

Book of Abstracts

**2008 Health Canada Science
Forum**

Foreword

As this year's host and the Department's Champion for Science, I am pleased to greet all of you and to offer my appreciation for your participation in the seventh annual Health Canada Science Forum, the most important science event on the department's calendar.

This year's overall theme "*Mobilizing Science and Technologies for Stronger Policies and Regulations*" recognizes that Health Canada, as a federal science-based department, relies on sound science to make its regulatory and policy decisions. For these decisions to be optimal, the interface between the science work of the department and its regulatory and policy functions must be as open and communicative as possible. Presentations and discussions will be structured around four sub-themes: 1) Emerging Challenges and Opportunities for Science and Policy Development; 2) Environmental Health Science and Technology and its Impact on Policy and Regulation; 3) Food and Product Safety and its Impact on Policy and Regulation; and, 4) Enablers for a Stronger Science Contribution to Decision-Making. The Organizing Committee has put much thought in identifying topics that will, I trust, generate fascinating presentations and animated discussions.

I would like to thank the Organizing Committee and the Abstract Review Committee, as well as the staff in the Science Policy Directorate, for their dedication and outstanding work in planning this event. I hope you will find this year's Forum interesting and a valuable opportunity to develop and strengthen collaborations in support of departmental priorities.

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Note: In this publication, Health Canada branches are represented by the following acronyms:

FNIHB: First Nations and Inuit Health Branch

HECSB: Healthy Environments and Consumer Safety Branch

HPFB: Health Products and Food Branch

HPB: Healthy Policy Branch

SPB: Strategic Policy Branch

PMRA: Pest Management Regulatory Agency

PACRB: Public Affairs, Consultation and Regions Branch

Other Acronyms:

PHAC: Public Health Agency of Canada

1.01 Emerging Technologies and Human Health: Biosafety Considerations for Biotechnology-Derived Animals

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SUMMARY: Safe handling, transfer, and use of biotechnology-derived animals require the development of containment and confinement guidelines that will describe basic physical, operational, transport, and disposal requirements. In consultation with stakeholders, containment and confinement guidelines will be developed or equivalent requirements may be referenced from the guidelines of other jurisdictions.

OBJECTIVE/BACKGROUND: Biotechnology, as an emerging technology, presents new challenges to biosafety professionals. Animals derived from biotechnology, where one or more genes are silenced or added through genetic modifications, are used extensively in research and development but little is known of their effects (allergenicity, toxicity, pathogenicity, or invasiveness) to human health and the environment. In Canada, most of these animals are currently being contained in fully enclosed laboratories and a few under field confinement but biosafety standards for their safe handling, transfer, and use are lacking. Therefore, as part of the ongoing review of the New Substances Notification Regulations (Organisms), the New Substances Program of Environment Canada and Health Canada plans to develop or adapt (by referencing) a set of containment and confinement guidelines that will spell out the basic requirements to properly contain biotechnology-derived animals.

DESIGN/METHOD/DESCRIPTION: The goal is to develop or adapt a set of containment and confinement guidelines for each group of animals (e.g., fish, insects, rodents, livestock, etc.) that will consider the risk level posed by the transgene or the animal to be contained as well as the nature of the activity to be performed. The guidelines will cover some elements of physical, operational, transport, and disposal requirements associated with each activity.

OUTPUTS/RESULTS: During previous consultations with biotechnology stakeholders including members of the academia, the industry, and the non-governmental organizations, there was an overwhelming support for the proposal for developing containment and confinement guidelines.

CONCLUSIONS/IMPLICATIONS: Containment and Confinement guidelines offer a level playing field among stakeholders with regard to safe handling, transfer, and use of biotechnology-derived animals and ensure protection of human health and the environment from hazards such as those associated with novel transgenes and the whole range of biotechnology-derived animals.

1.02 Potential Use of IgM in a Two-Site Quantitative Assay of Target Proteins in the ELISA as Well as the xMAP Format

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SUMMARY: Routine immunoassay based target protein quantitative studies utilize IgG antibodies extensively, while no standard protocols are available for IgM. An IgM-based ELISA as well as xMAP protocol was developed using Alt a1, major allergen of *Alternaria alternata*, as target protein model. Reliable standard curves were developed using both assay formats.

OBJECTIVES/BACKGROUND/ISSUE(S): While successful hybridoma production experiments consistently yield 80% IgM and 20% IgG monoclonal antibodies (MAb), routine immunoassay-based target protein quantitative studies on vulnerable populations extensively rely on IgG MAbs only with no standard protocols available for IgM. The size and complex pentameric structure of IgM (Mr 1000kDa) is generally speculated, by many investigators, to contribute towards steric hindrance, that may potentially nullify immunoassay results. There are no reports to confirm whether this speculation is a real occurrence or not.

DESIGN/METHOD/DESCRIPTION: A two-site IgM based immunoassay was tested in the ELISA and the xMAP formats with recombinant Alt a1(r-Alt a1) as the target protein. Various concentrations of r-Alt a1 were tested to determine whether reproducible standard curves could be generated. Concentrations between 250ng to 340pg/well of r-Alt a1 were tested in a standard ELISA test while two sets of concentrations, 10ng to 1pg/mL and 300ng to 3pg/mL were tested in the xMAP assay. Two microspheres, carboxylated microsphere (CAM) and LumAvidin microsphere (LAM) from LUMINEX™ Corporation, were compared in the xMAP assay.

OUTPUT/RESULTS: Reliable standard curves were generated in both assay formats confirming that IgM can indeed be used in two-site sandwich assays to quantify target proteins in the diagnosis of disease biomarkers among vulnerable populations as well as risk assessment due to environmental allergens.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Apart from the fact that this study for the first time opens up new possibilities to study allergens as well as antigens using IgM, it also alerts investigators that the IgMs produced during hybridoma experiments have huge potential in the diagnostic field.

1.03 Development of Congenic Mouse Lines for Use in the Analysis of Genetic Complexity in Caffeine Metabolism

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SUMMARY: Most individual variation in drug response is the result of complex interactions between several genes. This study demonstrates that mouse strains that isolate single components of variation in drug metabolism are an effective approach to studying genetically complex pharmacogenomic traits.

BACKGROUND: The cytochrome P450 CYP1A2 is an important enzyme in the metabolism of many drugs. The activity of this enzyme is monitored through the metabolism of caffeine. The regulation of this gene is not well understood, possibly as a result of the multi-genic nature of caffeine metabolism. We had previously mapped loci contributing to this phenotype to chromosomes 1, 4 and 9 in the mouse.

OBJECTIVES: The objectives of this study were: 1) To derive congenic mouse strains in which chromosomal DNA derived from the *C3H/HeJ* mouse strain, and corresponding to the previously mapped loci, were isolated on the genetic background of the *APN* mouse strain. 2) To identify the mechanism by which each of the regions affects caffeine metabolism. 3) To identify the specific genetic variation between the donor and recipient mouse strains in each of these regions.

DESIGN: A combination of phenotypic and genetic marker-assisted selection was used. Genomic DNA was screened with short tandem repeat markers spanning the genome. Mice were selected for preservation of the *C3H/HeJ* chromosome segment spanning the mapped locus, as well as for maximum decrease in residual donor strain genome. Breeders were selected based on the narrowing of the interval area with each successive mating.

RESULTS: We have produced congenic mice for each of the mapped intervals. Each of these congenic lines retains a caffeine metabolism phenotype, while only two lines show altered *Cyp1a2* gene expression. The other line shows altered expression of other phase I and phase II drug metabolizing enzymes, as determined by DNA microarray analysis.

IMPACTS: The congenic lines described are a valuable model for the study of genetic variation in drug metabolism. Our improved understanding of genetic complexity in pharmacogenomics contributes to the development of rational policies and guidelines pertaining to the generation, application and interpretation of genomic data.

1.04 The Development of a Coding Instrument to Assess Nutrition Messages on Four Micronutrients in Canadian Magazines

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SUMMARY: An instrument was pilot tested to assess the quantity, types and accuracy of messages related to calcium, vitamin D, iron and folate-nutrients of interest in women. Canadian magazines (n=16) were analyzed independently by two coders. Nutrient analyses of recipes were the most common source of information on these micronutrients.

OBJECTIVES/BACKGROUND/ISSUE(S): As the development of nutrition policies becomes more extensive, the dissemination of accurate information to consumers is very important. Data from the Canadian Community Health Survey (CCHS 2.2) has shown that women are not meeting Canada's Food Guide to Healthy Eating recommendations for vegetables and fruits, dairy products, and grain products, important sources for the nutrients folate, vitamin D, calcium, and iron. As a result, media messages regarding these nutrients must be examined, particularly as it relates to their portrayal to women. The objective of this project was to develop and pilot test a coding instrument used to assess the quantity, types and accuracy of messages related to calcium, vitamin D, iron and folate, for use in a larger Health Canada (HC) study.

DESIGN/METHOD/DESCRIPTION: An electronic coding sheet and codebook were created to capture the explicit and implicit messages related to the micronutrients of interest in three different magazines. Using content analysis methodology, a sample of magazine issues from *Chatelaine*, *Canadian Living* and *Homemakers* (n=16) were analyzed independently by two coders. Some of the criteria examined within each magazine issue included the quantity of messages, the micronutrients of focus in each message, and how the nutrients were presented (e.g., food, recipe analysis, supplement, etc.).

OUTPUTS/RESULTS: The number of messages presented in each magazine issue ranged from six to eighty. The nutrient analysis of recipes was the most common source of information on these micronutrients. Overall, not all messages accurately reflected Canadian nutrition policy, including nutrient content claims and nutrient recommendations for different age-sex groups.

IMPLICATIONS AND CONCLUSIONS: Based on the results of the pilot test, the coding instrument will undergo further revision for utilization within the larger HC study. The potential for consumer confusion could be reduced if media messages were more congruent with nutrition policy.

1.05 Evaluation of Cree Botanicals for Their Role to Potentiate Cytotoxicity in Human Caco-2 Intestinal Cells

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SUMMARY: The safety of six botanicals used by the Cree of Eeyou Istchee was evaluated using human intestinal cells. All six were evaluated for cytotoxicity and one was used to examine gene transcript changes using microarrays. All six appear to be safe at levels of normal consumption.

PURPOSE: To evaluate six botanicals used by the Cree of Eeyou Istchee, to treat type II diabetes (T2D), for their role to potentiate cytotoxicity in human Caco-2 intestinal cells and evaluate a priority botanical, AD09, for its role in modulating gene expression.

METHODS: Caco-2 cells were exposed to concentrations of ethanolic extracts ranging from 10 µg/mL to 200 µg/mL obtained from six botanicals for 24 hours. Cytotoxicity levels were then measured through Promega's CytoTox 96 Assay. Furthermore, Caco-2 cells were treated with AD09 at 100 µg/mL, and microarray experiments were performed using human 19K cDNA arrays to establish gene changes with the extract versus 0.1% DMSO control.

RESULTS: Out of the six botanicals examined for cytotoxicity, only AD11 showed moderate to significant toxicity at concentrations above 150 µg/mL. The other extracts appear to exhibit minimal to no toxicity. Microarray results for AD09 revealed no changes to the cytochrome P450 family of genes, however, Bayesian analysis yielded 304 significantly downregulated mRNAs including key transcription factors and members of different signaling pathways.

CONCLUSIONS: Extract concentrations above 150 µg/mL are extremely high and such levels would not be achieved in the body through normal consumption, thus we can speculate that these individual extracts will not be toxic, especially when it comes to first-pass metabolism. However, any possible gene transcript changes, particularly to the cytochrome P450 metabolic enzymes, that may occur, can lead to possible flags for drug interactions, since many drugs are metabolized by these enzymes. In the case of AD09, as no changes were observed to the cytochrome P450 family of genes, we can speculate that the individual botanical appears to be safe; however, we have no information on how these botanicals are formulated into traditional medicines. This project will support regulatory initiatives to identify potential interactions of concurrently administered traditional medicines and other therapeutic products in order to assist health care professionals and their patients make informed risk management decisions.

1.06 Assessing Health Vulnerabilities of a Changing Population of Canadians to a Changing Climate: Prescribing Interventions

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SUMMARY: Climate change refers both to a global warming trend and to increasing (less predictable) extreme weather events. This multi-year study produced a comprehensive inter-disciplinary report, detailing the vulnerabilities that we face. There will need to be changes in infrastructure and health protection systems, to preserve the health and safety of Canadians.

BACKGROUND: With its widespread environmental and human health impacts, climate change has become a major global health issue. A general warming trend in climate and its increasing variability (with more frequent extreme events - heat, precipitation, etc.) already affect (both directly and indirectly) the health of populations worldwide, causing physical and mental illnesses, injury and, in extreme cases death. Canada is no exception, as detailed most recently in the June 2008 United States Climate Change Science Program (USCCSP) product 3.3.

DESCRIPTION/METHOD: In 2003, the Climate Change and Health Office (HECS) launched an assessment of health impacts on Canadians from climate change. This health assessment utilized the integrated assessment approach advocated in *Methods of Assessing Human Health Vulnerability and Public Health Adaptation to Climate Change*, developed by the World Health Organization and Health Canada.

OUTPUTS: *Human Health in a Changing Climate: A Canadian Assessment of Vulnerabilities and Adaptive Capacity* reports on the scope and magnitude of current and anticipated health impacts of climate change in Canada related to air quality, natural hazards and a range of others. It also gauges the capacity of communities and governments to reduce health risks to Canadians. This assessment integrates information from a variety of perspectives including those of public health, the voluntary sector, emergency preparedness, meteorological services, air quality, and academia.

CONCLUSIONS/NEXT STEPS: If we are to thrive, we must establish preparedness for interventions (and their messaging) that are risk-prioritized. This would draw upon the existing science base and promote novel research as indicated by gap analysis, in order to first protect those who are most vulnerable.

By identifying populations and regions particularly at risk, the results of this assessment will assist public health decision makers to better intervene, maintain and protect (and perhaps, to enhance) the health of Canadians in a changing climate. The findings will be of interest to governmental agencies, health professional organizations, medical officers of health, public health inspectors, emergency managers, social service providers and health researchers, among others.

1.07 Modular Research Management System

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SUMMARY: This work describes modules of a research management approach that has been developed in the Inhalation Toxicology Laboratory of Health Canada over the past 15 years: staffing; equipment acquisition and maintenance; sample tracking and management; data integration, management and mining.

BACKGROUND/ISSUES: Laboratory Information Management Systems (LIMS) are available for corporate implementation. However, research laboratories vary in size and scope of work and no single system works for all. Our laboratory has developed a number of research management modules over the years that consist of databases and applications to facilitate and document the administration of research projects. These modules are adaptable and should be of interest to the general laboratory research community at Health Canada.

APPROACH: Specific areas of research activity management were addressed by these modules: 1) The Financial Database was designed to track budget, commitments and expenditures in real-time in order to provide an instantaneous reference on the budgetary status of projects and to provide historical expenditure data for planning new submissions. 2) The Staffing Database tracks initiation, termination, renewal of contracts, hazardous chemical exposures, etc. 3) The Equipment Database regroups information on equipment asset numbers, maintenance, service contracts, etc. 4) The Laboratory Alarm Monitoring System in conjunction with Labview application allows for integrated real time remote data acquisition and control of laboratory equipments. 5) The Research Samples Databases document conditions of generation of samples, and track their location, usage and remaining amounts. 6) The Research Results Databases integrate data from a number of research projects to facilitate data mining and ensure data security.

OUTPUT/RESULTS: The tools have been in use for several years in our laboratory and continue to be optimized. The Financial Database and the Equipment Database have now been cloned for application by other groups and are available as LAN applications within the Environmental Health Science and Research Bureau. We have now begun integration of the various modules and analysis/development of a common portal in order to facilitate navigation and mining of these databases.

CONCLUSIONS/IMPACTS: The modular nature of our tools has provided the flexibility of independent development, maintenance and implementation of specific modules in a research environment. Integration of these modules using a common access portal should present an invaluable research management system for widespread adaptation and use by other research laboratories inside and outside the government with similar operational requirements.

1.08 Delivery of the Aboriginal Diabetes Initiative in First Nations and Inuit Communities across Canada: An Overview

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SUMMARY: An analysis of community reports provides an overview of how the Aboriginal Diabetes Initiative is delivered in First Nations and Inuit communities.

OBJECTIVES/BACKGROUND/ISSUE(S): In Canada, Aboriginal people are three to five times more likely to develop diabetes than the non-Aboriginal population. Expanded in 2006, the Aboriginal Diabetes Initiative (ADI) is a community-based primary prevention, screening and treatment program. Currently, more than 600 First Nations and Inuit communities have access to health promotion and diabetes prevention activities. The main objective of ADI is to reduce type 2 diabetes in Aboriginal people through a range of program elements, including: health promotion; community based prevention, screening and treatment services; training and capacity building; research and surveillance; and evaluation and monitoring. Since the establishment of the ADI, limited analysis has been conducted on delivery of activities at the community level.

DESIGN/METHOD/DESCRIPTION: Annual reports provide descriptions of community-level activities supported by the ADI. 370 reports from 2006/2007 (N=137) and 2005/2006 (N=233) were analyzed from ADI projects across all eight regions. Total attendance at activities, frequency of activities and promising practices in communities were compiled on a regional basis.

OUTPUTS/RESULTS: A national summary was developed providing an overview of the 233 reports available for 2005/2006. Results indicate that diabetes education and awareness services were the most frequently delivered activities and present in 82% of the communities studied in 2005/2006. Lifestyle support workshops and events had the highest attendance rates, with nutrition workshops present in 53% of communities and 52% of communities having ongoing programs related to health.

IMPACT/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The diversity of methods by which communities organize and deliver activities, demonstrate the flexibility of ADI in serving Aboriginal communities. Increasing our knowledge of program delivery for diabetes education and support activities at the community level allows us to better understand how ADI is being implemented in First Nations and Inuit communities. This information will also contribute to the evidence base informing the renewal request for the ADI.

1.09 The Influence of Physical Activity and Eating Habits on Adult Obesity: The Canadian Evidence

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SUMMARY: This study quantifies the contributions of physical activity, eating habits, and other factors to obesity. After controlling for socio-economic, demographic and other factors, we find physical activity and healthy eating are significant factors, whereas caloric intake has little influence.

OBJECTIVES: The objective of this study is to quantify the influence of physical activity and diet on adult obesity while controlling for a host of socio-economic and demographic characteristics, and other unobservable confounders.

DESIGN: The study is unique in that it uses multivariate longitudinal and cross-sectional modelling techniques, including linear and logistic random-effects. The main data for this study come from the first six cycles of the National Population Health Survey (NPHS). Since caloric intake data were not collected in the NPHS, the Canadian Community Health Survey, cycle 2.2 cross-section data are analyzed to determine the effect of caloric intake on obesity while controlling for same control variables as in NPHS.

OUTPUTS/RESULTS: Our empirical analysis finds that, *ceteris paribus*:

- an additional kilocalorie expended per day reduces the likelihood of being overweight or obese by 13% and 28%, respectively for women; by 5% and 10%, respectively for men;
- compared to those reporting fair or poor eating habits, among those reporting their food habits as excellent, very good, or good reduce:
 - the probability of being overweight by 74%, 63% and 31% respectively, and the probability of being obese by 94%, 91% and 61% respectively for women;
 - the probability of being overweight by 52%, 39% and 0% respectively, and the probability of being obese by 85%, 85% and 58% respectively for men;
- caloric intake has no statistical significant influence on obesity based on CCHS 2.2 analysis;
- the higher the level of education or income, the lower the BMI and probabilities of being overweight or obese for women, but not for higher income men;
- compared to non-smokers, those who smoke daily or occasionally exhibit lower BMI and lower probabilities of being overweight or obese;
- the results for age, marital status, ethnicity and rural/urban are largely consistent with those found elsewhere;
- compared to Ontario, residents of British Columbia and Quebec have lower BMIs, while those in other provinces have higher.

IMPACTS/OUTCOMES/CONCLUSIONS: From a public policy perspective, promoting healthy food choices and adequate physical activity promise worthwhile returns.

1.10 Toxicity of CdTe Quantum Dot Nanoparticles and Their Effects on Immune Responses to Bacteria

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SUMMARY: Cadmium telluride quantum dots (CdTe-QDs) were investigated for their toxic effects. For this purpose, mammalian immune and intestinal cells were exposed to either CdTe-QDs or bacteria alone, or CdTe-QDs followed by bacterial exposures. Toxicity and immunological assays were used to test for differences between exposures.

OBJECTIVES: To study the toxicity of CdTe-QDs and their effects on the immune responses of mammalian cells towards *Pseudomonas* strains.

DESIGN: Murine macrophage J774A, human lymphocyte MOLT4, and human epithelial HT29 cells were exposed for 2hr to 24hr to different concentrations of CdTe-QDs (10^{-7} - 10^1 ug/ml). Media containing CdTe-QDs were then removed and replaced with fresh media. The cells were subsequently exposed to 10^6 cfu of *P.aeruginosa* 31480 (Pa 31480), *P.aeruginosa* O1 (PaO1) or *P.fluorescens* 13525 (Pf13525) for 2hr to 24hr. Levels of bioreduction (for cytotoxicity), nitric oxide (NO), and cytokines in test cells were measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, Griess method, and multiplex bead array, respectively. Localization of CdTe-QDs and bacteria within the cells were observed using confocal, electron, and atomic force microscopy. Statistical analyses were done by ANOVA followed by Dunnett's multiple comparison tests.

OUTPUTS/RESULTS: At a concentration of 0.01ug/ml or higher, CdTe-QDs alone caused a significant decrease in bioreduction activity in all test cells (40-90%, $p < 0.001$), but at lower concentrations (10^{-7} - 10^{-3} ug/ml), CdTe-QD exposures resulted in no observed cytotoxicity or changes in NO or cytokine production. These lower QD concentrations followed by *Pseudomonas* exposures caused ~10% greater cytotoxicity than that caused by *Pseudomonas*. As well, levels of NO, inflammatory cytokine (TNF-alpha), and neutrophil-attractant chemokines (IL-8 and KC) in test cells were reduced by 2-5 fold ($p < 0.001$), compared to *Pseudomonas* exposures alone.

IMPACTS/OUTCOMES/CONCLUSIONS: Data presented here provides important baseline information on the toxic nature of CdTe-QDs as part of Canadian Environmental Protection Act responsibilities. Specifically, the results show evidence that CdTe-QDs can induce cytotoxicity, as well as suppressing inflammatory mediators and affecting neutrophil recruitment signaling in response to bacterial exposures. Further work is warranted to assess whether CdTe-QD exposures may result in increased host's susceptibility to bacterial infection *in vivo*.

1.11 Superbugs from Food Animals: An Emerging Public Health Issue

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SUMMARY: By analyzing the published literature/surveillance data, we have identified the increasing occurrence of superbugs (e.g., methicillin-resistant *Staphylococcus aureus* [MRSA] and multi-drug resistant *Escherichia coli* and *Salmonella*) from food animals (including those from Canada) as an emerging public health issue with the need for development of possible risk mitigation measures.

OBJECTIVES: Analyze the current trends in superbugs (i.e., antimicrobial resistant and/or virulent bacteria) originating from food animals in order to identify potential issues of public health concern.

DESIGN: On the basis of published genotypic and susceptibility studies, we investigated the incidence of superbugs of food animal origins with an emphasis on MRSA and multi-drug resistant *E. coli* and *Salmonella* including those resistant to extended-spectrum cephalosporins or fluoroquinolones.

OUTPUTS/RESULTS: Superbugs pose a significant risk to public health. There are increasing reports of MRSA isolated from food animals and food products. These include the repeated isolations of MRSA from pigs and pork products in different geographical regions, including Canada. Pig-adapted MRSA isolates (e.g., ST398), which are characteristically different from the hospital- or community-associated MRSA, have colonized humans or resulted in human infections. These isolates also appeared resistant to tetracycline. To underscore this emerging issue, MRSA is increasingly being isolated from veterinarians and pig producers, raising an important question of the adverse impact of MRSA on population health. Besides MRSA, resistant *E. coli* and *Salmonella* are increasingly isolated from food animals/products. These zoonotic bacteria or foodborne pathogens are frequently resistant to multiple antimicrobials, as exemplified by isolates with plasmid-encoded CMY or CTX-M beta-lactamases as well as *qnr* plasmid-mediated fluoroquinolone resistance. The associated resistance genes often co-exist with other resistance determinants and can be associated with mobile genetic determinants (e.g., plasmids/transposons/integrans), highlighting the possible enrichment of multi-drug resistant bacteria by multiple antimicrobial agents and the dissemination of the resistance determinants among bacterial species including the potential to transfer to zoonotic pathogens.

IMPACT/OUTCOMES/CONCLUSIONS: The increasing occurrence of the superbugs of food animal sources highlights an emerging public health and animal health issue, which warrants further investigation of the adverse human health impact and the need for development of possible risk mitigation measures.

1.12 Snus Sold in Canada: Solution or Illusion?

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SUMMARY: This study evaluates the *in vitro* toxicity of snus and other smokeless tobacco products available in Canada. It demonstrates that snus, like other tobacco products, is a mutagenic, cytotoxic and genotoxic product.

CONTEXT/OBJECTIVES: During the past year, a new smokeless tobacco product, snus, has entered the Canadian market. This product, sold in little tea bags, is sucked after being placed between the upper lip and the gums. This study evaluates the toxicity of various types of smokeless tobacco products, including snus, to position this new arrival among the products already available in Canada.

METHODS: Snus sold in Canada and 11 other smokeless tobacco products were evaluated for mutagenicity (Health Canada Method T-501), cytotoxicity (T-502) and genotoxicity (T-503). These products were grouped into five types: snus, moist tobacco, semi-dry tobacco, tobacco in bags and chewing tobacco.

RESULTS: The mutagenicity results indicate that the number of revertants/mg of extract, for strains TA100 and TA102, is: snus (2-9; 7-12), moist tobacco (4-12; 8-9), semi-dry tobacco (9-12; 11-18), tobacco in bags (7-13; 2-19) and chewing tobacco (23-37; 32-39). Snus has an IC₅₀, a concentration that inhibits by 50% the activity in a cell population, at 21 µl of extract/ml media. This is the second-highest level of cytotoxicity after chewing tobacco. Finally, for genotoxicity, the percentages of micronuclei per ml of extract/ml media are: snus (28-35), moist tobacco (28-40), semi-dry tobacco (35-37), tobacco in bags (30-38) and chewing tobacco (40-47).

CONCLUSIONS: This study demonstrates that the snus sold in Canada, like other tobacco products (smokeless, cigarettes, cigars and so on) is a mutagenic, cytotoxic and genotoxic product. The obtaining of such toxicity results *in vitro* suggests the need for further, in-depth studies of this product, to increase the scientific evidence in support of development of regulations and policies.

1.13 Characterization of Mitogen-Activated Protein Kinase Phosphatase-3's Inhibitory Interdomain Binding Site

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SUMMARY: Mitogen-activated protein kinase phosphatase-3 (MKP3) is a key enzyme that attenuates cell proliferation in response to growth factors. How functional MKP3 levels are regulated in the cell, however, is not known. We show that the enzyme is capable of self-inhibition through formation of oligomers and characterize an interdomain binding site.

OBJECTIVES: MKP3 abrogates growth factor-mediated cell proliferation signals through specific dephosphorylation of activated extracellular regulated protein kinases (ERKs). However, we observed that pancreatic adenocarcinoma cells could concurrently express high levels of MKP3 and phosphorylated ERK. To account for the proliferation of cancer cells, we hypothesized that excess MKP3 was sequestered in an inactive state through an inhibitory interaction between MKP3's N- and C-terminal domains. The present study was designed to investigate this model and characterize MKP3's putative inter-domain binding site.

METHODS: MKP3 N- and C-terminal domain expression constructs were generated. Additional site-directed mutants were created within these domains by altering amino acids within locations possessing high net charge or hydrophobicity, characteristics displayed by protein-protein binding sites. Protein interactions were assessed using surface plasmon resonance and binding constants were determined. MKP3 enzymatic activity was quantified using a para-nitrophenol phosphate dephosphorylation assay.

RESULTS: MKP3 forms high molecular weight oligomers that do not affect cell viability when overexpressed in pancreatic adenocarcinomas. *In vitro* assay revealed that MKP3 activity was competitively inhibited by free N-terminal domain. To characterize N- to C-terminal domain binding, surface plasmon resonance was used on peptide sequences from the N-terminal domain. The data showed that a peptide corresponding to N-terminal amino acids 77-97 bound to the C-terminal domain. Analysis using the N-terminal peptide and C-terminal site-directed mutants showed that mutation N267A to the C-terminal domain abrogated binding. Taken together, the binding data provide a preliminary identification of MKP3's interdomain binding site.

CONCLUSIONS: MKP3 undergoes self-inhibitory binding that blocks its C-terminal catalytic site in the absence of ERK. However, this can lead to the formation of inactive oligomers via N and C-terminal interaction between MKP3 molecules. In this study we characterize MKP3's interdomain binding site. The data may lead to inhibitors of MKP3 oligomerization and potential biotherapeutics for pancreatic adenocarcinomas.

1.14 The Ethics of Informed Consent to Derive Human Embryonic Stem Cells: The Development of Regulatory Policy on the Use of *In Vitro* Embryos Under the *Assisted Human Reproduction Act*

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SUMMARY: This presentation will review the ethical issues and possible policy options related to informed consent for the use of *in vitro* embryos for research to derive human embryonic stem cells (hESCs) under the *Assisted Human Reproduction Act (AHRA)*.

The use of human embryos to derive hESCs may be valuable for the treatment of many diseases. In Canada, human embryo research is governed by many policies and more recently by the AHRA. Health Canada (HC) has a mandate to develop the components of the regulatory framework under the AHRA. The AHRA also provides authority to establish an Agency that will license and enforce the regulations.

OBJECTIVE: To identify ethical issues and develop possible policy options on informed consent for the derivation of hESCs.

DESIGN: Conceptual research and ethical analysis of the bioethics and international policy literature on issues of informed consent to derive hESCs is performed.

OUTPUT: Although *in vitro* embryos cannot be created specifically to derive hESCs under the AHRA, excess *in vitro* embryos that were originally created for the purpose of creating a human being can be donated for research through a process of receiving informed consent. Several issues related to informed consent will be addressed: 1) the avoidance of therapeutic misconception of the anticipated benefits of hESC research, 2) identifying the person most suitable to provide information to donors during informed consent, 3) stating the information to be provided to donors (i.e., the type of research project and the different uses for *in vitro* embryos: cryopreservation for future own reproductive use, destruction, and third party donation for another's reproductive use), and 4) the conduct for the right to withdraw consent (when and how) and knowing that a donor's participation is voluntary and will not affect their reproductive treatment.

OUTCOMES: The ethical issues and possible policy options on informed consent will be discussed in future consultations conducted by HC with multiple stakeholders for the development of regulatory policy on the use of *in vitro* embryos for research.

1.15 Maternal and Child Health Public Opinion Research 2006

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SUMMARY: Public Opinion Research was undertaken in 2006 to support a healthy pregnancy campaign targeting First Nations and Inuit. This research was important to the implementation of the Healthy Pregnancy Campaign in First Nations and Inuit communities and in tailoring the design and implementation of the Maternal Child Health Program in First Nations communities.

PURPOSE & RATIONALE: In February and March 2006 FNIHB supported public opinion research (POR) into First Nations and Inuit communities to determine:

- knowledge levels regarding healthy outcomes of pregnancy (healthy eating and physical activity, abstinence or reduction of use of alcohol and tobacco);
- community health resources (where to go for support and information); and
- barriers in accessing information regarding healthy pregnancy.

PROCESS/APPROACH: Ekos Research Associates conducted twenty-minute telephone interviews with 925 First Nations on reserve and 407 Inuit men and women between the ages of 18 and 40 during a four-week period. The response rate was 38%.

FINDINGS: The poster will highlight the levels of awareness of the practices required to support a healthy pregnancy - with good nutrition recognized as one of the most important things that a women could do to have a healthy baby (raised by 60%). Stopping the use of alcohol was noted by approximately 45% and tobacco use by over 40%. Emotional health, avoiding stress and resting were noted by only 2% of the respondents. There was a good understanding of some of the effects of behaviour during pregnancy - with approximately 80% identifying smoking as dangerous and 75% noted that even having a few alcoholic drinks during pregnancy could pose risks. The research also provides information on behaviour changes during pregnancy - specifically related to nutrition (73% indicated a change in behaviour), smoking (approximately) and alcohol consumption. Key sources of information during pregnancy were also explored. Over 50% of respondents indicated that they first looked for information on healthy pregnancy as soon as their pregnancy was confirmed. The usual sources of information were family doctors, nurses and health clinics. These same sources were recognized as providing the most useful information. Most women (55%) looked to their partner for support, as well as their mother (50%), will be outlined as well as the usefulness of specific sources. Supports for a healthy pregnancy will also be discussed.

IMPLICATIONS: The results of this POR were used to inform the development of programming and policies for First Nations and Inuit and as the focus of a social marketing campaign in late 2006 and early 2007. The campaign targeted pregnant First Nations and Inuit women, their partners and communities, but relied heavily on

distribution of information through nurses and health clinics, consistent with the research. The information has also been used in the development of the Maternal Child Health Program in First Nations communities.

1.16 Aboriginal Children's Health Research: A Scan of Peer-Reviewed Articles

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SUMMARY: A scan of published peer-reviewed research was undertaken to support the development of a research agenda related to Aboriginal children.

PURPOSE AND RATIONALE: Despite the many disparities between the health status of Aboriginal children and that of their Canadian counterparts, very little research has been done on Aboriginal children's health. This project was established to contribute to the development of an Aboriginal child health research agenda.

PROCESS/APPROACH: This poster presentation will highlight the results of a review of the published research on Aboriginal children in Canada, Australia, New Zealand and the United States between 1996 and 2005, organized schematically. The articles were identified by searching a variety of databases, using criteria for inclusion (qualitative, quantitative and descriptive studies; clear focus on Aboriginal child health; population focus prenatal to age 12; research conducted in the four countries noted above). The presentation will also outline primary research carried out with key informants in the research community and in relevant government departments.

FINDINGS: There were over 400 peer-reviewed research articles analyzed for the study. Almost half (48%) related to US indigenous populations; 22% were from Canada. Most were conducted by the university sector (65%). The research focused predominantly on school-aged children (40%) and children less than 2 years old (38%) with only 22% on preschool-aged children. In Canada, most of the research was centered in Quebec (38% of articles specifying a geographical location). Canadian research also focused predominantly on First Nations children (62%). Environmental exposure accounted for the largest number of research articles in Canada, while growth and development (including obesity) have clearly dominated the research agenda in Australia and the US. There were some clear gaps in the research, based on a comparison of the research issues and current health issues (e.g., injuries). Potential research priorities were also raised in the key informant interviews.

IMPLICATIONS: This research has identified potential gaps and priorities and led to a number of partnerships and potential areas of work, including discussions with the Canadian Institutes of Health Research (CIHR) on research related to injuries, obesity and secondary analysis of health data relating to child health. We have also recently supported work related to infant mortality rates in First Nations communities and development of a report on indigenous child health status across the four countries.

1.17 Sex and Gender Differences in Extreme Heat Events: Building a Collaborative Knowledge Base for Adaptation to Climate Change

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SUMMARY: This study analyses available literature as well as available mortality and morbidity data to assess sex and gender differences in vulnerability to extreme heat events and to determine the impact of gender sensitive mitigation strategies.

BACKGROUND: Global atmospheric temperature has risen over the last century, increasing the number of extreme heat events that can result in significant numbers of deaths, as in Chicago 1995 (~700 deaths) and Europe 2003 (70 000+ deaths).

Research suggests that men and women differ in how they perceive, experience, cope with and respond to such events. The differences are due to physiological, biological, social and behavioural factors including sex, age, chronic conditions, medication use, socioeconomic status, social roles and networks that can affect vulnerability to extreme heat.

OBJECTIVES: This collaborative project will identify and critically analyze research on differences in the effect of extreme heat events on men and women as well as in their responses to public health messages. The research will inform the implementation of Health Canada's Pilot Heat Alert Systems and Health Professional Interventions projects to support Canadians' adaptation to climate change.

DESCRIPTION: Critically appraise academic and grey literature concerning sex and gender differences in relation to extreme heat events (Canadian and international). Identify and compile sex-disaggregated mortality and morbidity data (Canadian and US), where available. Analyze and describe sex and gender patterns and trends in the data including limitations.

OUTPUTS/RESULTS: Provide evidence on the need to consider gender and sex-related factors in the design, implementation and evaluation of interventions during extreme heat events. Identify and recommend gender-sensitive practices and approaches, as appropriate.

CONCLUSIONS: Gender-based analysis will help clarify and elucidate sex and gender differences between and among women's and men's ability to cope with extreme heat, and the ability of response systems to accommodate these differences. Social networks and socio-economic status may play a critical role in determining the resiliency of a community, especially those most vulnerable to heat events as resiliency is related to access to resources, among other factors.

1.18 Performance of a Genotyping Array for *Bacillus cereus* (Bc) Group Organisms

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SUMMARY: For safety assessments, we are developing genetic methods to compare microbes used in environmental applications. Here we describe the performance of an array of DNA probes that can be used to score microbial genes. The array detected a reference strain properly and could discriminate it from more distantly related microbes.

OBJECTIVES: We are developing genomic-based methods for determining gene content of *Bacillus* biotechnology organisms covered under the *Canadian Environmental Protection 1999* and *Pest Control Products Acts*. The *B. cereus* (Bc) group contains closely related Bc, *Bacillus thuringiensis* (Bt) and *B. anthracis* (Ba). Our goal was to observe the performance of DNA probes, in microarray formats for fluorescent and electro-chemical detection, and determine their usefulness in assaying the DNA of several Bc group bacteria.

DESIGN: Bc ATCC 14579 (type strain) chromosomal and *Bacillus spp.* (Bs) plasmid and typing probes were selected from annotated genomes (National Centre for Biotechnology information database). Probes were rated for specificity, using a proprietary version of the Basic local alignment search tool (Combimatrix Corp.). Custom DNA microarrays carrying Bc 14579 chromosomal, specific plasmid, species, and control probes, were fabricated in formats for fluorescence and electrochemical detection. Microarrays were hybridized to genomic DNA (gDNA) from Bc group microbes and signals quantified.

RESULTS: Control Bc 14579 gDNA was detected by the chromosomal probes in both formats, with a Gaussian-like signal distribution. Signals were detected for Bc 14579 plasmid probes and in lesser degree to Ba and Bc 10987 typing probes. Hybridizations of Bs and other Bc group bacteria, in fluorescent detection, revealed less overall hybridization to the array, with some specificity towards the typing and plasmid probes.

IMPACTS/OUTCOMES/CONCLUSIONS: This analysis shows that the array of DNA probes can appropriately detect Bc 14579 chromosomal and plasmid genes and discriminate related from non-related *Bacillus* genomes. The arrays designed for electro-chemical detection featured a better signal distribution. The complete analysis of hybridization results to the arrays will provide information on the genetic content of *Bacillus* biotechnology microorganisms and which probes are best able to discriminate between these bacteria.

1.19 Comparison of Imaging Methods for Examining Nanoparticles

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SUMMARY: Nanoparticles such as quantum dots are being integrated into commercial products, which may result in environmental contamination and possible health effects. As part of a larger characterization and toxicity-screening endeavour, we compared various microscopy methods for their ability to give structural information about nanoparticles.

OBJECTIVES: Nanoparticles are physically and chemically diverse materials. These characteristics affect parameters such as aerosolization, absorption and depth of inhalation of the particles and thus have implications on their hazard potential. The objective of this study was to compare fluorescence/confocal microscopy (FM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) for capacity and limitations in detecting and characterizing (size, shape, aggregation) nanoparticles composed of various materials.

DESIGN: For FM and AFM, particles were serially diluted in either double distilled (dd) H₂O or ethanol and examined as a drop or air-dried onto pre-cleaned microscope slides. For TEM, the same dilutions were dried onto 300-mesh copper grids that were pre-coated with 0.5% formvar. Samples were examined with a Nikon C1 confocal or a T1 FM, a JOEL 1010 TEM and an Agilent 5500 AFM. Image analysis was done with NIS Elements, Gwyddion or Image J data analysis softwares.

OUTPUTS/RESULTS: Fluorescent polystyrene nanoparticles were detected by FM and AFM, but were not electron-dense enough to be detected by TEM, even after staining with various heavy metals. It was not possible to resolve individual polystyrene particles by FM. AFM analysis confirmed that the particles were 20nm in diameter and roughly spherical as purchased. Quantum dots (QDs) were detected with all microscopes, although structural analyses were only possible by TEM and AFM. Depending on the preparatory method, QDs had a tendency to form aggregates, which was apparent by both TEM and AFM. Monomers were determined to be approximately 7nm in diameter.

IMPACTS/OUTCOMES/CONCLUSIONS: FM provided low magnification visualization on aggregates and areas of high density. It was also well suited for detection of particles in solution, although it could not be used for sizing estimates. TEM was useful for electron-dense particles and metals and provided accurate sizing data. AFM detected all particles with minimal sample preparation. The most important parameter for AFM imaging was selection of appropriate probe. Data presented here fulfills requirements under the *Canadian Environmental Protection Act* (1999), and provides baseline information on the use of appropriate microscopy methods for nanoparticle characterization.

1.20 First Nations and Inuit Mental Wellness Teams

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SUMMARY: Health Canada is supporting First Nations and Inuit partners to develop and implement team approaches to mental health and addictions. Mental wellness teams include professionals and para-professionals meeting the needs of individual service users, as well as taking a determinants of health approach to the needs of the broader community.

OBJECTIVES/BACKGROUND/ISSUES: Cultural change and inter-generational trauma have contributed to high rates of mental health and addictions issues in First Nations and Inuit populations. Team approaches to mental health and addictions can enhance the effectiveness of care -- when applied to depression, shared care has demonstrated a 50% reduction in symptoms. Collaborative approaches support better access to care; referrals from family physicians rose from 8 to 73 in the first year of a collaborative program. When applied to major depression, shared care can reduce the cost of treatment (\$1783 vs. \$1940).

DESIGN/METHOD/DESCRIPTION: Health Canada is working with First Nations and Inuit partners to develop and pilot mental wellness teams that value traditional and mainstream approaches; build on community strengths; fill gaps in the continuum of care; and increase access to culturally-safe, community-based services. Teams include a variety of para-professionals (addictions worker, mental health worker, Elder, etc) and professionals (social worker, psychologist, nurse, etc). Teams include clinical and community development aspects, which are complementary processes applied from a micro and macro perspective. Teams work with individual service users and their immediate support network on the one hand, while at the same time working with the whole community through a determinants of health approach.

OUTPUTS/RESULTS: This project will support piloting, in First Nations and Inuit communities, mental wellness teams that value traditional and mainstream approaches; build on community strengths; fill gaps in the continuum of care; and increase access to culturally-safe, community-based services. Teams include a variety of para-professionals (addictions worker, Elder, etc) and professionals (social worker, psychologist, etc). Teams include clinical and community development aspects, which are complementary processes applied from micro and macro perspectives. Teams work with service users and their immediate support network, while at the same time working with the whole community through a determinants of health approach.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Evaluation and strengthening the evidence base for team-based approaches are the most significant next steps. Learnings from pilot projects will be disseminated broadly, to support teams in other jurisdictions and raise the quality of mental health and addictions services available across Canada. The flexible nature of team approaches suggests that teams could be developed and implemented successfully in a variety of other contexts.

1.21 The Extraordinary Use New Drug Project: How Science, Health and Regulatory Policy Can Work Together

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SUMMARY: Many drugs used to treat emerging diseases or health conditions caused by chemical, biological, radiological or nuclear (CBRN) agents cannot be tested in clinical trials. The Extraordinary Use New Drug (EUND) Working Group was tasked with developing a framework to allow the authorization of emergency-use drugs, based on limited human safety studies and supporting animal studies.

BACKGROUND ISSUES: The Food and Drug Regulations do not currently allow for the authorization of drugs that do not have sufficient information regarding the safety and efficacy of a drug under the recommended conditions of use. However, drugs used to treat many emerging diseases, such as pandemic influenza or Ebola, or life-threatening conditions caused by CBRN agents cannot be tested in clinical trials under the recommended conditions of use because of the sporadic or infrequent nature of the event leading to exposure. The main goal of the EUND Working Group was to develop a regulatory framework for the marketing approval of drugs that would be used in emergencies such as a pandemic or bioterrorist attack.

DESCRIPTION/CHALLENGES: Our multi-disciplinary working group consists of scientists, policy analysts, regulatory affairs specialists, medical doctors and legal counsel. We have representation from program areas representing all areas of drug approval, from clinical trial review to post-market surveillance. There was also participation from the Public Health Agency of Canada and the Department of National Defence. We each brought a unique set of needs and concerns to the table, and meetings have ranged from open-ended brainstorming sessions to very directed meetings with lawyers to discuss specific details of the proposed regulatory changes. There are many scientific uncertainties associated with the review of EUND submissions, which will rely on animal studies for efficacy and limited human studies of safety with surrogate endpoints for efficacy. Health Canada will be faced with the challenge of conducting risk-benefit analyses based on very limited information.

RESULTS: The working group wrote a paper identifying and analyzing the issues, and Health Canada sought public input by way of a Letter to Stakeholders posted on the departmental website in October 2007. A wide range of stakeholders responded to the consultation letter. Their concerns will be addressed in the Regulatory Impact Analysis Statement, which will accompany the proposed regulations when they are published in *Canada Gazette, Part I (CG I)*.

As of July 2008, the regulatory drafting process is still underway. The earliest possible date for prepublication in *CG I* is late fall of 2008. Some of the topics that have been considered and addressed during the process include, but are not limited to:

- the scientific requirements for an EUND submission
- the approach of other competent regulators to this matter
- the ethical considerations around the use of EUNDS
- the health and safety concerns of using EUNDS
- the tracking of potential adverse events after the use of EUNDS
- the collection of post-authorization data to support the clinical safety and efficacy of the EUND

DESIRED OUTCOMES AND NEXT STEPS: In the coming months, the WG will develop a guidance document for EUNDS for industry stakeholders and Health Canada staff, detailing the intent of the EUND regulations. The WG will also come up with an implementation plan, outlining the responsibilities of various government and industry stakeholders in the manufacture, stockpiling/storage, distribution, use and reporting of clinical outcomes.

The proposed regulations will go out for public consultation in *CG I*, and the WG will address any concerns or comments received at that point. The regulations will be revised, as necessary, and then published in *Canada Gazette, Part II*, at which point they will come into effect. The EUND regulations will serve to provide Canadians with access to authorized drugs for emergency use - ones which have undergone an appropriate level of scientific review and will be subject to the provisions of the *Food and Drugs Act* and Regulations.

1.22 Assessing the Impacts of the 2001 Clinical Trial Regulations on R&D in Canada

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SUMMARY: Using the number of clinical trial applications submitted to Health Canada as a measure of levels of R&D performed in Canada, this study evaluates whether the 2001 clinical trial regulations have achieved the objective of attracting and sustaining investment in R&D in Canada. Results indicate significantly increased levels of foreign investment in clinical research in Canada from European and American firms, although it seems more needs to be done to stimulate investment by Canadian firms.

OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada reformed its clinical trial regulations in 2001, which shortened the regulatory approval times for clinical trials to sustain and attract more R&D to Canada. The previous 60 day default review time for all trials is now changed into 7 days for bioequivalence (BE) and phase I trials involving healthy volunteers, and 30 days for other trials. Meanwhile, the new regulations also strengthened safety measures to protect participants through inspection and Research Ethics Board review, which may be seen by some as a barrier for clinical research to some extent. This study aims to assess the impacts of the new regulations on R&D activities in Canada.

DESIGN/METHOD/DESCRIPTION: This is the first formal study to evaluate the impacts of the 2001 new regulations. Records of all clinical trials applications from 1996 to 2007 were extracted from the Drug Submission Tracking System of HPFB. Trends in number of applications by phase, by sponsor type (Canadian, European, American or other) are compared between 1996-2001 and 2002-2007. In addition, we used a classical Chow test to verify if a structural break was present at the time of this policy change in the quarterly time series data.

OUTPUTS/RESULTS: A break point was detected around the time of the policy change in the time series data. Results indicate that the shortened approval time for clinical trials increased levels of foreign investment in all phases of clinical research in Canada, with increases in BE trials being the most significant. Canadian firms have apparently increased levels of BE studies as well, but appear to be performing fewer of more costly phase II & III trials in Canada.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The new regulations appear to have succeeded in attracting more foreign investment in clinical research to Canada, but it seems more needs to be done to sustain the levels of more costly confirmatory trials in Canada. A number of factors could have been behind this slight decline in phase II and III trials by Canadian firms, e.g. the insignificance of the change from 60 days to 30 days, perceived difficulties in dealing with the REBs, growing competition from developing countries, etc, but more research is needed to confirm.

2.01 Investigating Assumptions in Regulatory Toxicology: Comparison of *In Vitro* Versus *In Vivo* Toxicologic Response Using Laser Capture Microdissection

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SUMMARY: Validation of *in vitro* assays requires characterization of *in vitro* versus *in vivo* response in matched cell types. The FE1 cell line was derived from Muta™Mouse lung. The data suggest that FE1 cells are transitional, but have retained metabolic responsiveness to polycyclic aromatic hydrocarbons and could be used in studies examining metabolic transformation.

BACKGROUND: Thorough characterization of a cell line is essential prior to its application for *in vitro* toxicological assays; including the evaluation of potentially toxicologically-relevant changes in gene expression compared with corresponding parental tissue.

METHOD: We compared the effects of benzo[a]pyrene (BaP) on gene expression in FE1 cells with alveolar (AV) and terminal bronchiolar (TB) epithelial cells collected *in situ* by laser capture microdissection (LCM) from Muta™Mouse lungs along with whole tissue slices (TS) using Agilent 44K arrays. Differentially expressed probes between exposed and control samples were identified using the per gene variance estimator (F1 test) with sample shuffling using the maanova library in R. The p-values from statistical tests were adjusted using the FDR approach. Using analysis software (Genespring GX), significantly changing genes in each cell type were compared and biological significance based on response to exposure were inferred.

RESULTS: There were significantly more genes changing in the FE1 cell culture (30% of genome) compared to the lung LCM groups (less than 1%) in response to BaP exposure. In pairwise comparisons, FE1 shared 68 differentially expressed genes in common with TB cells (223 changing in total in the TB) and 20 with AV (of 65). Only one gene, Nrf2 (nuclear factor, erythroid derived 2, involved in stress response) was differentially expressed in all cell types. Within the stress response category, 449 genes response were differentially expressed in FE1 cultures compared to 11 in the LCM groups.

CONCLUSIONS: The data suggest that FE1 cells are transitional epithelial cells and have retained phase I metabolic responsiveness to polycyclic aromatic hydrocarbons. However, they also have additional responses that could be due to culture conditions alone. It is crucial to determine the general physiological responses of cells *in vitro* from chemical exposure before inferring their success as an assay to replace traditional animal models.

2.02 Investigating the Effects of Dissolution Media on Metal Solubility from Particulate Matter

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SUMMARY: The dissolution of particulate matter (PM) metals of concern to the Chemical Management Plan (CMP) and *Canadian Environmental Protection Act* (CEPA) is examined in various conditions. This investigation identifies factors influencing the interaction of PM metals with biological fluids as well as ways to improve exposure assessment to these metals.

OBJECTIVES/BACKGROUND/ISSUE(S): Solubility influences biological effects of metals. This study aims at understanding their behaviours when PM interacts with biological media.

DESIGN/METHOD/DESCRIPTION: All determinations (total and dissolved metals) were performed by ICPMS (Inductively Coupled Plasma Mass Spectrometry). The solubility was investigated by 4 sequential batch experiments with contact times of 1 h, 1 day, 4 days and 8 days. In addition to pure water, dissolution media containing the main physiological electrolytes were used. These dissolution media were adjusted at pH 7.3 and 4.5 to reflect the sequence of extra- and intra-cellular pH that could be encountered by the particles. Various acid-base systems were used for pH adjustment.

OUTPUTS/RESULTS: PM samples were examined for CMP/CEPA metals (e.g., Ni, Cu, Ag, Cd, Sb, Pb). For the first contact time (1 h), there were generally no large differences between the various conditions, with however exceptions regarding the media (pure water) or metals (e.g., Ag). For the subsequent contact times, the dissolution rates were largely different, depending on both experimental conditions and metals. At pH 4.5, metals presenting low solubility at pH 7.3 were also not completely dissolved after two weeks. A comparison of different buffering systems to adjust this pH indicated that the kinetics depended on the base used (EDTA, citrate, acetate, pyridine). For the same metal (e.g., Ni from smelter emissions), the base effects varied with the samples, resulting in similar partly dissolved amounts with different kinetics but also in smaller dissolved amounts.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The large difference in solubility results for pure water questions the pertinence of using water alone for monitoring purpose. The observation of variable behaviours for persistent insoluble metals at low pH suggests numerous questions about their biological fate and impact. These results contribute to understand the behaviours of PM metals and to assess exposure in the course of monitoring, toxicological or epidemiological studies.

2.03 Nutrient and Chemical Interactions: Mercury Exposure in a Costa Rican Population and the Potential for Risk Reduction with the Consumption of Antioxidant Food Items

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SUMMARY: A collaborative doctoral research project was established between Health Canada, the University of Ottawa, and the Environmental and Occupational Health Institute at the Universidad Nacional (UNA) in Costa Rica. The average levels of mercury (Hg) exposure in an urban Costa Rican population and the main dietary factors contributing to this exposure were determined. This epidemiological study builds upon concurrent mercury exposure research conducted at Health Canada.

OBJECTIVES: 1) To quantify Hg exposure in an urban Costa Rican population by hair sampling, and; 2) To explore the effect of nutrient factors on expected mercury exposure from fish consumption using a dietary questionnaire.

METHODS: Informed consent, hair samples and dietary questionnaires were collected from individuals at UNA (n=65). Commonly consumed fish were collected from the local supermarkets. Fish and hair samples were analyzed for total mercury (THg) by high temperature combustion atomic absorption spectrometry. Statistical analysis was conducted with SAS.

RESULTS: The average hair THg was 3.1 PPM. High average tuna consumption rates (1.5/week) and high average THg concentrations in tuna (0.85 PPM) both exceed Health Canada's guidelines. Based on each individual's consumption of fruits, vegetables, and teas they were grouped into low, medium, or high antioxidant consumers (0 to 13, 14 to 20, 21 to 28+ portions a week, respectively). Low antioxidant consumers had approximately two times higher hair mercury levels than high antioxidant consumers with the same mercury exposure from fish consumption.

CONCLUSIONS: High hair THg may indicate an elevated risk of developmental deficits in this population according to recent studies. Despite the small sample size, these preliminary results suggest the consumption of antioxidant food items were able to reduce the mercury body burden from fish consumption. This may provide an intervention to reduce the risk of mercury exposure while maintaining the nutritional benefits from fish consumption. A confirmatory study will be conducted in winter 2009.

2.04 Highlights from the 2007 Ontario Student Drug Use and Health Survey

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SUMMARY: In the 2007 OSDUHS report, alcohol was the most common drug used, followed by cannabis. Long-term trends show the peak years for drug use were 1979 and 1999, and most drug use was relatively lower in 2007, with a few exceptions. New concerns over prescription opioid misuse have emerged.

OBJECTIVES: To assess current drug use and related problems among Ontario students in 2007, and trends since 1977.

METHODS: Data are from the OSDUHS- a biennial ongoing student survey. In 2007, anonymous questionnaires were completed in-class by 6323 Ontario students in grades 7 to 12. Outcome measures included annual prevalence for alcohol, tobacco, and 22 other drugs, problem-use of alcohol and drugs, drinking-driving and cannabis-driving. Drug use estimates based on 16 cross-sectional surveys from 1977 to 2007 were also examined among grades 7, 9, and 11.

RESULTS: In 2007, alcohol (61%) was the most common drug used, followed by cannabis (26%), prescription opioid pain relievers (21%; non-medical use), and cigarettes (12%). Past year use of solvents, hallucinogens (i.e., mescaline or psilocybin), and tranquilizers was reported by about 6% of students. The remaining drugs were used by fewer than 5%. Past-month binge drinking was reported by 26% of students; drunkenness by 24%, and 19% indicated hazardous/harmful drinking. Eighteen percent may have a drug-use problem, and 3% of students indicated cannabis dependence. Among drivers, 12% reported drinking-driving in the past year, while 16% reported cannabis use and driving. Long-term trends show the peak years for drug use were 1979 and again in 1999, and most drug use was relatively lower in 2007, with the exceptions of solvent use and binge drinking. The prevalence of smoking and LSD use are at all-time lows.

CONCLUSIONS: A continuing decline in cigarette smoking was a positive finding. Other student drug use was currently lower compared to earlier decades, but problems still remain (e.g., risky drinking). New concerns over prescription opioid misuse have emerged. These results inform policy makers about current drug use trends among youth and help steer government prevention strategies.

2.05 Variation of Soil Radon Concentrations in Southern Ontario

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SUMMARY: This study reports on a transect survey of natural background variation in soil radon levels across southern Ontario from Ottawa to Sarnia. The results indicate that radon risk could be high in some areas of southern Ontario.

OBJECTIVES/BACKGROUND/ISSUE: Radon has been identified as the second leading cause of lung cancer after tobacco smoking. Information on indoor radon concentrations is required to assess the lung cancer burden due to radon exposure. However, radon data in highly populated southern Ontario are very limited. Since radon in soil is the main source of radon in homes, measurements of soil gas radon concentrations can be used to estimate variations in radon potential of indoor environments. A preliminary study of natural background variation in soil radon levels was carried out to assess the potential variability for indoor radon across southern Ontario from Ottawa to Sarnia.

DESIGN/METHOD/DESCRIPTION: Soil radon concentration and soil gas permeability were measured at 32 sites spaced at mean intervals of 40 km along a transect spanning southern Ontario between Ottawa and Sarnia. The sites were selected to represent bedrock, parent material, and soil characteristics typical of the region surrounding the sampling site. Soil radon was determined by measuring the radioactivity of soil gas samples extracted 80 cm below the ground surface. *In-situ* soil gas permeability measurements were performed at the same depth before extracting soil gas for radioactivity measurements. At most sites, soil gas permeability and radon concentration were measured at five different locations using probes at each corner and in the centre of the survey area about 10 x 10 m². A soil radon potential (SRP) index was defined to model the risk: $SRP = (C - C_0) / (-\log(P) + \log(P_0))$, where C is the radon concentration in soil gas in units of kBq m⁻³, and P is the soil permeability in units of m². C_0 and P_0 are set to 1 kBq m⁻³ and 1·10⁻¹⁰ m², respectively.

OUTPUTS/RESULTS: For each site, the *SRP* index was determined with the average soil radon concentration and average soil permeability measured on that site. The *SRP* indexes varied from 1 to 80 among the 32 sites on the transect from Ottawa to Sarnia.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Except for the city of Toronto, information on indoor radon concentrations is generally unknown for most areas in southern Ontario. Results from this study indicate that radon risk could be high in some areas of southern Ontario. This project provided important information and the results were used to prioritize areas of detailed survey in 2008.

2.06 Uranium Gastrointestinal Absorption Coefficients for Young Children

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SUMMARY: The absorption fraction (f_1) is an important parameter in risk assessment of uranium burdens from ingestion. An analysis of 73 bone-ash samples for young children (0 - 7 yr) has revealed the absorption coefficients of 0.093 ± 0.113 for infants (0 - 1 yr), and 0.050 ± 0.032 for children of 1 - 7 years of age.

OBJECTIVES/BACKGROUND/ISSUE: Uranium is ubiquitously found in drinking water and food. For risk assessments due to uranium ingestion, the International Commission of Radiological Protection (ICRP) recommended an f_1 value of 0.04 for infants and 0.02 for anyone more than 1 year of age. This recommendation was based on animal studies and some human studies in adults. The purpose of this study is to determine the f_1 values for chronic intake of uranium for young children of various age groups.

DESIGN/METHOD/DESCRIPTION: Human bone samples were collected by Health Canada between 1957 and 1980 as part of a program to monitor strontium-90 fallout from nuclear weapons testing. The samples were taken from autopsies performed at various hospitals across Canada. In order to determine uranium absorption coefficients, a total of 73 bone-ash samples were selected for children ranging in age from 0 to 7 years residing in a Canadian community known to have an elevated level of uranium in its drinking water supply. These were analyzed for total uranium by inductively coupled plasma mass spectrometry (ICP/MS). With estimated daily intakes, uranium in bone was calculated with the ICRP biokinetic model for uranium under the condition of unit absorption. The absorption fraction (f_1) can be determined as the ratio of the measured uranium to the calculated uranium.

OUTPUTS/RESULTS: The analysis of 73 bone-ash samples gives estimated absorption coefficients of 0.093 ± 0.113 for infants, and 0.050 ± 0.032 for young children of 1 - 7 years of age. The estimated uranium GI absorption coefficient in the first year is about a factor of 2 higher than that for the following years, however, they are also about a factor of 2 higher than the values recommended by the ICRP.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Since the ICRP recommended values were mainly based on animal studies and limited human data for adults, this study was able to provide direct estimates of human absorption coefficients for ingested uranium for young children of two age groups from 0 to 7 years of age.

2.07 An Overview of Radiation Doses Due to Cosmic Ray Exposure in Canada

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SUMMARY: Radiation doses due to cosmic ray exposure depend on solar activities and geographical locations. The population weighted average cosmic-ray annual effective dose is about $298 \pm 47 \mu\text{Sv}$ based on calculations for more than 1500 communities across Canada and more than 85% of the population.

OBJECTIVES/BACKGROUND/ISSUE: It was estimated for worldwide average that exposure to cosmic rays contributes to about 20% of the radiation dose from natural sources. Annual doses due to cosmic ray exposure depend on solar activities and geographical locations. Cosmic rays are more intense at higher altitudes and latitudes. Canada is a vast country. The intensity of cosmic rays can vary significantly for different locations. This study provides an overview of annual effective doses due to cosmic ray exposure in Canada in comparison with the worldwide average.

DESIGN/METHOD/DESCRIPTION: This study is designed to assess annual radiation doses due to ground level cosmic ray exposure for Canadians at various geographic locations. The detailed background information of this kind does not exist yet. This study aimed to cover more than 85% of the population. To reach the population coverage, a total of 1507 communities across Canada were identified. For each community, one central location (a town hall, a bank, a post office, or a school) was geo-coded with latitude, longitude and elevation. Calculations of the annual effective dose to ground-level populations were executed using the code PARMA. Six major particles were considered in the calculations. They were protons, alphas, neutrons, negative muons, positive muons, electrons and photons.

OUTPUTS/RESULTS: The population weighted average cosmic-ray annual effective dose is about $298 \pm 47 \mu\text{Sv}$ at solar maximum, with a range from $267 \mu\text{Sv}$ in Saint-Amable, Quebec to $721 \mu\text{Sv}$ in Mt. Lorne, Yukon. Detailed results are available for more than 1500 communities across Canada covering more than 85% of the population. The average annual effective dose differs from solar minimum to solar maximum by about 10%.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This study provided the first overview of cosmic-ray annual effective doses to Canadians at various locations. Since radiation can cause cancer, results of this study can provide information to identify areas of higher risk.

2.08 Guidelines for Chip-chip Pre-Processing and Analysis

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SUMMARY: We have used an innovative technique to identify transcription factor binding sites, the locations of DNA involved in up and down regulations of genes. The current report describes the optimization of bioinformatics and statistical processes to identify these regions, which will be gathered for further analysis.

BACKGROUND/OBJECTIVES: Many commercial substances (including brominated flame retardants, antimicrobial agents and their metabolites) and environmental contaminants (PCBs, dioxins) impair thyroid hormone receptor activation and thyroid hormone physiology. Given the importance of thyroid hormone action for normal development of diverse tissues, characterization of thyroid hormone response elements (TREs) in DNA will lead to novel understanding of the adverse impacts of these substances and to the development of rapid tools to screen for thyroid hormone disruption. The use of Agilent ChIP-chip promoter arrays required an investigation on the appropriate pre-processing and normalization methods. Secondly, an evaluation and adaption of existing analytical tools for use with Agilent ChIP-chip promoter arrays is required.

METHODS: Data were normalized using a variety of techniques including sequence-based normalizations, quantile normalization, mixed models, and no normalization. The processed data were then interrogated using Hierarchical Gamma Mixture Models (HGMM), Algorithms for Calculating Microarray Enrichment (ACME) and by Bayesian Analysis of Chip-chip (BAC) experiment.

RESULTS: Since the principle behind Chip-Chip is different than gene expression experiments, experimental designs well established for GE are not adequate for downstream analysis for Chip-chip. Studying the effects of normalization methods proved beneficial as it allowed us to identify the optimal method to correct for biases in our data and to eliminate spurious sequences. For detection of enriched regions, we found that BAC identified an unrealistically large numbers of sites while statistical assumptions underlying ACME are not valid for this type of data. However, HGMM was found to be an appropriate method, since the input parameters where customizable were customizable to our type of data.

CONCLUSIONS: Here, we established a standard procedure for future analysis. Conducting Chip-chip experimentation without proper experimental design, normalization and algorithms to identify enriched regions could result in biased analyses and spurious results. Therefore, it is critical that the analysis and interpretation of Chip-chip results for identifying targets for bioassays implement the appropriate approaches.

2.09 Mutagenicity and Dioxin-Like Activity of Biodiesel Emissions

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SUMMARY: Current efforts to reduce the adverse health effects of diesel emissions include the use of novel fuels. This study employed two assays to evaluate the toxicity of emissions generated using biodiesel fuels. The results obtained in this study support the hypothesis that biodiesel emissions are less mutagenic than diesel emissions.

OBJECTIVES: The aim of this study was to assess the toxicological properties of the soluble organic fraction of (bio)diesel emissions generated using various after-treatment technologies (i.e., diesel oxidation catalyst and exhaust gas recirculation) and diesel and biodiesel-blended fuels (i.e., derived from soy, canola, and animal fats).

DESIGN: Diesel emissions generated using diesel and biodiesel fuels were collected on Teflon-coated fiberglass filters and polyurethane foam plugs via a constant volume dilution tunnel. The soluble organic fractions were extracted using pressurized fluid extraction and the adsorbed organics were separated on open silica into polar aromatics and non-polar neutral compounds. Mutagenic (i.e., ability to induce mutations) and dioxin-like activities (i.e., induction of the aryl-hydrocarbon receptor pathway) were assessed using the *Salmonella* mutagenicity assay and the dioxin responsive (DR)-chemically activated luciferase expression (DR-CALUX) assay respectively.

OUTPUT/RESULTS: Results indicated that organic extracts of (bio)diesel particles contain direct- and indirect-acting polar aromatic mutagens as well as polar and non-polar Ah-receptor agonists. A reduction in the mutagenic activity of direct-acting compounds was observed for the polar aromatic fraction with increasing biodiesel content in the fuel (e.g., 48% reduction for biodiesel blend B20 compared to ULSD on *Salmonella* TA98 without metabolic activation). Conversely, an increase in dioxin-like activity with increasing biodiesel fuel content was observed for both the polar and non-polar fractions (e.g., 144% increase for the non-polar fraction and 111% increase for the polar aromatic fraction of biodiesel blend B20 compared to ULSD).

IMPACTS/OUTCOMES/CONCLUSIONS: The results obtained in this study support the hypothesis that the use of alternative fuels (e.g., biodiesel blends) and after-treatment devices reduce the risk of adverse health effects associated with diesel exhaust emissions. These results will provide a framework for evaluating the toxicological hazards of biodiesel emissions, and eventually identify fuel choice and engine design scenarios that minimize the risks of adverse health effects.

2.10 Selective Exposure of Humans to Ambient Contaminants: A Novel Approach to Isolate Effects of Air Pollutants

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SUMMARY: This work describes a novel method for selective removal of ambient toxic contaminants by using specific filters to investigate the role of air pollutants in acute human health effects.

BACKGROUND: In Canada, ambient air pollution is responsible for ~ 6000 cardiopulmonary deaths each year (exceeding deaths from breast cancer, ~5000; prostate cancer, ~4000; car accidents, ~1000) with societal costs estimated at \$1 billion a year. Agents of effects include particulate matter (PM), ozone, oxides of nitrogen (NO_x), and volatile organic compounds (VOCs). Furthermore, WHO estimates that 30% of new and remodelled buildings worldwide have poor indoor air quality leading to “sick building syndrome”. It is extremely difficult to attribute effects clearly to individual substances in such complex atmospheres. We urgently require an approach that allows to decompose exposures in human toxicological field studies.

APPROACH: We have evaluated an approach to isolate specific pollutants such as particulate matter (PM), organic vapours, acid gases, nitrogen dioxide and sulphur dioxide from ambient air by means of specific filters. We used a positive air pressure respiratory protection system (3M Breathe Easy Turbo PAPR Assembly) consisting of a helmet that is fed air at a flow rate of 4-15 cfm from a belt-mounted blower/filtration assembly holding 3 filters and a battery pack.

RESULTS: (1) HEPA filters removed 99% particulate matter and ultrafine particles but did not remove any of the gases (ozone, carbon monoxide, nitric oxide and nitrogen dioxide). This filter is useful to separate effects of PM from gas phase components. (2) Combined organic vapour, acid, chlorine and sulphur dioxide filter sets removed 98% of nitrogen dioxide (NO₂). Although this filter set intercepts 60% of particles, sufficient mass flow of fine and ultra fine particles remains to study PM effects without nitrogen dioxide (NO₂) interaction. (3) Combined organic vapour, acid, chlorine, sulphur dioxide and particulate matter filters removed 99% particulate matter, ultrafine particles and nitrogen dioxide. This filter set can be used to create a surrogate clean air environment devoid of PM, VOCs and nitrogen dioxide contaminants. (4) None of the filter sets could remove sufficient mass of carbon monoxide, ozone or nitric oxide. Custom-made cassettes may need to be developed to extract these components.

CONCLUSIONS: This approach allows us to investigate in the field several issues of enormous regulatory significance, such as the relative potency of ambient sources of particulate matter, their toxicological interaction with other criteria co-pollutants and the causes of the sick building syndrome.

2.11 Cytokine IL6/Stat3 Pathway is Induced in Response to Mainstream Tobacco Smoke in Murine Lungs: Validation of Genomics Data

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SUMMARY: High density DNA microarray analysis of lung tissues from mice exposed to mainstream tobacco smoke (MTS) revealed induction of inflammatory cytokine interleukin-6 (IL-6) and its antagonist suppressor of cytokine signalling (SOCS3) mRNA. Our findings suggest prevalence of one pathway against the other could determine the outcome of smoking in an individual.

OBJECTIVES: 1. Test the hypothesis that MTS induced transcriptional changes in lungs will be predictive of eventual health outcome of smoking. 2. Validate biological implications of Genomics data and its use in regulatory toxicology.

DESIGN: Male C57B1/CBA mice were exposed to MTS from two cigarettes daily, 5 days/week for 6 or 12 weeks. Mice were sacrificed immediately, or six weeks following the last cigarette. Left lung lobes were removed and flash frozen. Total RNA was isolated from a small part of the frozen lung and was hybridized against universal mouse reference RNA to Agilent Oligo DNA microarrays (Agilent Technologies) containing 22,000 transcripts. Microarrays were normalized using a global LOWESS approach and analyzed by MAANOVA 2.0 and SAM. Microarray results were validated by real time RT-PCR. Impact of alteration in expression of select genes was further validated by analysing their total protein levels in lung tissue homogenates.

OUTPUT/RESULTS: Seventy-five genes were significantly differentially expressed following exposure to MTS and are associated with a number of biological processes including xenobiotic metabolism, redox balance, oxidative stress and inflammation pathway. Interestingly, we found transcriptional upregulation of cytokine IL-6 and its antagonist SOCS3 in smoke exposed animals. Further analysis of total lung tissue extracts by immunoblotting revealed concomitant increase in IL-6 antigen and associated downstream targets including phosphorylated signal transducer and activator of transcription 3 (Stat3), basal cell-lymphoma extra large (BCL-XL) and myeloid cell leukemia 1 (MCL-1) protein in total lung tissue extracts.

IMPACTS/OUTCOMES/CONCLUSIONS: This work identifies a novel mechanism by which MTS influences inflammatory pathway and identifies several candidate biomarkers of potential adverse effects of MTS. Our findings also shed new light on role of IL-6 and SOCS3 in lung injury and inflammation in response to MTS and suggest how expression or suppression of select genes in a pathway determines the outcome of smoking.

2.12 Vapour Intrusion Guidance for Risk Assessment at Contaminated Sites: A Health Canada 2008 Update

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SUMMARY: The Contaminated Sites Program has developed written guidance and two spreadsheet tools in order to assist in the estimation of human health risks related to the vapour intrusion exposure pathway at contaminated sites.

OBJECTIVES: The transport of vapour phase organics from the sub-surface into buildings through vapour intrusion can pose a significant human health concern at federal contaminated sites. The Contaminated Sites Program objective was to develop guidance for assessing this pathway, consisting of written guidance and two spreadsheet tools.

DESIGN: The written guidance recommends an approach for assessing vapour intrusion and provides a methodology for calculation of groundwater and soil vapour screening levels. The spreadsheet tools are designed to facilitate the estimation of human health risks associated with the vapour intrusion pathway. Inherent in the written guidance and spreadsheet tools are key distinctions which have been made between default assumptions deemed appropriate at the preliminary quantitative as compared to the detailed quantitative risk assessment (PQRA; DQRA) levels. These may impact remedial options available to federal custodians of contaminated sites under the Federal Contaminated Sites Action Plan (FCSAP).

OUTPUT/RESULTS: The key guidance output was the derivation of semi site-specific attenuation factors using the Johnson & Ettinger model. It also establishes the minimum site investigation data needed to assess the vapour intrusion pathway while emphasizing the need for multiple lines of evidence. The guidance specifies site conditions deemed to be precluding factors for the assessment of vapour intrusion using modeled attenuation factors.

OUTCOMES/CONCLUSIONS: The primary outcome is the provision of guidance for assessing the vapour intrusion pathway. The guidance establishes a standard of quality for vapour intrusion assessment and facilitates the standardization of this type of assessment at federal contaminated sites.

Existing data gaps in the science of vapour intrusion pose challenges to decision-making for government, non-governmental and private sector stakeholders. Research undertaken by the Contaminated Sites Program to address data gaps in the area of biodegradation of petroleum hydrocarbons and the impact of northern climates is also presented as the secondary outcome.

2.13 Comparison of Cytotoxicity Bioassays for Toxicological Assessment of Environmental Contaminants

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SUMMARY: We compared a set of *in vitro* cytotoxicity bioassays based on different mechanisms of toxicity for toxicological assessment of a panel of chemicals including carcinogens. Toxicity thresholds, the doses at which toxicity started to appear, and toxic potencies calculated from the slope of dose-response curves varied for individual chemicals across all assay platforms. Existence of such variations should be considered during *in vitro* cytotoxicity screening and standardization of cell lines based on toxicity for downstream proteomic analysis.

BACKGROUND AND ISSUES: The use of cell culture based assays is increasingly common in the toxicological assessment of environmental contaminants, with good correlations often observed between *in vitro* cytotoxicity and acute lethal potency of a variety of chemical substances. Within the context of human health risk assessment, cell culture bioassays can be useful in toxicity screening of a large number of chemical substances including complex mixtures. In this work, we compared six different cytotoxicity assay platforms for the toxicological characterization of a set of chemicals including those of health relevance (*viz.* carcinogens) with an aim to increase our understanding of the application of these assay platforms as screening and standardization assays.

METHODS: An established human hepatocellular carcinoma cell line (HepG2) was exposed for 24 hr to selected chemical substances at a dose range of 0-1.6mM and cytotoxicity was assessed. The assays assessed toxicant effects on cell membrane integrity as measured by the leakage of intracellular lactate dehydrogenase (LDH assay) and lysosomal uptake of Neutral Red (Neutral Red Assay), on energy metabolism as measured by mitochondrial dehydrogenase activity (MTT assay), cellular levels of ATP (ATP assay), generation of extracellular reduced microenvironment (Alamar Blue Assay) and on cell proliferation (BrdU Assay).

RESULTS: Toxicity dose-responses of test chemicals summarized as toxic potencies generally ranked carcinogens higher than non-carcinogens, in spite of the variations in the ranking of individual chemicals by different assay platforms. The toxicity thresholds (doses at which toxicity started to appear) for individual chemicals on assays based on different mechanisms also varied.

CONCLUSIONS: The results suggest that the different cellular mechanisms of toxicity observed are a function of the chemistry and dose and this should be a consideration during selection of assay platforms for standardization of cell culture for downstream analysis, and for the toxicity screening of a large number of chemicals. Overall, this work provides an important assessment of the utility of cytotoxicity assay platforms in human health risk assessment of environmental contaminants.

2.14 Tools/Approaches to Address Human Health Risk Assessment of Data-Poor Chemicals

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SUMMARY: Regulatory risk assessment of substances with limited hazard and exposure data is challenging. Regulatory bodies are being required to develop efficient, innovative approaches for examining large numbers of data-poor chemicals, in lieu of traditional lengthy, resource intensive assessments. An overview of approaches used in Canada and other jurisdictions is presented.

OBJECTIVES/BACKGROUND/ISSUES: Existence of data-gaps pertaining to hazard and exposure potential of a large number of existing substances pose a major challenge for regulators undertaking human health risk assessment. Integration of novel international tools viz. OECD QSAR Application toolbox, Toxmatch, Ambit with in-house approaches, which combine various computational models and methodologies, can potentially help to fill in data-gaps as well as build a platform for carrying out robust risk assessments of data-poor substances.

DESIGN/METHOD/DESCRIPTION: The concepts and principles behind the existing in-house hazard and exposure assessment tools as well as those developed by other regulatory agencies are described. Each of these is evaluated for parameters including ease of operation, available features, and types of similarity analysis that could be performed. Methodologies to integrate the different approaches/tools for developing a robust risk assessment strategy are discussed.

OUTPUTS/RESULTS: The advantages of using these tools in an integrated manner as opposed to their independent use are evaluated in the light of traditional resource intensive and lengthy risk assessments conducted on chemicals which have sufficient empirical data on their hazard and exposure levels.

OUTCOMES/IMPLICATIONS: These approaches/tools will aid in filling data-gaps for data-poor chemicals. The information obtained is expected to serve as indicators of potential hazard and exposure to humans, and therefore supplement conventional risk assessment methodologies. This approach will further support decision making for risk management of such chemicals.

2.15 Potential Use of Shot-Gun Peptidomics/ Proteomics Analysis in Screening Particles for Potency Assessment

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SUMMARY: Toxicity of particles with varying physicochemical characteristics was investigated by *in vitro* exposure experiments with different lung cell lines. Cellular cytotoxicity tests and peptidome/proteome profiles implied marked differences that can be attributed to physicochemical characteristics of the particles and reflected potential toxicity of these materials.

OBJECTIVES: Exposure to particulate pollution has been well associated with adverse cardiovascular health outcomes through epidemiological studies. There are also new findings suggesting that exposure to ultra fine particulate matter (PM) can lead to neurodegenerative effects. Yet, particle toxicity mechanisms are still unclear. Particulate air pollutants exhibit complex chemical compositions based on source of emissions, atmospheric reactions and size characteristics. In order to understand particle toxicity in terms of their physico-chemical characteristics the most logical initial high-throughput assays to follow are *in vitro* exposure experiments. A peptidomic/proteomic analysis method was developed to screen cell lysates as a tool to map particle toxicity.

DESIGN: We selected 10 particulate materials including TiO₂, SiO₂, urban dust samples, diesel exhaust particles (DEP), carbon black and a PM_{2.5} sample, and exposed J774 (mouse alveolar macrophage) and A549 (human lung epithelial) cell lines to these materials. Exposure experiments were carried out for 24h at particle doses in the range of 0-100 µg/cm² (96-well plates) in serum-free medium. Shot-gun peptidomic/proteomic analyses of cell lysates were carried out by direct MALDI-TOF-TOF Mass Spectrometry using alpha-cyano-4-hydroxycinnamic acid as matrix. The mass spectral profiles were interrogated in the region <6kDa. This region was selected as we get very good resolution over in this region with MALDI-TOF-TOF-MS during mass spectral profiling applying the shot-gun peptidomic/proteomics method that we chose to use in this study.

OUTPUTS/RESULTS: Peptide/protein patterns generated using this method clearly indicated particle and cell-dependent changes in the mass spectral profiles. Even though, all particle exposures exhibited signature mass spectral profiles it was interesting to note some common features in the mass spectral data for particles with similar chemical profiles. For example, J774 cells exposed to urban dust particles exhibited increased expression of mass spectral peaks at m/z of 1.48 kDa and in the region of 1.8-2.0 kDa, and decreased intensities in the region of 2.2-2.8 kDa, when compared to control experiments in which the cells were not exposed to any particle. Analysis of macrophages exposed to DEP led to increased mass spectral peak intensities in the mass range of 1-3kDa compared to control. While urban particles exhibited similar biomarkers identified by data-mining using ClinPro Tools software, SRM 1650 and 2975 exhibited similar DEP specific markers. Such characteristic peptide/protein profile changes with respect to particle exposures can

shed light on particle-induced biological responses and assist in resolving early toxicity mechanisms.

IMPACTS/OUTCOMES/CONCLUSIONS: Characterization of these biomarker peptides/proteins and their inter-relationships along with particle chemistries should provide new insights into the toxicity mechanisms. This will allow estimation of toxicity potencies associated with these particles and can be useful as a screening tool for particles-related risk assessment studies.

2.16 A Novel Mutagenic Potency Ratio Method to Assess the Excess Lifetime Cancer Risk of Complex PAH Mixtures in Contaminated Soils

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SUMMARY: We employed a novel method to estimate cancer risk for chemical mixtures at contaminated sites, and showed that conventional methods used to estimate the excess lifetime cancer risk for polycyclic aromatic hydrocarbons (PAH) mixtures in contaminated soil appear to be conservative.

OBJECTIVES/BACKGROUND/ISSUES: The objective of this study was to use a novel mutagenic potency ratio (MPR) approach to assess the excess lifetime cancer risk posed by PAH-contaminated soils. Traditional cancer risk assessments for complex mixtures are based on either surrogate mixtures, or the concentrations and relative potencies of known carcinogens in the mixture. For the latter, risk is estimated as the sum of the risks attributable to each known carcinogen in the mixture.

DESIGN/METHOD/DESCRIPTION: The mutagenicity of non-polar and semi-polar aromatic fractions from ten soils was assessed in the *Salmonella* mutagenicity assay and the Muta™ Mouse FE1 *lacZ* transgene mutation assay. The MPR method used mutagenic potencies derived from these assays to infer the magnitude of exposure to a surrogate with known carcinogenic (oral) potency, namely benzo[a]pyrene. This value was then employed to estimate excess lifetime cancer risk for non-dietary ingestion by a construction worker. The estimated risk was then compared to risk calculated using the traditional additive method.

OUTPUTS/RESULTS: When mutagenicity data from the *lacZ* transgene mutation assay were employed, the MPR method consistently underestimated risk by at least 7-fold as compared to the traditional approach, suggesting that current risk assessment practices for complex PAH mixtures are conservative. However, when *Salmonella* mutagenicity data are used, the MPR approach overestimated risk for 9 of the 10 soils examined. This discrepancy may be attributable to the sensitivity of the *Salmonella* mutagenicity assay.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The MPR approach is attractive because it does not require a priori knowledge regarding the identity of mixture components, and does not invoke the assumption of dose additivity. However, it should only be used for complex mixtures containing chemicals with the same mode of action.

2.17 Acute Effects of Air Pollution on Pulmonary Function, Airway Inflammation and Oxidative Stress in Asthmatic Children

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SUMMARY: Using non-invasive methods including exhaled breath condensate, we filled a knowledge gap in air pollution-associated airway oxidative stress and inflammation in asthmatic children. Air pollution may increase airway oxidative stress and decrease small airway function of asthmatic children.

BACKGROUND: Air pollution is associated with respiratory symptoms, lung function decrements, and hospitalizations. However, there is little information about the influence of air pollution on inflammation and oxidative stress measured from exhaled breath samples.

OBJECTIVE: To investigate acute effects of air pollution on pulmonary function and airway oxidative stress and inflammation in asthmatic children using non-invasive methods.

METHODS: We studied 182 asthmatic children, age 9-14 years, for 4 weeks. Daily ambient concentrations of SO₂, NO₂, O₃ and particulate matter (<=2.5 µm, PM_{2.5}) were monitored from two central stations. Once a week we measured spirometry and fractional exhaled nitric oxide (FeNO), and collected exhaled breath condensate to determine concentrations of thiobarbituric acid reactive substances (TBARS), 8-isoprostane and interleukin-6. We tested associations using mixed-effects regression models, adjusting for confounding variables.

RESULTS: Interquartile-range increases in SO₂ (5.4 ppb), NO₂ (6.8 ppb) and PM_{2.5} (5.4 µg/m³), but not O₃ (5.3 ppb), were associated (p<0.05) with 2-4% decreases in percentage of predicted forced expiratory flow between 25% and 75% of forced vital capacity, and 24-35% increases in TBARS, an oxidative stress marker. Use of inhaled corticosteroids was a significant effect modifier; risk estimates appear to be stronger in children who did not take corticosteroids than in children who did. Forced expiratory volume in 1 second, FeNO, and 8-isoprostane and interleukin-6 in breath condensate were not consistently associated with air pollutants.

CONCLUSION/IMPLICATIONS: Air pollution may increase airway oxidative stress and decrease small airway function of asthmatic children. Inhaled corticosteroids may reduce oxidative stress and improve small airway function. Using non-invasive methods including exhaled breath condensate, we improved the knowledge on air pollution-associated airway oxidative stress and inflammation in asthmatic children. Our results were consistent with decrements in small airway function.

2.18 Drinking Water Advisories in First Nations Communities in Canada

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SUMMARY: Drinking water advisories (DWA) are preventive measures to protect public health from confirmed or suspected contaminants in drinking water. This analysis provides a national overview of DWA in First Nations communities. Trends for the period 2003-2007 and seasonal variability were examined.

BACKGROUND: Through the Drinking Water Safety Program, Health Canada works in partnership with First Nations communities in Canada to assist First Nations establish a monitoring program for drinking water quality as per the Guidelines for Canadian Drinking Water Quality. Drinking water advisories (DWA) are preventive measures to protect public health from waterborne contaminants that could be, or are known to be present in drinking water. They include "boil water", "do not consume" and "do not use" advisories.

OBJECTIVE: To describe the frequency, duration and reasons for issuance of DWA in First Nations communities in Canada.

METHOD: Reported regional DWA data from the years 1995-2007 were compiled to form a national dataset. Advisory variables included type; reason(s) for issuance; date set; date lifted. Summary statistical analyses were performed in June 2008. As reporting is more consistent since 2003, trends were examined for the period 2003-2007.

RESULTS: The median duration of DWA was 38.5 days. The most commonly cited reasons for issuance of DWA were unacceptable microbiological quality, inadequate disinfection and equipment malfunction. Most remedial actions required to rescind DWA were related to operational issues such as disinfection and equipment malfunction. The number of DWA in effect increased during the period 2003 and appears to stabilize in 2007. Implementation of increased water sampling and other interventions emanating from the First Nations Water Management Strategy since 2003-04 may contribute to this increase. Preliminary analysis suggests seasonal effects in DWA issuances.

IMPLICATIONS: It is anticipated that this and planned regional DWA analyses will help inform drinking water policy, program and First Nations decision makers in where best to focus resources in addressing the underlying causes of drinking water advisories.

2.19 The Mutagenic Activity of High-Energy Explosives, Contaminants of Concern at Military Training Sites

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SUMMARY: The genotoxicity of energetic compounds found in contaminated soils at military training sites has not been rigorously tested. TNT and tetryl were clearly mutagenic in *Salmonella*, as were soil samples from CFB Petawawa. RDX and HMX did not appear to be mutagenic in *Salmonella*. TNT and RDX elicited some mutagenic activity in Muta™Mouse FE1 cells. Testing of TNT-degradation products is underway.

OBJECTIVES: To evaluate the mutagenic activity of energetic compounds (i.e., high explosives), that commonly occur, in contaminated soils at military training sites.

DESIGN/METHOD: The *Salmonella* reverse mutation assay, and the Muta™Mouse *in vitro* transgene mutation assay were employed to examine selected energetic compounds and contaminated soil samples. *Salmonella* strains TA98 (frameshift), TA100 (base-pair substitution), and metabolically enhanced strains YG1041 (TA98 background) and YG1042 (TA100 background), with and without exogenous metabolic activation (S9), were employed for the *Salmonella* analyses.

OUTPUTS/RESULTS: TNT elicited a significant positive mutagenic response in all *Salmonella* strains without S9. Mutagenic potencies ranged from 0.87 ± 0.05 to 29.38 ± 1.72 revertants/ μg TNT, with the highest values obtained using TA100 and YG1042. Tetryl elicited a significant positive response in all strains with and without S9. Potencies ranged from 1.27 ± 0.09 to 47.32 ± 2.73 revertants/ μg tetryl, with the highest values obtained using TA100 and YG1042 without S9. RDX and HMX did not exhibit mutagenicity in any *Salmonella* strain. Soil samples obtained from CFB Petawawa exhibited considerable mutagenic activity in strains TA98 and YG1041, with and without S9. Potencies ranged from 3.48 ± 0.11 to 19.25 ± 1.13 revertants/mg soil, with the highest values obtained without S9. Despite variability in the response obtained from the Muta™Mouse assay, there is evidence that TNT and RDX can induce mutations at the *lacZ* locus in FE1 cells. In contrast, tetryl does not appear to elicit a response. HMX was highly toxic to this cell line. Mutagenicity testing of soil samples obtained from CFB Petawawa on the Muta™Mouse assay is underway.

IMPACTS/OUTCOMES/CONCLUSIONS: Results to date indicate that TNT and tetryl are primarily direct-acting, base-pair substitution mutagens. Soil samples obtained from CFB Petawawa elicit direct-acting and S9-dependant responses, particularly in the frameshift strains. This project indicates the importance of continued testing of energetic materials (i.e., TNT-degradation products), as well as explosives-contaminated soil samples from other military training sites, in order to determine the mutagenic hazard and subsequent human health risk posed to Canadians (i.e., explosives manufacturers and soldiers in training exercises).

2.20 Skin Absorption of Mercury and Nickel Salts from Contaminated Soil: Are Water Soluble Chemicals Absorbed?

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SUMMARY: Skin absorption of toxic metals in soil is of concern for people at contaminated sites. Our lab measured absorption of mercury and nickel in human skin *in vitro*. Results showed mercury and nickel can be absorbed following dermal exposure to spiked soil, and contrary to popular opinion, these polar water soluble chemicals are absorbed.

BACKGROUND/ISSUE(S): It is commonly held that ionic polar species of chemicals such as metals are not absorbed into skin although literature exists to the contrary. Our project investigated dermal exposure to mercury and nickel in soil.

METHOD: Breast skin surgical waste was tested *in vitro* using Teflon[®] flow-through cells with Hanks' modified saline (pH 7.4) as blood surrogate. Testing was conducted with wet gardening soil spiked with chloride salts of radioactive mercury (Hg²⁰³) or nickel (Ni⁶³) with concurrent controls without soil. Samples were analyzed by Liquid Scintillation Counting.

RESULTS: There was 78 % ± 8.1 (n = 4) total absorption of Hg²⁰³ with 77% still present in the skin depot after soap-washing and 1% in the blood surrogate in controls without soil, and 47 % ± 9.4 (n = 4) total absorption with 45% in the depot and 2% in blood surrogate with soil. In contrast the tests with Ni⁶³ gave 23 % ± 8.1 (n = 6) total absorption with 21% in the depot and 2% in blood surrogate without soil, while tests with soil gave only 1% ± 0.4 (n = 6) total absorption, with 0.5% in the depot and 0.5% in the blood surrogate.

OUTCOMES/NEXT STEPS: Contrary to the paradigm that water soluble chemicals are not absorbed into skin, the data for tests with soil showed 47% and 1% total absorption of Hg²⁰³ and Ni⁶³, respectively. These levels are higher than the defaults suggested for use in risk assessment by the Risk Assessment Information System (RAIS) of only 0.1% absorption of mercuric chloride or nickel salts and more data are required to address this issue.

2.21 Identification of Exposure Biomarkers for Mutagenic Carcinogens in Complex Environmental Matrices

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SUMMARY: Occupational or environmental settings are often associated with exposures to complex mixtures. Biomarkers indicating exposure are generally identified for single chemicals. This project investigated alterations in gene expression profiles elicited in response to a mixture. Differential gene expression will be compared to proteome profiles to identify candidate biomarkers of exposure.

OBJECTIVE: The purpose of this project is to illustrate that gene expression profiles can be used as unique, precise markers of complex exposures.

METHODS: Murine lung epithelial cells were exposed *in vitro* to coal tar, a complex mixture of carcinogenic PAHs. After 6 hours cells were collected for RNA extraction and genomic analysis, and the cell media was collected for proteomic analysis. Total RNA was extracted and hybridized to Agilent 22k DNA oligonucleotide mouse microarrays. Concurrently, secreted proteins were purified and analyzed using 2-D gel electrophoresis coupled to MALDI-Tof/Tof mass spectrometry. Subsequent bioinformatic analysis identified elements of genomic signatures and peptide mass fingerprints of secreted proteins were identified using the MASCOT database.

OUTPUT: Genomic and proteomic analyses are ongoing, however, the results obtained to date indicate differential expression of genes involved in energy and xenobiotic metabolism (e.g., cyp1A1), apoptotic pathways, and tumour induction. Approximately 250 spots of secreted proteins were visible on 2-D gels, with forty-five of the spots differentially displayed across treatments. Spots were excised, and identification of the corresponding proteins (i.e., biomarker candidates) is currently underway.

IMPACTS: Source-specific biomarkers for complex environmental mixtures could improve exposure and risk assessment. Results from this *in vitro* pilot project are promising, and indicate that the strategy employed can be used in an *in vivo* setting. However, additional validation would be required before the biomarkers could be used for human biomonitoring.

2.22 Assessment of Sub-Clinical, Toxicant-Induced Hepatic Gene Expression Profiles After Low-Dose, Short-Term Exposures in Mice

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SUMMARY: ToxArray™ (our in-house microarray) provides a sensitive measure of low-dose, adverse changes in gene expression relevant to informing mechanism-of-action, and dose-response issues in risk-assessment. Effects on gene expression induced by the neurotransmitter-mimetic, isoproterenol, were measured at oral doses below those with acute effects on classical toxicological endpoints. Significant effects on gene expression relevant to chronic toxicities were revealed at all doses, using an unconventional multivariate statistical analysis of gene response groups with known biological and toxicological significance.

OBJECTIVES/BACKGROUND/ISSUES: We sought refine dose-response characterizations by using array data as groups of genes within functional categories, rather than as individual genes. Further, we sought to incorporate, into hazard assessments, existing knowledge of adverse effects resulting from gene expression changes.

DESIGN/METHOD/DESCRIPTION: The experiment used five animals per treatment group and five treatments, consisting of a control and four isoproterenol dose groups (0.5 to 250 mg/kg, by gavage). Liver tissue was harvested after 8 hrs. ToxArray™ two-colour hybridizations were conducted with experimental and reference RNA samples. Hybridization results were tested statistically for differences between control and exposed samples, then grouping significantly affected genes by biological function (acute-phase response, angiogenesis, protein synthesis). Analyses used Euclidean distances between the means of the control and exposed samples. The critical region for the test was determined using permutation analysis, making no assumptions about data distribution. For each gene set, if the overall test for treatment differences was significant (i.e. $p < 0.05$) *post hoc* tests were conducted on individual genes. Group testing in this manner weakly controls the type 1 error rate and additionally, this can reveal differences not discovered by per gene tests.

OUTPUTS/RESULTS: The noradrenaline mimetic, isoproterenol, induced significant gene expression changes among members of critical toxicological pathways at doses lower than those required to alter traditional biochemical endpoints. Assessment of likely adverse phenotypic consequences (e.g. atherosclerosis) resulting from changes in RNA expression profiles were made by incorporating information from published functional genomic studies. On this basis, an assignment of a “Lowest Observed Adverse Transcriptional Expression Level” (LOATEL, 0.5 mg/kg) for isoproterenol was made.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: These results support the contention that expression profiles of genes belonging to toxicologically-relevant pathways adversely modifying phenotype, can provide useful tools for reducing uncertainty in establishing no-effect levels and for

discerning relevant end-points to measure in longer-term toxicity studies involving unknown chemicals. In particular, the results provide possible insights into adverse physiological effects resulting from chronic disruption of neurotransmitter levels due to environmental or psycho-social stresses.

2.23 Does Methanol Produced From Biodiesel Metabolism Pose a Health Hazard?

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SUMMARY: Studies were conducted to examine effects of exposure to methanol produced from biodiesel metabolism. Rats fed high doses of biodiesels had minimal increases in blood methanol but no elevation in formic acid, the toxic metabolite. It was unlikely that methanol from biodiesels will produce adverse effects in rats under ordinary exposure settings.

OBJECTIVES: Biodiesels are predominantly methyl esters of fatty acids derived from animal and vegetable oils. Humans are exposed to biodiesels through inhalation, dermal contact, contaminated groundwater, and accidental ingestion. Because of the similarity to cooking oil in appearance and odour, the U.S. DOT (1995) identified oral intake of biodiesel as a potential health hazard. It is not known if methanol released through biodiesel metabolism poses a health hazard. We conducted studies to explore the ability of key body tissues to hydrolyze biodiesels to methanol and evaluated the extent of *in vivo* production of methanol and formic acid from biodiesels.

METHODS:

In vitro studies: Different concentrations of biodiesels (canola, soy, fish, frying oil) were incubated with rat serum, rat liver, and porcine pancreas extract. Methanol produced over a 1-hr period was measured.

Animal study: Rats were fed biodiesels (canola, soy, frying oil) at 5, 50 and 500 mg/kg/day for 4 weeks. At termination, blood levels of methanol and formic acid were measured.

RESULTS: Pancreas extract was the most active in hydrolyzing biodiesels to methanol, followed by the liver and then serum. The ability of biodiesels to serve as a substrate for pancreas extract was canola > soy, frying oil >> fish. After the 4-week treatment, methanol was detected (<0.1 mM) in the blood of some rats on low dose biodiesels. Interestingly, methanol was not detected in animals receiving the high dose biodiesels. Formic acid was not elevated in any of the treated animals.

CONCLUSION: Because pancreas is a main source of digestive enzymes, it is likely that biodiesels entering the GI tract will be effectively hydrolyzed to methanol. Biodiesels entering by dermal or inhalation route will be hydrolyzed at a slower rate in the liver and blood. Although low levels of methanol (<0.1 mM) were detected in some treated animals, none showed an elevation in formic acid, the toxic metabolite. The most sensitive endpoint for methanol reported was developmental toxicity in mice following inhalation exposure. The NOAEL was 1000 ppm which resulted in a serum methanol of approx. 3 mM. Taken together, in non-acute exposure scenarios, methanol from biodiesel is not likely to produce adverse effects. It should be cautioned that humans are more sensitive to methanol than rodents.

2.24 The Study of the Radon Equilibrium Factor in Ottawa Dwellings

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SUMMARY: A study was conducted to observe variations in the “F” factor, or equilibrium ratio between the concentrations of radon progeny and radon gas, in several Ottawa dwellings, some of which were known to have high radon concentrations. Calculated F values ranged between 0.20-0.52 compared to an internationally accepted average of 0.40.

OBJECTIVES: It is reported in 1998 SRA-Europe Annual Meeting that most of the radiation dose is not from radon itself, but from the short-lived alpha particle-emitting progeny. Inhalation of these short-lived decay products at home or work and in outdoor yields the greatest amount of natural radiation exposure to the human. The aim of this pilot study was to establish a realistic radon equilibrium factor “F” in Ottawa dwellings. The study involved the measurement of indoor radon and its progeny and the determination of effective radiation dose.

DESIGN: The study was conducted in the suburban Ottawa area. The basement floor and first floors of detached houses were selected for study. In order to estimate the F value, portable radon monitoring devices AB-4 (Pylon Electronics Inc.) and Alpha GUARD (Genitron Instrument GmbH), were deployed with a portable Working Level (WL) monitor (Tracerlab Ltd.) for the measurement of hourly variation of the Equilibrium Equivalent Concentration (EEC) of Radon. All these monitors were operated continuously for four days or more.

RESULTS: The mean F value for the dwellings was 0.34 ± 0.10 . This value is lower than the UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation) suggested world average value of 0.4 for indoors. The effective dose with the true F values ranged from 0.73 to 2.69 mSv/ year with one outlier of 43.82 mSv/ year. The indoor air environment was carefully monitored for temperature, air pressure and relative humidity. It was noted that the F value was roughly proportional to the temperature and relative humidity and inversely proportional to the air pressure.

CONCLUSION: The time variation of the F value in the basements and 1st floor of several detached houses in Ottawa was estimated. The conclusions are: (1) the F value = 0.34 ± 0.10 for the tested dwellings was less than the UNSCEAR value of 0.40. (2) The effective dose depends strongly on the F value. (3) If it is necessary to determine the radon effective dose over a shorter period than one year, the time variation of each physical or environmental parameter including the true F value should be taken into account. This project will support the implementation of the Health Canada program to reduce radiation exposures from radon in the home and workplace.

2.25 Investigations into Oxidative Changes in Human Plasma Under Normal and Pathological Conditions Using a HPLC-EC Array Technique

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SUMMARY: There exists an association between increased oxidative stress and patho-physiological conditions in humans. Hence several biomarker compounds were investigated to assess the oxidative stress status in various disease conditions. We have developed a high pressure liquid chromatography-electrochemical array (HPLC-EC array) method for the simultaneous analysis of multiple oxidative stress biomarkers in plasma. This method was validated by analyzing commercially available plasma samples from individuals having different pathologies. Our results showed that m-tyrosine, 3-nitrotyrosine and 8-OH-dG levels increase significantly under pathological conditions.

OBJECTIVES: The primary objective of this work is to validate the HPLC-EC array method for the analysis of suite of oxidative stress biomarkers in human plasma samples. The secondary objective is to assess the levels of these biomarkers under different pathological conditions.

DESIGN: A suite of oxidative stress biomarkers were analyzed using HPLC-EC array method in commercially available human plasma samples with pre-existing pathologies such as asthma, hypertension, obesity, diabetes. Plasma samples from healthy individuals were analysed and served as controls to assess the baseline levels. Five male and female plasma samples were analyzed in each group. Our target analytes include biomarkers such as *o*-, *m*-, *p*-tyrosines, 3-nitrotyrosine, 3-chlorotyrosine, 8-OH-dG and catecholamine such as epinephrine, norepinephrine.

OUTPUT/RESULTS: The concentrations of m-tyrosine, 3-nitrotyrosine and 8-OH-dG were significantly higher in plasma samples under diseased condition compared to that of control samples. There is relatively high biological variation in the concentration of these compounds in diabetic plasma samples indicating a necessity to increase the sample size.

IMPACT: The HPLC-EC array method was validated to measure oxidative stress biomarkers in human plasma. This method will be very useful in monitoring these biological endpoints as indicators of health measures in monitoring and surveillance studies. For instance, as a part of MIREC study this method will be used to measure these biomarkers in a cohort of pregnant women to understand the effects of environmental chemicals on pregnancy outcomes.

2.26 Characterization of Pen b 26, a Major Allergen of *Penicillium brevicompactum* Expressed in *Escherichia coli*

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SUMMARY: Pen b 26 allergen of *Penicillium brevicompactum* was cloned and expressed as N- and/or C-terminal hexa-histidine (His₆) tagged fusion proteins and characterized by proteomic methods. The C-His₆ tagged Pen b 26 produced several fold greater yield and sensitivity than the N-His₆ tagged Pen b 26. The prevalence of the allergies to Pen b 26 was determined to be 73%.

OBJECTIVES/BACKGROUND/ISSUE(S): *Penicillium* species have been identified as major source of indoor mold allergies and asthma. *Penicillium brevicompactum* is one of the most prevalent indoor mold of *Penicillium* species. Up to date, very few allergens have been isolated and cloned from *Penicillium* species. Pen b 26 allergen of *P. brevicompactum* was previously cloned and determined to be a 60S acidic ribosomal phospho-protein P1 by sequence analysis. This study focuses on the expression, purification and the characterization of Pen b 26.

DESIGN/METHOD/DESCRIPTION: The purified Pen b 26 was cloned into the expression vectors pTrcHisTOPO (N-terminal His₆ tagged) and pTrcHis2Topo (C-terminal His₆ tagged). Both fusion proteins were purified by immobilized Ni²⁺-affinity chromatography. The purified proteins were characterized by immunological, biochemical and biophysical methods. Polyclonal antibodies were also produced against Pen b 26 in NZ rabbits.

OUTPUTS/RESULTS: The C-His₆ tagged Pen b 26 produced 4-5 fold greater yield, and sensitivity for the specific antibody than the N-His₆ tagged Pen b 26 as determined by Enzyme Linked ImmunoSorbent Assay (ELISA). The prevalence of the Pen b 26 was determined to be 73% by immunoblot analysis using the C-His₆ tagged Pen b 26.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The purified allergen Pen b 26, preferably the C-His₆ tagged Pen b 26 because of its higher yield, and sensitivity can be used as a reagent to screen the sera of the population for the presence of allergies to *Penicillium* species. The purified Pen b 26 in small quantities could also be used for safer desensitization of *Penicillium*-allergic patients. Furthermore, presence of autoantibodies against Pen b 26 in about half of the auto immune patients makes it an attractive target for the development of ELISA detection and screening kits. In addition, the polyclonal antibodies raised against Pen b 26 may also be used for the detection and quantitation of *Penicillium* species in household dust samples.

2.27 Monte Carlo Simulations of Semi-infinite Radioactive Clouds of Noble Gases

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SUMMARY: Conversion factors between air KERMA rate (a quantity related to radiation dose rate) and radiation activity concentration have been calculated. These conversions are important for atmospheric transport modelers to determine who is exposed to radioactive clouds in an emergency.

OBJECTIVES: Health Canada maintains a number of detector networks. One of these networks consists of NaI(Tl) detectors that measure air KERMA (Kinetic Energy Released in the Media). Located beside the NaI(Tl) detector in Ottawa is a radioxenon analyzer (which is part of Canada's contribution to preparation for the International Comprehensive Nuclear-Test-Ban Treaty), that measures the activity concentration of ^{131m,133m,133,135}Xe directly. The published conversion factor between these two quantities does not agree with our measurement. We focus on the NaI(Tl) detector to understand this discrepancy.

DESIGN: Two Monte Carlo methods are used. Firstly, a brute force method where a detector immersed in a semi-infinite radioactive source is modelled. The geometry of the series of simulations is like a "Russian Doll" where smaller dolls are encased in larger dolls. All the simulations' results are added together yielding the total KERMA; this is similar to putting all the dolls into each other without any gaps. This method has also been used to determine the detector's pulse height spectrum. Secondly, a reciprocal transformation of the brute force geometry is applied. This transformation constructs a condensed elongated source surrounded by a ring detector.

OUTPUTS/RESULTS: The reciprocal transform method proved to be an acceptable estimate of the total KERMA. It decreases the computation time from months to hours. It may be used at much greater distances than the brute force method can achieve. The results have helped explain the discrepancy. Previous work on ¹³³Xe and ⁴¹Ar has been presented at the Health Canada Science Forum in 2006; the work presented here has been extended to study other radioactive noble gases, namely ⁸⁵Kr, ^{85m}Kr, ^{131m}Xe, ^{133m}Xe, and ¹³⁵Xe.

IMPACTS/ CONCLUSIONS: Greater accuracy in the conversion value between the air KERMA and activity concentration will help meteorological modellers verify their models. In the event of an emergency, Environment Canada will be able to model the movement of a radioactive cloud from the air KERMA value measured by Health Canada's NaI(Tl) network. From this meteorological model, a more accurate radiological dose assessment towards the public may be developed.

2.28 Personal Activity and Microenvironmental Contributions to Daily Personal PM_{2.5} Exposure for Susceptible Populations

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SUMMARY: Two studies were conducted to find out where the major contributions of a person's daily PM_{2.5} exposure occurred. This was done by utilizing time activity diaries and portable light-scattering devices that continuously record PM_{2.5} exposure. The results will provide focus for policies designed to decrease exposure to PM_{2.5}.

OBJECTIVES/BACKGROUND: A recent study investigating the cardiovascular health effects of PM_{2.5} on COPD (Chronic Obstructive Pulmonary Disease) patients found that when their 24 hour integrated personal PM_{2.5} (particulate matter that is equal to or less than 2.5µm in diameter) measurements were separated into ambient and non-ambient generated components, adverse health outcomes were significantly associated only with the ambient component. To identify different PM_{2.5} exposure contributions in between microenvironments and activities, we conducted experiments in Windsor and Hamilton.

DESIGN/METHOD/DESCRIPTION: We chose children and pregnant women to be representative of susceptible populations. Subjects were asked to utilize time activity diaries (TADs) and the pDR-1200 (personalDataRAM), each co-located with an integrated, gravimetric sample for calibration. Windsor participants (48 asthmatic children aged 8-11) were monitored for 5 consecutive days each in winter and summer 2006. Hamilton participants (19 third trimester pregnant women) were monitored for 48 hours each between September and December 2007. Generalised Estimating Equation (GEE) models will be used to examine associations between particle exposures and time spent in various microenvironments and activities. The data collected in these studies will estimate the contributions of microenvironments, such as time spent indoors, outdoors and in transit along with activities upon a person's daily PM_{2.5} exposure.

RESULTS: Preliminary analyses demonstrated the Hamilton group spending more time indoors than the Windsor group. There were also differences for time spent in transit, outdoors and at work/school. Integrated PM_{2.5} measurements demonstrated mean exposure levels of 10.7µg/m³ for the Hamilton group and 8.7µg/m³ for the Windsor group.

CONCLUSIONS/NEXT STEPS: These results will provide information for intervention strategies and policy development for reducing personal exposures to particulates for susceptible populations. The next step will be to utilize recursive model infiltration rates for each participant and then apply them to derive the ambient components of each 'indoor at home' measure. This will refine our estimates of microenvironment contributions.

2.29 Design of an *In Vitro* Alpha Irradiation System for the Study of the Biological Effects of Radon Exposure

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SUMMARY: Naturally occurring radon gas is emitted into homes from the soil, water, and building material where it poses serious health risks. In 2007, Health Canada reduced their residential radon guideline to 200 Bq/m³. Further research is required to understand the mechanisms leading to the adverse health effects related to radon exposure.

OBJECTIVES/BACKGROUND/ISSUE(S): Radon gas (²²²Rn), an alpha particle emitter with progeny that also emit high energy alpha particles, is ubiquitous in our environment. Epidemiological studies have shown that exposure to ²²²Rn can significantly increase the risk of developing lung cancer. To assess the biological impacts of alpha exposure and determine the relative biological effectiveness (RBE) of the particles on human lung epithelial cells, an alpha radiation exposure system (ARES) was constructed.

DESIGN/METHOD/DESCRIPTION: The ARES consists of a 5.08 cm ²⁴¹Am electroplated stainless steel disc with radioactivity of 68.04 kBq. For stable growth of the cell-lines and transmittance of the alpha particles, "in house" cell culture dishes were constructed. The dishes consist of 2.5 µm gauge Mylar membrane stretched across the bottom opening of a plastic cylinder secured by a plastic sleeve. Initial validation of the ARES was conducted with the use of a cell-line that displays properties of normal human type II alveolar epithelial lung cells (A-549). The dosimetry of the system was calculated by Monte Carlo simulation using GEANT4 v.9.1. An example package was adapted to represent the geometry of the ARES. The dosimetry was validated using SRIM2008, which was used to calculate the energy deposited by alpha particles in the material and, therefore, the dose to the cells.

OUTPUTS/RESULTS: The resulting ARES was found to be reliable, accurate and able to deliver a uniform dose to the cells. Growth curve analysis and clonogenic assays showed that the A-549 cell line was able to self establish efficiently on the Mylar surface of the "in house" dishes relative to commercial dishes. Survival curves were generated on the A-549 cell lines and compared to those irradiated by ¹³⁷Cs to determine the RBE of the alpha particles.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: By using this robust ARES the underlying mechanisms of alpha particle-induced effects on chromosomal and gene expression changes, DNA damage and repair, and cell survival can now be studied in a more relevant cell culture system.

2.30 On-line Enhancement of Eight Inorganic Anions in Water Using Constant Pressure Assisted Electrokinetic Injection-Capillary Electrophoresis

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SUMMARY: An on-line enrichment technique, constant pressure assisted electrokinetic injection (PAEKI), was applied to enhance the injection amount of eight trace inorganic anions, bromate, bromide, nitrate, nitrite, selenate, selenite, arsenite and arsenate, in water samples in capillary electrophoresis (CE). The sensitivities were enhanced up to several thousands folds without compromising the CE separation efficiency.

OBJECTIVES/BACKGROUND/ISSUE: The concerns of inorganic anions in drinking water in trace level on human health impacts are kept increasing due to their carcinotoxicity and genotoxicity. It is a challenge to develop a sensitive and selective method to measure those anions but without changing their species in the sample pre-treatment process. Capillary zone electrophoresis (CZE) is a fast, low cost, and effective separation technique and has been applied to analyze various analytes, especially charged analytes. However, the small injection amount of samples (nL) in CZE limited its applications. The aim of this study is to apply the PAEKI method [1] to enhance the injection amount of the selected eight anions in water so as their analytical sensitivities in capillary electrophoresis.

DESIGN/METHOD/DESCRIPTION: The 20 mM ammonium carbonate buffer was filled into the capillary column. While a negative potential was applied to inject the analytes in water sample at the inlet end of capillary, a 50-mbar of positive external hydrodynamic pressure was applied to the inlet end at the same time to balance the opposite electroosmotic flow (EOF). After the PAEKI process finished, a positive potential was applied to the inlet end to separate the analytes, which were monitored by a DAD detector at the outlet end of the capillary.

OUTPUTS/RESULTS: Conditions of injection voltage, injection time, buffer concentration and pH for PAEKI were optimized. With 20mM buffer concentration at pH 9.5, the injected amount of eight analytes can be enhanced up to several thousands folds by comparing to the hydrodynamic injection of them. The separation efficiency was not compromised by the PAEKI injection. It was observed that enhancement factors of analytes increased with increase of their mobility.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The method is very useful in sensitively analyzing charged analytes in water samples due to needs of sample treatment. Study on expanding the method to analyze more charged contaminants in drinking water is undergoing, and this project will better support the survey activities of drinking water quality for anion species.

2.31 High Performed Environmental Gamma-Ray Spectrometry Analysis on Key Nuclides of ^{238}U Decay Chain for Soil Radon Survey in Southern Ontario

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SUMMARY: An improved gamma-ray spectrometry method for key radionuclides analysis of ^{238}U decay series products in large volume soil sample was developed. The method allows more accurate measurement of natural background radon in soil.

OBJECTIVES/BACKGROUND/ISSUE: Radiation Protection Bureau of Health Canada launched a radon concentration survey in soils of southern Ontario, as radon and its daughters are responsible for a major fraction of the dose received by humans from the naturally occurring internal emitters. In this survey, the soil radon concentrations and soil permeability were determined on site. The soil total released radioactivity is mainly from the ^{238}U family where radon's parent (^{226}Ra) and its daughters are most active. There is a need for a generic and validated analytical procedure for determining their activity concentrations.

DESIGN/METHOD/DESCRIPTION: A quantitative method to determine activity concentration of nuclides in ^{238}U decay series of soil sample was established using high performance environmental gamma-ray spectrometry. In this method, a semi-empirical calibration procedure was developed for volume soil sample counting efficiency calibration. It was achieved by the combination of experimental measurement and mathematical simulation. Mathematical calibration tool is Monte Carlo simulation software named Virtual Gamma Spectroscopy Laboratory. The Aatami software (CTBT Preparatory Commission) was used for gamma-spectrum baseline and gamma-ray peak fitting as well as multiplets deconvolution.

OUTPUTS/RESULTS: The ^{226}Ra was determined directly with doublet deconvolution method using Aatami. The method provides improved ^{226}Ra activity accuracy compared with radon progeny method by avoiding the error caused by radon diffusion through sample container sealing and walls. It has been roughly estimated that the fraction loss of radon by diffusion through plastic container is about 20%. The ^{226}Ra could therefore be underestimated via indirect determination with its grand daughters. The ^{238}U was also determined with its daughter ^{234}Th at 63.3keV; ^{210}Pb was determined with its 46.5keV peak. The correlations between the two nuclides and ^{226}Ra activity concentrations are significant. The activity concentration ratios of $^{210}\text{Pb}/^{226}\text{Ra}$ and $^{238}\text{U}/^{226}\text{Ra}$ were calculated for evaluation of the geochemical behaviour of radon daughters in soil samples collected in this survey.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The doublet deconvolution method allows more accurate ^{226}Ra measurement in soil samples using Aatami software. In soil sample analysis, if the escaped radon gas amount from sample container cannot be quantified, the ^{226}Ra activity cannot be determined by its grand-daughters. With well-established efficiency calibration

curve, evaluation of activity from different gamma-rays of the same nuclide offers the results accuracy crosschecking. The analysis of thirty-two Southern Ontario soil samples shows that there are two soil types were involved in the survey with different $^{210}\text{Pb}/^{226}\text{Ra}$ activity ratios.

2.32 Climate Change and Health Adaptation for Northern First Nation and Inuit Communities Program

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SUMMARY: This program is designed for Northern First Nations and Inuit communities. It will support community-based research activities, which are aimed at developing scientific expertise and relevant communication material to increase capacity/improve decision-making at the community, regional, and national levels with respect to human health in a changing climate.

OBJECTIVES/BACKGROUND/ISSUES: Over the last decade, scientists have become more aware of the magnitude of climate change in Canada, and its impacts on human health. The expected outcomes of a warmer planet are numerous and complicated and will have both direct and indirect health implications, particularly for Northern and remote communities. Although there are differences to the capacity to adapt to a changing climate, no one person, community or region can accomplish this alone.

Much of the existing climate change research and program development in Canada has focused on impacts to natural and built physical environments and on ways of mitigating or reducing green-house gases which contribute to climate change. Very little research has been done on its implications to human health. To fill the gap, Health Canada - FNIHB is developing a community-based research program, which aims to integrate both scientific studies and traditional knowledge, to help northern First Nations and Inuit increase their knowledge and capacity to develop health-adaptation strategies.

The ultimate objective of this program is to develop community relevant scientific expertise, build capacity and relevant messaging that will help in decision-making with respect to human health and a changing environment in Canada's First Nation and Inuit communities.

DESIGN/METHOD/DESCRIPTION: Northern First Nations and Inuit communities apply for funding from the program. Eligible proposals are those that are community-based, have a strong dissemination plan and that will examine, but are not limited too, the following: exposure models for health risk assessment, health impact assessment, identification of health risks including those effecting vulnerable peoples, risk management, and adaptation approaches to climate change impacts.

The program works closely with various stakeholders such as the Assembly of First Nations, Inuit Tapirrit Kanatami, and the Arctic Health Research Network who work directly with communities and have the expertise needed to get the program running on the ground. They can help communities with the application process and provide key information with regards to the program. As well, we work closely with our federal counter-parts such as EC, INAC, NRCan, PHAC to discuss the program, proposed projects, and steps forward.

OUTPUT/RESULTS: Summary of projects undergoing funding this year are underway and will be presented.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Much of the Northern research is conducted by “southerners; this program is an opportunity to allow Northern community-based researchers to develop their own research and find ideal solutions to their particular climate change and health issues.

This program will provide opportunities for First Nation and Inuit communities apprehensive about the potential impacts of climate change, and to conduct community-based research on environmental health concerns related to their specific situation. Next steps include issuing the call for proposals for FY 2009-2010; ensuring continued promotion of the program and the call; selection of projects and initiation of funding agreements.

3.01 Sodium Intakes of Canadians-Focus on Food Sources

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SUMMARY: In October 2007, the Department of Health announced the establishment of a working group (WG) to develop and oversee the implementation of a strategy for reducing sodium intake among Canadians. As part of the information requirements for the WG, Health Canada is compiling baseline data and the primary sources of sodium in the diet of Canadians.

OBJECTIVE: To determine the sources of the sodium consumed by Canadians.

METHOD: Data from the 2004 Canadian Community Health Survey (CCHS) 2.2 Nutrition-focus was used to explore the contribution that different kind of foods make to the sodium intake of Canadians. A total of 8874 foods and recipes were assigned to 52 main food groups based on similarities in nutrient content or use. The food groups were based on an existing classification system used for previous Nutrition Surveys, including CCHS 2.2.

RESULTS: The results from the food groups' analysis indicate that sandwiches, breads, red meats and poultry, soups, vegetables, pasta dishes, burgers and hotdogs, and processed meats accounted for around 57% of all sodium consumed by Canadians. Food contribution to the total sodium intake depends on sodium content of the food and also on the popularity of the food. Indeed, the order of contribution of the food groups to total sodium intake depends on the age group and is different in men and women.

IMPACTS: The data of our analysis would be useful to the task of the WG with regard to focus on major food contributors to sodium intake and also in developing targets for sodium reduction according to food groups with characteristics in common.

3.02 Significance of the Observed Cytoplasmic Immuno-Localization of Proliferating Cell Nuclear Antigen (PCNA) in Studies to Evaluate the Safety of a Chemical Food Contaminant

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SUMMARY: Emerging chemical food contaminants must be assessed for their toxicity and potential health effects in order to regulate, make policy decisions and minimize health risks to Canadians. Various validated short-term tests are also applied for determining the potential for carcinogenicity without resorting to conducting long-term cancer bioassays. One such test is the cell proliferation index, which is routinely determined in target organs of the exposed test animal using PCNA as an immunohistochemical indicator. PCNA is normally expressed in the nuclei of cells in S-phase of the cell cycle. Our focus in this study was to understand the preferential localization of PCNA in the cytoplasm when rats were exposed to the food contaminant, perfluorooctane sulphonate (PFOS).

OBJECTIVES/BACKGROUND/ISSUE(S): To maximize food safety for Canadians, an emerging chemical food contaminant, PFOS, was recently assessed in our laboratory for its toxicity, including its potential to cause cancer. When evaluating the cell proliferation index, PCNA staining was predominantly localized to the cytoplasm instead of the nucleus where PCNA is normally expressed. Other toxicity data from the same study showed that PFOS affects lipid metabolism in rat liver, and behaves like a weak peroxisome proliferator (PP). The significance of cytoplasmic expression of PCNA in relation to the PP-like activity of PFOS has, therefore, been investigated by comparing the cellular localization of PCNA in livers of rats treated with ciprofibrate (CPF), a known strong PP and a non-genotoxic carcinogen.

DESIGN/METHOD/DESCRIPTION: Rats were exposed to either PFOS in feed (0 - 100 mg PFOS/kg diet) for 28 consecutive days, or to CPF in the diet, according to a tumour-inducing regimen for 20 weeks. At necropsy, livers were fixed in formalin, and then processed for PCNA immunohistochemistry.

OUTPUTS/RESULTS: In control diet rats, nuclear PCNA labeling remained at a base-line steady state level. In contrast, in hepatocytes from both PFOS and CPF treated livers, PCNA-specific staining was primarily localized to the cytoplasm.

3.03 Comparative Effects of Low and High Glycaemic Diet on Body Weight and Metabolic Control of Rats with Dietary-Induced Obesity

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SUMMARY: The effects of low and high glycaemic diet on body weight gain and metabolic control were investigated in obese rats. The low glycaemic diet reduced food intake, body weight gain, and blood glucose concentrations when rats had free access to food, but not when they were energy restricted.

OBJECTIVES/BACKGROUND/ISSUES: To test the hypothesis that a low-glycaemic, high resistant starch (RS) diet improves body weight (BW) and metabolic control in rats with dietary-induced obesity (DIO).

DESIGN/METHOD/DESCRIPTION: Male Sprague-Dawley rats (BW=510-520 g; n=10-12/group) with established DIO were fed for 4 weeks one of the following *ad libitum* (ad lib) or energy restricted (ER) modified AIN-93G diets: waxy cornstarch (WX/high glycaemic/low RS) or high amylose cornstarch (HACS/low glycaemic/high RS). ER rats were fed 70% (as calories) of the intake of an age-matched, non-obese group (n=16, BW=440 g) fed a regular AIN-93G diet. Food intake and BW were measured regularly. At the end of the 3rd week, 7-8 rats/group underwent an oral glucose tolerance test (OGTT). Blood was collected from the tail vein at selected times. At the end of the 4th week, overnight-fasted rats were euthanized, and blood and tissues were harvested.

OUTPUTS/RESULTS: When fed ad lib, rats on the WX diet gained more weight, had higher total energy intake (TEI) and higher fat pad mass ($p < 0.01$) than, but similar feed efficiency to, rats on the HACS. Conversely, ER rats on the WX diet lost more weight ($p < 0.01$) than, but had similar TEI and fat pad mass, to those on HACS. Two thirds of the difference in BW among the ER animals can be attributed to higher caecum weight of rats fed the HACS diet ($p < 0.001$). After the OGTT, ER rats had a lower glycaemic response than those fed ad lib ($p < 0.01$); a similar trend was observed after the HACS vs WX diet only when given ad lib ($p = 0.06$).

IMPACTS/OUTCOMES/CONCLUSION: A low glycaemic, high RS diet improves BW, adiposity and metabolic control in DIO rats only when fed *ad lib*, but not when energy restricted. The results of this study will help develop dietary guidelines aimed at managing body weight in obesity.

3.04 Abundance and Distribution of Halophilic *Vibrio* Species in Molluscan Shellfish Harvested in Canada: Impact on Food Safety and Consumer Health

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SUMMARY: Molluscan shellfish are known to accumulate pathogenic bacteria, such as *Vibrio* spp., in their tissue from the marine environment. This study investigates the composition of *Vibrio* spp. in molluscs harvested in Canada and how the proportion can reduce, or increase, human health risk due to the bacterial hazards.

OBJECTIVES: To monitor the presence and abundance of pathogenic *Vibrio* species in molluscs harvested in Canada and construct a database of *Vibrio* species inhabiting the eutrophic regions of the ecosystem.

DESIGN: Molluscan shellfish were harvested from sites in the coastal waters of British Columbia (B.C.), the Gaspé Peninsula and two sites in Nova Scotia (N.S.) between May and October, 2006 - 2007. Molluscs were shucked in laboratories near the harvest sites, or packed live with aeration and moisture, and shipped overnight under refrigeration to the analytical laboratory in Ottawa. Biochemical (standard diagnostic kits, including API-20E) and molecular (Polymerase Chain Reaction) assays were used to identify and characterize the presumptive isolates of *Vibrio* spp.

RESULTS: Molluscan samples from the Pacific coast were more frequently positive for *Vibrio* species as compared to those from the Atlantic coast. *V. alginolyticus* (Va) was the predominant species followed by *V. parahaemolyticus* (Vp); 94% and 63% of west coast (Pacific) samples were positive for Va and Vp, respectively, while east coast (Atlantic) samples yielded 82% Va and 48% Vp during the study period. Levels of Vp in molluscan tissue were higher in the east coast samples than in bivalves from the west coast. Pathogenic Vp strains, positive for thermostable direct haemolysin (*tdh*), were isolated from oysters harvested in B.C. (2 sites) and N.S. (one site) in 2007. In addition to Vp and Va, *V. vulnificus* was detected in oysters from Eel Lake in N.S.

DISCUSSION AND IMPACT: The composition of halophilic *Vibrio* species identified in molluscs harvested in Canada is likely representative of the ecology of *Vibrio* species in the Canadian estuaries. Colonization by the less virulent Va may help to prevent the harmful *Vibrio* species, such as Vp, from being the dominant microflora on bivalve molluscs, and thus may reduce the risk of illness(es) in consumers of raw and undercooked shellfish. In contrast, Va may acquire virulence genes from Vp and become an emerging threat, thereby increasing the risk of illness(es). Information available from a sustainable monitoring programme will help us to support activities, which will provide more accurate and helpful information to consumers on seafood safety.

3.05 Investigation of Endogenous Formation of Furan in Fisher-344 Rat

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SUMMARY: Possibility of formation of furan, a possible carcinogen, from common substances found in a rat diet was investigated using male Fisher-344 rat model.

OBJECTIVES/BACKGROUND/ISSUES: Furan is known to form in trace amounts (ng/g) in foods. Precursors such as ascorbic acid, polyunsaturated fats and related compounds, and carbohydrates are known to form furan via processes, which involve oxidation and Maillard-type reactions. Animal studies have shown that furan is a potent hepatocarcinogen in both sexes of mice and rats. Based on animal feeding studies by the International Agency for Research on Cancer and National Toxicology Programme, furan is considered a possible human carcinogen. In our previous animal studies, the livers of sentinel and control animals were found to have trace amounts of furan. In addition, rat chow also contained furan. Therefore, there was uncertainty if the furan present in the liver comes from the diet.

DESIGN/METHOD/DESCRIPTION: In order to determine the origin of furan in the liver, male Fisher rats were orally administered uniformly ¹³C labelled compounds (ascorbic acid, linoleic acid ethyl ester, glucose) in two 3 hr intervals, after fasting for 16 hrs. After 6 hrs from first dose, animals were euthanized. The liver and blood were analysed for the presence of furan using headspace Solid Phase Micro Extraction Gas Chromatography/Mass Spectrometry.

OUTPUT/RESULTS: No significant formation of ¹³C-labelled furan was observed. Traces of ¹³C-labelled furan were detected in blood and liver (less than 0.005 ng/g) of rats fed ascorbic acid and linoleic acid ethyl ester, but not glucose. Traces of native furan were observed in tissues of all animals tested. However, animals gavaged with deuterium labelled furan (d₄-furan) at doses similar to those expected from the contribution of native furan in rat feed showed no detectable level of d₄-furan in blood or liver.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Our results suggest a possibility of an alternate pathway of endogenous furan production from substrates other than those tested. Endogenous formation of furan could impact assessments of risks associated with the consumption of furan-containing foods.

3.06 Effect of Calcium on Iron Absorption in Women with Marginal Iron Status

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SUMMARY: The inhibitory effect of calcium on iron absorption was shown in a group of women, with pre-existing marginal iron status, using a stable isotope technique. Large inter-individual variation in iron absorption was found, suggesting that iron status is probably not the main physiological factor determining iron absorption under conditions of identical dietary intakes.

OBJECTIVES/BACKGROUND/ISSUES: To evaluate the effect of calcium on iron absorption in a short-term study in women with marginal iron status, a condition typical of some 15% of Canadian women of child-bearing age. The study design also permits the evaluation of the effectiveness of pre-selecting participants within a narrow range of iron status as a possible approach to reduce inter-individual variability in iron absorption measures.

DESIGN/METHOD/DESCRIPTION: Iron absorption was measured, with and without calcium, in 13 healthy women with marginal iron status, defined as a haemoglobin between 120-160 g/L and a serum ferritin 12-24 g/L and a C-reactive protein <7 mg/L. This study is part of a larger ongoing study on the effect of long-term calcium supplementation on iron absorption.

Each subject received 2 stable iron isotope-labelled breakfasts on consecutive days. One of the meals was labelled with ⁵⁷Fe and the other, with ⁵⁸Fe, and consumed with 500 mg calcium (as CaCO₃ tablet). Isotopic analyses were performed by multi-collector inductively coupled plasma mass spectrometry as reported at the 2006 Health Canada Science Forum. Label incorporation into enriched blood samples was determined from measurements of ⁵⁷Fe/⁵⁶Fe and ⁵⁸Fe/⁵⁶Fe isotopic ratios before and after administration of the labels, assuming 80% of incorporation of absorbed iron into red blood cells. Fractional iron absorption was calculated as the ratio of the amount of the isotope labels incorporated to the amount ingested.

OUTPUTS/RESULTS: In these women, fractional iron absorption ranged from 2.2 to 40% and 0.7-18.9% in the absence and presence of 500 mg of calcium, respectively. The results of this study confirm the inhibitory effect of calcium on non-heme iron absorption in a short-term study (by 53%, P=0.0009). A significant inverse correlation (-0.67, P=0.01) was found between serum ferritin concentrations and iron absorption. Large inter-individual variation in iron absorption was found suggesting that iron status may not be the main physiological factor determining iron absorption under conditions of identical dietary intakes.

IMPACTS/OUTCOMES/CONCLUSIONS: The results of this short-term human study will help to inform food fortification and calcium supplementation policies for one of the population groups most at risk for iron deficiency.

3.07 Assessment of Iron Bioavailability in the Diets of 7-8 Year-Old Boys Living in Southwestern Ontario

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SUMMARY: The amount of dietary iron that is absorbed into the body is dependent on several factors. Using dietary information and a modified iron absorption algorithm, this study estimated the amount of bioavailable iron in the diet of young boys living in southwestern Ontario.

OBJECTIVE: Iron is an essential micronutrient. Adequate consumption of iron is especially important for children as evidence indicates that iron deficiency early in life can cause irreversible neurological abnormalities. Presently, the Recommended Dietary Allowance (RDA) for iron is based on an assumption that overall iron bioavailability in the mixed North American diet is 18%. Few studies have investigated the amount of bioavailable iron in the diets of children. The objective of this work was to estimate the amount of bioavailable iron in the diet of young boys.

METHODS: Dietary information for 7-8 year-old boys living in southwestern Ontario was collected through 3-day food records. Using the Nutrition Survey System, an in-house Health Canada dietary analysis software that uses nutrient composition data from the Canadian Nutrient File, dietary intakes of total iron, vitamin C and meat (red meat, poultry and fish) were calculated. A modified Mosen algorithm that uses dietary variables (total iron and vitamin C intake) and provides assumptions for absorption based on body iron stores was used to estimate bioavailable dietary iron. Where possible, published heme iron values were used rather than assuming that all meat, fish and poultry contains 40% heme iron.

RESULTS: Dietary records from 32 boys indicated a mean iron intake of 14.5 ± 5.4 mg/d. Calculated bioavailable iron ranged from 0.28 to 2.7 mg/d (mean 1.0 ± 0.48 mg/d) representing 4.0 to 12.4% of total iron intake.

CONCLUSIONS: Although total iron intakes appeared sufficient to meet the needs of this group, the bioavailable iron was less than the 18% assumption of availability used to calculate the RDA. Further research should examine the effect of consuming low iron bioavailability diets on iron status in this population. This work may influence future revisions of the Dietary Reference Intakes for iron for children.

3.08 The Effect of Background Flora on the Isolation and Detection of *Shigella* spp. from Food Samples

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SUMMARY: Isolation of *Shigella* spp. from outbreak samples remains difficult. When using Health Canada MFLP-25 and MFLP-26 methods, PCR can detect a virulence marker of *Shigella* from spiked and naturally-contaminated foods, with or without isolation of the organism. Our work demonstrates why it is difficult to isolate viable *Shigella* from PCR-positive samples.

OBJECTIVES/BACKGROUND/ISSUES: *Shigella* spp., human specific pathogens, cohabit the gut with other bacteria and are transferred to food by faecal contamination. Gut microflora, i.e., *E. coli*, produces antibiotic substances, which can inhibit or inactivate *Shigella* spp. Conventional methods are unable to detect *Shigella* in foods. The objectives of this work were to elucidate the effects of antagonistic bacteria on the isolation of *Shigella* spp. from foods.

DESIGN/METHOD/DESCRIPTION: For this study carrots and celery were inoculated with two clinical outbreak strains of *Shigella sonnei* against *E. coli* strains to observe the potential antagonistic effects of the background flora. To further confirm our hypothesis, 95 strains of *E. coli* were tested against clinical and reference strains of *Shigella* spp., which included *Shigella sonnei* (Greek Pasta salad outbreak, 2003), *S. sonnei* (carrot outbreak, 2007), *S. sonnei* ATCC 29930, *Shigella boydii* ATCC 25930, *Shigella flexneri* ATCC 12022, and *Shigella dysenteriae* LCDC 00-3278. In parallel, three food borne isolates, including *Pseudomonas* spp, *E. coli*, *Bacillus* spp. as well as three reference *E. coli* strains and *Bacillus thuringensis* ATCC 21322, were used as indicators for the inhibition of *Shigella* spp. and other Enterobacteriaceae.

OUTPUTS/RESULTS: The inhibition of *S. sonnei* by *E. coli* ranged from 49 to 56 % with higher inhibition to *S. sonnei* strain isolated from Greek pasta salad. Thirty-six and six percent of *E. coli* inhibited *S. flexneri* and *S. boydii*, respectively. None of the *E. coli* was able to inhibit *S. dysenteriae*. The *E. coli* and *Pseudomonas* spp. strains isolated from outbreak samples, inhibited 100 % of *S. sonnei* and *S. flexneri*. The rate of inhibition of Enterobacteriaceae by *E. coli* and *Pseudomonas* spp. ranged from 57 to 74%. *Bacillus* spp. isolated from a spiked sample of strawberries inhibited just 1% of Enterobacteriaceae while the reference strain ATCC 21922 inhibited 8%.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This study demonstrates the impact that certain background flora could have on the isolation of *Shigella* spp. from food involved in *Shigella* outbreaks. Considering the difficulties and the lack of appropriate selective culture media for *Shigella*, especially when *E. coli* is present, we would highly recommend the use of PCR method (MFLP-26) as well as possibly screening to identify antagonistic bacteria in the food samples.

3.09 Migration of Bisphenol A from Polycarbonate Baby and Water Bottles into Water under Severe Conditions

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SUMMARY: Migration of bisphenol A from polycarbonate baby and water bottles into water was investigated at 70°C, and level of bisphenol A in water contained in a polycarbonate bottle was as high as 521 ppb after migration at 70°C for 6 days.

OBJECTIVES: To investigate migration of bisphenol A (BPA) from polycarbonate (PC) bottles into water under severe condition.

DESIGN/METHOD/DESCRIPTION: US FDA recommends to test repeated-use food-contact articles under severe conditions (for example at high temperatures for extended period of time) using the pieces from the article to simulate the worst-case migration scenario. Although this approach is simple, the results obtained may not reflect the real situation. In this work, the whole bottles, rather than pieces cut from these bottles, were used to investigate migration of BPA into water. Polycarbonate baby and reusable water bottles were filled with boiling water and heated at 70°C. Samples were taken at different times and analysed by headspace solid-phase microextraction and gas chromatography-mass spectrometry without derivatization.

OUTPUT/RESULTS: Migration of BPA from the PC bottles heated at 70°C was found to increase over the time in the quadratic equations. Migration levels of BPA in water varied from 228 to 521 $\mu\text{g L}^{-1}$ or 0.26 to 0.90 $\mu\text{g cm}^{-2}$ after being heated at 70°C for 6 days. The results are higher than the migration levels of BPA from PC bottles measured with the pieces cut from the bottles as reported in the literature. The average migration rates of BPA from the PC bottles into water at 70°C ranged from 1.84 to 4.83 $\text{ng cm}^{-2} \text{h}^{-1}$.

IMPACT/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This project supported the activities of government of Canada's chemical management plan (CMP) by providing information on migration of BPA from PC bottles under severe conditions. Migration experiments with PC bottles should be conducted with the whole bottles whenever possible rather than with the pieces cut from the bottle in order to reflect a realistic scenario and obtain more accurate migration data. In order to minimize the exposure to BPA, the contact time of infant formula with the PC bottles should be kept to a minimum if they have to be warmed up in a microwave, and the leftovers should be discarded to avoid accumulation of BPA in the formula. For PC reusable water bottles, filling with hot water or heating in the microwave to high temperatures will increase the level of BPA migration, and thus is not encouraged.

3.10 Propylene Oxide in Foods?

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SUMMARY: A headspace GC-MS method was developed and used to investigate degradation of propylene oxide in water and determine propylene oxide in selected food samples from Canadian Total Diet Study.

OBJECTIVES: To investigate degradation of propylene oxide in water and determine levels of propylene oxide in foods.

DESIGN/METHOD/DESCRIPTION: Propylene oxide is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals. It is used primarily as a chemical intermediate in the production of polyurethane polyols, propylene glycols, glycol ethers, and specialty chemicals. It is also used in fumigation chambers for the sterilization of packaged foods, used for modifying food starches, which are used as thickening agents or stabilizers in the manufacture of foodstuffs such as canned soups, deserts, microwavable dinners and puddings.

Propylene oxide is listed in Batch 1 for further assessment under CMP. Although it is unlikely that propylene oxide will exist in foods as consumed since it has been known that propylene oxide reacts with water to form propylene glycol, there is no report or publication to confirm this. In this work, a headspace GC-MS method was developed for determination of propylene oxide in food samples, effect of time and temperature factors on the reaction of propylene oxide with water was investigated, and selected samples from the 2007 total diet study were analyzed for propylene oxide.

OUTPUT/RESULTS: Instrument linearity was demonstrated over the concentrations from 2 to 40 µg/L, $R^2 > 0.999$. Method detection limit of was estimated to be 0.6 ppb. Repeatability of the method was investigated at three levels, 5, 20, and 40 ppb, and the relative standard deviations (7 replicates at each level) were 6.0, 7.6, and 2.2%, respectively.

It is observed that propylene oxide degraded in water at room temperature, and degraded rapidly at higher temperatures. Propylene oxide was not detected in the 40 food composite samples from the 2007 Total Diet Study.

IMPACT/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The results demonstrated clearly that propylene oxide degrades in water, rapidly at higher temperature, due to reaction with water. Since water is involved in food preparations and after consumption, and foods are prepared quite often at high temperatures (boiling, roasting, frying etc.), it is very unlikely that propylene oxide will be found in foods. This has been confirmed by the analysis of food samples from the total diet study.

3.11 Aniline in Vegetable and Fruit Samples from Canadian Total Diet Study

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SUMMARY: A method based on solvent extraction followed by GC-MS analysis was developed and used to determine levels of aniline in vegetable and fruit samples from the Canadian Total Diet Study.

OBJECTIVES: To investigate the presence of aniline in vegetable and fruit samples from Canadian Total Diet Study.

DESIGN/METHOD/DESCRIPTION: Aniline is one of the chemicals evaluated under the Canadian Environmental Protection Act first Priority Substance List (PSL 1). There is no regulation on the acceptable level for aniline in food. The final PSL1 assessment report was published in 1993, and concluded that there was insufficient information on exposure of the Canadian population to determine whether the substance constitutes a danger in Canada to human life or health. While there was some suggestion that aniline may be present in fruits and vegetables, there was no Canadian specific data. In order to determine levels of aniline in Canadian vegetables and fruits and thus provide the dietary exposure data for further assessment of aniline, a liquid-liquid extraction method in conjunction with GC/MS analysis was developed and used to determine aniline in the vegetable and fruit samples collected from the Canadian Total Diet Study.

OUTPUT/RESULTS: 40 vegetable and fruit samples from the 2005 Total Diet Study (in Toronto) were analyzed for aniline. Aniline was detected only in apple samples at level of 0.278 mg/kg. Apple samples from total diet studies conducted in 2001 (St. John's), 2002 (Vancouver), 2003 (Montreal), 2004 (Winnipeg), 2006 (Halifax), and 2007 (Vancouver) were also analyzed for aniline, and aniline was detected at 0.468, 0.085 mg/kg in 2001 and 2004 apple samples, respectively. The average aniline concentration in the apple samples from 2001, 2004 and 2005 is 0.277 mg/kg.

IMPACT/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Among the 40 vegetable and fruit samples from the 2005 Total Diet Study, aniline was detected only in apple samples. Aniline was also detected in apple samples from Total Diet Studies conducted in 2001-2004 and 2006-2007. The exact sources for aniline detected in apples are unknown. One hypothesis is that diphenylamine, a fungicide used for post-harvest protection of pome fruit against fungal attack, in the apple samples could be degraded to form aniline during the sample extraction procedure, but this could not be confirmed by our preliminary experiment. A targeted survey should be conducted to investigate aniline in different types of apples (peeled, unpeeled) in more detail. Degradation of diphenylamine and other potential pesticides should also be investigated further in order to find out the sources of aniline in apples.

3.12 Monitoring and Evaluation of the Potential Risk of Excessive Iodine Intakes in the Canadian Population

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SUMMARY: Routine Food Directorate monitoring through the Canadian Total Diet Study has identified a potential concern with high iodine intakes in the Canadian population. We have proactively mounted a multi-pronged approach to assess exposures and potential health risks that may result from excessive iodine intake. The knowledge gained will be used to identify appropriate risk management options.

OBJECTIVES/BACKGROUND/ISSUE: Analyses in 2002 of food composites in the Canadian Total Diet Study (TDS) collection from Ottawa (2000) brought attention to potentially high intakes of iodine through the consumption of milk and dairy products.

DESIGN/METHOD/DESCRIPTION: The Food Directorate has mounted a multi-pronged approach to informing an appropriate risk management response(s):

- On-farm analyses of iodine levels through stages of collection and processing of milk.
- Continued monitoring of subsequent collections in the Canadian TDS.
- Population intake modelling based on food intake data from the Provincial Nutrition Surveys and CCHS 2.2 (Nutrition).
- Laboratory investigation of the role of excessive iodine intakes in the development of auto-immune thyroiditis in an appropriate animal model.
- Population iodine status measurements in the Canadian Health Measures Survey (CHMS).

OUTPUTS/RESULTS: Significant outputs of the ongoing activities include:

- a national/multi-regional on-farm milk sampling network has been established and 500 samples delivered to Quebec Region Food Directorate laboratories for analysis of iodine levels.
- Quebec Region analysts have developed and validated analytical methods using microwave digestion and ICP-MS quantification of iodine in milk and other food matrices.
- analyses of iodine levels in selected food composites from Canadian TDS collections from 2001 (St. Johns), 2002 (Vancouver) and 2003 (Montreal) have been conducted; analyses in ensuing years are planned.

- population intake modelling has been conducted using Provincial Survey Data and is planned for summer 2008 using the CCHS 2.2 data.
- a pilot study in laboratory rats to characterize auto-immune thyroiditis development in response to high levels of dietary iodine exposure has been done.
- a high-throughput microplate method for urinary iodine analysis has been established for monitoring of population iodine status in the CHMS.

IMPACTS ON POLICY/REGULATION: The described activities are addressing key knowledge gaps. Knowledge gained is being used to identify risk management options that may include regulatory, non-regulatory or a combination of both to address the potential risk of excessive iodine intakes in the Canadian population.

3.13 *Enterobacter sakazakii*. Phenotypic Study and Interaction with the Blood-Brain Barrier

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SUMMARY: *Enterobacter sakazakii* has been implicated in outbreaks causing meningitis and enteritis. An organism of concern due to its prevalence in powdered infant formula, it remains unknown what factor(s) play a role in the transfer across the human blood-brain barrier, or whether they are present in all strains of *E. sakazakii*.

OBJECTIVES: This study was intended to attain two main objectives. First, strains of *E. sakazakii* isolated from various clinical, food, and environmental sources were tested for their ability to adhere to and invade human brain microvascular (blood-brain barrier) endothelial cells. The second objective is to identify putative factor(s) responsible for adhesion, invasion and intracellular survival.

DESIGN: Adhesion of 30 different *E. sakazakii* isolates (10 each from clinical, environmental, and food sources) to endothelial cells was measured by adding bacteria to confluent endothelial cell monolayers followed by incubation for 1.5 hours. Monolayers were then washed twice with phosphate buffered saline (PBS) and lysed with 1% Triton X-100. Adherent bacteria were enumerated by serial dilution and plating on trypticase soy agar plates. Invasion was measured by incubating the monolayers for 30 minutes with medium containing gentamicin to kill extracellular bacteria, followed by two washes with PBS prior to lysis with Triton X-100. A transposon mutant library was screened in the same manner to identify isogenic mutants deficient in adherence or invasion.

OUTPUTS/RESULTS: All strains of *Enterobacter sakazakii* were able to adhere to the endothelial cells, and all but 2 clinical strains were able to invade. There was no significant relationship with adherence and invasion when compared to source of the *E. sakazakii* strain. Interestingly, 70% of strains isolated from clinical sources were either positive or indeterminate for capsule production, compared to 40% and 30% for food and environmental isolates, respectively.

IMPACTS/OUTCOMES/CONCLUSIONS: Since the source of the *E. sakazakii* strains did not affect adherent or invasive abilities, it is possible that adherence and/or invasion-related factor(s) are constitutively expressed. Further investigation into the transposon insertion sites in the genomes of non-adherent and non-invasive mutants should help shed some light onto the identity of the factor(s). Capsule formation may be important in the blood-brain barrier pathogenesis.

3.14 Timely Assessment by Health Canada of Potential Contamination of Heparin Medicinal Products

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SUMMARY: Collaboration between the Biologics and Genetic Therapies Directorate (BGTD), the Inspectorate and the Therapeutic Products Directorate (TPD) of the Health Products and Food Branch (HPFB) led to the rapid recall of contaminated heparin products on the Canadian market.

BACKGROUND: Heparin is very widely used as an anticoagulant in many procedures, including surgeries and dialysis. In January 2008, as a result of a dramatic increase in numbers and severity of reported adverse reactions (ADRs), there was a voluntary recall of nine lots of heparin products in the US. A previously unknown impurity identified by FDA scientists was suspected as the cause of the ADRs. The FDA and numerous collaborators identified the impurity as over-sulfated chondroitin sulfate (OS-CS). None of the American lots being recalled were on the Canadian market, however some Canadian products did have the same American supplier. Relevant samples were obtained by the Inspectorate and analyzed by BGTD's Centre for Biologics Research (CBR). TPD coordinated dealings with manufacturers and provided necessary information to the Canadian public and health care professionals.

OBJECTIVES: The critical objective was to ensure that heparin products on the Canadian market were free of the suspect impurity, without causing product shortages of a life-saving drug. Since the manufacturers did not have in-house lab capacity, Health Canada labs provided the interim analysis and expertise. Nuclear magnetic resonance and capillary electrophoresis methodologies were rapidly set-up, validated and executed.

RESULTS: One hundred and seventy heparin samples were analyzed by NMR and CE methods. OS-CS was unequivocally identified in a single heparin sample and that lot was rapidly recalled. Another impurity, dermatan sulfate, which was already known to be present in heparins, was also identified.

IMPACTS: The well-coordinated and efficient dealings within Health Canada on this issue resulted in a rapid response and thus, potentially harmful products were removed from the Canadian market. Also, Health Canada has been involved in three follow-up international meetings to optimize and harmonize laboratory methods and standards to be used in the US and European pharmacopoeial monographs which are intended to ensure ongoing heparin safety.

3.15 Development of a Food Composition Laboratory Network

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SUMMARY: A collaboration has been initiated this year between the Nutrition Research Division (NRD) and the Health Canada (HC) Regional laboratories (RLs) to build a Food Composition Laboratory Network. Food samples, which form a nationally representative sample set, are collected and sent to the various RLs for subsequent processing and analysis.

BACKGROUND / OBJECTIVES: Canada's food composition database, the Canadian Nutrient File (CNF) has traditionally been based upon USDA's Nutrient Database for Standard Reference. These data are modified to reflect the Canadian market and regulations. However this does not allow us to tailor the database to the research, surveillance and decision making activities specific to HCs goals. This new lab network will empower HC to possess its own food sampling and analysis program to analyze priority foods for key nutrients.

METHOD: According to sample designs prepared by NRD, food samples, which form a nationally representative sample set, are collected and sent to four RLs for subsequent processing and analysis. The results are then set back to NRD for compilation and entry in the CNF. For this fiscal year, flour and granola bars were chosen as the priority foods for a variety of reasons. Flour is a staple and a simple matrix for the setup phase of the project. Granola bars formulations are uniquely Canadian, have changed in response to the trans fat monitoring program, and are highly consumed by specific age groups such as children.

OUTPUTS: This ambitious project was initiated this year and is still in the development phase. We are beginning with flour and granola bars as the identified priority foods. We are determining the exact costs and logistics for streamlining the process and organizing the budget. A full profile of 96 nutrients for 4 types of flour and 12 types of granola bars will be compiled from the results and entered into the CNF this year with an aim to continuing this into an ongoing program resulting in new profiles for 10 priority foods per year. The exact number of foods to be included in this database will be dependent on future funding received for this project.

NEXT STEPS: Depending on the budget allocations for next year we have set priorities for yogurts, cheddar cheese, and processed meats. We also are in discussions to collaborate with the Canadian Turkey Marketing Agency where they would provide the representative samples and we would undertake the analysis.

3.16 Prevalence of Prominent Bacterial Pathogens in Canadian Food Supply from 2004 to 2007

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SUMMARY: A multi-year study was undertaken to establish a national data bank on the prevalence of pathogens of significance in the food supply along with their antibiotic resistance profile. This data will be helpful in hazard identification and exposure assessment activities in conducting risk assessment studies.

OBJECTIVES: This study was undertaken to establish a national database in order to provide data on the incidence and antibiotic resistance of medically important bacterial pathogens such as *Salmonella spp.*, *Listeria monocytogenes*, *Campylobacter spp.*, and *E.coli* O157:H7 in retail foods. There is a need for such data since little is known on the prevalence of those pathogens at retail. Furthermore the extensive on-farm use of antibiotics in the meat industry and in the production of fresh fruits and vegetable is a public health concern. Samples tested in our study included raw meat, fresh fruits and vegetables and dry commodities. The summaries of antimicrobial profile aspects are not presented in this paper, which focuses only on the prevalence aspects.

METHOD: Fifteen samples per month were collected at retail in four Canadian regions (British Columbia, Ontario, Quebec and Atlantic (4 provinces)). Samples were shipped by courier to the Health Canada Longueuil Microbiology Laboratory and were maintained between 4° and 10°C during transport. For each of the 4 parameters, 100 g analytical units were weighted. Samples were analyzed using the following Health Canada's Compendial methods: *Salmonella spp.* by MFHPB-20 and MFHPB-24, *Listeria monocytogenes* by MFHPB-07 and MFLP-33, *E.coli* O157:H7 by MFLP-80 and finally *Campylobacter spp.* by the MFLP-46.

RESULTS: Over a 28 month period, 1292 samples were tested. Of this number 317 samples were positive for one or more pathogens, for an overall positive rate of 24.5%. The positive rate by pathogens is as follows *Listeria monocytogenes* 13.5% (175/1292), *Salmonella spp.* 9.3% (121/1292), *Campylobacter spp.* 1.2% (21/1292). No *E.coli* O157:H7 was detected in this study. *Salmonella spp.* and *Campylobacter spp.* were mostly detected in raw meats. However *Listeria monocytogenes* was detected mostly in fresh vegetables.

CONCLUSION: This study demonstrates that the prevalence of *Listeria monocytogenes* in ready-to-eat vegetables in Canada may still be a concern to food safety authorities.

3.17 Effects of Dietary Plant Sterols and Stanols on Serum Antioxidant and Inflammatory Markers in Wistar Kyoto Inbred (WKY) and Spontaneously Hypertensive Stroke-prone (SHRSP) Rats in the Presence or Absence of Salt

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SUMMARY: Effects of dietary plant sterols and stanols on circulating biomarkers related to vascular disease and hypertension were examined in SHRSP versus WKY rats in the presence or absence of salt. Combination of plant sterols or stanols with salt may increase the risk of vascular disease and hypertension by lowering circulating antioxidant levels.

OBJECTIVES: Consumption of plant sterol and stanol supplements has been widely used to treat hypercholesterolemia. However, epidemiological studies revealed an association between elevated plasma plant sterols and increased risk of vascular disease. In addition, plant sterols and stanols have been found to increase blood pressure and promote stroke in salt-loaded SHRSP rats. To further clarify the health benefit/risk of dietary plant sterols and stanols, their effect on serum antioxidant and inflammatory markers related to vascular disease and hypertension were examined in SHRSP as compared to WKY rats in the presence or absence of salt.

DESIGN: 35-day old WKY and SHRSP rats were put on a dietary treatment consisting of a control diet or diets containing plant sterols or stanols at 2 g/kg diet in the presence or absence of 1% salt in their drinking water. After 35 days, rats were exsanguinated and serum samples were collected and analyzed for oxidized LDL (Ox-LDL), paraoxonase 1 (PON1) activity, total antioxidant capacity (TAC), vitamin D (V_D), vitamin E (V_E), and intercellular adhesion molecule 1 (ICAM-1).

OUTPUTS/RESULTS: In SHRSP rats, the combination of salt and sterols or stanols significantly decreased circulating levels of V_D and V_E , which are known protective factors of vascular disease. In WKY rats, the combination of salt and sterols but not stanol, significantly decreased circulating levels of antioxidants, PON1, TAC, and V_E . In the absence of salt, plant sterols significantly increased serum V_E in both strains, implying a potential health benefit. Neither sterols nor stanols significantly increased circulating inflammatory markers (Ox-LDL and ICAM-1) at the conditions used.

IMPACTS/OUTCOMES/CONCLUSIONS: The results of this project indicate that consumption of plant sterols and stanols, in combination with salt, may pose a potential risk for vascular disease and hypertension by decreasing circulating antioxidant levels. This project will support Health Canada to review and evaluate health claims for plant sterols and stanols in foods and natural health products.

3.18 An Updated Weight-of-Evidence Evaluation of Lead Acetate in Progressive Hair Dyes: the HC Cosmetics Policy Decision

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SUMMARY: Progressive hair dyes containing lead acetate had been on the North American market for decades. A recent health risk assessment of lead acetate determined that use in cosmetics presented unforeseen risks to users and other household members. As such it was prohibited from the Canadian market.

OBJECTIVE: Chronic exposure to low levels of lead has been an ongoing public health issue. There has been gradual reduction of acceptable levels of lead exposure as the understanding of lead's toxic effects has progressed from symptomatic clinical lead poisoning to subtle, sub-clinical effects. A primary objective of the work was to reassess the health effects of lead acetate based hair dyes and minimize consumer health risks, which might result from exposure to lead from the use of these products.

DESIGN: Evidence of the toxicological effects of low doses of lead was reviewed and summarized. Available literature on the chemistry, toxicokinetics, and absorption of lead, particularly for the dermal route, was also reviewed and summarized. Two models were used to estimate chronic lead exposure doses to users of lead acetate hair dyes. Model parameters were varied to quantify the influence of scientific uncertainty on model predictions. Estimates of chronic lead exposure from use of lead acetate hair dyes were compared to Health Canada's provisional tolerable daily intake (pTDI) for lead and the estimated daily intake (EDI) from the common sources of environmental lead exposure in Canada.

OUTPUTS/RESULTS: The results showed that relatively small incremental exposures, such as those which would occur with regular use of hair dyes containing lead acetate, could result in the accumulation of potentially harmful body burdens of lead.

IMPACTS/OUTCOMES/CONCLUSIONS: Use of lead acetate dyes would add to the cumulative population exposure for lead, which has already been found to be within the range of potential effects for some end-points and sensitive sub-populations. Given the conclusions of the report, companies were asked to remove lead acetate-containing hair dyes from the market. Since January 2008 lead acetate hair dyes have not been available for sale in Canada.

3.19 Application of the QuEChERS Extraction Method for the Analysis of Pyrethrins and Pyrethroids in Fin and Non-Fin Fish

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SUMMARY: The QuEChERS (quick, easy, cheap, effective, rugged and safe) method was adapted for the analysis of pyrethrins and pyrethroids in fish tissues. These products are not registered for use in the Canadian aquaculture industry and a survey was performed to confirm presence and levels of these compounds in fish products from the Canadian marketplace.

BACKGROUND: The aquaculture industry requires methods to control pests, such as sea lice, yet very few studies have been conducted to determine the levels of pesticides in fin and non-fin fish raised in aquaculture relative to those observed in wild fish. Residue testing for compliance is generally limited to determinations of whether compounds exceed maximum residue levels (MRL). Exposure of Canadians to compounds used in the aquaculture industry via dietary uptake is a data gap, particularly in light of the rapidly expanding aquaculture industry internationally and the increasing availability of farmed fish and seafood products to the Canadian consumer.

DESIGN: The development and validation of a method to determine the levels of natural pyrethrins and two pyrethroids, cypermethrin and deltamethrin at the sub-part per billion (ppb) level was required. The QuEChERS method, a new technique for the extraction of pesticides in fresh fruits and vegetables was adapted and validated for the analysis of these compounds in fish tissues employing gas chromatography-mass spectrometry. The validated method was applied to the analysis of 142 samples, purchased from retail stores across Canada.

OUTPUTS/RESULTS: Cypermethrin was the only pesticide observed and was present in seven samples of salmon at concentrations ranging from 0.3 ng/g to 6.5 ng/g with a mean level of 1.9 ng/g. In all cases, cypermethrin residues were well below the general Maximum Residue Limit (MRL) of 100 ng/g established in the *Canadian Food and Drug Regulations*.

IMPACTS: An improved method was developed and validated for the determination of trace levels of the natural pyrethrins and synthetic pyrethroids in fish tissues. As a result, low levels of cypermethrin, a pesticide not registered for use in aquaculture in Canada, were confirmed in seven samples of domestic farmed salmon. These data will be available for the Pest Management Regulatory Agency to use in exposure assessments during re-evaluation of these pesticides.

3.20 Agricultural Risk Reduction at the PMRA

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SUMMARY: The Pesticide Risk Reduction Program is a joint initiative of Health Canada's Pest Management Regulatory Agency (PMRA) and Agriculture and Agri-Food Canada (AAFC). The program is designed to support the development, availability and adoption of sustainable pest management tools and practices in agriculture and reduce the risk associated with pesticide use in agriculture through the development and implementation of cross commodity risk-based risk reduction strategies.

OBJECTIVES/BACKGROUND/ISSUES: The Pesticide Risk Reduction program's objective is to help reduce the risk associated with agricultural pesticide use and promote the use of sustainable pest management. For the past 5 years the program has worked closely with AAFC in developing commodity-based risk reduction strategies. Commodities were chosen based on a comparison of the current pest management practices and pesticide use on individual crops. The criteria used to compare crops included, environmental and occupational health risk assessment, the percent of the food in children's diet, risks associated with major pests not controlled, risk associated with the loss of the pesticide, trade issues associated with the pesticide and the availability of IPM solutions. Pest management gaps were identified through consultations with commodity stakeholders and strategies were created to help develop pest management solutions.

These risk reduction strategies have encouraged the registration and use of low risk or biopesticide products in substitution of traditional pesticides, and through research, have promoted the development of new integrated pest management tools and practices.

DESIGN/METHOD/ DESCRIPTION: This year the program is changing emphasis from a commodity-based to a risk management focused process. The risk reduction strategies designed under this new process are initiated based on one of two criteria. The first criterion is the loss and phase-out of an older pesticide which has been re-evaluated based on current standards and determined by the PMRA to be no longer acceptable for use. The loss and phase-out of these pesticides will create many gaps in the pest management program of various Canadian commodities. The second criterion is the identification of higher risk pesticides that are commonly used in agricultural production. The higher risk pesticides will be identified through the Canadian Pesticide Risk Indicator (CaPRI), a risk indicator database built by the PMRA which provides a relative ranking of potential risk associated with common agricultural pesticides.

IMPACTS/OUTCOMES: The impacts of this initiative include developing sustainable pest management in Canada through lowering risks associated with pesticide use in agriculture, improved understanding of risk indicators related to pest management tools and improved transparency of the risk management process. The final outcome being a decreased in human health and environmental risks associated with agricultural pesticides in Canada.

3.21 The Heat Inactivation of the Hepatitis A Virus in Mussels

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¹

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SUMMARY: One significant source of foodborne Hepatitis A outbreaks is mussels. Adequate cooking of mussels is required to inactivate the virus. Our goal is to determine accurate time/temperature recommendations for inactivation of Hepatitis A virus (HAV) in mussels to protect the health and safety of Canadians.

OBJECTIVES: To determine the heat inactivation profile of HAV in mussel homogenate and use this information for risk assessment and to recommend safe cooking procedures.

DESIGN: Preliminary heat resistant studies were carried out in phosphate buffer, which is used as a reference medium for comparison between different food systems. Mussel homogenate or phosphate buffer was artificially contaminated with HAV and heated at temperatures ranging from 55°C to 80°C in 2.5 degree intervals, with samples taken at variable time intervals. The presence of HAV was evaluated by cell culture infection, using Fetal Rhesus Monkey Kidney (FrhK) cells and plaque assays were performed in order to obtain virus titre in PFU (plaque forming units) per ml. Virus reduction at each temperature was determined and used to calculate the heat inactivation parameters (D and z values) for this virus. The D-value is the time required at a certain temperature to kill 90% of HAV; while the z-value is the temperature required to change the D value by a factor of 10.

OUTPUTS/RESULTS: We developed an efficient method for virus inoculation, extraction and assay in mussel homogenate. HAV in mussels was found to be more heat resistant than HAV in buffer alone - the D-value in mussels was larger than the D-value in phosphate buffer at the same temperature. D-values have been obtained for temperatures where there was at least a 3 log₁₀ reduction of HAV.

IMPACTS/OUTCOMES/CONCLUSIONS: The results obtained from these experiments will define time and temperature limits that will assist regulators to determine safe cooking procedures for potentially contaminated mussels. The guidelines developed from this study will provide regulators, industry and consumers with evidence-based safe cooking instructions.

3.22 Using Comparative Genomics to Investigate Why Clinical Isolates of *L. monocytogenes* Are Not as Prevalent in Food Matrices

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SUMMARY: *Listeria monocytogenes* causes the foodborne illness listeriosis. A gene discovered in lineage II strains, commonly isolated from cases of human listeriosis, may enhance survival of the microorganism in the host. Lineage II isolates had a reduced ability to grow in media currently used to isolate the microorganism from food matrices.

OBJECTIVES: The purpose of this project is to identify genomic differences in strains of *L. monocytogenes* to explain why strains causing human illness are not isolated from foods at an equal frequency.

METHODS: A mixed genome array, composed of PCR products from a genomic library of 15 *L. monocytogenes* isolates, was used to compare the genome content of different strains. A deletion mutant for a glycosyltransferase 65 (GT65) gene was created from strain HPB#3. Forty-five strains from different sources and the deletion mutant were studied in Mueller-Hinton broth with ampicillin, vancomycin or acriflavine.

RESULTS: A GT65 gene was found to be present exclusively in strains belonging to lineage II (more prevalent in human illness). The presence of this gene may provide strains with an increased ability to survive the host environment and thus become more prevalent in cases of human listeriosis. The GT65 deletion mutant and strains belonging to lineage I showed a reduced ability to proliferate in vancomycin and ampicillin. Conversely, lineage II isolates showed a reduced ability to proliferate in the presence of acriflavine, an essential component of Listeria Enrichment Broth. This may partially account for the apparent serotype prevalence of lineage I isolates in foods.

IMPACTS/CONCLUSIONS: This study has provided new insights regarding the tropism of *L. monocytogenes* for food, environment or the human being. The GT65 gene is involved in cell wall biosynthesis and could be providing an adaptation mechanism for clinical strains in the human host. The discovery that lineage I strains, isolated from foods, are able to out-compete clinical strains in laboratory media used to isolate the organism may provide an explanation as to why these strains are more readily isolated from foods. This information will aid in refining the Health Canada Policy for the isolation and detection of *L. monocytogenes* from ready-to-eat foods.

3.23 Evaluation of a PCR Method for Species Identification of *Campylobacter jejuni* and *C. coli* as an Alternative to Conventional Biochemical Tests

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SUMMARY: The speciation of foodborne *Campylobacter* is a costly and time-consuming process that requires the skill of a trained microbiologist. We have implemented a method for identifying signature DNA sequences within common species of *Campylobacter* that is simple, inexpensive and can be completed within hours rather than days. Using this method, it will be possible to reduce cost and time for the generation of data required for risk assessment analyses.

BACKGROUND: The objective of this study was to incorporate a PCR-based method into Health Canada's official method MFLP-46 (Identification of thermophilic *Campylobacter* from food) for rapid species identification of *C. jejuni* and *C. coli* from presumptive positive *Campylobacter* colonies isolated on selective plates.

METHODS: Several published primer sets were initially tested for specificity and ease of use with DNA derived from pure cultures. The multiplex PCR system described by Persson and Olsen (2005) was selected for further evaluation. This consisted of primer pairs for the *C. jejuni* hippuricase gene, the *C. coli* aspartokinase gene and a universal 16S rDNA sequence used as an internal positive control for the PCR. This primer set was tested with *Campylobacter* strains isolated from foods and identified using the traditional biochemical tests described in MFLP-46, and the results of both methods were compared.

RESULTS: A total of 230 *Campylobacter* strains were analyzed. Of these, 204 were identified as *C. jejuni* and 26 were identified as *C. coli* using biochemical tests. All strains that were identified as *C. jejuni* biochemically were also identified as *C. jejuni* using the multiplex PCR method. Seven of the strains identified as *C. coli* using biochemical tests were found to be *C. jejuni* using the PCR method. The PCR identification was confirmed using a second primer set with different gene targets. Misidentification by biochemical tests may have been due to variability in expression of the hippuricase gene.

CONCLUSIONS: The PCR identification of *C. jejuni* and *C. coli* was generally in concordance with biochemical tests and in some cases appeared to give more reliable results. Addition of this PCR method to MLFP-46 will decrease the time and cost of *Campylobacter* species identification in positive samples, enabling rapid data accumulation for use in risk assessment in cases of food contamination with *Campylobacter*.

3.24 Development of a Novel Carbohydrate-Based Detection Method for Norovirus

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SUMMARY: Norovirus is a leading cause of gastroenteritis and is commonly associated with contaminated food products. A method to detect norovirus contamination in food samples is being developed based on the known interaction between norovirus capsids and human blood group antigens. If successful this method will be rapid and sensitive enough for routine testing.

OBJECTIVES/BACKGROUND/ISSUES: Norovirus is a highly contagious human virus, which causes acute gastroenteritis. This organism has a very low infectious dose (10-100 viral particles) and spreads easily via the fecal oral route. It is estimated that 40% of norovirus infections are caused by contaminated foods. Noroviruses have been shown to specifically bind to histo-blood group antigens (HBGA) present on the surface of red blood cells and epithelial cells of the digestive tract. This specific interaction can be used to concentrate virus particles before detection. This is extremely important because of the lack of a cell culture system to amplify the virus *in vitro*.

DESIGN/METHOD/DESCRIPTION: This study will attempt to develop a method for detecting noroviruses in food samples using the specific binding interaction between norovirus capsids and HBGAs. Streptavidin magnetic beads will be coated with biotinylated HBGAs, which can subsequently be used to separate norovirus particles from the sample. The amount of norovirus captured by the beads will be quantified using reverse transcriptase real-time PCR.

OUTPUT/RESULTS: A real-time reverse transcriptase PCR assay with a standard curve has been developed to detect norovirus binding. Using this assay we have demonstrated that our system of magnetic beads and carbohydrates is able to bind noroviruses. Current work is focused on optimizing the protocol. Different types of HBGAs were tested to determine the best type for this assay. Multivalent HBGAs were found to bind more norovirus than an equal amount of univalent HBGAs.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Preliminary results have suggested that this method has the potential to be used for routine detection of norovirus. We will continue working to optimize this method to reach a detection limit of 10-100 viral particles. Once the conditions have been optimized we will begin testing spiked food matrices. The end goal of this project is to develop a standardized method for detecting norovirus contamination of food products.

3.25 Characterization of Noroviruses in Swine and Cattle

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SUMMARY: Norovirus causes severe foodborne outbreaks and is the leading cause of viral gastroenteritis worldwide. A strain type (GII.4) was detected in pooled swine and cattle feces that had previously only been shown to infect humans. Whole genome sequencing is underway to determine if these strains are human, animal or recombinant.

OBJECTIVES/BACKGROUND/ISSUE(S): The objective is to determine if zoonotic transmission of Norovirus (NoV) is possible. If human/swine or human/bovine recombinant NoV exist, then zoonotic transfer and/or anthro-zoonotic transfer may be implicated in the spread of NoV to food sources.

DESIGN/METHOD/DESCRIPTION: A total of 120 swine samples were tested for NoV in 2005/06, 179 samples from dairy cattle in 2006/07 and 77 samples from beef cattle in 2007/08. RNA was extracted from fecal filtrates and cDNA was synthesized using reverse transcription. Primers specific to the region B of NoV RNA polymerase were used to detect NoV using polymerase chain reaction. Presumptive positive samples were sequenced. The genomic sequence of the confirmed NoV positive samples was determined using the random amplification of cDNA ends (RACE) system.

OUTPUT/RESULTS: Twelve human GI.4 NoV strains were isolated and specific primers were designed for genome amplification. Amplification products from the successful reactions were cloned and sequenced. Sequence data to date indicates that the viruses only contain a short region of identity with the human strains and that they are likely to be novel NoV types. Additional sequencing using primer walking is underway.

IMPACTS/OUTCOMES/CONCLUSION/IMPLICATIONS/NEXT STEPS: In order to determine the most effective food handling practices and infection control regulations for norovirus, the suspected zoonotic transmission of norovirus to humans from swine and cattle must be confirmed.

3.26 Development of Unique Bacterial Strains for Use as Positive Controls in Food Microbiology Testing Laboratories

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SUMMARY: Food microbiology testing laboratories routinely utilize positive control strains of target pathogens to address method performance and quality control. To help overcome possibilities of contaminating test samples through routine testing, unique strains, readily distinguishable from naturally occurring counterparts were developed to retain salient characteristics typical of the pathogens in question.

OBJECTIVES: Modified strains of Canadian priority pathogens (*Salmonella enterica*, *Escherichia coli* O157:H7) were created to bear a stably inherited growth characteristic permitting their differentiation from naturally occurring pathogens in various food matrices. For *Listeria monocytogenes*, a strain bearing unique genotypic and phenotypic features was identified to serve as a distinguishable control.

DESIGN: Nalidixic acid-resistant (Nal^R) mutants of *Salmonella enterica* serovar Berta and *E. coli* O157:H7 were derived from wildtype laboratory cultures for routine use in food microbiology testing programs as control strains. For *L. monocytogenes*, a strain bearing a unique ribotype and rare serotype was identified by the Listeriosis Reference Service for Canada (LRS).

OUTPUTS/RESULTS: The Nal^R phenotype was verified using panels of related and unrelated strains based on the ability to grow vigorously on plating media containing nalidixic acid and to be stable in over several generations in the absence of selective pressure. Nal^R mutants were easily recovered on plating media after inoculation into foods at different levels using standard culture techniques described in The Compendium of Analytical Methods. Control strains, readily identified using the Nal^R phenotype, enabled their differentiation from wildtype bacteria in foods due to their ability to grow on plating media containing nalidixic acid. This approach was not possible for *L. monocytogenes* because of the inherent resistance of this organism to nalidixic acid. Instead, a *L. monocytogenes* isolate with rare genotypic and serologic features was selected from the LRS strain collection. The unique ribotype and rare serotype, 4c, easily assayed for, can serve as a unique and distinguishable positive control strain.

IMPACTS/OUTCOMES/CONCLUSIONS: The availability of these strains, along with a method for their selective recovery and identification without needing to change current methodology, will enable national and international food microbiology laboratories to distinguish positive results obtained with samples due to the presence of naturally occurring pathogens from incidents of in-house contamination with control strains.

3.27 Presence of *Listeria monocytogenes* in Beef and Chicken Manure Samples to Address Farm-to-Fork Transfer of Foodborne Pathogens

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SUMMARY: C-EnterNet is a surveillance project designed to help reduce the burden of enteric disease by comprehensive sentinel site surveillance implemented through local public health units. In this study beef and chicken manure were analyzed for the presence of *Listeria monocytogenes* as a possible source for human cases of listeriosis.

OBJECTIVES: To determine the level of risk and potential for zoonotic transmission of the foodborne pathogen, *Listeria monocytogenes* in manure samples from beef and chicken operations in the Ontario pilot sentinel site were analyzed.

DESIGN: In 2007 (9 chicken and 21 beef) and 2008 (9 beef and 6 chicken) farm samples were analyzed using the Health Canada method (two novel chromogenic media were evaluated). Phenotypic (motility, acid production from fermentable sugars, blood hemolysis, serology) and genotypic [pulsed-field gel electrophoresis (PFGE) and ribotyping] were done with PFGE profiles compared against the PulseNet Canada database.

OUTPUTS/RESULTS: In 2007, 51 out of 76 pooled beef manure samples (67%) were positive for *L. monocytogenes*. Twenty-nine isolates were serotype 1/2a (44%), 14 were 1/2b (22%), 5 were 4a (8%), 14 were 4b (22%) and 3 were 4c (4%). Pooled chicken manure samples had a single (3%) positive, 1/2a serotype. In 2008, 23 of 36 beef samples (64%) tested positive. Seventeen isolates were serotype 1/2a (46%), 9 were 1/2b (24%), 9 were 4b (24%) and 2 were 4c (5%). Six of 24 chicken samples were positive. Four isolates were serotype 1/2a (57%) and 3 were 1/2b (43%). Multiple serovar per sample were observed.

PFGE analyses grouped 110 strains into 47 clusters using AscI (52% similarity), with the more discriminatory ApaI putting 102 (8 untypeable) strains into 49 types (63% similarity). Eight of the 110 strains were from chicken manure, one having indistinguishable PFGE patterns to those isolated from beef manure. Novel PFGE patterns previously not seen in PulseNet Canada were found.

IMPACTS/OUTCOMES/CONCLUSIONS: The presence of *L. monocytogenes* in beef and chicken manure samples emphasizes the widespread nature of this pathogen and the potential for zoonotic transmission to humans. Special attention at the farm level should be in place to avoid its persistence in the farm-to-fork continuum.

3.28 Black Cohosh: A Collaborative Natural Health Product Program Approach from a Domestic Case Report to Regulatory Action

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SUMMARY: Identification of specific compounds at the raw material or finished product stage is a key step in the quality control of black cohosh (BC) (*Actaea racemosa* L.) products. This study describes the first instance that an adverse reaction associated with a BC product was linked to plant species mis-identification.

OBJECTIVES/BACKGROUND/ISSUE: Health Canada (HC) received 4 serious domestic cases of hepatotoxicity suspected to be associated with the use of BC products. Three cases were assigned “possible” causalities, while one case was “probable”, due to the lack of identifiable clinical confounders. Samples of the BC product involved in the “probable” domestic case were analysed for phytochemical markers to confirm the authenticity of the BC species.

DESIGN/METHOD/DESCRIPTION: Chemical analysis was conducted by liquid chromatography-mass spectrometry, using U.S. Pharmacopoeia BC powder as a standard. Samples were analysed in both full scan and single ion monitoring mode to identify phytochemical markers specific to authentic BC as well as markers present in other *Actaea* species.

OUTPUTS/RESULTS: Samples of the product tested positive for actein and 23-epi-26-deoxyactein, phytochemical markers common to *Actaea* species including authentic BC, and for cimifugin, a marker for other Asian or North American *Actaea* species. However, the product samples tested negative for cimracemoside-C, a marker for authentic BC. It was concluded that the BC product associated with the “probable” domestic case of hepatotoxicity did not contain authentic BC.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This study is the first instance in which a “probable” domestic case of hepatotoxicity was associated with a BC product found to contain other Asian or North American *Actaea* species, instead of authentic BC. Thus, herb misidentification may be a contributing factor in reports of hepatotoxicity, although other contributing factors such as use of the incorrect plant part, inherent toxicity, contamination, adulteration, and idiosyncratic reactions cannot be ruled out. HC has communicated the analytical findings to the manufacturer of the suspect product, and has requested quality control data to ensure future products from this manufacturer contain only authentic BC. Additionally, HC is currently taking actions to ensure that other BC products marketed in Canada contain authentic species of BC as well.

3.29 Modification of an *In Vitro* Method for Detecting Residual Pertussis Toxin Binding Activity in Vaccines

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SUMMARY: A simple test is described which can detect residual pertussis toxin activity in vaccine samples. This test may be an alternative to animal testing currently required to ensure vaccine safety.

OBJECTIVES/BACKGROUND/ISSUE(S): Current safety testing for residual pertussis toxin (PTx) activity in vaccine preparations requires a large number of animals, is difficult to conduct and interpret, and has a wide variability of outcome. A recent *in vitro* quantitative binding assay was described in the literature which could differentiate between the active PTx and the inactive toxoid (PTd) component based on the greater binding affinity of PTx to fetuin and its detection with a polyclonal anti-PTx antibody.

DESIGN/METHOD/DESCRIPTION: We have introduced this binding assay into our lab to further characterize its limits of detection, reproducibility, and range of acceptable operating conditions. In order to improve the specificity of the assay and reduce background binding, various buffer and reagent conditions were tested.

OUTPUT/RESULTS: Contrary to published results, PTd bound to fetuin at an affinity, which reduced the specificity of the assay to an extent that would prevent its use for screening vaccines. Increasing detergent concentration in the wash buffer had limited effect on PTd binding as did buffer type. Neither sodium, nor potassium ions in the preparation inhibited or improved binding of either PTx or PTd to fetuin. Increasing pH of the buffer from 7.2 to 10.2 during the binding step was found to significantly decrease PTd binding without affecting PTx binding to fetuin. Using a monoclonal antibody to PTx was also found to enhance specificity and sensitivity in this assay.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Using this modified assay it is now possible to quantitate residual PTx in PTd preparations. This method is currently being evaluated for use with combination vaccine preparations as a possible alternative to animal testing.

3.30 Living with a Gluten-Free Diet Study in Canada

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SUMMARY: Celiac disease (CD) is a common inherited autoimmune disease triggered by gluten - a collective name for the storage proteins in wheat, rye barley and related cereals. The only treatment for CD is to maintain a strict gluten-free diet (GFD) for life. Following this diet is essential to reduce the risk of long-term complications including anemia, osteoporosis, infertility and cancer. Strict compliance with this diet impacts on daily food related activities including: shopping, cooking, eating away from home and traveling. Little has been published on adapting to a GFD, particularly in Canada. To this effect, Health Canada (HC), in collaboration with the Canadian Celiac Association (CCA) and in consultation with the Quebec Celiac Foundation (QCF) has initiated a pan-Canadian study entitled "Living with a Gluten Free-Diet (LGFD)". The methodology and available data from the pretest of this study are presented.

DESCRIPTION: The LGFD study will include adult (>18 years) members of both the CCA & QCF with CD who are on a GFD. The objectives are to evaluate GFD management difficulties and strategies as well as the emotions experienced by CD individuals following a GFD. Quality of life, perceived health status and symptoms of recovery will also be assessed. Approval by the HC research ethics board has been undertaken. This study is sponsored by the Bureau of Chemical Safety, Food Directorate and the J. Alexander Campbell Research Fund of the Canadian Celiac Association.

ANTICIPATED OUTCOMES: The data generated by this study will be used to develop strategies and educational programs for more successful management of the lifelong dietary and lifestyle changes required for compliance with the GFD. It will enhance awareness and understanding of the GFD by health care providers, policy makers and the food industry; thus, helping to improve the health and quality of life of those with CD. Furthermore, this study will provide HC with crucial background data to support its review of the Canadian regulations on gluten-free foods.

October is "Celiac Awareness Month" in Canada.
Come and visit HC booth for more information.

3.31 Celiac Disease: What Is Health Canada Doing To Help Support Canadians With This Condition?

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SUMMARY: Celiac disease (CD) is an inherited disorder in which gluten, a protein present in wheat, rye and barley, damages the small intestine and reduces the absorption of essential nutrients. CD is an immune-mediated chronic condition with a wide range of manifestations of variable severity. It is estimated that CD affects between 0.5 % and 1% of Canadians. The only treatment for CD is to maintain a strict gluten-free diet (GFD) for life. If untreated, CD carries the risk of long-term complications including osteoporosis, reduced fertility and cancer.

HEALTH CANADA (HC) GOALS: HC recognizes that avoiding dietary gluten is challenging for those with CD. For those following a GFD, HC's goal is to minimize the risks associated with the consumption of gluten-containing or gluten-contaminated food and to maximize available choices of safe and nutritious foods.

HC INITIATIVES: The Bureau of Chemical Safety is collaborating with the Canadian Celiac Association, Quebec Celiac Foundation and professionals in the field to better serve the needs of Canadians with CD. These initiatives include: the creation of a HC webpage for CD, information pamphlets, a major review entitled "*The Safety of Oats and Celiac Disease*", funding and co-authoring a pan-Canadian study entitled "*Living with a Gluten-Free Diet*". HC is also initiating a research study to determine levels of undeclared gluten in foods, and of gluten contamination and nutritional values in a typical GFD.

ANTICIPATED OUTCOMES: Sharing this information will increase awareness about CD and the GFD amongst health care providers, policy makers, and the food industry. This data will provide a scientific basis for revisions to Canadian regulations on gluten-free foods. The ultimate goal is to increase the quality of life of those with CD by increasing food safety, dietary compliance, and reducing health complications and their associated health care costs in Canada.

**October is "Celiac Awareness Month" in Canada.
Come and visit HC booth for more information.**

3.32 Soy Isoflavones Inhibit Growth of DLD-1 Human Colon Adenocarcinoma Cells *In Vitro* and Are Associated With an Increase in Estrogen Receptor-beta

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SUMMARY: Food constituents such as soy isoflavones are known endocrine disruptors and modulators of colon carcinogenesis. We report the effect of soy isoflavones on the growth of a human colon cancer cell line and the role of estrogen receptor, a molecule implicated in the pathogenesis of endocrine-influenced cancers including colon cancer.

OBJECTIVES: We have previously demonstrated that soy isoflavones elicit a colon cancer preventive effect when exposed throughout the lifetime of rats, inclusive of *in utero* and post-natal stages using an experimental model. Soy isoflavones increased the expression of tumor estrogen receptor (ER)- β , one of the main candidates in endocrine disruption during colon carcinogenesis. To further understand the relationship between the role of soy isoflavones and ER- β in colon carcinogenesis, we aimed to examine the effects of soy isoflavones in DLD-1 human colon adenocarcinoma cells in the presence of ER- β or when expression was decreased by RNA interference (siRNA).

DESIGN: DLD-1 cells were administered increasing concentrations of soy isoflavones composed of genistein, daidzein and glycitein at a ratio of 1:1:0.2. Cytotoxicity was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, viability by the trypan blue exclusion method, and the signalling molecules were assessed by Western blotting or semi-quantitative RT-PCR techniques.

RESULTS: Soy isoflavones inhibited the growth of DLD-1 cells, with an IC₅₀ for cytotoxicity of 24.82 μ g/mL and an IC₅₀ for cell viability of 17.01 μ g/mL. At sub-cytotoxic doses, soy isoflavones modulate the expression of markers associated with MAP kinase, AKT and TNF- α signalling pathways, cell proliferation, cell cycle regulation and apoptosis conducive to a growth restrictive effect. A knockdown of ER- β at the gene and protein level was achieved in DLD-1 cells using siRNA, causing a differential expression of the molecular markers studied above.

CONCLUSIONS: Our results suggest that ER- β appears crucial in mediating the growth suppressive effects of soy isoflavones. ER- β may play an important role in the action of several endocrine disruptors including food chemical contaminants during colon carcinogenesis and thus requires further scrutiny.

3.33 Two years of Enteric Pathogen Surveillance Within a Community (C-EnterNet): Integrating Human and Exposure Components for Decision-Making

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SUMMARY: This presentation highlights the most interesting outputs that have been identified from an innovative sentinel site surveillance related to both the sources and occurrence of enteric pathogens in an Ontarian community. This enhanced, integrated surveillance provides a comprehensive picture of pathogen risks, to inform local, provincial and federal policies and regulations.

OBJECTIVES/BACKGROUND/ISSUES: Integrated surveillance has been advocated in Canada and abroad to reduce the burden of infectious enteric diseases in general. In Canada, C-EnterNet, a multi-partner integrated sentinel site surveillance program, was launched in 2005 in its first sentinel site for the surveillance of various enteric pathogens in both the human population and from various exposure sources.

DESIGN/METHOD/DESCRIPTION: Since 2005, active monitoring of those pathogens on farms, retail raw meats and untreated surface water has been initiated within the sentinel site boundaries. Culture-based detection (and molecular-based methods for water only), speciation, antimicrobial resistance testing, and enumeration (in food only) were performed. In parallel, enhanced epidemiological and microbiological data were collected for the human cases in the community.

OUTPUTS/RESULTS: Taking *Campylobacter* as an example, 134 and 177 campylobacteriosis cases were reported in 2006 and 2007, respectively. 19% and 26%, respectively, (higher in 2007) were related to travel abroad Canada. For both years, the incidence of endemic cases was higher in summer and in fall and higher for men, especially boys <5 yrs. In both years, *Campylobacter* were detected in 30 % of retail chicken meat, the bacterial load was usually low (<0.3 cell/g) and the contamination was the most frequent in fall and the least frequent in winter. *Campylobacter* was rarely detected in retail pork and beef. *Campylobacter* was detected in swine, dairy, beef and chicken farms. *Campylobacter* cells were present in raw surface water samples (between 9 and 98% depending on the year and the laboratory method) more frequently in 2007. The species distributions of *Campylobacter* found in farms and in water matched partially with the distribution seen in human patients.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This sentinel site surveillance program has generated a comprehensive database that is needed to inform the evaluation and development of food and water safety policies in Canada related to infectious enteric diseases.

3.34 Persistent Organic Pollutants (POPs) in Canadian Chicken Eggs

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SUMMARY: Polychlorinated biphenyl (PCB), dioxin/furan (PCDD/F) and polybrominated diphenyl ether (PBDE) concentrations were measured in egg yolks from four different hatchery types. Eggs were collected at grading stations in B.C., Quebec and the Maritimes. Levels of these legacy persistent organic pollutants (POPs) were similar regardless of hatchery type, although regional differences were observed.

BACKGROUND: Foods of animal origin are recognized as the major source of POPs to humans. European researchers have reported that eggs produced by chickens with access to the outdoors (free range chickens) have elevated levels of dioxins/furans and PCBs and frequently the concentrations of these POPs exceed the established European guideline levels. Eggs are both an important food source and commodity in Canada and monitoring of contaminant levels in eggs is performed. Focussed studies to determine POP levels in eggs from different Canadian hatchery types, however, have not been reported.

DESIGN: Ten eggs from each of the four main hatchery types (conventional, free range, omega-3 enriched, organic) were collected at grading stations in B.C., Quebec and the Maritimes (n=120) to ensure that no regional biases were introduced. Eggs were frozen as received until analysis. Because egg yolks are lipid rich and the POPs of interest accumulate in lipid tissues, yolks were extracted, prepared for analysis and analyzed using gas chromatography-mass spectrometry.

RESULTS: PCB, PCDD/F and PBDE levels ranged from 250 to 11700 pg/g lipid, 2.37 to 102 pg/g lipid and 18 to 3590 pg/g lipid, respectively in eggs analyzed from Quebec and B.C. PCB concentrations in free range eggs were not elevated relative to conventional, omega-3 enriched or organic eggs. PCB concentrations in omega-3 enriched and organic eggs from B.C. were lower than similar eggs from Quebec. In free range egg yolks, the variation in PCDD/F concentration was lower than observed in other egg types, in contrast with PBDE results.

IMPLICATIONS/NEXT STEPS: Although the Canadian guidelines for PCDD/Fs and PCBs in foods are under review, the eggs measured in the present study were generally found to have POP concentrations below the European guideline levels. Egg yolks from the Maritimes will be analyzed and POP levels will be compared to the results obtained in eggs from the other regions of Canada. The data developed in the present study will be provided for use in guideline development and exposure assessment.

3.35 A New HPLC Method for Analysis and Quality Control of Pandemic Influenza Vaccines

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SUMMARY: A physicochemical method using size exclusion high-performance liquid chromatography (SE-HPLC) was developed for the identification of haemagglutinin (HA) protein in monovalent influenza vaccines. This novel approach is advantageous in that it can be rapidly performed and does not rely on reagents.

OBJECTIVES/BACKGROUND: Single Radial Immunodiffusion Assay (SRID) is the currently accepted method for the determination of influenza vaccine potency (HA content) and is dependent on specific reference and antiserum reagents. However, these reagents may not be readily available during an influenza pandemic. As part of the pandemic preparedness plan, alternate methods for determining the potency of influenza vaccines are required. The utility of the SE-HPLC method, which is highly reproducible and independent of specific reagents, was investigated for assessing HA content as a measure of quality control of influenza vaccines.

METHODS: A TSK-GEL G4000SWxl (Pore size, 450 Å) column was coupled to a G3000SWxl (Pore size, 250 Å) column in series on a Varian Prostar HPLC system. Several untreated monovalent bulks of influenza vaccines, including those for candidate pandemic influenza strains, were injected into the columns system. Optimal conditions for separating the proteins were determined using different mobile phases, pH of mobile phases, and column temperatures. The HPLC profiles of those vaccines were analyzed for retention time, peak area and peak height. The identification and quantification of HA in the HPLC fractions were confirmed by SRID, BCA protein assay and SDS-PAGE, and assessed against the product's Certificate of Analysis.

RESULTS: The HPLC chromatogram demonstrated several peaks with the peak from 15-20 minutes containing HA. The HA profiles for different seasonal and H5N1 influenza strains were similar. Linearity was observed as a function of sample loading. Analysis of the HA peak areas by SRID confirmed the presence of HA which correlated with the HA protein values as reported on the manufacturer's Certificate of Analysis.

CONCLUSIONS/IMPLICATIONS: This SE-HPLC method has considerable potential for widespread use as a physicochemical method for HA identification and quantification, in quality control testing for seasonal and pandemic influenza vaccines, and as a practical alternative for potency measures by the reference reagent-dependent SRID assay. Further method development will facilitate the identification of compositions of the HA peak area and standardization of this method.

3.36 Experimental Challenge of Cattle with Atypical Bovine Spongiform Encephalopathy Isolates

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SUMMARY: The aims of this international collaborative study are to describe and to determine the disease process and the tissue distribution of BSE abnormal prion protein (PrPres) of two newly discovered atypical forms of BSE found in Canadian cattle. The results will be used in assessing the current federal SRM policy.

INTRODUCTION: To establish public health protection measures for BSE risks, it is important to precisely define the tissues of infected BSE cattle that can transmit the disease. These tissues are defined in the *Food and Drugs Act* as specified risk materials (SRM) and are banned for use in food.

At the time of promulgation of the SRM regulations, only the classic form of BSE had been diagnosed in Canadian cattle, but recently two new atypical forms of BSE called H-type and L-type have been confirmed. Currently, it is not known if the scientific basis used to define the current listing of SRM tissues similarly applies to these new forms.

OBJECTIVES:

Phase 1:

To experimentally infect cattle with H-type and L-type atypical forms of BSE.
To elicit information on the pathogenesis of H-type and L-type BSE.

Phase 2:

To determine the distribution of H-type and L-type BSE in tissues of pre-clinical and clinically affected cattle.
To compare the infectivity of H-type and L-type BSE in tissues of pre-clinical and clinically affected cattle to the classic BSE strain.

DESIGN: In Phase 1, five and six cattle were inoculated intra cranially under narcosis with the H-type and L-type homogenates respectively. The 10% brainstem homogenates were prepared from one H-type and from one L-type isolate.

Three animals per group were killed in the preclinical stage. An extensive group of tissue samples from all the major body systems were collected, processed and stored. The remaining animals were kept until they developed clinical symptoms. The animals were examined for behavioural changes every four weeks throughout the experiment.

RESULTS: All animals of both groups developed clinical symptoms and had to be euthanised within 16 months post-inoculation. The clinical picture differed from that of classical BSE, as the earliest signs of illness were loss of body weight and depression. However, the animals later developed hind limb ataxia and hyperesthesia, predominantly at the head. Analysis of brain samples confirmed the BSE infection and the atypical Western blot profile were maintained in all animals. Samples from the central nervous, peripheral nervous, musculo-skeletal, alimentary, respiratory, reproductive, and lymphoreticular systems of the study animals are now being examined, to describe the disease process and to assess the distribution of these novel BSE types in the various tissues.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The successful experimental transmission of both atypical forms in this phase of the study permits the analysis of the detection and distribution of PrP^{Res} in cattle tissues. An ongoing study to determine the infectivity of these tissues using a transgenic mouse model is currently underway.

Results will be used to help evaluate the current definition of SRM.

3.37 Characterization of Norovirus Capsid Stability

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SUMMARY: Norovirus is the leading cause of infectious gastroenteritis worldwide. In order to control the spread of infection, one must understand viral stability in its environment. Since noroviruses do not grow in cell culture, we are generating a virus-like particle (VLP) model to compare the stability of various norovirus strains.

OBJECTIVES: Since noroviruses assemble their capsid from a single structural protein (VP1), the objective of this study is to generate VLPs consisting entirely of VP1. The assembled VLPs will be selectively purified and biochemical tests carried out in order to characterize their stability under varying conditions of temperature and pH. The VLPs are morphologically similar to authentic virions, making them a potential model to study the stability of norovirus in its environment

DESIGN: Reverse transcription polymerase chain reaction (RT-PCR) was used to amplify the VP1 genes from four representative strains: the current surrogate, murine norovirus (MNV); a well studied previous surrogate, feline calicivirus (FCV); and the unculturable GI and GII human noroviruses. The VP1 genes were introduced into an insect cell line by means of a recombinant baculovirus. The recombinant VP1 monomers secreted by the insect cells spontaneously assembled into VLPs, which were isolated, purified and subjected to various pH and temperature conditions. Degradation of the VLPs was monitored using electron microscopy (EM).

OUTPUTS/RESULTS: At present, the VP1 capsid genes from MNV, FCV, GI and GII have been successfully amplified by RT-PCR and introduced into an insect cell line by recombinant baculovirus infection. Also, we have obtained presumptive FCV-VLPs as visualized by EM. This discovery has established the potential of the baculovirus expression system for the production/study of norovirus-VLPs.

IMPACTS/OUTCOMES/CONCLUSIONS: The baculovirus expression system has been successful in generating presumptive FCV-VLPs. With a little more time, we will be able to generate VLPs for MNV, GI.1 and GII.3 norovirus strains. The relative stabilities of these VLPs will be assessed under various temperature and pH conditions, and these data will contribute towards the development of anti-viral agents and safe cooking guidelines to control norovirus transmission on surfaces and in foods. Also, by relating our eventual findings to the prevalence of different noroviruses in Canada, this information may be used determine if risk assessment profiles should incorporate strain-specific information.

3.38 Furan Toxicity Testing *In Vitro*: Conquering the Problem of Volatility

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SUMMARY: The volatile chemical furan, a food contaminant, has proven very difficult to examine *in vitro* due to quick loss from culture media. To obtain reliable cell toxicity data, all components are kept on ice for chemical addition, followed by sealing the culture plates with plastic film before transfer to 37°C.

OBJECTIVES/BACKGROUND/ISSUE(S): Furan has been isolated from cooked or heated foods, arising through reactions of amino acids, fatty acids, and ascorbic acid. The volatility of furan (bp 31°C) has led to difficulties in toxicity assessments *in vitro* because of losses during pipetting and incubation. Therefore, a modified cell culture approach was needed to stabilize chemical exposure.

DESIGN/METHOD/DESCRIPTION: Rat H4IIE and human HEPG2 hepatoma cells, and 1064SK normal human diploid fibroblasts were allowed to attach overnight in 96-well plates. The next day, dilutions of chilled furan in cold medium were made in iced scintillation vials and rapidly capped. Growth medium was removed from the culture plates, the plates set on ice, and 300 µl of test chemical dilution added per well to minimize dead volume. The plates were sealed with plastic film and returned to the 37°C incubator.

OUTPUTS/RESULTS: After 24 and 48 hours of incubation, cell viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. In contrast to previous results of U-shaped dose-viability responses, the chilled conditions and sealing of plates produced well defined viability curves for all cell lines. HEPG2 and 1064SK cells exhibited similar sensitivity to furan with LD₅₀'s of 0.96 and 0.81 µl furan/well after 24 hours while H4IIE cells were somewhat more resistant (LD₅₀ 1.8 µl furan/well). All cell lines showed increased sensitivity after 48 hours of exposure.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: While the cold shock and lack of gas exchange are not optimal for cell culture, the treatment appears to be tolerated over short culture durations (24 to 48 hr). The approach has produced credible data for furan toxicity and will be used for assessments of furan effects in gene expression and genetic toxicology assays, thus contributing towards health risk assessments of furan exposure. Finally, the approach will also be applicable for future assessments of other volatile compounds.

3.39 FoodSHIELD: An International Tool for Responding to Food Safety Emergencies

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SUMMARY: FoodSHIELD is a unique, web-based platform designed to foster communication and collaboration between governments departments and agencies involved in food safety and security. The Canadian Food Emergency Laboratory Network has been formed to support the Canadian food safety system, and is working to establish a Canadian presence on FoodSHIELD.

OBJECTIVES/BACKGROUND/ISSUE(S): Governments at all levels have recognized the need for improved awareness of food safety and ability to deal with food safety and security issues. The ability to respond to food safety incidents and emergencies is complicated by the variety of government departments with some level of responsibility for food safety. FoodSHIELD has been created under the sponsorship of the US Dept. of Homeland Security to foster communication and collaboration between laboratories and regulatory agencies in the food and agricultural sectors.

DESIGN/METHOD/DESCRIPTION: Features of FoodSHIELD are presented. Application to everyday use by laboratories, as well as in food safety incidents and emergencies is discussed.

OUTPUTS/RESULTS: The Development of a Canadian Food Emergency Laboratory Network, with a presence on the FoodSHIELD website is underway.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Expansion of the Canadian Food Emergency Laboratory Network to include laboratories from other government departments and agencies will foster communication and collaboration within the food safety community. Inclusion of international research partners in workgroups within the FoodSHIELD website will provide access to a wider knowledge base. Thus, the use of FoodSHIELD will strengthen governments' ability to respond to food safety incidents and emergencies.

3.40 Phytosterol-/Phytostanol-Induced Diastolic Blood Pressure is Associated with Increased Ace1, Nos1, Nos3, Cox2, And Spon1 mrna Expression in SHRSP Rats

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SUMMARY: Whether or not dietary phytosterols or phytosterols elicit a net beneficial effect on cardiovascular disease remains controversial. Our data indicate that consumption of phytosterols or phytosterols increases blood pressure and alters the expression of blood pressure regulatory genes in spontaneously hypertensive stroke-prone rats (SHRSP).

OBJECTIVES/BACKGROUND/ISSUE(S): The aim of this study was to elucidate the molecular mechanisms that result in increased diastolic blood pressure due to dietary supplementation of high levels of phytosterols or phytosterols in SHRSP rats.

DESIGN/METHOD/DESCRIPTION: In total, sixty male SHRSP and Wistar Kyoto (WKY) *inbred* rats (10/group) were fed a control diet or a diet supplemented with phytosterols or phytosterols (2 g/kg diet) for five weeks. The blood pressure of each rat was measured at the beginning and end of this feeding phase. Subsequently rats were killed and kidney sterol levels were measured by gas chromatography and the expression of several renal genes known to be involved in blood pressure regulation was assessed by real-time quantitative PCR.

OUTPUTS/RESULTS: SHRSP rats fed the phytosterol or phytosterol diet demonstrated a significant increase in diastolic blood pressure compared to rats fed the control diet. Dietary supplementation of phytosterols or phytosterols induced phytosterol or phytosterol accumulation in kidney, respectively, but had no effect on renal cholesterol levels. SHRSP rats had lower renal levels of cholesterol, phytosterols, and phytosterols than WKY *inbred* rats. *Angiotensinogen*, *angiotensin I converting enzyme (peptidyl-dipeptidase A) 1 (Ace1)*, *nitric oxide synthase (Nos) 1*, *Nos3*, *cyclooxygenase 2 (Cox2)*, and *THUMP domain containing 1 (Thumpd1)* were expressed at higher levels in SHRSP compared to WKY *inbred* rats. *Renin* and *angiotensin II receptor type 1 (At1)* were expressed at lower levels in SHRSP than WKY *inbred* rats. Phytosterol supplementation up regulated the expression of *Ace1* and *Nos3* in SHRSP rats. Phytosterol supplementation increased mRNA levels of *Nos1* and *spondin 1 (Spon1)* in SHRSP and WKY *inbred* rats. *Cox2* mRNA levels were elevated in both phytosterol and phytosterol supplemented SHRSP and WKY *inbred* rats.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The increased diastolic blood pressure observed in phytosterol or phytosterol supplemented SHRSP rats may be due to altered renal expression of blood pressure regulatory genes. This project contributes to Health Canada regulatory and policy activities to evaluate the safety of phytosterol- or phytosterol-enriched foods. These results also warrant future studies to determine if foods fortified with

phytosterols or phytostanols will have similar effects in humans predisposed to hypertension.

3.41 Role of Surfaces in Disinfection of Human Norovirus

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SUMMARY: Many disinfectants claim to be highly effective against viruses; however we show that virucidal activities are somewhat dependant on the surface that is contaminated. The outcome of this research will directly lead to safer food preparation.

OBJECTIVES: To define the combinatorial effects of drying and disinfection on the inactivation of the human norovirus (NoV) and the murine norovirus (MNV).

DESIGN: NoV and MNV were inoculated on brushed stainless steel, ceramic tile, laminate and cotton fabric. Each virus was treated with accelerated hydrogen peroxide or bleach. Virus was recovered from the discs at various time points and the remaining viral titre was determined by plaque assay and reverse-transcription polymerase chain reaction (RT-PCR) for MNV or RT-PCR alone for NoV. In order to obtain RT-PCR data more representative of the remaining viral infectivity, samples were treated with proteinase K and RNase H to eliminate damaged particles and free RNA.

RESULTS/OUTPUT: MNV is a better NoV surrogate than the widely used feline calicivirus. The enzymatic treatment used here will improve the data on disinfection of human noroviruses. We found that virus dried on surfaces is more resistant to disinfection than virus in liquid medium.

IMPACTS/OUTCOMES/CONCLUSIONS: Bleach and Virox are currently used as effective disinfectants; however their virucidal efficacy on various surfaces was not tested until this study. Here, we show that MNV is a good substitute for NoV in disinfection studies and that drying on a variety of surfaces increases the resistance of noroviruses to disinfection. These differences can be attributed in part to surface porosity, which provides hiding places for viruses and can be inaccessible to disinfectants. This highlights the importance of testing disinfectants using the conditions where they are most often used. The outcome of these studies will be useful in developing standards for norovirus inactivation in various settings, particularly health care and food preparation environments.

3.42 Do Canadian Children Aged 1-8 Years Meet Their Nutrient Requirements Through Food Intake Alone?

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SUMMARY: Results from the Canadian Community Health Survey (CCHS) 2.2 (Nutrition 2004) showed that only few dietary nutrients are of concern among Canadian children. Potassium and sodium are the only two nutrients that require attention in the diet of the 1-8 years old.

BACKGROUND/OBJECTIVES: A nutrient dense diet helps children to reach full growth potential. Moreover, current evidence indicates that vitamins and minerals are important for immune function and cognitive development including the learning capacity of children. However, until now no national data were available to examine the nutrient intake of Canadian children.

Objectives: 1) Assess the nutrient adequacy of the diets of Canadian children aged 1-8 years and 2) Encourage program and policy makers to use CCHS 2.2 results.

DESIGN: Mean and median usual nutrient intakes from food were derived from 24-hour recalls of the CCHS 2.2 (n=27 424). Intakes were assessed against appropriate Dietary Reference Intake (DRI) values: Estimated Average Requirement (EAR) or Adequate Intake (AI) when appropriate. Results did not include nutrient intakes from vitamin and mineral supplements.

RESULTS: The prevalence of inadequate intakes for nutrients (coming from food) with an EAR was very low (<5%) for niacin, riboflavin, thiamine, folate, vitamin A, vitamin B₆, Vitamin B₁₂, Vitamin C, magnesium, zinc, phosphorus as well as iron. Based on the AI, the proportion of children with a low risk of inadequacy is high for vitamin D (>60%) and calcium intake (>80%). However, only a small proportion of boys and girls were at low risk of inadequacy for potassium (5-15%). Finally, a high proportion of this age group (>75%) showed a usual sodium intake above the Tolerable Upper Intake Level (UL).

CONCLUSION: Canadian children aged 1-8 years have an overall satisfactory intake of most nutrients when food consumption alone is considered. Only potassium and sodium need more attention for that age group. As a second step, a closer look at the sources of nutrients and the energy level of the diet of children will provide information on the quality of their diet.

3.43 Do Canadians Aged 51 and Older Meet their Nutrient Requirements Through Food Intake Alone?

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SUMMARY: Results from the Canadian Community Health Survey (CCHS) 2.2 (Nutrition 2004) showed that Canadians aged 51 and older have less than satisfactory intakes of vitamins A, C and D, zinc, folate, magnesium, sodium, calcium and potassium when food intake alone is considered

BACKGROUND/OBJECTIVES: There are many challenges to healthy eating during middle-age and beyond. Studies have shown that many older adults do not achieve their nutrient requirements through food intake alone. No national data were available until now to examine nutrient intake of this sub-population in Canada.

Objectives: 1) Summarize the prevalence of adequacies and inadequacies in selected nutrient intakes from food among Canadians aged 51+ years, 2) Encourage program and policy makers to use CCHS 2.2 results.

DESIGN: Mean and median usual nutrient intakes from food were derived from 24-hour recalls of the CCHS 2.2 (n=27 424). Intakes were assessed against appropriate Dietary Reference Intake (DRI) values: Estimated Average Requirement (EAR) or Adequate Intake (AI). Results did not include nutrient intakes from vitamin and mineral supplements.

RESULTS: In men and women, the prevalence of inadequate intakes for nutrients with an EAR varied from less than 10% (niacin, riboflavin, thiamine, phosphorus, iron), to 10-30% (vitamin B₆), to more than 40% (vitamin A, magnesium). Based on the AI, only a small proportion of men and women were at low risk of inadequacy for calcium (5-15%), vitamin D (<20%), and potassium (<10%). A large proportion had usual sodium intakes above the Tolerable Upper Intake Level. Between 10-25% of men consumed inadequate amounts (below the EAR) of folate whereas more than 25% consumed inadequate amounts of vitamin C and zinc. Between 15-20% of women had usual vitamin C intakes below the EAR. More than 25% of women had usual intakes of folate and zinc below the EAR.

CONCLUSION: Canadians aged 51 years and older have less than satisfactory intakes of certain nutrients when food consumption alone is considered. Further investigation of the contribution of supplements to the total nutrient intakes would provide a more complete picture of nutrient adequacy among this subgroup of the Canadian population.

3.44 Food Security Reference Group: Building the Evidence to Support Decision-Making at Policy and Community Planning Levels and Improve Food Security for First Nations and Inuit

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SUMMARY: The Food Security Reference Group (FSRG) is chaired by the First Nations and Inuit Health Branch, Health Canada, and brings together First Nations and Inuit partners, the federal government, academia and other key organizations to review evidence and set collective action for improving food security for First Nations and Inuit.

OBJECTIVES/BACKGROUND/ISSUE(S): Available data demonstrate that food insecurity is much higher among Aboriginal populations than non-Aboriginal populations in Canada. To date, there has been little conceptualization of what food security might mean for First Nations and Inuit, and little consideration of what policies or practices might enable the achievement of this important social determinant of health. The objective of the FSRG is to review and build the evidence and set priorities for collective action to improve food security in First Nations and Inuit communities.

DESIGN/METHOD/DESCRIPTION: The FSRG brings together the Assembly of First Nations, Inuit Tapiriit Kanatami, the federal government (including Health Portfolio partners and Indian and Northern Affairs Canada), academics and others in a forum for sharing information, discussing strategies and planning.

OUTPUTS/RESULTS: Since its inception in 2005, the FSRG has developed a comprehensive literature review and an evidence-based interventions framework, and has begun to review and document community-based initiatives. These outputs have helped better define food security issues for First Nations and Inuit, and highlight gaps in policy and research. This work has also helped conceptualize how food security can be promoted in First Nations and Inuit communities.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: There are unique considerations and implications for food security specific to First Nations and Inuit that must be taken into account in research and policy development. Future directions of the FSRG include identifying opportunities for advancing food security at policy and community planning levels through positioning and building on the tools developed and information gathered by the FSRG, and continuing to build the evidence to support decision-making at these levels.

3.45 Optimization of Growth Conditions for the Rapid Identification of Bacteria by Use of Focal Plane Array - Fourier Transform Infrared (FPA)-FTIR Imaging Spectroscopy

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SUMMARY: The food industry needs rigorous microbiology controls at practically all levels of its endeavours. Our group has demonstrated that the potential utility of FTIR-based bacteria identification is enhanced through the use of focal plane array (FPA)-FTIR imaging spectroscopy, which provides a means of simplifying sample preparation and improving accuracy, yet a standard protocol needs to be developed and validated in order to widely adopt this alternative method.

BACKGROUND: The objective is to optimize and standardize the protocol for bacterial growth prior to FPA-FTIR spectroscopic identification by establishing the type of media bacteria should be grown on, the optimal growth time the specimens should be grown before analysis, including an adequate method for inactivating the samples if these need to be transported for analysis. Bacterial identification using FTIR relies on the fact that whole cell infra red spectra of a bacteria culture represents the totality of biochemical compounds present in this at a given time; in fact the FTIR spectra of a bacteria is considered a biochemical fingerprint and is independent of the bacteria size or shape. It has been demonstrated by us, and others, that this information is enough to separate bacterial spectra according to taxonomic species.

METHODS: These objectives will be tackled by testing 13 different growth agars, 9 growth times and 9 inactivation methods. By employing a collection of 10 food-borne bacterial strains, a detailed examination of the effects of all of these different treatments on the accuracy of FTIR-FPA methods for bacterial discrimination and identification will be conducted. Based on the results of these investigations, a standardized protocol will be developed and tested in a preliminary internal validation study involving 25 food-relevant bacteria.

RESULTS: Preliminary results indicate that media type and brand as well as growth time before deposition influence the bacterial spectra; this effect is reflected in the way spectra segregate from each other when analyzed with a variety of mathematical methods. Nevertheless, in general, spectra of the same species tend to cluster together regardless of the mathematical method used to analyze the spectra or the bacterial growth conditions.

CONCLUSIONS: This project will support risk assessment and regulatory activities by providing a shorter turnaround time for bacterial identification in data collection projects, inspection of food commodities, but mostly in outbreaks or emergency situations where fast results are required from large numbers of samples.

3.46 Determination of Low Molecular Weight Volatile Cyclic Siloxanes in Cosmetics Sold on the Canadian Market

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SUMMARY: Levels of four low molecular weight (LMW) volatile cyclic siloxanes, namely octamethylcyclotetrasiloxane (D3), octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6) were determined in 253 cosmetic products sold in Canada using gas chromatography-mass spectrometry (GC/MS). These four siloxanes can be found in 1%, 4%, 14%, and 10% of the products, respectively. The highest concentration determined was 683 mg/g (D5).

OBJECTIVES: LMW volatile cyclic siloxanes have been identified in the categorization of the Cosmetic Substances List as a high priority for action under the Chemicals Management Plan (CMP) in Batch 2. These siloxanes are widely used in cosmetics due to their unique spreadability, cleansing and lubrication properties. There is no regulation so far on siloxanes in cosmetics. However, based on animal tests D4 can impair fertility, and D5 has potential carcinogenicity. Once released into the environment, D4, D5, and D6 can be persistent and bioaccumulative. Although there is a general knowledge on the use of siloxanes in cosmetics, the specific information on the levels of siloxanes in cosmetics sold in Canada is not well understood. This study is aimed at providing such specific information to support government's efforts in assessing and regulating these chemicals as outlined in CMP.

DESIGN: 253 cosmetic products were collected throughout Canada by the Consumer Product Safety (CPS). Each product was accurately weighed and mixed with hexane. After centrifugation, the hexane solution was analyzed by GC/MS.

RESULTS: The results indicated that 14% of the total cosmetic products contain at least 1 siloxane at concentrations up to 683 mg/g (D5). In addition, 3% of the baby products contain at least 1 siloxane at concentrations up to 150 mg/g (D5). Most of the antiperspirants, a subgroup of deodorants, contained relatively high concentrations compared to other types of products.

CONCLUSIONS AND FUTURE RESEARCH: The product specific data on the presence of siloxanes in cosmetics will help generate accurate Canadian exposure data for risk assessment and risk management. Further study on the determination of the siloxane emissions in cosmetics for estimating inhalation exposure, and on dermal exposure estimates associated with the use of these products will be conducted.

3.47 Soy Consumption Has Coordinated Effects with Dietary Iodine Levels on Hepatic Iodine Contents and Serum Thyroid Hormone Levels in Rats

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SUMMARY: This study reveals that both soy proteins and associated isoflavones (ISF) have anti-thyroid effects, which can be eliminated by increasing dietary intake of iodine, suggesting that sufficient iodine intake might be essential for soy consumers to prevent thyroid disorders. However, this needs to be confirmed in humans.

OBJECTIVES: Soy intake causes thyroid disorders in certain populations. However, the involved mechanism(s) and anti-thyroid component(s) in soy remain controversial. This study examined the potential coordinated effects of dietary soy and iodine status on thyroid metabolism.

METHOD: Weanling Sprague-Dawley rats were fed diets containing either 20% casein or alcohol-washed soy protein isolate (SPI) in the absence or presence of supplemental ISF (250 mg/kg diet) with varying iodine levels for 60 days. Iodine contents in diets and livers as well as serum thyroid hormone (T3 and T4) levels were measured.

RESULTS: In the rats fed casein diets, hepatic iodine contents were not affected by dietary iodine levels. However, supplemental ISF significantly reduced iodine content in liver of the rats fed low iodine casein diet. Hepatic iodine contents in male rats fed normal or low iodine SPI diets and in females fed low iodine SPI diet were markedly lower than those fed casein diets with the same iodine levels, respectively. This effect of SPI was eliminated by high dietary iodine supplementation. Dietary SPI significantly reduced serum free T3 in males and increased total T3 in both sexes compared to casein diet. Supplementation with ISF and high level of iodine reduced free T3 in rats fed casein diets compared to normal iodine casein diets alone. Low dietary iodine remarkably decreased free and total T4 levels in males fed SPI diet but had no effect in the rats fed casein diet.

CONCLUSIONS: This study shows that when dietary iodine levels were normal or marginally deficient, intake of soy proteins or soy-derived ISF reduces hepatic contents of iodine, the essential element for thyroid hormone biosynthesis, and serum thyroid hormone levels. The anti-thyroid action of soy can be prevented by increasing dietary iodine levels, suggesting that consumption of soy products may increase the iodine requirement to prevent thyroid disorders. This information is important for Health Canada in making Dietary Requirement Intake levels of iodine for soy consumers.

3.48 Germination Changes the Protein Profiles in Soybeans and May Produce Bioactive Peptides with Beneficial Effects on Diabetes

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SUMMARY: This study shows that the major storage proteins in soybeans are quickly degraded or cleaved to smaller fragments or peptides during early germination. This process may produce bioactive components responsible for the hypoglycaemic actions of the germinated soybeans observed in diabetic subjects.

OBJECTIVES: Soaked and germinated soybean seeds are reported to be highly effective in regulation of blood sugar and even more effective than oral hypoglycaemic drugs in treatment of type 2 diabetes patients. However, the mechanism of action and bioactive components produced during germination are not fully understood. This study was conducted to examine the changes of the major storage proteins in soybean seeds during early germination and to establish an effective method for detection of proteins or peptides in soy products.

METHOD: Soybean seeds were washed and soaked in distilled water (dH₂O) for 14 hours at room temperature, and then drained and wrapped in cotton cloth. To germinate, the seeds were kept in the dark at room temperature and misted with dH₂O. The germination process was allowed to proceed for 0, 2, 4, or 6 days. Germinated seeds were dehulled and then dried and grounded. The soybean flours were isolated for proteins through defatting, dissolving, precipitation, centrifugation, and freeze-drying. The protein isolates were characterized by gel electrophoresis referring to defined standards.

RESULTS: The protein profiles were similar in soaked and dry beans. Lipoxygenase content was lower in germinated beans compared to soaked or dry beans, but remains constant throughout the early germination process. The contents of all 3 subunits (', , and) of -conglycinin decreased with germination time, ' and subunits completely disappeared at day 6 of germination. Meanwhile, several new bands of lower molecular weight were detected. The abundances of all acidic subunits (A1, A2, A3, A4, and A5) of glycinin were decreased quickly with germination time, notably A3 and A5 disappeared at day 4 of the germination.

CONCLUSIONS: This study shows that most of the major storage proteins in soybean seeds markedly changed, probably through degradation or specific cleavage catalyzed by activated enzymes, during early germination. The low molecular weight proteins or peptides produced may play a role in modulating the development of diabetes. Further investigation will provide useful information for Health Canada in making dietary recommendation for Canadians with diabetes.

3.49 A Risk Assessment Framework to Guide the Screening Assessment of Micro-Organisms on the Domestic Substances List

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SUMMARY: The New Substances Program developed a three-step Risk Assessment Framework to guide the risk assessment of micro-organisms on the Domestic Substances List, and applied it to *Pseudomonas aeruginosa*. *P. aeruginosa* is a ubiquitous bacterium found in nature which is intentionally introduced at hydrocarbon-contaminated sites due to its capacity to degrade hydrocarbons. This raises human health and environmental safety concerns because *P. aeruginosa* is an opportunistic pathogen. A Technical Expert Group, recruited by the Program, will refine the Framework based on its review of the *P. aeruginosa* assessment.

BACKGROUND: The New Substances Program (NSP) of Environment Canada and Health Canada will conduct screening assessments of micro-organisms on the Domestic Substances List (DSL) to determine whether they are “toxic” or could become “toxic” as defined in the Canadian Environmental Protection Act, 1999 (CEPA 1999). Under the guidance of a Technical Expert Group (TEG), the NSP developed a framework to guide the screening assessment of DSL micro-organisms.

DESCRIPTION: A three-step risk assessment model for DSL micro-organisms, based on current practices in the assessment of organisms regulated as New Substances under the CEPA 1999, was developed. Step 1 is a hazard-based risk assessment, assuming conservative (“worst-case”) exposure scenarios. If at Step 1 the risk is considered negligible, the organism is declared not CEPA-toxic. Otherwise, Step 2 is applied: a refined risk assessment based on more precise exposure scenarios. The organism may be declared not CEPA-toxic or CEPA-toxic at Step 2, if the risk is low or high, respectively. For medium or uncertain risk, the assessment goes on to Step 3; a more detailed exposure analysis is used to better characterize the risk for a final decision. Also included in the Framework are discussions on the weight-of-evidence approach used in decision-making, the manner in which the NSP proposes to manage scientific uncertainty and the use of the precautionary principle.

OUTPUT: The Framework was accepted by the TEG as a working document. To test its worth, it was then applied to the screening assessment of *Pseudomonas aeruginosa*, a Risk Group 2 pathogen currently on the DSL. Review of this assessment will allow TEG members to better judge how well the Risk Assessment Framework meets the following criteria: it includes all the factors considered in a complete microbial risk assessment; it is flexible enough to accommodate the wide variety of DSL organisms (including microbial consortia); and it can accommodate missing information such that a risk assessment conclusion can still be reached using surrogate data and a weight of evidence approach.

NEXT STEPS: The NSP expects the TEG to suggest refinements to the Framework based on its review of the pilot assessment. The revised Framework will be applied to the screening assessment of other DSL micro-organisms.

4.01 Development of Cell-Based *In Vitro* Bioassay Platforms for Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL/Apo2L)

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SUMMARY: Recombinant soluble human TRAIL/Apo2L (rhTRAIL), a highly selective and promising biological therapeutic, is presently under clinical assessment for the treatment of various forms of cancer. We describe progress towards the development of cell-based *in vitro* bioassay platforms that use light emission and fluorescence for the determination of rhTRAIL potency.

OBJECTIVES: TRAIL [tumor necrosis factor (TNF)-related apoptosis-inducing ligand] induces rapid apoptotic death in malignant cell lines and tumors of diverse origins. Although selective, responses vary appreciably across and within tumor types, thus posing a challenge for the evaluation of TRAIL bioactivity. To address this issue, we set out to develop cell-based *in vitro* bioassay platforms that take into account the mechanism of action of TRAIL.

METHODS: Human SK-MES-1 lung carcinoma cells were chosen as substrates. Cell survival and caspase 3/8 activation assays were performed using CellTiter- and Caspase-Glo kits (Promega). ProCaspase and rhTRAIL-mediated apoptotic cleavage of PARP and BID, along with profiling of TRAIL receptors and ligand-dependent apoptotic pathway components, were done by semi-quantitative chemiluminescent Western blot hybridization. Authenticity and genetic integrity were ascertained by STR genotyping, 500K SNP chip and microarray analysis. Gross chromosomal anatomy was surveyed by spectral karyotyping.

RESULTS: SK-MES-1 cells expressed TRAIL receptors DR4, DR5 and the full complement of relevant and responsive apoptotic pathway components. Survival assays optimized for 96-well plates (3×10^4 cells/well; 3 wells/dose; 0-1000 ng/mL rhTRAIL dose range, 24h endpoint) could detect as few as 50 live cells. LD50 survival (~ 5 ng/mL) increased slightly (~ 8 ng/mL) as a function of *in vitro* passage. Caspase 8&3 assays corroborated survival data but gave lower LD50 values (0.5-0.1 ng/mL). Incorporation of a common lysis buffer allowed survival and caspase 3 activation assays to be run in parallel from a single plate. SK-MES-1 variants resistant to rhTRAIL were generated and incorporated as negative controls for TRAIL receptor activation. Cells expressing secretable luciferase or fluorescent reef coral proteins were also generated to provide flexible assay alternatives.

CONCLUSIONS: These bioassay platforms, which incorporate endpoints reflecting the molecular mechanism of biotherapeutic action in well-characterized indicator cells, provide a relevant foundation for the development of highly selective rhTRAIL potency assays.

4.02 Assessment of the Conformation of Interferon Alpha in Various Formulations Using the NMR Fingerprint Assay

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SUMMARY: Recombinant protein therapeutics, such as hormones or interferons, are formulated with additives (excipients) that either maintain or enhance biological activity while providing long term stability or shelf life. This paper presents a study of the effects of excipients on the structure of the active ingredient.

BACKGROUND: Recombinant protein therapeutics, such as hormones or interferons, are formulated with excipients or additives to either maintain or enhance biological activity and to provide long term stability or shelf life. In the context of comparison studies between a subsequent entry biologics (SEB) with the innovator's product, or a recognized standard, assessment of the structure of the active ingredient can be challenging in formulated preparation. Recently, we reported a powerful method based on nuclear magnetic resonance spectroscopy (NMR), termed the NMR Fingerprint Assay (Aubin and co-workers, *Anal. Chem.* 2008). This method assesses the bioactive conformation of recombinant protein therapeutics in solution. It is applied in this study to understand the effects of excipients on the structure of the protein and whether it can be de-formulated without irreversibly affecting its conformation.

OBJECTIVE: Assess the three-dimensional structure of interferon alpha 2a (IFN-a2a) and interferon alpha-2b (IFN-a2b) in various formulations. Compare the material produced in-house with the EDQM (European Directorate for Quality Medicine) reference standard.

DESIGN: Using the NMR Fingerprinting assay we have investigated the effects of various formulations on the conformation of IFN-a2a and IFN-a2b. The genes of human IFN-a2a and IFN-a2b were cloned, expressed in *E. coli* in ¹⁵N-labelled media as inclusion bodies, and refolded to their active conformation. The activity of the two proteins is inferred by comparing their NMR spectra with spectra recorded for EDQM reference standards of IFN-a2a and IFN-a2b. The protein is then subjected to freeze-thaw cycles, lyophilisation, pH variations and various solution conditions that reproduce formulations used for innovator products of Roferon-A and Intron® A. The structure is monitored by 2D-NMR spectroscopy before and after every treatment.

OUTPUTS/RESULTS: Near physiological pH (6.5), analysis of 2D-NMR spectra of formulated human ¹⁵N-IFN-a(2a and 2b) shows that excipients interact with the protein to maintain solubility. Under these conditions, IFN-a (2a and 2b) harbour a folded conformation in the presence of excipients, such as polysorbate-80 or human serum albumin. At lower pH (4.0), excipients no longer interact with the protein. This

is shown by comparing NMR spectra of IFN- α in the presence and absence of excipients. De-formulation experiments were performed at pH 4.0 with no detectable effect on the conformation.

IMPACT/OUTCOMES/CONCLUSION: Development of analytical techniques for the characterization of recombinant protein therapeutics is sometimes complicated by the presence of excipients in a given product. These may interfere with the method and may require their removal. In the latter case, a validation of the method may be required to correlate results in the absence of excipients with the complete product. By monitoring the structure of the protein (active pharmaceutical ingredient) in the presence of excipients, after and before de-formulation, the fingerprinting assay is more than a new method for product analysis, it can be used as a tool for method validation.

4.03 Extending Utilization-Based Physician Demand Models: Methods, Applications and Relevance

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SUMMARY: Main components of Health Human Resource (HHR) planning are demand and supply. Planners use supply-side policy levers (Medical school admissions, immigration, etc) to meet demand. It is imperative that demand be well forecasted. Our enhanced model incorporates other factors (technology, epidemiology etc) in addition to demographics and provides improved forecasts that enable better decision-making.

OBJECTIVE: A criticism of utilization-based models is that the forecast of Health Human Resources (HHR) demand is based on population growth and its distribution only. Many *other* factors such as technological innovation, changes in people's preferences and epidemiological changes affect the demand for healthcare providers. We build an improved version of the utilization-based model (*enhanced* model), which incorporates these *other* factors and apply the model to project the family physicians (FPs) and specialists in FTEs (full-time equivalents) for Nova Scotia.

METHOD: We develop a theoretical framework of the model for any type of health care provider followed by an empirical model for physicians and demonstrate how to quantify all the variables used in the model. Finally we apply this *enhanced* model to project the required number of FPs and specialists in terms of FTEs in Nova Scotia using Physician Billing data from that province.

RESULTS: The projected requirement of FPs FTEs increased from 902 in 2003/2004 (base year) to 1,114 in 2011/2012, an increase of 212 FTE in eight years. Of the total increase, only 22% is driven by the change in population and its distribution, 78% is driven by the *other* factors.

For physician's specialities also, the *other* factors plays a significant role. The results demonstrate the importance of this model: despite declining proportion and number of children, the model forecasts increasing requirements of paediatrician FTEs due to the positive effect of *other* factors offsetting the negative effect of population decline. We observe similar counterintuitive results among obstetricians, gynaecologists, anaesthesiologists, and psychiatrists.

IMPACTS: Availability and access to physicians is important, as is having the right mix of specialists. HHR planning models must use the available information and innovative techniques to project future HHR requirements. By providing policy makers access to better forecasts, our *enhanced* model strengthens the analytic body of evidence available to them for better decision-making.

4.04 The Application of Uncertainty Factors and the *Pest Control Products Act* Factor in the Human Health Risk Assessment of Pesticides

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SUMMARY: The Pest Management Regulatory Agency (PMRA) recently reviewed its use of uncertainty factors in the health risk assessment of pesticides. A new framework was developed for the application of uncertainty factors and the *Pest Control Product Act* (PCPA) factor (the latter specifically addressing the protection of infants and children).

BACKGROUND: The enactment of the new PCPA obliged the PMRA to provide additional protection to infants and children during risk assessment. The PMRA undertook a review of the application of uncertainty factors in concert with ongoing harmonization initiatives, involving principally the US EPA. The objective of the review was to develop a revised policy that facilitated the consistent application of uncertainty factors in accordance with internationally recognized regulatory practices and the obligations specified in the Act.

DESCRIPTION: The PMRA reviewed historical and current risk assessment practices both domestically and internationally. This overview was published in July 2007 along with questions designed to elicit stakeholder feedback. Responses, received from industry, government, academia and the public health sector were discussed at an open workshop in December 2007. This information was used to inform the development of a new policy on the application of uncertainty factors.

RESULTS: Under the new framework, uncertainty factors for interspecies extrapolation and intra-species variability are required for every risk assessment. Uncertainty factors for extrapolating from a Low Observed Adverse Effect Level (LOAEL) to a No Observed Adverse Effect Level (NOAEL), for extrapolating from short-term toxicity data to a chronic scenario or for database deficiencies are applied where necessary. The PCPA factor is applied in dietary and residential risk assessment taking into account the completeness of the database with respect to the toxicity to infants and children and prenatal and postnatal toxicity associated with a pesticide.

OUTCOMES: The new policy will ensure that the application of uncertainty factors and the legally-required PCPA factor will be applied in a consistent and transparent manner thereby strengthening the rigor of the risk assessment process and facilitating international work sharing. This policy, which is closely aligned with the practices of pesticide regulators at the US EPA, will be published by the summer of 2007.

4.05 Preventing Suicide in Aboriginal Communities

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² National Aboriginal Youth Suicide Prevention Strategy

SUMMARY: The Government of Canada is investing \$65M over 5 years to implement the National Aboriginal Youth Suicide Prevention Strategy (NAYSPS). The presentation will focus on the ongoing contributions of science and research to the Strategy, and its current evaluation and research initiatives.

OBJECTIVES/BACKGROUND/ISSUE(S): In Canada's Aboriginal population, suicidal behaviour is an urgent and preventable public health challenge. Suicide and self-inflicted injuries are the leading causes of death for First Nations youth and adults up to 44 years of age (Health Canada, 2003). Suicide rates among Inuit are among the highest in Canada.

Evidence-based suicide prevention initiatives implemented through NAYSPS require the blending of western and traditional Aboriginal knowledge to identify what works best in Aboriginal communities. Current research initiatives include Canadian Institutes of Health Research projects on understanding and acting on Aboriginal suicide; an adaptation of Dr. Chandler and Dr. Lalonde's research on cultural continuity and First Nations suicide to the context of Manitoba; and a review of national suicide surveillance data and identification of improved methods of collection and distribution.

DESIGN/METHOD/DESCRIPTION: The National Aboriginal Youth Suicide Prevention Strategy (NAYSPS), designed in partnership with the Assembly of First Nations and the Inuit Tapiriit Kanatami, is based on the best available evidence and on recommendations from groups such as the Suicide Prevention Advisory Group - a team of experts commissioned in 2001 to closely examine the issue and make practical recommendations to stem the tide of Aboriginal youth suicide.

OUTPUTS/RESULTS: Root causes of Aboriginal youth suicide are multi-faceted and multi-generational. The main purpose of NAYSPS is to reduce the risk factors associated with suicide and strengthen protective factors against suicide such as resiliency. An initial scan has examined NAYSPS's outputs including: new community-based initiatives; new tools/curricula; community/youth engagement; training/skills development; program and government linkages and partnerships; and more.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Reducing actual suicide rates is a long term goal that will require many years to effectively address the many factors that make Aboriginal youth vulnerable to suicide. To reach this goal, the Strategy will continue to support new research and evaluation activities that strengthen the Aboriginal youth suicide prevention evidence base. Plans for further research and evaluation will be discussed, as well as the renewal of the Strategy beyond 2010.

4.06 Revision of the National Nutrition Pregnancy Guidelines: Dietary Intake Modelling Supports a Low Level of Iron Supplementation for Pregnant Canadian Women

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SUMMARY: For many pregnant Canadian women, usual iron intakes from food alone appear inadequate, compared to the Dietary Reference Intakes (DRIs). Intake modelling to find a level of iron supplementation for acceptably low risk of both apparently inadequate and apparently excessive intakes supports a recommendation of 15-20 mg iron/day in supplement form during pregnancy.

OBJECTIVES/BACKGROUND/ISSUE(S): To determine a level of iron supplementation for pregnant women in Canada with an acceptably low risk of both apparently inadequate and apparently excessive intakes, for use in Health Canada guidelines.

DESIGN/METHOD/DESCRIPTION: The distribution of usual dietary iron intakes was estimated using 24-hour dietary recalls of pregnant women aged 19-50 from the Canadian Community Health Survey (CCHS), Cycle 2.2, Nutrition. The prevalences of usual intakes below the Estimated Average Requirement (EAR) for pregnancy (22 mg/d) or above the Tolerable Upper Intake Level (UL, 45 mg/d) were estimated. Iterative modelling with inclusion of supplemental iron in increments of 1-2 mg/d was done to determine desirable levels of supplementation. Apparent adequacy of combined intake (usual dietary intake + supplement) was judged in accord with the DRIs. Predicted prevalences of <3% for both apparent inadequacy of intake and apparent excessive intake were accepted as a working target.

OUTPUTS/RESULTS: The prevalence of apparently inadequate iron intakes fell below 5% at a level of 15 mg of daily supplemental iron. The level needed to achieve a prevalence of <3% could not be defined with precision but was unlikely to be much more than 15 mg/d. The prevalence of iron intakes above the UL became detectable above a supplement level of 20 mg/d.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: It was concluded that, based on the DRI requirement estimates for iron, a supplement of 15-20 mg/d throughout pregnancy is justified as both effective and safe for the general population. It is assumed that all pregnant women will be under the care of a qualified health care provider, and that therapeutic levels of iron will be prescribed if and when indicated by the condition of the patient. This work was undertaken to inform the revision of the National Nutrition Pregnancy Guidelines. Revising the iron supplement recommendation based on this work fits within Health Canada's mandate to implement the DRIs within its policies and guidelines.

4.07 Enabling More Effective Enforcement Decision-Making and Enhanced Compliance Through Analytical Testing of Workplace Hazardous Products

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SUMMARY: Effective decision-making for enforcing science-related legislation requires a knowledge of science and the scope of the legislation. Preliminary results for developing legally defensible and scientifically valid standard procedures and analytical methods to determine the veracity of information disclosed on MSDSs, in terms of ingredient identity and concentration, are reported.

OBJECTIVES: Enable more effective enforcement decision-making and enhanced compliance through legally defensible standard protocols for sampling, submitting, and analytical testing of workplace products by: 1) Determining the veracity of the disclosure on Material Safety Data Sheets (MSDSs) of the identity and concentration of hazardous ingredients and develop standard protocols/methods to monitor compliance with MSDS requirements established under the *Hazardous Products Act (HPA)* and the *Controlled Products Regulations (CPR)*; and, 2) Determining the effectiveness of GC-MS and GC-FID in monitoring compliance with such requirements.

METHODS: Standard protocols, developed in this work, were used to maintain a chain of custody throughout the process of the sampling, submitting and analytical testing of workplace chemical products. A validated GC-MS method for detecting, identifying and quantifying solvents in paint matrices was used to analyze commercial products sold to workplaces. GC-FID methods were used for certain substances when the MS was not the suitable means of detection.

RESULTS: Validated analytical methods, involving the use of GC-MS and GC-FID, were developed to test the presence of more than 40 organic solvents in commercial paint lacquers. This preliminary study of products sold to Canadian workplaces identified some discrepancies between the ingredients disclosed on MSDSs and those identified in this work at concentrations above the regulatory cut-off limits for disclosure.

CONCLUSIONS/IMPLICATIONS: Ensuring the safe use/handling of chemicals in Canadian workplaces requires the enforcement to ensure accurate ingredient disclosures on MSDSs as required by the WHMIS requirements of the *HPA* and *CPR*.

The standard protocols and the analytical methods developed through this pilot study showed a potential to be used as a (a) primary enforcement tool by the regulator and (b) means whereby downstream suppliers (such as blenders) and importers of WHMIS controlled products could ascertain the veracity of information transmitted to them by their suppliers.

4.08 Synthetic Receptors of the Influenza Hemagglutinin to Differentiate Human and Avian Viruses

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SUMMARY: We have used chemi-enzymatic protocols to synthesize analogues of the avian and human influenza virus receptors. Conjugated forms of the synthetic influenza receptors can be used to distinguish human and avian strains. Soluble forms of the synthetic receptors could inhibit virus binding and have potential therapeutic applications.

OBJECTIVES: To synthesize analogues of the avian and human influenza virus receptors that can be used to differentiate human and avian viruses based on the hemagglutinin specificity.

DESIGN AND METHOD: The influenza virus binds to sialylated glycans on host cells using the hemagglutinin on its surface. The specificity of a strain for a host depends on the specificity of its hemagglutinin. Human strains bind to NeuAca2,6Gal only. Avian strains bind predominantly to NeuAca2,3Gal. We have used an -2,6- and an -2,3-sialyltransferases to synthesize azidoethyl derivatives of NeuAc-2,6-lactose and NeuAc-2,3-lactose, respectively. The azidoethyl tails of the two sialyllactose derivatives were chemically reduced and used to conjugate the compounds to mouse serum albumin. The glycoconjugates were coated to ELISA plates and used to capture human (H1N1, H3N2 and Influenza B) and avian (H5N1) influenza strains.

RESULTS: We have successfully used bacterial sialyltransferases to synthesize preparative amounts (100 mg) of NeuAc-2,6-lactose-azidoethyl and NeuAc-2,3-lactose-azidoethyl. These sialyllactose derivatives were conjugated to mouse serum albumin and coated to ELISA plates. A H3N2 and a B strains bound to the NeuAc-2,6-lactose-conjugate while a H1N1 and a H5N1 strains bound to the NeuAc-2,3-lactose-conjugate.

CONCLUSIONS: This project could have diagnostic and therapeutic applications. NeuAca2,6Gal-1,4-Glc-azide could be used as a diagnostic tool to detect the human influenza virus. It could be used to differentiate human and avian influenza viruses. It could also help identify avian strains that have acquired the ability to bind to NeuAca2,6Gal; which have a higher potential for human-to-human transmission. Soluble forms of the synthetic receptors could inhibit virus binding and have potential therapeutic applications. Since the influenza virion carries a neuraminidase, the synthetic receptors need to be improved by making non-cleavable forms of the NeuAc-2,6-lactose and NeuAc-2,3-lactose. A promising approach is to synthesize S-linked sialyllactosides. This work should provide insightful information pertinent to the development of synthetic receptors useful for the detection of human and avian influenza viruses and related quality control methods for pandemic influenza vaccines.

4.09 Obesity, Depression and Social Support

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SUMMARY: We explore possible relationships between obesity and depression, as well as the role of social support as a potential moderating factor.

BACKGROUND/OBJECTIVES: Obesity and depression are serious and growing public health problems, each with substantial impacts on health outcomes, functional capacity, quality of life, and economic productivity. Recent research suggests that the two conditions are associated, although the specific mechanisms underlying this relationship remain unclear. Other research has linked high social support with a reduced risk of both weight gain and depression, suggesting that social support may warrant attention as a potential moderator of the obesity-depression relationship. We explore (i) the possible relationship(s) between obesity and depression; and (ii) the role of social support as a potential moderating factor.

DESIGN: Using data on Canadian adults aged 18 to 64, from the Canadian Community Health Survey and the National Population Health Survey, logistic regressions identify independent variables, including social support, significantly associated with the dependent variables (i) depression; and (ii) obesity. Analyses of the NPHS data investigate the role of social support in (i) moderating the associations between obesity and depression, (ii) predicting the likelihood that a person who is obese is trying to lose weight, and (iii) predicting the likelihood that the attempted weight loss is successful.

RESULTS: Controlling for gender, race, family income and age group, logistic regressions of the CCHS data show that obesity and depression have a significant bi-directional association with each other. Any significant moderating influence of social support is currently being investigated.

CONCLUSIONS: As the prevalence of obesity in Canada continues to rise, we can expect a significant increase in the prevalence of depression. Social support may potentially serve as a buffer against this risk. Dissemination of this knowledge among policy makers, fellow researchers, and other stakeholders can contribute to the development of effective mental health promotion policies and interventions.

4.10 Determining the Structure of Biologics in the Presence of Protein Excipients with Far U/V Circular Dichroism Spectroscopy

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SUMMARY: The correct structure of a therapeutic biologic is critical for its safety and proper efficacy and can be dramatically influenced by formulation excipients. Here we present a simple method, using far U/V circular dichroism spectroscopy (CD), to assess the structure of a biologic in the presence of protein excipients.

BACKGROUND: The increased structural complexity of therapeutic biologics as compared to small molecule drugs suggests that not only does a biologic require the correct chemical structure (amino acid sequence) but also the correct secondary structure (3 dimensional fold). Failure of a therapeutic biologic to meet either of these requirements can result in reduced efficacy and/or adverse drug reactions. Although numerous techniques are available to assess a biologic for chemical and secondary structures, difficulties arise when assessing secondary structure of a biologic in the presence of other proteins such as human serum albumin (HSA). HSA due to its unique amphiphilic properties is widely used as a formulation excipient to prevent protein aggregation and adsorption onto surfaces of glass vials. One such important biologic which is widely prepared with HSA is therapeutic formulations of interferon.

METHODS: Far U/V CD was investigated as a potential technique to assess the influence of HSA on the secondary structure of the EDQM interferon α 2a reference standard and other proteins (RNAaseA/Lysozyme) at a variety of protein concentrations and HSA/protein ratios.

RESULTS: The far U/V CD spectrum from EDQM interferon α 2a can be successfully isolated from the combined HSA/interferon spectrum. By doing so, it can be demonstrated the secondary structure of EDQM interferon α 2a remains intact in the presence of HSA at a variety of protein concentrations. Increasing HSA/interferon ratios were also examined and the interferon spectrum could be isolated with the structure remaining intact at ratios up to 5:1.

CONCLUSION: Far U/V CD is a suitable technique to assess the secondary structure of therapeutic biologics in the presence of protein excipients. As many manufacturers currently offer the same biologic with variations in formulation, this technique may be of importance in current and future assessments of similar biologics with varying formulations from the same or different manufacturers.

4.11 Smokeless...But Still Hazardous!

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SUMMARY: Recently, a Swedish snus-like smokeless tobacco product (STP) was introduced in Canada. In order to characterize this product, Health Canada investigated the presence of 22 constituents in 32 STPs available on the Canadian market, including snus. Results indicate that snus sold in Canada contain the same harmful chemical substances as other STPs.

OBJECTIVES/BACKGROUND/ISSUE(S): The objectives were to characterize and to compare constituents of snus and other STPs available on the Canadian market. STPs do not undergo combustion and are being promoted both for use where smoking is prohibited and as a smoking cessation aid. STPs are known to contain 28 carcinogens (IARC 2007). They are traditionally used in the Southern U.S. states and are widely used in Sweden, where they are known as "snus". Numerous factors influence the composition of STPs such as tobacco type, curing and method of preparation. STPs are available in different forms (pouch, loose or plug) and have different methods of preparation; some undergo fermentation while others, like snus, are pasteurized. Depending on their method of preparation, the levels of toxic substances present vary.

DESIGN/METHOD/DESCRIPTION: Thirty-two smokeless tobacco products were analyzed using Health Canada official methods (Tobacco Reporting Regulations) and the CDC method for nicotine.

OUTPUTS/RESULTS: The results indicate that fermented STPs have higher levels of benzo[a]pyrene (BaP), of tobacco specific nitrosamines (TSNAs), ammonia and nitrate than those that are pasteurized. The pH of the products, which affects the concentration of free-base nicotine, ranged from 4.90 to 9.68. Snus sold in Canada contained lower levels of TSNAs, ammonia, nitrate and BaP and a pH of 7.39, with close to 19% of the nicotine in its free base form.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Results from this study reveal that snus products sold in Canada contain the same harmful chemical substances that other STPs available on the Canadian market. Moreover, the snus products available in Canada have a slightly lower level of toxic substances when compared to other STPs. Nevertheless, the presence of toxic substances in the snus products sold in Canada can potentially lead to the same health risks as other STPs.

4.12 DNA Adducts Analysis Using LC/MS

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SUMMARY: A hydrolysis method has been developed to transform oligonucleotide or genomic DNA adducts into single nucleotides. The single modified nucleotides were identified and analyzed by a LC/MSD system after cleaning-up the unmodified nucleotides with solid phase extraction (SPE).

OBJECTIVES/BACKGROUND/ISSUE(S): DNA adducts study is one of the important ways to understand how chemicals exhibit their cytotoxic and carcinogenic properties. However, the capability of identifying structures of the DNA adducts and their corresponding parent chemicals depends on not only analytical tools but also the sample clean-up procedure due to the interference of unmodified nucleotides. Benzo(a)pyrene diol (BPDE), a metabolite of benzo[a]pyrene (B(a)P); Phenyl glycidyl ether (PGE), an important industrial epoxide; and Styrene epoxide (SO), a metabolite of styrene; are three active agents known to form covalent DNA adducts. The objective of this study is to develop a global screening method to identify both DNA adducts and corresponding parent chemicals through enzymatic hydrolysis of DNA adducts, clean-up by solid phase extraction (SPE), and identification by LC/MS.

DESIGN/METHOD/DESCRIPTION: BPDE, PGE and SO were incubated, respectively, with a 20 mer single-stranded oligonucleotide at 37°C for 48 hours. After removal of unreacted chemicals by liquid-liquid extraction, the mixture of oligonucleotide and adducts was hydrolyzed with enzymes. The hydrolysate was cleaned-up using ISOLUTE C18 (EC) cartridge to remove unmodified single nucleotides. The modified nucleotides were identified by an Agilent liquid chromatography mass spectrometer with ESI source.

OUTPUTS/RESULTS: The enzymatic hydrolysis conditions were modified to achieve a complete hydrolysis of oligonucleotide and adducts to expected single nucleotides. Unmodified single nucleotides were successfully removed by a SPE clean-up procedure. BPDE, PGE and SO were found to covalently adduct with more than one kind of bases such as guanine and adenine in the oligonucleotide. The MS spectra of single nucleotide adducts were used to elucidate the chemical structures bonded to the base in adducts so as to identify the corresponding parent chemicals in environment.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The method might be applicable to identification of DNA adducts and global screening of possible exposure parent chemicals in mixtures such as vehicle exhaust and indoor air. The current method is only verified by the known chemicals and for unknown chemicals the method needs more study. Application study to expand the method to environmental monitoring and human exposure is underway.

4.13 NAFTA Soil Crosswalk Project

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SUMMARY: The NAFTA Soil Crosswalk project, in collaboration with the U.S. and Mexico, proposes a matrix of the three soil classification systems currently used in North America and provides guidance on the acceptability of use of world soils in laboratory studies required for pesticide registration.

BACKGROUND: Under the North American Free Trade Agreement (NAFTA), Canada, Mexico and the United States (U.S.) are working together to develop common approaches to predicting the potential for pesticides to pose an unreasonable risk to human health and the environment to support the joint review processes. Pesticide registrants, in response to country-specific data requirements, conduct environmental fate and transport studies on soils representative of growing regions for each country. In order to accommodate the use of foreign studies in country-specific environmental risk assessments, uniform guidance for determining the applicability of a study conducted on foreign soils is necessary. A consistent and harmonized approach to characterizing the comparability of foreign soils used in environmental fate studies is needed to facilitate joint reviews and to maximize the value of information collected elsewhere in the international community.

OBJECTIVE: To create a soil crosswalk of the three soil classification systems used in North America: the Canadian Soil Classification System; the U.S. Soil Taxonomy and the World Reference Base (WRB); and Food and Agriculture Organization-United Nations Education, Scientific and Cultural Organizations (FAO-UNESCO) World Soil Resources to facilitate harmonized standards on world soil suitability. Also create a guidance document on how to use the crosswalk.

METHODS: Review soil classification systems and previous soil crosswalks with the Canadian Soil Classification System and the criteria used to define classification levels. Establish a matrix that correlates similar soils under each classification system.

OUTPUTS: A Soil Crosswalk for the Canadian, U.S. and FAO-UNESCO soil classification systems and a guidance document on how to use the Crosswalk to determine a soil's acceptability to the reviewing country.

IMPACTS: Provides a standard and harmonized framework for evaluating the acceptability of soils in laboratory studies, which will facilitate both NAFTA and OECD joint reviews.

4.14 Revision of the Booklet Nutrient Value of Some Common Foods

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SUMMARY: The booklet Nutrient Value of some Common Foods (NVSCF) is a condensed version of Canada's food composition database, the Canadian Nutrient File. Last published in 1999, this revised edition updates a valuable reference tool for health professionals and Canadians with special dietary requirements.

BACKGROUND / OBJECTIVES: NVSCF is a resource providing nutrient data on the most commonly consumed foods in Canada. The objective was to first determine what modifications would most benefit the users and then implement changes that would best reflect these needs.

METHOD: Feedback obtained from the NVSCF users indicated that they wanted format changes including better graphics, and improvements to font and shading to make reading easier. Content issues concentrated on more specific nutrients as well as an increase in new food items to reflect changes in eating and shopping practices.

RESULTS: The booklet Nutrient Value of Some Common Foods (NVSCF), is a tool that has been improved to provide values for 19 key nutrients found in over 1000 of the most commonly eaten foods in Canada. Nutrients vary for different food groupings since nutrients relevant to one specific food group may not be as important to another. When nutrients do not contribute significantly, emphasis is now directed towards components more specific to the group. In addition to updating the data contained in the booklet based on the latest scientific information, the new edition of the NVSCF booklet now focuses on multi-ingredient and pre-prepared foods rather than individual ingredients.

OUTCOMES: The Nutrient Value of Some Common Foods, always a popular resource, came to the attention of the Honourable Tony Clement, Minister of Health who has issued a news release. In the first month of its publication, there were orders for 2086 copies in English and 1935 copies in French. The booklet, which previously sold for \$9.50 can now be downloaded from Health Canada's website at www.healthcanada.gc.ca/nvscf or ordered through Health Canada Publications free of charge.

4.15 Integration of Science and Policy in Decision-Making for and Governance of Science-Embedded Regulatory Programs: The WHMIS FPT Partnership

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SUMMARY: Effective decision-making for administering a science-based regulatory program requires the integration of science into policy. Using the example of the National FPT WHMIS partnership, this work describes, discusses and provides key elements to achieve better outcomes for the application of science and science integrated policy for program implementation and governance.

OBJECTIVES: Effective decision-making for administering a science-based regulatory program requires the integration of science into policy. The objective of this work is the following:

1) describe the role of science and policy in a science-embedded regulatory program; 2) propose an approach for the integration of science and policy for decision-making; and 3) illustrate the integration of science and policy for the governance of a national federal-provincial-territorial (FPT) regulatory program using the example of the national FPT partnership of the Workplace Hazardous Materials Information System (WHMIS).

DESIGN/METHOD/DESCRIPTION: A framework to describe the use of science and application of science to policy for regulatory decision-making and governance was developed. The generation, exchange and utilization of knowledge and information are used as the three cornerstones to describe different elements related to the integration of science and policy. The role of science and policy in each of these elements is identified for the administration and governance of the WHMIS FPT partnership. Key elements include credibility and veracity of and/or accessibility to information.

OUTPUT/RESULTS: The application of science to policy and the integration of science with policy for decision-making are demonstrated for the administration and governance of WHMIS. The interplay of policy and science is demonstrated with respect to the generation, exchange and utilization of knowledge and the communication of hazards. It is found that the embedding of science-based specifications in law and the retaining of scientific rigour in policy instruments results in individual benefits to all stakeholders.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS: Science-embedded regulatory programs require the application of scientific principles and methods in policy development, decision-making and governance. Policy integrated with science has been successfully applied to the governance and administration of the national WHMIS FPT partnership with a mandate to ensure the protection of Canadian workers from the adverse effects of hazardous materials through the provision of relevant information while minimizing the economic impact on industry and the disruption of trade.

4.16 Free-Base Nicotine Trend in Canadian Cigarettes from 1969 to 2007

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SUMMARY: Free nicotine, also known as free-base nicotine, was measured with a newly developed method. An increase of the concentration of free nicotine in the mainstream smoke of Canadian cigarettes manufactured from 1969 to 2007 is observed.

INTRODUCTION: Nicotine, which can be found both in free-base or acid forms, is responsible for tobacco addiction. The free-base form of nicotine (or free nicotine) is more rapidly absorbed through the physiological membrane than the acid form.

The World Health Organization has listed free nicotine as a substance of interest in the product regulation article of the Framework Convention on Tobacco Control.

Traditionally, free nicotine was estimated from the total amount of nicotine in tobacco smoke and smoke pH. A method for the direct measurement of free nicotine in tobacco smoke was recently developed by Health Canada and used in this study.

OBJECTIVE: The concentration of free nicotine in tobacco smoke of 7 brands of cigarettes is reported. The results are analyzed to identify trends over time and parameters influencing the concentrations of free nicotine.

METHOD: Free nicotine concentrations in tobacco smoke of 7 brands of cigarettes manufactured at 5 different time points over the 1969-2007 period were measured using solid-phase microextraction techniques and analyzed by GC/MS. Tobacco smoke was collected under ISO and Health Canada modified smoking conditions. Smoke pH, tar, carbon monoxide, nicotine, paper porosity, water, pressure drop and tip ventilation were also measured using the official Health Canada, ISO and AOAC methods.

RESULTS: The concentration of total nicotine in tobacco smoke showed very little change over time. The concentration of free nicotine increased from an average of 11.6 µg/cig to 28.9 µg/cig over the past decades for 5 of the 7 brands analyzed. Trends of water, pH and paper porosity were similar to free nicotine.

CONCLUSION: An increase of the concentration of free nicotine in the smoke of Canadian cigarettes manufactured from 1969 to 2007 was observed. Parameters influencing free-nicotine levels are identified. A better knowledge of the product will lead to both an increased product regulations capacity and a better-informed consumer.

4.17 Development of the Canadian Pesticide Risk Indicator

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SUMMARY: The Canadian Pesticide Risk Indicator (CaPRI) serves as an analytical tool for relative ranking of common agricultural pesticides registered for use in Canada. In this project, the relative ranking of potential risk and risk trends over a 7 year period (2001 - 2007) were determined for 102 active ingredients.

OBJECTIVES/BACKGROUND/ISSUE(S): To develop a Canadian Pesticide Risk Indicator (CaPRI) for ranking registered agricultural pesticides according to their relative risk. The CaPRI model is a work in progress, designed to provide ranking results for the potential health and environmental risks associated with the use of pesticides and is a means of determining relative risk trends over time at National and Provincial levels. When integrated with use and sales information, it will allow the measurement of trends in pesticide risk reduction over time, thus providing a tool to quantify the results of various risk reduction initiatives implemented by the PMRA.

DESIGN/METHOD/DESCRIPTION: Pesticide usage data for the years 2001-2007, as well as eco-toxicology, physico-chemical characteristics, environmental fate, acute/chronic toxicity, and occupational exposure data were used to rank the selected pesticides according to 2 different modules. Whereas a somewhat modified version of the Norwegian Pesticide Risk Indicator (NPRI) was used in the Environmental Module, a substantially modified version of the NPRI, incorporating multiple exposure parameters, was used in the Human Health Module.

OUTPUTS/RESULTS: One hundred and two active ingredients (158 end-use products) with uses in nine provinces had complete data to run the model. Relative risk trends and pesticide rankings will be presented.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The next steps will be to expand CaPRI to include human and environmental health parameters for a further 190 commonly used registered active ingredients. A relative risk indicator associated with the use of pesticides will allow for performance assessment and policy guidance in regards to the new *Pest Control Products Act*. CaPRI is a tool, which will help the Pest Management Regulatory Agency measure progress towards meeting pesticide risk reduction goals.

4.18 Innovative Research in Canada's North

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SUMMARY: There is a need for relevant, long-lasting research and monitoring in Northern First Nation and Inuit communities. There are certain health variables that are more influential in Northern, isolated communities than in Southern Canada. Research and monitoring programs will be considered for their innovations in technology, community involvement, and project longevity. A map will be developed to visually display the scope of research across the North.

OBJECTIVES: To identify, locate, and map research projects in Canada's North, which for this purpose represents the continuous permafrost zone. This will focus broadly on climate change and its impacts on health determinants of direct relevance to many Northern Aboriginal communities.

DESIGN: Human health can and must be examined through a number of outlooks. There are certain determinants of health in Northern communities that can be easily overlooked in Southern-based health studies. In 2004, Nunavik completed its first regional health study "Qanuippitaa," which was specifically designed to measure health indicators of particular relevance to its communities. Some examples include the presence of contaminants in community food/water sources, coastal and sea ice diminution, and increasing frequencies of formerly unfamiliar animals and diseases. Projects of interest will be unique in science and technology, but also in their logistic planning and rationale. Research that engages communities proves to be a win-win situation for all parties involved. Research organizations of interest include International Polar Year, Arcticnet, Nassivik Centre, and others. With the help of Inuit Tapiriit Kanatami (ITK), a geographic map will be developed containing locations and descriptions of some of these projects.

OUTPUTS: This project is aimed at highlighting research that focuses on climate change and its ongoing effects on certain health indicators. The ultimate goal is to create an up-to-date map of existing research, with descriptions and explanations of particularly innovative projects. Some projects of interest include "Qanuippitali?" Inuit health survey (based on Nunavik's "Qanuippitaa?" study), the Amundsen research-icebreaker "Schools on Board" training initiative, and the "Arctic Biodiversity of Chars Network." These and other relevant studies are proving to be innovative in several ways, ranging from technological to logistical. With the help of ITK, these programs will be presented on a map of Northern Canada.

OUTCOMES: A map of Canada's North will include descriptions and locations of identified research programs. Such a tool has yet to be developed that encompasses Canada's entire North and could be useful to government departments when considering future research funding possibilities. Innovative science and technology is crucial for future Arctic planning, but this must be coupled with community engagement in order to maximize research efficiency, for the present and future.

4.19 Implementing a Knowledge Cycle for Evidence-Informed Practice and Practice-Based Learning in the Canadian Best Practices Initiative

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SUMMARY: Addressing root causes of chronic disease will require affecting system change and bridging gaps between science, policy and practice. This work provides an overview of the development and implementation of a Knowledge Cycle to guide efforts to enhance evidence-informed practice and practice-based learning through the Canadian Best Practices Initiative.

OBJECTIVE: The Knowledge Exchange component of the Canadian Best Practices Initiative (CBPI) seeks to increase use of and contribution of content to the Canadian Best Practices Portal for Health Promotion and Chronic Disease Prevention. This presentation will present a Knowledge Cycle conceptual framework, related needs assessment findings from across Canada and implementation of action strategies to date.

METHODS: Knowledge Cycle framework development was informed by literature and policy document reviews, and refined through consultation with knowledge exchange and public health leaders across Canada. Several needs assessment activities were also undertaken to gather information from a variety of intended user groups including: a synthesis of environmental scans and reports, focus groups with resource organizations across Canada and Public Health Agency staff; and web surveys of CBPI Portal users and stakeholders.

RESULTS: The resulting Knowledge Cycle includes the following components: Needs assessment, Knowledge Creation, Knowledge Translation, Dissemination, Adoption and Uptake, and Evaluation. The needs assessments identified jurisdictional roles, assets, gaps and opportunities to support pan-Canadian knowledge exchange activities and related capacity building. Three strategies were developed to address these findings: 1. *partnership strategy*- to facilitate existing resource organizations to undertake joint activities across jurisdictions; 2. *capacity building strategy*- to provide relevant tools, training and technical assistance; and 3. *exchange support strategy*- to develop tools and processes to facilitate knowledge co-creation to support evidence uptake and content contribution to the CBPI Portal.

CONCLUSIONS/IMPLICATIONS: The Knowledge Cycle framework has contributed to a common culture of knowledge exchange within the CBPI and beyond. The CBPI's Knowledge Exchange strategies reflect an interactive approach to integrating knowledge and action. The presentation will close with reflections on how the Knowledge Cycle might be used by others to affect systems change through better exchange between practice, policy and research.

4.20 On Considering the Advice of Others: Using the Challenge Advisory Panel and Stakeholder Advisory Council to Inform Decision-Making Under the Chemicals Management Plan

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SUMMARY: The Government of Canada's Chemicals Management Plan is a world-leading program to assess and manage approximately 200 high priority substances. Advice from two advisory bodies is incorporated into the traditional risk assessment process.

BACKGROUND: The Chemicals Management Plan (CMP) relies on advice from independent scientific experts, as well as input from multiple sectors, in order to implement the CMP successfully and achieve the overall goal of protecting health and the environment for all Canadians. The challenges and benefits of incorporating advice and input representing diverse interests into decision-making are discussed.

DESCRIPTION: The Challenge Advisory Panel provides advice to government on the CMP Challenge to industry and other stakeholders. The Panel considers specific questions involving the application of precaution and a weight of evidence approach to assessments of high priority substances. Panel members are independent experts from such fields as the precautionary principle, chemical policy, environmental and health risks, health and Aboriginal communities, and health care planning and delivery.

A second advisory body, the Stakeholder Advisory Council, is a multi-stakeholder committee that provides input on the implementation of the CMP and helps to foster dialogue between stakeholders and government, and among different stakeholder groups. Members represent Aboriginal, Consumer and Labour groups; Environment and Health Non-Government Organizations; as well as Industry associations, producers, and users.

OUTPUTS: The Panel membership consists of a wide range of expertise in many subject areas. This provides both benefits and challenges to bureaucracy in incorporating advice into a program bounded by legislative directives. While the discussion and advice provided in Panel meetings serve to broaden as well as refine the considerations involved in outlining proposed conclusions on chemical assessments, it is sometimes difficult to address certain aspects of the Panel feedback, as it may not meet or fit within the bounds inherent in the Challenge.

Additionally, the Stakeholder Advisory Council is an engaged group of informed representatives from a range of backgrounds, concerned with making a difference while representing often disparate viewpoints. The benefit of the Council is that it

provides this coordinated forum in which these viewpoints may be aired and discussed among stakeholders. The challenge, therefore, is to carefully consider the disparate viewpoints of these stakeholders and attempt to incorporate these considerations in the larger context of the CMP.

While both advisory bodies are providing advice on diverse and important issues within the CMP, decision-making responsibilities still lie with the Government. The Government's challenge, therefore, is to balance the many viewpoints and advice that are received as well as its legislative roles and responsibilities.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS/

CONCLUSIONS: There are many challenges and benefits to integrating diverse public views in the traditional scientific assessment and management process for potential health and environmental risks from chemicals. The CMP has created two advisory bodies that may have a real impact on the lives of Canadians through public input to these scientific processes.

4.21 Child Maltreatment and its Correlation to Adolescent Substance Use/Abuse: A Review

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SUMMARY: Although substance use among adolescents is well established, less is known about adolescents with a history of child maltreatment. The purpose of the literature review was to identify and critically assess studies that address maltreatment and its correlation to adolescent substance use/abuse. Interventions and future research implications were also explored.

OBJECTIVES: To identify studies that address child maltreatment (emotional maltreatment, witnessing violence, neglect, physical and sexual abuse) among adolescents and its correlation with substance use/abuse (alcohol, cigarettes and drugs); to critically assess the quality of the identified articles; to explore interventions used and future research.

METHODS: CINAHL, PsycINFO, ERIC, Medline, Social Policy & Practice and PubMed were searched. Fifty articles comprising 38 studies met inclusion criteria. The studies included samples from: communities, high schools, detention centres, clinical settings, substance abuse treatment centres and prospective cohort studies.

RESULTS: Upon review, 35 of the 38 studies found a positive correlation between child maltreatment and substance use. Among these studies physical and sexual abuse were the forms of maltreatment most often studied. Similarly, alcohol use/abuse was the focus of the majority of studies.

CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Further research should be conducted on the relationship between neglect, emotional abuse and witnessing violence with substance use/abuse. Timely intervention is required considering the early initiation of substance use/abuse among adolescents who experienced maltreatment. Health education and promotion activities should focus on pre-adolescents to prevent future substance use/abuse and include, for example, the development of alternative coping skills.

4.22 Emerging Disinfection By-Products (NDMA, MX) in Canadian Drinking Water: A Survey of Fifteen Water Distribution Systems

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SUMMARY: The occurrence of new, ultra-trace level DBPs NDMA and Mutagen X (MX) in water treatment systems across six provinces was investigated. NDMA was detected at 6 out of 15 locations and MX at 11 out of 15 locations. This study serves as a pilot for the upcoming National Survey of Disinfection By-Products and Selected Emerging Contaminants in Canadian Drinking Water.

BACKGROUND/OBJECTIVES: During water disinfection, various compounds with known or suspected toxicity are formed. The challenge is minimizing the formation of disinfection by-products (DBPs) without compromising disinfection efficiency. The occurrence of emerging DBPs in drinking water challenges the basis of current mitigating strategies, designed to reduce the amounts of regulated DBPs. Solid scientific monitoring data, including data for the emerging DBPs, are essential for developing effective risk management strategies.

During the past few years, we have developed analytical capabilities for new ultra-trace level DBPs, including N-nitrosodimethylamine (NDMA) and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (also known as Mutagen X or MX), regulated by some jurisdictions (Ontario guideline for NDMA is 9 ng/L). This survey was designed to investigate the occurrence of NDMA and MX in water treatment systems in Alberta, Saskatchewan, Ontario, Quebec, Nova Scotia and Newfoundland and Labrador.

DESIGN: Fifteen water treatment plants and distribution systems were selected so as to include various source water quality characteristics and treatment processes. Samples were collected under both winter and summer conditions, due to seasonal variability. Raw water, treated water, and mid-point distribution system (D2) samples were collected for NDMA. For MX, only mid-point distribution system samples were collected. Operational parameters at the plants were documented through questionnaires.

RESULTS: NDMA was detected at six locations, with concentration exceeding 100 ng/L at one location. Where NDMA was detected, D2 concentrations tended to be slightly higher than concentrations in treated water. Higher concentrations of NDMA were found in winter compared with summer samples from the same location. The highest NDMA concentrations were found in treatment plants that used chlorination and the incidence of high NDMA values seems to correlate with higher organic matter content in the raw water. MX was detected at 11 locations, with the highest value found being 104 ng/L.

OUTCOMES/NEXT STEPS: Data obtained will be used in the preparation and revision of Guidelines for Canadian Drinking Water Quality.

This study serves as a pilot for the upcoming **National Survey of Disinfection By-Products and Selected Emerging Contaminants in Canadian Drinking Water**, which will investigate contaminant concentration levels (including emerging DBPs, pharmaceuticals, bisphenolA and perfluoroalkylated compounds) in Canadian drinking water. Sixty water distribution systems across Canada will be sampled. The new National Survey, funded by CMP, will generate data to be used for Health Canada's risk assessment/management activities under CEPA. The research team includes scientists from EBD/ HECSB, WACCB/SEP, as well as University of Waterloo, Laval University and University of Alberta.

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Vigneault, M. - 3.01, 3.12
Vijay, H. - 1.02, 2.26
Villeneuve, M. - 3.15, 3.31, 4.14
Vincent, R. - 1.07, 2.01, 2.10, 2.13,
2.15, 2.25, 2.26
Vu, D. - 3.14

W

White, P.A. - 2.09, 2.16, 2.19, 2.21
Wiebe, P. - 1.20
Wierdsma, J. - 2.05
Wilkins, R.C. - 2.29
Williams, A. - 2.01, 2.08, 2.11, 2.21,
2.22
Willsie, A. - 2.18
Wong, B. - 1.21

Wood, C.M. - 3.47, 3.48

X

Xiao, C.W. - 3.47, 3.48
Xiong, H. - 1.05

Xu, X. - 2.28

Y

Yagminas, A. - 2.22
Yambao, K. - 3.49
Yapici, T. - 2.02

Yauk, C.L. - 2.01, 2.08, 2.11, 2.21,
2.22
You, H. - 2.28

Z

Zalot, L. - 1.04
Zarkadas, M. - 3.30, 3.31
Zehaluk, C. - 3.31
Zhang, H. - 2.30
Zhang, S. - 1.22
Zhang, W. - 2.31

Zheng, J. - 2.22
Zhou, G. - 2.22
Zhu, J. - 2.30, 3.11, 3.46, 4.12
Ziegler, U. - 3.36
Zou, W. - 4.08

**Marriott Hotel - Conference Rooms/
Salle de conférence - Hôtel Marriott**

Lower Level - Sous-sol

Salons Cartier Salons - I, II and III
Salon Albion Salon
Salon York Salon
Salon Elgin Salon
Salon Albert Salon
Salon Laurier Salon

2nd Floor - 2^e étage

Salle de bal Victoria Ballroom (North and South / nord et sud)
Salon Alta Vista Salon
Salon Capital/Carleton Salon
Salon O'Connor Salon

3rd Floor - 3^e étage

Victoria Ballroom Gallery/Mézzanine de la salle de bal Victoria
Salon Rideau Salon
Salon Dalhousie Salon
Salon Wellington Salon