44th Annual Symposium / Le 44e symposium annuel

December 2 – 4, 2012
New Residence Hall
McGill University

Frontiers of Toxicology: New Questions, New Methods and New Approaches

Les Frontières de la Toxicologie: Nouvelles Questions, Nouvelles Techniques et Nouvelles Approches

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

Programme Committee / Comité du programme
Sami Haddad, Université de Montréal, Chair
Jayadev Raju, Health Canada, Member
Andrew Winkley, Member

Sunday Dec 2 PM

2:00 – 5:00 STC Board meeting - New Residence Hall, McGill University

7:00 Student mentoring session – President's Suite, Best Western Ville-Marie Hotel & Suites, 3407 Peel – Montreal, Quebec

Frontiers of Toxicology: New Questions, New Methods and New Approaches

Monday Dec 3 AM

7:30 Registration / Continental Breakfast

8:30 **Louise Winn**, Queen's University, President STC
Opening remarks and Introduction

Session I: **Nanotoxicology**
Chairperson: Sami Haddad, Université de Montréal

8:40 **Introduction**

8:45 **Jim Riviere**, Kansas State University, Manhattan, KS
Biodistribution and pharmacokinetics of nanoparticles

9:25 **Sabina Halappanavar**, Health Canada, Ottawa, ON
The use of toxicogenomics for characterization of hazard of nanomaterials and calculation of transcriptional bench mark doses

10:05 **Coffee Break and Poster Session**

10:35 **Virginia Walker**, Queen's University, Kingston, ON
There may be more to nanoparticles than meets the eye: Investigations on microbes, insects and mammals

11:15 **Selected Highlights from the Posters**

Michela Zago, McGill University, Montreal, QC
Aryl hydrocarbon receptor-dependent retention of nuclear HUR suppresses cyclooxygenase-2 expression independent of DNA-binding [Poster #11]

11:30 **Judit Smits,** University of Saskatchewan, SK
Health benefits of Saskatchewan lentils in arsenic-exposed rats [Poster #7]

11:45 **Kim Babin**, INRS Institut Armand-Frappier, QC
Modulatory activity of TiO_2, CeO_2 and ZnO on human neutrophil degranulation [Poster #10]

Monday Dec 3 PM

12:00 **Lunch and Poster Session**

Session II: **Brominated Flame Retardants**
Chairperson: Andrew Winkley

1:10 **Introduction**

1:15 **Virginia Moser**, U.S. Environmental Protection Agency, NC, USA
Neurotoxicity of brominated flame retardants

1:55 **Larissa Takser**, Université de Sherbrooke, QC
Brominated flame retardants at low doses: Connection between developing rodents and humans

2:35 **Coffee Break and Poster Session**

3:05 **John Giesy**, University of Saskatchewan, Saskatoon, SK
Anthropogenic and naturally occuring brominated compounds

4:00 **Vendor Workshop**
Tools and strategies in the development of biomarkers in toxicology
Laura McIntosh, Caprion Proteome
Development of a mutiplexed MRM assay for the detection and quantification of organ toxicity biomarkers during pre-clinical safety studies
Marcus Kim, Agilent Technologies
Highly sensitive, rapid and robust method for detection of carboxy-THC in hair

4:00 **Annual Business Meeting**

5:00 **President's Reception, Poster Session & STC awards**
ToxQuiz – an animated challenge to your knowledge of toxicology, risk assessment and the posters at STC-2012

7:00 **STC Dinner**
L'Assommoir Notre-Dame (additional cost over registration fee)

Tuesday Dec 4

8:00 Continental Breakfast

8:30 **Gabrial L. Plaa Award of Distinction Lecture:**
Kannan Krishnan, Université de Montréal, QC
Molecules, models and memories from my diary

Session III: ***In Vitro*** **Technologies in Predictive Toxicology**
Chairperson: Jayadev Raju, Health Canada

9:00 **Introduction**

9:05 **Rebecca Laposa**, University of Toronto, Toronto, ON
Stem cells as targets and tools: Neural stem cells in neurotoxicity and stem cell-derived hepatocytes to model drug metabolism

9:45 **Coffee Break and Poster Session**

10:15 **Shang-Tian Yang,** Ohio State University, Columbus, OH
Microbioreactors and 3D fluorescent cell-based high-throughput screening (HTS) for drug discovery

10:55 **Tim Schrader,** Health Canada, Ottawa, ON
Regulatory use of in vitro data for risk assessment

11:35 **Poster Session**

12:00 **Lunch**

Session IV: ***In Vitro-In Vivo*** **Extrapolations**
Chairperson: Sami Haddad, Université de Montréal

1:00 Introduction

1:05 **Patrick Poulin**, Consultant, Quebec City, QC
In vitro-to-*in vivo* extrapolation of pharmacokinetics of drugs

1:45 **Russel Thomas**, Hamner Institutes for Health Research, Research Triangle Park, NC
Incorporating high-throughput *in vitro* screening, dosimetry, and exposure into toxicity testing and risk assessment

2:25 **Louise Winn**, Queen's University, President STC
Concluding remarks

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

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

Speaker abstracts and biographies

Monday Dec 3 AM

Session I: Nanotoxicology

Biodistribution and pharmacokinetics of nanoparticles

Monday, December 3, 8:45 – 9:25 AM

Jim Riviere, *Kansas State University, Manhattan, KS*

The biodistribution and pharmacokinetics of nanomaterials are both similar to yet fundamentally different from those that describe small molecule disposition in the body. They share similar routes of absorption, tissue sites of distribution, and modes of elimination, yet nanomaterial size, shape and surface chemistry modulate how these materials are handled by the body. Nanomaterial ADME and pharmacokinetic studies are now appearing in the literature, but the lack of consistent material characterization and appropriate metrics that mirror biological disposition hinders their interpretation. One initial level of nanomaterial characterization is whether their structure is uniform or complex (e.g. polymers, ground powders, cores with shells of different materials, rigid manufactured structures, etc). Potential contaminants from their manufacture may have to be taken into account. Once in the systemic circulation, the key factors governing disposition relates to size and shape of the particle as well the interaction between a nanomaterial's surface and the biological molecules it encounters, a process that results in so-called corona formation. Association with certain proteins results in opsonization and removal by elements of the reticuoendothelial system and localization in the liver and spleen. Association with other proteins may target the particles to endothelial cells and the vasculature. Ligands may be complexed to a particle's surface to further target a specific pharmacologic endpoint. For some materials, disposition may be a function of lymphatic trafficking which makes monitoring of particles in the systemic circulation less than optimal and possibly subject to hysteresis. Nanomaterial pharmacokinetic studies often use traditional modeling techniques but may need to be interpreted differently. For example, short plasma half-life may be related to reticuloendothelial clearance rather than elimination from the body. Because of fundamentally different mechanisms of cellular uptake in tissues (e.g., endocytic pathways versus active molecular transport pumps and diffusion), physiological based pharmacokinetic (PBPK) models may offer an advantage. A framework of nanomaterial ADME properties is beginning to be defined and will be presented, as will pharmacokinetic schemes for nanomaterial pharmacokinetic models and tissue biodistribution.

Dr. Jim Riviere holds the MacDonald Endowed Chair of Veterinary Medicine, is a University Distinguished Professor, and a Kansas Bioscience Authority Eminent Scholar at Kansas State University where he is Director of the Institute of Computational Comparative Medicine. Riviere earned his bachelor's degree in biology summa cum laude and a master's degree in endocrinology with distinction from Boston College, Chestnut Hill, Mass. He earned a DVM and a Ph.D. in pharmacology from Purdue University where he was also awarded an honorary D.Sc. in 2007. He is an elected member of the Institute of Medicine of the National Academies, and chaired their Committee on Strengthening Core Elements of Regulatory Systems in Developing Countries last year. He previously was the Burrough's Welcome Fund Distinguished Professor of Pharmacology and the director of the Center for Chemical Toxicology Research and Pharmacokinetics at North Carolina State University in Raleigh, NC where he was a faculty member for three decades. He has authored/edited 10 books and 500 scholarly publications in pharmacokinetics, toxicology and food safety, and received $20 million as principal investigator on extramural research grants. He is a fellow of the Academy of Toxicological Sciences,

and has served on the Science Board of the Food and Drug Administration. Among his honors are the 1991 Ebert Prize from the American Pharmaceutical Association, the Harvey W. Wiley Medal and FDA Commissioner's Special Citation, and the Lifetime Achievement Award from the European Association of Veterinary Pharmacology and Toxicology. His current research interests relate to applying biomathematics to problems in toxicology, including the risk assessment of chemical mixtures, pharmacokinetics of chemicals and nanomaterials, absorption of drugs, chemicals and nanoparticles across skin; and the food safety and pharmacokinetics of tissue residues in food-producing animals.

The use of toxicogenomics for characterization of hazard nanomaterials and calculation of transcriptional bench mark doses

Monday, December 3, 9:25 – 10:05 AM

Sabina Halappanavar**,** *Health Canada, Ottawa, ON*

Human exposure to engineered nanomaterials (NM) is expected to increase due to their widespread use in consumer products and biomedical applications. However, hazard characterization and risk assessment of NM has been challenging due to their diverse physical-chemical properties and lack of valid toxicity test methods. Moreover, there have been very few in vivo chronic studies conducted to date and possibilities of testing each material in a long-term toxicity study is almost not possible. In the present study using nano-sized titanium dioxide as a model NM, we investigated the applicability of high-content genomics tools to identify hazard associated with NM and inform risk assessment. Female C57BL/6 mice were exposed to nanoparticles of rutile titanium dioxide (primary size of 20.6 nm and surface area of 107.7 m2/g) via single intratracheal instillation of 18, 54 and 162 µg/mouse and sampled at 1, 3 and 28 days post-exposure. Using whole mouse genome microarrays, gene expression was profiled in lung (all doses and time points), liver and heart (high dose, all time points) tissues. Using gene ontology and pathway analysis tools, adversely affected genes and associated biological responses were identified. Microarray results were validated using quantitative real-time PCR. Deposition of particles in the lungs and translocation of particles to other organs was assessed using Cytoviva's Hyperspectral microscope system. Genes clearly associated with the phenotype and showing dose response were used to calculate Bench mark doses. The presentation will show the details of the process involved in using genomics data for risk characterization and estimating point of departure for non-caner endpoints for NM.

Dr. Halappanavar is a Research Scientist in the Mechanistic Studies Division of the Environmental Health Science and Research Bureau of Health Canada, Ottawa. She obtained her Masters degree in Experimental Medicine and Doctoral degree in Cellular and Molecular Biology from Université Laval, Quebec in 2003. After completing a year of postdoctoral fellowship at the Ottawa Health Research Institute, she moved to Health Canada in 2006 as an 'Office of the Chief Scientists' postdoctoral fellow. In 2007 she obtained an acting Research Scientist position and started her independent scientist career. She serves Health Canada since that time. Dr. Halappanavar is an adjunct professor in the Department of Molecular Biology, Medical Biochemistry and Pathology at Université Laval, Quebec. Her current research is focused on validation and application of advanced molecular biology and systems biology tools, such as genomics and proteomics in hazard identification, understanding mechanisms of action (cardiopulmonary toxicity and tissue carcinogenesis), risk calculation and deriving regulatory oversights for human risk of exposure to environmental toxicants including complex chemical mixtures and nanoparticles.

There may be more to nanoparticles than meets the eye: Investigations on microbes, insects and mammals

Monday, December 3, 10:35 – 11:15 AM

Virginia K. Walker, *Queen's University, Kingston, ON*

Human exposure to engineered nanomaterials (NM) is expected to increase due to their widespread use in consumer products and biomedical applications. However, hazard characterization and risk assessment of NM has been challenging due to their diverse physical-chemical properties and lack of valid toxicity test methods. Moreover, there have been very few in vivo chronic studies conducted to date and possibilities of testing each material in a long-term toxicity study is almost not possible. In the present study using nano-sized titanium dioxide as a model NM, we investigated the applicability of high-content genomics tools to identify hazard associated with NM and inform risk assessment. Female C57BL/6 mice were exposed to nanoparticles of rutile titanium dioxide (primary size of 20.6 nm and surface area of 107.7 m2/g) via single intratracheal instillation of 18, 54 and 162 μg/mouse and sampled at 1, 3 and 28 days post-exposure. Using whole mouse genome microarrays, gene expression was profiled in lung (all doses and time points), liver and heart (high dose, all time points) tissues. Using gene ontology and pathway analysis tools, adversely affected genes and associated biological responses were identified. Microarray results were validated using quantitative real-time PCR. Deposition of particles in the lungs and translocation of particles to other organs was assessed using Cytoviva's Hyperspectral microscope system. Genes clearly associated with the phenotype and showing dose response were used to calculate Bench mark doses. The presentation will show the details of the process involved in using genomics data for risk characterization and estimating point of departure for non-caner endpoints for NM.

Dr. Virginia K. Walker received her PhD (medical biochemistry) from Univ. Calgary and did her NSERC Postdoctoral Fellowship-funded research at Univ. Cambridge (genetics). Her lab's research interests concern stress genes and the molecular basis of resistance. This is a central question for scientific goals as diverse as envisaging the outcome of cancer chemotherapy, predicting the impact of global temperature fluctuations and the mechanisms of freeze tolerance, or the effect of industrial activities on our environment.

Selected Highlights from the Posters

Monday, December 3, 11:15 – Noon

Michela Zago, McGill University, Montreal, QC
Aryl hydrocarbon receptor-dependent retention of nuclear HUR suppresses cyclooxygenase-2 expression independent of DNA-binding [Poster #11]

Judit Smits, University of Saskatchewan, SK
Health benefits of Saskatchewan lentils in arsenic-exposed rats [Poster #7]

Kim Babin, INRS Institut Armand-Frappier, QC
Modulatory activity of TiO_2, CeO_2 and ZnO on human neutrophil degranulation [Poster #10]

Monday Dec 3 PM

Session II: Brominated Flame Retardants

Neurotoxicity of brominated flame retardants

Monday, December 3, 1:15 – 1:55 PM

Virginia Moser, *U.S. Environmental Protection Agency, Research Triangle Park, NC, USA*

Polybrominated diphenyl ethers (PBDEs) have been commonly used as commercial flame retardants in a variety of products including plastics and textiles. Despite their decreasing usage worldwide, congeners continue to accumulate in the environment, including soil, dust, food, animals and humans. Concern has been raised regarding their potential developmental effects, especially to the nervous system, since children may be differentially exposed to PBDEs and have higher blood levels. Studies of PBDEs in laboratory animals during different periods of development show a variety of adverse effects, including delayed behavioral ontogeny, alterations in motor activity, neurotransmitter changes, and reductions in learning and memory. Despite this growing literature, as yet there has been little consistency in terms of exposure paradigm, experimental procedures, test animal, or chemical congener; these variables may contribute to some contradictory findings. *In vitro* studies suggest sensitivity of multiple neurotransmitter systems, especially dopaminergic; however, an underlying biological substrate for developmental neurotoxic effects remains unclear. Recent human epidemiological data have also raised concerns for public health, suggesting neurological, thyroid, and metabolic effects in children. The emerging concordance between laboratory and human data support further investigations of both exposure and outcomes.(*This abstract does not necessarily reflect USEPA policy*)

Dr. Moser has been at the US EPA since receiving her Ph.D. in pharmacology and toxicology from the Medical College of Virginia, and is now a senior toxicologist in the Toxicity Assessment Division of the National Health and Environmental Effects Research Laboratory. Throughout her tenure at EPA she has focused on the use of neurobehavioral test methods for both toxicity screening and mechanistic research of a wide variety of environmental chemicals (including pesticides, persistent organic pollutants, and drinking water contaminants) following acute, repeated, and developmental exposures in both rats and mice. She is a diplomate of the American Board of Toxicology (ABT), served on the ABT Executive Board of Directors, and is a Fellow of the Academy of Toxicological Sciences (ATS). She has received many honors from the EPA, including the Scientific Achievement Award for Human Health Research and Gold Medal for Exceptional Service. As an active member of numerous scientific societies, she has held numerous officer positions, served on planning committees, and organized meetings. She has several adjunct faculty positions, is section editor for two journals, and serves on editorial boards for two more toxicology journals. She has over 130 peer-reviewed manuscripts and book chapters published or in press.

Brominated flame retardants at low doses: Connection between developing rodents and humans

Monday, December 3, 1:55 – 2:35 PM

Larissa Takser, *Université de Sherbrooke*, *QC*

Adequate protection of the developing child from environmental hazards is a public health priority. The general objective of our research is to detect subtle toxic effects at low doses environmental exposures *in utero*, especially brominated flame retardants, in human, by integrating knowledge obtained from experimental research. The talk will explain the state of knowledge on low-dose toxicity of flame retardants, available human data, main research challenges, and some aspects of decision making process related to fetal health.

Dr. Larissa Takser is a Professor at the Department of Pediatrics, the Université de Sherbrooke, Canada, since 2005. She received the M.D. degree from Setchenov Moscow Medical Academy, Russia, and the Ph.D. degree from the Université Paris XI, France, in 1996 and 2001, respectively. From 2002 to 2005, she was a Postdoctoral Fellow at the Université du Québec à Montréal and Université de Montréal, Canada. In 2006, she was the recipient of the Junior I Award from Fonds de Recherche en Santé du Québec. In 2009, she was a recipient of the Canadian Institutes of Health Research (CIHR) New Investigator Award and the CIHR Price Rising Star in Perinatal Research. Her research on in utero exposures to environmental contaminants and child development is supported by Canadian and international grants.

Anthropogenic and naturally occuring brominated compounds

Monday, December 3, 3:05 – 3:45 PM

John Giesy, *University of Saskatchewan, Saskatoon, SK*

There are nearly 3200 known naturally occurring organo-halogen compounds, and more than 1600 of these contain bromine. Little is known about the sources, transformations and biological activities of many of these compounds. Anthropogenic brominated compounds, especially brominated flame retardants (BFRs), have attracted recent interest due to their large production volumes and ubiquitous occurrence in the environment. Among the brominated compounds, polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBBs) are of major concern. Several recent studies have described the presence of naturally occurring organo-bromine compounds, such as methoxy-PBDEs (MeO-PBDEs), hydroxy-PBDEs (OH-PBDEs) bromo-phenols (BRPs), polybrominated dibenzo-*p*-dioxins (PBDDs) in the marine environment. These classes of naturally occurring compounds have in some cases been measured at concentrations greater than that of the anthropogenic brominated contaminants. Some OH-PBDEs and BRPs have been reported to be degradation products of anthropogenic organo-bromines. OH-PBDE's are of particular concern due to their greater toxicities relative to synthetic PBDEs. To clarify the relationships between anthropogenic and naturally occurring brominated compounds, environmental investigations and *in vitro* metabolism studies of these compounds have been conducted. The results demonstrated the metabolic production of OH-PBDEs from naturally occurring MeO-PBDEs as a previously unidentified mechanism that could be an important contributor for the occurrence of OH-PBDEs found in marine wildlife. To further

assess the contribution of anthropogenic organo-brominated compounds to total bromine in samples, a mass balance of total bromine was conducted. Total bromine in samples and organic sample extracts was determined by neutron activation spectroscopy. Combined concentrations of PBDEs, MeO-PBDEs, OH-PBDEs, BRPs and PBBs accounted for 0.01-0.04% of total bromine present the in the livers of tuna, albatross, and polar bears collected from marine environments worldwide. Furthermore, extractable organic bromine accounted for only 7.03-16.81% of total bromines in the samples. Ongoing studies are exploring the nature origins and toxicities of the predominant brominated compounds in these samples. These studies highlight the need to consider potential natural sources of organo-halogen compounds when assessing environmental and human health risk assessments of the marine environment.

Prof. Giesy obtained his Masters and Doctor of Philosophy Degrees in Limnology from Michigan State University in 1971 and 1974, respectively. Currently, he is Professor and Canada Research Chair at the University of Saskatchewan and Professor of Zoology Emeritus at Michigan State University. He is also Chair Professor at Large of Biology & Chemistry, at City University of Hong Kong, Honorary Professor in the School of Biological Sciences of the University of Hong Kong and Concurrent Professor of Environmental Science at Nanjing University, China and a *Distinguished Professor* of King Saud University. Prof. Giesy has published 838 articles and is a highly cited author, ranking among the top 0.001% of active authors in the world (ISI) and the 2nd most cited author in the world in the combined fields of Ecology and Environmental Sciences. He has an h index of 68 on over 19,500 citations. Prof. Giesy has received a number of distinctions and awards including: the *Vollenweider Medal for Aquatic Sciences* from the National Water Research Institute of Canada for his work on contaminants in the North American Great Lakes and the *Founders Award*, which is the highest award given by the Society of *Environmental Toxicology and Chemistry* (SETAC) for continued excellence in research and education. He is the recipient of the *SCOPE-Zhongyu Environmental Sciences Life Achievement Award,* which is presented by The Scientific Committee on Problems of the Environment (SCOPE). He is fellow of the *Royal Society of Canada* (National Academy of Science) and *Einstein Professor* of the Chinese Academy of Sciences. For more information about Prof. Giesy, you may visit his website at http://ww.usask.ca/toxicology/jgiesy

Vendor Workshop

Tools and strategies in the development of biomarkers in toxicology

Monday, Dec 3, 4:00 – 5:00 PM

Laura McIntosh, Caprion Proteome
Development of a mutiplexed MRM assay for the detection and quantification of organ toxicity biomarkers during pre-clinical safety studies

Marcus Kim, Agilent Technologies
Highly sensitive, rapid and robust method for detection of carboxy-THC in hair

Tuesday Dec 4 AM

Gabriel L. Plaa Award of Distinction Lecture

Molecules, models and memories from my diary

Tuesday, December 4, 8:30 – 9:00 AM

Kannan Krishnan, *Université de Montréal, QC*

This year's recipient of the ***STC Gabriel L. Plaa Award of Distinction,*** Kannan Krishnan, obtained his B.Sc. (Agriculture '83) from Annamalai University (India), M.Sc. (Agricultural Chemistry '87) from McGill University and Ph.D. (Public Health; Option Toxicology '90) from Université de Montréal. After completing his post-doctoral training (1990-'92) at The Hamner Institutes (formerly CIIT Centers for Health Research), Research Triangle Park, NC, he started his teaching carrier at Département de santé environnementale et santé au travail of Université de Montréal where he is currently full professor. An expert in physiologically-based pharmacokinetic (PBPK) modeling and health risk assessment methods, Dr. Krishnan has held visiting scientist/faculty appointments at the Karolinska Institutet, Sweden (2004), Toxicology Excellence for Risk Assessment (TERA, Cincinnati, OH) (2007), Environmental & Occupational Health Sciences Institute of UMDNJ-Rutgers University, NJ (2007), Sri Ramachandra University, Porur, India (2010-) and National Center for Environmental Assessment, U.S. EPA, Washington, D.C. (2011-'12). His panel and committee memberships has included: U.S. National Academy of Sciences' Sub-committee on Acute Exposure Guideline Levels; Risk Assessment, Mixtures and Biological Modeling Specialty Section councils of the Society of Toxicology (SOT); International Programme on Chemical Safety (IPCS) of the World Health Organization; Human Studies Review Board and FIFRA SAB of U.S. Environmental Protection Agency; Comité d'experts specialise (Evaluation des risques liées aux substances chimiques) de l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, France; as well as Workgroup of the International Agency for Research on Cancer (Lyon, France). Currently he is on the roster of experts for the joint meeting on pesticide residues (JMPR) of the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (2010-'15), and the roster of toxicological and epidemiological experts of the Joint FAO/WHO Expert Committee on Food Contaminants & Additives (JEFCA; 2011-'15). He has served on the editorial boards of *Toxicological Sciences*, *International Journal of Toxicology*, *Journal of Applied Toxicology, Journal of Toxicology, Current Computer Aided Drug Design, Frontiers in Predictive Toxicology* and *Journal of Child Health*. Listed as an educator in Canadian WHO's WHO since 2006, other recognitions include: the *Veylian Henderson Award* (2000) of the Society of Toxicology of Canada, the *Best paper award in Toxicological Sciences (2003)* of SOT as well as Science and Technological Achievement Award of EPA (2009; Level II). In this lecture, Kannan will briefly highlight the molecules, models and memories relating to his lab's research work on: prediction of determinants of volume of distribution, modeling of higher order interactions in mixtures, structure-pharmacokinetic relationships and PBPK modeling in health risk assessment of environmental contaminants.

Session III: *In Vitro* Technologies in Predictive Toxicology

In vitro technologies in predictive toxicology

Tuesday, December 4, 9:05 – 9:45 AM

Rebecca Laposa, *University of Toronto, Toronto, ON*

Within the field of toxicology, stem cells can be considered as critically important cellular targets for xenobiotic toxicity. Our laboratory has focused on the effect of genotoxic stress in neural precursors. Two cardinal features of stem cells are self-renewal and multipotency. We have assessed neural precursor self-renewal by the neurosphere assay, and multipotency using *in vitro* differentiation to neuronal and glial lineages, and have employed both embryonic and adult mouse models. We have observed that the multifunctional DNA repair protein Cockayne syndrome B (Csb) maintains neural precursor function, and that DNA damage impairs both neural stem cell self-renewal and neuritegeneis in newly-born neurons in this genotype. We suggest that neurotoxicity assessment of drugs and environmental chemicals should also consider effects on neural stem cells, since these cells are crucial to the development of neurons and glia in the embryo, as well as during ongoing adult neurogenesis. Human stem cells are also useful tools to model drug toxicity and drug metabolism of novel agents during preclinical drug development and during risk assessment of environmental chemicals. I will summarize the progress of a Stem Cell Network of Canada team project focused on human stem cell-derived hepatocytes. This project is lead by Dr. Gordon Keller at the University Health Network, Toronto and team members include Dr. Denis Grant, Dr. Rachel Tyndale and myself at the University of Toronto. This team has successfully differentiated hepatocyte-like cells from both human embryonic stem cells and human induced pluripotent stem cells. The derivation of these cells, along with the expression and functionality of drug metabolizing enzymes in these cells, will be presented.

Dr. Laposa earned her Ph.D. in Pharmaceutical Sciences at the University of Toronto under the supervision of Dr. Peter Wells. She followed this with postdoctoral training in cancer research at the University of San Francisco, California, under the guidance of Dr. James Cleaver. Dr. Laposa started her own laboratory as a principal investigator at the University of Toronto in 2008 in the Department of Pharmacology and Toxicology. Current work in her laboratory focuses on genomic integrity of both the nuclear and mitochondrial genomes, with an emphasis on developmental neurotoxicity. Infrastructure in the Laposa laboratory is funded by a Canadian Foundation for Innovation award in Developmental Neurotoxicity. Laboratory research is funded by NSERC, CIHR and the Stem Cell Network of Canada National Centre of Excellence. Dr. Laposa's teaches an undergraduate course on the topic of Drug Discovery. Dr. Laposa is a member of the Stem Cell Network of Canada.

Microbioreactors and 3D fluorescent cell-based high-throughput screening (HTS) for drug discovery

Tuesday, December 4, 10:15 – 10:55 AM

Shang-Tian Yang, *Ohio State University, Columbus, OH*

To accelerate the discovery of chemicals with biological activities, we have developed fluorescence-based live 3D cell cultures in microwell plates and microfluidic biochips for high-throughput cytotoxicity and proliferation assays using enhanced green fluorescent protein (EGFP)-expressing cells. This is the first cell-based high-throughput system in 3D that can mimic in vivo environment and be used reliably for screening chemicals and biomolecules as potential drug targets. Drug responses of the cells in this 3D system can be monitored in real time and noninvasively. The microbioreactor arrays can also be used for medium optimization and cell culture process development, and thus will have wide applications in drug discovery, cell culture process development, and tissue engineering. This presentation will outline the principles and methods used in this 3D HTS system and provide several application examples in cytotoxicity and embryotoxicity assays, drug efficacy study, and biomolecule discovery, particularly in screening herbal medicines for potential effects on stimulating stem cell growth and/or inhibiting cancer cells.

Dr. S. T. Yang is Professor of Chemical and Biomolecular Engineering at the Ohio State University, where he has been on the faculty since 1985. He is also the director of Ohio Bioprocessing Research Consortium and has worked with many companies in commercial technology development. Dr. Yang received his B.S. degree in Agricultural Chemistry from National Taiwan University and M.S. and Ph.D. degrees in Biochemical Engineering from Purdue University. Dr. Yang has broad research interests in bioengineering. His current research involves biocatalysis, cell culture, tissue engineering, functional genomics, and microfluidic biochips for high-throughput cell-based assays and biodiagnostics. He has more than 150 scientific publications and 12 patents in the bioengineering field. Dr. Yang is also a co-founder of two biotechnology startup companies. He is an elected fellow of American Institute of Medical and Biological Engineering, Associate Editor for the journal Process Biochemistry, past chair of Division 15 Food, Pharmaceutical and Bioengineering of American Institute of Chemical Engineers (AIChE) and an active member of American Chemical Society (ACS).

Regulatory use of in vitro data for risk assessment

Tuesday, December 4, 10:55 – 11:35 AM

Tim Schrader, *Health Canada, Ottawa, ON*

The regulatory assessment process has been adopted to protect human life as well as economic interests, and data requirement variations exist across pharmaceutical, food, animal and environmental areas. A chemical health risk assessment reflects a comprehensive and coherent description of the toxicological properties of the chemical in question and requires that as much evidence as possible related to chemical effects be scrutinized. Epidemiological, in vivo, as well as in vitro data all assist in establishing allowable safety limits of exposure; however, limitations in the abilities of in vitro systems to accurately model the in vivo situation hinder the extrapolation of in vitro data to real life exposures. In many instances, data gathered through well-designed epidemiological or in vivo studies demonstrating a NOAEL or characterizing a dose-response relationship is

considered as primary, with in vitro data providing a secondary, supportive role. Nevertheless, in vitro approaches have demonstrated their usefulness in high throughput screening and mechanistic assays, and hold potential for at least partial replacement or supplementation of in vivo work. In this seminar, some of the factors limiting the replacement of in vivo studies by in vitro studies will be presented. In addition, major classes of in vitro assays and primary uses of in vitro data, including chemical detection and delineation of mechanism of action, will be discussed. Included in the presentation will be the contributions of national/international organizations toward the identification and validation of useful assays resulting in standardized assay protocols and international mutual acceptance of data.

Dr. Schrader graduated from the University of Western Ontario with a BSc (Honours Biochemistry) and PhD (Biochemistry, specializing in DNA excision repair). His interest in cancer biology continued with postdoctoral fellowships at the Cancer Research Laboratories, Queen's University (Dr. Roger Deeley) and at the Mutagenesis Section, Environmental Health Centre of Health Canada, (Dr. Craig Parfett) studying the molecular biology of estrogen gene regulation and tumour promoter activity, respectively. In 1993, he accepted a Research Scientist position within the Toxicology Research Division, Food Directorate, Health Canada where he has since supervised an in vitro toxicology laboratory with a major focus on the development and use of in vitro approaches for genetic toxicology assessments of food additives, contaminants and processing-induced chemicals. A particular interest of the laboratory has involved the reintroduction of metabolic capabilities into cell lines. Recent efforts have concentrated upon the genetic and epigenetic consequences of possible test chemical modifications resulting from processes such as nitrosylation, occurring in the acidic environment of the gut, and oxidative/nitrosative reactions occurring as a result of the inflammatory response. Dr. Schrader has also served on the OECD Validation Management Group, Non Animal in the development of test guidelines for the in vitro characterization of endocrine disrupters.

Session IV: *In Vitro-In Vivo* Extrapolations

In vitro-to-*in vivo* extrapolation of pharmacokinetics of drugs

Tuesday, December 4, 1:05 – 1:45 PM

Patrick Poulin, *Consultant, Quebec City, QC*

Various methods are available to predict human pharmacokinetics (PK) with some based on preclinical *in vivo* data and others utilizing human *in vitro* data. *In vitro* methods are most convenient because they require minimal amount of compound and do not necessitate animal studies. However, the predictivity of *in vitro* methods depends on the models used to scale the PK properties under *in vivo* conditions. Factors influencing the predictive performance of an *in vitro-in vivo* extrapolation (IVIVE) method for drug tissue distribution, and, hence, the resulting volume of distribution (*Vd*), are mainly related to drug permeation limitation in the *in vitro* cell systems compared to *in vivo* condition. For drug clearance (*CL*), the factors influencing the predictive performance of IVIVE methods are related to differences between the incubation systems *in vitro* and *in vivo* conditions; namely, drug ionization differences between the incubation systems and liver cells *in vivo*, protein binding correction to the intrinsic clearance *in vitro* (CL_{int}), and the chosen liver model to scale to *in vivo*. Finally for accurate oral absorption prediction from IVIVE methods, the categorization of the compounds depending of the biopharmaceutics drug disposition classification system (BDDCS) and/or bioclassification system (BCS) is essential. Diverse examples will be

demonstrated for prediction of ADME, including those from the literature and in house from the consultant.

Dr Poulin obtained a BScA degree in Food Sciences and Technology at the Université Laval in Québec City, and a PhD degree in Toxicology at the University of Montréal. Following his academic experiences, he conducted a postdoctoral fellowship in pharmaceutical sciences at the company F. Hoffmann-La Roche located in Basel, Switzerland. After the fellowship, Patrick became a senior scientist at Roche where his main responsibility consisted of making the Physiologically-Based Pharmacokinetic (PBPK) models more useful for drug discovery. These PBPK models are used to help the selection of clinical candidates based upon more rationale and effective screening efforts of PK-PD relationships by considering the interrelationships between in silico, in vitro and/or in vivo data. Therefore, an important part of Patrick's work is to improve in vitro-in vivo extrapolation (IVIVE) methods. Consequently, he develops mechanism-based prediction tools for the volume of distribution, tissue:plasma partition coefficients and liver metabolism, which are essential input parameters for PBPK modeling. At present, Patrick's main activity is to act as a consultant for several industries in the pharmaceutics and food technologies. Beside his research work, Patrick was the leader consultant of an international study of a PhRMA initiative, which consisted of evaluating the impact of drug properties on human PK prediction of a novel large and diverse dataset. Patrick is also currently developing commercial and versatile simulation software for prediction of PK in animals and humans in association with the AEGIS company. Finally, Patrick contributes to several review activities in different journals. He is a co-author of two general book chapters and several papers on the topic of PBPK modeling and PK prediction from IVIVE methods. He is active in the academia by teaching students.

Incorporating high-throughput *in vitro* screening, dosimetry, and exposure into toxicity testing and risk assessment

Tuesday, December 4, 1:45 – 2:25 PM

Russel Thomas, *Hamner Institutes for Health Research, Research Triangle Park, NC*

Over the past five years increased attention has been focused on using high-throughput *in vitro* screening for identifying chemical hazards and prioritizing chemicals for additional *in vivo* testing. The U.S. Environmental Protection Agency's (EPA) ToxCast program has generated a significant amount of high-throughput screening data allowing a broad-based assessment of the utility of these assays for predicting *in vivo* responses. However, efforts have generally relied on nominal assay concentrations for prioritization which may misrepresent potential *in vivo* effects of these chemicals due to differences in bioavailability, clearance, and exposure. This seminar will briefly cover a series of studies that evaluates incorporating high-throughput *in vitro* screening, dosimetry, and exposure into toxicity testing and risk assessment. The first study is a comprehensive cross-validation model comparison to evaluate the predictive performance of the more than 600 *in vitro* assays from the ToxCast Phase I screening effort across 60 *in vivo* endpoints using 84 different statistical classification methods. The predictive performance of the *in vitro* assays was compared to that of chemical structure descriptors. The results showed that the current suite of ToxCast high-throughput toxicity assays have limited applicability for predicting in vivo chemical hazards using standard statistical classification methods. However, if viewed as a survey of potential molecular initiating events and interpreted as risk factors for toxicity, the assays may still be useful for chemical prioritization. The second study involves the development of high-throughput methods for estimating pharmacokinetics for *in vitro* studies. Hepatic metabolic clearance and plasma protein binding were experimentally measured for a subset of the ToxCast Phase I chemicals using rat hepatocytes and plasma. Computational *in vitro*-

to-*in vivo* extrapolation models used these results to estimate the rat daily oral dose necessary to produce steady-state *in vivo* blood concentrations equivalent to the AC_{50} values obtained in the 600 *in vitro* ToxCast assays. The estimated rat daily oral doses associated with the *in vitro* assays were compared to the low effect levels (LEL) for apical responses obtained from *in vivo* rodent studies. The results showed that the daily oral dose equivalent for the most sensitive *in vitro* assay provided a conservative estimate of the *in vivo* LEL value. The third study extended the high-throughput pharmacokinetic measurements to include hepatic metabolic clearance and plasma protein binding for 239 of the ToxCast Phase I chemicals using human hepatocytes and plasma. Computational *in vitro*-to-*in vivo* extrapolation models used these results to estimate the human daily oral dose necessary to produce steady-state *in vivo* blood concentrations equivalent to the AC_{50} values obtained in the 600 *in vitro* ToxCast assays. The estimated human daily oral doses associated with the *in vitro* assays were compared with chronic aggregate human exposure estimates to assess whether *in vitro* bioactivity would be expected at the dose-equivalent level of human exposure. Less than 10% of chemicals for which human oral exposure estimates were available had oral equivalent doses at levels equal to or less than the highest estimated U.S. population exposures. Ranking the chemicals by nominal assay concentrations would have resulted in different chemicals being prioritized. The incorporation of dosimetry and exposure provide necessary context for interpretation of *in vitro* toxicity screening data and are important considerations in determining chemical testing priorities.

Dr. Russell Thomas is the director of the Institute for Chemical Safety Sciences at The Hamner Institutes for Health Sciences. Dr. Thomas maintains an adjunct faculty appointment in the Division of Pharmacogenomics and Individualized Therapy at the University of North Carolina at Chapel Hill. His laboratory has diverse interests that range from basic research in cancer biology to applied research in chemical risk assessment. Dr. Thomas completed his M.S. in radiation ecology and Ph.D. in Toxicology at Colorado State University. Following his doctoral studies, Dr. Thomas performed postdoctoral research in molecular biology and genomics at the McArdle Cancer Research Laboratory at the University of Wisconsin. Prior to coming to The Hamner, Dr. Thomas worked in the biotech and biopharmaceutical industry.
Academic and professional honors of Dr. Thomas include the Agilent Thought Leader Award (2011), Society of Toxicology Achievement Award (2009), Honorable Mention for Society of Toxicology Board of Publications Best Paper Award (2009), Best Papers Advancing the Science of Risk Assessment by the Risk Assessment Specialty Section (2007, 2008, and 2011).

Session d'Affichage
Poster Session

HEAVY METALS IN FISH, WATER, *CERATOPHILLYUM DEMERSUM* PLANT AND SEDIMENT SAMPLED FROM WADI EL-RAYAN LAKE, EGYPT

Kh. A. Abdou, A.A.B. Sharkawy, M.M. Manal, O.H. Ehdaa,
Department of Toxicology and Forensic Medicine, Faculty of Veterinary Medicine, Beni Suef University, Beni Suef, Egypt
Department of Toxicology and Forensic Medicine, Faculty of Veterinary Medicine, Assiut University Assiut, Egypt
Biochemistry Department, Animal Health Institute, Cairo, Egypt
Animal Health Institute, El-Fayoum, Egypt

Background: Environmental pollution is a worldwide problem, heavy metals belonging to the most important pollutants. The progress of industries has led to increased emission of pollutants into ecosystems. Wadi Al Raiyan lakes are three man-made lakes in a depression connected to the agricultural drainage system of El-Fayoum province near Cairo, Egypt which receives the agricultural waste water drainage from El-Wadi drain.

Objectives: In this investigation, concentrations of seven heavy metals (Cd, Pb, Fe, Mn, Zn and Cu) in two species of the most consumed fishes (*Tilapia nilotica* and *Claries lazera*), water, aquatic plant *Ceratophillyum demersum*, and sediment have been determined.

Methods: Samples were collected from ten different stations along the southern coast of the lakes, in summer and winter 2010-2011. Heavy metal concentrations were measured by Atomic Absorption Spectroscopy M6, Thermo Scientific 2009. Data were analyzed by means of statistical software package GraphPad InStat Version 2.

Results: The metals studied were detected in all samples examined. Zn had the highest concentration among the metals detected in water. Pb, Cd, Zn, Fe, Cu, and Mn concentrations were below the permissible limit. Cu, Pb and Cd concentrations in water samples during the summer season are much higher than their concentrations in water during winter season; Zn and Fe concentrations in water samples during winter are much higher than their concentrations in water during summer season; Mn concentrations in water samples were the same during winter and summer seasons. Iron concentrations in *Ceratophyllum demersum* L. were above the permissible limit. Cd, Zn, Cu, and Pb concentrations in plant samples during summer season were much higher than their concentrations in winter season. Fe concentrations in plant samples during winter season are much higher than concentrations in summer season. The observed heavy metals concentrations in the sediment and tissues of *Tilapia nilotica* and *Claries lazera* fish samples were above the recommended limits.

Conclusions: The investigation showed elevated levels of heavy metals in the environment. The high concentrations of these metals in water, aquatic plants and fish in the El Ebrahimia canal may be the result of both anthropogenic activities producing industrial, agricultural and domestic waste and accidental pollution incidents.

HIGH-RESOLUTION GENOME-WIDE MAPPING OF ARYL HYDROCARBON RECEPTOR AND ARYL HYDROCARBON RECEPTOR NUCLEAR TRANSLOCATOR BINDING SITES BY CHIP-SEQ

Raymond Lo, Jason Matthews
Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, Canada

Background: The aryl hydrocarbon receptor (AHR) and AHR nuclear translocator (ARNT) activated complex regulates genes in response to the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). AHR has also emerged as a potential therapeutic target for the treatment of human diseases and different cancers, including breast cancer.

Objective & Methods: To better understand AHR and ARNT signaling in breast cancer cells, we used chromatin immunoprecipitation linked to high throughput sequencing to identify AHR- and ARNT-binding sites across the genome in TCDD treated MCF-7 cells.

Results: We identified 2,594 AHR-bound, 1,352 ARNT-bound and 882 high confidence AHR/ARNT co-bound regions. No significant differences in the genomic distribution of AHR and ARNT were observed. Approximately 60% of the co-bound regions contained at least one core AHRE, 5'-GCGTG-3'. AHR/ARNT peak density was the highest within 1 kb of transcription start sites (TSS); however, a number of AHR/ARNT co-bound regions were located as far as 100 kb from TSS. *De novo* motif discovery identified a symmetrical variation of the AHRE (5'-GTGCGTG-3'), as well as FOXA1 and SP1 binding motifs. Microarray analysis identified 104 TCDD responsive genes where 98 genes were up-regulated by TCDD. Of the 104 regulated genes, 69 (66.3%) were associated with an AHR- or ARNT-bound region within 100 kb of their TSS.

Conclusions: Overall, our study identified AHR/ARNT co-bound regions across the genome, revealed the importance but not absolute requirement for an AHRE in AHR/ARNT interactions with DNA, and identified a modified AHRE motif, thereby increasing our understanding of AHR/ARNT signaling pathway.

RING-SUBSTITUTED ANALOGS OF 3,3'-DIINDOLYLMETHANE (DIM) INDUCE APOPTOSIS AND NECROSIS IN ANDROGEN-DEPENDENT AND – INDEPENDENT PROSTATE CANCER CELLS

AA Goldberg[1], VI Titorenko[2], A Beach[2], S Safe[3,4], JT Sanderson[1].
[1] INRS – Institut Armand-Frappier, Université du Québec, Laval, QC, Canada
[2] Department of Biology, Concordia University, Montreal, QC, Canada
[3] Veterinary Physiology and Pharmacology, A&M University, College Station, TX
[4] Institute for Bioscience and Technology, Houston, TX

Background: We have recently shown that novel ring-substituted analogs of 3,3'-diindolylmethane (ring-DIMs) have anti-androgenic and anti-proliferative effects on androgen-dependent prostate cancer cells.

Objectives: The objectives of this study were to compare the activity of these compounds to inhibit cell growth and induce apoptotic and necrotic cell death in androgen-dependent (LNCaP) as well as -independent (PC-3) prostate cancer cells.

Methods: Prostate cancer cells were treated with increasing concentrations of DIM and ring-DIMs (0.3-30 μM) and effects on cell growth were measured in real-time using an xCELLigence cellular analysis system. Chromatin condensation and loss of membrane integrity were determined by Hoechst and propidium iodide staining, respectively. Apoptotic protein markers were determined by immunoblotting and activation of caspases by using selective fluorogenic substrates. Intra- and extracellular concentrations of DIM and ring-DIMs were assessed by electrospray ionization tandem mass spectrometry.

Results: Ring-DIMs inhibited androgen-stimulated LNCaP cell proliferation and induced apoptosis and necrosis in LNCaP and PC-3 cells with 2-4 fold greater potencies than DIM. DIM and the ring-DIMs increased caspases-3, -8 and -9 activity and induced PARP cleavage in both cell lines. The cytotoxicity of the most potent ring-DIM, 4,4'-dibromoDIM, but not the other compounds was decreased by an inhibitor of caspase-3. 4-4'-dibromoDIM was primarily found in the extracellular medium, whereas all other compounds were concentrated to a much larger extent in the cell.

Conclusion: Ring-DIMs inhibited prostate cancer cell growth and induced cell death in LNCaP and PC-3 cells with higher potencies than DIM and also activated different cell death pathways that were structure-dependent, suggesting that these compounds have clinical potential as chemopreventive and chemotherapeutic agents in prostate cancer, regardless of hormone-dependency.

HUMAN EXPOSURE TO BISPHENOL A DURING PREGNANCY IS ASSOCIATED WITH CHANGES IN GENE EXPRESSION IN PLACENTAL AND FETAL LIVER TISSUE

I. Curran[1], A. Nunnikhoven[1], C. Qiao[1], Xu-Liang Cao[1], C.G. Goodyer[2], G.M. Cooke[1]
[1] Food Directorate, Health Canada, Ottawa, ON, Canada.
[2] Montreal Children's Hospital Research Institute, McGill University, Montreal, QC, Canada

Background: Bisphenol A (BPA) is a known endocrine disruptor and is a human health concern. Several animal studies have shown BPA affects reproductive and developmental endpoints. In earlier reports, we have determined the levels of BPA in human fetal liver and placenta samples obtained from the Greater Montreal Area.

Objectives: To assess the possibility that BPA influences global gene expression in human fetal liver and placenta using microarray technology and to identify possible target pathways of BPA.

Methods: Residue analysis of each sample was done to determine BPA levels and classify them into 3 groups. For fetal liver: Low (<1.0 ng/g, n=11), Med (1.0-10.0 ng/g, n=14) and High (>10 ng/g, n=6). For placenta: Low (<1.0 ng/g, n=11), Med (1.0-30.0 ng/g, n=78) and High (>30.0/g, n=10). Gestational age ranged from 11-20 wks. Microarray analysis was performed for 28 fetal liver and 99 placenta samples. Normalized microarray data was filtered for p-value of 0.05 and fold change +/-1.3.

Results: For fetal liver, 40 genes were found to be differentially expressed between Low or Med or High BPA groups. For placenta, 58 genes were differentially expressed between Low or Med or High BPA groups. Tissue morphology, cell movement and cellular growth and proliferation were consistently featured as biological pathways significantly represented in both fetal liver and placental gene lists. Genes associated with the estrogen receptor, such as VEGFC, CAV1 and TGFB2, were up-regulated in fetal liver. By comparison, placenta tissue showed significant up-regulation of four major histocompatibility class I genes (A,B,C,E) as well as immune system and inflammation response pathways. CYP19A, a known target of BPA, was down-regulated in placenta.

Conclusions: This study has identified potential gene/pathway targets in the human fetal liver and placenta which correlate with environmental BPA exposure during critical fetal developmental stages.

Supported by CMP.

ARSENIC INCREASES ATHEROSCLEROSIS BY LXRα-DEPENDENT AND LXRα-INDEPENDENT MECHANISMS

Maryse Lemaire[1], Catherine A. Lemarié[2], Manuel Flores Molina[1], Koren K. Mann[1]
[1] Dept. Oncology and [2] Medicine, Lady Davis Institute for Medical Research, McGill University, Montreal, Canada

Background: Arsenic exposure has been linked to atherosclerosis; however, molecular mechanisms involved in arsenic-enhanced atherosclerosis are unknown. Previously, we have shown *in vitro* and *in vivo* that arsenic inhibits transcriptional activation of the liver X nuclear receptors (LXR), a key regulator of macrophages lipid homeostasis.

Objective: We evaluated here the role of LXRα in arsenic-induced atherosclerosis using the $ApoE^{-/-}$ mouse model of atherosclerosis.

Methods: $LXR\alpha^{-/-}ApoE^{-/-}$ mice were exposed to 200 ppb arsenic for 13 weeks or maintained on tap water. Atherosclerosis lesion areas were then studied.

Results: We have previously shown that 200 ppb arsenic increases atherosclerosis plaque size in $ApoE^{-/-}$ mice after 13 weeks. In contrast, $LXR\alpha^{-/-}ApoE^{-/-}$ mice do not show increases in plaque size following arsenic exposure. Also, because we previously saw significant changes in plaque composition in the $ApoE^{-/-}$ mice, we assessed plaque staining in $LXR\alpha^{-/-}ApoE^{-/-}$ of: 1) lipid deposition and macrophages content and 2) collagen composition and smooth muscle cells content. Interestingly, arsenic decreases macrophages in $LXR\alpha^{-/-}ApoE^{-/-}$, but no change was observed in $ApoE^{-/-}$ exposed mice. However, arsenic increases lipids in both genotypes, suggesting impairment in the macrophages cholesterol efflux capacity and a subsequent lipid accumulation. Secondly, we observed that arsenic decreases collagen content in $LXR\alpha^{-/-}ApoE^{-/-}$ and $ApoE^{-/-}$ to the same extend, but arsenic increases smooth muscle cells in the in $LXR\alpha^{-/-}ApoE^{-/-}$, while it decreases it in $ApoE^{-/-}$. This indicates that LXRα may be involved in maintaining matrix integrity. In fact, arsenic-exposed $LXR\alpha^{-/-}ApoE^{-/-}$ plaques showed increases matrix metalloproteinase (MMPs) activity compare to both control $LXR\alpha^{-/-}ApoE^{-/-}$ and $ApoE^{-/-}$, which could be responsible for both the decrease in plaque collagen and the smooth muscle cells invasion.

Conclusions: Our observations suggest that arsenic maybe increasing atherosclerosis formation through LXR inhibition, but it alters plaque composition in a LXR independent manner.

DIFFERENTIAL INFLAMMATORY EFFECTS OF 20 nm AND 70 nm SILVER NANOPARTICLES ON HUMAN NEUTROPHILS

Michelle Poirier, Jean-Christophe Simard, Denis Girard
Laboratoire de recherche en Inflammation et Physiologie des Granulocytes, INRS Institut Armand-Frappier, Université du Québec, Laval, QC, Canada

Background: Inflammation is highly regulated by elimination of apoptotic neutrophils by professional phagocytes. In certain conditions, someNPs (TiO_2 for example) are able to delay human neutrophil apoptosis. Because the effects of only few NPs on the apoptotic rate of neutrophils have been investigated, it is plausible that some NPs will delay and some others will accelerate apoptosis in neutrophils. This could be affected not only by the nature but also by the size of a given NP.

Objectives: The aim of this study was to determine whether or not silver (Ag) NP could alter the apoptotic rate of human neutrophils and to verify the role of the size of the AgNP on apoptosis.

Methods: Neutrophils were isolated from healthy volunteers and incubated in vitro with increasing concentrations of AgNP with a size of 20 nm (AgNP20) or 70 nm (AgNP70) for up to 24h. Cell cytotoxicity was determined by trypan blue exclusion assay as well as by the release of lactate dehydrogenase (LDH). Apoptosis was evaluated by cytology and the production of interleukin (IL)-6 and IL-8 was determined by ELISA.

Results: No cell necrosis was observed when neutrophils were incubated for 24h in the presence of 5, 10, 20, 50 or 100 µg/ml AgNP70. The same results were observed for AgNP20 except that ~10% of cells were necrotic at the highest concentration of 100µg/ml. No secretion of LDH was observed for all experimental conditions tested. Interestingly, the apoptotic rate was similar with that of controls when cells were incubated with 10 µg/ml independently of the size of the AgNP (~45%). However, at 100 µg/ml, AgNP20 increased apoptosis (81.8 ± 4.1%) while AgNP70 delay the apoptotic rate (19.3 ± 4.8). Both AgNP20 and AgNP70 did not increase the production of IL-6, despite the fact that the positive control, lipopolysaccharides (LPS), markedly increased the production of this cytokine. However, while the basal production of IL-8 was 146 ± 65 pg/ml, we observed an increase of more than 22000 pg/ml IL-8 in response to 100 µg/ml AgNP20 and 1000 pg/ml when neutrophils were treated with 100 µg/ml AgNP70.

Conclusions: We conclude that AgNP can alter the apoptotic rate of human neutrophils and that opposing effects are observed according to the size of the NP. The fact that AgNPs are not cytotoxic for neutrophils, and that no increase of LDH secretion was observed (even at 100 µg/ml), as well as IL-6, indicate that the modulatory activity of the AgNPs on apoptosis at not due to the release of nonspecific molecules simply caused by cell necrosis. Whether the very elevated concentration of IL-8 induced by AgNP20 is related to its ability to increase apoptosis remains to be determined. In future, it will be important to understand how the particle size is involved in the modulatory activity of AgNP on human neutrophil apoptosis.

HEALTH BENEFITS OF SASKATCHEWAN LENTILS IN ARSENIC-EXPOSED RATS

Shweta Sah[1], Albert Vandenberg[2], Judit E.G. Smits[1]
[1] University of Calgary, Faculty of Veterinary Medicine, Department of Ecosystem and Public Health, Calgary, AL, Canada
[2] NSERC Industrial Research Chair, Plant Sciences Department, University of Saskatchewan, Saskatoon, SK, Canada

Background: Arsenicosis, or chronic arsenic (As) toxicity affects an estimated 140 million people world-wide which is concentrated in the Indo-Gangetic Plains and mountainous areas of China, but is growing in other regions as well. An As selenium (Se) complex is formed in the liver allowing excretion through hepatic biotransformation and subsequent urinary and fecal elimination. Saskatchewan (SK) grown lentils, relative to lentil produced elsewhere in the world, have the high, yet nontoxic Se concentrations. Diets containing high Se lentils may reduce body burdens of As, and offer health benefits such as supporting a healthy immune response and increasing antioxidant defenses.

Objectives: The objective of this study was to examine the potential benefits of selenium-manipulated lentil diets in counteracting chronic As toxicity in rats.

Methods: 40 young male Wistar rats were exposed to As in drinking water, and were fed selenium manipulated diets for 14 weeks. Rats were randomly assigned to treatments per drinking water; control (0 ppm As) and exposed (40 ppm As) groups. They were fed i) Se deficient (<0.01 ppm) US lentil diets or ii) Se fortified (0.3 ppm) SK lentil diets. To assess As-induced liver damage and oxidative stress, malondialdehyde (MDA) lipid peroxidation assays (liver) and glutathione (GSH) (whole blood) assays were performed. Whole blood, urine and feces collected monthly, and kidney and liver collected after euthanasia were analyzed for total arsenic and selenium concentrations. Immune function was evaluated by testing the antibody response.

Results: The high Se lentil diets increased urinary and fecal As excretion and decreased renal As residues ($p < 0.01$). Rats fed high Se diets had higher glutathione levels regardless of As exposure, and had higher antibody responses in both As-exposed and control groups ($p=0.04$). Selenium deficiency caused higher hepatic peroxidative damage ($p<0.05$).

Conclusions: These findings indicate that damage from chronic As exposure can be reduced by feeding high Se lentils, compared with lentils that are produced in regions in the world with Se-deficient soils.

2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN-INDUCIBLE POLY(ADP-RIBOSE) POLYMERASE IS A NEGATIVE REGULATOR OF DIOXIN-INDUCED ARYL HYDROCARBON RECEPTOR TRANSACTIVATION AND A MONO-ADP-RIBOSYLTRANSFERASE.

L. MacPherson, S. Rajendra, L. Tamblyn, J.P. McPherson, J. Matthews.
Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario, Canada

Background: The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates the toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). AHR regulates the expression of hundreds of genes including TCDD-inducible poly(ADP-ribose) polymerase (TiPARP, PARP7). TiPARP is a member of the PARP superfamily, which is an enzyme family that mediates poly(ADP-ribosyl)ation of protein targets. Poly(ADP-ribosyl)ation is post-translational modification associated with a number of biological functions including DNA repair, transcription, and apoptosis. TiPARP contains a C-terminal PARP catalytic domain, a conserved WWE (tryptophan-tryptophan-glutamate) domain and a zinc-finger domain. However, the biological role of TiPARP and whether TiPARP modulates AHR action is unknown.

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

Methods: We investigated the effects of loss and overexpression of TiPARP on TCDD-induced cytochrome P450 1A1 (CYP1A1) and CYP1B1 gene expression in T47D human breast carcinoma and HuH7 hepatoma cell lines. PARP catalytic assays were used to determine TiPARP catalytic activity.

Results: RNAi-mediated knockdown of TiPARP significantly increased TCDD-induced CYP1A1 and CYP1B1 expression. TiPARP knockdown also reduced TCDD-induced AHR protein degradation following 24 h treatment. TiPARP overexpression decreased TCDD-induced *CYP1A1*- and *CYP1B1*-regulated reporter activity in a dose-dependent manner. Interaction studies demonstrated that overexpressed GFP-tagged TiPARP co-localized and co-immunoprecipitated with AHR. TiPARP truncations and point mutants revealed TCDD-induced inhibition required the zinc-finger and catalytic function. Catalytic assays demonstrated TiPARP possesses mono-ADP-ribosyltransferase (mART) activity rather than poly(ADP-ribosyl)ation activity. Auto-mART activity required both the catalytic domain and central portion (residues 275-328).

Conclusions: Collectively, these results demonstrate TiPARP as a mART and a negative regulator of AHR transactivation.

INDUCTION OF AMINOGLUTETHIMIDE-MEDIATED PROTEIN RADICAL FORMATION BY *N*,*N*-DIMETHYLGLYCINE THROUGH A HOMOCYSTEINE-DEPENDENT MECHANISM: POTENTIAL IMPLICATIONS FOR DRUG-INDUCED AGRANULOCYTOSIS

Argishti Baghdasarian, Karim Michail, Arno G. Siraki
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton T6G 2E1, Canada

Background: Aminoglutethimide (AG), an anti-cancer drug in clinical use, has been shown to induce agranulocytosis (dangerously low neutrophil count); however, the mechanism of this reaction is unknown. AG-derived free radical metabolites are generated by myeloperoxidase (MPO), a major bactericidal peroxidase abundant in neutrophils; we have proposed that these metabolites subsequently initiate adeleterious modification of the proteins, especially MPO, into protein radicals. Our lab has previously demonstrated that native polyunsaturated fatty acids can attenuate myeloperoxidase protein radical formation and might therefore mitigatethe toxicity of AG free radical metabolites. This led us to investigate the opposite scenario, that is, if endogenous biomolecules augment the effect of AG free radical metabolites. Several lines of evidence confirmed that high levels of *N*,*N*-dimethylglycine (DMG),a byproduct of choline metabolism, inhibit betaine-homocysteine-S-methyl-transferase (BHMT), which resulted in the accumulation of homocysteine (Hcy). Furthermore, hyperhomocysteinemia has been repeatedly demonstrated to correlate with oxidative stress, which could potentially drive the peroxidative metabolism of AG.

Objectives: 1) To determine the modulatory effect of DMG on AG-induced myeloperoxidase protein radical formation. 2) To assess the ensuing cytotoxic and apoptotic events in MPO-containing human promyelocytic leukemia (HL-60) cells.

Methods: Oximetric analysis to determine real-time oxygen consumption, electron paramagnetic resonance (EPR) spectrometry to detect primary and secondary free radical metabolites, immuno-spin trapping and/or SDS-PAGE electrophoresis to detect MPO radicals and BHMT in cell lysates and intactHL-60 cells were used. Cell viability, cytotoxicity and caspase activation assays were performed using ApoTox-Glo Triplex kit (Promega). Intracellular glutathione (GSH) and Hcy levels were measured using the DTNB assay and HPLC, respectively.

Results: DMG significantly enhanced oxygen consumption by oxidation of GSH through nitrogen-centered radicals generated by the AG/MPO/H_2O_2. There was a significant increase in MPO protein radical formation in both HL-60 cell lysates and intact cells in presence of DMG, which correlated with cytotoxicity, apoptotic signals and GSH depletion. HL-60 cells express BHMT (HepG2 cells were used as positive control). Levels of Hcy were markedly higher in HL-60 cells treated with DMG vs. untreated controls.

Conclusions: Our findings suggest that DMG dose-dependently increased AG-induced MPO protein radicals which correlated with glutathione depletion, caspase activation, cytotoxicity and hyperhomocysteinemia in HL-60 cells. Protein radicals could suggest an autoimmune mechanism of drug-induced agranulocytosis, which may be enhanced in the presence of oxidative stress.

MODULATORY ACTIVITY OF TiO_2, CeO_2 AND ZnO ON HUMAN NEUTROPHIL DEGRANULATION

Kim Babin, Denis Girard
Laboratoire de recherche en Inflammation et Physiologie des Granulocytes, INRS Institut Armand-Frappier, Université du Québec, Laval, QC, Canada

Background: Nanoparticles (NPs) are used in a vast array of applications, including personal products, household commodities, food and in medicine. Because the number of products containing NPs has increased rapidly, humans will be increasingly exposed to NPs. Therefore, the effects of NPs on human health need to be determined. Recently, human neutrophils have been found to be targets to NP exposure. Knowing the importance of neutrophils in inflammation, investigating the ability of NPs to modulate neutrophil functions is an excellent way to evaluate potential nanotoxicity of a given NP. Degranulation is one of the most important functions exerted by neutrophils for the defense of an organism against an infection; it consists in a rapid release of potent degradation enzymes, several receptors involved in the recognition and ingestion of pathogens, etc. These molecules are localized in three different kinds of granules: azurophil, specific/gelatinase and secretory granules.

Objective: The objective of the present study was to determine whether the three NPs, TiO_2, CeO_2, and ZnO, can alter the degranulation process.

Methods: Neutrophils were isolated from healthy volunteers and incubated in vitro with 100 µg/ml of TiO_2, CeO_2, or ZnO NP (concentration selected according to previous preliminary experiments). Degranulation was assessed by flow cytometry, by monitoring cell surface expression of CD63, CD66b, and CD35, for azurophil, specific/gelatinase and secretory granules, respectively). Western blot experiments were conducted to identify proteins secreted from the granules into the extracellular milieu. Zymography was used to determine enzymatic activity in the extracellular milieu allowing visualization of gelatine degradation by gelatinases contained in the specific/ gelatinase granules.

Results: The cell surface expression of CD66b was significantly increased by CeO_2 and TiO_2 while the expression of CD63 and CD35 was not significantly altered. The protein expression of myeloperoxidase (azurophil granules) and matrix metallopeptidase 9 (MMP9 or gelatinase B) (specific/gelatinase granules) was significantly increased by the three NPs. Expression of albumin was significantly increased by CeO_2 and TiO_2, but, although its expression was increased by ZnO, this was not significant. Zymography revealed that the enzymatic activity in the supernatant of CeO_2 and TiO_2-induced neutrophils was significantly increased, but not ZnO.

Conclusion: We conclude that the NPs tested can induce degranulation in human neutrophils. However, the cell surface expression of specific markers for each type of granules does not appear to be the best way to determine potential effect on degranulation, except for CD66b, since the results also correlate with the detection of the protein (gelatinase B) in the supernatants, as well as the gelatinase enzymatic activity detected by zymography.

ARYL HYDROCARBON RECEPTOR-DEPENDENT RETENTION OF NUCLEAR HUR SUPPRESSES CYCLOOXYGENASE-2 EXPRESSION INDEPENDENT OF DNA-BINDING

M. Zago[1], J.A. Sheridan[1], P. Nair[2], A. Rico de Souza[1], I.E. Gallouzi[1], S. Rousseau[1], S. Di Marco[1], Q. Hamid[1], D.H. Eidelman[1], C.J. Baglole[1]
[1] McGill University, Montreal QC, Canada; [2] McMaster University, Hamilton ON, Canada

Background: The aryl hydrocarbon receptor (AhR) has emerged as an endogenous suppressor of cyclooxygenase-2 (Cox-2). Cox-2 is an immediate-early gene that is robustly increased by cigarette smoke exposure. We have published that the AhR suppresses cigarette smoke-induced Cox-2 protein but not mRNA, suggesting post-transcriptional regulation as a mechanism. The AhR may destabilize Cox-2 mRNA by retaining the RNA-binding protein (RBP) HuR in the nucleus. There is no known association between the AhR and HuR.

Objectives: The objectives of this study are to determine if the AhR suppression of cigarette smoke-induced Cox-2 protein is due to Cox-2 mRNA destabilization independent of DNA binding and to investigate whether AhR-dependent retention of nuclear HuR is responsible for Cox-2 mRNA destabilization.

Methods: $AhR^{-/-}$, $AhR^{+/+}$, $AhR^{DBD/DBD}$ (harbouring a mutant AhR unable to bind DNA) and $AhR^{DBD/B6}$ mouse lung fibroblasts were exposed to cigarette smoke extract (CSE) for 3 h, followed by actinomycin D (ActD) for 30 min, 1 or 3 h. Cox-2 protein and mRNA were analyzed by western blot and qRT-PCR, respectively. HuR expression was assessed by western blot and immunofluorescence. $AhR^{-/-}$ cells were transfected with HuR siRNA and exposed to 1% CSE for 3 h with or without ActD for an additional 3 h. Cox-2 mRNA was then assessed by qPCR.

Results: Steady-state Cox-2 mRNA levels significantly declined upon ActD treatment in $AhR^{+/+}$, $AhR^{DBD/DBD}$ and $AhR^{DBD/B6}$ cells, suggesting that the AhR destabilizes Cox-2 mRNA by a DRE-independent mechanism. Cox-2 mRNA instability was due to the nuclear retention of HuR. CSE did not alter HuR expression, but induced cytoplasmic HuR shuttling only in $AhR^{-/-}$ cells. Knockdown HuR in $AhR^{-/-}$ cells significantly decreased Cox-2 mRNA expression after exposure to ActD.

Conclusions: AhR-dependent retention of nuclear HuR suppresses cigarette smoke-induced Cox-2 protein by a mechanism that is independent of DNA-binding activity. These important findings open the possibility that a DRE-independent AhR pathway may be exploited therapeutically as an anti-inflammatory target.

TUNGSTEN EXPOSURE INCREASES LUNG METASTASES IN AN ORTHOTOPIC MURINE BREAST CANCER MODEL

Alicia M. Bolt, Valérie Sabourin, Jerome Janiak, Alexander Kelly, Maryse Lemaire, Giuseppina Ursini-Siegel, Koren K. Mann
The Lady Davis Institute for Medical Research, McGill University, Montreal, Canada

Introduction: Tungsten is a strong, flexible metal that until recently had been thought to be an "inert" metal. These properties led to its incorporation into the manufacturing of medical devices. In a recent clinical trial, a tungsten-based shield was used in the treatment of breast cancer patients undergoing intraoperative radiotherapy. Following the procedure, the women were left with residual tungsten in their breasts. Elevated tungsten levels in the blood and urine indicate that tungsten is not remaining in the breast tissue. Tungsten was detected in the urine of patient even 8 months after mastectomy, indicating another reservoir has been created. Based on previous data, we hypothesize this reservoir to be the bone. Animal studies suggest that tungsten may contribute to carcinogenesis and can alter development and increase DNA damage in the immune system.

Objective and Methods: In order to evaluate the effect of tungsten on breast cancer, female Balb/C mice were exposed to 15 ppm of sodium tungstate for 1 month followed by injection of 66cl4 cancer cells into the fat pad. The size of primary tumor, the extent of lung metastases and immune parameters were evaluated.

Results: Tungsten did not alter the growth of the primary tumor. However, the number and average size of lung metastases was significantly greater in the tungsten-exposed animals. This model is not known to metastasize to the bone, but we found that tumor-bearing mice had 3-fold more tungsten in the bone than non-tumor bearing mice. Tungsten increased the peripheral blood leukocyte count in non-tumor bearing mice, but decreased the massive granulocytosis associated with tumor growth.

Conclusions: These data suggest that tungsten increases the extent of breast cancer metastasis to the lung, which could have a significant impact on individuals who have cancer and are also exposed to tungsten. The levels of tungsten deposition within the bone and immune cell parameters were also altered, which could also impact metastasis in this model.

ALTERATION OF SEROTONIN TRANSPLACENTAL TRANSPORT BY EXPOSURE TO LEAD.

Marc Fraser[1], Joey St-Pierre[1], Céline Surette[2], Cathy Vaillancourt[1].
[1]INRS – Institut Armand-Frappier and BioMed Research Centre-UQAM-INRS-UQTR, Laval, QC, H7V 1B7;
[2]Département de chimie et de biochimie, Université de Moncton, Moncton, NB, E1A 3E9

Background: Maternal exposure to metals may interfere with placental functions and affect the fetus development. Our laboratory has observed a negative correlation between the expression of placental serotonin transporter (SERT) and levels of lead (Pb) measured in cord blood (fetal blood). We suggest that Pb exposure decreases SERT expression and activity in trophoblast, the functional unit of the placenta expressing the SERT.

Objectives: The aim of this study is to determine the effect of exposure to low Pb levels on the expression and activity of SERT in a model of villous trophoblast, the cell line BeWo.

Methods: BeWo cells were exposed to increasing doses of Pb (0.01 to 1000 nM) for 2 h (SERT activity) or 24 h (expression). The expression of SERT was analyzed by Western blotting (proteins) and real time RT-PCR (mRNA). SERT activity was measured by capture of 3[H]-5HT in the absence or presence of a specific inhibitor of SERT, fluoxetine (10 μM). The cytotoxicity was analyzed with xCELLigence system and by the activity of lactate deshydrogenase (LDH, Roche).

Results: No cytotoxic effect is observed at all doses of Pb. The results shows a maximum decrease of SERT protein expression at 100 nM Pb (30%) compared to control (0 nM Pb), whereas RT-qPCR analysis shows a 40% increase in the expression of SERT mRNA at 100 nM Pb. The activity of SERT is reduced by 50% at 0.01 nM Pb and reached a maximum decrease of 75% at 0.1 nM Pb compared to untreated cells untreated.

Conclusions: These results show that low concentrations of Pb, acceptable by current standards of Health Canada and having no effect on the viability of the placental trophoblast, alter SERT activity, suggesting that current acceptable level of Pb exposure should be revised. The mechanism by which Pb affects transplacental transport of serotonin remains to be studied.

PRENATAL EXPOSURE TO GROUP B STREPTOCCOCUS, PHTALATES, AND FLAME RETARDANTS: A NEW ANIMAL MODEL FOR ASD?

S. Degroote, J. Bergeron, ME Brochu, G. Sébire, L. Takser
Département de Pédiatrie, Faculté de Médecine et des Sciences de la Santé, Université de Sherbrooke, QC, Canada

Background: Epidemiological data suggests that: (i) prenatal exposure to phthalates and flame retardants (FRs) can affect mental and motor development, and provoke internalizing behavior; (ii) there is a clear link between ASD and prematurity, frequently associated with chorioamnionitis, for which Group B Streptoccocus (GBS) is one of the most frequent causes. We hypothesize that the combination of these two frequent aggressions (GBS and FRs/ phthalates) during a critical perinatal period can lead to ASD through a perinatal neuroinflammatory response. In numerous rodent studies, valproic acid, a frequently prescribed antiepileptic drug, has been shown to induce ASD-like behavior including lower exploratory activity, deficit in social behaviors, diminished acoustic prepulse inhibition, delayed nest-seeking response. We therefore used valproic acid as a positive control in our study.

Objective: To determine the extent to which prenatal exposure to GBS and/or phthalates/FRs induces the full spectrum of autism relevant behavior.

Methods: The study was carried out in 25 pregnant Lewis rats exposed to: GROUP 1: a mixture of selected FRs and phthalates at low dose (three phthalates: DEHP, DBP, DiNP; and 2 FRs: BDE-47, BDE-99) (n=7) in peanut oil by gavage from GD15 to delivery; GROUP 2: inactivated GBS y i.p. injections from GD19 to GD21 and the same mixture of contaminants as in GROUP 1 (n=7) by gavage; GROUP 3: 600mg/kg of valproic acid by i.p. injection at GD12, a positive control group (n=5); GROUP 4: peanut oil vehicle by gavage from GD15 to delivery (n=6), a negative control group. The following behavioral tests were administered to offspring: recording of ultrasonic vocalizations (PND7 and PND14), nest-seeking behavior (PND8), auditory startle (PND11 to PND13), Open Field (PND20), Elevated Plus Maze (PND25), prepulse inhibition (PPI) of the acoustic startle (PND35), and test of social interactions (PND40).

Results: Our preliminary results show that offspring exposed to GBS and contaminants, as well as those exposed to valproic acid, had significantly more difficulty to find the maternal compartment in nest-seeking behavior and were less active in the Open Field test than control animals. Animals exposed in utero to the mixture of FRs and phthalates were more active in the Open Field and social interactions tests. In addition, we observed a dramatic effect of valproate acid on gestation in three of the five dams (non delivery and spots of embryo implantation on the uterus) and on developmental landmarks (decreased birth weight, delayed eye opening and fur growth) in the two delivered litters.

Conclusions: Our results suggest that simultaneous prenatal exposure to GBS, phthalates, and FRs induces long-term behavioral effects in rat offspring, similar to the features of ASD, including a very attenuated response to maternal presence and substantially less explorative behavior.

LOSS OF PPARγ EXPRESSION IN MAMMARY SECRETORY EPITHELIAL CELLS CREATES A PRO-BREAST TUMOURIGENIC ENVIRONMENT.

A. Apostoli[1], G. Skelhorne-Gross[1], R. Rubino[2], M. Schneider[1], S. SenGupta[1], C. Nicol[1,2].
[1]Dept. of Pathology & Molecular Medicine; [2]Division of Cancer Biology & Genetics, Cancer Research Institute, Queen's University, Kingston, ON, Canada K7L 3N6.

Background: Breast cancer is the leading cause of new cancer diagnoses among women, with 1 in 29 Canadian women dying from breast cancer metastases. Interactions between many identified, and other still unknown, risk factors can influence the onset and spread of this disease. These risk factors can be subtyped into *environmental*, e.g. exposure/activation of cancer causing chemicals such as 7,12-dimenthylbenz[a]anthracene (DMBA); and *genetic*, e.g. loss/mutations in genes that normally prevent tumour growth such as PTEN. Improved understanding of these interactions may help to more effectively diagnose, treat and/or prevent breast cancer. We previously showed, using peroxisome proliferator-activated receptor (PPAR)γ heterozygous mice, that normal expression of PPARγ was critical to stop DMBA-induced breast tumour progression. Mammary secretory epithelial (MSE) cells are among the many PPARγ-expressing cell types associated with breast tissue. MSEs proliferate as required during pregnancy, and undergo apoptosis or reversible transdifferentiation during involution once lactation is complete.
Objectives: To test the hypothesis that *in vivo* loss of PPARγ in MSE cells enhances DMBA-mediated breast tumourigenesis.
Methods: MSE cell-specific PPARγ knockout (PPARγ-MSE KO) and control (PPARγ-WT) mice were generated, mated and allowed to nurse their pups for three days. One week after the start of involution, dams were treated with DMBA to initiate breast tumours, and randomized on week 7 to continue receiving a normal chow diet (DMBA Only: PPARγ-WT, n=15; PPARγ-MSE KO, n=25) or one supplemented with a PPARγ activating drug (DMBA+ROSI: PPARγ-WT, n=17; PPARγ-MSE KO, n=24), and monitored for changes in breast tumour outcomes. Protein (Western blot) analysis was performed on untreated mammary glands and mammary adenocarcinomas (AC) and squamous cell carcinomas (SCC) generated in PPARγ-WT and PPARγ-MSE KO mice across both treatment groups.
Results: PPARγ-MSE KOs had significantly lower overall survival and decreased mammary tumour latency compared to PPARγ-WT controls. PPARγ activation significantly reduced DMBA-mediated malignant mammary tumour volumes irrespective of genotype. MSE-specific PPARγ loss resulted in decreased mammary gland expression of PTEN and Bax. Moreover, PPARγ activation down-regulated cyclin D1 in mammary tumours. Interestingly, compared to similarly treated PPARγ-WT controls, Cox-2 expression increased ~3-fold in SCCs from DMBA. Only-treated PPARγ-MSE KOs. Cox-2 expression was decreased ~2-fold in SCCs from DMBA+ROSI-treated PPARγ-WTs compared to DMBA Only tumours; however, this effect was not observed in mammary tumours from DMBA+ROSI-treated PPARγ-MSE KOs.
Conclusions: Together, these studies are the first to show the protective role of MSE-specific PPARγ expression and signaling in breast tumourigenesis, and suggest PPARγ loss may contribute to the increased breast tumour risk following childbirth. This may be due to decreased mammary tumour latency from disrupted Bax and PTEN signaling in the mammary gland prior to tumourigenesis, and COX-2 and cyclin D1 overexpression in mammary tumours. This also adds further support for a novel chemopreventive role of PPARγ activation in breast cancer.

CO-CULTURE OF HUMAN ADRENOCORTICAL AND TROPHOBLAST CELLS TO STUDY THE REGULATION OF FOETO-PLACENTAL STEROIDOGENESIS.

Andrée-Anne Hudon Thibeault, Cathy Vaillancourt, Thomas Sanderson.
INRS-Institut Armand-Frappier and BioMed Research Center-UQAM-INRS-UQTR

Background: During pregnancy, estrogen production requires a perfect cooperation between mother, placenta and foetus. Foetal androgen precursors are converted to estrogens by the placental enzyme CYP19 (aromatase). A disruption of this communication and an alteration of estrogen production might cause adverse pregnancy outcomes and disrupt foetal development.

Objectives: To develop an *in vitro* co-culture system of BeWo (human trophoblast) and H295R (human foetal-like adrenocortical) cells to study the regulation of steroidogenesis of the foeto-placental unit. To validate our system, we determined the effect of the co-culture on BeWo and H295R cell phenotypes and the biosynthetic capacity of the co-culture to produce estrogens.

Methods: H295R cells (bottom of wells) and BeWo cells (in the insert) were cultured in plates with transwell permeable polycarbonate inserts (0.4 μm pores) in a modified co-culture medium: DMEM/F-12 HAM without phenol red, supplemented with 1.2 g/L NaHCO3, 2 mg/L pyridoxine-HCl, 1% FBS, 2.5% Nu-serum and 1% ITS+1. Cell proliferation and doubling time were evaluated with an xCELLigence^{TM} continuous cell monitoring system. Aromatase activity was determined by tritiated water-release assay and human chorionic gonadotropin (hCG) production by enzyme-linked immunoabsorbent assay (ELISA).

Results: The co-culture medium did not have major effects on proliferation of the two cell lines, except that H295R cell doubling time was reduced by 14% in the co-culture medium (29.6 ± 0.7 h) compared to standard cell culture medium (34.4 ± 1.2 h). The co-culture medium had no impact on hCG production in BeWo cells suggesting that their differentiating capacity was not affected. Aromatase activity was four times higher in BeWo than in H295R cells, but was not significantly altered in the presence of co-culture medium. After co-culturing BeWo and H295R cells for 24 hour, 17β-estradiol and estriol production were 152.8 and 520 pg/mL, respectively, in comparison with 33.4 and 40 pg/mL by H295R cells alone and with 7.6 and 140 pg/mL by BeWo cells alone.

Conclusions: These results demonstrate that a BeWo and H295R co-culture is suitable to study the regulation of foeto-placental steroidogenesis. We have demonstrated that the enzymes of the two components of the foeto-placental unit are complementary and work in synergy to produce estrogens such as the pregnancy-relevant estriol, thus confirming that this co-culture model mimics physiological conditions. This co-culture model provides a unique and valuable research tool to evaluate effects of drugs and toxic compounds on the steroidogenic interactions of the foeto-placental unit and consequently on pregnancy and foetal development.

THE UPREGULATION OF REPAIR OF AFLATOXIN-B_1-N^7-GUANINE ADDUCTS THAT OCCURS FOLLOWING CHRONIC DIETARY EXPOSURE TO AFLATOXIN B_1 IS ATTENUATED IN HETEROZYGOUS P53 KNOCKOUT MICE.

J.E. Mulder[1], R. Mehta[2], G.S. Bondy[2], T.E. Massey[1].
[1] Pharmacology& Toxicology Graduate Program, Queen's University, Kingston, Ontario, K7L 3N6;
[2]Toxicology Research Division, 2202D, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario, K1A 0K9.

Background: Aflatoxin B_1 (AFB_1) is produced by *Aspergillus*species,moulds that grow on grains, oilseeds and spices. AFB_1 is biotransformed *in vivo* into the highly reactive AFB_1-*exo*-epoxide, which binds preferentially to the N^7 position of guanine residues in DNA, forming AFB_1-N^7-Gua adducts. These adducts, which are normally excised by nucleotide excision repair (NER), can cause mutations that can lead to cancer if not repaired. *p53* is a tumour suppressor gene implicated in both AFB_1 carcinogenesis and the regulation of DNA repair.

Objectives: To assess the effects of low-dose chronic dietary AFB_1 exposure on NER activity in lung and liver of heterozygous *p53* knockout and wild type mice.

Methods: Male heterozygous*p53*knockout (*p53* (+/-); B6.129-*Trp53*^{tm1Brd}N5, Taconic) and wild type mice were exposed to0, 0.2 or 1.0ppm AFB_1 in AIN 93M semi-purified diet for 26 weeks. NER activity of lung and liver nuclear protein extracts was assessed with an *in vitro* assay, using adducted plasmid DNA as a substrate.

Results: In wild type mice, repair of AFB_1-N^7-Guaadducts was 124% and 96% greater than control in lung extracts from mice exposed to 0.2 ppm or 1.0ppm AFB_1 respectively, and 224% greater than control in liver extracts from mice exposed to 0.2ppm AFB_1($p<0.05$). No effect was observed compared to control in liver extracts from mice exposed to 1.0 ppm AFB_1. In contrast, in *p53*(+/-)mice, repair of AFB_1-N^7-Gua was only 45% greater than control in extracts from lungs of mice exposed to 0.2ppm AFB_1($p<0.05$), and no difference in repair was observed between extracts from lungs of mice treated with 1.0ppm AFB_1 versus control or extracts from livers of mice treated with either AFB_1 concentration. When comparing AFB_1 effects on NER activity after normalizing to untreated tissue, the induction of NER activity was significantly attenuated in*p53*(+/-) mice compared to wild type controls in both lung and liver.

Conclusions: The increase in NER activity seen in wild type mice following chronic AFB_1 exposure suggests a homeostatic response to DNA damage. This response was diminished or lost in *p53*(+/-) mice, which is consistent with *p53* having a major role in regulating NER. (Supportedby CIHR Grant No. MOP-89698 andGRDI)

FOOD–BORNE ACRYLAMIDE EXPOSURE NEITHER PROMOTES THE GROWTH OF HT-29 HUMAN COLON CANCER XENOGRAFTS IN ATHYMIC NUDE (*NU/NU*) MICE, NOR ALTERS TUMOR SUPPRESSOR GENE PROMOTER METHYLATION STATUS AND EXPRESSION OF CANCER-RELATED GENES

Jayadev Raju[1], Jennifer Roberts[1], Chandni Sondagar[1], Kamla Kapal[1], Syed A. Aziz[1], Don Caldwell[1], Stephen Hayward[2]and Rekha Mehta[1]
[1] Bureau of Chemical Safety and [2] Bureau of Biostatistics and Computer Applications; Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, ON

Background: Acrylamide, a known rodent and possible human carcinogen, is formed in certain carbohydrate-rich foods processed at high temperature. Two recent experimental studies suggest that acrylamide elicits tumorigenic effects in the colon.

Objectives: We evaluated if dietary acrylamide, at doses reflecting upper levels found in human foods, promoted the growth of human colon cancer xenografts.

Methods: Male athymic nude (*nu/nu*) mice bearing HT-29 human colon adenocarcinoma cells-derived tumor xenografts received AIN-93G diets without (control) or with acrylamide (0.5, 1.0 or 2.0 mg/kg diet); tumor growth was monitored and mice were killed 4 weeks after. Using real-time qPCR arrays, we assessed the methylation status of 22 tumor suppressor gene (TSG) promoters, and the expression profile of 84 genes, representing nine biological pathways involved in tumorigenesis, in the colon tumor xenografts from control and acrylamide-treated (2.0 mg/kg diet) mice.

Results: There were no differences in the palpable tumor size between control and acrylamide-treated groups at any of the four weekly time points. Dietary acrylamide did not alter the size or the wet weights of the colon tumor xenografts by comparison to the control at termination. There were no changes in the ratio of the labeling indexes of mitotic and apoptotic cells between control and acrylamide-treated groups. The methylation status of TSG promoters and the expression patterns of the 84 genes we targeted were not significantly different between control and the 2.0 mg acrylamide/kg diet groups.

Conclusions: These results suggest that dietary acrylamide does not aid in the progression of established colon tumors and is corroborated by the methylation and gene expression data. Thus exposure to food-borne acrylamide, at upper human exposure levels, is not a risk for promoting the growth of existing colon tumors of human origin.

INDUCTION OF NADPH DEHYDROGENASE QUINONE 1 (NQO1) BY SULFORAPHANE IN CD-1 MOUSE FETAL LIVER CELLS.

Nikki A. Philbrook, Louise M. Winn.
Department of Biomedical and Molecular Sciences, Graduate Program in Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada

Background: Sulforaphane (SFN) is a phytochemical derived from cruciferous vegetables, such as broccoli, that has been shown to protect against numerous types of cancers. Among many suggested mechanisms, SFN has been demonstrated to induce numerous phase II enzymes, including NADPH dehydrogenase quinone 1 (NQO1), that are involved in detoxification of many carcinogens, such as benzene and its metabolites.

Objectives: The objective of the present study was to determine whether SFN induced NQO1 activity in cultured gestational day (GD) 14 CD-1 mouse fetal liver cells or in maternal and fetal tissue following *in utero* exposure.

Methods: GD14 fetal livers were extracted from pregnant CD-1 mice and cultured at a density of 4 million cells/well, in a 6-well plate, in supplemented media. At the time of plating, cells were treated with 0, 0.25, 0.5, 1, 2.5, or 5 μM SFN for twenty-four hours. In the *in vivo* studies, on GD14, pregnant CD-1 dams were exposed to 0, 50, or 100 mg/kg SFN for 2, 6, or 24 hours. Following each time point, maternal liver, heart, bone marrow, as well as placenta, and fetal livers were removed. NQO1 activity was measured using a colorimetric assay involving the use of dichloroindophenol as a competitive inhibitor of the NQO1 enzyme for both *in vitro* and *in vivo* studies.

Results: Twenty-four hour treatment of fetal liver cells with 2.5 or 5 μM SFN led to a significant increase ($p < 0.05$) in NQO1 activity compared to cells exposed to vehicle alone. *In vivo*, SFN treatment increased activity of NQO1 in fetal livers 24 hours after treatment, however no increase was observed in any other tissue examined.

Conclusions: This is the first time that SFN has been investigated for use in fetal tissue and pregnant animals. The results observed in this study suggest that SFN could be implemented in protecting against *in utero* initiated cancers, including that associated with *in utero* benzene exposure.

ALTERATION OF PLACENTAL TYPE 2 11B-HYDROXYSTEROID DEHYDROGENASE EXPRESSION AND ACTIVITY BY LEAD.

Joey St-Pierre, Marc Fraser, Cathy Vaillancourt.
INRS – Institut Armand-Frappier, Université du Québec et Centre de Recherche BioMed-UQAM-INRS-UQTR, Laval, QC, H7V 1B7

Background: An important function of the placenta is to protect the foetus from the transfer of maternal stress hormone, cortisol. The placenta is regulates the transfer of maternal stress by the type 2 11β-hydroxysteroid dehydrogenase (11β-HSD2) enzyme, which converts maternal cortisol to inactive cortisone. Prenatal maternal stress may influence the expression and activity of 11β-HSD2 and foetal development. Lead (Pb), an environmental stressor, increases the risk of obstetrical complications and alter foetal brain development. Unfortunately, the effects of Pb on placental 11β-HSD2 have never been studied.

Objectives: The objective of this study is to determine the effect of increasing concentrations of Pb on the expression and activity of 11β-HSD2 in the placental the cell line BeWo, model of villous trophoblast, the functional unit of placenta expressing the 11β- HSD2.

Methods: BeWo cells were exposed 24h to different Pb concentrations with or without forskolin (potent trophoblast differenciation agent). Concentrations were established from literature and a cohort where environmental contaminants were previously measured. Gene expression was analysed by RT-qPCR and protein expression by Western blot. 11β- HSD2 activity was assessed by radioenzymatic assay (conversion of cortisol to cortisone).

Results: Western blot results demonstrate a reduction in protein expression by 45% at 0.01 nM, 55% at 0.1 nM and 45% at 1000 nM Pb compared to untreated cells, while no effect on the expression of mRNA (RT-qPCR) was observed. Radioenzymatic assays show a significant reduction in cortisol to cortisone conversion rate at 0.01, 1, 10, 100 and 1000 nM Pb. No concentrations of Pb analyzed affected villous trophoblast viability parameters (no effect on cell viability, cell proliferation or production of hCG (differentiation capacity)).

Conclusions: This study shows that Pb alters the activity of placental 11β-HSD2 and suggests that current exposure standards should be revised.

VALPROIC ACID INDUCES P53 HYPERACETYLATION AND ACTIVATION LEADING TO INCREASED CELLULAR APOPTOSIS IN MURINE LIMB BUDS

F.H. Paradis, B.F. Hales
McGill University, Montreal, QC, Canada

Background: Valproic acid (VPA) is commonly used in the treatment of epilepsy and bipolar disorders and is in clinical trials as an anticancer agent. It is also an established human teratogen, causing spina bifida and limb malformations. Although its mechanism of action remains unresolved, histone deacetylases (HDACs) involved in chromatin remodeling and cellular signaling are known to be its targets. HDAC inhibitors have been shown to cause apoptosis in cancer cell lines. We hypothesize that VPA-induced HDAC inhibition triggers cellular apoptosis, leading to limb teratogenesis.

Objective: To test this hypothesis, we compared the effects on protein and gene expression of VPA and its inactive analog valpromide (VPD), using an in vitro limb bud culture system.

Methods: Gestational day 12 murine embryonic forelimbs were cultured in the absence or presence of VPA or VPD (0.6, 1.8 or 3.6 mM) for 6 days, stained with toluidine blue, and scored according to their morphology. Limbs were cultured for 1, 3, 6 or 12h and used for Western blot quantification of histone 4 acetylation, p53 acetylation and cleaved-caspases 9 and 3 or for the mRNA quantification of p53 downstream targets, Bcl2 and Survivin, by qRT-PCR.

Results: VPA significantly increased limb malformations that included oligodactyly and missing digits. At 3h, VPA induced the hyperacetylation of both histone 4 (0.6 mM, 2.0-fold; 1.8 mM, 2.7-fold; 3.6 mM, 3.4-fold) and p53 (0.6 mM, 3.4-fold; 1.8 mM, 6.6-fold; 3.6 mM, 6.8-fold). At 6h, both Bcl2 and Survivin were down-regulated (3.6 mM, 30 and 20%, respectively). At 12h, caspases 9 and 3 were activated (caspase 9: 1.8 mM, 2.7-fold; 3.6 mM, 4.7-fold; caspase 3: 1.8 mM, 4.6-fold; 3.6 mM, 6.9-fold). In contrast, VPD caused a small significant effect on limbs only in the group exposed to the highest concentration; no changes were observed in the acetylation of histone 4 or the cleavage of caspase 3.

Conclusions: Together, these data suggest that, in the midorganogenesis limb, VPA induces p53 activation by hyperacetylation, triggering apoptosis via the intrinsic pathway. We propose that this pathway plays a role in VPA-induced teratogenesis.

These studies were supported by CIHR and FRSQ.

POSTNATAL ALTERATIONS IN RAT CARDIAC FUNCTION AFTER *IN UTERO* EXPOSURE TO DIMETHADIONE, THE N-DEMETHYLATED METABOLITE OF THE ANTICONVULSANT TRIMETHADIONE

E. Purssell, K. Aasa, M.A. Adams, T.R.S. Ozolinš
Department of Biomedical and Molecular Sciences, Queen's University, Kingston ON K7L 3N6

Background: Dosing pregnant rats with dimethadione (DMO), the N-demethylated teratogenic metabolite of the anticonvulsant trimethadione, induced a high incidence of structural defects in the heart and outflow tract. *In utero* exposure to DMO also caused functional deficits (bradycardia, dysrhythmia and reduction in cardiac output and ejection fraction in embryos and foetuses), even in the absence of detectible structural defects. These data supported the hypothesis that *in utero* chemical exposure to DMO causes persistent post-natal functional deficits, even in the absence of detectable cardiac structural anomalies.

Objectives: Generate and identify a cohort of rats that were exposed to DMO in utero, but that did not present with structural defects of the heart post-natally; compare the functionality of *in utero* DMO-exposed hearts with control hearts, using high-resolution ultrasound, electrocardiography (ECG) and surgically implanted radiotelemeters.

Methods: Pregnant Sprague-Dawley rats were administered six 300 mg/kg doses of DMO every 12 hours beginning on the evening of gestational day 8. This dosing regimen has been shown to induce both structural and functional deficits in foetal rat heart. Dams were allowed to deliver pups naturally, and, upon reaching 150 grams, six control and five DMO-treated rats were surgically implanted with radiotelemeter transponders that measured physical activity levels and blood pressure. After a recovery period, animals were assessed with radiotelemetry for a month under basal conditions. One month later, the rats were given a high-salt dietary challenge. Finally, animals were placed under anaesthesia and cardiac structure and function assessed with high-resolution ultrasound and ECG.

Results: Surprisingly, animals exposed to DMO in utero displayed higher basal activity levels. Thus, comparisons of telemetric-generated cardiac measures were normalised for activity levels. At low activity levels, control and DMO-exposed rats had similar mean arterial pressure, but at elevated activity levels, mean arterial pressure was significantly elevated in the DMO-exposed group. DMO-treated rats had increased sensitivity to high salt load. Under anaesthesia, high-resolution ultrasound revealed increased cardiac output and a larger left ventricular volume during systole in DMO-treated rats. *In utero* DMO exposure increased the incidence of dysrhythmia at high levels of activity as well as under anaesthesia, which ECG suggested was due to disturbances in the electrical conduction in the heart. At the time of testing, only one of the five DMO-treated hearts had a structural defect (ventricular septation defect).

Conclusions: *In utero* exposure to DMO causes deficits in the cardiac conduction system that persist into postnatal life in the rat, even in the absence structural anomalies. We speculate this is not unique to DMO, indicating there may be long-term postnatal health implications for infants with unrecognized gestational chemical exposures. (Supported by Garfield Kelly Cardiovascular Research Grant).

AN EFFICIENT NOVEL METHOD FOR ANALYSIS OF THYROID HORMONES AND DERIVATIVES IN RAT SERUM WITH LC/MS/MS AS BIOMARKERS OF EFFECTS OF EXPOSURE TO ENVIRONMENTAL STRESSORS

Nanqin Li, Mike Wade, Sonia Johnson
Hazard Identification Division, Environmental Health Science and Research Bureau, HECSB, Health Canada, Ottawa, ON K1A 0K9

Background: Thyroid hormones (THs) regulate multiple processes of growth and development in humans and other vertebrates, and exposure to environmental pollutants and other stressors may disrupt the production or action of THs in body. As there is growing recognition of the value of measuring TH levels in standardized toxicity assays to assess hazards to thyroid physiology and the disadvantages of the current methods, there is a need to develop novel methods to simultaneously assess multiple THs and their breakdown products.

Objectives: To develop an efficient method for simultaneous measurement of multiple thyroid hormones and their metabolites in rat serum. The method will be applied to toxicity studies to evaluate effects on thyroid physiology.

Methods: Solvent extract of rat serum – containing the target molecules (T4, rT3, T3, 3′,3-T2, 3,5-T2, 3-T1 and thyronine) was subjected to tandem solid-phase extraction with reversed-phase, anion- and cation-exchange cartridges. The sample is eluted, concentrated and re-constituted prior to liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis. Stable isotope tracers were added to correct for extraction efficiency and improve quantitation. The method was validated for detection and qualification limit, linearity of calibration curves, recoveries and variability of replicate analyses, and ruggedness.

Results: All of the THs and thyronine could be independently quantified. THs were effectively extracted from serum, while proteins, lipid and other biomolecules were separated by organic solvent precipitation and SPE cartridges. With this method, a very clean sample is obtainable, which is beneficial to LC/MS/MS analysis. The method is sensitive, with analyte concentrations as low as 0.1 ng/mL of THs in serum being detected. The use of isotope-labelled TH internal standards allowed correction for inefficiencies in analyte extraction and LC/MS/MS detection.

Conclusion: A sensitive and reliable LC/MS/MS method was developed for measurement of THs in rat serum. The tandem cartridge system is effective in separating the interfering substances in serum, which is essential for high sensitivity of LC/MS/MS detection.

EFFECTS OF PESTICIDES ON CYTOCHROME P450 17 (CYP17) AND ANDROGEN RECEPTOR (AR) FUNCTION IN H295R ADRENOCORTICAL AND LNCAP PROSTATE CANCER CELLS.

Christina N. Robitaille, Patricia Rivest, Thomas J. Sanderson.
INRS-Institut Armand-Frappier, Laval (Qc)

Background: Exposures to endocrine disrupting chemicals, including pesticides, are thought to be involved in the increased incidence of certain endocrine-related cancers in the human population, including prostate cancer. Prostate cancer growth is initially androgen-dependent and under control of the nuclear androgen receptor (AR), which increases the gene transcription of proteins involved in cell proliferative, such as prostate specific antigen (PSA). CYP17 is a key enzyme in the biosynthesis of androgens and increased expression is associated with increased prostate cancer risk.

Objectives: We evaluated the effects of several pesticides suspected or known to modulate hormonal function in androgen-dependent LNCaP human prostate cancer cells and an *in vitro* steroidogenesis model, the H295R human adrenocrotical cancer cell line.

Methods: CYP17 and AR genic expression was evaluated by RT-qPCR and protein expression by Western blotting. Dihydroepiandrosteron (DHEA) was measured, by ELISA, to evaluate CYP17 activity.

Results: Benomyl, vinclozolin and prochloraz reduced dihydrotestosterone-(DHT)-stimulated LNCaP cell proliferation and nuclear AR protein accumulation concentration-dependently. Levels of the active phosphorylated form of AR, pAR-Ser81, were increased by 10 nM (DHT), whereas benomyl, vinclozolin and prochloraz decreased these stimulated levels (after a 1 or 6 h exposure). All three pesticides reduced DHT-stimulated PSA secretion. We found AR to be expressed in H295R cells but levels of AR and pAR-Ser81 were not affected by DHT, although they were increased by 30 µM atrazine. Benomyl and vinclozolin (10 and 30 µM) and prochloraz (1 and 3 µM) decreased CYP17 mRNA expression in H295R cells at sub-cytotoxic concentrations and prochloraz strongly inhibited CYP17-catalyzed conversion of pregnenolone to DHEA (24h exposure). In LNCaP cells, CYP17 protein expression was increased by atrazine (30 µM).

Conclusions: Certain pesticides exert combined anti-androgenic effects at the level of CYP17 and AR, the latter by reducing AR phosphorylation at serine 81.

BENZOQUINONE INDUCED INCREASES IN C-MYB ACTIVITY IN HD3 CELLS.

S. Stokes[1], L.M. Winn[1,2].
[1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, K7L 3N6;
[2]School of Environmental Studies, Queen's University, Kingston, Ontario, K7L 3N6

Background: Benzene is a known carcinogen and is found throughout the environment. Chronic
exposure to benzene has been associated with an increased risk of the development of various hematological disorders such as leukemia. *In utero* exposure to benzene has been associated with an increased risk for the development of leukemia in children. C-MYB is an oncoprotein that is important in normal hematopoiesis is often overexpressed in some cancers such as leukemia. Our laboratory has previously shown that the protein expression of C-MYB is increased following *in utero* exposure to benzene in a mouse model and that both the activity and protein expression of C-MYB is increased in an *in vitro* and cell culture model following exposure to the benzene metabolite benzoquinone. However, it is unknown what cellular signalling pathway(s) is involved in mediating this increase in expression. NF-κB is a redox sensitive transcription factor involved in cell signalling that might play a role in this sequence cascade. Additionally, due to its known interactions with NF-κB, p38-MAP kinase may also be involved in mediating benzene-induced increases in C-MYB expression.

Objectives: The objective of this research is to determine whether or not NF-κB and/or p38-MAP
kinase signaling leads to increased C-MYB activity and protein expression following benzoquinone exposure in HD3 cells.

Methods: To evaluate this objective, HD3 chicken erythroblast cells were transfected with either a C-MYB or NF-κB luciferase linked reporter plasmid and then exposed to 25 μM benzoquinone for 2-24 hours. Luciferase activity assays were then conducted to determine the activities of both C-MYB and NF-κB, and Western blotting was conducted to assess changes in protein levels in non-transfected cells.

Results: Preliminary results demonstrate increased C-MYB activity following benzoquinone exposure in HD3 cells. Ongoing studies are evaluating NF-κB activity and C-MYB and NF-κB protein expression. Future studies will evaluate the effects of the NF-κB inhibitor, SN50, and the p38-MAPK inhibitor, SB203580 on alterations on the C-MYB signalling pathway following benzoquinone exposure.

Conclusions: Evaluating the cell signalling pathways that are altered due to toxicant exposure is vital to our understanding of mechanisms of xenobiotic-induced toxicity.
Support: CIHR.

EFFECT OF BENZO(A)PYRENE EXPOSURE DOSE ON LEVELS OF EXPOSURE BIOMARKERS, DNA ADDUCTS AND GENE EXPRESSION IN RATS.

Marjory Moreau[1], Pierre Ayotte[2], Michèle Bouchard[1].
[1]Department of Environmental and Occupational Health, and Chair in Toxicological Risk Assessment and Management, University of Montreal, Montreal, QC, Canada; [2]Université Laval, QC, Canada

Background: Exposure to benzo(a)pyrene (BaP), a polycyclic aromatic hydrocarbon (PAH) classified as a known carcinogen in humans (International Agency for Research on Cancer IARC), is of great health concern for both workers and the general population.

Objective: The project aims to document the effects of multiple doses of BaP on levels of biomarkers of exposure (3-and 7-OHBaP, 4,5-and 7,8-diol-BaP, tetrol, 1,6 -, 3,6- and 7,8-dione-BaP) and effect.

Methods: The effect of benzo(a)pyrene (BaP) doses on levels of several biomarkers of exposure and early effects was studied in rats intravenously injected with 0.4, 4, 10 and 40 µmol/kg of BaP. Blood, tissues and excreta were collected 8 h and 24 h post-treatment. BaP and several of its metabolites, 3- and7-OHBaP, 4,5- and 7,8-BaP diols, tetrol, 1,6-, 3,6- and 7,8-BaP-diones, were simultaneously measured in blood, tissues and excreta by UHPLC/fluorescence. BaPDE-DNA adducts in lungs were quantified in parallel using an ultrasensitive immunoassay with chemiluminescence detection. Expression of various genes in lungs of treated rats (lung RNA) compared to control rats was also assessed by qRT-PCR.

Results: There was a dose-dependent increase in blood and tissue levels as well as excretion of BaP metabolites. At 8 hand 24 h post-injection, BaP and 3-OHBaP were found in higher concentrations in blood and tissues compared to the other analytes. However, BaP diols were excreted in greater amounts in urine and apparently more quickly than hydroxyBaP. Mean percentages (± SD) of injected dose excreted in urine as4,5-diol-BaP during the 0-8 and 0-24-h period post-treatment were 0.16 ± 0.03% and 0.14 ± 0.08%, respectively. Corresponding values for 3-OHBaP were 0.004 ± 0.001% and 0.026 ± 0.014%. Diones were not detectable in blood, tissues and excreta using the developed method and BaP-7,8-diol and 7-OHBaP were found to be more minor metabolites. There was also a dose-dependent increase in DNA adduct formation. Analysis of gene expression further showed a modulation of cyp1a1, cyp1b1, nqo1, nrf2, fos andAhr expression at the 10 and 40 µmol/kg doses, but not at the lower doses.

Conclusion: This study confirms the interest of measuring multiple metabolites in combination with DNA adducts and alteration of gene expression for a more comprehensive assessment of links between biomarkers of BaP exposure and early effects.

BIRTH WEIGHT IS NOT CORRELATED WITH ENVIRONMENTAL EXPOSURE TO ORGANOHALOGENS AND METALS IN PREGNANCY.

Laetiscia Lavoie[1], Nadia Abdelouahab[1], Jean-Charles Pasquier[2], Larissa Takser[1].
[1]Université de Sherbrooke, Faculté de Médecine, Département de Pédiatrie;
[2]Université de Sherbrooke, Faculté de Médecine, Département de Gynécologie-Obstétrique

Background: The effect of environmental contaminants on pregnancy, and especially fetal development, is one of hottest topics in public health. The potential for environmental contaminants to induce fetal growth restriction as a result of *in utero* exposure has been suggested by several epidemiological studies, including lead (Pb), cadmium (Cd), manganese (Mn), mercury (Hg) and polychlorinated biphenyls (PCB) as well known examples. However, the effects of emerging pollutants, such as flame retardants including polybrominated diphenyl ethers (PBDE), on fetal growth remain unknown. Birth weight is influenced by a multitude of factors including maternal smoking, maternal body mass, pregnancy complications, or infections. The majority of epidemiological studies does not consider all these important risk factors for low birth weight and analyze environmental contaminants one by one.

Objectives: To determine the correlation between environmental exposure to PBDE, PCB and metals (Pb, Mn, Cd, Hg) in pregnant women and birth weight, considering known evidence based risk factors for low birth weight.

Methods: This study is done using our prospective GESTE birth cohort, in which 397 pregnant women were enrolled at <20 weeks of pregnancy, at their first visit. At this visit, maternal blood was collected for PBDE, PCB and metal analyses. Socio-demographic information was collected using an interview-administered questionnaire, including information on age, education, income, alcohol consumption, smoking and drug habits, occupational and recreational exposure to chemicals, medical history, and medication. All data on maternal health and obstetrical history, medication and delivery were obtained from medical records.

Results: Blood metal and PCB levels in pregnant women in our study were very low, while PBDE concentrations were similar to those reported in the US population and higher than in Europe. There was no significant correlation between contaminants in early pregnancy and birth weight.

Conclusions: Our findings suggest that low dose environmental contaminants, including PBDE have no or little impact on fetal growth compared to other risk factors.

TISSUE-SPECIFIC CARCINOGENIC MODES OF ACTION OF BENZO(A)PYRENE: A TOXICOGENOMICS PERSPECTIVE.

Sarah Labib[1,2], Charles Guo[2], Andrew Williams[2], Paul A White[1,2], Sabina Halappanavar[2].
[1]Department of Biology, University of Ottawa, Ottawa; [2]Mechanistic Studies Division, Environmental and Radiation Health Sciences Directorate, Health Canada, Ottawa.

Background: Forestomach is the primary site of exposure and directly targeted for carcinogenesis in experimental animals orally exposed to benzo(*a*)pyrene (BaP). However, tumours in tissues remote from the site of contact, including the lung and liver, have also been reported following BaP exposure. The carcinogenic mode of action is primarily attributed to the ability of its metabolites to form DNA adducts, which if left unrepaired can be fixed as mutations. However, the underlying molecular mechanisms and biological pathways involved in this tumourigenesis have not been elucidated at a tissue-specific level.

Objective: Use toxicogenomics tools to understand the underlying tissue dynamics involved in tumor formation in the liver, lung, and forestomach tissues.

Methods: Adult male Muta^{TM}Mouse were exposed daily to three doses of BaP (25, 50, and 75 mg/kg/day) for 28 days by oral gavage. Mice were sacrificed three days after the final exposure and lung, liver, and forestomach tissues were collected. Total RNA was isolated from a portion of each tissue and global gene expression changes were profiled using oligonucleotide microarrays. Bioinformatics and pathway analysis tools were used to identify adversely affected pathways in each tissue. Microarray results were validated using quantitative real-time PCR and western blotting.

Results: In the forestomach, the majority of the genes were associated with antigen processing and presentation, the immune and inflammatory response, and keratinocyte differentiation. These processes are all implicated in an inflammatory-mediated carcinogenic transformation specific to the stomach and gastrointestinal tract. The major genes affected included Nlcr5 (transactivator of major histocompatibility class I genes) and killer cell lectin-like receptor (activator of natural killer cells) at the lowest dose, and inflammatory response genes, cystatins and proteases at the higher doses. In the lungs, altered genes were mainly associated with the DNA damage response at all three doses regulated primarily by the p53 signaling pathway. Cellular proliferation, angiogenesis, negative regulation of B cell receptor signaling, and evasion of apoptosis were also observed at the high doses, which are hallmarks of cancer. In contrast, the liver showed a very weak response with few DNA damage response genes altered only at the highest dose. This low response can be explained by the function of the liver as the primary site for detoxification and chemical metabolism, making it well equipped for dealing with toxic exposures.

Conclusions: Our study provides toxicogenomic perspectives on the influence of tissue cell types and tissue function in determining carcinogenic outcome following exposure to BaP. The underlying mechanisms will be discussed in detail.

PARTICULATE MATTER COLLECTED NEAR INDUSTRIAL SITES DISPLAYS DIFFERENTIAL TOXIC AND INFLAMMATORY POTENTIAL

Errol Thomson[1], Subramanian Karthikeyan[1], Dalibor Breznan[1], Prem Kumarathasan[2], Jeff Brook[3], Ewa Dabek-Zlotorzynska[3], Jean-Pierre Charland[3], Renaud Vincent[1]
[1] Inhalation Toxicology Laboratory, Hazard Identification Division, Environmental Health Science and Research Bureau, Environmental and Radiation Health Sciences Directorate, HECSB;
[2] Proteomics Laboratory, Mechanistic Studies Division, Environmental Health Science and Research Bureau, Environmental and Radiation Health Sciences Directorate, HECSB;
[3] Emission Research and Measurements Section, Air Quality Research Division, Science and Technology Branch, Environment Canada

Background: Particulate air pollution is associated with increased cardiopulmonary morbidity and mortality. Air quality standards for particulate matter are expressed as mass concentrations for a given size range (i.e. PM_{10} and $PM_{2.5}$; particles with aerodynamic diameter < 10 μm and 2.5 μm respectively). It is clear, however, that biological responses are dependent not only on particle size, but also on other physicochemical factors including elemental and organic composition, which are impacted by mode of generation, and therefore source.

Objective: Our objective was to better understand the contribution of specific sources to the toxicity of airborne particles. We investigated determinants of toxicity by comparing the potency of particles from geographical areas impacted by distinct pollution emission sources.

Methods: Size-fractionated particles ($PM_{0.1\text{-}2.5}$, $PM_{2.5\text{-}10}$, $PM_{>10}$) were collected on filters using a ChemVol High Volume Cascade Impactor at sites impacted predominantly by specific sources. These were: a steel mill (Hamilton, ON), petrochemical refineries (Sarnia, ON, and Montréal, QC), a copper smelter (Montréal, QC), and an aluminium refinery (Shawinigan, QC). Particle effects on a panel of endpoints (including cytotoxicity and cytokine release) were assessed in two cell lines (human lung epithelial-like A549, murine macrophage-like J774A.1).

Results: Particle composition varied greatly among sites, particularly for the fine $PM_{0.1\text{-}2.5}$ fraction (e.g. predominance of zinc in Hamilton, aluminium in Shawinigan, copper in Montréal). This was reflected in differing cytotoxic and inflammatory responses across particle size fractions and sites. Cytotoxicity tended to decrease with increasing particle size, whereas inflammatory potential increased. For most sites, cytokine profiles for $PM_{2.5\text{-}10}$ and $PM_{>10}$ fractions were similar and generally reflected the pattern of cytotoxicity. In contrast, the $PM_{0.1\text{-}2.5}$ fraction provoked the greatest variability in cytotoxic responses but little inflammatory response.

Conclusions: Particles of varied composition exhibited differential cytotoxic and inflammatory potential. Identification of particle constituents driving these responses should assist prioritisation of sources for regulatory action.

MAPPING SYSTEMIC EFFECTS OF INHALED POLLUTANTS: ACTIVATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

Errol Thomson[1], Djordje Vladisavljevic[1], Susantha Mohottalage[2], Prem Kumarathasan[2], Renaud Vincent[1]
[1] Inhalation Toxicology Laboratory, Hazard Identification Division, Environmental Health Science and Research Bureau, Environmental and Radiation Health Sciences Directorate, HECSB
[2] Proteomics Laboratory, Mechanistic Studies Division, Environmental Health Science and Research Bureau, Environmental and Radiation Health Sciences Directorate, HECSB

Background: Air pollution is recognised as a risk factor for respiratory and cardiovascular disease. Recent epidemiological studies have also found associations between common air pollutants and stroke, neurodegenerative disease, appendicitis, low birth weight, aggression, depression, and suicide, among others, but the underlying mechanisms are not known.

Objectives: Our objective was to gain insight into biological mechanisms underlying acute effects of inhaled pollutants by mapping perturbation of biological pathways in a number of organs.

Methods: Male Fischer-344 rats were exposed by inhalation for 4h to particulate matter and ozone, and euthanized immediately or 24h after exposure. Real-time polymerase chain reaction was used to assess the expression of genes involved in a number of biological pathways in the lungs, heart, liver, kidney, spleen, cerebral hemisphere, and pituitary.

Results: Pollutant exposure provoked changes in the expression of genes involved in a number of pathways, including antioxidant response, xenobiotic metabolism, inflammatory signalling, and endothelial dysfunction. The pattern of gene expression, though exhibiting some inter-organ differences, was remarkably similar across organs for a set of genes, including transiently increased expression of the redox and glucocorticoid-sensitive genes metallothionein-II and hypoxia inducible factor-3α and decreased expression of inflammatory genes, suggesting a possible hormonal effect. Hypothalamic-pituitary-adrenal axis activation was confirmed by detection of increased levels of the glucocorticoid-signalling adrenocorticotropic hormone. Expression of glucocorticoid-inducible leucine zipper, an important mediator of glucocorticoid action, was increased in all organs, consistent with higher glucocorticoid levels.

Conclusions: Changes in the regulation of stress responses are implicated in a number of adverse health outcomes. Our results indicate that both particulate and gaseous pollutants can activate transcriptional pathways in a number of organs, including the brain and pituitary, that are consistent with activation of the hypothalamic-pituitary-adrenal axis and glucocorticoid action. Given the emergence of new health effects associated with pollutant exposure, these observations of systemic effects warrant further investigation.

THE REGULATION OF *TIPARP* BY ARYL HYDROCARBON RECEPTOR, PLATELET DERIVED GROWTH FACTOR RECEPTOR AND ESTROGEN RECEPTOR α.

S. Rajendra, L. MacPherson, J. Matthews.
Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario, Canada

Background: The toxicity of a number of environmental pollutants such as 2,3,7,8 – tetrachlorodibenzo-*p*-dioxin (TCDD) is mediated by the aryl hydrocarbon receptor (AHR). Upon activation by ligand binding, AHR induces the transcription of many genes including TCDD-inducible poly(ADP-ribose) polymerase (TiPARP). TiPARP mediates the suppression of hepatic gluconeogenesis, a symptom of TCDD-induced lethal wasting syndrome. Our laboratory has recently shown that TiPARP acts as a negative regulator of AHR transactivation. In addition to being regulated by AHR, *TiPARP* is regulated by the platelet derived growth factor receptor (PDGFRαβ) and the estrogen receptor (ERα). While AHR and PDGFRαβ crosstalk has not been reported, AHR and ERα crosstalk has been demonstrated extensively in the literature with AHR inhibiting ERα-mediated responses. Although these distinct pathways have been demonstrated to up-regulate the expression of *TiPARP*, the molecular mechanisms governing this regulation have not been studied.

Objectives: The aim of this study is to investigate the regulation of *TiPARP* by AHR, PDGFRαβ and ERα, and to explore the interplay among these distinctive pathways.

Methods: We examined the temporal mRNA expression profile of *TiPARP* following treatments with TCDD, PDGF BB, and 17β-estradiol (E2) using quantitative real-time PCR in mouse embryonic fibroblasts (MEFs) and MCF-7 human breast cancer cells. Chromatin immunoprecipitation assays (ChIP) were performed to determine changes in transcription factor recruitment to the *TiPARP* regulatory region.

Results: Peak induction of *TiPARP* mRNA levels was observed following 1 h treatment with TCDD or PDGF BB alone, while co-treatment demonstrated enhanced *TiPARP* expression at 3 h in wildtype MEFs. Additional mRNA studies demonstrated TCDD-dependent induction of *TiPARP* in *Pdgfrαβ-/-* MEFs as well as PDGF BB-dependent induction of *TiPARP* in *Ahr-/-* MEFs. ChIP studies revealed that TCDD induced AHR recruitment to the *TiPARP* regulatory region, and co-treatment with PDGF BB did not significantly alter TCDD-induced AHR recruitment levels. In MCF-7 cells, both TCDD-dependent and E2-dependent *TiPARP* induction was observed, and co-treatment displayed significantly enhanced *TiPARP* expression. Additional mRNA studies demonstrated E2-dependent *TiPARP* induction in MCF-7 *Ahr null* cells, and TCDD-dependent *TiPARP* induction in the ERα-negative human breast cancer cell line, MDA-MB-231. ChIP studies revealed TCDD-dependent recruitment of AHR and E2-dependent recruitment of ERα to the *TiPARP* regulatory region. Co-treatment significantly enhanced ERα recruitment relative to E2 alone and AHR recruitment relative to TCDD alone.

Conclusions: Collectively, these results suggest that AHR can regulate *TiPARP* independent of PDGFRαβ and ERα, and PDGFRαβ and ERα can regulate *TiPARP* independent of AHR. Further, AHR and ERα crosstalk has a positive effect on *TiPARP* regulation.

IN VITRO TOXICOLOGICAL PROFILE OF OECD TiO_2 NANOPARTICLES IN EXPOSURES OF HUMAN AND MURINE CELL LINES.

D. Breznan[1], D. Das[2], C. MacKinnon-Roy[1], D. Jurgens[1], P. Kumarathasan[2], R. Vincent[1].
[1] Inhalation Toxicology Laboratory, Hazard Identification Division, HECSB, Health Canada, Ottawa, ON;
[2] Analytical Biochemistry and Proteomics Laboratory, Mechanistic Studies Division, HECSB, Health Canada, Ottawa, ON

Background: TiO_2 nanoparticles (NPs) are finding increasing use in biomedical, electrical and consumer applications such as photocatalysts, photovoltaics, textiles, UV-resistant paints, sunscreens, water treatment agents and anticancer treatments. TiO_2 nanopowders are one of the most widely used nanomaterials, with projected market growth estimated at $1.4 billion by 2017. However, emerging evidence shows the potential for nano-scale TiO_2 to cause adverse effects on human health and the environment. Determinations of the relationship between material properties of TiO_2 and toxicity require extensive study. *In vitro* toxicity screening approaches are ideal for toxicity evaluations due to their relative low cost, ease of use and higher throughput, in comparison to animal studies.

Objective: Evaluate the relative toxic potency of TiO_2 NPs using an *in vitro* integrated bioassay.

Methods: The variants of TiO_2 NPs tested were obtained through OECD, WPMN. The materials have well-defined physicochemical characteristics. Human A549 lung epithelial cells, THP-1 macrophages (differentiated with 10 ng/ml PMA) and mouse J774A.1 macrophages were exposed to multiple doses of TiO_2 NPs in cell culture media for 24h. The bioassay simultaneously assessed cell metabolic capacity (CTB reduction), energy metabolism (ATP assay) and cell membrane damage (LDH assay) in supernatants and cell lysates from each well of a 96-well plate. Additional aliquots were kept for future analyses. Relative potency of the particles was represented by the slope of the dose response curves and applied to derive potency ranking.

Results: The relative potency of the particles varied across cell lines and assays. Macrophages were more sensitive to TiO_2 NPs than epithelial cells, as exemplified by decreased cellular ATP levels, and increased LDH leakage from cells with perturbed plasma membrane. Overall cell viability of the three cell lines was mostly intact after exposure to most of the TiO_2 NPs, with murine cells being more sensitive than the human cell lines, as shown by a decrease in cell viability (CTB reduction) in response to a number of the TiO_2 NPs tested.

Conclusion: The study revealed cell type- and endpoint-specific variability in *in vitro* response of cells to TiO_2 NPs, illustrating the complexity of biological response to the materials and emphasizing the need to conduct more comprehensive toxicological screening assessments. Future work will include the assessment of additional endpoints and cell culture models. The data will provide comprehensive information for more informed safety assessments of TiO_2 NPs.

TARGETED ARYL HYDROCARBON RECEPTOR KNOCKOUT IN MCF-7 AND MDA-MB-231 CELLS USING ZINC FINGER NUCLEASES

Shaimaa Ahmed, Raymond Lo, Annie Wang, Trine Celius, Jason Matthews
Department of Pharmacology & Toxicology, University of Toronto, Toronto, Ontario, Canada M5S 1A8

Background: The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that is implicated in breast cancer through its inhibition of estrogen receptor α (ERα) signalling, and by upregulating drug metabolizing enzymes, such as cytochrome P450 1B1 (CYP1B1). CYP1B1 catalyzes estrogen hydroxylation and the activation of potential carcinogens.

Objective: Here we used zinc finger nucleases (ZFNs) targeting AHR to create ERα-positive (MCF-7 AHR$^{-/-}$) and ER negative (MDA-MB-231 AHR$^{-/-}$) AHR knockout human breast cancer cell lines to assess the role of AHR.

Methods: To determine the functional significance of AHR we used, Western analysis, chromatin immunoprecipitation, and RNA expression.

Results: Loss of AHR resulted in the gene-specific reduction in constitutive CYP1B1 levels, which was greater than that achieved with RNAi-mediated CYP1B1 knockdown. CYP1B1 levels were rescued with overexpression of wildtype but not a DNA binding domain mutant of AHR. Chromatin immunoprecipitation assays showed that the constitutive and ligand-induced recruitment of AHR, ARNT and ERα to *CYP1B1* was abolished in MCF-7 AHR$^{-/-}$ cells. *AHR*$^{-/-}$ also prevented TCDD- and E2-dependent increases in CYP1B1 mRNA levels. *AHR*$^{-/-}$ resulted in increased constitutive and E2-inducible regulation of TFF-1, but not GREB1. Constitutive ERα protein levels were unaffected by *AHR*$^{-/-}$ but TCDD-induced ERα degradation was abolished. Loss of AHR reduced the proliferation of both MCF-7 and MDA-MB-231 cells compared to controls and prevented the inhibitory effects of TCDD on E2-dependent MCF-7 cell proliferation.

Conclusions : These findings show the key role AHR plays in the regulation of CYP1B1 and in the proliferation of breast cancer cells, supporting AHR as a therapeutic target for breast cancer.

IMPACT OF CHRONIC PRENATAL ETHANOL EXPOSURE ON PERIPHERAL INSULIN AND IGF SIGNALLING OF ADULT OFFSPRING.

C. Dobson[1], D. Mongillo[1], M. Poklewska-Koziell[1], A. Winterborn[3], R. Stepita[4], A.C. Holloway[4], J.F. Brien[1,2], J.N. Reynolds[1,2].
[1]Pharmacology and Toxicology Program, Department of Biomedical and Molecular Sciences; [2]Centre for Neuroscience Studies; [3]Office of the University Veterinarian, Queen's University, Kingston, Canada; [4]Department of Obstetrics and Gynecology, McMaster University, Hamilton, Canada

Background: Maternal alcohol (ethanol) consumption during pregnancy can cause a range of adverse developmental outcomes in children, termed fetal alcohol spectrum disorder (FASD). Central nervous system (CNS) injury is the most widely studied manifestation of FASD. However, studies in children and animal models suggest that prenatal ethanol exposure also leads to metabolic dysregulation, including impaired glucose metabolism, increased gluconeogenesis, and insulin resistance. The mechanisms underlying prenatal ethanol-induced metabolic dysfunction remain poorly understood, but are thought to involve alterations in insulin signaling. This study will test the hypothesis that impaired insulin signaling in the periphery is critical to ethanol metabolic teratogenicity.

Objectives: To examine the impact of chronic prenatal ethanol exposure (CPEE) on insulin and IGF signaling in the periphery and to correlate these changes with metabolic deficits in adult offspring.

Methods: Pregnant Dunkin-Hartley-strain guinea pigs received ethanol (4 g/kg maternal body weight/day throughout gestation) or isocaloric-sucrose/pair-feeding (nutritional control). Body weight was measured in offspring from birth until euthanasia. On postnatal day (PD) 100-140, offspring underwent structural magnetic resonance imaging (MRI) to measure visceral and subcutaneous lipid volumes. Fasting blood glucose concentration was measured prior to euthanasia at PD 150-200, when liver, skeletal muscle and pancreas were excised. Expression of genes involved in insulin signaling, including insulin receptor, insulin receptor substrate (IRS) types 1 and 2, PI3K, PDK-1, and PKB, will be examined using real-time PCR. Expression levels will then be correlated with metabolic parameters.

Results: CPEE offspring, compared with nutritional control, were growth restricted at birth. However, in young adulthood, MRI analyses revealed increased visceral and subcutaneous adiposity in CPEE offspring. At PD 150-200, CPEE offspring demonstrated dysregulated fasting blood glucose concentration, increased body weight, and increased liver weight, all of which may be manifestations of altered insulin signaling. CPEE decreased the number of insulin-like immunoreactive â-cells in islets and increased the amount of adipose tissue in the pancreas of adult offspring. Ongoing quantitative RT-PCR analyses will assess IGF-1 expression in peripheral tissues.

Conclusions: The data demonstrate that this chronic maternal ethanol regimen produces neurobehavioural and metabolic dysfunction in guinea pig offspring, which appears to be related to altered insulin and IGF signaling. (Supported by CIHR grants MOP84553 and ELA80227).

ENHANCED MITOCHONDRIAL AUTOPHAGY PROTECTS AGAINST ROTENONE SUSCEPTIBILITY IN CELLS WITH IMPAIRED MITOCHONDRIAL GENOMIC INTEGRITY

Fernando Bralha, Rebecca R. Laposa
Department of Pharmacology and Toxicology, University of Toronto

Background: Mitochondrial genome integrity maintains the function of the electron transport chain and is crucial for proper mitochondrial activity. Mitochondrial DNA is exclusively replicated by the mitochondrial DNA polymerase gamma (POLG). Genetic variants of POLG are common in the human population. Mouse embryonic fibroblasts (MEFs) from mice with a proofreading-defective version of Polg ($Polg^{m/m}$) and consequently abundant random point mutations in mitochondrial DNA have increased susceptibility in the form of reduced survival and proliferation when exposed to the electron transport chain complex I inhibitor rotenone.

Objectives: In order to elucidate the mechanisms responsible for susceptibility to rotenone in $Polg^{m/m}$ MEFs, we attempted to rescue this phenotype by modulating the mitochondrial quality control processes of mitophagy and mitochondrial fusion/fission. In mitophagy, autophagy machinery actively destroys damaged non-functional mitochondria. Mitochondrial fusion and fission serve to sustain healthy function by mixing mitochondrial contents.

Methods: Wild type and $Polg^{m/m}$ MEFs were exposed to rotenone, and cell survival was assessed by colony forming assays and by MTT reduction. Mitophagy was modulated by treating cells with the mitophagy inducers Rapamycin and LiCl. Mitochondrial fusion was enhanced by over-expression of mitofusins 1 and 2 (Mfn1,2). Mitochondrial fission was inhibited pharmacologically using mdivi-1 (mitochondrial division inhibitor).

Results: In MTT reduction assays, 10 mM LiCl significantly rescued cell viability of $Polg^{m/m}$ MEFs exposed to 5 nM and 10 nM rotenone for 48h. 1 μM rapamycin did not recue cell viability in wild type or $Polg^{m/m}$ MEFs. Colony forming assays in MEFs treated with rotenone did not show increased viability in cells with mitofusins over-expression. Over-expression of Mfn1 and 2 was confirmed by western blot analysis. In MTT reduction assays, inhibition of mitochondrial fission by 1-50 μM mdivi-1 did not rescue rotenone sensitivity observed in $Polg^{m/m}$ MEFs.

Conclusions: Our results suggest that enhanced mitochondrial autophagy, but not increased mitochondrial fusion or decreased mitochondrial fission, protects against rotenone susceptibility in cells with impaired mitochondrial genomic integrity.

IN VITRO MODELS FOR THE STUDY OF ENDOCRINE DISRUPTERS EFFECTS ON BREAST CANCER INITIATION AND PROGRESSION

A. Kawata, E. Atlas
HID, HECSB, Health Canada, Ottawa, ON

Background: The incidence of breast cancer has increased steadily over the last 20 years achieving 15% in the female population in the western world. Both genetic and life style choices are likely to contribute to the development of the disease however, chemical exposureis likely to also be a factor. To date, most *in vitro* models examining the effects of endocrine disrupters such as bisphenol-A (BPA) on breast cancer cells use two-dimensional cultures, cells grown on plastic dishes. These models do not take into account the contribution of the cell's microenvironment, such as the extracellular matrix to the effects of endocrine disrupters on the breast epithelium, which provides a highly physiological *in vitro* model.

Objective: To establish a physiologically relevant *in vitro* model to study carcinogenic effects of endocrine disrupters on the breast epithelium and to assess the response of breast epithelial cells in this physiologically relevant context to the effects of endocrine disrupters. To determine morphological changes and changes in gene expression in this model system.

Methods: We have developed an *in vitro* model using the MCF-10A human breast epithelial cells. We have introduced the Her2 oncogene using retroviral vectors (MCF-10A-Her2) or a control retroviral vector (MCF-10A-Puro). The cells were grown in 3D cultures in Matrigel for 8 or 14 days, treated with BPA or untreated as controls, stained by immunofluorescence and examined by confocal microscopy.

Results: First, we have confirmed the overexpression of Her-2 by immunofluorescence and Western blots. Confocal microscopy analysis showed that control cells formed an organized and polarized architecture in which cell-cell interaction and cell basement interactions occur. These structures were polarized, as assessed by integrin-6 staining and formed a lumen. The Her-2 overexpressing cells grew in a disorganized fashion, did not form a lumen and had a less polarized staining for integrin-6. Furthermore, our preliminary data indicate that treatment with bisphenol-A induced a phenotypic change in the control cells reminiscent of further progression into an aggressive phenotype, and affected the characteristics of the MCF-10A-Her2 cells.

Conclusions: Using the *in-vitro* 3D model system, we showed that the MCF-10A cells form structures that resemble the mammary gland ducts. This model system provides a good model system for the study of endocrine disrupters such as BPA, and has the potential to better inform us of the risks involved in the exposure to endocrine disrupters and breast cancer initiation and progression. Furthermore, this model system enables us to reveal molecular markers for the assessment of potential risk for breast cancer development in response to chemical exposure.

EPIGENETIC PROGRAMMING OF UTERINE TISSUE BY BISPHENOL A: WHAT EVIDENCE IS RELIABLE?

Alexander Suvorov, Andy Rampersaud, Kerri A. Stanley, Jeannette Connerney, David J. Waxman
Dept. of Biology, Boston University, Boston, MA 02215

Background: Bisphenol A (BPA) is an estrogenic substance produced in billions of pounds annually. Exposure of the general population to this compound is widespread, but the consequences of this exposure are poorly understood. Another estrogenic substance, diethylstilbestrol, induces abnormalities in uterine tissue development and ultimately causes cancer in pubertal girls exposed *in utero*. There is an urgent need to understand whether exposure to BPA at a critical window of uterus development may reprogram this organ for reproductive disease susceptibility later in life. A substantial number of experimental studies have been carried out in animal models, but results are often inconsistent. Our review of 24 relevant publications shows that seven of the studies in which mice or rats were treated with BPA between developmental days E11 and P15 (time window for organogenesis and differentiation of uterus) did not control for the estrous cycle. The study reported here suggests that the failure to control for uterine tissue rearrangement during the estrus cycle may be a major source for the discrepant reports regarding adult uterine effects of early xenoestrogen exposure.

Objectives: To analyze gene expression and histone modification changes in mouse uterus in estrus versus proestrus.

Methods: Changes in the CD-1 mouse uterine transcriptome between proestrus and estrus were identified using whole-genome gene expression microarrays. Three activating histone marks (H3K4me1, H3K36me3, H3K27ac) were mapped in the epithelial layer of uterine endometrium in proestrus and estrus, using ChIP-seq.

Results: Microarray analysis identified 2,730 genes differentially expressed during estrus and proestrus (|fold-change| >2, p <0.005). Significant genomic regions showing differential histone modification marks between proestrus and estrus were identified, as follows: 1,487 regions for H3K27ac, 4,767 regions for H3K36me3, and 954 regions for H3K4me3.

Conclusions: Mouse uterus undergoes remarkable remodelling during the estrous cycle. This is seen both at the level of gene expression and histone modification, and affects ~10% of all protein-coding genes. Thus, it is crucial to control for the effects of the estrous cycle when investigating uterine effects of xenoestrogen exposure. There is robust evidence for uterine effects of early BPA exposure in adult animals, as seen in a variety of models based on studies that do control for estrous cycle stage. However, many experimental studies of BPA effects fail to control for stage of the estrous cycle. Epigenetic findings in these studies, which include studies of trans-generational effects of BPA exposure, may include false-positives. There is an urgent need for high throughput analysis of the uterine effects of early BPA exposure, including both its short-term and its longer-term effects, and to elucidate any long-lasting epigenetic signatures of these exposures. Supported by NIH grant RC1 ES018332 (to D.J.W.).

EFFECTS OF IN UTERO EXPOSURE TO BROMINATED FLAME RETARDANTS (BFRS) ON PREGNANCY AND FETAL DEVELOPMENT IN SPRAGUE-DAWLEY RATS

Yi-Qian Ma[1], Sheila R. Ernest[1], Robert G. Berger[1], Pavine L.C. Lefèvre[1], Mike G. Wade[3], Bernard Robaire[1,2], Barbara F. Hales[1]
[1] Depts of Pharmacology and Therapeutics and [2] Obstetrics and Gynecology, McGill University, Montreal, QC, Canada H3G 1Y6;
[3] Health Canada, Environmental Health Science and Research Bureau, Radiation and Research Directorate, Ottawa, ON K1A 0K9

Background: Brominated flame retardants are widely used in consumer products and are ubiquitous in the environment. Ingestion of BFRs occurs through exposure to contaminated household and workplace dust. Presence of BFRs has been detected in mid-gestation human fetal liver and placenta and elevated levels have been positively correlated with abnormal fetal development.

Objective: The aim of the study is to determine whether exposure of Sprague-Dawley rats to a representative mixture of the BFRs found in North American household dust during premating and gestational periods affects fetal development.

Methods: Females were exposed to nominal doses of 0, 0.06, 20 or 60 mg of the BFR mixture/kg body weight/day from two weeks prior to mating until gestation day 20. Dam fertility, reproductive parameters and fetal developmental endpoints were evaluated. The activities and mRNA expression of hepatic cytochrome P450 drug-metabolizing enzymes (DMEs) were quantified.

Results: Fertility and fecundity indices, litter sizes, fetal viability and growth measures were not significantly affected by BFR exposure. In the dams, significant increases in the activity of several drug metabolizing enzymes (DME) were seen at the two highest doses. BFR exposure had significant differential effects on expression of genes responsible for these activities in both the dam and fetal livers at all doses. Fetal digit malformations were induced in the litters exposed to the low (5.76%) and high (6.4%) BFR doses compared to controls (2.2%) (Chi-square, $p<0.05$). Decreased ossification of the sternum was detected in all BFR exposed fetuses (Fisher Exact test, $p<0.014$).

Conclusions: These results suggest that *in utero* exposure to environmental relevant BFR doses induces hepatic DMEs in the dams and fetuses and developmental abnormalities in the fetuses.

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EFFECT OF SELENIUM ON ARSENIC INDUCED EXPRESSION OF INFLAMMATORY MEDIATORS AND ENDOTHELIAL DYSFUNCTION

Regina M. Krohn, Judit E. G. Smits
Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary

Background: Arsenic is a toxic metalloid element to which millions of humans are exposed in their environment. Alongside other severe diseases, chronic arsenic exposure has been associated with cardiovascular disease, such as atherosclerosis and hypertension, but the molecular mechanisms are not fully understood. The micronutrient selenium is a known arsenic antagonist, and protects tissue from damage caused by oxidative stress. Selenium deficiency has been identified as the cause for heart disease (Keshan disease). To date, there are no studies investigating the effect of selenium supplementation on arsenic induced atherosclerosis.

Objective: This *in vitro* study aims to determine whether selenium can inhibit the molecular mechanisms that lead to arsenic induced cardiovascular disease, by looking at the expression of inflammatory markers in human aortic endothelial cells (HAEC), the generation of reactive oxygen species (ROS), and endothelial integrity of HAEC after treatment with sodium arsenite (As) and sodium selenite (Se).

Methods: The endothelial expression of chemokines, such as IL-8 and MCP-1 in response to different concentrations of As with and without Se is determined by ELISA. The expression of intercellular adhesion molecule ICAM-1 on HAEC and ROS generation are measured by flow cytometry, for the latter HAEC are loaded with the non-fluorescent dye CM-H_2DCFDA, which becomes fluorescent after cellular oxidation. In order to determine endothelial dysfunction, HAEC are grown on a transwell filter. After incubation with As and/or Se, FITC-labeled albumin is added to the upper chamber, and its leakage through the endothelial monolayer into the bottom well, as a sign for reduced endothelial integrity, is measured in a fluorometer.

Results: The exposure of HAEC for 24 hr with As (concentrations ranging from 100 ng-1.5 µg/ml) lead to an increase in IL-8, which were significantly reduced when cells were co-incubated with Se. Preliminary results also suggest that treatment with Se decreases As-induced ICAM-1 expression. Experiments to assess ROS generation and endothelial integrity are currently in progress.

Conclusion: According to our current findings, we propose that Se antagonizes As-induced atherosclerosis by decreasing the expression of inflammatory mediators, such as IL-8 and ICAM-1. In the near future, this study will also examine the effects of Se and As on endothelial integrity and ROS generation, as well as As-induced leukocyte recruitment.

ENZYMATIC PROCESSING OF S-ARYL AND S-BENZYL CONJUGATES THROUGH THE MERCAPTURIC ACID PATHWAY

Anita A. Agblor, James C.H. Poon, P. David Josephy
Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada

Background: The metabolism of a xenobiotic to a mercapturic acid requires a series of enzymatic steps, beginning with bioactivation to an electrophilic species (such as a quinone or an epoxide) and glutathione transferase-catalyzed conjugation with GSH, followed by: (i) hydrolysis of the γ-glutamyl isopeptide bond of the GSH conjugate, catalyzed by γ-glutamyltransferase (γ-glutamyltranspeptidase; GGT); (ii) hydrolysis of the peptide bond of the resulting cysteinylglycine conjugate, catalyzed by a dipeptidase; (iii) N-acetylation of the cysteine conjugate to give the mercapturic acid. Although this metabolic pathway is enshrined in standard textbooks of pharmacology and toxicology, the identities and properties of the enzymes catalyzing the individual steps remain surprisingly poorly characterized. Most biochemical studies have been performed using surrogate substrates chose for assay convenience (*e.g.*, chromogenic assays).

Objectives: To characterize the enzymatic processing of aryl and benzylic conjugates of glutathione, as representatives (or analogues) of the detoxication products of polycyclic aromatic xenobiotics.

Methods: We have synthesized 4-nitrobenzylglutathione, 2,4-dinitrophenylglutathione, 4-methylbiphenylglutathione, 1-menaphthylglutathione, and 9-methylanthracenylglutathione. The corresponding cysteinylglycine conjugates were also obtained, by partial hydrolysis of the glutathione conjugates. Enzyme assay protocols were developed for all of these conjugates, using HPLC-UV methods for separation and quantitation.

Results: We measured the transpeptidation activity of bovine kidney GGT for each of these glutathione conjugate donor substrates, with glycylglycine as the GGT acceptor substrate. All substrates tested were hydrolyzed by GGT, with a trend towards higher Km values for bulkier aromatic substrates. To examine the hydrolysis of cysteinylglycine conjugates, we purified porcine kidney cortex membrane dipeptidase (MDP) to homogeneity, via phosphatidylinositol-specific phospholipase C-mediated cleavage of the protein's membrane anchor and cilastatin affinity chromatography. The homodimeric structure of the MDP protein was confirmed by mass spectrometry. MDP catalyzed the hydrolysis of all conjugates tested (1-(chloromethyl)- naphthalene, 4-nitrobenzyl chloride, and 1-chloro-2,4-dinitrobenzene), but the rate of hydrolysis was strongly dependent on the nature of the substituent on the cysteine sulfur atom. We are also examining the activity of the hepatic cytosolic dipeptidase leucyl aminopeptidase with respect to the same substrates.

Conclusions: Enzymes of the mercapturic acid pathway can act on bulky aromatic substrates, but the nature of the S atom substituent can have a substantial effect on enzyme activity.

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ASSESSMENT OF BLOOD LEVELS OF ETHANOL RESULTING FROM ETHANOL EXPOSURE BY INHALATION: STUDIES IN VOLUNTEERS AND TOXICOKINETIC MODELING.

J. Dumas-Campagna, G. Charest-Tardif, R. Tardif, S. Haddad
Department of Environmental Health and Occupational Health, Université de Montréal

Background: Today, most of people are exposed to ethanol via inhalation – during gas tank filling or use of hydro-alcoholic solutions for disinfection - at concentrations highly variable ranging from 10 $mg.m^{-3}$ to 758 $mg.m^{-3}$. These exposure concentrations are considered low because they are below the exposure time weighted average (TWA) 1880 $mg.m^{-3}$ (1000 ppm) in force in Canada. Toxicological information is currently lacking in regards to chronic inhalation exposure to low concentrations. We developed a physiologically based pharmacokinetic model (PBPK) for inhaled ethanol based on exposed volunteers only based on levels above 5000 ppm but uncertainty still remains about the validity of this model to predict the blood levels of ethanol (BE) for exposures below TWA.

Objective: This project aims to determine the BE resulting from exposures to low concentrations (<1000 ppm) in order to adjust/validate the PBPK model.

Methods: Ten volunteers (five men and five women) were exposed for 4 consecutive hours to vapors of ethanol (125, 250, 500, 750 and 1000 ppm) in resting conditions in an inhalation chamber of 18 m^3. An additional exposure to 750 ppm that included four periods of 12 minutes of exercise at 50W was performed. Blood samples and alveolar air were collected during and after the exposure.

Results: Results show that there is a linear relationship between the ethanol inhaled air concentrations and (i) BE (women: $r^2 = 0.98$/men: $r^2 = 0.99$), as well as (ii) ethanol concentrations in the alveolar air at end of exposure period (men: $r^2 = 0.99$/women: $r^2 = 0.99$). Furthermore, exercise resulted in a significant increase (2 to 3 times) in BE after each period of 12 min of exercise. Overall, the PBPK model predictions overestimated BE at exposure levels < 2616 ppm for men and < 2300 ppm for women. At lower exposure concentrations, limiting the clearance to the liver compartment was insufficient to account for total ethanol clearance. Adjusting the model by adding extra-hepatic biotransformation of high affinity and low capacity associated with the richly perfused tissues allowed the model to fit adequately measured BE at the low and high exposure levels.

Conclusions: These new toxicokinetic data and improved PBPK model for ethanol will facilitate the refinement of risk assessment for chronic inhalation exposure to low levels of ethanol. (Project funded by ANSES, France)

RATS PULMONARY EFFECTS TO ACUTE INHALATION OF 5 NM TIO_2 SHOWING TWO DISTINCT AGGLOMERATION STATES.

Alexandra Noël[1], Karim Maghni [2], Yves Cloutier[3], Chantal Dion[3], Kevin James Wilkinson[4], Stéphane Hallé[5], Robert Tardif[1], Ginette Truchon[3].
[1]Département de santé environnementale et de santé au travail, Université de Montréal; [2]Centre de recherche de l'Hôpital du Sacré-Coeur de Montréal, Université de Montréal; [3]Institut de recherche Robert-Sauvé en santé et en sécurité du travail (IRSST); [4]Département de chimie, Université de Montréal. 5Département de génie mécanique, École de Technologie Supérieure (ÉTS).

Background: At the nanoscale, quantum effects give nanoparticles (NP) new physicochemical properties compared to particles of identical chemical composition, but of larger size. These same characteristics are a matter of concern since they might also induce health effects. NP agglomeration in aerosols can lead to micron-sized particles. Thus, the intrinsic properties of NP, the structure and size of agglomerates, and a number of exposure metrics (e.g., mass, volume, number, surface area, size distribution) are all necessary parameters for understanding the relationships between NP dose and biological effects. Few studies have investigated how agglomeration influences the relationship between the NP exposure dose and the induction of pulmonary toxicity. According to current literature, nano-aerosols composed of large agglomerates (LA) (> 100 nm) are more likely to promote pulmonary clearance via macrophages phagocytosis, while small agglomerates (SA) (< 100 nm) seem to escape this first defense mechanism and are more likely to interact directly with biological material. These different mechanisms can influence pulmonary toxicity.
Objectives: This hypothesis was evaluated by comparing the relative pulmonary toxicity induced by aerosolized nano-TiO_2 showing two different agglomeration states: SA (< 100 nm) and LA (> 100 nm) at mass concentrations of 2 or 7 mg/m^3.
Methods: Four groups of Fisher 344 male rats (n = 6) were nose-only exposed to different agglomeration states 5 nm TiO_2 aerosols for 6 hours. Two control groups were exposed to clean air. Dry generation methods (fluidized bed, TSI; Palas, GmBH) and a wet method (Collison, BGI) were used to aerosolize the NP powder. The average exposure concentrations in the inhalation chamber were measured gravimetrically and median number aerodynamic diameters (MNAD) were provided by an electrical low pressure impactor (Dekati). Rat broncho-alveolar lavage fluids (BALF) collected 16 hours after the end of the exposure were analyzed for pulmonary inflammation cells (total and differential cell count) and biomarkers (IL-1α, IL-6, TNF-α, MIP-1α and MCP-1), cytotoxicity responses (lactate dehydrogenase (LDH), alkaline phosphatase (ALP), protein concentration) and oxidative stress responses (HO-1, 8-isoprostane and glutathione).
Results: The MNAD were 30 and 185 nm at 2 mg/m^3, and 31 and 194 nm at 7 mg/m^3. At 2 mg/m^3, the pulmonary toxicity profiles were similar for the two exposure groups and the controls. A significant 2.1-fold increase in the number of neutrophils ($p < 0.05$) was found in BALF of the group exposed to the 7 mg/m^3 LA nano-aerosol, suggesting a mild inflammatory response. Rats exposed to the 7 mg/m^3 SA nano-aerosol showed a 1.8-fold increase in LDH activity and 8-Isoprostane concentration in BALF, providing evidence for cytotoxic and oxidative stress effects.
Conclusions: Acute inhalation of 5 nm TiO_2 with two distinct agglomeration states, smaller or larger than 100 nm, induced mild pulmonary effects at 7 mg/m^3, but via different mechanisms. An acute inflammatory response followed the exposure to LA (> 100 nm). Clear trends showing both cytotoxic and oxidative stress effects without apparent activation and recruitment of immune cells measured in BALF was observed for SA (< 100 nm). Our study provides the first evidence that biological responses to NP might depend not solely on the dimension of the NP but also on the dimension of the NP agglomerates.

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