41st Annual Symposium / Le 41e symposium annuel

Nov 29 – Dec 1, 2009
Delta Centre-Ville
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Toxicology of drug and chemical mixtures: from mechanisms to risk assessment

Toxicologie des mélanges de médicaments et de contaminants chimiques: des mécanismes à l'évaluation du risque

Organised by / Organisé par
SOCIETY OF TOXICOLOGY OF CANADA
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Programme Committee / Comité du programme
Kannan Krishnan, Université de Montréal, Chair
Michael Wade, Health Canada, member
Douglas Bryant, Intrinsik Science Inc., member

Sunday Nov 29 PM

2:00 – 5:00 STC Board meeting

7:00 Student mentoring session

Toxicology of Drug and Chemical Mixtures: From Mechanisms to Risk Assessment

Monday Nov 30 AM

7.30 Registration / Continental Breakfast

8:30 **Genevieve Bondy**, President STC
Opening remarks and Introduction

Session I: Overview and Mechanisms

Chair person: Kannan Krishnan, Université de Montréal

8:40 Introduction

8:45 **Rick Burnett**, Health Canada
Air pollutant mixtures and health effects: progress and challenges

9:30 **R. Scott Obach**, Pfizer
Prediction of cytochrome P450-based drug-drug interactions *from in vitro* information

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

10:45 **Sami Haddad**, Université du Québec à Montréal
Interactions between human pharmaceuticals and environmental chemicals: Occurrence and mechanisms

11:30 **Poster Session**

Monday Nov 30 PM

Session II: Current Advances and Challenges

Chairperson: Douglas Bryant, Intrinsik Science Inc.

12:55 Introduction

1:00 **Richard Kim**, University of Western Ontario
Transporters and drug-drug interactions

1:30 **Frank-Peter Theil**, Genentech
PK-based drug-drug interactions and protein therapeutics

2:00 **Current advances in Toxicology and Environmental Health, I.**
Chairperson: **Thomas Sanderson**, INRS-Institut Armand-Frappier

- **D. Breznan, M. Phaneuf, Y. Siddiqui and R.Vincent**. Inhalation Toxicology Laboratory, Environmental Health Science and Research Bureau, Environmental Radiation and Health Sciences Directorate, HECSB, Health Canada
  Biological effects of air particulate matter: human in vitro co-culture model

- **A.S.Long[1], C.L. Lemieux[1], S. Lundstedt[2] and P.A. White[1].** [1]Mechanistic Studies Division, Environmental and Radiation Health Sciences Directorate, HECSB, Health Canada. [2]Department of Chemistry, Umeå University, Sweden.
  In vitro mammalian mutagenicity and Ah receptor agonism of complex PAH mixtures in contaminated soil

- **T. Peyret and K. Krishnan**. Dép. de santé environnementale et santé au travail, Université de Montréal.
  In vitro-in vivo extrapolation of the dose-response relationship for cellular perturbations of a binary mixture of toluene and n-hexane

- **E. Thomson[1], R. Vincent[1] and P. Kumarathasan[2]**. [1]Inhalation Toxicology Laboratory, and [2]Proteomics Laboratory, Environmental Health Science and Research Bureau, HECSB, Health Canada.
  Additive and antagonistic effects of co-exposure to air pollutants on biological pathways in the lungs

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

3:00 **Current advances in Toxicology and Environmental Health, II.**
Chairperson: **Roger Keefe**, Imperial Oil

- **F. Antoine, J. Ennaciri et D. Girard**. INRS-Institut Armand-Frappier, Université du Québec
  Le rôle de SYK dans la toxicité du trioxyde d'arsenic sur les neutrophiles

- **S. Aziz, G. Bondy, M. Barker, K. Kapal, P. Bellon-Gagnon, E. MacLellan, I. Curran and R. Mehta**. Toxicology Research Division, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch,Health Canada.
  BCL-2, BAX, CASPASE-9, and proliferating cell nuclear antigen (PCNA) expression, and apoptosis in rat livers exposed to the food contaminant, perfluorooctane sulphonate (PFOS)

- **X. Jin, M. Coughlan, S. Ulhaq, J. Yan, J. Roberts, R. Mehta and J. Raju.** Toxicology Research Division, Bureau of Chemical Safety, Food Directorate, HPFB, Health Canada
  Effects of dietary acrylamide on systemic oxidative stress and inflammatory markers in rats

- **N. Philbrook[1], V.K. Walker[1,2] and L.M. Winn[1,3]**. [1]School of Environmental Studies, [2]Department of Biology, [3]Department of Pharmacology & Toxicology, Queen's University
  Observations of the effects of nanoparticles on reproduction and development in Drosophila melanogaster and CD-1 mice

- **P. Kumarathasan, D. Das, S. Mohottalage, D. Breznan, E. Blais and R. Vincent.** Healthy Environments and Consumer Safety Branch, Health Canada.
  Mechanistic studies to verify toxicity of carbon-based nanomaterials in murine macrophages

- **S. Jones and D.G. Cyr**. INRS-Institut Armand-Frappier, Université du Québec.
  Localization and induction of the xenobiotic efflux transporter, P-glycoprotein (ABCB1), in the rat epididymis

- **J. Chi-Jen Lin[1], S. Talbot[2], K. Lahjouji[2], J-P. Roy[1], J. Sénécal[2], R. Couture[2] and A. Morin[1].** [1]Imperial Tobacco Canada Ltd, Montréal. [2]Department of Physiology, Université de Montréal
  Cigarette smoke-induced kinin B1 receptor expression in rat lung slices is mediated by IL-1ß

- **R. Lo[1], A. Forgacs[2], T. Celius[1], L. MacPherson[1], P. Harper[1], T. Zacharewski[2] and J. Matthews[1]**. [1]Dept of Pharmacology & Toxicology, University of Toronto. [2]Dept of Biochemistry & Molecular Biology Michigan State University.

Genomewide analysis of aryl hydrocarbon receptor binding targets in dioxin-treated mouse hepatic tissue

4:00 **Workshop:**
Benchmark dose (BMD) modeling
Instructor: **Jay Zhao**, U.S. EPA

4:00 **Annual Business Meeting**

6:00 **President's reception & STC awards**

ToxQuiz – an animated challenge to your knowledge of Toxicology, risk assessment and posters at STC-2009
Co-hosts: **Kannan Krishnan**, Université de Montréal & **Jamie Nakai**, Health Canada

Tuesday Dec 1 AM

7:00 Continental Breakfast

Session III: Evaluation of Combined effects: Data and the Debate

Chairperson: Michael Wade, Health Canada

8:05 Introduction

8:15 **Earl Gray**, U.S. EPA
Effects of mixtures of phthalates and other toxicants on sexual differentiation in rats: a risk framework based on disruption of common developing systems

9:00 **Nigel Walker**, National Toxicology Program, U.S. National Institute of Environmental Health Sciences
Assessing the cumulative effects of mixtures: Lessons learned from NTP chronic bioassays of mixtures of dioxins and PCBs

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

10:15 **Christopher J. Borgert,** Applied Pharmacology and Toxicology, Inc
Combination effect models, mode of action, and risk: is there any relationship?

11:00

STC Debate

"Additivity should be the rule for assessing the health risks of chemical mixtures in the environment"

Moderator: Jules Brodeur, M.D., Ph.D.
For: Claude Viau, Université de Montréal
Against: Raymond Yang, Colorado State University

12:00 **Lunch and poster session**

Tuesday Dec 1 PM

Session IV: Safety and Risk Assessment Approaches

Chair person: Carol Drury, Shell Canada

1:25 Introduction

1:30 **Bette Meek**, University of Ottawa
Risk assessment of combined exposures to multiple chemicals: A WHO/IPCS framework

2:00 **Moiz Mumtaz**, Agency for Toxic Substances and Disease Registry
Application of mixture risk assessment methods to hazardous waste and contaminated sites

2:30 **Deborah A. Nicoll-Griffith,** Merck
Drug-drug interactions and safety assessment: current approaches

3:00 **Genevieve Bondy**, Health Canada, 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 Nov 30 AM

Session I: Overview and Mechanisms

Air pollutant mixtures and health effects: Progress and Challenges.

Monday, November 30, 8:45 AM – 9:30 AM

Richard T. Burnett, Population Studies Division, Environmental Health Sciences and Research Bureau, Environmental and Radiation Health Sciences Directorate, Healthy Environment and Consumer Safety Branch, Health Canada.

It is now widely recognized that exposure to combustion related outdoor air pollution is a contributing risk factor to the exacerbation of cardiopulmonary disease and death at concentrations currently observed in Canada. Pollution in both the particle and gas phase has been linked to health effects. Atmospheric pollutants covary in both space and time due to common sources and meteorology. The independent role of individual pollutants and sources of pollution have thus not been clearly identified, making the development of cost-effective mitigation strategies challenging. Epidemiological approaches to understanding the role of atmospheric pollutant mixtures will be discussed. Some recent results based on studies of both short and long term exposure on mortality are presented.

Richard Thomas Burnett received his Ph.D. from Queen's University in 1982 in Mathematical Statistics. He is a senior research scientist with the Healthy Environments and Consumer Safety Branch of Health Canada, where he has been working since 1983 on issues relating to the health effects of outdoor air pollution. Dr. Burnett work has focused on the use of administrative health and environmental information to determine the public health impacts of combustion related pollution using non-linear random effects models, time series and spatial analytical techniques.

Prediction of cytochrome P450-based drug-drug interactions from *in vitro* information.

Monday, November 30, 9:30 AM – 10:15 AM

Obach RS, Pharmacokinetics, Pharmacodynamics, and Drug Metabolism, Pfizer, Inc., Groton, CT

A frequent mechanism that underlies pharmacokinetic-based drug-drug interactions (DDI) is through inhibition of cytochrome P450 enzymes that are responsible for the metabolic clearance of a majority of drugs. With our understanding of the multiplicity of human P450 enzymes and their substrate and inhibitor specificities, we have been able to use in vitro data to predict the magnitude of DDI for new drugs that inhibit or inactivate these enzymes. Prediction of DDI from in vitro data requires a conceptual picture of drug disposition, with particular emphasis on the liver and intestine, such that in vitro inhibition or inactivation data can be used in equations that model these drug disposition properties for the inhibitor and the drug affected by the inhibitor. The process by which

this is done will be reviewed in this presentation and the inherent assumptions and shortcomings will be described. This is important because the vitro tools used to determine which P450 enzymes can be inhibited by a new drug have become commonplace, and such experiments have become an expectation for supporting the development and registration of new drugs.

Scott Obach is a Senior Research Fellow in the Pharmacokinetics, Dynamics, and Drug Metabolism Department at Pfizer in Groton, CT. He earned his Ph.D. in biochemistry from Brandeis University in 1990, followed by a post-doctoral fellowship in 1990-1992 at the New York State Department of Health Research Laboratories. In 1992, Scott joined the Drug Metabolism Department at Pfizer Inc. as a Research Scientist. He currently serves on the editorial boards of Drug Metabolism and Disposition, Chemico-Biological Interactions, Drug Metabolism and Pharmacokinetics, and Xenobiotica and is a member of the Drug Metabolism Technical Group of PhRMA. He is an author or coauthor on over one hundred research publications and has given invited oral presentations at over thirty scientific conferences. His research interests include application of in vitro approaches to study drug metabolism, prediction of human pharmacokinetics and drug interactions, mechanisms of cytochrome P450 catalysis and other biotransformation reactions, including generation of chemically reactive metabolites.

Interactions between human pharmaceuticals and environmental chemicals: occurrence and mechanisms.

Monday, November 30, 10:45 AM – 11:30 AM

Haddad, S. Department of biological sciences, centre TOXEN, University du Québec à Montreal, Montreal, Canada.

A number of factors can potentially modify the toxicity of chemical pollutants to which humans are exposed. Among these factors are the genetic background, hypersusceptibility, age, sex, lifestyle, and toxicological interactions resulting from exposure to multiple chemicals such as pharmaceuticals. In our modern society, consumption of pharmaceutical drugs has reached unequalled proportions which are still increasing. Many people consume drugs on a daily basis in variable quantities and drug combinations are not infrequent. Consumers of pharmaceuticals are also simultaneously exposed to chemical pollutants present in the environment or at work. This raises the following questions: Can consumption of these drugs on a regular basis affect the health risk associated with exposure to occupational or environmental chemical pollutants? In other words, can they toxicologically interact with chemical pollutants, and hence, modulate the ensuing risk? Although combined exposures are a reality, very few studies have looked at this question. An overview of existing evidence for drug-pollutant interactions will be presented. This will be followed by our recent in vitro studies on : i) drug interactions with metabolism of alkylphenols (bisphenol A and n-nonylphenol) and trichloroethylene; ii) bisphenol A displacement on plasma albumin by selected pharmaceuticals; as well as iii) modulation of benzo[a]pyrene DNA-adduct formation rates caused by various pharmaceuticals. Finally, the use of modeling approaches/strategies to enable the prediction of potential impacts of pharmaceuticals on environmental chemical risk assessment will be discussed.

Dr Sami Haddad is an associate professor in the Dept of Biological Sciences of the Université du Québec à Montréal and member of the TOXEN research center since 2004. He graduated in 1994 from McGill University with a major in biochemistry and minor in environmental studies. He then pursued his graduate studies at the Université de Montréal. He completed his M.Sc. in occupational and environmental health in 1996 with his study on pyrene pharmacokinetic modeling and metabolism. This was followed by his Public Health Ph.D thesis (2000) on physiologically-based modeling of chemical mixtures. He then pursued his research in Switzerland at Hoffmann-La Roche Ltd first as a post-doctoral fellow for and then as a specialist in Biomathematical Modeling in preclinical research. He has been involved in mixture toxicology research for more than 10 years now. He has authored several publications on PBPK modeling of mixtures as well as on their application in health risk assessment. In 2003, one of his publications was selected for the *Best Paper in Toxicological Sciences Award* by the SOT Board of Publications. His current research activities in mixtures include various aspects of multichemical pharmacokinetic interactions such as in vitro characterization of mechanisms of interactions, in vitro-in vivo extrapolations, physiologically-based pharmacokinetic modeling, interindividual variability, and impacts of drug consumption in the risk assessment of chemical contaminants.

Session II: Current Advances and Challenges

Transporters and drug-drug interactions.

Monday, November 30, 1:00 PM – 1:30 PM

Kim R. Department of Medicine, University of Western Ontario, London, Canada.

Drug transporters are now increasingly recognized as clinically relevant determinants of variable drug responsiveness and unexpected drug-drug interactions. A number of uptake and efflux transporters expressed in organs such as the liver and kidney, include members of the organic anion transporting polypeptide (OATP), Organic Anion Transporter (OAT), Organic Cation Transporter (OCT), as well as certain ATP-Binding Cassette (ABC) families. These appear to be particularly important to the disposition of many drugs in clinical use today. We now know much more regarding the extent of their substrate specificity and the effects of genetic variation in such transporters to substrate drug disposition in humans. Not surprisingly, drug drug-drug interactions relating to the inhibition or induction of these transporters are now increasingly recognized as a clinically relevant problem. The mechanisms of drug uptake and efflux transporter-related interactions will be outlined and key examples of clinically relevant transporter associated drug-drug interactions will be provided.

Dr. Kim received his medical degree from the University of Saskatchewan in 1987. After completing an internship and residency training in Internal Medicine at Royal University hospital in Saskatoon in 1991, he went on to carry out postdoctoral fellowship training in Clinical Pharmacology at Vanderbilt University. In 1994, upon completion of his fellowship training, he remained at Vanderbilt University in the Division of Clinical Pharmacology as a faculty member where he rose to the rank of Professor. In July of 2006, Dr. Kim and a number of his colleagues and post-doctoral fellows from his group moved from Vanderbilt University to the University of Western Ontario. He is currently Professor and Chair of the Division of Clinical Pharmacology in the Department of Medicine at the Schulich School of Medicine & Dentistry, University of Western Ontario. He is leading a program of excellence in the field of Drug Transporters and Metabolizing Enzymes of relevance to Personalized Medicine.

PK-based drug-drug interactions and protein therapeutics.

Monday, November 30, 1:30 PM – 2:00 PM

Theil F-P, Girish S and Joshi A. PKPD Science, Genentech Inc, South San Francisco, U.S.A.

In the last two decades, protein therapeutics have become important treatment alternatives to conventional small molecule drugs (SMD) in several therapeutic areas. Depending on the therapeutic modalities available for a given disease, protein therapeutics are potentially combined with other protein therapeutics or SMD. Since combinations of drugs can result into drug-drug interactions (DDI), clinical pharmacology strategies during drug development should assess the risk of pharmacokinetic and pharmacodynamic-based DDIs. In particular for SMD, serious side effects caused by PK-based DDI resulted in restricted use of those drugs and even into market withdrawals. For protein therapeutics, therapeutically relevant PK-based DDI were not expected, since relevant elimination pathways of proteins are of large capacity. Furthermore, they are clearly distinct from the smaller capacity clearance processes of SMD. Consequently, DDIs were not formally studied in the past and regulatory submissions for marketing approval did not provide information on DDI assessments. Safety information collected during development and post-approval indicates that there was no evidence for therapeutically relevant PK-based DDI for protein therapeutics, which would have required black box warnings or market withdrawals like for SMD – this further supports limited relevance of PK-based DDIs. However, the scientific literature provides some examples where protein therapeutics modulate the expression of metabolizing enzymes like P450 resulting in changes in CL for SMD. In particular for cytokines and antibodies targeting cytokines, it has been shown that the expression of metabolizing enzyme (e.g. CYP450) can be altered. The most often cited example, interferon-alpha, demonstrates down modulation of several CYPs including CYP1A2. DDI studies could consequently show that drugs primarily metabolized by CYP1A2 had reduced clearance during comedication with interferon-alpha. Since endogenous cytokine levels are also altered in certain diseases such as inflammation, infection and cancer, changes of expression and alteration in functional activities of metabolizing enzymes might also cause changes in clearance of drugs just because of the disease progression. Only few examples are available illustrating that small molecule drugs can also change the clearance of protein therapeutics. For instance, the clearance of the anti-TNFalpha antibody, adalimumab exhibited lower clearance given together with methotrexate. However, so far the available clinical evidence on PK-based DDI of protein therapeutics suggests that the changes in clearance are rather moderate. Nonetheless, in case of narrow
therapeutic index compounds even moderate changes in clearance caused by PK-based DDI might become relevant for drug therapy.

Frank-Peter Theil obtained his PharmD and PhD from Humboldt University in Berlin, Germany. After 3 years at the Regulatory Agencies in Germany, he has been working in the pharmaceutical industry including biotech and multinational pharma companies for 20 years. His expertise includes DMPK, PKPD, clinical pharmacology and Modeling & Simulation aspects of biotech products as well as chemically derived small molecule drugs. He is currently leading the Early Development PKPD

Department at Genentech Inc., where his focus is on PKPD characterization of protein therapeutics as part of the pre-(non-)clinical development of potential clinical candidates. His current responsibility at Genentech includes the development of risk-based strategies for the assessment of PKbased drug-drug interactions of protein therapeutics with other proteins and small molecules. In the last years, he was involved in the organization of conference round table discussions and symposia on this subject to initiate a dialogue between representatives from FDA, biotech and pharma companies.

Hands-on Workshop: Benchmark dose (BMD) modeling

Monday, November 30, 4:00 PM – 6:00 PM

Instructor*: Jay Zhao,* U.S. EPA, Cincinnati, OH.

This workshop is designed to provide participants with an overview of benchmark dose modeling for cancer and noncancer dose-response assessment, and to provide a hands-on experience in using the latest EPA's BMDS software in chemical risk assessment.

Jay Zhao, M.P.H., Ph.D., D.A.B.T., is a board certified toxicologist with U.S. EPA, National Center for Environmental Assessment (NCEA) and is responsible for developing chemical risk assessment documents such as Toxicological Reviews for Integrated Risk Information System (IRIS) and Provisional Peer Reviewed Toxicity Values (PPRTV). He obtained a medical degree and M.P.H. from Shanghai Medical University, and a Ph.D. in Toxicology from University of Cincinnati. He has extensive experience in toxicology, including mutation research, biomarkers for exposure to environmental pollutants, tumor epidemiology, pulmonary toxicology and chemical risk assessment. Before joining U.S. EPA in 2007, he worked in Toxicology Excellence for Risk Assessment (TERA) for 10 years and he was the program manager leading *TERA*'s chemical assessment program, and organized all the chemical assessment activities within the organization. He specializes in dose-response analysis and human health risk assessment, and has extensive experience in using and teaching the use of Benchmark Dose (BMD) modeling, dosimetric adjustment, and chemical-specific adjustment factor (CSAF) methods in risk assessment. He has developed and provided training on these topics to risk assessors repeatedly at national SRA and SOT, local and regional toxicology and risk assessment conferences, as well as invited workshops.

Tuesday Dec 1 AM

Session III: Evaluation of Combined Effects: Data and the Debate

Effects of mixtures of phthalates and other toxicants on sexual differentiation in rats: a risk framework based upon disruption of common developing systems.

Tuesday, December 1, 8:15 AM – 9:00 AM

Gray, LE.[1]; Rider, C.[2, 1]; Howdeshell, K.[1]; Hotchkiss, A.[1]; Furr, J.[1]; Lambright, C.[1]; Foster, P.[3];Wilson, V.[1]
[1]United States Environmental Protection Agency, RTP, NC, USA. [2]Duke University, Durham, NC, USA. [3]NTP, NIEHS, Research Triangle Park, NC, USA.

Since humans are exposed to more than one chemical at a time, concern has arisen about the effects of mixtures of phthalates and other chemicals on human reproduction and development. We are conducting studies to determine 1) what effects are associated with in utero phthalate exposure, 2) which phthalates disrupt sexual differentiation, and 3) how mixtures of phthalates behave when combined with other phthalates or with other toxicants. In the mixture studies we have examined the postnatal development of male rat offspring after in utero exposure to 1) pairs of AR antagonists (vinclozolin and procymidone), 2) pairs of phthalates (DBP and DEHP; DBP and BBP), 3) phthalates with AR antagonists (Linuron and BBP; Procymidone and DBP), 4) five phthalates (DEHP, DBP, DiBP, BBP and DPP), 5) seven chemicals (four pesticides (vinclozolin, procymidone, prochloraz and linuron) and three phthalates (DBP, BBP, DEHP)), 6) ten chemicals (four pesticides (vinclozolin, procymidone, prochloraz and linuron)) and six phthalates (DBP, BBP, DEHP, DiBP, DPP, DiHP) and 7) the potent Ah receptor agonist 2,3,7,8-tetrachlorodibenzodioxin plus a phthalate (DBP). We also have examined the effects of these chemicals on fetal male rat hormone levels and testicular gene expression levels. Results of these studies demonstrate that only Dose Addition models accurately predict the effects of these mixtures on male rat sexual differentiation. For example, when ten chemicals were administered in utero, 100% of the males displayed reproductive tract malformations as predicted by Dose Addition models whereas Response Addition models predicted that none of the males would be malformed. We propose that the regulatory framework for cumulative risk assessments should not be based upon common mechanisms of toxicity, as this under-predicts the effects of mixtures of chemicals with dissimilar mechanisms of toxicity. Rather, the framework should be based upon the disruption of common fetal targets or systems during development regardless of the mechanism of toxicity. This abstract does not necessarily reflect EPA policy. NTP, NIEHS/EPA Interagency Cooperative Research Agreement HHS Y1-ES-8014-01; EPA RW75922.

Leon Earl Gray Jr. is senior reproductive toxicologist at EPA's National Health and Environmental Effects Research Laboratory in the Office of Research and Development. He obtained his B.S. (Biological Science) from Cornell University (1967) and Ph.D. (Zoology) from North Carolina State University (1976). After completing his postdoctoral training in the Duke University/EPA-IPA program,

Dr. Gray joined EPA as a research biologist in the Reproductive Toxicology Branch where he then became team leader and section chief. His research has focused on how individual toxicants and mixtures induce alterations of mammalian reproductive development. Current interests include investigations of how chemicals with divergent mechanisms of action interact during sexual differentiation. He has received more than 15 EPA Scientific and Technological Achievement Awards for research publications as well as two gold and seven bronze medals from EPA for his work. Dr. Gray is listed as Highly Cited scientist by the Citations Indices and his work has been presented at various national and international symposia as well as in several legislative hearings held by governmental agencies. Dr Gray has published over 180 journal articles and book chapters, including some in prestigious journals such as *Science* and *Nature.*

Assessing the cumulative effects of mixtures: Lessons learned from NTP chronic bioassays of mixtures of dioxins and PCBs

Tuesday, December 1, 9:00 AM – 9:45 AM

Walker NJ. National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA.

The dioxin Toxic Equivalency Factor (TEF) approach is currently used worldwide for assessing and managing the risks posed by exposure to mixtures of polychlorinated dibenzodioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs). Use of the TEF approach assumes that the combined effects of these dioxin-like compounds in a mixture can be predicted based on a potency adjusted dose additive combination of constituents of the mixture. While the TEF approach is generally only applied to dioxins, furans and dioxin-like PCBs, studies have shown the potential for interactions between different classes of dioxins and PCBs for nonneoplastic responses. To evaluate the TEF concept for carcinogenesis and interactions between dioxin-like and non-dioxin-like PCBs for carcinogenic responses, the National Toxicology Program conducted multiple 2-year rodent bioassays in female Harlan Sprague Dawley rats examining the toxicology and carcinogenicity of TCDD, PeCDF, PCB126, PCB118, PCB126, PCB153 and three mixtures; TCDD/PeCDF/PCB126, PCB126/PCB153 and PCB126/PCB118. This talk will present an overview of these studies, and the results and implications of statistically based, dose-response modeling approaches used to evaluate the interactions between compounds within mixtures and interactions between dioxin-like and non-dioxin like PCBs (This abstract does not represent NIH policy).

Nigel Walker, Ph.D., D.A.B.T., is Deputy Program Director for Science for the National Toxicology Program (NTP) at the U.S. National Institute of Environmental Health Sciences (NIEHS). Dr. Walker currently is leading several efforts for the NTP, including the Nanotechnology Safety Initiative and the Dioxin Toxic Equivalency Factor Evaluation, a series of studies evaluating relative potency factors for the carcinogenicity of dioxin-like compounds in female rats. In 2004, Walker received an NIH Merit Award for his activities on the TEF project.

Prior to his current position, Dr. Walker was a Staff Fellow and Postdoctoral Fellow (IRTA) at the Laboratory of Computational Biology and Risk Analysis for the Environmental Toxicology Program at

the National Institute of Environmental Health Sciences.

He received his B.Sc. in Biochemistry in England from the University of Bath in 1987 and his Ph.D. in Biochemistry from the University of Liverpool in 1993. Following postdoctoral training in environmental toxicology at the Johns Hopkins School of Hygiene and Public Health in Baltimore MD, he moved to the NIEHS, where he has been since 1995.

His research interests are environmental toxicology, quantitative dose response modeling and risk assessment, mixtures toxicology and nanotoxicology. He has published over 70 peer-reviewed publications and reports and two of his recent publications received Best Paper awards by the by the Risk Assessment Specialty Section of the Society of Toxicology.

He currently serves on the editorial boards for Environmental Health Perspectives and Toxicology and Applied Pharmacology. He is an adjunct associate professor in the Curriculum in Toxicology at the University of North Carolina at Chapel Hill, was President of the North Carolina Society of Toxicology in 2005, and is a board-certified toxicologist.

Combination effect models, mode of action, and risk: is there any relationship?

Tuesday, December 1, 10:15 AM – 11:00 AM

Christopher J. Borgert, Applied Pharmacology and Toxicology, Inc., and C.E.H.T, University of Florida College of Veterinary Medicine, Department of Physiological Sciences; Gainesville, FL, USA.

Methods for assessing toxicologic risks from combined exposure to multiple chemicals have relied on assumptions that relate the presumed mode or mechanism of action of individual chemicals to dose-response phenomena for a mixture. In short, dose addition is assumed to correctly predict adverse effects from combined exposure to chemicals with similar mechanisms of toxicity, i.e., to share a mode of toxic action, whereas response addition (independence) is assumed to correctly predict adverse effects of combined exposure to chemicals with dissimilar mechanisms of toxic action. Although there is theoretical and empirical support for such assumptions, we previously exposed theoretical inconsistencies and empirical exceptions and challenged their broad applicability. Nonetheless, most recent laboratory research aims to support the assumptions rather than to explore their ramifications and limitations. Recently, some have argued for a broader application of the dose additional model in toxicologic risk assessment and have published data interpreted as supporting the extension of the dose addition assumption to chemicals with dissimilar modes of action. This interpretation, however, ignores the possibility that rather than testing the validity of extending the dose addition model, such data might instead imply that the underlying assumptions about mechanistic similarity were incorrect or overly simplistic. In order to improve the theoretical and empirical basis of risk assessment, it is important to consider alternative interpretations consistent with the data and to consider relevant work performed in related fields. In this regard, pharmacological research has recently demonstrated non-linear isoboles for drugs that differ only in their efficacy at a receptor, and linear isoboles for drugs acting differently at receptor sites, indicating that the dose-additive assumption

of potency ratios cannot be applied broadly. These results are also inconsistent with the assumption that combined action can be predicted based on a few similar mechanistic features. Beyond these issues, an even greater challenge for toxicology is devising experimental methods to test the assumption that models of combined action consistent with mixture effects in a high dose range are applicable to doses orders of magnitude lower. Toxicologists' ability to respond objectively and scientifically to these issues and challenges will largely determine the scientific validity of mixture risk assessment in the future.

Christopher J. Borgert, Ph.D. is President of APPLIED PHARMACOLOGY AND TOXICOLOGY, INC. (APT), a consulting firm that specializes in applied research in the areas of causation analysis, safety assessment and study design. He also holds a courtesy faculty appointment in the Department of Physiological Sciences, University of Florida College of Veterinary Medicine. He received a bachelor of arts from Kenyon College, Gambier, Ohio, a doctorate in Pharmacology and Therapeutics from the University of Florida College of Medicine, and completed a postdoctoral fellowship in toxicology at the University of Florida Center for Environmental and Human Toxicology. He has served on the U.S.EPA Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) as the general representative for Small Business stakeholders and on numerous national and international expert and peer-review panels, including the Society of Toxicology Expert Panel on Chemical Mixtures. He is past President of the International Society of Regulatory Toxicology and Pharmacology (ISRTP), 2007-2008. His recent publications address methods for evaluating chemical mixtures in human milk, cumulative risk assessments for human exposure to drugs and chemicals, the pharmacology and toxicology of dietary supplements and interactions with drugs, and mechanistic dose-response evaluation for chemicals in human tissues, as well as conceptual and basic research papers that address the use of interaction data in mixture risk assessment and clinical medicine. He has recently contributed commentaries and editorials on the debate over conflict of interest and the peer-review process.

STC Debate

Tuesday, December 1, 11:00 AM – 12:00 PM

"Additivity should be the rule for assessing the health risks of chemical mixtures in the environment"

Moderator: Jules Brodeur, M.D., Ph.D.

For: Claude Viau, D.Sc.
Université de Montréal

The cornerstone of toxicology is the dose-response relationship. The latter is itself the very first expression of the rule of additivity in toxicology. A dose of two milligrams of a given xenobiotics has twice the effect of one milligram (provided of course there is no saturation in the toxication or detoxication mechanisms), as defined by the nature of the dose-response relationship. In occupational health, many organisations have had the wisdom of following the recommendation of the American Conference of Governmental Industrial Hygienists regarding exposure to mixtures: "In the absence of information to the contrary, different substances should be considered as additive where the health effect or target organ or system is the same." Therefore, unless patent information exists to prove two or more substances act independently, the risk assessor should consider the default assumption of additivity rather than play Russian roulette with public health.

Against : Raymond Yang, Ph.D.
Colorado State University

Former Secretary of Defense Donald Rumsfeld once said, regarding intelligence reports, "There are known knowns. There are things we know we know. We also know there are known unknowns. That is to say, we know there are some things we do not know. But there are also unknown unknowns, the ones we don't know we don't know." Rumsfeld's wisdom on intelligence appears to apply perfectly to the state of chemical mixture toxicology. Among the three categories, the unknown unknowns are the ones that we worry about the most in the area of chemical mixture toxicology. In this debate, examples will be given on the unknown unknowns and some of the recent advances of biological concepts to emphasize the potential dangers of assuming "Additivity" in chemical mixture toxicology.

Jules Brodeur obtained a MD degree from Université de Montréal in 1961 and a PhD degree (pharmacology) from the University of Chicago in 1964. From 1964 to 1996, he was Professor at Université de Montréal, first at the Department of pharmacology and later at the Department of occupational and environmental health. Upon his retirement, he was named Professor Emeritus. He now occupies the position of Administrative Judge at the Tribunal administratif du Québec, part-time.

Dr. Brodeur has received several distinctions : Prix Léon-Lortie from the Société Saint-Jean-Baptiste in 1989; Prix de la recherche from the Institut de recherche en santé et en sécurité du travail in 1990; Merit Award from STC in 1996; Education Award from SOT in 1999; Prix Antoine-Aumont from the Association québecoise pour l'hygiène, la santé et la sécurité du travail in 2001. A Diplomate of the American Board of Toxicology since 1980, Dr. Brodeur served as President of the Society of Toxicology of Canada from 1987 to 1989.

Claude Viau is professor in the Department of Environmental and Occupational Health at the University of Montreal; from 1994 to 2002, he served as chairman of the department. He was also senior risk management advisor for the Healthy Environment and Consumer Safety Branch of Health Canada during 2004-2006. In June 2006, he was appointed as the Chair in Toxicological Risk Analysis and Management at University of Montreal. He lectures in Environmental Toxicology and in Risk Management. His current research focuses on biomonitoring of polycyclic aromatic hydrocarbon exposure. Dr. Viau is a member of the ACGIH BEI Committee, of the Occupational Exposure Limits Experts Committee of the French Agency AFSSET and he is the former chair of the Scientific Committee on Occupational Toxicology of the International Commission on Occupational Health.

Raymond S. H. Yang is a Professor of Toxicology and Cancer Biology, and the leader of the Quantitative and Computational Toxicology Group, at the College of Veterinary Medicine and Biomedical Sciences, Colorado State University (CSU). Between October 2007 and July 2009, Dr. Yang had also been a Visiting Scientist at the National Center for Environmental Assessment, USEPA, Cincinnati, to work on TCDD and chemical mixture toxicology and risk assessment, among other projects. Dr. Yang's research focuses on physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling, and other biologically-based computer modeling with a special emphasis on the toxicology of chemical mixtures. Dr. Yang has had extensive research and administrative experience in academia, chemical industry, and the federal government. At CSU in the last 19 years, Dr. Yang had served in the capacity as a Department Head, a Center Director, and the Director for a NIEHS Quantitative Toxicology Training Program. Dr. Yang publishes extensively in biomedical journals and is the editor/co-editor of two books; Toxicology of Chemical Mixtures: Cases Studies, Mechanisms, and Novel Approaches (1994), and Physiologically Based Pharmacokinetics: Science and Applications (2005). Dr. Yang is a Fellow of Academy of Toxicological Sciences and served on many prestigious national and international committees and panels.

Session IV: Safety and Risk Assessment Approaches

Risk assessment of combined exposures to multiple chemicals: A WHO/IPCS Framework.

Tuesday, December 1, 1:30 PM – 2:00 PM

Meek, M.E., McLaughlin Centre for Population Health Risk Assessment, University of Ottawa, Ottawa, ON.

Terminology and methodology for assessing the impact of combined exposures to multiple chemicals has recently been considered in a project of the World Health Organization (WHO) Programme on Chemical Safety (PCS). This project, which is part of the WHO initiative on Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals, is being coordinated with additional initiatives of the

International Life Sciences Institute (ILSI) and the European Centre for Ecotoxicology and Toxicology of Chemicals.

Following an international workshop to review advances in this area, a draft framework has been developed with the objective to promote more consistent and efficient assessment of combined exposures to multiple chemicals. The framework includes delineation of explicit criteria for initial consideration of the bounds of appropriate grouping for assessment of combined exposures, followed by stepwise consideration of both exposure and hazard in several tiers of increasingly data-informed analyses.

The extent of assessment and nature of recommendations for generation of additional data are dependent upon the extent of the knowledge base, the magnitude of public health concern (i.e., taking into account margins between exposure and effect), and the objective of the risk assessment (e.g., implications of potential risk management decisions). The approach involves, then, decision-based analysis that takes into account relevant information at an early stage as a basis to scope the need or not for additional assessment and recommend any required data generation. Approaches range from predictive methodologies and conservative assumptions in early tiers to more realistic estimates of risk and rigorous descriptions of uncertainties in later tiers, based on increasingly data-informed and probabilistic approaches.

The framework has been illustrated through application to a series of case studies for pesticides and environmental contaminants selected to illustrate iterative consideration of exposure and hazard, and the nature of assessment in tiers of increasing complexity. The status of development of the framework, its content and application will be described and illustrated, through examples.

Bette Meek, is currently the Associate Director of Chemical Risk Assessment with the McLaughlin Institute of the University of Ottawa on interchange from Health Canada, where she managed the Existing Substances Division in the Safe Environments Programme. Her responsibilities in this capacity related to development and implementation of process and methodology for the assessment of the effects on human health of Existing Substances under the Canadian Environmental Protection Act, including setting priorities for assessment from among all 23, 000 commercial chemicals used in Canada by September, 2006 (i.e., categorization).

She has considerable experience in the development of methodology for and evaluation of health-related data on environmental contaminants, having also managed previously programmes within Health Canada on contaminants of drinking water and air. She acts as an advisor to several international organizations and has authored over 150 scientific publications in this area.

Specific areas of experience include development of frameworks to increase transparency in the assessment of human relevance of animal modes of action, increasing incorporation of biological data in dose-response as a replacement for default, development of predictive exposure and hazard modelling and increasing efficiency in assessment through effective problem formulation and early and continuing peer engagement.

Application of mixture risk assessment methods to hazardous waste and contaminated sites.

Tuesday, December 1, 2:00 PM – 2:30 PM

M. Mumtaz, M. Johnson, and D. Mellard, Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta, GA, U.S.A.

The issue "Do chemical mixtures pose added risk?" has been a long time concern of health assessors. The ATSDR guidance manual for the toxicity assessment of chemical mixtures was put forth to analyze its relevance to hazardous waste sites (ATSDR, 2001). This guidance is designed to be used in conjunction with ATSDR's public health assessment guidance manual that is inclusive of all pertinent risk assessment issues. The mixtures guidance recommends a series of steps that include simple calculations that allow a systematic analysis of data. Initially hazard quotients (HQ) are calculated for each chemical component of the mixture, followed by the hazard index of the mixture (HI Mix). The HQ and HI values dictate which additional steps of the guidance, that are data driven, need to be applied to draw conclusions for a given site. ATSDR analysis, using the guidance, has shown that for several waste sites, no additional health hazard is posed by mixtures. Results from three hazardous waste sites are presented. The first site (Endicott) did not pose an additional mixtures hazard while the second site (Conrail) did. At the Endicott site, exposures were via municipal water to low levels of several volatile organics chemicals including vinyl chloride, per-chloroethylene, and 1,1-dichloroethane. The HQs for individual chemicals were below 0.1, the cut-off value recommended by the guidance, thus the HIMix calculation and further analysis was not needed. On the contrary, at the Conrail site, exposures were to high levels of trichloroethylene and carbon tetrachloride. The HQs for individual chemicals were very high (Inhalation HI Mix = 151), target organ specific toxicity (neurological HIMix = 135; hepatic HIMix= 52) and weight of evidence determinations for synergistic interactions were performed. The third site, Palmerton, was a zinc smelter site that had high soil levels of zinc, lead, and substantial levels of other metals. As mixtures, these pollutants can potentially cause neurological, hematological, hepatic and testicular toxicities. The issue at this site was can the soil cleanup-goals for lead be relaxed because of the presence of zinc that inhibits other toxicities. The recommendation for this site was that additional toxicity data for testicular toxicity were needed and until then the clean-up levels could not be lowered. For the Endicott site the recommendation was the water supply was safe for drinking and bathing. At the Conrail site, safe water was provided, vapor mitigation systems were installed, and health education was provided to residents and health care providers.

Moiz Mumtaz is Science Advisor, Division of Toxicology, Agency for Toxic Substances and Disease Registry (ATSDR), Centers for Disease Control and Prevention (CDC) and an adjunct faculty at the Environmental Occupational Health Department, Emory University. His involvement in several agency wide activities has led to a) the establishment of a mixtures research program for determining significant human exposures to environmental chemicals, b) the establishment of a computational toxicology laboratory for characterizing the behavior of chemicals after they enter the human body or estimating the toxicity of chemicals based on structure-activity relationships (SAR), and c) the revision of ATSDR guidelines and policy for clearing publications. Dr. Mumtaz obtained his Ph.D. in toxicology from the University of Maryland and received his M.S. in chemistry/entomology from Oregon State University.

Dr. Mumtaz started his professional career as a chemist after completing his M.Sc. in analytical chemistry from Osmania University, India. Dr. Mumtaz has actively published his research findings in several peer-reviewed journals. These publications have covered a wide range of research areas pertinent to medicine and human health that included but were not limited to dopamine metabolism and mental health; chemical analysis of xenobiotics and environmental chemicals; health risk assessment of chemicals and susceptible human populations. Dr. Mumtaz is a member of the Society of Toxicology (SOT), and the past- president of the SOT Mixtures Specialty Section. He represents ATSDR on several inter-agency workgroups such as the Department of Health and Human Services (DHHS) Interagency Coordinating committee on the validation of alternative methods (ICCVAM), and Mixed Exposures Work Group, National Occupational Research Agenda, NIOSH.

Drug-drug interactions and safety assessment: current approaches

Tuesday, December 1, 2:30 PM – 3:00 PM

Deborah A. Nicoll-Griffith and *Raymond Evers*. Global Drug Metabolism and Pharmacokinetics, Merck & Co., Inc., Rahway, NJ, USA.

Drug-drug interactions, in particular those involving inhibition of drug metabolizing enzymes or transporters, may cause significant exposure increases of the therapeutic agent. For example, clinical plasma concentrations may increase to the point that safety margins established during pre-clinical safety assessment studies no longer hold and unwanted side effects or toxicity may become manifest. Ideally, drug development will focus on drug candidates that are not predisposed to drug-drug interactions as a way to mitigate this risk. The key elements governing these drug-drug interaction processes include drug metabolizing enzymes such as cytochrome P450, glucuronosyl transferases and transporter proteins. Another confounding element is the interplay between induction of enzymes and transporters through nuclear receptors, and the inhibition of these same enzymes and transporters by the therapeutic agents. Given that preclinical animal models often have significant differences in homologs of drug metabolizing enzymes and transporters, in vivo studies often provide little information that can drive confident decision making around the predictions of clinical drug-drug interactions. Therefore, human in vitro systems are commonly used to predict the likelihood and magnitude of drug-drug interactions. This is still a developing area but progress is being made towards more accurate predictions. There is currently also a big driver in the use of in silico tools and models to enhance our predictive capabilities. Several case studies will be shown to demonstrate issues that the Pharma industry faces in this area and tools that are currently being employed to aid decision making.

Deborah A. Nicoll-Griffith, Merck & Co., Inc.: Debbie is a Senior Scientific Director in Global DMPK at Merck, Rahway, NJ . She previously held positions at Merck Frosst Canada Inc, a subsidiary of Merck located in Montreal, Quebec where she led the Discovery DM group. Debbie completed her Ph.D. degree in 1986 at the University of British Columbia in Synthetic Organic Chemistry. Her research interests include in vitro, in vivo and in silico approaches to the drug discovery and development. She has authored over 50 peer reviewed publications and has served on the committees of drug metabolism organizations and conferences, including serving as the Drug Metabolism Gordon Research Conference chair.

Session d'Affichage
Poster Session

LE RÔLE DE SYK DANS LA TOXICITÉ DU TRIOXYDE D'ARSENIC SUR LES NEUTROPHILES.

Francis Antoine, Jamila Ennaciri et Denis Girard. (SPON : Patrick J. Devine)
INRS-Institut Armand-Frappier, Université du Québec, Laval, Québec, H7V 1B7.

L'anhydride arsénieux (As2O3), ou trioxyde d'arsenic (TA), est grandement utilisé en industrie pour blanchir le verre. L'arsenic est depuis longtemps catalogué comme un poison pour ses effets toxiques. Paradoxalement, le TA est une drogue utilisée en clinique qui est très efficace pour traiter les leucémies promyélocytique aiguës (APL). Son efficacité chimiothérapeutique réside dans le fait que le TA élimine les promyélocytes cancéreux par induction de l'apoptose. Nous nous sommes intéressés à l'étude portant sur l'interaction entre le TA et les neutrophiles principalement pour les deux raisons suivantes: *i)* les promyélocytes sont les précurseurs des neutrophiles, des phagocytes grandement impliqués dans l'inflammation et *ii)* des neutropénies ont été notées chez les patients traités avec le TA, présageant la possibilité que le TA induit l'apoptose des neutrophiles. Dans une étude récente, nous avons démontré que le TA induit l'apoptose des neutrophiles humains par la production d'H2O2, l'activation des caspases et la synthèse *de novo* des protéines. Un autre aspect du projet de recherche est d'étudier le rôle du TA dans la physiologie des neutrophiles afin de vérifier si cet agent peut moduler d'autres fonctions que l'apoptose et, dans l'affirmative, d'en déterminer les mécanismes d'actions. Nous présentons ici des résultats démontrant que le TA augmente la phagocytose et la dégranulation des neutrophiles par un mécanisme qui dépend de la protéine tyrosine kinase Syk. De plus, l'approche pharmacologique ainsi que l'approche de déplétion par nucléotides antisens ont montré que Syk est impliqué dans la régulation de l'apoptose. À l'aide d'immunobuvardages, nous avons d'abord montré l'activation de Syk par le TA, ainsi que sa réversibilité par le piceatannol (un inhibiteur pharmacologique spécifique). De plus, l'inhibition pharmacologique de Syk par le piceatannol permet de renverser l'augmentation de la phagocytose et de la dégranulation des neutrophiles par le TA. Présentement, nous pouvons conclure que le TA possède la capacité de moduler l'apoptose, la phagocytose et la dégranulation, de façon Syk-dépendante. Malgré le nombre élevé d'études visant à élucider les voies signalétiques du TA chez les cellules cancéreuses, notre étude est la toute première montrant que le TA active la voie de Syk. De plus, le TA induit un stress du réticulum endoplasmique ainsi que la production de cytokines, dont IL-8, mais l'implication de Syk reste à déterminer. Aussi, de récents résultats ont montré l'activation de la caspase-4, une caspase impliquée dans l'induction de l'apoptose par la nouvelle voie du réticulum endoplasmique. En conclusion, notre approche expérimentale à permis de découvrir une nouvelle voie de signalisation transmettant les effets toxiques du trioxyde d'arsenic.

Abstract / Résumé #1

DERIVATION OF BIOMONITORING EQUIVALENTS FOR DI(2-ETHYLHEXYL) PHTHALATE (DEHP)

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Recent efforts worldwide have resulted in a growing database of measured concentrations of chemicals in blood and urine samples taken from the general population. However, few tools exist to assist in the interpretation of the measured values in a health risk context. Biomonitoring Equivalents (BEs) are defined as the concentration or range of concentrations of an environmental chemical or its metabolite in a biological medium (blood, urine, or other medium) that is consistent with an existing health-based exposure guideline, and are derived by integrating available data on pharmacokinetics with existing chemical risk assessments. This study reviews available health based exposure guidance values for di(2-ethylhexyl)phthalate (DEHP) from Health Canada, the United States Environmental Protection Agency (U.S. EPA), the Agency for Toxic Substances and Disease Registry (ATSDR), the European Chemicals Bureau (ECB), and the European Food Safety Authority (EFSA). BE values corresponding to the oral reference dose (RfD), minimal risk level (MRL) or tolerable daily intake (TDI) estimates from these agencies were derived based on data on excretion fractions of key urinary metabolites. BE values based on the sum of a) the three most commonly measured metabolites (mono-2-ethylhyexyl phthalate [MEHP], mono-(2-ethyl-5-hyd roxyhexyl) phthalate [M EH HP] and mono-(2-ethyl-5-oxohexyl) phthalate [MEOHP]), and b) the sum of the five most predominant metabolites (MEHP, MEHHP, MEOHP, mono-(2-carboxymethylhexyl) phthalate [2cx-MMHP] and mono-(2-ethyl-5-carboxypentyl) phthalate [5cx-MEPP]), are presented. These values may be used as screening tools for evaluation of biomonitoring data for DEHP metabolites in the context of existing risk assessments and for prioritization of the potential need for additional risk assessment efforts for DEHP relative to other chemicals (supported by Health Canada).

Abstract / Résumé #2

BCL-2, BAX, CASPASE-9, AND PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) EXPRESSION, AND APOPTOSIS IN RAT LIVERS EXPOSED TO THE FOOD CONTAMINANT, PERFLUOROOCTANE SULPHONATE (PFOS)

Syed Aziz, Genevieve Bondy, Michael Barker, Kamla Kapal, Pascale Bellon-Gagnon, Ellen MacLellan, Ivan Curran and Rekha Mehta.

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

Introduction: An emerging food contaminant, PFOS, was recently assessed in our laboratory for toxicity in adult rats fed a diet containing 2 to 100 mg PFOS/kg diet for 28 days. Pathological examination revealed the liver as the major target organ.

Purpose: The purpose of the study is to further evaluate (A) significant rise in the apoptotic index with increasing PFOS dose in both male and female rat livers, (B) unusual cytoplasmic PCNA stained hepatocytes was observed in both sexes with increasing PFOS dose.

Methods: The expression of the mitochondrial network proteins, Bcl-2, Bax, and Caspase-9, cellular apoptosis, was further evaluated in normal and treated livers by IHC using their respective antibodies.

Results: Bcl-2, Bax and Caspase-9 were expressed in control and treated livers very prominently, and not restricted to any specific area of the liver. Bcl-2 staining exhibited a significantly higher intensity in the control compared to the treated livers; however, no significant difference (p=0.3 10). Bax expression varied greatly, within and between male and females and for different dose groups, but with no significant difference (p=0. 106). A trend in increasing Caspase-9 expression with PFOS dose in both males and females was also not significant (p=0.3 10). However, an important difference in the pattern of Bcl-2, Bax and Caspase-9 staining between the control and treated livers was noted, in controls, staining appeared as patchy deposits in the cytoplasm whereas in the treated liver, uniform intense staining covered the entire cytoplasm.

*Significance: O*ur observed unusual immuno-localization of PCNA in the cytoplasm, and intense uniform cytoplasmic expression of Bcl-2, Bax and Caspase-9 in PFOS-treated livers may be explained by chemical properties of PFOS that cause dysregulation of lipid metabolism and peroxisome proliferation, and which result in disruption of cellular/ nuclear/ mitochondrial membrane structures, including mitochondrial bioenergetics, with eventual consequences such as elevated hepatocytic apoptosis.

ROLE OF NITRITE IN GLYCERYL TRINITRATE (GTN) INDUCED MICROPHTHALMIA IN QUAIL EMBRYOS.

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Microphthalmia or the presence of a small eye may be caused by genetic or environmental factors. Rat whole embryo culture experiments have provided evidence that nitro compounds cause microphthalmia; structurally similar compounds without the nitro moiety did not cause this malformation. These data suggest that nitrite (NO2~) may be a key mediator in the induction of microphthalmia. There is increasing evidence that NO2 ~ can modify proteins and modulate gene expression. We hypothesize that enzymatic metabolism of GTN, a well characterized nitro compound, by the embryo releases NO2 ~which nitrates macromolecules and triggers ectopic aberrant signalling in the embryo, leading to eye malformations. This hypothesis necessitates: (1) description of the effects of GTN in the quail *(Coturnix coturnix japonica)* embryo *(in~ovo* and *ex~ovo),* (2) identification of embryonic enzyme(s) capable of GTN metabolism to nitrite and (3) demonstratation of the modification of proteins and gene expression as a result of nitrite release. To test this hypothesis, we performed two in ovo egg injection studies. The rationale for the first study was to target critical periods of development, one of which is optic development. In the first study, eggs (n=10~11 eggs/group) were injected with 10 ul of GTN (0, 1, 10 or 100 ug/ul) at 0, 33 and 72h of embryonic development. In the second study, embryos at 33h of development were injected with GTN (0, 0.1, 0.5, 1, 5, or 50 ug/ul; n=50 eggs/group) and examined 24, 48 or 72h post injection. Finally, one side of the developing eye field in the quail embryo was exposed ex ovo to GTN (10 ug/ul). We identified the NG metabolites formed in quail embryos and purified the metabolizing enzymes using LC/MS and reverse phase (RP) HPLC, respectively. We determined if proteins were subject to nitration using western blot analysis. An increased incidence of micropthalmia was observed in the 33h treatment group (40% with 10ug/ulGTN) following 7d of incubation. In the follow up study 72h post injection microphthalmia (1ug/ul: 3%; 5ug/ul: 20% or 10ug/ul: 9%) was accompanied by developmental delay, craniofacial and neural tube defects. Embryos treated ex ovo with GTN presented with an undeveloped eye. Using LC/MS we found that quail embryos metabolized GTN to both 1,2 and 1,3 glycerol dinitrate (1,2 and 1,3 GDN); this metabolism was catalyzed by a GSH~dependant cytosolic enzyme. Further, GST isozyme purification and identification, using reverse phase RP~HPLC, SDS~PAGE and MS, revealed the presence of multiple α and μ type GSTs; GSTα was the predominant family member responsible for producing nitrite from GTN. Western blotting analysis, using a monoclonal anti 3~NT antibody, demonstrated that multiple proteins were nitrated in treated embryos. These data demonstrate that GTN induces micropthalmia in the quail embryo and that nitrite is released from GTN, via GSTαmetabolism, and modifies proteins that may be a key initial step in the aberrant ectopic activation genes. Supported by an FRSQ studentship to G. Bardai, and the National Research Council of Canada.

MODELING THE IMPACT OF WORKLOAD ON THE BIOLOGICAL EXPOSURE INDICATORS OF STYRENE: COMPARISON BETWEEN SINGLE EXPOSURE AND BINARY EXPOSURE WITH ACETONE

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Physical exertion (workload) has been recognized as a major determinant of the absorbed dose for many solvents. This study was undertaken to assess the impact of workload on the biological levels of unchanged styrene or styrene metabolites used as biological exposure indices (BEIs). Physiologically based toxicokinetic models were adapted and validated in order to simulate a typical weekly occupational exposure (8 h/day, 5 days) to styrene alone and combined with acetone at their current threshold limit values (ACGIH) of 20 ppm and 500 ppm, respectively. Simulations were then conducted under workload levels corresponding to rest (12.5 W), 25 W and 50 W, and the impact on the levels of styrene in venous blood (STY-B) and on urinary mandelic acid (MA) and phenylglyoxylic acid (PGA) at the end of the last work shift of a week was examined for a typical worker. The predicted values were compared to results of both experimental and field studies which supported the adoption of the current BEIs for styrene. For an exposure to 20 ppm, the end-of-shift values of STY-B for a workload of 50 W showed a 3-fold increase compared to the value at rest (0.17 mg/L), whereas the sum of MA and PGA in urine was 2.7 times higher than at rest (144 mg/g creatinine). The model predicted slight effect of co-exposure to acetone on biological levels of styrene at these exposure levels. Based on the relation between physical activity and the values of BEIs predicted by the model, the average workload level in field studies was approximately 50W. Overall, the model described well the impact of workload on biological levels of styrene and showed that workload needs to be taken into account to avoid underestimation of the internal exposure to styrene and health risk. (Supported by Afsset, France and IRSST, Canada)

Abstract / Résumé #5

LACK OF INVOLVEMENT OF THE RENIN-ANGIOTENSIN SYSTEM IN AMIODARONE AND DESETHYLAMIODARONE CYTOTOXICITY

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Amiodarone (AM) is a potent antidysrhythmic agent which can cause potentially life-threatening pulmonary fibrosis, and desethylamiodarone (DEA) is a metabolite of AM that may contribute to the toxicity of AM *in vivo*. Recent evidence has implicated the involvement of the renin-angiotensin system (RAS) in the initiation and progression of amiodarone-induced pulmonary toxicity (AIPT). In HPL 1 A human peripheral lung epithelial cells, we found AM to be converted to DEA minimally (<2%) after 24 h of incubation, indicating that the HPL1A cell culture model can be used to observe the effects of AM and DEA independently. Apoptotic cell death was assessed by annexin-VFITC and by TUNEL, while necrotic cell death was determined by propidium iodide staining. The percentage of necrotic cells increased over six-fold after 24 h treatment with 20 μM AM (80.8%) compared to control (12.0%), while the percentage of apoptotic cells decreased from 8.26% (control) to 1.56% ($p<0.05$). In contrast, incubation with 3.5 μM DEA for 24 h increased the percentage of necrotic cells two-fold, from 10.8% (control) to 20.4%, and increased the percentage of apoptotic cells from 9.86% (control) to 22.0% ($p<0.05$). As determined by TUNEL, increased apoptosis was detected after 24 h treatment with 5.0 μM DEA (26.7%) compared to control (4.2 1%). Treatment with angiotensin II (100 pM – 1 μM) alone or in combination with AM or DEA did not alter cytotoxicity. Furthermore, pre-treatment with the angiotensin converting enzyme inhibitor captopril did not protect against AM or DEA cytotoxicity. In conclusion, AM activates primarily necrotic pathways, whereas DEA activates both necrotic and apoptotic pathways, and the RAS does not seem to be involved in AM or DEA cytotoxicity in HPL 1 A cells. Multiple mechanisms may contribute to the initiation of lung damage observed clinically, due to actions of both AM and its metabolite DEA. (Funded by CIHR Grant No. MOP-13257).

BIOLOGICAL EFFECTS OF AIR PARTICULATE MATTER: HUMAN *IN VITRO* CO-CULTURE MODEL

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In vitro studies are an important tool for elucidating the mechanisms by which air pollutants damage cells. However, a limitation of *in vitro* studies is that cells are removed from their complex *in vivo* environment in which they interact with neighbouring cells. We examined the role of lung cell interactions in modulating the biological effects of particles collected from ambient air. We evaluated the toxic effects of six air pollution particles with varied physicochemical properties by utilizing a co-culture model of human cell lines. Human lung epithelial (A549) and macrophage (THP-1) mono- and co-cultures were incubated with particles (EHC-93, EHC-6802, DWR1, SRM-1650, TiO_2 and SiO_2) at various concentrations for 24 hours. Additionally, pulmonary artery endothelial cells (HPAE) were incubated for 24 hours with conditioned media obtained from particle-treated A549 and THP-1 mono- and co-cultures. Cellular interactions were revealed in the differential release of cytokines and other factors in response to particle exposure. Epithelial A549 and macrophage THP-1 cells were shown to synergistically modulate their production of cytokines (IL-1ß, IL-6, IL-8, MCP-1 and TNF-α), ICAM-1 and VEGF in response to particle exposure. Cellular mediators from particle-exposed co-cultures activated lung endothelial HPAE cells and induced their production of cytokines (IL-6, IL-8, GM-CSF, MCP-1) and adhesion factors (ICAM-1, VCAM-1 and E-selectin). A subset of the particles (EHC-6802, DWR1 and SRM-1650) were instilled intratracheally in BALB/c mice. After 2 and 24 hours the mice were euthanized and lungs were lavaged with saline. Intratracheal instillation of the particles in mice led to neutrophilia and elevated cytokines (IL-6, KC, MIP-1α and TNF-α). The *in vitro* cell interaction model correlated with lung inflammation in mice after intratracheal instillation of particles. Thus a human cellular co-culture model was used to simulate the complexity of the *in vivo* lung micro-environment by enabling interactions between various cell types. It was shown to represent a useful approach for toxicological screening of air pollution particles and for studying the mechanisms underlying the adverse biological effects of air pollution.

MODULATION OF TRICHLOROETHYLENE METABOLISM BY DIFFERENT DRUGS. Cheikh Rouhou M and Haddad S . Département des sciences biologiques, centre TOXEN, Université du Québec à Montréal, Montréal, Québec.

Trichloroethylene (TCE) is a volatile organic solvent to which humans are frequently exposed. Toxicokinetic interactions on TCE have been reported with aspirin and acetaminophen but no information can be found for other drugs. The purpose of this study is to identify drugs that could potentially affect the metabolism of TCE. Fifteen widely used drugs were selected: acetaminophen, ibuprofen, acetylsalicylic acid, mefenamic acid, naproxen, diclofenac, sulphasalazine, cimetidine, ranitidine, valproic acid, carbamazepine, amoxicillin, erythromycin and gliclazide. Suspensions of rat hepatocytes were exposed to TCE alone and in presence of each of these drugs (10x therapeutical maximal plasma levels) in closed vials. The concentrations of TCE and its metabolites trichloroethanol (TCOH) and trichloroacetic acid (TCA) were measured by headspace gas chromatography coupled to mass spectrometry (GC-MS). Our results were statistically assessed using T-Test and the interaction is considered significant when $p < 0.05$. Our results show the drugs can be separated into 3 groups. Group 1 is composed of drugs causing no significant interactions (i.e., carbamazepine, ibuprofene, mefenamic acid and ranitidine). Group 2 includes drugs significantly affecting both TCOH and TCA levels: decreased levels by valproic acid (3-fold), acetaminophen (2-fold) and gliclazide (2-fold); increased levels by acetylsalicylic acid (1.5-fold) and naproxen (2-fold). While decrease in metabolite levels may be explained by inhibition of CYP activity, increases may result from inhibition of glucuronidation. Finally group 3 includes drugs that affect either TCOH or TCA levels. TCA concentrations were decreased by amoxicillin and sulphasalazine and to lesser extent by cimetidine and diclofenac. This may be explained by aldehyde oxidase inhibition (the enzyme catalyzing chloral hydrate oxidation into TCA). Whereas the decrease of TCOH concentration noticed with erythromycin may result from alcohol dehydrogenase inhibition (the enzyme catalyzing chloral hydrate reduction into TCOH). Our results confirm the existence of interactions between the TCE and a variety of widely used drugs. These interactions can result in the modulation of the formation of one or even two metabolites. Further studies in human material are needed to assess implications for human health.

Abstract / Résumé #8

A PILOT STUDY TO DEVELOP QUANTITATIVE STRUCTURE-PROPERTY RELATIONSHIP (QSPR) MODELS OF OCCUPATIONAL EXPOSURE LIMITS (OELS) FOR SOLVENTS

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In occupational hygiene, Overexposure Potential Indices (OPIs), e.g. the Vapour Hazard Ratio (VHR), represent the intrinsic propensity of a substance to cause an unacceptable exposure, based on the ratio between its volatility and its 8-hr occupational exposure limit (OEL). Due to the lack of legal or recommended OEL for a large number of contaminants, it is not possible to calculate an OPI in all cases. The animal-alternative methods in the literature require substance-specific data such as LC_{50}, LD_{50}, LOAEL, NOAEL, Kow (n-octanol:water partition coefficient) and Kaw (Henry's law constant), which are not available for all substances. In this regard, fragment-constant approaches may be useful. The objective of this study therefore was to investigate a new quantitative structure-property relationship (QSPR) model for estimating OELs. The frequency of occurrence of 13 fragments (CH_3, CH_2, CH, C, C=C, H, OH, -O-, C=O, AC, H-AC, Cl and N) was used to describe 90 solvents (aliphatic, aromatic and halogenated hydrocarbons, alcohols, glycols, ketones, esters, ethers and glycol ethers). OELs listed in the American Conference of Governmental Industrial Hygienists (ACGIH) TLV book, which take into consideration all the non-carcinogenic critical effects, were used. Multilinear regressions against ln-transformed OELs (ppm) were conducted using SPSS (version 17.0, Chicago, IL) to identify the contributions of individual fragments. Regression coefficients for each of the 13 fragments were as follows: 1.7 for CH_3, 0.36 for CH_2, -0.67 for CH, -1.1 for C, 0.56 for C=C, 1.7 for H, 0.95 for OH, -0.22 for -O-, - 0.36 for C=O, -3.5 for AC, 0.98 for H-AC, 0.98 for Cl and -2 for N. Only the contributions of CH_3, CH_2, CH, C, H, OH and Cl fragments were statistically significant (t value > 1.8). The predictive residual sum of squares (PRESS) over the sum of squares of the response values (SSY) of the QSPR model has a value of 0.13 and the R^2 was 0.86. The mean ± SD of the ratio of true vs predicted ln-transformed OEL values was 1.12 ± 0.52. For chemicals with OEL less than 50 ppm, the model predictions were more accurate than for those with much higher OELs. Work in progress focuses on improving the current performance of the model and its application for risk ranking purposes. Overall, the QSPR method should be useful to provide a relative ranking of OELs and might, upon further evaluation and validation, help occupational hygienists calculate OPIs for solvents for which no OEL exists (Supported by NSERC, IRSST, AFSSET).

DEVELOPMENT OF PYROSEQUENCING ASSAYS TO ASSESS CpG METHYLATION CHANGES IN THE PROMOTER REGION OF THE GENES FOR THE GLUCOCORTICOID RECEPTOR AND THE TUMOR SUPPRESSOR PROTEIN $p16^{INK4a}$.

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Disadvantageous early life environment can permanently alter gene expression patterns and predispose individuals to an increased risk of adult onset diseases. Changes in the CpG methylation status of genomic DNA could comprise a mode of action through which the altered phenotypes are maintained throughout life, and thereby could provide an indelible biological marker for early adverse events. Methylation at individually targeted CpG sites can be accurately and reproducibly quantified using bisulfite pyrosequencing. Therefore, to investigate links between exposure to environmental contaminants, adverse health effects, and DNA methylation, this presentation describes the development of three pyrosequencing assays targeting genomic sequences whose environmentally altered methylation status have been linked to reduced gene expression and disease states. The first assay, at the alternative exon 1_7 of the rat glucocorticoid receptor (GR) promoter, measures methylation at seven consecutive CpG sites within the neuronal growth factor 1A (NGF1A) binding domain. Maternal care in early life modifies the methylation level in these hippocampal CpG sites (Weaver et al., 2004). A second assay was designed to span twenty CpG's within exon 1_10 of the GR promoter, the most abundantly transcribed promoter in the liver, where CpG methylation is modified by the diet (Lillycrop et al., 2005). The third assay analyzes six CpG's within the *cdkn2a* coding region of $p16^{INK4a}$ previously found hypermethylated in various tumors including silica-induced lung tumors of rats (Badia et al., 2007). These assays were optimized for PCR conditions, and quantity of biotin labelled amplicon, using standard mixtures of unmethylated and highly methylated rat genomic control DNA. The PCR annealing temperature that created the least biased methylation standard curve was determined. The data for the measurements of methylation levels highly correlated with the dilution of methylated standards with ranges of r_2 value of 0.83 1 to 0.862 across 8 individual CpG's and 0.973 to 0.982 across 7 individual CpG's for exon1_7 and 1_10 of the GR respectively, and 0.994 to 0.997 across 6 individual CpG's for *cdkn2a*. In all cases the sensitivity of the assays were below 2.5%. These assays are being used to (1) screen rat cell lines of different tissue origins and disease states to discover sources of in vitro positive controls for pyrosequencing and DNA methylation arrays, and (2) to determine any specific CpG methylation differences in the tissues of rats treated with mixtures of environmental contaminants. (Funded by Health Canada, Chemical Management Plan).

CHARACTÉRISATION DE L'EFFET DE L'EXPOSITION MULTIVOIE SUR LA DOSE INTERNE DU 2,2,4-TRIMETHYLPENTANE CHEZ LE RAT

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Les composés organiques volatiles (COV) présents dans l'eau potable peuvent être absorbés non seulement par la voie orale, mais aussi par les voies d'inhalation et percutanée. L'objectif de ce projet était de caractériser l'effet de l'exposition multivoie au 2,2,4-trimethylpentane (ou *iso*octane; TMP), sur la dose interne chez le rat à partir d'un modèle expérimental animal. Des études préliminaires ont d'abord montré que l'absorption du TMP par la voie percutanée n'était pas significative. Conséquemment, des groupes de 5 rats Sprague-Dawley ont reçu une dose unique de TMP par voie orale (40 ou 163 mg/kg; ORA) ou ont été exposés à 300 ou 1200 ppm (2h) par inhalation (INH). Dans un deuxième temps, des groupes supplémentaires ont été exposés simultanément par les deux voies (orale et inhalation) aux faibles (ORA: 40 mg/kg et INH: 300 ppm) et fortes doses (ORA: 163 mg/kg et INH: 1200 ppm). À partir de prélèvements sanguins (25-100 µl) effectués, les cinétiques du TMP lors des expositions par voie unique et multivoie ont été comparées. Les niveaux sanguins (moyenne ± écarts-types) mesurés lors de l'exposition multivoie aux faibles doses étaient de 1,8 ± 0,2 mg/L et de 0,49 ± 0,04 mg/L à 2h et 4,5h post-administration. Ces niveaux étaient similaires (soit ~1,0 – 1,2 fois) à l'addition des niveaux sanguins obtenus lors de l'exposition au TMP par chacune des voies : 1,7 et 0,4 mg/L pour les mêmes temps. De la même manière, les résultats expérimentaux de l'exposition multivoie aux fortes doses (7,4 ± 1,0 mg/L et 2,0 ± 0,2 mg/L, à 2h et 4,5h post-administration) étaient comparables aux prédictions faites sur la base de l'additivité des résultats pour les voies ORA et INH (6,9 et 1,9 mg/L, à 2h et 4,5h). De plus, les surfaces sous la courbe (SSC) expérimentales (11 et 2,4 h•mg/L) étaient équivalentes aux prédictions basées sur les résultats des voies individuelles (11 et 2,4 h•mg/L), et ce, tant pour l'exposition multivoie aux faibles et aux fortes doses. Ces résultats suggèrent que, pour les doses évaluées dans cette étude, la dose interne résultant d'une exposition multivoie peut être prédite correctement en additionnant les doses internes mesurées après exposition par voie unique. Projet financé par ExxonMobil et CRSNG (CRD-335163).

Abstract / Résumé #11

TITANIUM DIOXIDE (TiO_2) NANOPARTICLES ACTIVATE HUMAN NEUTROPHILS IN VITRO AND INDUCE NEUTROPHIL INFLUX IN VIVO.

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Nanotoxicology is an emergent discipline due to the substantial developments in nanotechnology and the increased handling of nanoparticles (NPs). Titanium dioxide (TiO_2) NPs are extensively used as industrial nanomaterial. The role of these NPs in neutrophils, key players in inflammation, as well as their ability to induce neutrophil influx in vivo remain poorly studied. In this study, incubation of human neutrophils with TiO_2 (0-100 Pg/ml) did not cause apparent cell necrosis after and incubation period of 24h. However, TiO_2 induced important neutrophil cell shape changes in a concentration-dependent manner over time, indicating its potential to activate these cells. To further support this, we demonstrated that TiO_2 markedly and rapidly induced general tyrosine phosphorylation events in these cells, including the two key enzymes p38 mitogenactivated protein kinase (MAPK) and extracellular signal-regulated kinases-1/2 (Erk-1/2). We next determined the effect of TiO_2 on two neutrophil functions requiring longer period of time for measurement such as apoptosis and cytokine production. Interestingly, at concentrations ~20 Pg/ml TiO_2 inhibited neutrophil apoptosis in a concentration-dependent manner after 24h of treatment. Supernatants from TiO_2-induced neutrophils were harvested after 24h and tested for the presence of 36 different analytes (cytokines, chemokines) using an antibody array assay. We found that the production of 13 (36%) analytes was increased by TiO_2 among which the production of IL-8 was the most important with a fold increase ratio of ~1 6 vs control cells. This increased production of IL-8 was confirmed by ELISA. These results suggest that TiO_2 NPs possess important neutrophil agonistic properties in vitro opening the possibility that these NPs induced neutrophil influx in vivo. Using the murine air pouch model of neutrophillic inflammation, we present recent data indicating that TiO_2 NPs induce neutrophil influx in vivo. Therefore, our results clearly indicate that TiO_2 NPs can influence neutrophil cell physiology and possess pro-inflammatory effect in vivo.

Abstract / Résumé #12

Anti-proliferative and anti-steroidogenic effects of pomegranate compounds in human LNCaP prostate and H295R adrenocortical cancer cells

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Prostate cancer is the second leading cause of cancer related deaths among men in many Western countries. It is a hormone-dependent cancer and its proliferation is stimulated by endogenous steroid hormones. Aromatase (CYP19) and 5α-reductase (SRD5A) are the key enzymes that synthesize these hormones. Aromatase converts androgens to estrogens and 5αreductase converts testosterone to DHT. Natural compounds present in the pomegranate delay prostate cancer progression. These compounds may act as inhibitors of steroidogenesis. Pomegranate compounds (punicic-, quinic-, gallic, *trans* vaccenic-, and *cis*-vaccenic acid, pelargonidin-, cyanidin-, malvidin- and delphinidin chloride, epicatechin gallate, epicatechin, kaempferol, and epigallocatechin) were tested *in vitro* in a hormone-dependent prostate cancer (LNCaP cells) and steroidogenesis (human adrenocortical H295R cells) model. Cells (5000 cells/well) in 96-well culture plates were exposed to various concentrations of pomegranate compounds for 5 days with a fresh re-exposure after 48h. For cytotoxic effects, H295R were exposed once to pomegranate compounds for 24h. Cytotoxicity and antiproliferative effects were measured with WST- 1 reduction assay. Catalytic activity of CYP 19 was determined in H295R cells by tritiated water-release assay. Punicic acid had an antiproliferative effect in LNCaP cells reducing cell growth by 78, 89 and 89 % at 10, 30 and 100 µM respectively. Kaempferol reduced proliferation by 18, 30, 44 and 65 % at 3, 10, 30 and 100 µM respectively. Punicic acid was not cytotoxic in H295R cells, and at 30µM, decreased aromatase activity by 77 % compared to control. CYP 19 was not expressed in LNCaP cells. Preliminary results show that various natural compounds found in the pomegranate have an antiproliferative effects in LNCaP cells and punicic acid acts as an aromatase inhibitor in H295R cells.

DIETARY NICKEL INTAKE IN THE CANARY ISLANDS (SPAIN): A TOTAL DIET STUDY

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Introduction: Nickel (Ni) is a widely distributed environmental metal. Diet and drinking water are the main sources of nickel for human beings. Foods usually contain less than 0.2 mg/kg but there are certain exceptions like tea leaves, cocoa grains and nuts with high Ni levels. Even if dietary exposure to Ni is generally safe and very rarely causes toxic effects, there is more and more concern about allergic reactions (dermatitis) after dietary exposure to Ni. Moreover, the alteration of Ni metabolism may be responsible for pathological responses. Finally, this metal can modify the metabolism of certain essential metals like Fe (II), Mn (II), Ca (II), Zn (II) and Mn (II), affecting the human health.

Objectives: To determine the Ni content in the 22 different food groups most commonly consumed by the population of the Canary Islands and to estimate the total dietary intake of Nickel in the population of the Canary Islands.

Materials and Method: The Ni content of a total of 440 samples has been analysed by Atomic Absorption Spectrometry with graphite furnace (GF AAS) after dry incineration of the samples.

Results: The mean Ni concentration in foods and drinks was found to be 0.179 mg/kg. All food groups showed detectable Ni concentrations within a range of 0.002 mg/L (in drinking water) and 2.348 mg/kg (in the nuts group). Considering the food consumption of each one of these 22 food groups published in the last official nutritional survey in the Canary Islands (ENCA; 2000) for the different sexes and age groups, the highest Ni intake among women would be observed for those between 11 – 17 years old (0.08953 mg/day) and the lowest Ni intake would be detected in women between 65 and 75 years old (0.07632 mg/day). Ni dietary intake in men showed that 45-54 year old men showed the highest intake, at 0.10845 mg Ni/day, and as was the case for women, men between 65 and 75 years old showed the lowest intake (0.08675 mg/day).

Conclusions: Safe recommended levels of metal intake can only be established after determining and monitoring the metal contents in foods and drinks. Very few studies have been conducted about overall dietary intake of Ni, therefore this study offers a new perspective about dietary Ni from a nutritional, toxicological and food safety perspective, not only for the population of the Canary Islands but for may other populations as well.

Effects of Dietary Acrylamide on Systemic Oxidative Stress and Inflammatory Markers in Rats

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Objectives: The health risks of acrylamide from dietary sources have become a public concern since the discovery of acrylamide in potato chips and other foods that are cooked at high temperature. A recent study in human volunteers suggested that chronic intake of potato chips (157 mg acrylamide/1 60 g chips/day for 28 days) increased acrylamide-hemoglobin adducts, and may increase the risk of atherosclerosis by increasing production of reactive oxygen species and inducing a proinflammatory state. To explore the role of dietary acrylamide in cardiovascular disorders, we examined the effects of dietary acrylamide on systemic oxidative stress and inflammatory markers in rats under low and high fat diet conditions.
Methods: Male F344 rats were fed diets based on AIN-93G semisynthetic formula modified to contain (wt/wt) low (7%) or high (23.95%) fat corn oil, and 0, 5, 10, or 50 mg/kg diet acrylamide for 8 weeks. Rats were housed in metabolic cages 24h prior to necropsy for urine sample collection. Blood samples were collected from the abdominal aorta at necropsy. Urine samples were analyzed for 8-hydroxydeoxyguanosine (8OHdG) and isoprostane (IP), and serum samples for oxidized LDL (Ox-LDL), paraoxonase 1 (PON1) activity, c-reactive protein (CRP), homocysteine (HC), intercellular adhesion molecule-1 (ICAM-1), thromboxin 2 (TBX2), and Nε-(carboxymethyl)lysine (CML) using ELISA.
Results: Dietary acrylamide at 10 and 50 mg/kg significantly increased urinary 8OHdG in rats fed either the low or high fat diet. At the same doses, dietary acrylamide significantly decreased serum CRP and ICAM-1 in the rats fed the high, but not the low, fat diet. In the rats fed the low fat diet, acrylamide at all doses significantly increased serum PON1 activity and at 50 mg/kg decreased serum HC. Although urinary IP was higher in the rats fed the high than low fat diet, acrylamide did not affect this parameter. Nor did acrylamide affect serum Ox-LDL, TBX2, and CML.
Conclusions: At the higher doses used, dietary acrylamide increased oxidative DNA damage regardless of dietary fat level, suggesting a role for oxidative DNA damage, in addition to DNA adduction, as a mode of action for genotoxicity by this chemical. Under the conditions used, dietary acrylamide did not induce a proinflammatory state. However, in the presence of a high fat diet, it altered inflammatory pathways. In addition, the high fat diet hindered the compensation mechanism of an antioxidant enzyme in response to acrylamide exposure. . Oxidized lipid may contribute to the proinflammatory state in humans, induced by chronic consumption of potato chips containing acrylamide.

LOCALIZATION AND INDUCTION OF THE XENOBIOTIC EFFLUX TRANSPORTER, P - GLYCOPROTEIN (ABCB1), IN THE RAT EPIDIDYMIS

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The epididymal epithelium and maturing spermatozoa are susceptible to xenobiotics that diffuse out of the systemic circulation. The blood-epididymis barrier is necessary to attenuate the entry, and facilitate the elimination of harmful substances. In addition to providing a sophisticated immunological barrier, the epididymis also provides a specialized environment where sperm transit, are concentrated and acquire both motility and the ability to fertilize. The epididymal epithelium contains numerous specialized transporters that selectively regulate the compounds present in the epithelium and lumen. abcb1a and abcb1b are rodent orthologs of the human P - glycoprotein (ABCB1), a member of the ATP-binding cassette (ABC) efflux proteins capable of regulating the excretion of xenobiotics in normal tissues. The objective of this study was to characterize the previously unknown expression profile, localization and inducibility of abcb1a and abcb1b in the rat epididymis. abcb1a mRNA levels, as determined by real-time PCR, were significantly higher in the cauda epididymidis when compared to initial segment, caput and corpus epididymidis. abcb1b mRNA levels were similar throughout the epididymis. Immunohistochemistry using an antisera against both isoforms of abcb 1 revealed a gradient distribution of the protein along the epididymis. Minimal immunostaining was observed in the epithelial cells or spermatozoa in the caput epididymidis, and progressively increased in the corpus and cauda. Interestingly, the immunoreaction in spermatozoa was detected in the lumen of the distal caput, corpus and cauda. This gradient was further confirmed by western blot analysis of sperm protein extracted from the head and tail regions of the epididymis. To assess whether or not the system was inducible by xenobiotics, cells from an immortalized rat epididymal cell line (RCE) were exposed to different concentrations of nonylphenol (NP), an industrial surfactant, and doxorubicin, an anti-cancer agent and known inducer of abcb1. RCE cells exposed to 20 uM NP and 500 ng/ml DOX revealed a significant induction of both abcb1a and abcb1b mRNA and abcb1 total protein, suggesting that ABC efflux transporters are inducible in the epididymis. The unique expression profile and induction of abcb1a and abcb1b in the epididymis suggests an important role for these proteins in regulating barrier function and xenobiotic defence mechanisms in the male reproductive tract. Supported by Environment Canada, CIHR and the Armand-Frappier Foundation.

TOXIC POTENCY OF URBAN PARTICULATE MATTER COLLECTED ACROSS THE GREAT LAKES BASIN: IMPACT OF ATMOSPHERIC TRANSPORT

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This study is aimed at understanding the impact of atmospheric transport of ambient particles in modulating toxic potencies and at identifying specific determinants of toxicity. PM 2.5 samples were collected at six sites across the Great Lakes Basin chosen based on their location along the path of predominant north-easterly wind: Vermillion (Ohio), Ann Arbor, Sanillac (Michigan), Tiverton, Egbert, Dorset (Ontario). Particles were analyzed for elemental and polyaromatic hydrocarbon composition, and acidity. The cytotoxic potencies were assessed by using human epithelial (A549) cell lines. For each location and sampling period, backward wind trajectories were calculated in order to assess the contribution of sources along the wind path to particle composition and potency. Cytotoxicity analyses showed large variations in the toxic potency of particles collected at different sites, and within those collected on the same site on different days. Six out of the 9 particle samples collected on different dates at Egbert, Ontario ranked the most toxic among all particles (40) tested. In general, the direction of wind trajectories varied for different locations and days. On days when the air mass moved north-easterly across the Great Lakes Basin over the multiple sampling sites, no consistent increases in acidity or potency were noted. The absence of effects on potency attributable to air mass transport across the Great Lakes Basin suggests that the observed particle potencies are largely impacted by local atmospheric factors and emission sources. Regression of cytotoxic potencies on particle chemistry, and correlation of particle chemistry to wind trajectory directions and potential sources along the trajectory should enable quantitation of the contribution of local events and sources versus atmospheric transport to the modification of particle toxic potencies.

Abstract / Résumé #17

BIOLOGICAL EFFECTS OF INHALED DIESEL EXHAUST IN ANIMALS AFTER TREATMENT OF EMISSIONS WITH A DIESEL PARTICULATE FILTER

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This work was aimed at understanding the contribution of diesel particulate matter to the cardiovascular toxicity and inflammatory potential of total diesel exhaust. Rats were exposed by inhalation in a custom-built Mobile Inhalation Toxicology Exposure System (MITES) for 4 hours to dilute diesel exhaust from a heavy-duty diesel engine (Cummins, 2004 emission standard) operated either in the “engine out” (no after-treatment) or diesel particulate filter (DPF) after-treatment configuration in a steady-state mode at ~75% engine load. Animals exposed to HEPA filtered ambient air and naïve unexposed animals were maintained as control groups. Necropsies were conducted either at 4 or 20 hours after exposure. Tissues were recovered for analysis of specific biochemical endpoints relevant to inflammatory and cardiovascular status. Analysis of emission data showed that deployment of the DPF reduced particle mass concentrations by 85% with corresponding large reductions in the particle number concentrations. CO and total NOx levels were also reduced (80% and 25% respectively) but NO_2 was increased 4-fold by the operation of DPF. There was a significant (p0.01) increase in neutrophil infiltration in animals exposed to DPF treated exhaust compared to animals exposed to whole exhaust. Diesel exposure and emission treatment also impacted a number of haematological parameters. Significant neutrophilia observed in this study with DPF emission after-treatment may be linked to the higher levels of NO_2 generated. Additional analyses relevant to cardiovascular and other inflammatory markers are in progress in order to understand the continuum of biological responses and relate results to potential health risks.

Abstract / Résumé #18

STIR BAR SORPTIVE EXTRACTION (SBSE) COUPLED WITH GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) TO CHARACTERIZE THE DILUTED TOBACCO SMOKE GENERATED FROM THE BORGWALDT RM20S® WHOLE SMOKE EXPOSURE SYSTEM

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The Borgwaldt RM-20S® smoke delivery instrument, in combination with Transwell™ technology, is a relatively recent *in vitro* technique that allows for direct whole cigarette smoke exposure to cells at the air-liquid interface. To date, the smoke dose delivered to the cell exposure chamber by the RM-20S® system has not been characterized. Earlier studies from our laboratory indicated that the repeatability (% RSD) of the smoke dilution by the RM20S® whole smoke exposure system was 1.5% using a CO gas standard, and 8.4% using solanesol, a particulate phase marker. The average percentage error (accuracy) associated with the dilution was 6.3% (using the CO standard). The objective of the current study was to develop a method to analyze the chemical composition of the smoke's vapor phase that is directly in contact with cells during exposure. SBSE was applied to collect the volatile/vapor phase components in the exposure chamber. A thermal desorption system directly connected to a GC/MS allowed for the vapor phase components on the stir bar to be directly desorbed, re-focused and analyzed. SBSE coupled with GC/MS was successfully applied to a diluted tobacco smoke sample and a variety of volatile compounds were observed. This collection of techniques allows for the analysis of the volatile phase to which cells are exposed and could enable direct dosimetry applications in the future.

Abstract / Résumé #19

ANTIANDROGENIC AND ANTIPROLIFERATIVE EFFECTS OF 3,3'-DIINDOLYLMETHANE (DIM) AND RING-SUBSTITUTED ANALOGS (RING-DIMS) IN LNCAP HUMAN PROSTATE CANCER CELLS

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Cruciferous vegetables have been found to protect against prostate cancer. Indole-3-carbinol (1 3C) and its dimeric product 3,3 '-diindolylmethane (DIM) exhibit anti-tumor activities both in vitro and in vivo. Previous studies have shown that DIM inhibits androgen receptor (AR) nuclear translocation, leading to down-regulation of target genes. In this study, we observed structure-dependent differences for the effects of the synthetic 4,4'-, and 7,7'-dihaloDIMs on AR and prostate specific antigen (PSA) expression in LNCaP cells. We also report that DIM and ring-substituted analogs (ring-DIMs) 4,4'-dibromo-, 4,4'-dichloro-, 7,7'-dibromo- and 7,7'-dichloro-DIM, inhibit the proliferation of androgen-sensitive LNCaP prostate cancer cells at 10 and 30 µM. Western blot analysis showed that 4,4'- and 7,7'-dibromo-DIMs reduced AR protein levels, whereas 4,4'- and 7,7'-dichloro-DIMs had little effect. RT-PCR analysis clearly indicated that the 4,4'-dihaloDIMs and 7,7'-dihalo-DIMs (24 h exposure) inhibited the expression of AR at the mRNA level. The 4,4'-and 7,7'-dihalo-DIMs also significantly decreased PSA mRNA expression and cellular protein secretion levels at 10 and 30 µM. These anti-androgenic effects of the dihalo-ring-DIMs suggest they may be interesting as (or form the basis for) novel agents for the treatment of hormone-sensitive prostate cancer, either alone or in combination with other therapeutics. This work will be continued with the study of apoptotic effects of ring-DIMs in LNCaP as well as hormone-insensitive PC-3 human prostate cancer cells.

Abstract / Résumé #20

MECHANISTIC STUDIES TO VERIFY TOXICITY OF CARBON-BASED NANOMATERIALS IN MURINE MACROPHAGES

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Proteomic and metabolite analyses can be valuable in obtaining information on free radical-mediated modifications and protein profile changes caused by pollutant exposures. Here, we have compared the biological responses of J774. 1 murine macrophage cells induced by exposure to four types of carbon-based particles: pristine and oxidatively-modified carbon nanotubes (single-walled and multi-walled), carbon black particles, and diesel exhaust particles. Cells were exposed to the particles at doses of 0-100 $\mu g/cm^2$ in 96-well plates and in serum-free medium for 24h. Physicochemical characteristics of the carbon nanomaterials were compared (surface area, porosity). Cell culture supernatants were analyzed for oxidatively-modified protein metabolites by HPLC with coulometric array detection method. Shotgun proteomic analyses of cell lysates were also performed by direct MALDI-TOF-TOF-MS with saturated alphacyano-4-hydroxycinnamic acid served as the matrix. The mass spectral profiles of the cell lysate proteins for each exposure condition were interrogated in the region <6kDa using k-nearest neighbor clustering algorithm. Our results clearly indicated that J774. 1 cells exposed to the various particle preparations exhibited characteristic mass spectral signature profiles. In particular, the MS data has identified the dose-dependent elevation of the cellular levels of the peptide endothelin- 1, which is a known descriptor of inflammatory status of macrophages. The association of the physicochemical characteristics of these particles with induced biological changes provide new insights into mechanisms of particle toxicity.

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Abstract / Résumé #21

CIGARETTE SMOKE-INDUCED KININ B1 RECEPTOR EXPRESSION IN RAT LUNG SLICES IS MEDIATED BY IL-1ß

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Pulmonary inflammation is an important pathological feature of tobacco smoke related lung diseases such as chronic obstructive pulmonary disease (COPD). Bradykinin type 1 and type 2 receptors (B1R, B2R) have been reported to be involved in lung inflammation and also in other organs. Among these two bradykinin receptors, B1R is more associated with the chronic phase of inflammation and is normally absent in healthy tissues. In this study, a rat lung slice model was used to determine whether B1R played a role in the cigarette smoke-induced pulmonary inflammation which was monitored by the expression of B1R, IL-1ß and TNF-α genes. Rat lung slices treated with 5 µg/ml cigarette smoke condensate (CSC) for 24h increased B1R and IL-1ß gene expression by 5-fold and 12-fold, respectively in comparison to vehicle treatment (dimethyl sulfoxide). No significant increase of B2R and TNF-α gene expression was observed. Higher doses of CSC failed to induce B1R. Expression of B1R in rat lung slices was further confirmed by Western blot analysis. CSC treatment at 5µg/ml for 24 h resulted in a 2-fold increase of B1R protein expression which was totally blocked by a co-treatment with an IL-1ß receptor antagonist (IL-RA, 2 ng/ml). In summary, the results demonstrate the involvement of B1R in CSC-induced parenchymal inflammation through a mechanism which is mediated by the pro-inflammatory cytokine IL-1ß.

Abstract / Résumé #22

IN VITRO MAMMALIAN MUTAGENICITY AND AH RECEPTOR AGONISM OF COMPLEX PAH MIXTURES IN CONTAMINATED SOIL

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Traditional risk assessments of polycyclic aromatic hydrocarbon (PAH) - contaminated matrices assume that total risk is the incremental sum of the contributions from each monitored PAH. However, involvement of unidentified compounds and alterations in the metabolic capacity of the receptor will affect actual risk. The objective of this study was to compare the mutagenic activities and arylhydrocarbon receptor (AhR) activities of complex PAH-containing soil fractions, synthetic PAH mixtures and individual priority PAHs in an effort to determine if discrepancies in observed mutagenic activity can be explained by alterations in the metabolism of PAHs. First, organic material from two contaminated soils was extracted and analysed for PAH content. Two types of synthetic mixtures were prepared to mimic each soil extract: (i) a mixture containing all 16 priority PAHs measured in the soils, and (ii) a mixture containing only the 5 priority PAHs known to induce *lacZ* mutations in Muta™Mouse FE1 cells. We then measured induction of *lacZ* mutations by the PAH-containing soil fractions, both types of synthetic PAH mixtures, as well as individual priority PAHs, in FE1 cells using the PGal positive selection assay. Predicted mutagenic activities were calculated for the complex PAH-containing soil fractions by assuming dose additivity of the mutagenic PAHs. The actual mutagenic activity of the soil fractions was found to be far less than that of both types of synthetic mixtures, as well as predictions based on dose additivity of mutagenic PAHs. AhR agonism was assessed using the DR-CALUX assay in order to investigate the possibility that the differences in observed mutagenicity could be explained by alterations in enzyme induction. Twelve of the 16 priority PAHs elicited a significant AhR response. Both types of synthetic mixtures induced a similar AhR response, and the complex soil fractions elicited only a slightly higher response than the synthetic mixtures. These results correspond with relative mutagenicity and indicate that the reduction in the expected mutagenic response is likely due to competitive inhibition of AhR binding. The results stress the importance of examining the whole mixture (actual or surrogate) for reliable risk assessment, and suggest that the current risk assessment paradigm for complex mixtures containing indirect-acting mutagenic carcinogens is conservative.

Abstract / Résumé #23

GENOME WIDE ANALYSIS OF ARYL HYDROCARBON RECEPTOR BINDING TARGETS IN DIOXIN-TREATED MOUSE HEPATIC TISSUE.

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor which mediates the toxic effects of 2,3,7,8-tetrachlorodibenzo-ρ-dioxin (TCDD). Genome-wide, promoter-focused ChIP-chip analysis of hepatic AHR binding sites were examined in 8 week old female C57BL/6 mice that received a single i.p. injection of 30 μg/kg TCDD at 2 and 24 h post dose. Using a 1% false detection rate, 1642 and 508 AHR-bound regions were identified at 2 h and 24 h, respectively. 434 AHR-bound regions were in common between the two time points, corresponding to 403 unique genes. Conventional ChIP assays confirmed the ligand-dependent recruitment of AHR to many of the identified regions. However, significant ligand-independent binding to a select number of regions was also evident. Computational binding motif analysis revealed an over-representation of dioxin response element (DREs) in the isolated regions. For example, 49% and 61% of the AHR bound regions contained at least one DRE at 2 and 24 h, respectively. Moreover, 70% of the regions in common between 2 and 24 h contained at least one DRE. Comparison of ChIP-chip data to hepatic gene expression profiles obtained from 3 week old ovariectomized, immature C57BL/6 mice orally gavaged with 30 μg/kg TCDD for 2, 4, 8, 12, 18, 24, 72, 168 h using Agilent 4 x 44K whole genome microarrays. Of the 1787 unique differentially expressed genes (|fold change| > 1.5, p1(t) > 0.95) identified across all times points, 202 regions exhibited corresponding AhR enrichment within the 2 and 24 h ChIP-chip data sets. Although there were differences in models (age), hormone status and routes of administration between ChIP-chip and microarray studies, a strong correlation was observed between known TCDD responsive genes, computationally identified putative DREs, and evidence of AhR enrichment in their regulatory regions. Funded by CIHR MOP-82715 and SBRP P42E S049 11.

Abstract / Résumé #24

INVESTIGATING THE ROLE OF DNA DOUBLE~STRAND BREAKS AND DNA RECOMBINATION IN BENZENE~METABOLITE INDUCED HEMATOTOXICITY.

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Chronic benzene exposure has been associated with bone marrow toxicities including various forms of leukemia. It is suggested that in utero exposure to environmental carcinogens such as benzene may lead to the initiation of childhood cancers. Benzene and its metabolites have been shown to cross the placenta and lead to bone marrow aberrations in fetal tissues that may persist post~natally. Benzene metabolite induced DNA double strand breaks may undergo erroneous repair leading to chromosomal aberrations including chromosomal inversions and translocations commonly seen in childhood leukemias. This study investigated the abilityof the benzene metabolite benzoquinone (BQ) to induce DNA recombination and double strand breaks in an *in vitro* model of mutagenesis. The pKZ1 transgenic mouse model contains a DNA construct allowing for the detection of intrachromosomal recombination events. Immunocytochemisty probing for H2A.X phosphorylation was used to assess the abilityof BQ to induce DNA double strand breaks in hematopoietic cells. Primary cultures of gestational day 14 fetal livers were exposed to 5, 10, 25, or 50 PM BQ and stained for recombination. A significant increase in recombination events was observed following treatment with 25 and 50 PM BQ at all time points with the 10 PM treatment displaying a significant increase over control at 24 hrs. Hematopoietic cells treated with 25 and 50 PM BQ showed as increased number of fluorescent J~H2A.X foci at 8 and 24hrs but this increase was found to be non~significant likely due to the small sample size. These results indicate that BQ is able to induce intrachromosomal recombination in fetal hematopoietic cells possibly through the creation of DNA double stand breaks. Support: CIHR.

INHIBITION OF ARYL HYDROCARBON RECEPTOR-DEPENDENT TRANSCRIPTION BY RESVERATROL OR KAEMPFEROL IS INDEPENDENT OF ESTROGEN RECEPTOR ALPHA IN T-47D HUMAN BREAST CANCER CELLS

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Resveratrol (RES) and kaempferol (KAE) modulate a number of different pathways acting as aryl hydrocarbon receptor (AHR) antagonists, but also as estrogen receptor alpha (ERD) agonists. Since ERD is present in the transcriptionally active AHR complex at AHR target genes and that there are controversial reports regarding the ability of ERD to modulate AHR signalling, we examined the role of ERD in RES- or KAE-mediated inhibition of AHR-dependent transcription. We observed that 10 PM of KAE efficiently antagonized dioxin-induced cytochrome P450 1A1 (CYP1A1) and CYP1B1 gene expression which compared to RES. Time course studies demonstrate that co-treatment with either RES or KAE inhibited CYP1A1 and CYP1B1 expression as early as 1.5 h after treatment. Both compounds effectively inhibited dioxin-dependent increase in CYP1A1 and CYP1B1 expression even after an initial 2 h pre-treatment with dioxin followed by 6 h co-treatment with RES or KAE. Chromatin immunoprecipitation assay reveal that the reduced CYP1A1 and CYP1B1 mRNA expression was due to reduced recruitment of AHR, ARNT and co-activators to the enhancer and promoter regions of these genes. Both compounds also induced recruitment of ERD to the ERD target gene, GREB1, at a level similar to that of E2. The TCDD-induced ERD recruitment to CYP1A1 and CYP1B1 was also inhibited by both compounds and RNAi-mediated knockdown of ERD had no effect on the ability of RES or KAE to repress AHR-dependent transcription. These data show that the estrogenic action and ERD do not contribute to the AHR-inhibitory properties of RES and KAE.

Abstract / Résumé #26

EFFECT OF ENVIRONMENTAL CONTAMINANTS ON BETA CELL FUNCTION

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Type 2 diabetes (T2D) is a chronic metabolic disorder characterized by impaired insulin secretion from the pancreatic β cells and reduced insulin-stimulated glucose uptake in the peripheral tissues. The etiology of T2D is unclear, although, growing evidence suggests that increased exposure to environmental contaminants may contribute to the development and progression of this disease. Support for this hypothesis comes from epidemiological studies demonstrating that populations with high exposure to arsenic, persistent organochlorine pollutants (including dioxins, PCBs and organochlorine pesticides) and air pollution have an increased incidence of T2D. However, little experimental evidence exists to support a direct effect of these chemicals on β cell function. The first goal of this project was to test the hypothesis that the commonly encountered environmental pollutants: 1,1 -dichloro-2,2-bis (*p*-chlorophenyl)-ethylene (DDE), benzo[a]pyrene (BaP), perfluorinated alkyls (PFOA & PFOS), parabens (methyl and propylparaben) and bisphenol A (BPA) can impair β cell function. To examine the ability of the selected compounds to affect Ecell function, βTC-6 cells (a mouse Ecell line) were treated for 1 hour with DDE, BaP, PFOA, PFOS, methylparaben, propylparaben and BPA (0.1-1000 ng/ml) under low (3.3 mM) and high (16.7 mM) glucose conditions. Insulin release (a measure of E cell function) was determined by radioimmunoas say (RIA). There was no effect of BaP, methylparaben, propylparaben or PFOA on insulin secretion under either glucose condition. DDE (0.1ng/ml) resulted in a significant ($p=0.02$) reduction in insulin secretion under high glucose. PFOS significantly ($p=0.016$) reduced insulin secretion under both glucose conditions at 2 concentrations (1μg/ml and 1 ng/ml), however, this effect did not appear to be dose dependent. BPA significantly stimulated insulin secretion under both low and high glucose conditions. Because mitochondrial function is highly correlated with Ecell function and estrogens have been shown to affect mitochondrial activity, we examined whether BPA could stimulate insulin secretion via altered mitochondrial electron transport chain (ETC) activity. We treated βTC-6 cells with 100ng/ml BPA, a dose shown to stimulate insulin secretion, and evaluated citrate synthase (CS) and cytochrome *c* oxidase (COX) activity following 1, 6, 24 and 48 hours of treatment. There was no effect of BPA at any time examined on either CS or COX activity suggesting that the BPA-induced changes in insulin secretion occur independently of changes in mitochondrial ETC function. In summary, we have demonstrated that both DDE and PFOS can inhibit β cell function *in vitro,* however the mechanisms remain undetermined and the effect does not appear to be dose dependent. Conversely, BPA treatment results in significant stimulation of insulin secretion through a mechanism that does not appear to involve the mitochondria. The long-term consequences of the β cell overstimulation as a result of BPA exposure may ultimately result in β cell failure, however this remains to be determined.

A PRELIMINARY STUDY ON THE EFFICACY OF MEDICINAL PLANTS FROM LAWACHERRA RAIN FOREST USED AGAINST ALL FORMS OF CANCER Md. Ariful Haque Mollik

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Bangladesh is a land of forests. The health, wealth, and happiness of Bangladesh depend on her forests. Lawacherra Rain Forest is one of the important and well-reserved forests in Bangladesh. The Forest is home to a fantastic variety of plants, birds & animals and is considered to be of great ecological importance. Botanists will be happy to note that the incredibly rare Chloroform tree can be found here – the only one in Asia. There is also plenty of other vegetation in the area as the hilly terrain is just covered with a variety of plants. Two important ethnic groups can be found living within the boundaries of the forest – the Manipuri and Khasia. Their location on top of the hilltops is absolutely breathtaking and has often been called a 'piece of paradise'. The present study were conducted an ethnomedicinal survey amongst the Manipuri and Khasia ethnic healers of the area and noted that their formulations contain a number of medicinal plants not usually used by traditional healers in other regions of Bangladesh. Information was collected after obtaining informed consent with the help of a semi-structured questionnaire and guided field-walk method, where the informant pointed out the various medicinal plants and described their uses to treat all forms of cancer. Medicinal plant samples were collected and identified at the Bangladesh National Herbarium. Information on fifty two medicinal plant species are grown and consumed as summer, rainy, winter, and spring seasons because there are six seasons in Bangladesh, distributed in fifty two genera and thirty nine families. The medicinal plants used to treat all forms of cancer included *Abrus precatorius, Holarrhena antidysenterica, Plumbago rosea, Ocimum gratissimum, Aloe vera, Polygala venusta, Thunbergia grandiflora, Azadirachta indica, Stephania japonica, Madhuca latifolia, Lasia spinosa, Ipomoea mauritiana, Tabebuia argentea, Curculigo orchioides, Term inalia chebula, Achyranthes aspera, Withania somnifera, Rauvolfia serpentina, Barringtonia acutangula, Sansevieria cylindrica, Torenia polygonoides, Hygroryza aristata, Curcuma longa, Cannabis sativa, Hyptis suaveolens, Coccinia cordifolia, Borassus flabellifer, Tamarindus indica, Alisma gram ineum, Acrostichum aureum, Drynaria quercifolia, Euphorbia antiquorum, Randia dumetorum, Zingiber oficinale, Calotropis gigantea, Cajanus cajan, Aegle marmelos, Citrus acida, Alpinia galanga, Allium sativum, Carica papaya, Datura stramonium, Mimosa diplotricha, Lawsonia inermis, Cuscuta reflexa, Acorus calamus, Trigonella foenum-graceum, Wedelia chinensis, Ricinus communis, Piper betle, Kalanchoe pinnata,* and *Areca catechu.* A number of these medicinal plants are becoming highly endangered. It was further noted that leafs formed the major plant part used followed by roots and whole plant. It was clearly observed that medicinal plants from Lawacherra Rain Forest did have a positive healing effect upon many cancer cases. It was also shown to produce some encouraging effects in terms of several important factors like: delaying the tumor progression, pain reduction, enhancing the immune response & life expectancy, and over all well-being of the patients.

RAT HEPATIC CYTOCHROME P450 OXIDOREDUCTASE IS UP-REGULATED BY LOW DOSE DEXAMETHASONE AND DOWN-REGULATED BY ADRENALECTOMY

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Cytochrome P450 (CYP) enzymes are involved in both normal physiological functions, such as steroidogenesis, and xenobiotic metabolism. Electron donation by NADPH-cytochrome P450 oxidoreductase (POR) is required for all microsomal CYP-catalyzed reactions. The level of POR expression can affect the activity of CYP enzymes, although relatively few endogenous and exogenous compounds have been shown to regulate its expression. Hypophysectomized rats display decreased levels of hepatic POR and thyroid hormone was identified as the primary pituitary-dependent hormone responsible for hepatic POR maintenance [Ram & Waxman. J Biol Chem 267: 3294-301, 1992]. The hormonal regulation of hepatic drug metabolism, particularly by glucocorticoids, is an interest of our laboratory. Some CYP-catalyzed enzyme activities, such as aryl hydrocarbon hydroxylase, are decreased in the liver of adrenalectomized (ADX) rats relative to SHAM. In the context of a larger study examining the effects of ADX and exogenous glucocorticoids on the aryl hydrocarbon receptor and its CYP target genes, we also assessed POR expression because of its important contribution to xenobiotic metabolism. Adult, male Fischer 344 rats underwent bilateral ADX or SHAM-ADX, followed by treatment with dexamethasone (DEX), a synthetic glucocorticoid receptor (GR) agonist that is selective for the GR at low doses, or corn oil vehicle. DEX was administered by intraperitoneal injection once at 6 h before euthanasia, 4 days after ADX (acute), or once daily for seven consecutive days, starting 2 weeks after ADX (subacute). A DEX time-course study (3,6,12,27 h) was also performed with intact rats. Hepatic POR expression was analyzed at the mRNA level by real-time quantitative RT-PCR (qPCR) and at the protein level by immunoblot analysis. A reduction in hepatic POR protein was observed following ADX, both at 4 days and 3 weeks following surgery, with no corresponding change in POR mRNA levels. Subacute treatment using low doses of DEX (1 mg/kg) was sufficient to return hepatic POR protein in ADX rats to SHAM levels. While a single low dose of DEX (1.5 mg/kg) showed a similar trend to increase POR protein in ADX rats, this difference did not reach statistical significance. This stimulatory effect of DEX was also observed at the mRNA level; DEX induced hepatic POR mRNA in SHAM and ADX rats by 2 to 3-fold following the subacute protocol and by up to 7-fold with the acute protocol. While DEX is a selective GR agonist at low doses, higher doses (5 0-80 mg/kg) are known to activate the pregnane X receptor (PXR), a nuclear receptor that regulates several target genes involved in xenobiotic metabolism and transport. High dose DEX (80 mg/kg) is known to up-regulate POR expression [Simmons et al. J Biol Chem 262: 326-32, 1987]. To ascertain whether PXR was activated by low dose DEX in our acute and subacute protocols, we measured CYP3A23 mRNA, a PXR target gene, by qPCR. Hepatic CYP3A23 mRNA was not induced by DEX at any time point in the time-course study. There was a modest 30% induction of CYP3A23 mRNA by DEX following subacute dosing in SHAM but not ADX rats. This suggests that PXR is not activated by low dose DEX with acute dosing in intact rats or in ADX rats following subacute dosing; any PXR activation by low dose DEX in SHAM rats following subacute dosing appears marginal. Our findings implicate the GR, and possibly PXR, as putative regulators of POR expression. A specific role for the GR in POR regulation has not been reported. Bioinformatic analysis of glucocorticoid-response elements in the rat POR 5 ’-flank together with studies of GR recruitment to such elements in the absence and presence of a GR antagonist will be important in determining the mechanism by which low dose DEX acts to induce the expression of the obligate redox partner for all microsomal CYPs. [Support: CIHR, OGS, University of Toronto, and Peterborough K.M. Hunter Studentship]

GENERATION AND CHARACTERIZATION OF TIO2 NANO-AEROSOLS OF DIFFERENT AGGLOMERATION STATES.

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Introduction: The increasing use of nanostructured materials in the industrial workplace has raised concerns on human health risks. However, in the literature, little is known on the qualification and quantification of these risks. The non-harmonized research protocols in nanotoxicology and the controversies concerning the metric used to express the dose of nanoparticles (NP) (mass, number, surface area), are among the reasons why few information is currently available on health effects caused by NP exposure. Therefore, in nanotoxicology, there is a need to develop standardized methods to characterize and assess exposure to NP in the workplace and in animal inhalation experiments since the state of agglomeration is a key factor in the generation of nanopowders that may represent a critical parameter when assessing the toxicity of NP. The main idea supporting this research is that the agglomeration of titanium dioxide (TiO2) NP will decrease the adverse pulmonary effects. At equal mass concentration, aerosols composed of TiO2 NP of the same primary particle size but with two distinct agglomeration states, one with a number median aerodynamic diameter (NMAD) above 100 nm and one with an NMAD below 100 nm, will produce different pulmonary toxicity profile in rodent exposed by inhalation. The aim of this presentation is to describe two different methods of generation of NP used to produce different agglomeration states and to investigate the physicochemical characteristics of the nano-aerosols produced. This is the initial and critical step in the investigation of pulmonary toxicity induced by NP in an animal inhalation experiment allowing a correlation between the characteristics of the NP dose and the adverse biological effects. Methods: Generation of 5 nm primary particle size TiO2 nano-aerosols were conducted in a 500 L inhalation chamber. Nano-aerosols were generated by different techniques. A six-jet Collison (BGI Inc.) was used to atomize NP suspensions and a Palas RBG-1000 (Palas GmbH) was used to generate aerosols from dry NP powder. The real-time measurements of number concentrations in the inhalation chamber were made with an electrical low pressure impactor (ELPI) (Dekati Inc.). The stability of mass concentrations was monitored in real-time using a Dust Trak (TSI Inc.) and absolute mass concentrations were determined by weight measurements using cassettes. NP generated were collected on copper grids and pre-metalized polycarbonate substrates placed in cassettes. Grids and substrates were examined by transmission electronic microscopy (TEM). Results: Both generation techniques used in the study produced aerosols with different size profiles. The size profile in number obtained for the aerosol generated by the Collison had a NMAD of 30 nm and a total number of 1 187 491 /cm^3. The size profile of NP obtained for the aerosol generated by the Palas had a NMAD of 185 nm and the total number reached 161 898 /cm^3. These generation techniques produced aerosols at the same mass concentrations (2.02 and 1.96 mg/m^3 respectively). TEM characterization of NP collected from the aerosols showed agglomerates. Conclusion: Nano-aerosols presenting different distribution profiles in size and number can be generated from the same 5 nm TiO2 NP at an equal mass concentration. The control of generation parameters and the characterization of nano-aerosols allow the determination of NP physicochemical properties and are essential for conducting suitable **in vivo** nanotoxicological studies.

NONINVASIVE, HIGH-RESOLUTION ULTRASOUND IMAGING TO EVALUATE CARDIAC FUNCTION IN TERATOGEN-EXPOSED RAT FOETUSES.

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Congenital heart defects (CHDs) are the most common human birth defect (Hoffman and Kaplan, 2002), and have been associated with numerous genetic disorders and environmental factors. CHDs also occur in laboratory rats, and are commonly identified in regulatory pre-clinical developmental toxicity studies. Accordingly, there is a critical need to improve methods to identify and characterize the functional changes resulting from CHDs during pre-clinical safety testing. We assessed the utility of the Vevo660 high-resolution ultrasound biomicroscope (VisualSonics, Toronto, ON) for evaluating cardiac defects in Sprague-Dawley rat embryos/foetuses exposed to dimethadione, a known cardiac teratogen (Salomon et al., 1997; Fleeman et al., 2004). Doppler, m-mode and b-mode images were collected from foetuses ranging from gestational day (GD) 14 to post-natal day (PND) 1, both in utero and following externalization of the rat uterus. Foetal exposure to dimethadione was associated with quantitative changes in morphological and functional (haemodynamic) parameters indicative of improper heart development, and consistent with post-mortem histology. The ability to monitor cardiac anomalies longitudinally was limited by an inability to control in situ embryo/foetal orientation and to accurately identify a given embryo within a litter over multiple days. Although externalization of the uterus enabled greater control over image orientation, the difficulty in controlling physiological parameters, combined with its invasiveness, made longitudinal studies non-feasible for our purposes. Despite these limitations, imaging in all three modes provided quantitative measures of parameters that were previously only assessed qualitatively. These included ventricular septal defects, persistent truncus arteriosis (PTA), thinning of ventricular walls and ventricular hyperplasia. In addition, high frequency ultrasound permitted the monitoring of functional (haemodynamic) changes not typically measured in post-mortem developmental toxicity safety studies. We conclude that high frequency ultrasound is an excellent tool to assess cardiac structure and function following in utero exposure to teratogens. (Funded by Pfizer Global Research and Development)

Abstract / Résumé #31

CHARACTERIZATION OF HEPATIC GENE REGULATION FOLLOWING SHORT-TERM PERTURBATIONS IN THYROID HORMONE LEVELS IN JUVENILE MICE

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Thyroid hormones (THs) play a critical role in growth, development and metabolism primarily through transcriptional regulation of targeted genes. We are currently investigating the effects of hyper- and hypothyroidism on gene expression in juvenile mice liver to develop a stronger understanding of the mechanisms by which thyroid disrupting chemicals impair development. Hypothyroidism was induced from post natal day (PND) 13 to 15 by adding model thyroid toxicants methimazole and sodium perchlorate to drinking water of pregnant females. Hyperthyroidism was induced by intraperitoneal injections (i.p.) of THs at PND 15, 4 hours before decapitation and tissue collection. Gene expression was examined by hybridization of hepatic RNA to Agilent mouse microarrays for hyper-, hypo- and euthyroid animals. MAANOVA has identified over 400 genes that are differentially regulated with a false discovery rate-adjusted p-value less than 0.05 in at least one treatment condition. Regulation of well characterized TH-responsive genes was observed, including up-regulation of deiodinase-1 and spot-14 in hyperthyroid animals with concomitant down-regulation in hypothyroid animals. In addition, hundreds of novel candidate genes, potentially directly regulated by THs, are being validated using alternative assays. Primary affected pathways were involved in oxidative stress response, xenobiotic metabolism, glutathione metabolism and thyroid receptor/retinoid x receptor activation. These results provide insights into the thyroid hormone-regulated transcriptome of the juvenile mouse liver. This research is part of an ongoing project aimed at establishing a tissue specific model to assess hepatic effects of environmental chemicals.

Abstract / Résumé #32

VALPROIC ACID ALTERS THE EXPRESSION OF HDAC1 AND HIF1A IN MURINE LIMB BUDS.

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Valproic acid (VPA), a drug used widely for the treatment of epilepsy and bipolar disorders, increases the incidence of neural tube defects and limb malformations in the fetus. The molecular mechanism of VPA teratogenesis remains unresolved. VPA is a histone deacetylase (HDAC) class I and II inhibitor. HDAC1, a class I histone deacetylase, directly regulates the activity of hypoxia~inducible factor 1 alpha (HIF1a) through post~translational modifications, yet little is known about the transcriptional regulation of either HDAC1 or HIF1a. HDAC inhibitors induce various birth defects; furthermore, HIF1a, a key mediator of the cellular response to hypoxia, plays a pivotal role in embryonic development. The goal of this study was to test the hypothesis that exposure of limbs in culture to VPA concentrations that disrupt limb development will alter the expression of HDAC1 and HIF1a. Timed~pregnant CD1 mice were euthanized on gestation day 12 and the embryonic forelimbs were excised. The limbs were cultured in absence or presence of VPA (0.6, 1.8 or 3.6 mM) for 6 days, stained with toluidine blue, and scored according to their morphology, or cultured for different period of time for RNA and protein isolation and quantification by qRT~PCR and western blot.The transcripts and proteins were normalized to the amount of 18s and Actin respectively. VPA induced a concentration dependant decrease in limb score. Although the ulna and radius were minimally affected, the digits and metacarpals showed a marked decrease in differentiation and growth; oligodactyly was observed. VPA exposure increased Hdac1mRNA transcripts significantly at 1h in the high dose group, at 3h in all groups ($p<0.05$), returning to control at 6h. In contrast, VPA exposure downregulated Hif1a gene expression; decreased expression was observed at 3h after exposure to 1.8 or 3.6 mM VPA and at 6 h for all concentrations ($p<0.05$). HIF1a protein level were also downregulated after 12h exposure. However, transcription of the vascular endothelial growth factor (VEGF), a downstream target of HIF1a was unaffected by VPA exposure. Thus, exposure of limbs to teratogenic doses of VPA disrupted regulation of both Hdac1and Hif1a; the effect on Hdac1occurred prior to that on HIF1a. Disturbances in the regulation of Hdac1and Hif1a expression may contribute to the teratogenicity of VPA. Further studies are needed to examine the upstream regulation of these genes as well as the downstream effectors of HIF1a which could lead to limb malformations. These studies were supported by CIHR and FRSQ.

Abstract / Résumé #33

EFFECT IVE PERFORMANCE OF THE C3H/10T1/2 CELL TRANSFORMATION ASSAY IN A DEFINED, SERUM-FREE MEDIUM

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Cell transformation assays (CTAs), based upon primary Syrian hamster cells or established Balb/c and C3H/10T1/2 cell lines, have been employed as tools for discovery and characterization of carcinogens of chemical, physical and biological origin. These in vitro assay systems are sensitive to both genotoxic and non-genotoxic modes-of-action and therefore highly predictive of rodent cancer bioassay outcomes. Efforts toward validation of standardized methodologies for conducting CTAs have been and are being undertaken; however, variability of the serum component of tissue culture media remains an impediment hampering reproducibility in interlaboratory validation exercises. We sought to circumvent this source of variability by investigating the behaviour of the C3H/10T1/2 CTA, using chemical carcinogens in culture medium with defined components as replacements for serum. RESULTS: An optimized set of protein growth and attachment factors enabled growth at clonal density, permitted rapid growth to confluence, and sequential passages of stock cultures if the trypsin concentration used to release cells were minimized. In the defined medium, the well-organized confluent monolayer remained intact for the period of time required for transformed foci to form and overgrow normal monolayer cells. Transformed foci were easily recognized and scored in as little as three weeks, compared to 6 to 7 weeks in standard, serum-containing culture medium. Moreover, the number of foci produced by low concentrations of 3-methylcholanthrene (MCA, 56-49-5) was increased. In addition to MCA, a second polycyclic aromatic agent forming bulky adducts

(7,1 2-dimethylbenzanthrance, DMBA, 60-11-7) and an alkylating agent (**N**-Nitroso-**N**-methylnitroguanidine, MNNG, 70-25-7), were also effective transforming agents in the defined medium. Tests with other categories of carcinogens and noncarcinogens are in progress.

CONCLUSIONS: The results demonstrated that a defined medium effectively supported the performance of the C3H/10T1/2 CTA, providing increased sensitivity while reducing the required time by half. In addition, the replacement of serum in the culture medium eliminated need for this animal-derived product, indirectly reducing the use of animals in chemical testing. It is expected that use of a fully defined culture medium will enable increased CTA reproducibility among laboratories and thereby contribute to eventual regulatory acceptance.

In vitro-in vivo extrapolation of the dose-response relationship for cellular perturbations of a binary mixture of toluene and **n**-hexane

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There is an increasing interest in the development and application of in vitro toxicity testing using human cell culture. Nevertheless there is a lack of tools to interpret such in vitro data for risk assessment purposes. The objective of this study was to predict the in vivo dose-response relationship in humans based on in vitro concentration-response data for chemical mixtures. An in vitro dosimetry model was developed to calculate the concentration of toluene and n-hexane in the cultured Jurkat-T cells (McDermott et al. 2008. Toxicol. Sci. 101:263-274). The in vitro model consisted into two compartments, namely the culture medium (CM) and the lymphocyte. The in vivo human PBPK physiologically based pharmacokinetic (PBPK) model has been developed previously for toluene and n-hexane, based on uncompetitive metabolic interaction (Ali et Tardif 1999 J. Occup. Health 41, 95-103). In order to determine the concentration of toluene and n - hexane in the T-cells, lymphocyte:blood and lymphocyte:CM partition coefficients (PCs) were estimated and incorporated into both in vitro and in vivo models. The later PCs were calculated for toluene and n-hexane on the basis of matrix (i.e. CM, plasma, erythrocyte and lymphocytes):water PCs. For both chemicals, the CM:water PC was calculated by dividing the experimental CM:air PC (McDermott et al. 2008 Toxicol. Sci. 101:263-274) by the experimental value of water:air PC (Gargas et al. 1989 Toxicol. Appl. Pharmacol. 98, 87-99). The other matrix:water PCs were estimated using an algorithm based on the composition of the matrix in water, neutral lipids, phospholipids and proteins along with the solubility and extent of uptake of toluene and n-hexane in the components of the matrix. The in vitro concentration of both chemicals in lymphocytes was determined by multiplying the published CM concentration by the lymphocyte:CM PC. The in vivo concentration of toluene and n-hexane in lymphocytes was calculated as the product of the venous blood concentration by the lymphocyte:blood PC. The human exposure concentrations of toluene and n-hexane that yield the same lymphocyte concentrations as in the in vitro Jurkat-T cell toxicity study were determined. The results indicated that, for toluene alone, in vitro exposure concentrations of 3.77, 5.09 and 10.2 µM would correspond to in vivo inhaled concentrations of 105, 125 and 193 ppm, respectively. Similarly for n-hexane, the in vitro exposure to 1.22, 2.04 and 4.08 µM would compare with continuous human inhalation exposure to 60, 100 and 195 ppm. In the case of mixed exposures, for LDH leakage and intracellular Ca^{++} perturbation, the lowest concentrations at which supra-additive effects were observed in vitro (1.22 µM n-hexane and 3.77 µM toluene) would correspond to in vivo co-exposures of humans to 58 ppm n-hexane and 87 ppm toluene, as determined with the interaction-based PBPK model. Overall, this study demonstrates the use of human PBPK models in the conduct of in vitro-in vivo extrapolation of the dose-response relationship for mixtures obtained in high throughput assays. (Supported by AFSSET).

OBSERVATIONS OF THE EFFECTS OF NANOPARTICLES ON REPRODUCTION AND DEVELOPMENT IN DROSOPHILA MELANOGASTER AND CD~1MICE.

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The excitement surrounding the multiple uses of nanoparticles continues to increase, while information about their potential toxicity lags behind. Because of the small size of nanoparticles (>100nm), their chemical properties can change allowing them to cross cellular membranes and to potentially interfere with cellular processes. Silver (Ag) and titanium dioxide (TiO_2) nanoparticles are becoming widely used in popular commercial products such as foods and packaging, cosmetics and medical devices. To investigate any effect on reproduction and development, these two nanoparticle types were assessed using bothDrosophila melanogaster and mice as models. Male and female Drosophila were housed together and exposed to varying concentrations of either type of nanoparticle or a vehicle control in their food (0.005% w/v to 0.5% w/v). The exposure period was 14 days and during this time, males and females were allowed to reproduce while female fecunditywas recorded daily. Information taken included both oviposition and overall fertility. Both Ag and TiO_2 nanoparticles significantly reduced female fecundity, particularly at 0.1% and 0.5% concentrations. In mice, pregnant CD~1dams were orally dosed with either nanoparticle (10, 100or 1000 mg/kg) or a vehicle control on gestational day (GD) 9. Fetuses were removed from dams on GD19, and were examined for both incidence of resportions and the incidence of morphological defects. Defects were observed in mouse fetuses particularly with TiO_2 nanoparticles, though to a lesser extent than in the invertebrate studies. Together, these studies shed light on the potential toxicological implications of nanoparticles and future studies will investigate the mechanisms of this toxicity.

Abstract / Résumé #36

CIGARETTE SMOKE CONDENSATE UP REGULATES MMP1 GENE EXPRESSION IN HUMAN LUNG FIBROBLASTS IN THE PRESENCE OF TGF-ß1

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Deficiency in repairing the degraded extra cellular matrix following chronic cigarette smoke exposure has been hypothesized to be the cause of human emphysema and of alveolar destructions observed in animal models. MMP1, one of the emphysema-related and cigarette smoke inducible proteases, mediates extra cellular matrix destruction which consequently leads to lung inflammatory as well as immunological responses. TGF-ß1 is a key cytokine promoting alveolar repair; it inhibits MMP1 and induces **α**-SMA gene expression through SMAD 3 pathway. The objective of our study was to examine whether MMP1 was still induced by cigarette smoke condensate (CSC) in the presence of its inhibitor TGF-ß1. Confluent human lung fibroblasts were treated with TGF-ß1 alone or co-treated with CSC plus TGF-ß1 for 3 days; MMP1 and **α**-SMA gene expression were assessed by real-time PCR. This in vitro model was validated by showing that TGF-ß1 inhibits MMP1 and induces **α**-SMA gene expression. Co-treatments with TGF-ß1 and CSC up-regulated MMP1 gene expression in a dose dependent manner without affecting the expression of **α**-SMA and other SMAD downstream genes. These results suggest that CSC up regulates MMP1 gene expression through a pathway that does not interact with SMAD 3. Pulmonary parenchymal cells adapted to cigarette smoke environment might result in altered cellular physiology and behave differently against xenobiotics challenges. We therefore examined whether CSC pre-treatment could alter the cellular responses to TGF-ß1 stimulation. CSC pretreatment for 7 days did not alter the **α**-SMA inducing capacity of TGF-ß1 but upregulated MMP1 gene expression. Again, qPCR array analysis showed that expression of SMAD 3 and its downstream genes were not changed. In summary, current data show that either CSC co-treatment or pretreatment with TGF-ß1 is able to induce MMP1 expression in the human lung fibroblasts. This finding indicates a possible counter action of cigarette smoke against parenchymal repair following extra cellular matrix damage.

Abstract / Résumé #37

INVESTIGATION OF INDUCED AND PERSISTENT GENETIC INSTABILITY IN MICE EXPOSED TO PARTICULATE AIR POLLUTANTS *IN UTERO*

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Particulate Air Pollutants (PAPs) are widespread. Previous work has shown that PAPs from industrial environments cause germline mutations in mature male mice. It is unclear how PAP exposure will impact the developing germline during critical periods of gametogenesis. This work investigates transgenerational effects arising following exposure of males to DEP **in utero** during critical stages of gametogenesis. In mice, expanded simple tandem repeat (ESTR) loci exhibit the highest rates of mutation measured to date, and provide valuable tools for studying inherited mutation and genomic instability. Mutations are measured directly in germ cells, but also persist in unexposed descendents of exposed males, a phenomenon known as transgenerational genetic instability. The objective of this study was to quantify rates of induced ESTR mutation in sperm of the descendants of male mice exposed to DEP **in utero.** C57Bl mice were exposed **in utero** to 20mg/m^3 NIST 2975 (a diesel exhaust particle [DEP]) for 1 hour daily from gestational day 7 until birth, alongside sham controls. Male offspring were collected and mated with unexposed mates. F2 males were sacrificed at maturity and DNA extracted from sperm. ESTR mutation frequencies for 6 exposed and control males were determined using single molecule PCR (SM-PCR) as described previously (1). No increase in mutation frequency was found in the descendents of exposed male mice (p=0.5521). The findings suggest that exposure in utero to 20mg/m3 NIST 2975 (DEP) may not cause transgenerational mutation mediated via the male germline in the F2 generation. However, a larger sample size, exposure through the female lineage, and different doses should all be examined.

1- Yauk CL, Dubrova YE, Grant GR, Jeffreys AJ. A novel single molecule analysis of spontaneous and radiation-induced mutation at a mouse tandem repeat locus. Mutat Res - Fundam Mol Mech Mutag 2002 MAR 20;500(1-2):147-56.

EFFECTS OF PESTICIDES ON AROMATASE EXPRESSION IN BIOLUMINESCENT MICE AND GSK-3BETA/BETA-CATENIN SIGNALLING IN LNCAP HUMAN PROSTATE CANCER CELLS

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Certain pesticides are known to disrupt the endocrine system in humans and wildlife and are suspected of causing endocrine-related diseases, such as reduced fertility, impaired fetal/child development and hormone-dependent cancers of the breast, ovary, testis and prostate. Several hormone-dependent cancers are associated with dysfunction of aromatase (CYP19), the enzyme responsible for converting androgens to estrogens; its inhibition can lead to infertility and osteoporosis in women, and decreased sperm production in men. Overexpression of aromatase is associated with pro-proliferative effects, most notably in breast cancer. It is also suggested that hormone-independent mechanisms, such as the GSK-3beta/beta-catenin pathway are involved in the development/progression of certain cancers, such prostate cancer. In vitro studies suggest that atrazine induces aromatase, but little evidence exists in vivo. We studied the effects of atrazine in a bioluminescent Cyp19-luciferase transgenic mouse model (Caliper LifeSciences; line 125), which expresses luciferase under control of the gonadal pII Cyp19-promoter; the mice can be scanned in real-time without need for sacrifice. In males, forskolin (10 mg/kg, ip, single injection), a potent inducer of pII-mediated aromatase in vitro, increased bioluminescence 3-5 days after exposure, but only in a few individual mice. Atrazine (100 mg/kg, once, or 30 mg/kg, daily for 5 days) did not increase bioluminescence for up to 7 days after initial exposure. Ex vivo tissue analysis (testis, epididymis) found a statistically significant increase in bioluminescence in forskolin-, but not atrazinetreated mice. In our hormone-dependent LNCaP prostate cancer model, >1 **μ**M of vinclozolin inhibited nuclear androgen receptor and beta-catenin accumulation in the presence of 10 nM DHT. Together, these finding suggest atrazine may not be an effective gonadal aromatase inducer in vivo and that vinclozolin may act as antiandrogen via AR-dependent and -independent mechanisms.

Abstract / Résumé #39

SENSITIVITY OF AN EX VIVO MODEL TO TOBACCO SMOKE

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The chemistry of cigarette smoke (CS) is complex and due to the absence of appropriate experimental model, its relationship to chronic obstructive pulmonary disease (COPD) is difficult to study. In vitro studies using cell lines are relatively easy to perform but interpretation of their results cannot be translated to what occurs in the lung. Although more representative, in vivo studies are expensive to perform. We have proposed to use cultured rat lung slices and the Borgwaldt RM20s Smoke Exposure System™ to study the effect of CS on the lung (Lin et al. 2008, STC poster). In the present study, we report on the sensitivity of our ex vivo model in CS toxicity as well as gene expression assessment. Rat lung slices were maintained and exposed to whole smoke (WS) or vapor phase (VP) in the liquid-air interface. Following a 3-day exposure regimen (30 minutes/day, 70ml/puff, at the doses of 2, 5 and 10% WS and sham air control), lung slices were harvested at 24 hours following last exposure for toxicity (MTT assay) or gene expression (real time-PCR array) assessment. We observed a dose response where 2% WS did not exhibit significant toxic effect to the lung slices. Exposure to 5 and 10% WS showed statistical significance ($p < 0.0001$) in comparison to sham air control and to each other dose ($p < 0.0001$). No difference in toxicity was observed between the WS and VP exposure. However, cyp1a1 was induced 103- and 10-fold following exposure to 2% WS and 2% VP, respectively. To test further the sensitivity of the ex vivo model, we exposed lung slices to 5% WS of 1, 8 and 14 mg tar level cigarettes and obtained 105, 64 and 18% relative survival, respectively. Such differential toxicity was statistically significant ($p=0.027$). In conclusion, the ex vivo model was sensitive enough to study the effect of different types and doses of smoke on either the survival or expression of cyp1a1 of rat lung slices. This tool could help in understanding the toxicity of CS and its link to the chemistry of tobacco smoke.

FLUORIDE IN DRINKING WATER: RISK CHARACTERIZATION

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Fluoride (F) has been described as a conditionally essential dietary element involved in the maintenance of good oral health, due to its capacity to prevent the formation of cavities. Nevertheless, the ingestion of high levels of this element above the Recommended Dietary Intake (RDI) for adults (1.5-4 mg/day) is a hazard that may result in dental fluorosis and skeletal fluorosis (both usually endemic), thyroid function alterations, stunted growth, and other serious health problems. Food safety has always been an important issue, and currently it is high on the political agenda of many countries. Risk assessment is a specialized field of applied science that involves reviewing scientific data and studies in order to evaluate risks associated with certain hazards. As drinking water is considered the main dietary source of F, and therefore a potential hazard, the World Health Organization (WHO) suggests a maximum fluoride guideline of 1.5 mg/L. This maximum level of F is also codified in the law in many countries, including Spain (RD 140/2003).

The high prevalence of dental fluorosis is well known since 1960 and its etiology has been linked to high levels of fluoride, above the legislated limit, in the natural drinking water sources of the volcanic island of Tenerife. Samples from springs in the northern part of the island have shown F levels as high as 6,4 mg/L. Although the Government has forbidden, in certain municipalities, the consumption of contaminated waters in children under 8 years and has attempted to manage this health risk by building reversible electrodyalisis treatment plants to reduce F levels, public drinking water still contains more F than recommended in certain areas.

Objectives

- Determine the F concentration in the public drinking water of each of the 18 municipalities in the island of Tenerife.
- Estimate the daily intake of F from drinking water for the population of Tenerife and the contribution of these intakes to the recommended dietary intake of this element.
- Characterize the toxicological risk to human health based on the estimated intake levels of contaminated water for the different municipalities.

Materials and Method

A potentiometric method based on a fluoride selective electrode has been used for the quantitative analysis of fluoride in water samples.

Results

A total of 236 drinking water samples from the public supplies of 18 municipalities of Tenerife were analyzed. Among the 30 points sampled, 20 presented F levels over the legislated limit of 1.5 mg F/L. All these 20 areas were near the “Barranco de Vergara” sprin, water source that originates in the Teide volcano. The high levels of F in waters have been observed for municipalities that did not suffer this natural contamination problem before. The problem of high levels of fluorides and the potential risk of dental fluorosis has spread from being a local problem in the municipal of La Guancha and its neighbours to an almost island wide, which effects the greater part of the north coast, and has not left the southern shores

unaffected. Three northern municipalities (Adeje, El Tanque and Icod de Los Vinos) showed the highest F levels in drinking water (2.59– 4.89; 0.35–4.38 and 2.22–3.94 mg F/L, respectively). When a 2 L water/day consumption is assumed the estimated daily intake from water in these populations reaches levels of 9.78; 8.76 and 7.88 mg F/day, values far above the Daily Recommended Dietary Intake.

Conclusions

The high fluoride levels detected in the drinking water of Tenerife continues to be a public health problem that affects the population, and the policies established to manage the problem have been unsatisfactory. The public should be informed about health risks of a high daily intake of fluoride, and the government should undertake a risk assessment study about fluoride levels in drinking water, not only in Tenerife, but also in other volcanic areas.

Abstract / Résumé #41

DEVELOPMENT AND VALIDATION OF A LIQUID HANDLING PROGRAM FOR HIGH THROUGHPUT INTEGRATED CYTOTOXICITY BIOASSAYS

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Screening the toxicity of a large collection of environmental materials, often only available in limited quantities, requires analytical platforms capable of performing several assays from the same source material in a high throughput manner. In the present work, we have developed and validated an automated liquid handling protocol to perform an **in vitro** integrated cytotoxicity assay. The integrated cytotoxicity assay allows the simultaneous assessment of cell viability (Alamar Blue), cell proliferation (BrdU ELISA), membrane damage (LDH), and energy metabolism (ATP). As an initial step, a program was written using the liquid handling station software that replicated the manual assay protocol. Optimization of a number of parameters, including aspiration and dispensing speed and height, resulted in a functional program. In order to validate the program for use in screening of environmental materials, parallel experiments were conducted using the traditional manual approach and the new automated procedure. J774A.1 murine macrophage cells were exposed for 24 hours to a panel of particulate materials, comprising standard reference air pollution particles (SRM-1649, SRM-1649A, SRM-1650, SRM-2975), Ottawa urban particles (EHC-93, EHC-96, EHC-98, EHC-2000, EHC-6802), and mineral dusts (TiO2, SiO2) at four concentrations (0, 30, 100, 300 $\mu g/cm^2$). Performance of the integrated assay using the liquid handling approach yielded results comparable to the manual approach. Mean values for both approaches were highly correlated. Our results indicate that the integrated cytotoxicity platform protocol can be automated, enabling increased throughput for assessment of environmental materials and chemicals.

Abstract / Résumé #42

DOES 2,2',4,4'-TETRABROMODIPHENYL ETHER INTERACT DIRECTLY WITH THYROID HORMONE RECEPTORS?

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Background: Polybrominated diphenyl ethers (PBDE) are a group of bioaccumulative and persistent environmental contaminants increasing in biota. Thyroid and neuro-developmental disruption remain the most susceptible known endpoints of toxicity. In the vertebrate brain, thyroid hormones (THs) play key roles in regulating neural development and functioning. Thyroid signalling in brain is mediated through thyroid hormone receptors (TRs) and TRβ1 is expressed in brain and predominant in liver. PBDEs are structurally similar to THs, and it has been hypothesized that PBDEs might interfere with THs signaling at different levels. No data is available on interaction of the most prevalent PBDE congener found in maternal and cord blood, 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), with TRs. In this study we used a variety of approaches to examine BDE-47 interaction with TRβ. Methods: In vitro thyroid hormone binding assay was performed using human TRβ1, synthesized in rabbit reticulocyte lysate, [^{125}I]T3, and different concentrations of T3 or BDE-47. Luciferase assay was performed using QB1-HEK293 cell line transfected with pSG5 plasmid with human TRβ1 and TRETK construct containing two copies of an idealized positive thyroid response element (TRE) arranged as a palindrome, upstream of a minimal thymidine kinase promoter and fused to the luciferase gene in the PSVO vector. Analysis of expression profile of thyroid responsive genes was performed using total RNA extracted from liver and brain of offspring of Wistar rats which were exposed to 0.2 mg/kg body weight of BDE-47 from GD 15 to PND 20 every 5 days. cRNA was hybridized to the whole-genome RNA expression BeadChips RatRef-12 (Illumina) containing 22,523 50-mer probes. The chips were scanned and raw data were analyzed using FlexArray 1.2 software. Differentially expressed genes in each dataset were determined under thresholds of FDR corrected $p \sim 0.05$ and expression fold change ~ 1.5. The lists of thyroid-responsive genes were determined on the base of literature data for brain tissues and liver. The enrichment of these lists with differentially expressed genes for each studied tissue was done by Fisher exact test. Results: In binding assays, % bound-[^{125}I}T3 was unaffected by BDE-47 even at 4000 fold higher concentrations (10 μM), showing lack of ability to compete for TRβ1 even at high concentrations . In luciferase assay activation by BDE-47 does not exceed basal activity and BDE-47 does not interfere with T3-signaling. The list of thyroid responsive genes in brain was not enriched significantly while the list of thyroid responsive genes in liver was overrepresented significantly. Among both genes known from literature to be positively or negatively regulated by thyroid hormone, we found over- and down-expressed genes. Discussion: The main finding of our study consists in the lack of interaction between BDE-47 with TRβ demonstrated in in vitro binding assay and study of transcription regulation of thyroid responsive elements. Developmental exposure of rats to BDE-47 leads to differential expression of thyroid responsive genes in liver and brain due to unknown mechanism.

SIZE- AND SITE-SPECIFIC DIFFERENCES IN THE POTENCY OF AIRBORNE PARTICULATE MATTER COLLECTED IN WINDSOR, ONTARIO

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Canadians are exposed to a range of concentrations of ambient particulate matter contributed by local industrial point sources, commercial and residential area sources, transportation sources, and long-range transport. Health effects of exposure likely relate to the chemical constituents and physical properties of the particles, which differ across locations and over time. Characterisation of the potency of airborne particles at specific locations is important to identify sources contributing to their toxicity. To address the need for source-specific toxicity data, we evaluated the cytotoxic potencies of airborne particles collected at sites with contrasting emission sources within Windsor predominantly influenced by the Detroit urban area, steel mills, or local transportation, including diesel heavy-duty engines. Simultaneous PM0-1, PM1-2.5, and PM2.5-10 size fractions were collected at selected sites over several weeks and recovered from filters by aqueous extraction and sonication followed by vacuum-evaporation. The cytotoxic potency of particulate matter samples was determined in human lung epithelial cells (A549) using bioassays for energy metabolism, cell proliferation, and membrane integrity. Potency (β) was determined from Fold-effect = $(\text{Dose}+1)^{\beta}$. Expression of genes representing inflammation, oxidative stress, heat shock, and xenobiotic metabolism pathways was measured by RT-PCR for a subset of samples with contrasting potencies. Particle potency was impacted by location, day of sampling, and size range. The average cytotoxicity ranking was PM1-2.5 > PM2.5-10 > PM0-1. Quantification of relative transcript levels of key genes revealed site- and size-specific differences in potency, with striking differences in the activation of inflammatory genes (TNF-α, IL-6, IL-8; $p<0.05$). Expressing risk of toxicity as the product of ambient concentration and particle potency revealed that days with relatively low levels of airborne particulate matter but with high potency still exhibited relatively high toxicity. The data show that the relative potency of particles collected within a small geographical area such as Windsor display a range of cytotoxic potencies, reinforcing the need for investigation of source-specific toxicity.

Abstract / Résumé #44

ADDITIVE AND ANTAGONISTIC EFFECTS OF CO-EXPOSURE TO AIR POLLUTANTS ON BIOLOGICAL PATHWAYS IN THE LUNGS

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Air pollution is a complex mixture of gaseous and particulate constituents associated with a range of health effects, including respiratory and cardiovascular morbidity and mortality. Although actual exposures always involve mixtures, pollutants are generally studied and regulated as individual agents. As a result, we lack insight into the significance of multi-pollutant interactions on health. In the present study we monitored activation of biological pathways in the lungs in response to common air pollutants to investigate whether effects of individual exposure are predictive of effects of co-exposure. A factorial design was used to evaluate the effect of exposure to urban particulate matter (0, 5, 50 mg/m^3 EHC-93), ozone (0, 0.4, 0.8 ppm), or combinations of particles and ozone. Fisher-344 rats were exposed by inhalation for 4 h and euthanized immediately or 24 h post-exposure. Expression of genes involved in a number of biological pathways, including inflammation, oxidative stress, metal-response, xenobiotic metabolism, chemotactic factors, adhesion factors, vasoconstriction, and vasodilation, was assessed by real-time RT-PCR in cells recovered by bronchoalveolar lavage and in lung tissue homogenates. Compared with effects of individual pollutants, effects of co-exposure in bronchoalveolar lavage cells were either additive (metallothionein-II, macrophage inflammatory protein-2, endothelin- 1, heme oxygenase- 1, intercellular adhesion molecule-1) or antagonistic (interleukin- 1 ß, tumour necrosis factor-**α**, cyclooxygenase-2, monocyte chemoattractant protein-1, inducible nitric oxide synthase) (Particle x Ozone interaction, $p<0.05$). Comparison of responses in cells recovered by bronchoalveolar lavage and lung parenchyma revealed distinct responses to the pollutants in these two lung compartments. The gene expression data confirm that effects of individual contaminants are not necessarily predictive of effects of co-exposure, extending our previous observations for lung injury and cardiovascular impacts, and suggest that further research is warranted to examine how health effects attributed to specific pollutants are altered in mixtures.

Abstract / Résumé #45

LOCALIZATION OF THE CELL CYCLE PROTEIN P21 IN EMBRYOS EXPOSED TO VALPROIC ACID IN UTERO. Emily W.Y. Tung[1] and Louise M. Winn[1,2]. [1]Department of Pharmacology and Toxicology and [2]School of Environmental Studies, Queen's University, Kingston, ON, Canada.

Exposure to the anticonvulsant drug valproic acid (VPA) in utero is associated with a 1-2% increase in neural tube defects (NTDs), however the molecular mechanisms by which VPA induces NTDs is unknown. Previous studies in our laboratory demonstrated that VPA, a histone deacetylase inhibitor, caused an increase in reactive oxygen species (ROS) formation in a whole embryo culture system. Furthermore, we have also demonstrated that VPA may be inhibiting DNA replication 6 hours after VPA exposure in an in vivo murine model. The purpose of this study was to examine the localization of two regulators of cell cycle progression, p53 and p21, in mouse embryos exposed to VPA in utero. In addition, immunohistochemical staining was also performed for NF-κB, as it is activated by oxidative stress and acetylation, and inactivated by histone deacetylase 3. On gestational day (GD) 9.5 (vaginal plug = day 1), dams were injected with a teratogenic dose (400 mg/kg) of VPA or saline subcutaneously and embryos explanted 6 hours after exposure and processed for immunohistochemical staining. Preliminary results demonstrated that p21 staining was increased in the cranial region of VPA-exposed embryos, specifically in the neuroepithelium, when compared to controls. Differences in NF-κB staining were not observed between embryos treated with VPA and saline. Currently, p53 staining is being optimized as well as cell cycle analysis by flow cytometry. (Support: CIHR).

Abstract / Résumé #46

EVALUATION OF THE MAGNITUDE OF THE INTERINDIVIDUAL VARIABILITY FACTOR (IVF) FOR CYP2E1 AND ADH SUBSTRATES AS A FUNCTION OF CHEMICAL AND SUBPOPULATIONS CHARACTERISTICS

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The toxicokinetic component of IVF (IVF-TK) used in non-cancer risk assessment corresponds to a default value of 3.16. But the magnitude of IVF-TK for specific subpopulation as a function of pathway-specific metabolism and physicochemical/ biochemical properties of toxicants has barely been studied. The objective of this work was to characterize the IVF-TK as a function of physico-chemical/biochemical properties for CYP2E1 and ADH substrates in various subpopulations. A steady-state algorithm (SSA) was used to compute the internal dose (blood concentration $_{(Cblood)}$ and rate of metabolite produced/L liver (RAM)) of 22 CYP2E1 (acetone, acrylamide, BDCM, benzene, bromoform, 1,3 -butadiene, chloroform, chloroprene, carbon tetrachloride, DBCM, dichloromethane, 1 ,4-dioxane, ethylbenzene, n-hexane, furan, trichloroethylene, tetrachloroethylene, styrene, toluene, 1,1,1,trichloroethane, vinyl chloride, m-xylene) and 4 ADH (isopropanol, ethylene glycol, 2-butoxy and 2-methoxy ethanol) substrates in four human subpopulations (neonates, adults, elderly, pregnant women) following continuous inhalation exposure. In order to solve the SSA, data on body weight and height, hepatic content of CYPs and ADH, liver blood flows and volumes, renal function and alveolar ventilation rates were obtained from the literature or P3M software. In 12 out of 13 cases, the Cblood output of SSA was within a factor of 2 of published experimental values resulting from steady-state exposure to VOCs. Using Monte Carlo simulations, IVF-TK (as the ratio of the 95^{th} percentile value for each subpopulation over the 50^{th} percentile value in adults) was computed for $_{Cblood}$ and RAM and depicted as a function of substrate's blood:air partition coefficient (Pb) (range: 1 - >33,000) and hepatic extraction ratio in adults (Ea) (range: 0.01 – 0.99). Based on $_{Cblood,}$ IVF-TKs were greatest in neonates for both CYP2E1 and ADH pathways. For CYP2E1 substrates, it exceeded the default value in neonates (up to 4.24) only for substrates that had Pb > 250 and Ea < 0.1. For ADH substrates, IVF-TKs exceeded the default value based on $_{Cblood}$ in neonates (up to 5.29) only for chemicals with a Pb > 7900 and an Ea in the range of 0.08 – 0.77. The IVF-TKs based on RAM never exceeded the default value, and were greater in elderly for CYP2E1 substrates with Ea < 0.05 (max. = 2.07) as well as for 2-methoxyethanol (Pb = 32800, Ea = 0.08, IVF-TK = 1.9). For all other substrates, pregnant women presented the highest RAM-based IVF-TKs (max. = 1.83), irrespective of the metabolic pathway. Overall, this study has elucidated the critical impact of Pb and Ea on the magnitude and adequacy of the default IVF-TK for different subpopulations exposed by inhalation.

Keywords: Risk assessment, Interindividual variability factor, toxicokinetics

A PROTEOMIC ANALYSIS OF CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN RAT LIVERS

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Abstract

Objectives: To identify potential liver proteome marker proteins for detection of carbon tetrachloride (CCl4) induced hepatotoxicity by a comparative proteomic approach.

Methods: A comparative proteomic approach with two-dimensional polyacrylamide gel electrophoresis (2D PAGE) coupled with mass spectrophotometry (MALDI-TOF/TOF MS) was used to investigate the change of liver proteomes in male Fischer 344 rats (N=20) treated without CCl4 (control), received subcutaneously of CCl4 (Merck KGaA, Darmstadt, Germany) diluted (1:1 v/v) with pure olive oil at a dose of 0.2 mL/100 g of body weight twice a week for 3 and 5 weeks, respectively. Liver protein spots were visualized by SYPRO Ruby staining and scanned with Molecular Imager PharosFX Plus System (Bio-Rad, CA, USA). Protein spots were matched and analyzed by using the PDQuest 8.0 software for windows (Bio-Rad). Protein spots with differential expression were subjected for peptides identification by MALDI-TOF/TOF MS and NCBI protein database searching for identity.

Results: Around 500 protein spots were clearly detected in the rat liver by 2-DE. 10 proteins with significant ($p < 0.05$) differential expression between normal control and CCl4 treatment groups were identified. These were methionine sulfoxide reductase A, catechol-O-methyltransferase, carbonic anhydrase 3, protein disulfide isomerase precursor, hemoglobin alpha 2 chain, hemopexin precusor, catalase, glutathione S transferases m1, calcium binding protein 1, and mitochondrial aldehyde dehydrogenase). These proteins are involved in various parts of cellular functions, including hormone regulator, protein transport, protein synthesis, calcium metabolism, lipid metabolism, cell proliferation, detoxification and inflammatory response during oxidative stress.

Conclusion: Proteomic technology provides a powerful tool for fast identification and confirmation of proteins with significant roles in the pathogenesis of liver injury during toxic chemicals exposure. The data present facilitate not only the molecular mechanistic investigation of CCl4-induced liver injury but also provide direction for early stage liver fibrosis and cirrhosis study.

THE EFFECT OF SMOKING CESSATION PHARMACOTHERAPIES ON BETA CELL FUNCTION

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Recent studies suggest that exposure to nicotine, the addictive component in cigarette smoke, causes impaired pancreatic function, which may explain the increased risk of type 2 diabetes (T2DM) in smokers (Holloway et al. 2005; Somm et al. 2008). Many individuals are exposed to nicotine via cigarette smoking; however individuals who use nicotine replacement therapy (NRT) for smoking cessation are also exposed to nicotine. If nicotine is indeed responsible for pancreatic damage, then these smoking cessation interventions may also be increasing the risk of developing T2DM. Currently in Canada there are two smoking cessation pharmacotherapies, bupropion (Zyban®) and varenicline (Champix®) that do not involve nicotine replacement, but rather exert their actions by reducing or blocking activation of the nicotinic acetylcholine receptor (nAChR). Therefore, it is important to determine whether these products have a better safety profile than NRT with respect to adverse metabolic outcomes. The goal of this study was to examine the effect of nicotine, varenicline (an α4β2 nAChR partial agonist), and bupropion (a nAChR antagonist) on beta cell function *in vitro*. INS-1E cells (a rat beta cell line generously donated by Dr. Claes Wollheim) were treated with vehicle or the test compounds (1nM and 1μM) under both low (3.3mM) and high (16.7mM) glucose conditions for 1 hour. Following the incubation period, media was collected and insulin secretion, a marker of beta cell function, was measured by radioimmunoassay. The effects of these test compounds on beta cell viability were then assessed. Previous work from our group suggests that nicotine impairs beta cell function via the mitochondria. Therefore, mitochondria were isolated from INS-1E cells treated for 24 or 48h with the test compounds to assess the impact of these pharmacological agents on mitochondrial function (i.e., electron transport chain enzyme activity). As has been previously reported, nicotine treatment decreased insulin secretion under low glucose conditions. However, there was no effect on insulin secretion in cells treated with 1 μM of nicotine under high glucose. Varenicline inhibited insulin secretion under both low and high glucose conditions, whereas bupropion had no effect on insulin secretion in low glucose and inhibited insulin secretion under high glucose conditions. None of the treatments tested significantly altered cell viability. All 3 compounds significantly decreased mitochondrial function following 48 hours of treatment ($p<0.05$). Taken together, these results suggest that in lower glucose environments, bupropion may have the least effect (ie no effect) on beta cell function, and therefore, may be the best smoking cessation pharmacotherapy with respect to metabolic outcomes. However, in a hyperglycaemic environment, there is no difference in the effect of these pharmacological agents on beta cell function, despite their different mechanisms of action for smoking cessation.

Abstract / Résumé #49