analyses of cell lysates were then performed by direct MALDI-TOF-TOF-MS. Mass spectral profiles were data-mined by k-nearest neighbor analysis to investigate for potential markers of peptide/protein changes. Our results clearly indicated particle-dependent changes in MS profiles. Even though all particle exposures exhibited signature MS profiles, common candidate biomarkers were observed between specific particle exposures, especially with DEP and urban particle exposures. Furthermore, the MS data revealed an elevation of cellular endothelin- 1 peptide in J774 cells in relation to the doses of particles. This peptide is implicated in the inflammatory status of macrophage. Characterization of proteomic biomarkers and their association with the physicochemical characteristics of particles can provide new insights into mechanisms of toxicity.

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A Study to Examine Induction of Multiple Inflammatory/TH1/TH2 Cytokines and Chemokines Expression in the Albino Mouse Using Different Mitogens

S. Lavallée, R. Falvo, J. Dubois, N. Corsillo, M. Brugerie, I. Veillette, L. LeSauteur (Charles River Laboratories Montreal)

In the study, the Lipopolysaccharide (LPS), Concanavalin A (Con A) and Phytohaemagglutinin (PHA) were formulated with 0.9% Sterile Saline for Injection USP to achieve dose levels of 0.8, 20 and 25 mg/kg respectively. The formulations were administered by an intravenous injection (via the tail vein) at dose volumes of 1 mL/kg. Blood samples were processed to yield serum and were analyzed for IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-17, TNF-α, IFN-γ, GM-CSF, MCP-1, MIP-1α, MIP-1β and RANTES using a Luminex method. Samples were collected from a first subset of study animals at approximately 90 minutes post mitogens administration and from a second subset of study animals 3 hours post mitogens administration from the abdominal aorta after isoflurane anaesthesia.

There were no clinical observations noted in any animal following administration of the LPS, Con A or PHA with the exception of one mouse given Con A. The observations noted included skin pallor along with decreased activity and respiratory rate.

For the animals injected with LPS, increases of various intensity in IL-6, IL-10, IL-12, TNF-α, GM-CSF, IFN-γ, MCP-1, MIP-1α, MIP-1β and RANTES concentrations were observed 90 minutes and/or 3 hours post LPS injection. No increases in IL-1β, IL-2, IL-4, IL-5 and IL-17 were observed after administration of LPS.

For the animals injected with Con A, increases of various intensity in IL-4, IL-6, IL-2, IL-12, IL-17, GM-CSF, TNF-α, IFN-γ, MCP-1, MIP-1α and MIP-1β concentrations were observed 90 minutes and/or 3 hours post Con A injection. No increases in IL-1β, IL-5, IL-10 and RANTES were observed after administration of Con A.

No change in cytokine or chemokine concentration was observed after administration of PHA, except for IL-6, MIP-1β and MCP-1 for which increased levels were observed at both time points for IL-6 and at the 3 hours post injection of PHA for MIP-1β and MCP-1.

The results have shown robust cytokine release after LPS and Con A challenges and both models

can be used to determine and define a test article's inhibitory effect on cytokine release.

THE USE OF LUNG SLICE CULTURE IN THE ASSESSMENT OF CIGARETTE SMOKE TOXICITY

James C. J. Lin*, J-P Roy*, N. Poirier*, N. Kaur*, M. Lacasse*, A. Morin*
"(SPON: Michel Charbonneau)"
Imperial Tobacco Canada Ltd, Montréal, Canada, H4C 3P6
Email: *jlin@itl.ca*

Neutral red uptake (NRU) assay is required by Health Canada as one of the regulatory tests to evaluate the cytotoxic effect of cigarette smoke. The chemistry of the test articles in such assay, *i.e.* cigarette smoke condensate and vapor phase-bubbled PBS, is different from that of whole cigarette smoke (WS); in addition the outcome of the NRU assay is not related to any pulmonary disease. The current work assesses the feasibility of applying lung slice culture as an alternative test system based on the concept of mimicking real exposure (WS on target organ). The lung slice system maintains the lung structure as well as the cell-cell and cell-matrix interactions which can provide better insight into the tobacco smoke related pulmonary toxicity and diseases.

Lung slices of roughly 1mm in thickness, purchased from a CRO, were prepared by inflating 0.6% low melting agarose into the lungs of Sprague-Dawley rats (250-300 g) followed by manually slicing. They were maintained on Transwell™ culture plates overnight before being subjected to either WS exposure in a smoking machine that can dilute smoke, cigarettes smoke condensate treatment (CSC) or cadmium chloride treatment. The latter was used as a control for lung slice toxicity assessment. Three consecutive day exposures were performed and the lung slices were harvested for MTT assay as well as histological assessment at the end of exposure.

Dose responses in MTT assay were observed in the lung slices treated with CdCl2 and those exposed to WS but not in those treated with CSC. CSC treatment up to 1 60µg/ml for 6 days did not show significant reduction in MTT assay. Histology examination revealed that active re-epithelization can be observed within three days of 2.5µM CdCl2 treatment and such repair can be inhibited by dexamethasone. Loss of alveolar epithelium integrity was observed in most part of the lung slices treated with WS at higher doses (10 and 20-fold dilution). Milder alterations were also observed in those with lower dose (50 and 4000-fold dilution). Clear alveoli alteration was observed in the lung slices treated with 80 and 160 µg/ml CSC and DMSO alone after 6 days of treatment. However, CSC might contain components that interfere with MTT reading as the histological result did not correspond with the results of MTT assay. Lack of bronchiolization in the lung slices treated with WS at lower doses indicated the distinct alveolar damage and repair mechanisms induced by WS in comparison to CdCl2.

ESTROGEN RECEPTOR REGULATES CYP2B6 LEVELS IN HUMAN BREAST CANCER CELLS.

R. Lo*, S. Ahmed*, L. MacPherson*, A. Pansoy*, & J. Matthews
Department of Pharmacology & Toxicology, 1 King's College Circle, Medical Sciences Building, University of Toronto, Toronto, Ontario, M5S 1A8.

Epidemiological data suggest a trend for higher Cytochrome P450 CYP2B6 expression in estrogen receptor alpha (ERα) positive breast tumors. CYP2B6 metabolizes many compounds including tamoxifen and testosterone; therefore, understanding its regulation could impact the effectiveness of breast cancer therapy. ChIP-chip of anti-ERa immunoprecipitated protein-DNA complexes isolated from T-47D human breast cancer cells identified significant recruitment of ERα to regions near and upstream of *CYP2B6*. Conventional ChIP assays confirmed the ChIP-chip data and subsequent computational for transcription factor binding sites identify an ERE ~ -1.7 kb from CYP2B6 and a cluster of 3 EREs located ~ 68 kb upstream of CYP2B6. RNAi-mediated knockdown of ERα resulted in a slight but significant decrease in CYP2B6 mRNA levels in T-47D cells. Analysis of MDA-MB-231 cells stably expressing ERα or ERβ showed that both CYP2B6 mRNA and protein levels were elevated compared to empty vector control cells. ChIP assays revealed that both ER subtypes bound to distal enhancer element (-68kb) and proximal (-1.7kb) regions of CYP2B6. Transient co-transfection of HuH7 human hepatoma cells with an 1.8 kb fragment of the CYP2B6 promoter containing the proximal ER-responsive region or the distal enhancer region cloned into the pGL3 promoter vector with pSG5-ERα resulted (i) in ERα-dependent increase in luciferase activity; and (ii) ERα regulation was abrogated when a mutation or a deletion of the ERE was introduced. Finally, transfection of a DNA-binding deficient ERα prevented its recruitment to both the distal and proximal estrogen responsive regions. Collectively, the results demonstrated an ERα-dependent regulation of CYP2B6, shedding light on the complex autoregulation of estrogen signalling through the metabolism of testosterone and its potential role in ERα positive breast cancer therapy through the metabolism of tamoxifen.

ROLE OF ESTROGEN RECEPTOR ALPHA AF2 DOMAIN IN ARYL HYDROCARBON RECEPTOR CROSSTALK

Laura MacPherson* and Jason Matthews
Department of Pharmacology & Toxicology, University of Toronto
Toronto, Ontario, M5S 1A8

Aryl hydrocarbon receptor (AHR) is a ligand activated transcription factor that mediates most of the toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and polycyclic aromatic hydrocarbons (PAHs). Estrogen receptor α (ERα), which belongs to the nuclear receptor family, is also a ligand activated transcription factor and mediates the biological actions of estrogens. Reciprocal crosstalk between AHR and ERα has been described in a number of different systems, and has been implicated to play a role in various human conditions such as breast cancer and endocrine disruption. Previous studies have reported AHR to have anti-estrogenic activity, suggesting an inhibitory role of AHR in ERα signalling. However, more recent reports have suggested AHR can mediate pro-estrogenic responses when activated by AHR ligands. A clearer understanding of the precise role of AHR/ERα crosstalk is needed to properly assign the magnitude of human health risk associated with AHR ligand exposure. My laboratory has reported an AHR ligand-dependent recruitment of ERα to AHR target genes, *CYP1A1* and *CYP1B1*. To gain better insight into molecular and functional importance of this new mechanism of AHR/ERα crosstalk, I characterized the ERα functional domains involved in this interaction. I used ERα mutants of the activation function 1 (AF1) (ERα-ΔAF1), AF2 (ERα-AF2) and DNA-binding (DBD) (ERα-DBD) domains and transiently transfected mutants as well as wildtype ERα into ERα-negative HuH-7 human liver hepatoma cells. Using chromatin immunoprecipitation (ChIP) analysis followed by quantitative real-time PCR (Q-PCR) I determined wildtype ERα was substantially recruited to classical AHR targets *CYP1A1* and *CYP1B1* as well as to a novel region of a less known AHR-responsive gene, *TiPARP* in response to TCDD and 3-methylchloranthrene (3 MC) treatment alone and in combination with E2. The ERα-AF2 mutant displayed

significantly lower recruitment to all three AHR targets in response to TCDD and 3MC treatment and co-treatments indicating the ERα AF2 is required for AHR ligand-dependent recruitment to AHR targets. Recruitment of ERα-ΔAF1 and ERα-DBD mutants displayed a less consistent pattern of recruitment suggesting that these domains are involved in this interaction in a context-specific manner. Pharmacological antagonism the ERα AF2 with selective estrogen receptor modulators (SERMs) inhibited the AHR ligand-induced recruitment of wildtype ERα and the ERα-ΔAF1 mutant to *CYP1B1;* further supporting the importance of AF2 domain in this recruitment. These results indicate that ERα is recruited to AHR-target genes in response to AHR agonist treatment through the AF2 domain and not through the AF1. These data also suggest that additional co-regulators may be involved in ERα recruitment to AHR target genes. Collectively, our findings provide new insight into the complex crosstalk between AHR and ER signalling pathways.

Development of a Short-term Fluorescent-based Assay to Assess the Toxicity of Anticancer Drugs on Rat Spermatogonial Stem Cells *in vitro*[1]

Ludovic Marcon[2], Xiangfan Zhang[3,*], Barbara Hales[2], Makoto Nagano[3,*] and Bernard Robaire[2,3,4]

Departments of Pharmacology and Therapeutic[2] and Obstetrics and Gynecology[3], McGill University, Montréal, Québec, Canada H3G 1Y6.

Abstract

Spermatogonial stem cell transplantation (SSCT) is the gold standard assay for unequivocally measuring the stem cell activity of a germ cell preparation, based on the restoration of donor-derived spermatogenesis within a recipient testis; however, this is a time-consuming and labor-intensive procedure not amenable for screening strategies. Recently, SSC culture systems were developed for various species and have proven to be excellent *in vitro* techniques to expand and maintain SSCs as germ cell clusters over long periods of time. In the current study, we demonstrate that once formed, these germ cell clusters can be expanded and pooled in a sufficient quantity for subsequent toxicological evaluation of anticancer agents. Testes from postnatal day 7/8 transgenic rat pups, that express enhanced green fluorescent protein (EGFP) only in the germ cell lineage, were used to isolate a pure population of undifferentiated spermatogonia by FACS. Sorted $EGFP^+$ spermatogonia that were cultured in a chemically defined serum-free medium supplemented with GDNF, GFRα1 and bFGF formed three-dimensional aggregates, termed germ cell clusters, after 6 days. Cultured rat $EGFP^+$ clusters were then exposed to cisplatin or etoposide to assess their cytotoxic effects *in vitro.* These drugs reduced the number and size of clusters in a dose-dependent manner, suggesting that SSCs may be affected. Importantly, the expression of EGFP in cluster cells allowed us to acquire data using an automated fluorescence microscope, making this approach very appealing for screening applications. Our results provide evidence that the SSC culture system is suitable for testing the cytotoxic effects of anticancer agents on survival and proliferation of SSC clusters. Furthermore, this culture system may offer a valuable *in vitro* model to screen libraries of small molecules for the identification of compounds that could potentially regulate self-renewal and differentiation of SSCs.
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FUNCTIONAL ANALYSIS OF AN α-HELICAL REGION PROXIMAL TO TRANSMEMBRANE HELIX 17 IN MULTIDRUG RESISTANCE PROTEIN 1

S.V. Molinski*, G. Conseil* and S.P.C. Cole.

Department of Pharmacology & Toxicology and Division of Cancer Biology & Genetics, Cancer Research Institute, Queen's University, Kingston, ON, K7K 3T1

Multidrug resistance protein 1 (MRP1/ABCC1) is a 190 kDa phosphoglycoprotein that mediates the ATP-dependent efflux of structurally diverse physiological toxins and chemotherapeutic agents across biological membranes. At present, the structure of MRP1 is hypothetical, and is substantially based on a recent model derived from the crystal structure of the *Staphylococcus aureus* ABC transporter Sav1866. Site-directed mutagenesis has been the predominant approach used to study MRP1 structure-function relationships thus far; this technique has identified specific regions of MRP1 that are critical for substrate-protein interactions. Previous studies have shown that mutation of certain residues in transmembrane helix 17 (TM 17) and the proximal membrane/cytosol interface, results in either a substrate selective, or global loss of transport activity. The goal of the present study was to extend our understanding of this region by examining the functional importance of three highly conserved acidic residues in the cytoplasmic α-helical region COOH-proximal to TM 17. Thus, Glu^{1253}, Glu^{1255}, and Glu^{1262} were individually replaced with Ala, and after expression in HEK293T cells, the properties of the mutant proteins were investigated. We found that Ala-substitution of all three residues had no effect on the expression of MRP1 protein. Transport assays using inside-out membrane vesicles prepared from transfected cells showed that Glu^{1253} is necessary for transport of 17β-estradiol 17-(β-D-glucuronide) (E217βG), but not leukotriene C4 (LTC4). However, mutation of Glu^{1255} had no effect on the transport of either substrate, while mutation of Glu^{1262} caused a significant loss of LTC4 and E21 7βG transport. Further mutations of Glu^{1253} to Asp and Lys showed that a negatively charged residue at position 1253 is required to maintain wild-type transport activity. These results indicate that Glu^{1253} is part of a specific subset of amino acids that are critical for E217βG binding and efflux, while Glu^{1262} contributes to a different subset of amino acids that are involved in both E21 7βG and LTC4 transport. These observations are somewhat surprising since earlier studies showed that amino acid substitutions causing substrate selective changes in activity were located well within TM17 (i.e. Thr^{1242}, Asn^{1245}, Trp^{1246}), while mutations of amino acids located in the more COOH-proximal TM17/cytosol interface (i.e. Arg^{1249} and Met^{1250}) caused a total loss of transport activity. Ongoing studies involve further analysis of the functional role of these and other amino acids located in this region of MRP1.*Supported by CIHR Grant MOP-10519.*

METHYL MERCURY DISRUPTS CALCIUM HEMOETASIS AND INDUCES NITRIC OXIDE SYNTHASE AND PROTEIN KINASE C CAUSING CELL DEATH IN HUMAN SH-SY 5Y NEUROBLASTOMA CELLS

Leopold Tchapda Ndountse, Hing Man Chan

Community Health Sciences Program, University of Northern British Columbia, Prince George, BC, Canada

ABSTRACT

Methylmercury (MeHg) is a widespread persistent environmental pollutant known to be neurotoxic for wildlife and humans. However, the mechanism of MeHg-induced neuronal damage is not totally clear. We investigated in this study the effects of MeHg on protein kinase C (PKC) and nitric oxide synthase (NO S), two important enzymes in mediating cellular transduction mechanisms and the regulation of neuronal plasticity. After treatment of human SH-SY 5Y neuroblastoma cells with MeHg, nNOS and PKC expression were measured by western blot, Nitrite was measured as indices of nitric oxide (NO) and the subsequent induction of cell death was quantified using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) and lactate dehydrogenase (LDH) release. The results showed increase of neuronal NOS (nNOS) and PKC isoforms α, γ and ε. PKC isoforms α was the most expressed. The expression of nNOS was calcium dependent. MeHg-mediated cell death was attenuated by intracellular calcium chelator 1 ,2-bis(2-aminophenoxy)ethane-N,N,N9,N9-tetraacetic acid (BAPTAAM; 1 μM) and by extracellular calcium chelator, ethylene glycol-bis[baminoethyl ether]-N,N,N9,N9-tetraacetic acid (EGTA; 1mM). MeHg-induced neurotoxicity was also inhibited using nNOS inhibitor (NG-Propyl-l-arginine; NPLA, 5 nM), or PKC inhibitor (staurosporine, 25 nM). These results demonstrated the importance of calcium, nNOS and PKC in MeHg neurotoxicity.

3-METHYLCHOLANTHRENE INDUCED GENOME-WIDE BINDING PROFILES OF ARYL HYDROCARBON RECEPTOR AND ESTROGEN RECEPTOR ALPHA

Andrea Pansoy*, Shaimaa Ahmed*, Laura MacPherson*, and Jason Matthews

Department of Pharmacology & Toxicology, University of Toronto, Toronto, Ontario, M5S1A8

In this study, chromatin immunoprecipitation followed by DNA microarray analysis (ChIP-chip) was used to determine the 3-methylcholanthrene (3MC)-induced genomic footprints of aryl hydrocarbon receptor (AHR) and estrogen receptor α (ERα). T-47D human breast cancer cells were treated with 1 μM of 3MC for 1 hour from which chromatin was isolated using specific antibodies against AHR and ERα. Isolated DNA was linearly amplified and hybridized to Affymetrix® human 1.0R promoter tiling arrays. Enriched regions were identified using CisGenome and TileMap v2 software. Of the 893 ERα-bound regions 225 overlapped (>50 % sequence identity) with the 734 AHR-bound regions (31%). Well described AHR and ERα target genes were among the identified regions. Recruitment of AHR and ERα to 35 of the identified regions was validated using conventional ChIP and changes in target gene expression were confirmed using quantitative real-time PCR. To determine if there were any temporal differences in the recruitment patterns, time course ChIP experiments were performed on cells treated with 1 μM of 3MC for 0 to 4.5 hours. Under these conditions 3MC induced oscillatory binding of AHR, ERα, aryl hydrocarbon receptor nuclear translocator (ARNT), and nuclear receptor co-activator 3 (NCoA3) to *CYP1A1* and *CYP1B1* enhancer regions. Peak binding occurred at 0.5 hr and rebounded again after 4 hours. These transcription factors were also recruited in a temporal manner to estrogen responsive enhancer regions but displayed distinct temporal recruitment patterns. We compared the 3MC-AHR bound regions to ChIP-chip data obtained for TCDD-AHR bound regions and there was surprisingly little overlap between the regions (30%), suggesting that AHR-ligands influence the chromatin binding profile of AHR. However, of the top 100 identified regions, 95% were identical between 3MC and TCDD. Overall, this study provides evidence for (1) overlapping chromatin binding patterns of AHR and ERα, (2) region-dependent oscillatory recruitment of AHR and ERα, and (3) ligand-dependent chromatin binding profiles for AHR.

ASSESSMENT OF SUB-CLINICAL, TOXICANT-INDUCED HEPATIC GENE EXPRESSION PROFILES AFTER LOW-DOSE, SHORT-TERM EXPOSURES IN MICE

Craig Parfett*, Jenny Zheng*, Andrew Williams*, Al Yagminas*, Gu Zhou* and Carole Yauk*

SPON: Mike Wade

Mechanistic Studies Division, Chemical Management Directorate, Health Canada, Environmental Health Centre 0803A, Tunney's Pasture,Ottawa, Ontario, CANADA K1A 0A9

We sought to refine dose-response characterizations in microarray experiments by using array data as groups of genes within functional categories, rather than as individual genes. Further, we sought to incorporate, into hazard assessments, existing knowledge of adverse effects resulting from gene expression changes. An in-house microarray, the HC ToxArray™, was employed which provides a number of quality-control and data normalization features. These features enable sensitive measures of low-dose, adverse changes in gene expression relevant to informing mechanism-of-action, and dose-response issues in risk-assessment. In this study, effects on gene expression induced by the neurotransmitter-mimetic, isoproterenol, were measured at oral doses below those with acute effects on classical toxicological endpoints. Adult male mice were exposed to 4 isoproterenol doses (0.5 to 250 mg/kg, by gavage) alongside controls. Liver tissue was harvested 8 hrs after exposure. Experimental and reference RNA was hybridized to the ToxArray™. The arrayed genes were tested individually for statistical differences between control and exposed samples, then by grouping significantly affected genes by biological function (eg. acute-phase response, angiogenesis, protein synthesis). Analyses used Euclidean distances between the means of the control and exposed samples. The critical region for the test was determined using permutation analysis, making no assumptions about data distribution. For each gene set, if the overall test for treatment differences was significant (i.e. $p < 0.05$) post hoc tests were conducted on individual genes. Group testing in this manner weakly controls the type 1 error rate and additionally, can reveal differences not discovered by per gene tests. We demonstrate that in contrast to traditional analytical approaches, significant effects on expression relevant to chronic toxicities were revealed at all doses using this unconventional multivariate statistical analysis of gene response groups with known biological and toxicological significance. An assessment of likely adverse phenotypic consequences (eg. atherosclerosis) resulting from changes in RNA expression profiles was made incorporated information from published functional genomic studies. On this basis, an assignment of a "Lowest Observed Adverse Transcriptional Expression Level" (LOATEL, 0.5 mg/kg) for oral isoproterenol was made.

MATERNAL FISH CONSUMPTION AND MERCURY: IMPLICATIONS TO FETAL HEALTH?

Katherine Schoeman* - MSc. Candidate, Department of Physiology & Pharmacology
John R. Bend, PhD - Departments of Pathology; Physiology & Pharmacology; and Pediatrics, Schulich School of Medicine & Dentistry, University of Western Ontario (UWO)
Dr. Gideon Koren - Departments of Medicine; Pediatrics; and Physiology & Pharmacology; Ivey Chair in Molecular Toxicology, UWO and the Motherisk Program, Hospital for Sick Children, University of Toronto

Abstract

Background: Fish provide essential nutrients such as n-3 polyunsaturated fatty acids, but may also contain toxic contaminants, such as methylmercury. Methylmercury is a well established neurotoxin that can have adverse effects on the development of the central nervous system. The main source of mercury exposure to humans is through consumption of predatory fish. Previous research has estimated that eating fish accounts for 80-90% of total mercury exposure, of which 75-100% is methylmercury. This raises the concern about whether or not women of reproductive age should consume fish. Dietary changes that affect essential protein and nutrient intake during pregnancy could prove to be as dangerous to the fetus as the poorly defined risk associated with exposure to trace amounts of methylmercury in fish. **Objectives:** 1) Examine the amount of fish consumed by women, as well as the concentration of mercury in the fish eaten; 2) Study women's perceptions on fish intake during pregnancy; and 3) Compare hair mercury content in women with moderate and heavy fish consumption. **Rationale:** Of greatest concern are the predatory fish that contain the highest levels of methylmercury. Most women however, consume small amounts of these fish and thus fall below the WHO provisional tolerable intake (PTI) of methylmercury of 1 .6µg Hg/kg body weight, established on the basis of protecting the developing fetus. **Methods:** Women (n=100) that had called Motherisk in 2006-07 for information on eating fish were contacted and asked to complete a telephone interview. Hair analysis will be conducted on willing subjects to estimate the amount of mercury exposure. Scalp hair has been widely used as a validated indicator of mercury exposure. The qualitative aspect of the questionnaire will focus mainly on the women's "chemophobia" towards eating fish during their pregnancy. **Partial Results:** The majority of women perceived fish as being harmful to their babies. Most were unable to describe the harmful effects of mercury, however they were able to acknowledge the benefits of eating fish during pregnancy. Women consumed less seafood while pregnant compared to when not pregnant. Women consumed low amounts of seafood high in mercury and therefore we presume they will have minimal mercury residues in hair. **Conclusion:** A significant proportion of women of reproductive age avoid eating fish during their pregnancy despite the necessary fatty acids found within the fish. By avoiding fish due to unjustified chemophobia, they will have missed any beneficial effects that come from eating fish.

IN VITRO EXAMINATION OF FURAN TOXICITY AND EFFECTS ON CELL CYCLE IN RAT AND HUMAN HEPATOMA CELLS.

T. Schrader and I. Langlois*,
Toxicology Research Division, HPFB, Health Canada, Ottawa, ON

Objectives: Furan arises in cooked or heated foods through reactions of amino acids, fatty acids, and ascorbic acid. Furan is carcinogenic in rodent models and has been classified as group 2B, possibly carcinogenic to humans, by the International Agency for Research on Cancer (IARC). In order to explore the carcinogenic mechanism involved and possible differences in species sensitivities, furan toxicity and cell cycle effects were compared in rat H4IIE and human hepG2 hepatoma cell lines.

Methods: Cells were plated into 96 multiwell plates (300 ul/well) and allowed to attach overnight. Due to volatility, chemical dilutions and additions were made on ice and the plates sealed with plastic film before further incubation. Cell viability, after 24 and 48 hours of incubation, was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Cells

with or without low serum synchronization were analyzed for cell cycle effects by flow cytometry (Guava Easycyte) with propidium iodide staining (Guava Cell Cycle Reagent) according to manufacturers instructions.

Results: H4IIE and hepG2cells exhibited somewhat different sensitivities to furan with LD50's of 1.8 and 0.96 µl furan/well respectively. With nonsynchronized cells, cell cycle effects were only observed at levels of furan showing toxicity, with the proportion of cells in G0/G1 decreased while S-phase cells were increased. A small initial increase in G2/M cells at 1 ul furan/well was followed by G2/M cell loss after treatment with 2-3 ul/well. Synchronized HepG2 cells also showed increased S and decreased G2/M populations beginning at 0.75 ul/well. However, furan decreased both S and G2/M populations in synchronized H4IIE cells beginning at 0.75 ul/well.

Conclusions: The increased sensitivity of human hepG2 cells to furan suggest that increased care is needed in developing health risk assessments. The increased proportion of S-phase cells found in both lines, at least under nonsynchronized conditions, may have implications for carcinogenesis.

VALPROIC ACID INDUCES HYPER-ACETYLATED HISTONES AND DNA DOUBLE STRAND BREAKS LEADING TO INCREASED HOMOLOGOUS RECOMBINATION REPAIR.

K. Sha[1*], L.M. Winn[1,2]
[1]Department of Pharmacology and Toxicology, [2]School of Environmental Studies.
Queen 's University, Kingston, Ontario, K7L 3N6

Use of the first-line antiepileptic agent, valproic acid (VPA) during pregnancy is associated with an increased incidence of major congenital malformations; however the molecular mechanism mediating VPA-initiated teratogenicity has not been elucidated. Our previous study showed an increase in the frequency of homologous recombination (HR) repair in Chinese hamster ovary 3-6 (CHO 36) cells after exposure to VPA (Defoort et al., 2006). HR is not an error free process and can result in detrimental genetic changes. Since proper development requires tight regulation of gene expression, changes leading to disruption of this process may underlie a mechanism of VPA induced teratogenticity. In this study we evaluated if and how VPA affects DNA double-strand break (DSB) repair. To investigate whether VPA affects the activity of DNA DSB repair, CHO 33 cells containing the neo direct repeat recombination reporter substrate were transfected with either the Saccharomyces cerevisiae mitochondrial endonuclease I-SceI to induce a site specific DSB within the recombination substrate or the empty plasmid, pGem. Cells were then exposed to 5 mM VPA for 24 hrs and two weeks later, the frequency of HR was determined by counting the number of functional neo expressing G41 8-resistant colonies per cells plated. A significant increase in the frequency of HR was observed in the presence (I-Sce1) or in the absence (pGem) of an articially created DSB after exposure to VPA; however there was no increase in the fold difference in HR between VPA and vehicle (media) exposed I-Sce1 transfected cells compared to cells transfected with pGem suggesting that VPA does not affect DNA repair activity. To determine whether VPA induces DNA DSBs to elicit repair, CHO 33 cells were exposed to 5 mM VPA for 10, 16 or 24 hrs and γ-H2AX foci, a marker of DNA DSBs, was measured by immunofluorscence microscopy. A significant increase in the number of γ-H2AX foci per cell was observed for all time points with the greatest increase at 16 hrs after exposure to VPA. Recently VPAs ability to inhibit histone deacetylase (HDAC) has also been proposed as a possible mechanism of teratogensis. Therefore, we also evaluated whether HDAC inhibition by VPA

contributed to the increase in HR. The same recombination assay was carried out with 10, 50 and 100 nM of trichostatin A, a known HDAC inhibitor. Similar frequencies of HR to VPA were observed for all TSA treatment. Western blot analysis was also carried out to assess acetylated H3 and H4 levels at the time of DNA DSBs and a significant increase in acetylated histones was seen at all time points except for H3 at 24 hrs. These results suggest inhibition of HDAC by VPA causes hyper-acetylation of histones leading to relaxed chromatin structure where DNA damage may occur in the form of DNA DSBs with subsequent increase in HR repair. [Support: CIHR]

APPLICATION OF AN *IN VITRO* MINIATURIZED BIOASSAY PLATFORM FOR DETECTION OF CYTOTOXICITY OF URBAN PARTICULATE MATTER

Yunus M. Siddiqui, Subramanian Karthikeyan, and Renaud Vincent

Inhalation Toxicology Laboratory, Environmental Health Sciences and Research Bureau, Safe Environments Programme, Health Canada, Ottawa, Ontario, K1A 0K9

Rationale: Individuals in geographic areas with high particulate matter (PM) in ambient air have increased risk of premature death from cardiovascular conditions. Assessing the biological plausibility of particle-mediated health effects and delineating the specific components of particulate matter that are responsible for effects require an integrated approach involving assessment of in vitro and in vivo toxicity, and reconciliation of the biological and epidemiological evidence. In general, screening cytotoxicity involves a battery of bioassays requiring a large amount of test-material. However, most PM sample collection techniques generally yield only a limited mass of material. This is a particular concern for collection of ultra-fine and nano-particles.

Objective: The objective of the current study was to simulate and validate an integrated in vitro cytotoxicity assay platform that incorporates a number of classical bioassays based on varying mechanism of action, with more detailed genomic and proteomic analyses.

Methods: Human lung epithelial cells (A-549) were seeded in black walled-plate (compatible for fluorescence and luminescence) for 24 hours. Cells were dosed with varying concentrations of the particles (SRM-1649, SRM-1650, SiO2, TiO2, EHC-93 as well as chemical standards) and after 24h of exposure, multiple bioassays were conducted using same plate: LDH release assay (test and total), Alamar blue reduction, level of ATP in cells and BrdU incorporation. In brief, supernatants (100µl) were collected from the culture wells for the detection of experimental LDH release and the cytokines. Wells were inoculated with a mixture of alamar blue dye and BrdU labelling reagent, and the plates were incubated for 4h. Alamar blue reduction was read in a Cytofluor® reader at 0 and 4h. Alamar blue dye was removed by incubating cells with fresh medium for 30 minutes after which the cells were lysed and this lysate was used to measure cellular LDH, ATP content, and to recover mRNA for gene expression analyses. Ice-cold 80% methanol was added to each well to fix the nuclei and the plates were processed for BrdU ELISA in order to assess cellular proliferation.

Results: All the above asssays were integrated into a single cell culture well on a 96-well platform. The data obtained from the integrated experiments was very much identical to the data that was obtained from individual assays.

Conclusions: The integrated assays are very consistent but requiring only 25% of the test material necessary for conduct of individual assays. Supported by Health Canada.

TRANSPORT OF GSH-CONJUGATED CATECHOL ESTROGEN METABOLITES BY MRP1/ABCC1 AND MRP2/ABCC2

Andrew J Slot, Terrence J Monks*, and Susan PC Cole.
Department of Pathology & Molecular Medicine, Queen's University, Kingston, Ontario Canada K7L 3N6

We have previously reported that GSH conjugated metabolites, including four hydroxylated estrogens, some of which are implicated in the carcinogenesis of estrogen responsive tissues, can inhibit estradiol glucuroninde (E217βG) and leukotriene C4 (LTC4) transport by MRP1 and MRP2 [1]. However, although all four GSH conjugated estrogen (GS-estrogen) metabolites tested were potent inhibitors of E217βG transport by MRP1 ($_{IC50}$ range 0.1 – 0.3 μM), only the 2-OH-1-GS-E2 and 2-OH-1-GS-E1 metabolites were potent inhibitors of MRP2 ($_{IC50}$ range 1.5 – 2 ìM) while 4-OH-2-GS-E2 and 2-OH-4-GS-E2 (IC50's ~ 600 and 115 μM, respectively) were not. Furthermore, two of the GS-estrogens inhibited photolabeling of MRP1 and MRP2 by [^{3}H]LTC4 in a concentration-dependent manner, a result that indicates these GS-estrogens inhibit LTC4 transport by competing for substrate binding. Thus, 2-OH-1-GS-E2 and 4-OH-2-GS-E2 inhibit MRP1 labelling by [^{3}H]LTC4 with $_{IC50}$ values <3 μM, while MRP2 labeling is inhibited with IC50 values <20 ìM. The objective of the current study is to identify if any of the GS-estrogen metabolites are possible substrates for MRP1 and MRP2 in vitro using a newly developed HPLC-ECD detection method. This method indirectly measures MRP-specific, ATP-dependent GS-estrogen uptake into inside-out membrane vesicles prepared from transfected HEK cells. We found that GS-estrogen transport into MRP1 and MRP2 containing vesicles is temperature- and time-dependent as well as MRP-specific and ATP/Mg^{2+}-dependent. Results obtained to date indicate that 2-OH-1-GS-E2 is transported by both MRP1 and MRP2 (12.2 ± 3.1 pmol/ug/10 min and 14.7 pmol/ug/20 min respectively), as is 2-OH-1-GS-E1 (8.4 ± 5.7 pmol/ug/10 min and 11.3 pmol/ug/20 min respectively). Furthermore, 2-OH-1-GS-E2 transport is inhibited by the substrate E217βG, and inhibitors MK571 and S-decyl-GSH. These findings are the first to demonstrate direct transport of GS-estrogens by MRP1 and MRP2 in vitro, and indicate that further studies of the role of MRP-related transporters in the disposition of these potentially toxic agents are warranted. In future, this method could be employed to screen for the possible disposition of drug conjugates by various drug transporters, using membrane vesicles or cell culture transwell inserts. Supported by CIHR grant (MOP-10519) and a CIHR doctoral award (AJS) [1] Slot et al. (2008) Drug Metab Dispos **36**:552-560

DEVELOPMENTAL EXPOSURE TO ENVIRONMENTAL BROMINATED FLAME RETARDANTS: AN INTEGRATIVE APPROACH TO TOXIC EFFECTS

Alexander Suvorov, Larissa Takser
Université de Sherbrooke, Québec, J1H 5N4

Traditional approaches to environmental toxicology do not provide data in a timely manner to inform decision makers. For example, it took over 50 years between the discovery of the adverse effect of lead and PCBs and their restricted usage. Firstly, experimental studies must aim at better simulating the exposure scenario estimated for humans. Few animal data are available, for example, for low-doses exposures which are found in humans. Second, in epidemiological studies there is an unmet need for relevant sensitive endpoints to correlate with individual exposure. Few studies provide the concentrations of pollutants in peripheral tissues and correlate them with effects. The extrapolation of experimental

results to human health is impaired by this lack of information. Thus, one priority for modern environmental toxicology is to bridge experimental approaches and epidemiological studies. Moreover, epidemiologic data are often criticized for lack of biological plausibility to support the causality of observed relationships. The simulation of human exposure in experimental models can provide these causal links. This integrative approach is applicable to substances with cumulative properties, such as polybrominated diphenyl ethers or PBDE. PBDE are used as fire-resistant additives in polymers, especially in the manufacture of electrical and household appliances. The fact that breast milk of Canadian women contains the second-highest levels is a growing concern for particularly sensitive populations such as developing children. This particular concern is addressed in our concurrent study (GESTE study) on the relationship between PBDE exposure during pregnancy and thyroid status in women and their newborns. The GESTE study, which will also assess the long-term effects of prenatal exposure to PBDE during childhood, requires appropriate endpoints usable in humans for future evidence-based risk assessment. Unfortunately, extensive analysis of experimental data on PBDE toxicity does not provide endpoints relevant to children from the general population, since most studies have used high-dose PBDE exposure. Two main toxic effects of high doses of PBDE, namely thyroid disruption and hyperactivity, have been detected in animals. **METHODS**: We experimentally reproduced the same range of exposure in rats and sheep, estimated by fat-PBDE concentrations in humans. We used PBDE doses 1000-fold lower than those published previously. **RESULTS**: We observed a significant decrease in total serum thyroxine (T4) in lambs exposed in utero to low doses of BDE-47 (0.2 µg/kg bw per injection), a level similar to estimated human exposure. A decrease in both total triiodothyronine (T3) and total T4 occurs at 2 µg/kg bw. Furthermore, we observed a similar decrease in total and free T4 in rat pups perinatally exposed to low-dose BDE-47. Our data obtained in rats suggest several additional neuroendocrine endpoints, such as body weight increase, anomalies in adrenal function and/or glucose tolerance, and long-term behaviour abnormalities, which could be assessed in our ongoing human birth cohort. **RELEVANCE** - Results are expected to indicate PBDE health hazards at exposure levels relevant to the human population. The unique knowledge gained from this study will provide groundwork for policy makers to ensure adequate protection for future generations from environmentally-induced hazards related to persistent organohalogens.

DEVELOPMENT OF A HIGH-THROUGHPUT PLATFORM FOR ASSESSMENT OF GENE EXPRESSION AND TOXICITY SCREENING

Errol Thomson, Alain Filiatreault, Yunus Siddiqui, Renaud Vincent
Inhalation Toxicology Laboratory, Environmental Health Science and Research Bureau, Health Canada, Ottawa, Ontario, K1A 0K9

There are increasing demands for rapid screening of chemicals for toxicity. Timely evaluation of effects of a large number of compounds, mixtures, and conditions requires a high-throughput approach. Here we report the combined use of a liquid handling unit coupled with a real-time PCR system for partial automation of gene expression analysis. Liquid handling protocols were developed for the distribution and mixing of reagents and cDNA, and were optimized for several variables related to aspirating, dispensing, and mixing of reagents, using both 96-well and 384-well platforms. Validated methods were then applied to the screening of effects of test materials (carbon black, titanium dioxide, cristobalite, benzo(a)pyrene, cadmium chloride, copper chloride, zinc chloride, lipopolysaccharide, and the urban particles EHC-6802) on gene expression using the human adenocarcinoma-derived alveolar epithelial cell line A549. Optimizing dispensing depth and mixing protocols reduced well-to-well variability. Assessment of reaction efficiency with serial dilutions of cDNA showed good reproducibility

of replicates. Comparison of standard curves from plates allowed to sit at room temperature for various periods of time showed no apparent loss of sensitivity, confirming the stability of reagents and cDNA and allowing preparation of multiple plates simultaneously by the liquid handler for PCR cycling at a later time. Application of the platform for toxicity screening showed dose-dependent effects of test compounds, confirming its utility. The overall performance of the 96-well plate assay indicated that the automated plate setup can provide results comparable to a manual approach, with reduced chance of user error or inter-user variability, and thus improved reproducibility. With appropriate optimization, the 384-well plate platform provides the capacity for increased sample throughput. Supported by Health Canada.

EMBRYONIC GLOBAL DNA METHYLATION IS NOT ALTERED BY IN UTERO EXPOSURE TO VALPROIC ACID.

Emily W.Y. Tung[1] and Louise M. Winn[1,2]. [1]Department of Pharmacology and Toxicology and [2]School of Environmental Studies, Queen's University, Kingston, ON, Canada.

Exposure to the anticonvulsant drug valproic acid (VPA) in utero is associated with a 1-2% increase in neural tube defects (NTDs), however the molecular mechanisms by which VPA induces NTDs are unknown. VPA is a histone deacetylase (HDAC) inhibitor and has been shown to increase histone acetylation in embryos 1 hour after maternal administration of the drug. Chromatin structure and DNA methylation are tightly correlated. Open chromatin is associated with unmethylated DNA, and HDAC inhibitors can trigger demethylation of hyperacetylated genes. Previously in our laboratory, we demonstrated that folic acid pre- and co-administrated with VPA was protective against the teratogenic effects caused by VPA, indicating that one-carbon metabolism may be disrupted. The purpose of this study was to evaluate the global methylation status of embryos exposed to VPA during gestation using the cytosine extension assay. On gestational day (GD) 9.0, CD-1 pregnant dams were injected with 400 mg/kg VPA or saline subcutaneously. Embryos were collected 1, 6, and 24 hours after VPA exposure and pooled from at least 2 dams. Embryos obtained 24 hours after exposure were categorized by treatment group and neural tube closure status. The cytosine extension assay was then used to assess the degree of hypomethylation in these embryos. Results indicate that methylation is unchanged in VPA treated embryos with closed neural tubes (n=6) and VPA treated embryos with exencephaly (n=6) when compared with saline treated embryos (n=5) 24 hours after VPA exposure. Global methylation is also unchanged amongst treatment groups 1 and 6 hours after VPA exposure. Currently, we are evaluating histone acetylation changes at the same time points by western blotting for acetylated histones H3 and H4. (Support: CIHR).

AN ASSESSMENT OF THE INTERINDIVIDUAL VARIABILITY FACTOR APPLICABLE TO MULTI-ROUTE EXPOSURE TO DRINKING WATER CONTAMINANTS

Mathieu Valcke,1, 2, * Kannan Krishnan[1]

1: Département de santé environnementale et de santé au travail, Faculté de médicine, Université de Montréal, Montréal, Québec, H3T 1A8

2: Institut national de santé publique du Québec, Montréal, Québec, H2P 1 E2 *ABSTRACT*

The toxicokinetic component of the interindividual variability factor (IVF-TK) used in non-cancer risk assessment corresponds to a default value of 3.16 for oral and inhalation routes. But the magnitude of the chemical-specific IVF-TK for simultaneous multi-route exposures have not been investigated. The objective of the current study was to assess the magnitude of the multi-route IVF-TK for three drinking water contaminants and compare it to IVF-TK measured for each route taken separately. A modified physiologically-based toxicokinetic (PBTK) model published in the literature was used to simulate a 24-hour multi-route exposure to trichloroethylene (TCE), chloroform (CHL) and bromoform (BRO) in drinking water. The simulated scenario included ingestion of drinking water, inhalation of indoor air concentrations resulting from domestic usage of water, as well as inhalation and dermal exposures during 15 min shower or bath. Area under the blood concentration vs time curve (AUC) and the amount metabolized/L liver (MET) were computed for adults, pregnant women, and infants (age 1-3). Biochemical data, partition coefficients, and the probability distributions for body weight, body height and CYP2E1 content were obtained from the literature or P3M software, while the data for surface area, blood flows and tissue volumes were based on morphological characteristics. For multi-route exposures as well as for each route considered separately, probability distributions of AUC and MET were generated using a Monte Carlo methodology and the ratio of the 95^{th} percentile value for infants and pregnant women over the 50^{th} percentile value in adults was calculated as IVF-TK. In infants, the magnitude of IVF-TK for TCE based on AUC was 1.53, 2.20, 1.33, and 1.53 for multi-pathway, drinking water ingestion, inhalation, and dermal exposure, respectively. Based on MET, the values were 1.19, 1.25, 1.11, and 1.34. In pregnant women, the corresponding values were 1.34, 0.40, 1.72 and 1.30 based on AUC; and 0.71, 0.91, 0.49, and 0.42 based on MET. Comparable values were obtained for CHL and BRO. In summary, the results of this study suggest that the chemical-specific IVF-TK varies according to the route of exposure and the dose surrogate considered. The multi-pathway IVF-TK value, however, is mostly influenced by the interindividual variability associated with the determinants of the most important route of exposure.

A VALIDATED PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR THE ESTIMATION OF POSTNATAL EXPOSURE TO POLYBROMINATED DIPHENYL ETHERS (PBDES) IN INFANTS

Marc-André Verner[1], Jonathan Chevrier[3]*, Asa Bradman[3]*, Brenda Eskenazi[3]*, Michel Charbonneau[2], Sami Haddad[1]

1 Département des sciences biologiques, Université du Québec à Montréal, Montréal (QC) Canada, H3C 3P8

2 INRS Instituts Armand-Frappier, Laval (QC), Canada, H7V 1B7

3 School of Public Health, University of California in Berkeley, Berkeley (CA), USA, 94704

Polybrominated diphenyl ethers (PBDEs) are ubiquitous flame retardants found in most human blood, tissue and breast milk samples. Postnatal exposure to these persistent compounds was shown to induce several deleterious effects in rodents such as altered neurodevelopment. Recent studies showed that infants are highly exposed to PBDEs, predominantly through the ingestion of contaminated breast milk. In this study, we aimed to characterize postnatal exposure to PBDEs for infants participating in the CHAMACOS longitudinal birth cohort through the use of a physiologically-based pharmacokinetic (PBPK) model. We previously validated this modeling approach in another longitudinal birth cohort using data on compounds sharing physico-chemical similarities with PBDEs such as polychlorinated biphenyls (PCBs). Ranges of maternal blood PBDE levels (ng/g lipids) in this cohort were 0.7-761 (BDE47), 0.2-298 (BDE99), 0.1-138 (BDE100) and 0.2-96.9 (BDE153). Using the PBPK model and these blood levels, we simulated infant toxicokinetics for the first year of life. Monte Carlo simulations were carried out based on probabilistic distributions of sensitive parameters (e.g., breastfeeding period,

infant physiology) and maternal exposure to estimate ranges of infant blood levels. Monte Carlo simulations generated lognormal distributions of blood PBDE levels in one year old infants. Obtained values (i.e., 50^{th} percentile - 95^{th} percentile in ng/g lipids) for each congener were 65-363 (BDE47), 6-61 (BDE99), 3-31 (BDE100) and 6-105 (BDE153). The predicted infant:mother blood level ratio for the global cohort was normally distributed and similar for all congeners with a mean value of approximately 1.5 (5^{th} percentile = 0.1; 95^{th} percentile = 3.7). However, when strictly considering infants assumed to have been breastfed for 12 months, this ratio was increased to approximately 3.0 (5^{th} percentile = 1.1; 95^{th} percentile = 5.8). Simulations showed that breastfeeding, as expected, has a major impact on infant PBDE blood levels which can potentially reach values in the ug/g lipids range for individual congeners within the CHAMACOS birth cohort. This work highlights the importance of generating individualized toxicokinetic profiles for infants exposed to PBDEs through breastfeeding. This novel approach will enable the assessment of potential associations between postnatal exposure to PBDEs and various health outcomes.

Toxicogenomics Analyses of Methoxyacetic Acid Effects on Spermatogenesis

Michael Wade, Alice Kawata, Andrew Williams and Carole Yauk
Environmental Health Science & Research Bureau, Chemical Management Program, Health Canada, 50 Columbine Driveway, Tunney's Pasture, Ottawa

Methoxyacetic acid, a metabolite of industrial solvent ethylene glycol monomethoxyether, has been known to be a potent disrupter of spermatogenesis for more than a quarter century. Exposure to this substance results in rapid induction of apoptosis in pachytene spermatocytes but has no apparent effect on testis germ cells at any other stage of differentiation. Despite diverse attempts to investigate the mechanism of toxicity that makes these cells uniquely vulnerable to MAA it remains unknown. We evaluated the possible mechanisms by which 2-methoxyacetic acid (MAA) disrupts spermatogenesis using high density microarrays. Levels of mRNA transcripts were determined in total RNA isolated from testes of MAA-treated or concurrent control rats sacrificed 4, 8, 12 or 24 hrs post exposure (PE). Germ cell death was examined in testis sections using in situ staining for DNA fragmentation. MAA treatment caused increased death of pachytene spermatocytes starting 8 hr PE and increasing dramatically at 12 and 24 hr PE. Microarray results indicated that at 4, 8 12 and 24 hr PE the testis transcript levels of 7, 6, 485 and 507 different genes, respectively were significantly altered by MAA treatment. At 4 hours post exposure, all 7 significantly altered genes were over represented in the testes of exposed animals while at other time points significant genes were altered in either direction. Analysis of function of the known genes significantly altered indicated that genes associated with meiosis, transcriptional regulation and post-transcriptional processing were under represented in MAA treated samples while transcripts for genes coding for receptor, cytoskeleton, cell adhesion and oxidative stress response proteins were over-represented in MAA treated samples at 12 and 24 hr PE. By examining web-based datasets from studies of germ cell specific gene expression we identified sets of genes expressed primarily in specific testis cell types. By comparing these sets with microarray data we observed that, in keeping with increased apoptosis of spermatocytes at 12 and 24 hours, most genes specific for spermatocytes were reduced at these times (although 3 were increased) in MAA-treated samples. Genes specific for somatic cells and spermatids tended to increase. Analysis of molecular pathways represented by significantly altered genes, performed using Pathway Studios software, indicated that pathways involved in control of somatic cell function (steroidogenesis, inhibin release), cell adhesion and membrane trafficking showed increased expression while pathways involved in meiosis were reduced. Our results indicate that genome wide transcriptional analysis of testis can provide data to indicate toxologically relevant responses.

EFFECTS OF *IN UTERO* AND/OR POSTNATAL EXPOSURE TO MIXTURES OF BLOOD CONTAMINANTS ON THE ADULTHOOD GLUCOCORTICOID STRESS RESPONSE IN MALE RAT.

G-H Xiao*, D Desaulniers*. (SPON: MG Wade). Health Canada, HECSB/EHSRB/HID. AL:0803D Tunney's Pasture, Ottawa, ON, K1A 0K9.

Rodent studies suggest that perinatal events could reprogram the expression of the glucocorticoid receptor for the entire lifespan, creating abnormal hormone levels and predispositions to diseases. As part of a larger study, we tested the hypothesis of a link between perinatal exposure to environmental contaminants and abnormal adulthood corticosterone (CS) stress response (CSR) in rats. The experiment included 9 treatment groups. From gestation-day 0 (day of vaginal plug) and until postnatal day (PND) 20, dams were fed daily cookies laced with corn oil (control) or a chemical Mixture (M: polychlorinated biphenyls, organochlorine pesticides, and methylmercury) at 0.5 or 1.0 mg/kg/day (0.5M, and 1M). At birth, some control (C) and 1M litters were crossfostered to create 4 groups of pups with the following ***in utero***/postnatal exposure: C/C, C/1M, 1M/C, 1M/1M. Other dams received cookies with a dose of 1.7 ng/kg/day of a mixture of aryl hydrocarbon receptor (AhR) agonists (AhR: non-***ortho*** PCBs, dibenzodioxins and furans) without or with 0.5M. A CSR was induced in male offspring at PND85 (10 min exposure to a heating lamp to induce vasodilation and 5 min bag-restraint to facilitate the collection of 500 microliter of blood from the tail vein). Then, the CS decay was assessed from the trunk blood collected at decapitation 30 min later (T30). The concentrations of CS returned to normal at T30 in the group C, 0.5M, 1M, C/C and 1M/C, but it remained elevated in the group AhR, AhR+0.5M, C/1M, and 1M/1M. Interestingly, 1M had no effect on its own but it prevented the CS drop in adulthood in the 1M/1M group in which the perinatal exposure is associated with the postnatal stress created by the crossfostering procedure, suggesting that rats can tolerate exposure to 1M with no consequences unless they are subjected to early postnatal stress. The liver is a metabolic target organ for corticosterone and the abundance of hepatic glucocorticoid receptor (GR) mRNA was significantly reduced by the C/M and MM treatments. In contrast, the abundance of the GR mRNA was significantly elevated by the AhR treatment compared with 1M. Globally, the crossfostering procedure permitted us to demonstrate that both the CSR and the abundance of the hepatic GR mRNA are modified by the postnatal period of exposure to 1M and not by ***in utero*** exposure, and that postnatal stress might modify the effects of exposure to chemicals. These results are important in our understanding of the perinatal influence of contaminant exposure on stress-induced diseases.