Book of Abstracts

Message from the Chief Scientist

It is a triumph for Health Canada and its science community that, for a fourth year in a row, the Health Canada Science Forum continues to elicit strong support and enthusiasm. As a showcase for departmental science, the Forum is a tribute to the high degree of collaboration, innovation and commitment of our scientists.

Each year we choose themes that allow us to demonstrate just some of the incredible science conducted at Health Canada. This year, under the able leadership of Dr. Pierre Charest, organizers put together a program that addresses selected constant and evolving priorities of the department. For example, the theme of *Science in Support of Health Policies and Regulations* demonstrates the relevance of the ongoing work of Health Canada's scientific community and its direct impact on Canadians. The sub-theme on *The Role of Civil Society* is highly relevant to the departmental priority of improving the health and safety of Canadians and it supports Minister Dosanjh's emphasis on openness and transparency.

I extend my appreciation to the numerous scientists and non-scientists who are giving of their time and effort to participate in Forum 2005. The Forum provides a platform where scientists, policy makers and managers can come together to discuss science-based problems. It is a unique opportunity that I hope will lead to better science and more effective science-based policy.

Pierre-Gerlier Forest, Ph.D.
Chief Scientist

Foreword

It has been a great pleasure for me to lead the organization of this year's program, which I hope you will find is rich in variety and quality. The number of abstracts received was almost as high as in previous years. From outside the department, over 60 portfolio partners from the science community were invited to participate. I am happy to point out that several of our PHAC colleagues will attend to present joint research conducted with Health Canada. Participants will note the involvement of international partners this year as we recognize the value of addressing common issues together. You will also find some different events and presentation formats as the organizers continue to seek ways to be inclusive of different kinds of information and aspects of interest to our scientific community. Furthermore, this year's forum truly encompasses the whole range of science that the department covers from social to biological and health sciences. I would like to recognize and thank members of the Organizing Committee, the Scientific Review Committee, the Office of the Chief Scientist and many others in Health Canada who worked hard to ensure that this event continues to be a success and continues to enhance the understanding and professional development of our scientists and non-scientists alike.

Pierre Charest, Ph.D.
Chair, Health Canada Science Forum 2005

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Table of Contents

Risk Assessment Methodologies

Building Health: Healthy Individuals and Healthy Communities

Emergency Issues and Product Developments

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

FNIHB: First Nations and Inuit Health Branch
HECSB: Healthy Environments and Consumer Safety Branch
HPFB: Health Products and Food Branch
HPB: Healthy Policy Branch
PMRA: Pest Management Regulatory Agency

1.01 Germline Mutation, DNA Damage and Epigenetic Modifications in Response to Exposure to Particulate Air Pollution in an Industrial Location

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OBJECTIVES: To investigate inherited genetic instability and DNA damage arising in the germline of mice exposed *in situ* to particulate air pollution. To characterize epigenetic modifications that result from exposure to particulate air pollution.

METHODS: Mature male C57Bl/CBA mice were housed in a shed erected 1 km from two integrated steel mills in Hamilton, Ontario. Mice were exposed to ambient urban air pollution (whole air) alongside mice in chambers with HEPA filters that removed inhalable air particulate (>0.3mm in diameter). Mice were housed for 3 and 10 weeks, or 10 weeks followed by a 6 week recovery period in the laboratory. Tandem repeat mutation (measured using single-molecule PCR), single strand breaks (random oligonucleotide-primed synthesis assay) and global methylation (cytosine extension assay) were evaluated in germ cell DNA.

RESULTS: A significant 1.5 fold increase in heritable non-coding tandem repeat mutation was found in mice exposed to whole air compared to HEPA-filtered air. DNA strand breaks were significantly elevated in the testes of 3 and 10 week males exposed to whole air (1.4 and 1.3-fold respectively), but were identical in both groups after a 6 week recovery period. Hypomethylation was observed in testes of animals exposed to urban air particles for 3 and 10 weeks, and these changes persisted following the 6 week recovery period (a consistent 1.3 fold decrease in cytosine methylation was observed for each time point).

CONCLUSIONS/IMPLICATIONS: This study demonstrates that exposure to ambient levels of particulate air pollution results in significant genetic and epigenetic consequences in the male germline. The repercussions of these modifications are unknown but may include increased and potentially persistent genetic disease in the offspring of exposed males.

1.02   Estimation of Pesticide Concentrations in Surface Water and Groundwater for Human Health and Ecological Exposure

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OBJECTIVES: Estimating concentrations of pesticides in water bodies that are potential sources of drinking water or environmental exposure is an integral part of the risk assessment process conducted by the Pest Management Regulatory Agency (PMRA). The PMRA has developed a methodology for estimating pesticide concentrations in relationship to proposed and registered pesticide application practices across Canada.

DESIGN: Computer simulation models are used to estimate concentrations of pesticides in surface water and groundwater. For surface water, PMRA uses the linked models Pesticide Root Zone Model (PRZM) and Exposure Analysis Modeling System (EXAMS). For groundwater, PMRA uses the Leaching Estimation and Chemistry Model (LEACHM). Pesticide concentrations in surface water and groundwater are estimated for a series of agricultural scenarios that are typical of the major crop-growing areas in Canada utilizing regional meteorological data, characterisation of soils and crops, and chemistry and environmental fate data identified or calculated within the PMRA review process. For surface water, PMRA currently uses three standard water bodies representing a small reservoir and a prairie dugout (for human dietary assessment) and a small pond (for ecological assessment).

OUTPUTS/RESULTS: Daily pesticide concentrations in surface water and groundwater are estimated over a multi-year period and summary statistics are computed and results formatted into reports for risk assessment. Modelled pesticide concentrations are compared to monitoring data, where available.

IMPACTS/OUTCOMES/CONCLUSIONS: These results are used to assess risks to human health and the environment in the context of registration decisions. Improvements to the modelling process are ongoing based on experience gained and consultation with other regulatory agencies.

1.03 A Global Gene Expression of Mouse Lung Cells *In Vivo* and *In Vitro*

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OBJECTIVES: To characterize global transcriptional differences between a spontaneously immortalized mouse lung cell-line (FE1), cultured primary lung epithelia, and parental murine lung tissue. To confirm the origin of the FE1 cell line, and evaluate its utility in a toxicological context.

METHODS: RNA from confluent and sub-confluent FE1 cells, cultured primary epithelia, and whole lung were hybridized to Agilent microarrays. LOWESS normalization, SAM[1], k-means clustering, and pathway analysis were carried out. Eight randomly least significant genes from SAM analysis were analyzed using RT-PCR. RT-PCR was also employed to assess expression of genes encoding surfactant proteins (SP) A, B and C.

RESULTS: We identified 1909 differentially expressed genes between confluent and sub-confluent FE1, primary cells, and whole lung. Gene ontology analysis revealed a number of genes involved in cell cycle regulation, DNA metabolism, substrate attachment, and intercellular communication. 75% of the 8 chosen genes showed similar microarray and RT-PCR expression patterns. Surfactant protein transcripts were detected by RT-PCR in all four samples.

CONCLUSIONS: *In vitro* growth, whether immortalized or primary cells, requires substantial changes in gene expression relative to the parental tissue *in vivo* as seen by both the RT-PCR and microarray results. Furthermore, the use of RT-PCR confirms, due to the presence of all 3 surfactant proteins, that the FE1 cell line is derived from type II epithelial cells.

IMPLICATIONS: This research increases our understanding of the functional relationships *in vitro* and *in vivo* models for toxicity assessment, and provides insight into the functional utility of extrapolations from *in vitro* to *in vivo* toxicity assessment.

[1] Significance Analysis of Microarrays: Tusher et al. Proc Natl Acad Sci U S A 98 (2001) 5116-5121.

1.04 Side Effects of Treatment with Lime Products on the Quality of Distributed Drinking Water

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OBJECTIVES: Improve the understanding of the effect of lime addition on drinking water quality.

DESIGN: Different parameters were monitored in drinking water systems when lime was used. Lime contributions to water media were also examined by dissolution experiments.

OUTPUTS/RESULTS: Increases in pH, turbidity and aluminium species were observed in treatment plants when lime was added after filtration. Changes were also observed after the plant outlet. Turbidity increases were followed by decreases in the distribution system. The aluminium changes were mostly in soluble form. In parallel, experiments performed directly on lime also showed that aluminium was mostly soluble. Slow dissolutions were observed for a part of aluminium and 15% generally remained insoluble after one day. The aluminium contents of lime from different sources varied over nearly an order of magnitude. By using the less contaminated lime, its contributions to water aluminium concentrations would have been 7 μg/L or less.

IMPACTS/OUTCOMES/CONCLUSIONS: There is a long tradition of lime use for water treatment. The present results (soluble contaminant, dissolution delays, particulate residuals) show that lime properties cause unwanted effects. In addition to contamination, these effects create supplementary problems such as uncertainties in defining the quality of finished water at the plant outlet (e.g., pH) and all operation difficulties linked to suspended particulate matter and its deposition in distribution system. A solution can be the use of alternative chemicals but these are generally more expensive. A considerable reduction of the problems caused by lime is also possible by choosing the best sources of lime and by improving the conditions of use.

1.05   Food Issues in Environmental Impact Assessments

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OBJECTIVE: To establish a Health Canada protocol for conducting risk assessments in environmental reviews to better protect human health. In this regard, human health risk assessments of contaminant levels in foods are an integral part of environmental impact reviews. Country foods can potentially become contaminated due to the activities undertaken in development projects such as constructing/operating a mine and in the area of contaminated sites.

DESIGN: A standardized procedure has been developed for risk assessment in regard to contaminants in country foods. This standardized risk assessment involves collecting analytical data consisting of the levels of contaminants found in country foods harvested from the project area.

OUTPUTS/RESULTS: Human health risk assessments can then be undertaken employing this Health Canada protocol. Contaminant intake estimates must be determined based on the data collected to undertake risk characterization. Toxicity reference values for the contaminants are required as well as food consumption data.

IMPACTS/OUTCOMES/RESULTS: It is anticipated that the scientific procedure outlined in this Health Canada protocol will serve to provide more in-depth and structured human health risk assessments of food contaminants in environmental reviews in Canada. Estimating exposure to contaminants using contaminant levels found in these foods will better identify potential human health impacts and can be used to establish mitigation procedures to reduce exposure. This document can also be used as a "template" for conducting these risk assessments.

1.06   Estimate of Radiation Doses to Embryo and Foetus During Commercial Flight

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OBJECTIVE: Assessing the radiation exposure from cosmic-rays to the embryo and foetus of pregnant aircrew and air-travelers.

DESIGN: Dose estimate received during commercial flights is based on neutron dosimetry. Monte Carlo code MCNPX 2.5f was used. Models recently developed at the Radiation Protection Bureau, Health Canada, for an embryo of 8 weeks (2 months) and for a foetus of 3, 6, or 9 months (first, second and third trimester) were used. An isotropic irradiation geometry is assumed. Neutron spectrum measured on board a Canadian Forces flight at an altitude of 11.3 km has been used in this analysis. ICRP-92 radiation-weighting factors for neutrons are implemented.

RESULTS: On average, a foetus could receive a neutron dose of 0.015 mSv over a 10-hour flight. Since neutrons contribute to about one-half of the radiation exposure, a 10-hour flight gives only 3% of the annual dose limit of 1 mSv for members of the general public. After 335 flight hours, the average neutron equivalent dose to the body of the foetus would be 500 Sv. The total radiation dose received by the conceptus would be approximately 1 mSv. This amount of flight time corresponds to approximately 33 round trans-Canada trips (e.g., Ottawa to Vancouver and return), or 20 round trans-Atlantic trips (e.g., Toronto to Paris and return).

CONCLUSIONS: For a casual air-traveller, the dose to the embryo/foetus from cosmic radiation exposure is trivial. However, for pregnant aircrew or frequent air-travelers, the foetus can exceed the annual dose limit of 1 mSv for the general public after 33 round trans-Canada trips (e.g., Ottawa to Vancouver and return) or 20 round trans-Atlantic trips (e.g., Toronto to Paris and return).

1.07   Vision for a Global Registry of Anticipated Public Health Studies (GRAPHS)

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OBJECTIVES: This paper addresses a number of problems in the current process of evidence-based decision making. First, the existing knowledge generation process is affected by the "false positive research cycle" and "circular epidemiology". Second, the current knowledge management process is retrospective and incomplete. They produce undesirable effects on the process of knowledge generation, exchange and uptake.

DESIGN: An international writing group was set up by the first author, which includes a number of multidisciplinary experts in evidence-based policy from Canada, UK, USA, Australia, Finland, WHO, China, Uruguay, Brazil, and Colombia. The writing group (23 members) exchanged ideas and information through the internet, and after iterations of revisions created a vision document.

RESULTS: The vision of a future knowledge-based information system, a Global Registry of Anticipated Population Health Studies (GRAPHS), is proposed to help resolve the current problems. It is a research notification system that is prospective and comprehensive. It minimizes the effect of the "false positive research cycle", provides a comprehensive evidence base, minimizes the faulty process of "circular epidemiology", and identifies "cold topics" for call for further research.

CONCLUSIONS: This paper points out a number of practical problems that need to be debated and resolved before such a knowledge-based information system can be put in place.

1.08 Safety Assessment of Conjugated Linoleic Acid: Limited Effects of Dietary CLA on Serum Biomarkers of Bone Metabolism in Young Rats

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OBJECTIVES: Conjugated linoleic acid (CLA) is promoted as having health benefits including reduction of risk for carcinogenesis and atherosclerosis, and decreasing body fatness. While generally considered to be safe, the potential for adverse effects of long-term CLA consumption, particularly through reproduction and early development, has not been thoroughly investigated. Previous studies have shown that weanling male rats given 1% dietary CLA had decreased bone formation rate (Li et al. 1999). Fetal, neonatal and young growing rats of both genders may be particularly sensitive to potential effects of CLA on bone development.

DESIGN: Mature female rats were fed diets containing 0%, 0.5% or 1% CLA, mated and on day 17 of gestation 5/group were killed to provide maternal and fetal samples. From the remaining litters, 5 pups/sex/group were killed at postnatal day 22 to provide weanling samples (primarily lactational exposure). At 150 days of age, 5 rats/sex/group (fed the same diets as their dams) were killed to provide young adult samples. Several serum biomarkers of bone metabolism were measured.

RESULTS: No significant differences were found in serum alkaline phosphatase activity, or levels of osteocalcin, magnesium, inorganic phosphorus, prostaglandin E metabolite or prostaglandin D2. Small changes were noted in serum parathyroid hormone and calcium levels in young adult male rats only.

CONCLUSIONS: CLA had only limited effects on serum biomarkers of bone development in young rats. Additional parameters (urinary biomarkers, histopathology) are being investigated in this study of potential CLA effects on developmental endpoints (including bone, neurological development and behaviour).

1.09 Prioritizing Disinfectant Ingredients for Safety Asessment

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OBJECTIVES: Pre-market approval requires that disinfectants meet quality, safety and efficacy standards. Accordingly, manufacturers must provide regulatory agencies with data to support product efficacy claims; safety data are assessed as needed. The present study is intended to determine which ingredients commonly found in disinfectants should be prioritized for toxicological risk assessment.

DESIGN: To create a priority ingredient list (PIL), both exposure and toxicity surveys were produced through literature search, and consulting Health Canada's Drug Product Database (DPD). The exposure survey focused on frequency of occurrence in DPD rather than total quantity used in Canada. The toxicity survey relied on oral, dermal, inhalation, and skin irritation data.

RESULTS: The exposure survey led to an ingredient list ranging from most to least commercialized: benzalkonium chloride (BAC), alkyl dimethyl ethylbenzyl ammonium chloride, didecyl dimethyl ammonium chloride (DDAC), dioctyl dimethyl ammonium chloride, o-phenylphenol (OP), chlorophene (CP), hydrogen peroxide (HP), etc. The toxicity survey listed, from most to least toxic, DDAC, peracetic acid (PA), glutaraldehyde (GLUT), HP, OP, BAC, CP and pine oil (PO). A PIL was obtained using the following formula: Exposure Rank + [∑(Toxicity Rankings) ÷ Number of Scores] = Priority Score. A lower priority score indicates greater need for safety assessment. Targeted for assessment were, DDAC with a score of 3.0, BAC 5.5, OP 7.3, HP 8.0, GLUT 8.7, PA 9.5, CP 10.0, and PO 13.3.

CONCLUSION: Preliminary attempts at assessing DDAC (first priority ingredient), suggest that further information, such as exposure models, is essential for performing the safety assessment of prioritized substances.

1.10   Some Causes of Degradation and Bio-Incompatibility of Bisphenol-A Polycarbonate in Medical Devices

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OBJECTIVES: This study was initiated by an incident report to HC, which had shown that 61 polycarbonate heads in battery-operated toothbrushes had broken and shattered in the mouths during use, in the USA. Our Division was concerned that the degradation of polycarbonate could be affecting other medical devices such as hemodialyzers. Understanding degradation and bio-incompatibility factors of polycarbonates in medical devices was necessary.

DESIGN: The study was made by conducting an evaluation of the information in the literature and incident reports.

OUTPUTS/RESULTS: The literature and reports revealed that there were no cases of toothbrush breakage in Canada. That bisphenol-A polycarbonate (PC) has been used in medical devices since 1960. Its major properties of clarity, high strength, rigidity, toughness, good heat resistance, low water absorption, and biocompatibility have enabled it to replace glass or metal in many medical products. It can withstand sterilization, by steam autoclaving, ethylene oxide, gamma irradiation, electron beam and isopropyl alcohol. Polycarbonates were not suitable for devices that are repeatedly autoclaved or reacted with solutions of free radicals. Accordingly, dentifrices containing baking soda, peroxide, and high pH caused the degradation of the reported toothbrushes. PC is stable to 100 kGy of gamma radiation, but, it undergoes yellowing, indicating another mode of failure. Temperatures higher than 121°C, cause distortion of PC. These factors would break bisphenol-A PC. Copolymerization with acrylonitrile-butadiene-styrene terpolymers, or polyesters produces stable blends.

IMPACT/OUTCOMES/CONCLUSION: This work provides a basic understanding of conditions associated with polycarbonate degradation in medical devices, e.g. repeated autoclaving or sterilization.

1.11 Experimental Study of Mechanism of Apoptosis Induced by TNF-α Neutralizing Drugs on Endothelial Cells

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OBJECTIVES: Tumour necrosis factor α (TNF-α) neutralizing drugs are widely used to treat diseases like arthritis and Crohn's disease. Both infliximab (RemicadeTM) and etanercept (Enbrel TM) are TNF-α neutralizing drugs. Etanercept is a soluble fusion protein consisting of the Fc portion of human IgG1 and the extracellular ligand-binding protein of p75 TNF-α receptor. Infliximab is a chimeric IgG1 monoclonal antibody to TNF-α Our previous studies have demonstrated that both drugs induce apoptosis *in vitro*. Here, we investigate the involvement of apoptotic proteins including executor caspases, DNA fragmentation factor (DFF45) and poly (ADP-ribose) polymerase (PARP) in the above process.

METHODS: Human endothelial cells (ECs*)* were treated with either etanercept or infliximab *in vitro* and Caspase 3/7 activity, as an indicator of early apoptosis, was assayed. RNAse protection assays and membrane gene arrays for either cytokine or apoptosis-related genes and Western blots were also performed.

RESULTS: TNF-· (positive control) increased Caspase 3/7 activity and induced the expression of IL-1 and IL-6 in both RNase protection assays and cytokine gene arrays. Etanercept also increased Caspase 3/7 activity in both unactivated and TNF-α-treated ECs. Caspase inhibitors, Z-VAD-fmk and Ac-DEVD-fmk inhibited this increased activity. Similar studies with infliximab showed comparable results. Microarray experiments for apoptosis-related genes showed that etanercept also increased DFF45 expression. Western blot analyses of Caspase 3/7 and PARP are underway to identify their involvement in these processes.

CONCLUSION/IMPLICATIONS: Our data suggest that etanercept and infliximab, approved biotherapeutics, induce apoptosis via a Caspase-dependent pathway, which may contribute to adverse reactions associated with these drugs.

1.12 Hepatic DNA Methylation as Target of Polychlorinated Biphenyl and Methylmercury, but not Pesticide Toxicity

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DNA methylation is one epigenetic mechanism that regulates gene expression, chromosome structure and stability. Abnormal DNA methylation has been associated with cancer, infertility, developmental, neurological, immunological, and age-related disorders. Our goal is to determine if early exposure to environmental contaminants could compromise long-term healthy living.

OBJECTIVE: To determine if the DNA methylation system could be a target of *in utero* and postnatal exposure to human blood contaminants.

DESIGN: Pregnant rats were dosed with two levels of pesticides, methylmercury (MeHg), polychlorinated biphenyls (PCBs), or a mixture including these chemicals. Livers from offspring were collected and mRNA expressions, and DNA methylation of the promoter regions (usually hypomethylated), for DNA methyltransferase-1 (DNMT1), -3a, and -3b, were investigated using real time RT-PCR and methylation specific (MS) PCR. The DNA methylation status of the abundant retrotransposon Long Interspersed Nuclear Element-1 (Line-1) (usually hypermethylated) has been analyzed by MS-PCR, sodium bisulfite treatment, and methylation sensitive restriction enzymes.

OUTPUTS/RESULTS: The PCB treatment decreased hepatic mRNA abundance for DNMT1, -3a and -3b to 3.9%, 53.5% and 12.6% of control, respectively. MeHg reduced DNMT1, and DNMT3b to 51%, and 18%, of control. While the promoter regions of Dnmt-1, and -3a were not affected, that of Dnmt3b appears hypermethylated and might explain its reduced expression. No effects were detected for Line-1.

IMPACTS/OUTCOMES/CONCLUSIONS: The DNA methylation system is a target of PCB and MeHg toxicity. Effects on DNA methylation are a novel area of investigation in toxicology that has potential in the development of bioassays that predict long-term effects.

1.13 Environmental Contaminants in Arctic Canada – Risks and Benefits of Traditional Foods

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INTRODUCTION: Since the late 1980s, environmental contaminants such as persistent organic pollutants (POPs), various toxic metals, and radionuclides have become a cause for human health concern for residents of arctic Canada. These contaminants come to the arctic primarily by long range atmospheric transport, travelling on air currents from southern regions where compounds such as DDT are used to control mosquitoes and contaminants such as PCBs are off gassing out of waste dumps.

OBJECTIVE: The objective of this research is to define the data needs for risk-benefit assessment of country foods in northern Canada. Few studies have examined the relationship between community health, environmental contaminants and country foods in northern communities. Country foods provide many benefits, such as physical, psychological, social, nutritional, economic, cultural, and spiritual benefits that play an important role in community health. These benefits must be balanced with the risk of contaminant exposure of eating country food when developing public health policies. This research relies on in-depth interviews with people living in Nunavut and a comprehensive literature review.

METHODS: The Canadian Northern Contaminants Program has undertaken a series of studies over the past 14 years which have outlined the levels of these contaminants in the abiotic and biotic environment and human beings. Levels of contaminants have been evaluated both in foods consumed by people and in the people of Canada's arctic and human health effects studies have been undertaken.

RESULTS: In the marine environment these contaminants build up in the food chain resulting in higher levels of these contaminants in top-level marine mammal predatory species (e.g., seals, beluga whales, and polar bears). The Inuit who consume these traditional marine foods have significantly higher levels of many contaminants in their tissues and fluids than the general population. Recent research has indicated that Inuit continue to exceed dietary intake guidelines for some contaminants such as chlordane and toxaphene. New community-based research on risk-benefit decision-making will be presented. This research makes a contribution to the benefit-risk management body of knowledge. Other human health research in the Canadian arctic has found subtle but significant effects on the growth, immune system and neurobehavioral development of Inuit children and infants.

DISCUSSION: In spite of these health risks, traditional foods are key to the cultural, social and spiritual identity of aboriginal peoples of arctic Canada. The nutritional benefits of traditional foods are also substantial as they are a key source of protein, essential fatty acids and a number of other important nutrients. A shift away from traditional foods has been associated with an increase in obesity and diabetes in the arctic. The concomitant risks and benefits of contaminants and traditional foods makes any public health advice much more complex than just simply reducing consumption of specific contaminated foods. Balancing the benefits and risks of traditional foods is very difficult. The development of public health advice needs to involve the community and take the benefits of traditional foods into account to help limit any adverse health effects in the Aboriginal community affected.

1.14   Plaminogen and Angiostatin Interact with Heat Shock Proteins

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BACKGROUND: Our previous studies have demonstrated that plasminogen and angiostatin bind to endothelial cell (EC) surface-associated actin in a specific manner. Heat shock proteins (hsps) like hsp 27 are constitutively expressed by vascular ECs and regulate actin polymerization, cell growth and migration.

OBJECTIVES: Since many hsps are abundant on cell surfaces, the purpose of this study was to determine whether plasminogen and angiostatin would interact with hsps.

METHODS: ELISA assays were developed to assess these interactions.

RESULTS: Plasminogen bound to hsps 27, 60 and 70 in a time- and concentration-dependent manner. Binding was inhibited (85-90%) by excess lysine indicating kringle involvement. Thirty-fold molar excess actin inhibited the interaction of plasminogen with all hsps. In contrast, angiostatin predominantly bound to hsp 27 in a time-, concentration- and kringle-dependent manner. Also, thirty-fold molar excess actin could inhibit the interaction of angiostatin with hsp 27 by only 15-20%. In addition, there was dose-dependent inhibition of angiostatin's interaction with hsp 27 by plasminogen. Collectively, these data indicate that (i) while plasminogen interacts with hsp 27, 60 and 70, angiostatin interacts predominantly with hsp 27; (ii) actin partially displaces plasminogen/angiostatin binding to hsps and (iii) plasminogen only partially displaces angiostatin's binding to hsp 27 possibly because of differences in binding avidity.

CONCLUSIONS: Hsps and actin present on cell surfaces could mediate the binding of these ligands to ECs. Further, since hsp 27 inhibits actin polymerization, its presence on cell surfaces may modulate the level of globular actin resulting in altered binding of the above ligands. Data derived from our study will contribute towards clarifying the risks associated with the use of blood and blood components.

1.15   Crystalline Silica: A Discussion of Current and Novel Methods to Meet WHMIS Requirements under the *Hazardous Products Act* and Associated *Controlled Products Regulations*

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OBJECTIVES: To assess the feasibility of detecting crystalline silica in hazardous materials at levels below detection limits of conventional techniques, to help WHMIS regulators determine the veracity of silica content in such materials, and to determine whether suppliers of such materials are complying with WHMIS requirements under the *Hazardous Products Act* (*HPA*) and the *Controlled Products Regulations* (*CPR*) in terms of the adequacy of labelling and the accuracy of MSDSs.

METHODS: This feasibility study was conducted by combining literature search with experimental assessment. The experimental portion of the feasibility study focused on thermal analysis and a new X-ray Diffraction (XRD) technique followed by Rietveld analysis.

RESULTS: The literature search showed that the most common way of quantifying crystalline silica is XRD followed by Fourier Transform Infrared (FTIR). Other methods included microscopy, solid state 29Si NMR (nuclear magnetic resonance), thermal analysis and wet chemistry. All of these methods are briefly discussed.

If Rietveld is used appropriately, it can compensate for line broadening associated with the instrumentation, crystallite size and strain. Results of this investigation, using this new approach, showed that Rietveld appears to be a promising new technology for detecting silica at a very low level in these complex materials. Measurements with thermal analysis proved to be difficult.

CONCLUSIONS/IMPLICATIONS: It was concluded that Rietveld offered a new research direction, which may lead to improvements in determining crystalline silica levels in complex materials. Such an improvement would greatly assist regulators assess compliance with WHMIS ingredient disclosure requirements of the *HPA* and *CPR*.

1.16 Activity of *Actrostaphylos uva-ursi* on Cytochrome P450 Family-Mediated Metabolism and P-Glycoprotein Function in Human Cell Lines

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PURPOSE: Interactions between Natural Health Products (NHPs) and drugs may affect their disposition, potentially affecting the safety and efficacy of these or other products. This study was undertaken to characterize the potential effect of *Arctostaphylos uva-ursi (A. uva-ursi)* on drug disposition by examining cytochrome P450 3A4/5/7, 2C19, and CYP19-mediated metabolism, and P-glycoprotein (Pgp)-mediated transport.

METHODS: Bulk (3) and capsulated (2) *A. uva-ursi* was obtained from commercial outlets. The capsules were batched to give a representative sample and herbal material was ground to a common consistency. Aqueous and methanolic extracts (25 mg/mL in dH2O and 5 mg/mL in methanol) were prepared prior to testing. CYP 3A4/5/7, 2C19 and 19-mediated metabolism was determined using *in vitro* fluorescence bioassays. Aqueous extracts were used to determine the effect on Pgp-mediated uptake of rhodamine 123 into human monocytes (THP-1) and Caco-2 cells. All products were analyzed by HPLC for arbutin, gallic acid, myrcitrin, isoquercetin.

RESULTS: Our data indicates that both aqueous and methanolic extracts of the five *A. uva-ursi* products show high potential to inhibit metabolism, with the exception of the methanolic extracts against 3A4 and 19 which had low to moderate activity. The aqueous extracts of *A. uva-ursi* showed an inhibitory effect on Pgp at 1 hr and an inductive effect at 18 hrs for both cell lines. With the exception of gallic acid, similar levels of the examined biomarkers were found in the five products.

CONCLUSIONS: These herbal products have pharmacological properties that may potentially affect drug safety and efficacy. Further studies are warranted against a wider range of cytochrome P450 isozymes and transport proteins to determine if these effects are clinically significant.

1.17 Assessment of Two Potency Tests for Smallpox Vaccine Using a Study Panel Obtained from the National Institute for Biological Standards and Control (NIBSC)

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OBJECTIVES: Identification of Smallpox as an A list bioterrorist pathogen has renewed interest in smallpox vaccine manufacturing and regulation. The Centre for Biologics Evaluation (CBE) has re-established the standard chorioallantoic membrane (CAM) potency assay, performed in embryonated eggs, and established a Vero cell plaque assay, to assess live vaccinia virus titres required for the vaccine. This study compares results from CBE's CAM and Vero assays to those from the UK NIBSC collaborative study designed to evaluate reference standards and assay methods for smallpox vaccine.

DESIGN: Seven blinded vaccine samples, including the international reference preparation (IRP), obtained from NIBSC were run in both CAM and Vero assays in three separate tests. Results from these assays were compared to those in the NIBSC panel study.

RESULTS: The CBE CAM potency estimates were similar to the mean estimates from the NIBSC study. The Vero cell plaque assay typically gave titres toward the lower end of the estimates from the collaborative study, and one CBE value fell 9.1% below the lowest panel study estimate.

The CBE Vero plaque assay titres were typically 0.5 logs lower than observed in our CAM assay. However, when normalized to the IRP, the plaque assay titres were generally higher than our CAM estimates with greater variability.

CONCLUSIONS: CBE's CAM assay results correlated well with those from the laboratories that participated in the NIBSC study. Our Vero assay also performed well but additional work is needed before it can substitute for our CAM potency assays.

1.18 Method to Estimate the Usual Intakes from Foods for Risk-Benefit Assessment

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OBJECTIVES: Methods have been developed in Food Directorate (FD) and elsewhere to estimate usual intake of nutrients and substances in foods using 24 hour recall data which remove the day-to-day variability. However, in the assessment of risks and benefits from agents in foods which are not consumed on a daily basis or consumed by everyone, these methods for deriving usual intake do not properly account for the consumption/non-consumption pattern and give invalid estimates of the distributions of intake. The objective was to extend the FD method to account for the consumption/non-consumption pattern.

DESIGN: A method has been developed that uses beta binomial estimation of the probability of intake and combines this with an adjustment of the positive intakes to derive a usual intake distribution. This method allows estimation of a population usual intake distribution that both removes the day-to-day variability and accounts for the probability of consumption. An example of the use of this method is the assessment of risks from the mycotoxin patulin found in apple juice and apple sauce. Apple juice and apple sauce intakes from the federal-provincial nutrition surveys along with lab results of mycotoxin analyses are used to develop and assess models and methods to estimate usual intakes for foods and agents not consumed on a daily basis. The results from this method are compared with an alternative approach.

OUTPUTS: Outputs include a description of the methodology and a set of programs to implement this procedure, as well as the exposure assessments using this methodology.

IMPACTS/OUTCOMES/CONCLUSIONS: This method of estimating usual intakes will extend our capabilities in the area of exposure assessment, which forms part of a complete risk-benefit assessment. This in turn will lead to better-informed policy decisions.

1.19 High Frequency Mutation of Functional DNA Repeat Sequences in Human Cells by Environmental Agents

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OBJECTIVES: To employ a green fluorescent protein (GFP) reporter assay to measure the susceptibility of functional human DNA tandem repeat sequences and biomarker loci, in human cells, to mutations induced by environmental carcinogens. To also demonstrate the ability of this mutation assay to detect disease etiology-relevant genetic damage in a variety of human cells.

DESIGN: The assay is based on activation of GFP. Engineered plasmids place the GFP gene sequence out-of-reading-frame via upstream attachment of tandem repeat sequences (TRS). The genetic targets included the *Ms6-hm* biomarker and human TRS in cancer-associated genes p53, transforming-growth-factor-beta-II-receptor (TβRII), and aromatase (P450arom). The reporter-constructs were permanently introduced into human HT29 colonic, MCF7 mammary and lung epithelial cells. The responsiveness of these constructs in human cells was assessed with a battery of standard test agents. Frame-shift mutations in the gene targets resulted in green fluorescent mutants, as determined by flow-cytometry. Using PCR analysis, mutation frequencies of the endogenous loci (TTTA and polyA tract in P450arom and TβRII respectively) were compared to results obtained from the reporter assay.

OUTPUTS/RESULTS: All tested constructs (*Ms6-hm*, TβII, P450arom) showed statistically significant increases in mutation rate after exposure treatments in MCF7 cells. Testing of the p53 construct, PCR of endogeneous TRS and responses in HT29 and lung epithelial cells is ongoing.

IMPACTS/OUTCOMES/CONCLUSIONS: Results from these experiments show that the GFP frameshift assay is an effective way to detect environmentally inducible states of genetic instability in human cells, especially in functional human TRS that are susceptible to cancer- and other disease-associated mutations.

1.20 Mathematical Tools for Infectious Disease Outbreak Control

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OBJECTIVE: Develop a set of mathematical tools for use in infectious disease outbreak management.

- The first is a preliminary model–work continues to add more realistic interaction among the susceptibles and their environment, and to make it possible to model such interventions as isolation, quarantine and vaccination.

- If the situation permits, we will show a different application for dynamic, real time literature surveillance, designed to show the relations among target concepts or words within a text corpus. This is useful in disease surveillance and scanning for possible outbreaks as they develop and are reported worldwide.

DESIGN: A team of mathematicians, epidemiologists, statisticians, and software engineers physicians have designed and are realizing a series of statistical and stochastic simulation tools for use by planners dealing with a variety of disease outbreaks and situations.

OUTPUTS: One [or hopefully two] interactive tools will be presented and offered for hands-on demonstration. These are to be used for outbreak simulation and contact tracing and for network analysis among infected individuals.

CONCLUSIONS: Such tools are useful, and they will become still more useful in interaction and demonstrations such as this, to knowledgeable audience that can make useful comments and suggestions.

1.21　Hepatic Gene Expression Profiles in Mice Exposed to Sub-Toxic Doses of Lead Acetate

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OBJECTIVES: (1) to examine and compare standard biochemical and histopathological endpoints against changes in hepatic genomic profiles following low-dose exposure to lead acetate; and, (2) to identify and validate specific gene expression signatures that could be used as a biomarker of lead exposure and disease-related changes.

METHODS: Adult male B6C3F1 mice were given lead acetate at 0.2, 2.0, 20, and 200 mg/kg/day in aqueous solution by oral gavage for 28 days. Controls received distilled water only. Mice were sacrificed twenty-four hrs after the final dose. Blood and tissue were collected for biochemical, histopathological, and gene expression analysis.

RESULTS: No clinical signs of toxicity or changes were found in body or organ weight between treated and controlled mice. Serum alkaline phosphatase levels were elevated by 19 and 40%, respectively, in the 20 and 200 mg/kg exposures indicating some hepatobiliary damage. No changes were seen in cytochrome P450 related EROD, BROD, and PROD activity and liver or thyroid pathology. Microarray analysis of liver identified potential biomarkers of toxicity not detected via conventional methods.

CONCLUSIONS: Analysis of classical toxicological endpoints did not reveal significant lead-induced biological responses at the lowest doses examined. In principle, microarray technology can detect underlying molecular events that occur at very low doses. Such responses will provide a better understanding of the underlying mechanism(s) of toxicity. The results obtained suggest a rational safe dose of lead acetate below which there is no toxicity-related modulation of gene expression. The approach should improve the accuracy and precision of toxicological risk assessments.

1.22 Comparison of *In Silico* Tehniques for Study and Prediction of Xenobiotic Metabolism

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OBJECTIVE: To review commercially available *in silico* expert systems that simulate real life metabolism and serve as important tools in the process of xenobiotic risk assessment.

DESIGN: Fully functional and/or evaluation versions of four expert systems namely TIMES, META, METEOR and MetabolExpert were obtained from respective firms. 4-nitrophenol, 2, 4-dichlorophenol and a host of other chemicals were used as model compounds in order to evaluate and compare their key features.

OUTPUT: Depending on the biotransformation rules, the knowledge-base and metabolite management mechanism integrated within the individual expert systems they could generate a metabolite tree for the query chemical giving their individual molecular structures. Some metabolites were common among the four expert systems while others were not. Some expert system can provide reasoning and access to additional information lacking in other systems.

CONCLUSIONS: This review highlights and compares the major features of these different metabolism prediction softwares in addition to providing information on their operational details. The review results can be used in better selecting tools in their application in predicting metabolites of environmental pollutants. An expert system that is user-friendly and, at the same time, able to fulfil the objective of predicting metabolites will be a useful tool in the assessment of chemicals.

1.23 Investigating the Mutagenic Hazards of Polycyclic Aromatic Hydrocarbons in Complex Mixtures

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OBJECTIVES: To evaluate risk assessment methods currently employed to predict the mutagenic hazard of complex PAH (polycyclic aromatic hydrocarbon) mixtures in contaminated soils.

DESIGN: Organic constituents of PAH-contaminated soils were extracted using pressurized fluid extraction and subsequently fractionated into non-polar neutrals and polar organics on silica gel. Synthetic mixtures containing 16 priority PAHs, quantified via GC-MS (gas chromatography/mass spectrometry), were compiled to correspond with the results of the chemical analyses. The mutagenic potencies of the complex soil extracts/fractions and the synthetic PAH mixtures were evaluated using the *Salmonella* mutagenicity assay.

OUTPUT/RESULTS: All soil extracts/fractions, and all synthetic PAH mixtures yielded significant mutagenic responses in *Salmonella* strains TA98, TA100 and YG1041. For *Salmonella* strains TA98 and TA100, the mutagenic potency of the synthetic PAH mixtures were approximately 1.6-fold higher than those of the soil fractions. However, in *Salmonella* YG1041, a strain that is particularly sensitive to N-containing heterocyclic compounds and aromatic amines, the mutagenic potency of the soil extract was 6.6-fold greater than that of the synthetic mixtures.

IMPACTS/OUTCOMES/CONCLUSIONS: These results indicate that a targeted approach focusing on 16 priority PAHs may be reasonable for assessing of mutagenic hazard of PAH-contaminated soils. However, use of a targeted priority substance approach for soils contaminated with aromatic amines or heterocyclic compounds may lead to significant underestimation of actual mutagenic (or carcinogenic) hazard. Testing of additional PAH-contaminated sites is currently being carried out to validate these findings.

1.24   Potential for Rat Liver Preneoplasia by Bovine Growth Promoter Residues Found in Meat

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OBJECTIVES: Trenbolone acetate, a synthetic androgen is often used in combination with 17-beta-estradiol (ED) to promote weight gain and feed efficiency during meat production. Violative residues of these growth promoters in meat has raised human health concerns, and trade barriers for Canadian beef in Europe. This study investigated the carcinogenic potential in rats of beta-trenbolone hydroxide (TBH) and ED administered individually or in combination.

DESIGN: Weanling SD rats were fed AIN76G diets containing various dose levels and combinations of TBH and ED for 28 days. At necropsy, livers were removed, weighed, and fixed in formalin for routine histopathology and immunohistochemistry for biomarkers indicative of rat liver preneoplasia: proliferating cell nuclear antigen (PCNA); glutathione S-transferase placental form (GSTP); and apoptosis.

OUTPUTS/RESULTS: Pre-cancer biomarkers visualized as immuno-stained protein-antibody complexes, were quantified as number of stained cells per section area using microscopic image analysis. With 16ppm TBH alone, only hepatocyte proliferation (PCNA) index in female rats, and GSTP in male rats increased significantly. The apoptotic index decreased significantly in all high dose groups except females fed 16ppm TBH alone. Histopathology indicated significant hepatocellular lipidosis, hypertrophy and anisocytosis in all high dose groups, with consistent, greater severity in female rats.

IMPACTS/OUTCOMES/CONCLUSIONS: These data suggest the potential for long-term adverse health effects in rat liver by TBH alone or in combination with ED. Cancer initiation-promotion studies are warranted to identify potential tumour promoting effects of these veterinary drug residues. This study will contribute valuable information for re-evaluation of growth promoter usage and regulation for Canadian meat production by the Veterinary Drugs Directorate.

1.25 Modulating Effects of Dietary [Selenium (Se) + Vitamin E (VE)] on Methylmercury (MeHg) Toxicity in Rats

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OBJECTIVES: The potential for dietary [Se + VE] to protect against MeHg toxicity was investigated with the objective to provide HC regulators with data for formulation of appropriate risk management strategies for Canadians exposed to unacceptable levels of MeHg through consumption of contaminated fish and marine mammals, which are also a rich source of Se and VE.

DESIGN: Male SD rats fed starch-based casein diet, with or without [Se (1 mg/kg diet) + VE (250 mg/kg diet)] for 28 days, were gavaged with 0, or 3 mg MeHg/kg BW for 14 consecutive days. Body weights were recorded. At necropsy, tissues were weighed, and blood and urine samples were analyzed for changes in hematology, clinical biochemistry, and oxidative stress.

OUTPUTS/RESULTS: MeHg significantly decreased body and relative liver weight; increased relative spleen and adrenal weights, serum creatinine, and monocyte count in both dietary groups, but to a lesser degree in the [Se + VE] group; decreased red blood cell counts, hemoglobin level and hematocrit; increased relative kidney weight and serum lipase activity in both dietary groups; increased urinary 8-hydroxydeoxyguanosine and protein in the casein group only; and increased serum cholesterol to a greater degree in [Se + VE] than casein group.

IMPACTS/OUTCOMES/CONCLUSIONS: Dietary [Se + VE] improved performance of some systemic markers in rats, but did not attenuate the effects of MeHg on markers related to lipid metabolism/catabolism. These data will aid HC Regulators in characterizing the modulatory effects of dietary constituents on MeHg toxicity for providing advice to Canadians on health risks associated with consumption of MeHg contaminated fish and marine mammals.

1.26 A Microarray Analysis of Transcriptional Responses Following Treatment with Three Promoters of C3H/10T1/2 Cell Morphological Transformation

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OBJECTIVES: To identify disruptions to gene expression contributing to cell transformation by conventionally non-genotoxic agents that promote focus formation in a short-term, *in vitro* assay for carcinogenesis

DESIGN: Alterations in gene expression potentially affecting assay outcome were thought to be those consistently increased or decreased in response to each of three chemically distinct promoting agents in C3H/10T1/2 cell cultures. Treatments were 100 nM 12-*O*-tetradecanoyl-13-acetate (TPA), 9 mU xanthine oxidase and 60 FM benzoyl peroxide. Agilent mouse-development microarrays were used to measure responses of 22 000 mRNA species over a period of 36 hours following exposure, in triplicate experiments. Interpretations were made of expression changes in relation to biological/biochemical hypotheses regarding promotion in this model system.

OUTPUTS/RESULTS: Treatments produced little toxicity, but slowed growth rates from 10 to 50%. TPA and xanthine oxidase affected expression (at $p < 0.05$ significance) of 4404 and 1428 mRNA species, respectively, at one or more times after treatment. mRNA species significantly affected by both treatments included those encoding proteins functioning in DNA replication, recombination, repair and stability, cell proliferation and apoptosis. Northern blot and RT-PCR confirmations of selected genes and statistical analysis of results from benzoyl peroxide treatments are ongoing.

IMPACTS/OUTCOMES/CONCLUSIONS: Numerous expression changes resulted from treatment with relatively non-toxic levels of three promoters of transformation in the C3H/10T1/2 carcinogenesis model. The results provide mechanistic insight into the actions of nongenotoxic carcinogens, aiding development and implementation of rapid screening assays for similar agents among priority chemical lists and environmental samples.

1.27 Developmental Neurotoxicity in Rats of a Persistent Organic Pollutant (POP) Mixture Mimicking the Exposure of Canadian Northern Populations: Effects on Pups Growth, Survival, Endocrine Functions and Brain Proteome

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OBJECTIVES: Neurodevelopmental toxicity was observed in rats following perinatal exposure to a "Contaminant Mixture" mimicking the exposure profile of Canadian Arctic populations. The present study was designed to investigate the toxicological contribution of the major components of Contaminant Mixture and to identify molecular targets of neurotoxicity.

DESIGN: The total Contaminant Mixture and its three major components, methylmercury (MeHg), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs), were administered to dams at comparable levels. Exposure to antithyroid agent 6-propyl-2-thiouracil (PTU) was used as a positive control for hypothyroxinemia. The negative control group received vehicle (corn oil) only. Growth rate, mortality, endocrine functions and brain proteome of pups were measured.

OUTPUTS/RESULTS: The effects of Contaminant Mixture on growth and mortality were attributed to MeHg, while the effect on thyroid hormone status was attributed to PCBs. The neurodevelopmental effect of PCBs was not entirely attributable to the induced hypothyroxinemia: PCBs and PTU toxicities differed and exposure to PCBs and OCs (which did not affect thyroid system) induced similar effects on the proteome profile of hippocampus. Results also supported antagonistic interactions between individual pollutants. The effects of MeHg, PCBs, or OCs administered alone on growth, mortality and brain proteomic profile were generally more severe than those of the complete Contaminant Mixture.

IMPACTS/OUTCOMES/CONCLUSIONS: We clearly identified distinct patterns of developmental toxicity for each of the three components of Contaminant Mixture. Accurate prediction of toxicity of contaminant mixtures based on their components will require a better understanding of the molecular mechanisms involved and of the interactions between pollutants.

1.28   Comparison of Three Real-Time PCR Mycoplasma Detection Kits

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OBJECTIVES: Three real-time PCR mycoplasma detection kits were tested to determine the suitability for tissue culture screening. The kits tested were the EZ PCR (MD Biosciences, whose PCR protocol was adapted for real-time applications), VenorGem-DI (Minerva Biolabs) and MycoSensor (Stratagene).

DESIGN: The same six mycoplasma species were used to test each kit and two types of preparations (inactivated mycoplasma and column purified DNA from the inactivated material) for each species were run in triplicate. Amplification detection was achieved with SYBR green dye for the EZ PCR and MycoSensor kits and with scorpion probes in the VenorGem-DI kit. Plus/minus screening criteria were based on the amplification growth curves for all the kits and combined with a melt curve analyses for the SYBR green kits. The real-time PCR products were also analyzed using agarose gels.

RESULTS: All three kits showed 100% detection with the purified DNA samples. The EZ PCR, VenorGem-DI and MycoSensor indicated 67%, 100% and 0% detection of the inactivated non-purified samples, respectively.

The VenorGem-DI kit had the largest separation between negative and positive control curves, while MycoSensor had the lowest separation.

The EZ PCR kit was determined to be the least consistent in terms of replicate reproducibility with the purified DNA samples. The percent consistency for the replicates with purified DNA was 83%, 100%, and 100% for the EZ PCR, VenorGem-DI and MycoSensor kits respectively.

Using a commercial mycoplasma quantification standard, preliminary estimates for consistent detection limits with the VenorGem-DI and real-time adapted EZ PCR kits were 5000 and 40 000 DNA copies respectively. Additional optimization and testing is required to verify these results, as well as evaluate the sensitivity of the MycoSensor kit.

CONCLUSIONS: The data indicate that the best results were obtained for all kits with purified DNA samples. With purified samples, both the VenorGem-DI and MycoSensor kits performed well and it is noted that the later is one seventh of the cost of the former and includes a purification column for each PCR test in the kit.

1.29   Rapid Extraction and Detection of Hepatitis A Virus from Food Samples

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Fruits, vegetables and ready-to-eat foods are increasingly implicated in Hepatitis A virus (HAV) outbreaks.

Since only limited research has been done on virus detection in these foods as compared to shellfish, this study aimed at developing a rapid method for the extraction and detection of HAV from foods of plant origin. The Pathatrix system, which relies on pumping and forceful circulation of immunomagnetic beads throughout the entire food sample, has been successfully used for the capture and extraction of bacteria from foods.

In this study, this system was adapted for HAV extraction from foods. Twenty-five grams each of lettuce and strawberries were inoculated with HAV and placed in a stomacher bag containing 225 mL of EBSS buffer and 100 uL of positively-charged magnetic beads. After thirty minutes of processing in the Pathatrix, HAV-conjugated beads were separated from the food and suspended in 140 uL of PBS buffer. RNA was extracted by using the QIamp Viral RNA Mini Kit (Qiagen), and 10 uL of RNA was run through RT-PCR cycles. Preliminary data indicated that the sensitivity of detection was 10 CFU/25g of lettuce. This method is also successful at extracting HAV from other foods but further testing must be done to determine detection limits. Initial data suggests that the Pathatrix system can be used for rapid virus extraction and detection (within 5 hours) in large volumes of foods.

This, we believe, is the first report demonstrating the use of the Pathatrix system for the detection of viruses in foods.

1.30   Development of a Common Rapid Method for Norovirus Concentration and Detection in Various Food Matrices

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Noroviruses (NoV) are the leading cause of viral gastroenteritis outbreaks in humans. The virus is highly infectious and easily spreads through foods and direct contact. Most current methods are geared towards detecting noroviruses in shellfish. With fruits, vegetables and ready-to-eat-foods becoming increasingly implicated in norovirus outbreaks, the aim of this project is to develop an efficient and rapid common method to detect these viruses in different food matrices. Feline calicivirus (FCV) was used as a surrogate for the noroviruses in initial studies.

FCV was concentrated and its RNA was extracted from artificially-seeded ham samples by TRIzol™ reagent. The extracted RNA was purified by Instagene Matrix™ prior to the RT-PCR and subsequent visualization by gel electrophoresis. After standardization, this methodology was successfully used to detect noroviruses from deli meat and salad samples implicated in a recent norovirus outbreak. However, Instagene Matrix™ was not capable of complete removal of inhibitors present in foods, which may hamper our detection limits.

To overcome this limitation, subsequent trials using Dynabeads Oligo d(T)25™ were done on FCV and demonstrated an improved sensitivity of detection to limits <103 PFU of FCV inoculated onto ham, bread and lettuce samples. This methodology is currently being used to detect noroviruses from food samples artificially-innoculated with norovirus-positive stool. Once standardized, this methodology would be ideal in an outbreak setting due to its common application to various types of foods, increased sensitivity of detection, and its rapidity (whereby results can be obtained within a day).

1.31 Genetic Damage Caused by a Variety of Carcinogens as Measured at a Tandemly Repeated DNA Locus

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OBJECTIVES: To investigate mutations at tandemly repeated DNA in the mouse genome towards developing a sensitive system for assaying induced genetic instability. To characterize the types of insult leading to mutation at tandem repeat loci.

DESIGN: Mouse pluripotent embryo fibroblast cells were treated with chemicals exhibiting a variety of carcinogenic potential. Cell cultures were exposed for five weeks to either 325 nM of 12-O-tetradecanoyl-phorbol-13-acetate (TPA; receptor mediated non-genotoxic tumour promoter), 0.5 nM okadaic acid (serine/threonine protein phosphatase inhibitor), 0.64 mM ethyl-N-nitrosourea (ENU; DNA alkylating agent), 3 nM etoposide (topoisomerase II inhibitor), 1 μM benzo[a]pyrene (B[a]P; forms DNA adducts), xanthine with 225 mU xanthine oxidase (generates active oxygen species) or 25 μM benzoyl peroxide (free radical generating tumour promoter) alongside solvent- and time-matched controls. Single molecule PCR (SM-PCR) analysis of DNA was used to measure the degree of size alterations at a non-coding tandemly repeated DNA locus (*Ms6-hm*) in exposed cells.

OUTPUTS/RESULTS: A greater than 2 fold increase in mutation at *Ms6-hm* was observed for the majority of both genotoxic and non-genotoxic carcinogenic agents tested. The spectra of size changes in the exposed cells correlate with observed mutation *in vivo*.

IMPACTS/OUTCOMES/CONCLUSIONS: Exposure to low levels of carcinogens induces quantifiable increases in mutations of an endogenous tandem repeat locus measured using SM-PCR. The induced mutations can be measured quickly and efficiently in cell cultures providing a sensitive assay for the detection of genetic instability. Chemicals exhibiting a variety of known effects induce this instability, whether or not they are considered directly genotoxic, and do so with very similar mutational spectra. It is possible that an underlying pathway might be in play amongst these stresses raising the possibility of previously unidentified genomic risk.

1.32 Development of Analytical and Sampling Protocols for Personal, Indoor and Outdoor Monitoring of Airborne Metals and PM2.5 in Windsor Residential Environments

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OBJECTIVES: A pilot study of 24-hr personal, indoor and outdoor levels of airborne metals and 2.5 micron particulate matter ($PM_{2.5}$) was conducted in Windsor residential environments in 2004, to determine optimum sampling and analytical protocols in preparation for a series of biannual personal exposure monitoring campaigns starting in 2005, under the Canada-US Border Air Quality Strategy.

DESIGN: Gravimetric analysis was performed inside a controlled environmental chamber with a method detection limit of 0.005 mg. Metals were then determined using ICP-MS on a preliminary subset of 24 pilot study filters loaded with a minimum particle mass of 0.060 mg, which included corresponding personal, indoor, and outdoor samples. Various extraction procedures were assessed, including $HF-HNO_3$ and microwave-assisted $HNO3_3-H_2O_2$ digestion. Testing of five replicate (co-located) air samplers assisted in identifying and quantifying sources of contamination of filters in the laboratory and in the field.

RESULTS: Of the three $PM_{2.5}$ sample types (personal, indoor and outdoor), the lowest elemental concentrations were generally observed in the indoor samples. Median concentrations (ng/m^3) for the indoor $PM_{2.5}$ subset were: 2.0 for Pb, 0.4 for As, and 0.1 for Cd.

CONCLUSIONS: Overall, the Windsor indoor residential air metal concentrations are three to five orders of magnitude lower than indoor occupational exposure limits. Extreme caution must be exercised to avoid contamination of filters during handling, as the introduction of metal contamination can easily exceed the contribution from the air particles being sampled. Subjecting the same filter to multiple analyses in different laboratories introduces further risk of contamination associated with increased handling of the samples, and will be avoided as far as possible.

1.33   Evaluation of a Toy Safety Protocol for Determination of Bioaccessible Metals in Dust and Soils of Contaminated Sites

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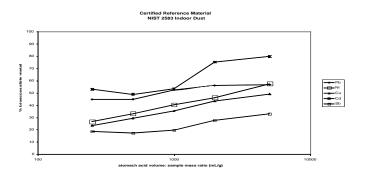
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PURPOSE: A simple yet reliable *in vitro* method of estimating the ingestion bioaccessibility of soil-borne and dust-borne contaminants is required to improve the accuracy and reliability of federal contaminated site risk assessments in Canada. In this study, the European Standard Toy Safety Protocol (EN 71-3:1994) was evaluated for its applicability to dust and soil samples.

DESIGN: The EN-71 protocol is a simulated stomach acid extraction in which the sample is mixed with 50 times its mass of a dilute HCl solution (pH 1.5) for 2 hours at 37°C. The solution is then centrifuged and analyzed by ICP-MS. Experimental work consisted of varying the acid volume to sample mass ratio over two orders of magnitude using certified reference material (NIST 2583), to determine the influence of this parameter on bioaccessibility. The modified method was then applied to different size fractions of indoor dust.

RESULTS: Relative bioaccessibility of metals in indoor dust increased as the acid volume to sample mass ratio increased (see graph). Bioaccessibility of cadmium, lead, nickel and copper in the fine size fraction of indoor dust (<36 micron) ranged from 66% to 80% (n=6), while bioaccessibility of these metals in the coarse fraction of dust from the same house (80 to 150 micron) ranged from 23% to 33% (n=6).

IMPACT/OUTCOME/CONCLUSIONS: The 50-fold acid volume to sample mass ratio prescribed by the EN-71 Toy Safety Protocol is inappropriate for testing indoor dust, due to the likelihood of underestimating metal bioaccessibility. Particle size is a key factor affecting metal bioaccessibility and is thus a critical parameter in residential dust and soil surveys.

Certified Reference Material NIST 2583 Indoor Dust

1.34 Safety Assessment of Conjugated Linoleic Acid: Supplementation of Diets with up to 1% Conjugated Linoleic Acid Does Not Alter the Levels of Arachidonic and Docosahexaenoic Acids of Milk and Brain of Rats at Different Stages of their Growth

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OBJECTIVES: Animal studies have indicated that conjugated linoleic acid (CLA) could replace linoleic acid from tissues. This might have negative consequences in infant nutrition, because acquisition of large amounts of arachidonic (AA) and docosahexaenoic acids (DHA), the primary metabolites of linoleic and αlinolenic acids respectively, is essential for the development of brain and the retina. This study was conducted to determine whether intakes of CLA by pregnant mothers and by their pups during lactation and post-weaning period would affect the AA and DHA levels of milk and brain of growing rats.

DESIGN: Female rats (n=20 per group) were fed diets containing 0%, 0.5% or 1% CLA and mated. On day 17 of gestation 5 dams/group were killed to provide fetal samples. The remaining dams were continued through pregnancy to full term. On postnatal days 21 and 23, milk samples from dams and brain from five pups per litter were collected. The remaining pups were fed the respective CLA diet of the mother for 150 days and were killed for collection of brain.

RESULTS: CLA treatment had no effect on milk fat content and on the levels of AA and DHA of milk fat and brain phospholipid (PL) fractions of fetuses and 150-day old rats. However, the brain PL fraction of 23-day old pups fed 1% CLA diet had slightly lower amounts of AA and DHA compared with the 0% and 0.5% CLA groups.

CONCLUSION: We found little evidence that dietary CLA influence the levels of AA and DHA of rat milk and brain of fetus, pups and growing rats in either positive or negative way.

1.35 Fatty Acid Composition of Canadian Margarines Prior to Mandatory Trans Fat Labelling

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OBJECTIVES: Trans fatty acids are an undesirable component of the diet and their consumption leads to adverse effects on the blood lipid profile and consequently increased risk of coronary heart disease. Margarines made from partially hydrogenated fats are a primary source of trans fats. This study determined the trans fat content of Canadian margarines prior to the new food labelling regulations coming in to effect on December 12, 2005. The new labelling regulations require that trans fat content be declared in the nutrition facts panel in all prepackaged foods.

METHODS: The top-selling twenty nine margarine brands in Canada which represented >95% of the market share were purchased from retail stores during the period of December 2004 to March 2005 and their fatty acid composition was analyzed by capillary-gas liquid chromatography.

RESULTS: The mean trans fatty acid content was 12.0% (of total fat). This value is substantially lower than the mean value of 23.1% reported by us for 1995 margarines. This large difference is primarily due to the current availability of fifteen brands of margarines made from non-hydrogenated vegetable oils. These 15 brands termed "zero-trans margarines" contained less than 2% trans fatty acids (mean 0.9 ± 0.3%) and accounted for more than 46% of the margarine market share. In contrast, in the other 14 margarines, which were made from partially hydrogenated fats, the mean trans content was 23.8% (range 16.1 to 43.5%).

CONCLUSIONS: During the last ten years in response to negative health effects of trans fats, margarine manufacturers have made considerable progress in reducing the trans fat content in Canadian margarines. However, still some of the best-selling margarines contain unacceptably high levels of trans fats.

1.36   *N*-Methyl Carbamate Pesticides in Infant Foods Available in the Canadian Marketplace

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OBJECTIVE: A survey of infant foods was performed to establish *N*-methyl carbamate levels in infant foods, for use in the exposure assessments as part of the ongoing re-evaluation of these compounds by the Pest Management Regulatory Agency (PMRA).

DESIGN: One hundred and fifty-seven samples of commercially prepared infant foods were purchased from retail stores across Canada. Samples were extracted using solid phase extraction columns and analyzed using high performance liquid chromatography with post-column derivitization and fluorescence detection.

OUTPUTS/RESULTS: Carbaryl was the most frequently (7. 6%) detected *N*-methyl carbamate in infant food samples studied, at concentrations ranging from 1 ng/g to 18 ng/g with a mean level of 5.4 ng/g. Carbaryl was generally detected in fruit samples. Methomyl, however, was detected in a single chicken sample at a level of 0.8 ng/g. In all cases, *N*-methyl carbamate residues were well below the maximum residue limit (MRL) established in the *Canadian Food and Drug Regulations* for Canadian foods.

IMPACTS/OUTCOMES/CONCLUSIONS: Children are thought to be more susceptible to the effects of pesticide exposure than adults, due to their higher metabolic rate, less mature immune systems and different dietary consumption patterns. Children, especially infants, consume more food per body mass than adults. The PMRA is currently re-evaluating carbamate insecticides and will consider effects to children, in addition to adults, as part of each risk assessment. Data from this survey indicate that although *N*-methyl carbamates can be detected in infant foods, concentrations are well below levels of concern, based on the current MRL established for Canadian foods. These data will be shared with the PMRA for inclusion in dietary exposure estimates for young children.

1.37 An Assessment of the Impact of Variation in Pathogen Virulence on Establishing Food Safety Objectives Using Quantitative Risk Models

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OBJECTIVES: To assess both the impact of variation in pathogen virulence across genetic subgroups on the establishment of Food Safety Objectives (FSO's) and the sensitivity of current methodologies to the assumption of low dose linearity.

DESIGN: Genetic subtyping data derived from two independent studies U.S. - one of clinical cases of *listeriosis,* the other a food survey, are combined with food consumption information and epidemiological surveillance to provide estimates of subtype virulence (defined as the average probability of illness resulting from exposure to a single organism). The method for combining information exploits the underlying belief network of a previous quantatative risk assessment model (QRA) by: (1) modifying the dose response relationship to account for genetic subtype difference; (2) incorporating a model for possible nonlinearity; (3) decomposing the risk function in terms of conditional probabilities; and, (4) defining a cumulative risk function which provides a more reliable basis for defining an FSO

OUTPUTS/RESULTS: The analysis indicates that there are significant differences in virulence attributable to genetic subgroups of *listeria monocytogenes*. As a result, traditional techniques of choosing FSO's give optimistic predictions with respect to the corresponding level of protection. Additionally, the assumption of low dose linearity is highly influential in determining FSO's.

IMPACTS/OUTCOMES/CONCLUSIONS: Results from the analysis demonstrate a more reliable method for using QRA's to identify FSO's. In addition, it emphasises the need for increased research in the area of dose response for microbial pathogens.

ABSTRACT: Quantatative Risk Assessment models are often proposed as a basis for establishing Food Safety Objectives. The efficacity of these thresholds depends critically on the appropriateness of the assumptions incorporated in the risk assessment model - most critically on the frequency on distribution of exposure levels, together with the dose response model. Recently, it has been suggested that there may be considerable variation in pathogen virulence across genetic subgroups, affecting both the assumed distribution of dose levels and the dose response model. This presentation summarizes recent research related to *listeria monocytogenes*, characterising variation in virulence and assessing the impact of various model assumptions on the predicted impact of various risk reduction strategies. The approach taken is to integrate genetic subtyping databases derived from both food surveys and clinical data with epidemiological data by using the belief network implicit in the corresponding risk assessment model. Based on this methodology, large variations in virulence (quantified as the average probability of illness from a single organism) were estimated. In addition, the predicted impact of various risk reduction scenarios was significantly altered when variation in virulence was accounted for. The assumption of low dose linearity (commonly assumed in microbial risk assessment) also affected predicted results.

1.38 Improved Method for the Extraction and Analysis of Isoflavones and their Metabolites in Rat Blood Using Liquid Chromatography Combined with Mass Spectrometry and Photodiode Array Detection

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OBJECTIVES: To study the safety and potential health benefits of soy isoflavones, a simple, rapid and reproducible method based on liquid chromatography combined with mass spectrometry (LC-MS) and photodiode array detection (PDA) was developed for the determination of isoflavones in rat plasma.

DESIGN: The analytes included aglycones (daidzein, genistein, glycitein), glycosides (daidzin, genistin, glycitin), equol, 4-ethyl phenol and Biochanin A over a concentration range of 0.001- 5.1 μM using 75 μl of rat plasma. Rat plasma samples were hydrolyzed by adding an enzyme mixture from *Helix pomatia* containing 400-600 units/mg of glucuronidase activity and 15-40 units/mg of sulfatase activity. A liquid-liquid extraction method using ethyl acetate as the extraction solvent was used to extract aglycones and the internal standards (phenolphthalein β-D glucuronide, 4-methylumbelliferyl sulfate) from digested plasma samples. The extract was evaporated to dryness under a nitrogen stream, reconstituted with 85:15 % water: acetonitrile with 0.1% formic acid and injected in to a Zorbax SB-CN column (4.6 X 75 mm, 3.5 μm particle size).

OUTPUTS/RESULTS: The chromatographic run time was 16.5 minute per injection with 20 minutes injection delay between samples. The inter-day precision and accuracy of the standard samples were < 4.6% relative standard deviation (RSD) and < 1.8% relative error (RE), respectively. Recovery of the isoflavones with this method varied from 85-100% based on their chemical characteristics.

IMPACTS/OUTCOMES/CONCLUSIONS: This state-of-the art LC-MS method will be used to analyze the level of isoflavones in blood from the Food Directorate multi-generation rodent study for assessing safety of dietary isoflavones.

1.39   Tissue-Specific Suppression of Acetyl-CoA Carboxylase Gene Expression by Dietary Soy Protein Isolate in Rats

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OBJECTIVES: Soy consumption is becoming more and more popular in Canada. Although U.S. FDA approved the health claims for soy protein in the prevention of coronary heart disease, the existing literature on the health safety and nutritional quality of soy components are conflicting. The objective of this study was to examine, using a rat as a model, the effect of dietary soy protein isolate (SPI) and soy-derived isoflavones (ISF) on plasma triglyceride (TG) level, a key risk factor for cardiovascular diseases, and gene expression of acetyl-CoA carboxylase (ACC), a rate-limiting enzyme in fatty acid synthesis, in different tissues.

DESIGN: Sprague-Dawley rats were fed diets containing 20% casein or 20% alcohol-washed SPI with or without supplemental ISF (250 mg/kg diet) for 70, 190, and 310 days. Plasma TG concentration and gene expression of ACCα and ACCβ in the tissues were determined.

RESULTS: Plasma TG levels in female rats fed the casein diet increased with age, and were significantly higher than those of male rats fed the same diet. SPI remarkably reduced plasma TG levels in female, but not in male rats compared to casein. Supplemental ISF had no additional effect. Hepatic ACCα and ACCβ mRNA and protein content were significantly lower in female rats fed SPI diets than those fed casein. ACCβ was the predominant isoform expressed in heart, but unaffected by either SPI or ISF. Neither ACCα nor ACCβ protein was detectable in kidney by Western blot.

CONCLUSIONS: SPI markedly lowered plasma TG level in female rats, which may be attributed to the suppression of hepatic ACC gene expression, suggesting that soy intake may benefit certain hypertryglyceridemic chronic diseases. This information will be important to Health Canada in the evaluation of health claims for soy products and health safety of soy-based infant formulas.

1.40   Characterization of the Predictive Ability of Transgenic Rodent Mutation Assays

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OBJECTIVES: To examine the agreement of results between transgenic rodent (TGR) mutation assays and other short-term mutagenicity assays, and to assess the predictivity of each assay for rodent carcinogenicity and the performance of these assays in a test battery.

DESIGN: A relational database was created containing the results of all published TGR experiments, as well as the results of common short-term genotoxicity tests (STT) and rodent carcinogenicity bioassays for 103 chemicals. Using this database, the operational characteristics of the TGR assay were determined. Measures included sensitivity, specificity, predictive values, and a coefficient of inter-assay agreement (kappa).

RESULTS: The data set contains a high proportion (~ 90%) of carcinogens. Within this group of compounds, the TGR assay exhibited high sensitivity and positive predictivity but, as was also the case with the other STT, low specificity and negative predictivity.

The best overall predictivity was obtained from the TGR assay alone, the *Salmonella* reverse mutation assay alone, and a battery where a positive result from either TGR or *Salmonella* was considered an overall positive and negative results from both assays considered an overall negative. Despite the lack of substantial increases in predictive values of the test batteries compared with the component assays alone, test batteries had lower false negative rates.

CONCLUSIONS: The development of TGR mutation models has provided the ability to directly detect mutations in a range of tissues. Current test battery approaches do not routinely employ an *in vivo* test for gene mutations as an adjunct to *Salmonella* because the existing gene mutation assays are difficult to conduct, expensive and not well validated. This analysis suggests that the TGR assay could potentially fill this void.

1.41   Incidence and Levels of Aflatoxins B1, B2, G1 and G2 in Breakfast and Infants Cereal Sold in Canada

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OBJECTIVES: The goal of the project is to collect surveillance data for aflatoxins B1, B2, G1 and G2 in breakfast and infant cereals sold in Canada. Information generated will provide baseline data for the occurrence of aflatoxins in breakfast and infant cereals and will be used for risk assessment.

DESIGN: 349 breakfast and infant cereal samples were collected at the retail level across Canada from 2002 to 2005. They included rice-, soy-, barley-based and mixed-grain infant cereals, corn-, wheat-, rice-based and mixed-grain breakfast cereals and were analysed for aflatoxins B1, B2, G1 and G2 using a modified AOAC International official method. An immunoaffinity column was used for cleanup and purification of extracts. Determination of aflatoxins was by HPLC using post-column derivatisation with pyridinium hydrobromide perbromide and fluorescence detection.

OUTPUTS/RESULTS: Results indicated that more than 50% of the breakfast cereals and 50% of the infant cereals analysed in this survey had detectable levels (LOD 0.002 ng/g) of aflatoxin B1, which is the most toxic of the four toxins. The levels found varied from 0.002 to 1.00 ng/g for aflatoxin B1, 0.002 to 0.14 ng/g for B2, 0.008 to 0.27 ng/g for G1 and 0.008 to 0.048 ng/g for G2.

IMPACTS/OUTCOMES/CONCLUSIONS: The incidence of finding low levels of aflatoxins in breakfast and infant cereals was high, but the concentrations found in this survey were well below international guidelines (i.e., EU legislative limits are 2 ng/g for B1 and 4 ng/g for total aflatoxins for cereals for direct human consumption). The data collected in this survey will provide the necessary information for risk assessment of aflatoxins in cereals consumed by Canadians.

1.42 A Rapid LC/MS/MS Screen Method for the Determination of Pharmaceutical and Personal Care Products in Drinking Water

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OBJECTIVES: The objective of the research is to develop a rapid LC/MS/MS screening method for the detection of the most common pharmaceuticals and personal care products (PPCPs) in drinking water, and to determine if any contamination of the PPCP reaches a level which may require further investigation in sewage influent and effluent.

DESIGN: Liquid chromatography-mass spectrometry was used. To improve the recovery and repeatability for a large number of targeted compounds, simple liquid-liquid extraction was performed instead of solid phase extraction. A vacuum solvent removal apparatus further concentrated the samples.

OUTPUTS: The developed method is simple and efficient. More than 50 real world samples can be processed in one day by a single chemist. With a limit of detection between 0.5 to 1 ppb of the developed screening LC/MS/MS method for 25 common PPCPs, no residues were detected in tap water samples collected from private homes in the Greater Vancouver area. However, a number of PPCP compounds were found in sewage water samples. The concentration was between 1 to over 100 ppb, which is a concern for the environment and eventually human health. Caffeine was detected in all of the influent sewage water samples at concentrations higher than 100 ppb.

IMPACTS/OUTCOMES/CONCLUSIONS: A LC/MS/MS screening was developed for the detection of PPCP in water. None of the 25 common PPCP detected in the method was found in drinking water above 0.5 ppb level. This work suggests that local urban drinking water is not contaminated with PPCP at ppb levels. It also demonstrated that Health Canada's regional lab is ideally located and suitable to deal with regional issues. The results from this research could provide the scientific basis for the development of regulations and policies regarding PPCPs in drinking water. Since caffeine was detected in all sewage samples, further work should be done to track caffeine and its metabolites in water samples using UPLC-QTOF to see the impact of human activities on water quality.

1.43 Analysis of Methylmercury in Fish Through Solid Phase Microextraction (SPME) and Isotope Dilution Assay

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OBJECTIVES: Use an adaptation of solid phase microextraction (SPME) to develop a fast and innovative method requiring little preparation for analysing methylmercury in fish.

DESIGN: The SPME technique developed by Dr. J. Pawliszyn in the early 1990s generated much interest in the analytical chemistry field. It is fast, requires little solvent, and extraction and purification of the analyte is done in one step. However, the technique allows for the preparation of only one sample at a time and is difficult to reproduce. In our project, an adaptation of SPME allowing for the preparation of 50 samples simultaneously was tested. Then, to improve the precision of the results, isotope dilution assay was also included in the study.

OUTPUTS/RESULTS: The results obtained using certified reference material (NIST 1946) show that the reliability of the method is very good. The SPME technique developed by Dr. Don Forsyth of Health Canada is therefore an interesting alternative to the traditional SPME technique using a syringe. Quantification through isotope dilution enabled us to obtain reproducible results. The method is simple and allows for the preparation of a number of samples, thereby increasing productivity.

IMPACTS,/OUTCOMES/CONCLUSIONS: Application of this method for testing for the presence of methylmercury in predatory fish and canned fish has been planned and is in the process of being carried out in the Food Directorate. This collection of data will be used to reassess the level of methylmercury to which Canadians are exposed and then to review the current Health-Canada-established standard of 0.5 ppm.

1.44 Modelling Consumption Intakes for Risk Assessment of Regulated Discretionary Fortification

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OBJECTIVES: The objective was to evaluate the impact on exposure of various vitamin and mineral fortification options for the proposed policy on regulated discretionary fortification. Modelling options considered which nutrients to add, at which levels and to which food vehicles.

DESIGN: The approach was intended to ensure that safe levels of addition resulted from the proposed policy, in order to protect the health of Canadians. Using food and nutrient intake data from Federal-Provincial nutrition survey, various levels of addition were applied to various selections of qualifying fortification vehicles. The intakes were then adjusted for day-to-day variability to estimate usual intakes resulting from fortification options.

OUTPUTS/RESULTS: For each option, the impact in the shape of the nutrient distribution curves, and impacts in terms of the percentages of the population exposed and the levels of exposure to excessive intakes were assessed. These risks were estimated by applying the new Dietary Reference Intakes from the Institute of Medicine, particularly by assessing the exposure to intakes over the Tolerable Upper Intake Levels. Additionally, simulations allowed consideration of varying the potential future market penetration for fortified foods. Results were presented in the form of intake distribution graphs, and tables estimating the percentage of the population at risk of excessive intakes. These assessments informed the decision-making process for the policy.

OUTCOMES: The modelling assisted in terms of the risk characterization and in setting limits on vitamin and mineral additions for regulated discretionary fortification.

1.45 Outdoor Air Pollution and Emergency Room Visits for Cardiovascular Disease and Stroke in Ottawa, Canada

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OBJECTIVES: While outdoor levels of air pollution have been associated with cardiovascular disease and stroke, the findings from pervious studies have been equivocal and typically been based on mortality outcomes. This study was undertaken to examine the relationship between meteorological events, outdoor air pollution and emergency room visits for stroke and cardiovascular disease in Ottawa, Canada.

METHODS: A case-crossover design was used to examine the relationship between daily levels of gaseous air pollutant and emergency room visits for cerebrovascular and cardiovascular disease. Daily measures of O3, NO2, CO and SO2 were obtained from two fixed monitoring sites from the National Air Pollution Surveillance (NAPS) Network. A total of 5597 emergency room visits were observed between January 1, 1993 and December 31, 2000. Control periods were used by selecting daily intervals one week before and one week after an emergency room visit for cerebrovascular disease. Conditional logistic regression was used to estimate the odds ratio and the corresponding 95% confidence interval associated with a daily increase in the interquartile range of each of the pollutants.

RESULTS: On warm days, NO2, which is a surrogate measure of traffic pollution was related to cardiovascular disease and stroke. Other meteorological events, for example, snowfall, were associated with an increased risk of emergency room visits for cardiovascular visits.

CONCLUSIONS: Our results suggest that air pollution exposure from traffic sources, particularly on warm temperature days, is an important risk factor for cardiovascular and cerebrovascular disease in areas with relative low levels of air pollution.

1.46 Activity and Learning Alterations Following Perinatal Exposure to a Chemical Mixture or its Major Components

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OBJECTIVES: Previous work using a mixture of chemicals based on blood levels in maternal blood of Arctic populations (Arctic mixture) showed that this mixture produced developmental and neurotoxic effects, including functional behavioural disturbances. The current study was conducted to determine: a) if the effects of the mixture could be attributed to specific chemical components of the mixture; and, b) if the toxicity of selected components of the mixture were affected by co-exposure to other mixture components. The overall objective was to determine the toxicity assessments based on individual chemicals adequately reflects toxicity associated with the more realistic exposure to multiple chemicals.

DESIGN: This study compared, in rats, the effects of peri-natal exposure to the Arctic mixture with the effects of specific major components of the mixture (polychlorinated biphenyls - PCB, organochlorine pesticides - OC, and methylmercury -MeHg). Functional neurotoxic effects were evaluated using tests of motor activity and learning/memory in the Morris Water Maze (MWM). Pregnant Sprague-Dawley rats were dosed from gestation to weaning with 0.05 or 5.0 mg/kg mixture doses, 0.01 or 1.1 mg/kg PCB, 0.019 or 1.9 mg/kg OC, 0.02 or 2.0 mg/kg MeHg doses. Offspring were tested at day 16 and 48 (activity) and days 60-100 (MWM).

RESULTS: The highest mixture and MeHg doses increased ambulation and decreased vertical activity at day 16. Both PCB doses and the low MeHg dose also decreased vertical activity. At day 48, ambulation was unaffected but MeHg and both OC doses decreased vertical activity. Learning was disrupted in the MWM (latency to locate target platform) by the high mixture dose. The high MeHg and PCB doses increased latencies in females, but decreased it in males. Memory was also disrupted by the high mixture dose, suggesting that development of new search strategies were altered by the mixture but not the components.

CONCLUSIONS: Results indicate that: 1) perinatal exposure of the mixture or some components alter exploration and learning activity and these effects persist into adulthood; and, 2) the behavioural effects of the mixture differ from the components indicating that toxicity of the mixture cannot be predicted from the effects of the individual components.

1.47 Safety and Efficacy Assessment of Natural Health Products Derived from Medicinal Plants with Psychological Activity: Some Perspectives of the New Canadian *Natural Health Products Regulations*

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OBJECTIVES: To overview some natural health products (NHPs) derived from medicinal plants that promote mental health and the perspectives of the Natural Health Products Regulations in their safety and efficacy assessment.

DESIGN: Review

OUTPUTS/RESULTS: Share author's experiences in product assessment.

IMPACTS/OUTCOMES/CONCLUSIONS: Some perspectives of the new *Natural Health Products Regulations* in safety and efficacy assessment of some NHPs that promote mental health and the usage of such NHPs in different medicinal paradigms will be revealed.

ABSTRACT: The *Natural Health Products Regulations*, which came into effect in January 2004, provides a new fundamental regulatory framework in assessing safety and efficacy of commercially marketed natural health products in Canada.

The biologically active metabolites synthesized in medicinal plants may play a significant role as readily available sources of therapeutic products. An overview of health products derived from some medicinal plants, which are in use to promote mental health in different medical systems, will be discussed. Recent scientific advancements in understanding health risks and efficacy assessment from policy perspectives will be presented for:

Ashwagandha (*Withania somnifera*)
Brahmi (*Bacopa monniera*)
Ginkgo (*Ginkgo biloba*)
Ginseng (*Panax ginseng*)
Kava (*Piper methysticum*)
Passion Flower (*Passiflora incarnata*)
St. John's Wort (*Hypericum perforatum*)
Valerian (*Valeriana officinalis*)

A comprehensive review of scientific research on gotukola (*Centella asiatica*), also as a medicinal plant with potential to promote mental health was presented at last year's Science Forum. Based on this review, a proposal to stop compliance actions on products containing whole gotukola herb and to de-list gotukola extracts and active principles from the prescription schedule (*Food and Drugs Act and Regulations*, Schedule F) has been approved by the NHPD's Expert Advisory Committee and is now proceeding through Health Canada review. As a follow-up, further findings on the potential of gotukola to promote mental health will be revealed.

1.48 Combining Multiple Scans to Improve the Detection of Differentially Expressed Genes

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OBJECTIVES: To empirically validate the effectiveness of detecting statistically differentially expressed genes from microarray data generated by incorporating two scans at different sensitivities.

METHODS: Three mathematical models (gamma saturation maximum likelihood approach, gamma saturation threshold approach and clipping saturation maximum likelihood approach) were used to combine the data from two scans at different sensitivities. The data used in this study were from three separate studies. Two of these studies used the Agilent platform and one study was performed using a custom oligonucleotide microarray (TOXARRAYTM). These studies examined hepatic and neural gene expression induced by exposure to phenobarbital and propylthiouracil. The number of differentially expressed genes was recorded for each model and compared to results based on individual scans.

RESULTS: Combining low and high intensity scans using the threshold approach in conjunction with a global error F-test provided an increased ability to detect differentially expressed genes over single scans and other mathematical models. However, the models are not as effective when there is little to no saturated signal on the high sensitivity scans.

CONCLUSIONS/IMPLICATIONS: Selecting the optimal photo-multiplier tube MT) scanner setting while minimizing the effect of photobleaching can be difficult. Maximum likelihood models (linear or gamma) provide a smooth transition between low sensitivity and high sensitivity scans at the cost of being more computationally expensive. However, incorporating two scans can significantly improve the ability to detect differentially expressed genes particularly with estimators that borrow strength across genes.

1.49   Methods for Identifying and Controlling Nuisance Parameters in Microarray Studies

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OBJECTIVES: To identify and control for sources of variability in microarray experiments and to study the impact on detecting differentially expressed genes when nuisance parameters are not controlled for.

METHODS: Multiple microarray datasets from different Health Canada investigators were used in this analysis. Heatmaps and hierarchical clustering were used to visualize and group the microarrays sharing similar expression profiles. Microarray analysis of variance (MAANOVA) was conducted to identify differentially expressed genes. All methods were conducted in a stepwise manner in R[1]. At each step the most influential parameter (based on the dendrogram) was controlled and the process halted when the microarrays clustered randomly.

RESULTS: The microarray data were dominated by sources of variability outside the experimental factors of interest. Additionally, clustering identified potential outliers in the data that were then removed. Detecting differential gene expression was impaired when factors such as day of hybridization were not controlled using a step-wise approach. In one dataset, the number of differentially expressed genes almost tripled when the day of hybridization was accounted for (based on the James-Stein shrinkage test). Other dominating sources of variability can be seasonal effects and the array batch effect.

CONCLUSIONS/IMPLICATIONS: When planning an experiment incorporating randomization, blocking and replication are very important. Estimation of fold change and other statistics can be influenced by uncontrolled nuisance parameters, which may lead to nonsensical estimates and failure to detect significantly expressed genes. Methods like MAANOVA can control for these factors provided that a sound experimental design is used.

[1] R is a language and environment for statistical computing and graphics.

2.01   In Search of an Optimal Tuberculosis (TB) Screening Strategy

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OBJECTIVES: Tuberculosis (TB) is an infectious disease responsible for severe morbidity and mortality in Canadian FNI communities.

Despite significant drops in TB rates in FNI population, they still remain significantly above the average for Canada.

Newborns are still being vaccinated with Bacille Calmette-Guerin (BCG). However, due to concerns about side effects, BCG is being withdrawn. Alternative TB containment strategy is required to protect FNI communities from TB and reduce TB prevalence to the average for Canada.

The strategy intended to replace BCG vaccinations consists of pre-school and school yearly screening of FNI children followed by prophylaxis treatment of infected individuals. The goal of this paper is to find the most effective way to implement pre-school screening.

DESIGN: We use MatLab to model all possible screening strategies consisting of one to six rounds of screening of all children between years one to six as well as at age eleven of life for each cohort. Six sets of strategies corresponding to the number of screenings between years 1 and 10 (set one contains all screening strategies consisting of one screening between years 1 and 10, set 2 contains all screening strategies consisting of two screenings between years 1 and 10, etc.) were generated. The number of cases and costs were estimated in order to identify the optimal strategy for a given time period and resources available.

OUTPUTS/RESULTS: Optimal strategies in each set were identified, and the effects of uncertainty in key parameters were evaluated.

IMPACTS/OUTCOMES/CONCLUSIONS: Optimal strategies in each set were identified. The strategies cover a wide range of options for policy intervention from the first best given the current epidemiological situation, to strategies optimal under other conditions. The model allows the decision maker to choose the best strategy given existing epidemiological conditions and budgetary constraints.

2.02   Beluga Whales: Botulism in Canada from 1985 to 2005

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OBJECTIVES: Summarize the incidence and causes of botulism in Canada over the past twenty years.

DESIGN: Review data maintained by the Botulism Reference Service for Canada, supplemented with data from the BC Centre for Disease Control.

RESULTS: In Canada, since 1985, approximately 4.4 outbreaks of foodborne botulism occur annually, with an average of 2.5 cases/outbreak. Traditional foods prepared in Inuit west coast First Nations communities were responsible for most outbreaks. Traditional foods involved were mainly raw meats from marine mammals, fermented meats such as muktuk (meat, blubber and skin of the beluga whale), raw fish or fermented salmon eggs. Type E was implicated in almost every case involving traditional foods. Commercial products have been implicated in two incidents. Commercial pate caused two cases of type B botulism, while a cooked boneless pork product caused a single case of type A botulism. Both products were temperature abused by the consumer. Garlic-in-oil, bottled mushrooms and a baked potato have been responsible for outbreaks involving food-service establishments. The fatality rate of botulism in Canada has decreased from greater than 45% in the 1960's to less than 3% in the 1990's, due mainly to the availability of antitoxin to type E neurotoxin.

CONCLUSIONS: Most botulism outbreaks that have occurred in the past 20 years in Canada have occurred in northern and west coast native communities. Occasional outbreaks continue to result from consumption of commercial foods and foods at food-service establishments.

2.03   Interprovincial Migration of Physicians in Canada: Who Moves Where and Why?

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OBJECTIVES: To identify key individual and provincial characteristics contributing to interprovincial migration of physicians in Canada.

DESIGN: We used Southam Medical Database (1974-2002) and National Physician Database (1989-2000) and estimated two models: 1) a logistic formulation of discrete-time hazard model to estimate yearly probability of moving from current province of residence with individual characteristics variables along with provincial dummy variables and time-periods; and, 2) McFadden's conditional logit discrete-choice model with yearly probability of choosing a province of residence as a dependent variable for physicians residing in each province (origin) separately. We included average annual income in a province as the main choice (province) characteristics variable.

RESULTS: Younger physicians (aged 26 to 30) are six times more likely to move than those aged 45 to 50. Medical and Surgical specialists are 57% and 49% more likely to move than family physicians. The interaction effect between language and Quebec as province of residence is also significant.

The effect of expected income in a province on the choice of province of residence is positive and statistically significant for physicians residing in Ontario and Saskatchewan. After the home province, Ontario and BC are the most preferred destination choices. New Brunswick comes at the bottom in most cases except for the physicians residing in Nova Scotia, and Quebec.

CONCLUSIONS: The connections our study has made between a physician's personal characteristics and their likelihood to migrate from one province to another would be useful in the policy development both in terms of managing ensuing gaps and creating incentives to steer movement of physicians. For example, knowing that younger physicians are more likely to migrate and settle as they age, could allow policy makers to create incentives attractive to young physicians to draw them to provinces where they are needed. This could help solve short-term supply gaps, and provide a settled and more predictable workforce for the future.

2.04　Utilization of Family Physicians Services in Canada: Can it be Explained by Need Alone?

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OBJECTIVES: Determine whether the utilization of family physician's services in Canada can be explained by need alone.

DESIGN: We used Canadian Community Health Survey, 2003. We define utilization of family physicians services as the number of consultations with a family doctor or a general practitioner. We estimated Negative Binomial regression with family physician's service utilization as the dependent variable. The explanatory variables included in the model are *self-perceived health status*, and dummy variables for prescription drug coverage, having regular medical doctor, having children less than 12 years of age in the household, urban/rural residence, province and sex.

We again estimated the model using two-stage method. First, we estimated Ordered Logit model to predict health status using age, age-squared, education, marital status, household income and the number of chronic conditions as explanatory variables. Then we used this *predicted health status* as one of the explanatory variables in our second stage Negative Binomial regression.

RESULTS: When controlled for provinces and all the other explanatory variables, the regression with *self-assessed health status* shows higher utilization for same health status compared to the model with predicted health status. Implying that the utilization cannot be explained by need alone.

CONCLUSIONS: The utilization of family physician's services is not solely based on need (proxy by predicted health status). Other factors such as having coverage for prescription drugs, having a regular medical doctor, having young children in the household, being female, and urban residence also increases the likelihood of utilization of such services. The identification of these factors, as a result of the research conducted, presents a real potential to continue research on this topic, and contribute new knowledge in the area of health services utilization and population health.

2.05   Children's Environmental Health Indicators in Canada

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OBJECTIVE: The project is to develop indicators of children's environmental health (CEH) in Canada and is intended to identify the priority issues for indicators development. An initial list of 42 issues related to children's environmental health has been developed based on best available scientific evidence.

DESIGN: A comprehensive list of key issues of CEH was developed and will be narrowed down using a ranking procedure, (Hanlon method), which considers the extent, severity, solubility of the issue and feasibility of the related intervention. The Steering Committee and additional experts in the field of CEH are working on the ranking process. The newly prioritized list will inform the development of indicators of CEH. However, the project will not populate the indicators at this point in time.

OUTPUTS/RESULTS: The project will guide better data collection, environmental monitoring and health surveillance. Also, the project demonstrates successful collaboration by many stakeholders (governments, academia, NGOs) to further advance the field of CEH. When populated, the indicators will allow for the tracking of trends over time on issues such as outdoor air quality, indoor air quality, pesticides, toxic substances and water quality, as they affect children's health in Canada. This project also contributes to Canada's international commitment as a participant in the World Health Organization's Global Initiative to develop Children's Environmental Health Indicators.

IMPACTS/OUTCOMES/CONCLUSIONS: The indicators will ultimately improve our current knowledge of the physical environment as a determinant of child health and development. The indicators are intended to be relevant to address health issues at the regional and community level in Canada. Also, they will help inform decision-makers and enable the development of environmental and health policies, programs, and regulations to address environmental threats to children's health.

2.06 Characterizing Treatment-Seeking OxyContin® Abusers

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OBJECTIVES: Increasing numbers of individuals using controlled-release oxycodone (OxyContin®) have been seeking detoxification at CAMH in Toronto. The purpose of this study was to determine the demographic, substance use and health status of these individuals; and to compare them with other opioid users.

DESIGN: This study is a retrospective chart review of patients admitted for opioid detoxification.

OUTPUT/RESULTS: Preliminary analysis indicated that in 2004 there were 167 opioid-related admissions for 162 individuals to the medical withdrawal service. Of these, 91 patients (56%) were using OxyContin®, 64 (40%) used other prescription opioids ('otherRx' group) (e.g., codeine, hydromorphone, morphine, other oxycodone products (e.g., Percocet®)), and 7 (4%) used heroin only. The OxyContin® users reported currently using an average of 405±305 mg daily, with an average duration of use of 2.4±2.2 years. Most reported using the product for recreational purposes (69%), with 20% for therapeutic purposes and 11% for both. Most were chewing the product (73%), with 13% crushing/snorting and 14% crushing/injecting. Many used other opioids and substances: 45% other oxycodone products, 19% morphine, 13% codeine, 8% hydromorphone, 6% heroin, 48% cocaine. A significant proportion indicated current psychiatric problems: 53% depression, 25% anxiety, and 46% were currently receiving psychiatric medications. Compared to the 'otherRx' group, the OxyContin® users tended to be younger (36±10 years vs 39±9 years, respectively, p=052; had a higher proportion of males (66% vs 47% males, respectively, p<0.02); were more likely to use street sources (55% vs 28% street source, 19% vs 27% physician source, 24% vs 33% both, respectively, p=0.02).

IMPACTS/OUTCOMES/CONCLUSIONS: Characterizing this population is important for creating effective prevention and treatment strategies.

2.07 Health Impact of Radon Exposure on the Canadian Population

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OBJECTIVE: Recent pooled epidemiological studies from North American and European residential radon studies have confirmed a small but significant increase in lung cancer risk at indoor radon levels. In order to determine whether our current radon guideline should be revised, we have undertaken an analysis of the health impact of radon exposures on the Canadian population.

DESIGN: The proportion of lung cancer deaths in Canada attributable to indoor radon were calculated using a lifetime relative risk model developed by the US Environmental Protection Agency. Canadian age-specific mortality rates from lung cancer and from all causes of death for the period 1996 to 2000 were utilized, along with smoking prevalence data for males and females for the year 2002. The distribution of radon concentrations in Canadian homes were obtained from a cross Canada radon survey of 14 000 homes carried out in the late 1970s.

RESULTS: Radon concentrations in Canadian homes are log-normally distributed, with a geometric mean of 11 Bq/m^3 and a geometric standard deviation of 3.9. About 3% of homes have concentrations above 200 Bq/m^3 and about 0.1 % have concentrations above the current Canadian guideline of 800 Bq/m^3. Our calculations show that the risk of lung cancer to a non-smoker is doubled for lifetime exposure at an indoor radon concentration of 200 Bq/m^3. At 800 Bq/m^3 the risk is increased fivefold. The risks to smokers are even greater because of a synergistic effect between exposures to radon and to tobacco smoke. The rigorous application of a radon action level at 800 Bq/m^3 would save only about 36 lives per year in Canada, due to the small number of homes above this level. However, the number of lives saved increases to 347 at an action level of 200 Bq/m^3, out of a total of 1589 radon-induced lung cancer deaths per year. This would represent a significant reduction in mortality and health care expenses.

CONCLUSIONS: Our analysis strongly supports lowering the current Canadian guideline for indoor radon from 800 to 200 Bq/m^3.

2.08 New and Fast Analytical Procedure for the Determination of Actinides in Field Samples Following an R/N Emergency

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OBJECTIVES: Develop new and fast laboratory protocols for the measurements of actinides in field samples (urine, air particulate and swipe samples) following a radiological/nuclear (R/N) emergency.

DESIGN: The analytical methods are based on fast sample digestion, fast and one-line matrix removal and sample preparation, and fast and sensitive measurement. The microwave-based autoclave can digest 50 small volume samples or 6 large volume samples in two hours. FIAS (flow injection analysis system) or HPLC (high performance liquid chromatography) based sample preparation system can remove the matrices and pre-concentrate the analytes efficiently and introduce the sample to ICP-MS (inductively coupled plasma mass spectrometry) via a desolvation system.

OUTPUTS/RESULTS: The protocols (for urine, air particulates and swipe samples) provide validated analytical methods for rapid and sensitive assessment of actinides in field samples following an R/N emergency. The sample throughput is 2-6 samples per hour, and the sensitivity for the most interested nuclides is around 1-100 fg per ml or gram (e.g., for Pu-239, mBq/L in urine).

IMPACTS/OUTCOMES/CONCLUSIONS: The protocols are the most practical (robust, fast and sensitive) to date in the world. They provide the nuclear cluster laboratories in Canada technically standardized solutions for emergency preparedness. Technologies developed throughout the project can be applied for other research and application projects.

2.09 Real Time Monitoring of Cs and U in Fresh Waters Using DGT Technique and Gamma Detector: A Preliminary Study

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OBJECTIVES: Develop real time radionuclide monitoring systems for Canadian fresh waters to keep alert to radioactive contamination.

DESIGN: The monitoring system was based on two techniques: diffusive gradients in thin films (DGT) and NaI gamma detector. DGT can pre-concentrate the nuclides from water efficiently and make the nuclide detectable by gamma detector, which was coupled to DGT. Measurement data can be transmitted to the home laboratory via technologies, which are currently using for our air monitoring program, so that the concentration of the nuclide in water and its variation can be monitored from home laboratory.

OUTPUT/RESULTS: To meet the specific requirement of radionuclide detection, a series of new binding layers, such as Chelex 100, UTEVA, P 81, SuperLig and AMP resin imbedded in polyacrylamide hydrogel, were developed and tested for this application. The newly developed DGT devices were investigated under varying conditions of pH, ionic strength, analyte concentration. Chelex 100, P 81, and SuperLig were the best among the tested for their high capacity of binding. Laboratory experiments have also confirmed the feasibility of the DGT/NaI detector combination.

IMPACT/OUTCOMES/CONCLUSIONS: This project will give Health Canada the capability of monitoring radionuclides in waters in a real-time manner. Such a capability is crucial for radiological or nuclear incidents caused by either technical failure or terrorist attack.

2.10 Solubility Modelling of Strontium Titanate and Uranium Dioxide in Simulated Lung Fluid

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OBJECTIVES: Study and model the solubility of the radionuclide-bearing particles in simulated lung fluid; evaluate their biological effects and study the solubility mechanism.

DESIGN: Batch method, filter sandwiched method and artificial lung device were tested for the solubility of radionuclide-bearing particles in simulated lung fluid (SLF). The dissolved radionuclides in SLF were measured by ICP-MS (inductively coupled plasma mass spectrometry) on-line through a desolvation system where most of the matrices were removed before being introduced into the MS.

OUTPUTS/RESULTS: The solubility of high fired $SrTiO_3$ and UO_2 by different experimental designs was compared. The mechanism of solubility was studied. Experimental results contributed to the modeling work for ICRP (International Commission for Radiation Protection). With the chemical speciation information of the radionuclides dissolved in lung fluid from another project, the solubility data obtained in this work help in experimental design of future research on lung/blood transfer of the nuclides.

IMPACTS/OUTCOMES/CONCLUSIONS: The results obtained from this work help in assessing risks associated with the inhalation of radionuclide-bearing particles following a radiological / nuclear incident. The introduction of new ways of studying lung dissolution/transportation using artificial lung device makes the experimental design closer to a real biological mechanism. This design can also be applied in research related to heavy metal inhalation and solubility.

2.11 Bioavailable Dietary Iron Estimations for Fast Food Dishes Contained in the Canadian Nutrient File

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OBJECTIVES: The Canadian Nutrient File (CNF), like most national food composition databases, lists only total iron values. The lack of heme/nonheme iron amounts in the database has limited the ability to accurately estimate total bioavailable iron in the Canadian diet. Our objective was to estimate iron bioavailability for fast foods, composed of multiple ingredients, some containing both heme and nonheme iron sources.

DESIGN: In order to estimate the bioavailable dietary iron (BDI), the ratio of food sources contributing to the total iron of fast food dishes was calculated using the Nutrition Survey System (NSS), which contains a recipe database developed for nutrition surveillance. The ingredient amounts in selected NSS recipes were modified to closely meet the iron, protein, carbohydrate, fat and energy content of the fast foods in the CNF, in order to approximate their iron bioavailability. A variance of 15-20% was accepted for most values when adapting the NSS recipes to approximate the CNF nutrient amounts.

RESULTS: Using a modified Monsen model (1978), BDI was calculated to be 0.05, 0.07, 0.08, 0.11, 0.15, 0.18, and 0.27 mg for salad, burrito, ham, egg and cheese sandwich, chicken fillet, taco, fish sandwich and hamburger, respectively, for an individual with 250 mg of stored iron (typical levels seen in Canadian premenopausal women). This sample of foods has 4.0-11.0% bioavailable iron, below the estimated 18% bioavailability for a mixed diet.

CONCLUSIONS: This novel approach for estimating fast food's BDI will provide a more complete database of iron bioavailability estimations for the CNF.

2.12   The Effect of Perinatal Hypothyroidism on Hepatic Gene Expression in Juvenile Mice

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OBJECTIVES: To examine gene expression changes in liver of neonatal hypothyroid mice and to identify novel thyroid hormone (TH) responsive genes and elucidate underlying mechanisms.

METHODS: To generate hypothyroid offspring, pregnant mice were treated with 0.1% propylthiouracil (PTU) from gestational day 13 to weaning. Liver and serum samples were collected. Hypothyroidism was confirmed by serum thyroxine (T4) levels. Liver RNAs from control and PTU-treated pups were hybridized to Agilent microarrays (~22 000 genes), normalized using LOWESS, and analyzed with MAANOVA. Candidate genes were confirmed by real-time (RT) PCR in liver tissue and in cultured HepG2 cells. Electromobility shift assay (EMSA) was used to determine the presence of TH-responsive elements (TRE) in the promoter region of one candidate gene.

RESULTS: Serum T4 levels and decreased expression of known TH-regulated genes (Dio1, S14 and ME) confirmed hypothyroidism in pups. Differential expression was found for 379 genes (158 up-regulated and 221 down-regulated), including known TH-regulated genes. Altered pathways were associated with lipid metabolism, apoptosis and signal transduction. Expression of six novel TH-regulated genes (3 up- and 3 down-regulated) was confirmed using RT-PCR. Expression regulation *in vitro* of two genes (Nr4a1, c-JUN) was similar to response *in vivo*. The promoter region of Nr4a1 was amplified and sequenced. Three half-TRE sites exist in the upstream -1188 to -1218 bp and binding by TH receptor was confirmed by EMSA.

CONCLUSIONS/IMPLICATIONS: We showed that disruption of maternal thyroid homeostasis resulted in disregulation of numerous pathways. Nr4a1 was negatively regulated by TH via binding to TREs providing a potentially new biomarker of TH-disruption.

2.13 Generic Predictors of the Effectiveness of On-Farm Food Safety Programs

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OBJECTIVE: To identify early predictors of the effectiveness of on-farm food safety programs, for use in detecting, monitoring and controlling a range of potential food-safety hazards of public health significance.

DESIGN: Part of a 5-year project funded by the Agriculture Policy Framework (APF), this work focusses on identifying predictors of effectiveness of on-farm food safety programs across a range of food commodities and hazards (microbiological, chemical, and physical). FD partners working on relevant APF projects were consulted and the scientific literature was systematically searched from 1995 forward. Database searches included Medline, Current Contents, FSTA Direct, Agricola and CABHealth. Search terms included "HACCP", "farm or harvest or agriculture" and "assessment or evaluation or effective or success" and all variations therein. Identified reports were reviewed and predictor information extracted. As needed, predictor wording was customized to the on-farm setting (see Figure).

OUTPUT/RESULTS: This work has resulted in a list of generic predictors of the effectiveness of on-farm food safety programs. Examples are presented below.

- Wide range of stakeholders consulted during program development
- Program pilot tested by representative on-farm producers
- Relevant training material readily available
- Program readily customized to farm-specific circumstances
- Required documentation is the minimum necessary to ensure food safety

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Figure: Sample Generic Predictors of Program Effectiveness

IMPACTS/OUTCOMES/CONCLUSIONS: Generic predictors of effectiveness can be expected to facilitate identification and early correction of any problems in on-farm food safety programs. Enhanced effectiveness can be expected to contribute to long term outcomes such as increased consumer confidence in the Canadian food supply and protection of human health.

2.14 Incidence of Cardiovascular Disease in Canada: Rural Verus Urban Areas
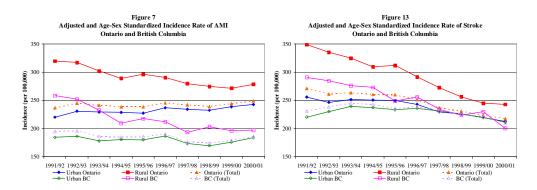
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OBJECTIVES: We examine variations in incidence of acute myocardial infarction (AMI) and stroke in rural and urban areas of British Columbia and Ontario from 1991/92 to 2000/01.

DESIGN: We calculate incidence as the number of patients admitted to hospital for the first time due to AMI or stroke, correcting for the number of patients who would have been readmissions after having been admitted in years prior to the study period. Ontario and BC are compared using age-sex-standardized incidence rates. AMI and strokes episodes are identified using the Discharge Abstract Database (CIHI) and population data are from Statistics Canada.

OUTPUTS/RESULTS: Incidence of AMI and stroke increases with age and is higher in Ontario than in British Columbia, although for stroke the difference between the two provinces has faded over time. Incidence of AMI is higher for men than for women and we find the opposite for stroke. Overall, incidence tends to be higher in rural areas than in urban areas, but there seems to be a trend toward a closing of the gap between the two, which is driven mainly by decreasing incidence in rural areas.

Figure 7
Adjusted and Age-Sex Standardized Incidence Rate of AMI
Ontario and British Columbia

Figure 13
Adjusted and Age-Sex Standardized Incidence Rate of Stroke
Ontario and British Columbia

IMPACTS/OUTCOMES/CONCLUSIONS: The decreasing difference between rural and urban incidence of cardiovascular disease, driven mainly by a decline in rural incidence, is an encouraging result. Further work would be needed to determine if this stems from policy interventions aimed at reducing risk factors and improving health status in rural areas.

2.15 Soy Protein Improves Bone Metabolism in Young and Old Female Rats Fed Low Dietary Calcium

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OBJECTIVES: Generally, Canadian women, especially elderly, consume diets low in calcium (Ca) that can promote bone loss. It has been suggested that soy protein (SP) and/or soy isoflavones (IFs) may slow the loss of bone. In this study, we examined the effect of feeding a SP or a SP plus IF diet on bone metabolism in rats fed low levels of Ca.

DESIGN: A group of 96 female rats, 54 weanling and 54 retired breeders (RB), were randomly assigned to 1 of 8 diets: 1) a casein-based diet; 2) a SPI-based diet; 3) a casein-based diet with low Ca; 4) a casein-based diet with low Ca and 150 mg IF/kg diet; 5) a casein-based diet with low Ca and 400 mg IF/kg diet; 6) a SPI-based diet with low Ca; 7) a SPI-based diet with low Ca and 150 mg IF/kg diet; and, 8) a SPI-based diet with low Ca and 400 mg IF/kg diet. Bone mineral density (BMD), bone mineral content (BMC), bone growth parameters, and biochemical markers of bone resorption were measured.

RESULTS: Young rats fed the soy-based diets had significantly smaller reductions in BMD and BMC from consuming a low Ca diet compared to rats fed a casein-based diet. The added IFs had no further benefits on any parameters measured. BMD, BMC and bone growth parameters of RB rats were unaffected by the SP diet. However, SP showed positive effects on bone turnover in both the young and the RB rats as determined by bone resorption markers (urinary deoxypyridinoline and pyridinoline).

CONCLUSIONS: Feeding a SP-based diet positively affects bone metabolism and minimizes the negative effects associated with low Ca intakes in young rats. These data merit further investigation on the effects of SP on bone growth, especially when Ca intake is inadequate.

2.16 Rate of Diisocyanate-Induced Asthma in Ontario: Possible Role of Surveillance

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OBJECTIVES: A key to chronic disease prevention and control is integrated policy and program development, surveillance, hazard communication and knowledge development and dissemination. Exposure to diisocyanates is a recognized cause of occupational asthma (OA). The objective of this study is to determine whether identification of cases of OA related to diisocyanates, through surveillance and early intervention with removal from further exposure, can prevent or reduce the incidence of permanent asthma.

DESIGN: Chart abstraction of compensation files of accepted Ontario Claims during 1980-1993, examination of the incidence of OA, and record linkage of those compensated for OA with the Health Information Discharge Abstract Data Base of the Ontario Ministry of Health to examine the severe end of health outcomes were used.

OUTPUTS/RESULTS: Chart abstraction of compensation files showed that the causes of OA accepted Ontario claims during 1980-1993 included diisocyanates (in 50.4% of claims); the examination of the incidence of OA revealed that there was an initial increase in numbers of diisocyanate claims through1991 more steeply than that for other causes. Record linkage of those compensated for OA showed that those with diisocyanate-induced OA were only half as likely to be hospitalized for asthma during the follow-up period (5.3% vs 11.1%; p=0.003).

IMPACTS/OUTCOMES/CONCLUSIONS: The study showed that surveillance related to chronic diseases allows a better understanding of diseases. This, in turn, facilitates improved targeting of WHMIS/OHS surveillance and hazard communication programs and research for the prevention and control of occupational asthma.

2.17 Analysis of DNA Adducts by On-Line Pressure Assisted Electrokinetic Injection Technique in Capillary Zone Electrophoresis – Electrospray Mass Spectrometry

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OBJECTIVES: Study on DNA adducts will help us better understand how chemicals such as environment contaminants specifically react with DNA. The objective of this study is to develop a quick, reliable and highly sensitive analytical method to determine DNA adducts.

DESIGN: Two oligonucleotides, one corresponding to an internal sequence within the serotonin transporter (5-HTT, 5'-TGGACCTGGGCAATGTCTGG-3') the other a brain-derived neurotrophic factor (H-BDNF, 5'-GGACTCTGGAG-3') half binding site, were separated by CE-MS and analyzed as single stranded, complementary and double-stranded structures. The complementary 5-HTT oligonucleotide was also incubated with benzo[*a*]pyrene-diol-epoxide (B[a]PDE) to obtain DNA adducts.

A reverse pressure to Electro-osmotic flow was applied to the capillary during the electrokinetic injection of samples. The DNA and DNA adducts were concentrated during this pressure assisted electrokinetic injection process (PAEKI) and subsequently monitored and identified by mass spectrometer.

OUTPUTS/RESULTS: Single-and double-stranded oligonucleotides were separated under the conditions of 20 mM of ammonium acetate. Sensitivity can be enhanced up to 1000 folds with the application of PAEKI technique. With the pre-concentration technique of PAEKI, adduct from 5-HTT complement incubated with BPDE was identified.

IMPACTS/OUTCOMES/CONCLUSIONS: Results from these experiments revealed that without a water plug, the sample analytes can be highly concentrated into a very narrow sample zone, which produced excellent separation efficiency on CE. Such approach dramatically increased detection sensitivity and enabled to directly measure DNA adducts in samples at a level of about 15 nmol. The method is very useful in evaluating potential DNA alteration as a result of exposing to environmental pollutants and industrial chemicals.

2.18   The Windsor, Ontario Children's Respiratory Health Study

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OBJECTIVES: This study aims to provide a comprehensive assessment of adverse respiratory health effects in Windsor elementary school children and relate them to measured levels of air pollution.

METHODS: In the first phase of the study (Nov, 2004), a questionnaire was distributed to grades 1 to 8 school students to collect information on health conditions and medical history. Lung function testing for phase 2 (February to June 2005) of this study was carried out among grade 4-6 students. Children underwent tests including spirometry a measure of ventilatory function, and exhaled nitric oxide and exhaled breath condensate, which are indicators of lung inflammation.

The 3rd phase of this study (September to December 2005) plans to measure lung function and air pollution on a daily basis for 30 days in about 200 subjects with asthma.

Seasonal levels of air pollutants (Nitrogen dioxide, sulphur dioxide and volatile organic compounds) collected in a study occurring concurrently with the WCRHS are being used. Land use regression modeling is being used to assign weighted exposure estimates to participants based on their home and school address.

RESULTS: Questionnaires from phase 1 of the study were returned by approximately 70% of students. Descriptive results including participation rates, and preliminary air pollution data will be presented at the Health Canada Science Forum.

CONCLUSIONS: The success of this large-scale survey was dependent on the support of school boards and families. Results from this study will help us understand the impact of ambient air pollution on the respiratory health of children, a vulnerable population.

2.19   Acquisition and Loss of Chronic Disease Risk Factors in Canadians from 1994 to 1998: Smoking, Risky Drinking, Physical Inactivity, Overweight and Hypertension

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OBJECTIVE: To estimate the baseline, within-person, population changes in modifiable risk factors for chronic diseases.

METHODS: National Population Health Survey longitudinal data were used to estimate changes over time of five risk factors in the Canadian population, aged 16 years and over, from 1994 to 1998. Percentages who acquired, or lost, risk factors were estimated from self-reports.

RESULTS: Percentages of risk factor losses in the population were estimated as follows: smoking daily, 4.5%; risky alcohol consumption, 3.7%; physical inactivity, 19.2%; overweight, 5.3%; and hypertension, 1.6%. Percentages of risk factor acquisitions were as follows: smoking daily, 3.9%; risky alcohol consumption, 3.9%; physical inactivity, 13.8%; overweight, 9.4%; and hypertension, 5.6%. More males acquired overweight (10.70%, 95% confidence interval (CI): 9.52 - 11.87) than did females (8.14%, 95% CI: 7.21 - 9.08) and more males reduced risky alcohol drinking (5.10%, 95% CI: 4.35 - 5.84) than did females (2.37%, 95% CI: 1.85 - 2.88). But more males also acquired risky alcohol drinking (5.28%, 95% CI: 4.48 - 6.09) than did females (2.67%, 95% CI: 2.04 - 3.30), in the same study period. Risk factor acquisition rates were in general the highest in people 16 to 29 years of age.

CONCLUSIONS: These figures fill a gap in the literature, since population-based, longitudinal surveys are very rare. They establish baseline change rates against which to compare results of health promotion programs and could inform strategies for such interventions.

2.20 Superoxide Dismutase Mimetic *AEOL 10150* Reverses Plasma Endothelin Levels in Rats After Inhalation of Air Pollutants

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OBJECTIVE: Epidemiological studies have associated cardiovascular and respiratory morbidity and mortality with air pollution, particularly in susceptible individuals with concurrent cardiovascular and pulmonary diseases. We have shown that inhalation of pollutants increases plasma levels of endothelin (ET)-1 and ET-3 in animals and humans. A surge of circulating endothelins after breathing air pollutants may account for acute cardiovascular symptoms and adverse events in susceptible individuals. Additional studies have shown that air pollutants can induce oxidative stress scenario in the lungs. Our objective was to block a potential superoxide surge during or after inhalation of pollutants with a superoxide dismutase (SOD) mimetic drug (*AEOL 10150*) in order to verify whether oxidative stress pathways play a role in the regulation of the endothelinergic system.

DESIGN: Male Fischer-344 rats (n=4) were injected subcutaneously with 2mg/kg of *AEOL 10150* two hours prior to inhalation exposure, followed by exposure for four hours to clean air, urban particles EHC-93 (50 mg/m3), or ozone (0.8 ppm). Control animals without the drug were also exposed to the pollutants. The animals were sacrificed immediately or 24 hours after inhalation exposure. Plasma samples were analysed for ET levels by HPLC-flourescence.

RESULTS: Plasma endothelins (Big ET-1& ET-1) were increased immediately after exposure to ozone and particles (p<0.05). Pre-treatment of the animals with the SOD mimetic decreased the plasma levels (BET-1: *DrugXexposure* p<0.079; ET-1:*DrugXexposure* p<0.01). After 24 hours recovery in clean air, plasma endothelin levels(Big ET-1& ET-1) were increased in animals exposed to ozone or particles (p<0.01), an effect that was reversed by pre-treatment with the SOD mimetic (*Drug X Exposure*, p<0.01). Similar decrease was seen in the lung and heart ET-1 mRNA expression with the drug. Significant changes were not noticed in Plasma ET-2 &ET-3.

CONCLUSIONS: These results indicate that drug *AEOL 10150* injected subcutaneously reduces plasma ET levels in pollutant exposed rats probably by downregulating ET-1 mRNA expression. Increased levels of ET-1 have been implicated in various heart diseases like congestive heart failure and reduction causes improvement of the symptoms. Oxidative stress has been implicated in enhancing ET-1 mRNA in human endothelial and vascular smooth muscle cells. Since this drug scavenges O_2. -species formed during pollutant exposure, this could lead to reduced oxidative stress and therefore reduced ET-1 production. This suggests that oxidative stress pathways contribute to the regulation of endothelinergic system. This drug *AEOL 10150,* may also be used therapeutically to reduce plasma endothelin levels during pathophysiological conditions.

2.21 Comparison of Two Data Sources on the Use of Prescription Medications

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OBJECTIVES: This study will compare and contrast drug utilization patterns as described in IMSight pharmaceutical data with CCHS health data. It will also examine the relationships between health indicators and whether a person is being treated, by medications, for any of seven leading chronic conditions: allergies (food or other); back problems (arthritis or other); high blood pressure; migraine headaches; and asthma.

METHODS: Using the Canadian Community Health Survey 2003 2.1 Share file and IMSight data, drug use data will be compared for the two data sources for each of seven medication groups, one per chronic condition. IMSight data will be used to show population trends in prescription medication use.

Health status indicators such as self-rated health, self-rated mental health and number of consultations with a medical doctor will be compared between those with any of the selected chronic conditions who are taking medications and those who are not.

RESULTS: Results from this study will enable readers to understand links between drug treatments for chronic conditions and differences in health status indicators.

CONCLUSIONS: This study will show how health status indicators are linked with a person's chronic condition state, and how use of medications can be beneficially or adversely associated with a person's chronic condition state.

IMPLICATIONS: This presentation can be used to gain a better understanding of associations between chronic conditions, medications and health status indicators. Thus it could be used to further develop targeted policy and programs in this area.

2.22 CCS is a Sensitive Biomarker of Zinc-Induced Mild Copper Deficiency

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OBJECTIVES: Small increases in dietary zinc (Zn) above the Recommended Dietary Allowance can reduce copper (Cu) status. Today, a large proportion of young children have Zn intakes exceeding the Tolerable Upper Intake Levels (ULs), stressing the need for sensitive biomarkers able to detect small reductions in Cu status. Here, we examine the value of copper chaperone for Cu/Zn superoxide dismutase (CCS), a novel molecular biomarker of Cu status, as an indicator of mild Cu deficiency induced by moderately high dietary Zn.

DESIGN: Weanling male rats (n = 12/diet group) were fed one of 4 diets varying in Zn content (30, 60, 120 or 240 mg Zn/kg diet) and normal in Cu for 5 weeks. CCS protein expression in liver, erythrocytes and white blood cells (WBCs) was determined by Western blot analysis and compared with changes in conventional indices of Cu status.

RESULTS: CCS protein content in liver, erythrocytes and WBCs was upregulated in rats fed high dietary Zn and showed a strong inverse correlation with common measures of Cu status (i.e., liver and plasma Cu levels and ceruloplasmin activity). When compared with conventional indices, increased CCS expression in liver and erythrocytes was determined to be the most sensitive measure of reduced Cu status.

CONCLUSIONS/IMPACTS: CCS expression is responsive to small reductions in Cu status and may be a better indicator of reduced Cu nutriture than commonly used measures. CCS is currently being used to evaluate Cu status of young boys in a human clinical trial of Zn supplementation. This study will provide valuable information for setting more accurate ULs for Zn for children and making sound recommendations regarding intakes of Zn and Cu.

2.23   A New Biological Role Claim for the Omega-3 Fatty Acids, EPA and DHA

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OBJECTIVES: The objectives were to evaluate the scientific evidence for biological functions of the long chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in cardiovascular health, and to identify the levels of intake associated with these biological functions. This information would be applied in setting conditions for the use of a biological role claim for EPA and DHA for use in food labelling and advertising.

DESIGN: A review of research linking EPA and DHA to cardiovascular function in healthy humans was conducted. This was done to address the requirements for a biological role claim as set out in the Food and Drug Regulations. The Regulations allow a food to carry a claim, referred to as a Abiological role claim for a nutrient it contains regarding a generally recognized role for the nutrient in maintaining health. Nutrients here refer to those that are recognized as essential in the diet, such as vitamins and minerals; biological role claims have been developed for most vitamins and minerals. The fatty acid, linolenic acid, is recognized as an essential nutrient, but its role in nutrition appears to be largely carried out through its longer chain derivatives, EPA and DHA.

OUTPUTS: A recent series of epidemiological studies in healthy populations as well as controlled studies in healthy subjects indicates that EPA and DHA reduce the risk of sudden death due to heart disease, and also lower serum lipids. The conversion of these fatty acids from their precursor fatty acid is limited, suggesting that these fatty acids contribute to meeting the requirement for omega-3 fatty acids.

IMPACTS: Based on the evidence of provisional essentiality and importance in maintaining heart function, Health Canada is proposing a provisional adequate intake level for these long chain fatty acids and a biological role claim to be used in food labelling and advertising.

2.24   Heterocyclic Aromatic Amines Contribution to the Mutagenic Activity of Tobacco Smoke

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OBJECTIVES: Epidemiology data show that cigarette smoking is the leading cause of preventable death in Canada. Different cancers including lung, throat, mouth, etc. account for up to 39% of those deaths.

Carcinogenic compounds such as Heterocyclic Aromatic Amines (HAAs) which are formed during tobacco combustion are considered carcinogenic by the International Agency for Research on Cancer (IARC) and individually present strong mutagenic activity. The chemical characterisation of Heterocyclic Aromatic Amines (HAAs) in mainstream tobacco smoke was performed. Harman, Norharman, Amino-carbolines: AαC, MeAαC, and Imidazo-quinolines: IQ and MeIQ concentrations were put into relation with the tobacco smoke mutagenic activity to determine the contribution of these Heterocyclic Aromatic Amines to the Ames test signal (Bacterial Reverse Mutation Assay).

DESIGN: HAAS are known to be responsible for part of the tobacco smoke Ames test signal (Bacterial Reverse Mutation Assay). It was formerly determined that strains TA98 and TA100 (+/- S9) presented a specific sensitivity to HAAs. These strains were used to determine the mutagenic activity of five cigarette brands representing a range of different tobacco blends, including the Kentucky Reference 2R4F.

RESULTS: HAAs concentration in tobacco smoke were determined. Harman and Norharman, were present at the highest concentration in mainstream tobacco smoke at respectively 1110-4307 ng/cig and 2706-9895 ng/cig. AαC and MeAαC were present at lower levels 100-550 ng/cig and 5-50 ng/cig, respectively. IQ and MeIQ were not detected.

The tobacco smoke condensate mutagenic activity of TA98 ISO+S9 shows the highest response for all cigarette brands. Positive relationships between the chemical concentration and the mutagenic activity were demonstrated. Results were presented in terms of relationships between Heterocyclic Aromatic Amines (HAAs) mainstream smoke concentration and; other toxic tobacco emissions and tobacco smoke mutagenic activity.

CONCLUSIONS: HAAs were characterized in tobacco smoke. Potential relationships between the chemical concentration and the mutagenic activity were demonstrated and indicated that Heterocyclic Aromatic Amines contribute to the mutagenicity of tobacco smoke.

2.25   Individual and Community Effects in the Relationship Between Self-Reported Health Status, Smoking and Obesity

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OBJECTIVES: To examine the relationship between community-level characteristics and health status, smoking, and obesity across genders and age groups while controlling for individual characteristics and behavior.

DESIGN: Using a finite mixture model and the Canadian Community Health Survey (CCHS) 2001, differences in community level effects on individual health status are examined.

PRELIMINARY RESULTS (currently work in progress): Daily cigarette smoking is the single most important variable for explaining health status in younger persons, although the effect declines with age. Number of years smoked is more important for older age groups. Body mass index is negatively related and physical activity is positively related to healths status. Alcohol use amongst older age groups is associated with better health. More education, higher household income, and being employed are also all associated with better health status. Individual characteristics, as opposed to behavior, account for the largest portion (65% to 79%) of the explained variation in reported health status in all but the youngest age group. While there are correlations between community-level (i.e., health region) variables and individual health status, these relationships are not systematic across gender and age groups.

IMPACTS/OUTPUTS/CONCLUSIONS: Using sub-sample analysis, substantial age and gender differences are found across individuals when examining health. We observe different community and individual level effects, indicating a need to control for both when analyzing individual health status. Individual characteristics like income and education explain more variation in health status than health behaviors themselves, this result presents avenues for policy makers to target sub-populations but it also suggest that groups most in need may be the most difficult to influence.

2.26 The Mutagenic Hazards of Polycyclic Aromatic Hydrocarbons in Settled House Dust from Ottawa Homes

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OBJECTIVES: As a potential sink for indoor contaminants, settled house dust (SHD) may be an important source for exposure to mutagenic and carcinogenic substances such as polycyclic aromatic hydrocarbons (PAHs). The objectives of this study were to evaluate SHD samples for mutagenic activity and PAH content, to investigate relationships between mutagenicity, PAH concentration and household attributes, and to assess the excess lifetime cancer risks posed by non-dietary ingestion of PAHs in SHD during pre-school years.

DESIGN: Vacuum cleaner bags were collected from homeowners participating in a Health Canada indoor air study conducted in Ottawa, Ontario. Fifty-one organic extracts of sieved SHD were evaluated for mutagenic activity using the *Salmonella* mutagenicity assay, and analyzed for 13 targeted PAHs via GC/MS.

OUTPUTS/RESULTS: Dust samples contained 1.5-325 μg/g of total PAHs, and yielded mean mutagenic potencies between 2300 – 23 000 revertants/gram. A significant positive correlation was identified between dust mutagenicity and PAH concentration, (r^2=0.23-0.43, p<0.005) and both mutagenicity and PAH concentration were negatively related to vacuum cleaning frequency. Risk assessment calculations revealed that exposure to PAHs at levels found in most dust samples (<30 μg/g) resulted in excess cancer risks that are considered acceptable under USEPA guidelines (i.e.,1-100 x 10^{-6}). However, exposure to higher levels of PAHs found in 5 homes yielded risks that could be higher than the maximum acceptable level.

IMPACTS/OUTCOMES/CONCLUSIONS: This study, although based on a limited number of homes, indicates that SHD is a potential source of mutagenic hazard; moreover, depending on levels of PAH contamination, SHD may increase lifetime cancer risk.

2.27 Reduction of Levels of Tobacco-Specific Nitrosamines (TSNs) in Canadian Cigarettes: Where Are We?

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OBJECTIVES: To evaluate whether the levels of TSNAs in Canadian cigarettes have been reduced between 2000 and 2004 following the change in the curing process of the tobacco leaves in 2001.

METHODS: Information about the TSNAs (NNK, NNN, NAT and NAB) in tobacco and in smoke emissions was extracted from manufacturer reports submitted under the requirements of the Tobacco Reporting Regulations (TRR) requirements. Nine Canadian brands were selected for this study.

RESULTS: The results indicate that, between 2000 and 2004, there have been reductions in all TSNAs found in tobacco. The average amount of NNK declined by 67% (1357 ng/cig to 445 ng/cig). The average amount of NNN declined by 56% (914 ng/cig to 399 ng/cig). The average amount of NAT declined by 65% (1406 ng/cig to 487 ng/cig). The average amount of NAB declined by 100% (281 ng/cig to NQ ng/cig). The decrease of the amount of TSNAs in maintream smoke for the nine Canadian brands was slightly smaller than in tobacco.

CONCLUSIONS/IMPLICATIONS: Results of the efforts by the manufacturers and growers to convert the direct-fire burners into an indirect heating system involving heat exchangers during the curing process have resulted in a slow decline of the TSNAs levels in tobacco and smoke of Canadian cigarette studied. As the data still represents the early stage in the conversion of Canadian tobaccos to reduced levels of TSNAs, larger decreases can probably be expected in the future. Nevertheless, TSNAs represent only one class of carcinogens found in tobacco and tobacco smoke and the impact of their decrease on individual/population health and health services cannot be estimated at this point.

2.28 Analgesics Use in Institutionalized Alzheimer Disease (AD) Patients in Canada

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OBJECTIVES: To compare pain medication use in Alzheimer Disease (AD) patients with that of other institutionalized chronic patients and to examine reasons for any differences.

DESIGN: The study population was derived from the health-related institutional component of the National Population Health Survey (NPHS), which began in 1995 with 1790 respondents over 60. Respondents were re-interviewed in 1996-1997 (N=1106) and 1999 (N=626) including those who had moved but excluding deaths. Drug use in last two days before the interview and other health related variables were available.

RESULTS: In 1995, 72% of the institutionalized population were female, 68% over 80 years old and 34% had AD. OR calculated by logistic regression adjusted for age and sex, indicated that non-AD patients were more likely to receive analgesics (OR1.9), especially opioids and NSAIDs, and anti-anxiety/sedative drugs (OR=1.5). AD patients were more likely to receive antipsychotics (OR=2.1). Patients categorized as extremely forgetful had similar patterns of medication use but this was less true for patients categorized as not being able to think. Adjusting for social support, health status, partner, education, immigrant only affect analgesic minimally. Prevalence of chronic diseases was similar in both groups.

CONCLUSIONS: Possible explanations for differences in analgesic use for AD patients include: 1) difficulty communicating pain; 2) fewer painful conditions; and, 3) experiencing pain to a lesser degree. Communication is obviously difficult for AD patients, but the influence of the other two will also be evaluated.

2.29 Does Uranium and Thorium Concentration in Bone Change with Age?

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OBJECTIVES: Study the health effects of uranium (U) and thorium (Th) and their relationship to intake from food and drinking water. Specifically, study the age dependence of the concentrations of U and Th in human bones.

DESIGN: Radiation Protection Bureau (RPB) in Health Canada had collected over 5000 human bone samples across Canada in the 1960's and 1970's. The samples were collected for radiological monitoring since there were intensive atmospheric nuclear tests in the two decades. They were ashed and measured for some radionuclides such as ^{90}Sr. Now the ash samples turned into a unique archive. In this study, samples representing different age groups and genders from two populations (samples from Regina where U concentration in drinking water was very high and samples from Winnipeg where U concentration in drinking water was low) were selected. The ash sample was digested by microwave and analyzed by ICP-MS (inductively coupled plasma mass spectrometry) to determine the concentration of U and Th. Results were compared with published data and interpreted using ICRP (International Commission for Radiological Protection) age-specific biokinetic model.

OUTPUTS/RESULTS: Preliminary study showed that U concentration in human bone decreases with age. More samples will be analyzed to verify the observation. Th will be included in the forthcoming analyses.

IMPACTS/OUTCOMES/CONCLUSIONS: The observations from this project will provide in-depth knowledge on the intake of U and Th from food and drinking water, and their health effects. The results from this research will help check the ICRP model since they are from real-life samples.

2.30   Validation of ICP-MS Determination of Ce, Sr and Ti in Ceramic Samples

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OBJECTIVES: Ceramic materials of different composition (CeO2 and SrTiO3) are used in explosive dispersal studies to determine material aerolosization during a "dirty bomb" attack. CeO2 was used to simulate actinide oxides and natural SrTiO3 was used to simulate 90SrTiO3. The objective of this study is to validate the ICP-MS (Inductively Coupled Plasma Mass Spectrometry) measurement method for cerium (Ce), strontium (Sr) and titanium (Ti) by comparison with NAA (Neutron Activation Analysis) and ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry).

DESIGN: ICP-MS is a destructive analytical method. To validate this method we developed, one destructive method (ICP-OES) and one non-destructive method (NAA, recommended by US EPA for ceramic material analysis) were selected to compare the analytical results for the same materials. For ICP-MS and ICP-OES measurement, cerium oxide (CeO2) samples were digested with HNO3 and H2O2 (1:2), while strontium titanate (SrTiO3) samples were digested with HNO3 and HF (18:1). For NAA measurement, there is no need to digest the samples.

OUTPUTS/RESULTS: Before the inter-comparison procedure take effect, a recovery test for CeO2 and SrTiO3 was conduced to ensure the viability of the digestion procedure and the determination by ICP-MS. For the recovery test, filters spiked with 0.100 g of SrTiO3 or CeO2 were digested in the microwave oven and determined by HR-ICP-MS after dilution. Each compound was digested and analyzed in triplicate. Preliminary results show a good recovery for the elements in both compounds. Results from ICP-OES and NAA will be available in June 2005.

IMPACTS/CONCLUSIONS: ICP-MS is much more sensitive, time efficient and cost effective compared to NAA, the method recommended by US EPA. Preliminary work shows that ICP-MS method can be a good choice for the measurement of the two ceramic materials. If the inter-comparison results did not show any significant difference between the methods, ICP-MS could be used for our explosive dispersal project, which generates many samples during each explosion test.

2.31 Oral Secretions as an Alternative to Serum for the Detection of Human Antibody Responses to Infection with Verotoxin-Producing *E. coli*

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OBJECTIVES: To determine the suitability of oral fluid as a non-invasive alternative to serum for evaluation of exposure and immunity to *E. coli* O157:H7 and other verotoxin-producing *E.coli* (VTEC).

DESIGN: Paired serum and oral fluid samples were collected from 80 subjects for testing by enzyme-linked immunosorbent assay (ELISA) and Western blotting for IgG and IgA antibodies to various antigens of *E. coli* O157:H7, namely Stx1, Stx2, EspA, EspB, Intimin, Tir and O157 LPS. The correlation between qualitative and quantitative test results for each sample type will be used to assess the reliability of oral fluid as an alternative to serum.

RESULTS: ELISA and Western blotting of sera have revealed variable rates of seropositivity to most of these antigens, with the highest frequencies noted for antibodies to Tir. Oral fluid samples have been tested for total protein, total IgG and total IgA, to allow normalization of the results of testing these samples for specific antibodies to *E. coli* O157:H7 antigens, which is presently under way.

OUTCOMES/IMPACTS: The main outcome will be evidence for (or against) the use of oral fluid for measuring antibody responses to VTEC infections. If oral fluid is suitable, as we predict, then it will greatly simplify assessment of population immunity and public health significance of VTEC infections, which now relies on testing of increasingly difficult-to-obtain serum samples. It will also have diagnostic and prognostic applications in individuals.

2.32   Windsor Children's Respiratory Health Study – Quality Assurance of Exhaled Breath Condensates and Saliva Interference with Biomarkers of Health Effects Assays

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OBJECTIVES: The Windsor Children's Respiratory Health Study explores the potential association between air pollutants and respiratory health of children. It employs epidemiological design to monitor air quality, and investigate health effects endpoints that include a questionnaire on medical history, respiratory function tests, and measurement of biomarkers in exhaled breath condensates (EBCs). An early part of the study focuses on the quality assurance of EBC collection, and impacts of contaminated specimens on biomarker assays.

DESIGN: Collection of EBCs was performed by technicians and respiratory technologists trained on the use of the collection kit (Rtube™), and on specimen storage and transportation. Post-collection review was performed on the collection technique and processes. An amylase assay was used to detect saliva contaminant in EBCs. Assays for H_2O_2 (biomarker of inflammatory response) and isoprostane (biomarker of oxidative stress) were done on EBCs.

RESULTS: Four out of 77 EBC specimens were highly contaminated with saliva. This corroborated the result of a post-collection review indicating that saliva had been introduced into these specimens. All remaining specimens had saliva content of <0.3%. Assay for H_2O_2 showed that only the saliva-contaminated specimens had negative H_2O_2 levels, indicating a negative interfering effect of saliva. Assay for isoprostane indicated that saliva-contaminated specimens had false-positive values, which were consistent with the ~tenfold higher isoprostane levels in saliva than EBC.

CONCLUSIONS: Adherence to quality assurance procedures for EBC collection and the use of amylase assay to detect saliva can screen out contaminated specimens, and ensure the reliability of results obtained from biomarkers measurements.

2.33

[WITHDRAWN]

2.34 Exploratory Factor Analysis of Canadian Community Health Survey (CCHS) Data Aggregated to the Health Region Level

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OBJECTIVES: Indicators to measure communities' health .

DESIGN: Starting with over 20 key variables from the CCHS database, we aggregate the data to the health region level. We then perform factor analyses to explore and name the common factors underlying the data.

OUTPUTS/RESULT: The factor analysis allows us to reduce the dimensionality from over 20 variables to 4 underlying common factors. These are: Mobility, Chronic Conditions, Weight, and Lifestyle. They can be used as indicators to rank the health regions. In particular, they help us to graphically identify health regions with extremely high or low factor scores. The analyses show, not only what factors correlate with each other, as well it shows which do not: viz, alcohol usage is independent of cardiovascular disease.

IMPACTS/OUTCOMES/CONCLUSIONS: A better understanding of health indicators. The identification of relatively healthy and unhealthy communities. The relationship between health determinants and their resulting outcomes.

2.35   Is There a Relationship Between Age of First Use of Smoking Tobacco and Marijuana?

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OBJECTIVES: Adolescence and young adulthood are periods of life often characterised by risk behaviours, which may have detrimental health effects. Examples of such behaviours include drinking alcohol, smoking tobacco or marijuana use. It is often at these ages that decisions regarding substance use are made. The objective of the presentation is to examine a possible relationship between age of first use of smoking tobacco and using marijuana with a focus on youth aged 12 to 25.

DESIGN: Results are based on data from the Canadian Tobacco Use Monitoring Survey (CTUMS) for ages 15 to 24 and from the Youth Smoking Survey (YSS) 2002 for students in grades 7 through 9 (ages 12 to 14). The CTUMS is a general population telephone based survey of Canadians aged 15 years old and over. Questions about marijuana use have been added to the CTUMS since wave two of data collection in 2003. The YSS is a paper and pencil survey completed in the classroom by students in grades 5 through 9. Questions about marijuana were asked of youth in grades 7 through 9.

OUTPUTS/RESULTS: Data on rates of use and age of first use will be presented according to combined patterns based on use and sequence of use (tobacco first, at same age, marijuana first).

IMPACTS/OUTCOMES/CONCLUSIONS: Smoking tobacco and marijuana are two behaviours frequently engaged in by youth. Findings from this analysis may help to inform the development of smoking cessation and marijuana prevention programmes and policies in young people.

2.36 Results from the Canada Addiction Survey (CAS)

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METHODS: The CAS is a random digit dialling telephone survey of 13,909 Canadians aged 15 years old and over covering the 10 provinces (the Territories are not included).

OBJECTIVES: One objective of the survey was to determine the prevalence and frequency of alcohol and other drug use in the Canadian population aged 15 years old and over and to assess the extent of harms associated with this use.

RESULTS: Results presented will focus on prevalence of use and of related harms. The CAS identified significant increases for almost all substance categories since 1994. This was mainly dominated by, but not limited to, a two-fold increase in rates of use of cannabis, both lifetime and current use. Among current users, 8.8% reported harm associated to their alcohol use and 17.5% to their other drugs use. However, 32.7% of Canadians, representing about 4.5 million people, reported some form of harm due to someone else's drinking. This clearly positions alcohol and other drugs use as a current significant threat to the health of Canadians.

CONCLUSIONS/IMPLICATIONS: In May 2002, the Government of Canada announced the renewal of Canada's Drug Strategy with investments of $245 million over the next five years. Results from this survey confirm the importance and timeliness of this investment. This survey enhances evidence-based decision-making capability to address the harms associated with alcohol and other drugs both nationally and provincially. The CAS acts as the centerpiece for accountability and monitoring related to Canada's Drug Strategy.

2.37   Inhibition of Cytochrome P450 Isozymes by the Polychlorinated Pesticides Chlordane and Related Constituents/Metabolites

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OBJECTIVES: Residues of the polychlorinated pesticide chlordane and related compounds have been identified in Northern samples of human blood and milk, apparently carried to the Arctic on prevailing winds. The possibility exists that detoxification and breakdown of these pesticides could interfere with the metabolism and detoxification of other compounds, including alcohol or prescription drugs. In order to examine this aspect, technical chlordane, individual components cis-chlordane, trans-chlordane, cis-nonachlor, trans-nonachlor and the metabolite oxychlordane were examined for effects on various cloned human cytochrome P450 isozymes.

DESIGN: Test chemical inhibition of cytochrome P450 isozymes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP19) was assayed in a microplate format using commercially available kits. The enzyme, fluorescent substrate and necessary cofactors were added to wells containing either a known inhibitor or polychlorinated test chemical and incubated. The reaction was stopped with acetonitrile and generation of fluorescent product determined.

OUTPUTS/RESULTS: When purified, cloned isozymes were incubated with the test chemicals up to 75 g ml-1, dose dependent inhibition was observed in most cases. With the exception of oxychlordane, CYP3A4 generally exhibited a biphasic response where an initial inhibition with an IC50 of 300-700 ng ml-1 was followed by an increase in activity at higher chemical concentrations. Trans-chlordane induced a similar biphasic response with CYP2C8.

IMPACT/OUTCOMES/CONCLUSIONS: Chlordane and related polychlorinated compounds which persist in wildlife and humans have been found to inhibit the activity of several purified human cytochrome P450 isozymes. However, the levels of chemicals needed to exert these effects are several-fold higher than those detected in blood and milk samples. Therefore there is little concern that chlordane contaminants have an effect on drug metabolism in the adult population.

2.38   Evaluation of Knowledge, Attitudes, Beliefs, Behaviours and Best Practices of Immunization Among On-Reserve Nurses

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OBJECTIVES: To evaluate the knowledge, attitudes, beliefs, behaviours and best practices of the on-reserve nursing community with respect to immunization.

DESIGN: A telephone survey of on-reserve nurses was conducted over a two and a half week. Out of a total of 327 on-reserve nurses called across the country, 205 participated in the survey, which represents a response rate of 63%. The margin of error on a sample of this size is +/- 4.2%, 19 times out of 20.

OUTPUTS/RESULTS: Most (88%) on-reserve nurses believed that their level of knowledge about immunization was good, although those with two years or less experience were somewhat less confident in comparison to those with more experience.

On average, on-reserve nurses estimated the immunization coverage of two year olds in First Nations communities to be about 85%. The main challenges faced by on-reserve nurses in getting two year olds in their community immunized were: convincing parents to bring in their children for immunization; transportation issues; getting parents to comply with appointments in a timely fashion; and a lack of education and understanding in the community about vaccines. However, about two-thirds of on-reserve nurses believed that the 97% immunization coverage rate for two year olds in Canada is a realistic target for First Nations communities.

IMPACTS/OUTCOMES/CONCLUSIONS: The two actions that would be of most use to on-reserve nurses to achieve an immunization target of 97% are improving community awareness and adding nursing staff, although differences do exist from region to region.

2.39   Workplace Smoking Restrictions and Smoking Behaviour

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OBJECTIVES: To document changes in smoking behaviour which may be associated with the implementation of smoking restrictions in the workplace.

METHODS: The Canadian Tobacco Use Monitoring Survey (CTUMS) is an ongoing random digit dialing telephone survey of over 20,000 Canadians 15 years and older. Employed daily smokers were defined as current daily smokers 15 + years who worked at a job or a business at any time during the last 12 months.

RESULTS: Approximately 70% of respondents reported working in the last 12 months and, of these, 89% reported some kind of smoking restriction in the workplace with 69% identifying that their workplace was completely smoke-free (up from 40% in 1994). Complete workplace smoking restrictions varied among provinces with the highest rates reported in Ontario and Manitoba while the lowest rates were in the Prairies. Since 2001, Nova Scotia experienced the largest percent change in complete workplace restrictions.

Differences between the number of cigarettes smoked per day varied according to the level of smoking restrictions in the workplace. Fewer cigarettes were smoked per day for those working in environments with a complete ban compared to those working where there was no ban.

CONCLUSION: Workplace smoking restrictions affect the number of cigarettes smoked daily. Nevertheless, there is no indication that it has an effect on smoking prevalence.

IMPLICATIONS: Smoke-free workplaces are but one of the tools in the continuum needed to provide healthy workplaces. Effort is needed to identify supports, resources and partnerships needed to lead toward healthier workplaces.

2.40   Air Pollution and Emergency Department Visits for Migraine

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BACKGROUND: Ambient air pollutants, as many studies have indicated, can affect the number of visits at the emergency department (ED). We present findings here of an analysis of emergency department visits for migraine at a Vancouver hospital between 1999 and 2003 and ambient air pollution levels.

OBJECTIVE: To assess the association between migraine ED visits and air pollution.

DESIGN AND METHOD: This study examines the relationship between emergency department daily visits for migraine and air pollution, adjusting for temperature and relative humidity. Two statistical methods are applied: time series (GAM methodology) and case-crossover. The pollutants included are nitrogen dioxide (NO2), sulphur dioxide (SO2), carbon monoxide (CO), ozone (O3) and particulate matter with diameter less than both 10 and 2.5 microns (PM10.0 and PM2.5.)

RESULTS: Both methods show statistically significant associations between ambient air pollution and the number of migraine ED visits. The results are a positive association between ozone levels (with a lag of 7 and 8 days) and number of migraine visits. For other pollutants (with a lag of 2 days), at least one applied statistical method shows significant association. For sulphur dioxide, significant results from both methods are obtained for a lag of 3 days. Relative risk is estimated between 1.14 – 1.27 for an interquartile change in air pollution concentration.

CONCLUSION: This study demonstrated that migraine ED visits are associated with air pollution. In particular, ozone and sulphur dioxide show special characteristics in their association with migraine ED visits.

2.41   Identifying Health Region Peer Groups by Main Health Characteristics of Population

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OBJECTIVES: Compare Health Regions by health indicators and place them into "peer" groups with similar health characteristics. Describe dissimilarity of the peer groups.

METHODOLOGY: Cluster Analysis will be performed to organize Health Regions into distinct groups. Number of health indicator variables for each Health Region, such as Self-Rated Health, Activity Limitations, and Chronic Conditions will be mapped into a p-dimensional space, where p is a number of variables used to represent each observation (Health Region (HR)). An optimum number of clusters will be suggested based on visual inspection of principal components, Projection Pursuit technique, and Gap-statistics. Hierarchical clustering method will perform grouping. The vector of differences between cluster means it will be used to interpret the direction of the gap between clusters in terms of chronic conditions.

IMPACTS: After peer groups with similar health characteristics are determined, it would be possible to provide health promotion and prevention activities for each group based on differences between groups.

RESULTS: Results from this study show that Quebec and all provinces outside of Quebec form two major clusters and differ in terms of health characteristics. All Health Regions excluding Quebec cluster together. However, we can identify 23 Health Regions that are located far from the "core" of the cluster. They are grouped into four separate clusters. Preliminary results show that the main difference between Quebec and other HRs can be explained by differences in Multiple Chemical Sensitivities, Thyroid Conditions and back problems. The differences between "outlying" clusters and the "core" cluster are also explored.

2.42   Understanding Community Capacity to Respond to an Extreme Heat Event: The Case of a Montreal Simulation Exercise

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OBJECTIVE: To test the effectiveness of Montreal's heat event response plan and to assess the institutional capacity to respond to the health hazards of a heat event.

DESIGN: Simulation exercises were examined as a tool to better understand individual and community-level adaptive capacity to climate-related extreme events. In June 2005, Health Canada and Montreal Public Health conducted a one-day tabletop exercise to test Montreal's heat event response plan and assess the capacity of public health and emergency preparedness organizations to respond to an extreme heat event. Through the use of scenarios and injects, participants provided input on how their organizations responds to climate-related health emergencies, in the areas of communication, operations, and emergency response.

OUTPUTS/RESULTS: Institutional capacity of the public health sector to respond to an extreme heat event and the effectiveness of the response plan was evaluated throughout the exercise. The exercise allowed practitioners to adjust and improve their emergency plans to the possibility of an extreme heat event and it allowed Health Canada to:

- determine that exercises are a good tool to evaluate institutional capacity to adapt to climate change; and

- understand what resources are necessary to respond to an extreme climatic event.

IMPACTS/OUTCOMES/CONCLUSIONS: This exercise will provide policy makers with information about ways in which urban communities can prepare for climate change and the projected increase in frequency and severity of heat events. Results of the exercise will be integrated into Health Canada's *Canadian Climate Change and Health Vulnerability Assessment 2007*.

2.43 Assignment of Portion Sizes Relating Canada's Food Guide to Healthy Eating Food Grouping Principles to Each of the Canadian Nutrient File Foods

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OBJECTIVES: Canada's Food Guide to Healthy Eating is an accepted and well-understood tool for dietary guidance. However, dissatisfaction existed with the limitations of food group and portion size definitions. Subgroup assignments will assist health professionals to apply the principles of the Food Guide in a standardized fashion and expand the opportunity to collect and link nutrient intake data within group assignments, to the nutrient database. The objectives were to: 1) standardize data reporting within food groups particularly for nutrition assessment and monitoring purposes; and, 2) allow for the computer-assisted breakdown of diets into Food Guide servings.

DESIGN: To maintain the four CFGHE basic food groupings while expanding these groups into 4 traditional food groups into over 40 additional subgroups. This expansion allowed for the development of thresholds facilitating subgroup assignment (e.g. high fat, low fat) and portion size calculation. Common sense eating patterns also came into account in developing portion size (e.g., 1 medium apple). Another additional 8 subgroups termed "other foods" reflect foods with little or no nutrient density.

RESULTS: The 2005 Excel tables are available online describing CNF food code, food description, CFGHE subgroup assignment, and portion size. The subgroup codes easily allow for collapse to broader categories where desired. Documentation reporting the rationales, thresholds and decision-making process accompany these files.

OUTCOMES: These tables have been applied to Canada's most recent provincial nutrition survey data to assess Canadian eating patterns. Reception from health professionals accessing the tables has been very positive and the availability of a standard now allows comparison from one survey to another.

2.44   Relating Canada's Food Guide to Healthy Eating to Food Consumption and Nutrition Surveys

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OBJECTIVES: Assess the dietary adherence of Canadians to Canada's Food Guide to Healthy Eating (CFGHE) using data from provincial nutrition surveys.

DESIGN: The classification of foods and recipes into CFGHE groups and portion size assignments was developed using the guiding principles and thresholds from previous Canadian Nutrient File (CNF) work. A number of additional steps were required to extend the decisions from the CNF to the data entry system used for the provincial nutrition surveys. Reported foods from the 24-hour recalls were coded either as basic foods or recipes with ingredients. Recipes were either classified in a single food group, or recipe ingredients were classified in their respective food groups. Total daily portions of each food group were estimated for each respondent. Population usual intake distributions for portions consumed in each food group separately were derived taking the day-to-day variability into account.

RESULTS: Published results from two provincial nutrition surveys showed that minimum recommendations for: (i) *fruits and vegetables* were not met by adults nor children; (ii) *dairy products* were not met by adults or girls 9 years and older; and, (iii) *grain products* were not met by most women. About half the women did not consume enough *meat and alternatives*. Also, approximately one quarter of the energy intake came from *other foods*. Compliance to the directional statements was often problematic.

OUTCOMES: This new approach has provided valuable information on dietary compliance to CFGHE guidance and has allowed a better understanding of the nutrient intake results.

2.45 Effect of Diabetes on Abcg5/Abcg8 Expression and Tissue Incorporation of Dietary Plant Sterols and Stanols in BB Rats

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OBJECTIVES: Plant sterols/stanols (PSS) are structurally similar to cholesterol and have long been known to reduce serum total and low-density lipoprotein cholesterol levels. Although absorption of dietary PSS is low (0.02-3.5%) compared to cholesterol (35-70%), accumulation of PSS in tissues may adversely affect physiological functions. Abcg5 and abcg8 (abcg5/8) are sterol transporters that are required for secretion of cholesterol into bile. The objectives of this study were to determine the effect of diabetes on the expression of abcg5/8 transporters and tissue incorporation of dietary PSS.

DESIGN: Control BioBreeding (BB) rats and BB rats diagnosed with overt diabetes and treated with insulin implants, were fed diets containing soybean oil (control diet), soybean oil supplemented with plant sterols (5 mg/g diet) or soybean oil supplemented with plant stanols (5 mg/g diet) for four weeks. Tissue levels of PSS were measured by gas chromatography. Expression of abcg5/8 in liver was assessed by Western blot and/or quantitative PCR.

RESULTS: Diabetic rats showed increased accumulation of PSS, especially plant sterols, in the aorta and red blood cells compared to control rats when fed diets supplemented with plant sterols or stanols, respectively. Liver abcg5/8 mRNA expression was lower in diabetic versus control rats fed the control diet. Abcg8 protein was also decreased in liver of diabetic rats.

CONCLUSIONS: Lower steady-state levels of abcg5/8 in liver of diabetic BB rats may, in part, account for the increased accumulation of plant sterols in tissues of these rats. These results warrant studies to examine whether human diabetic patients would accumulate plant sterols similar to that of BB rats and the potential health effects related to increased incorporation of plant sterols, as these data may impact food safety assessments of PSS.

2.46 Hepatic Gene Expression Profiles in Mice Treated with 3,3',4,4',5-Pentachlorobiphenyl (PCB 126)

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OBJECTIVES: To examine gene expression changes in the liver of PCB126-treated mice. To investigate the correlation between blood chemistry, hepatic metabolism and gene expression profiles.

METHODS: Male B6C3F1 mice (5 mice/group) were dosed by oral gavage daily for 3 days with vehicle or 0.3, 3, 15 and 30 µg/kg/day PCB126 and sacrificed on the 4th day. Serum samples were collected to measure standard clinical chemistry. Hepatic ethoxyresorufin-O-deethylase (EROD) and pentoxyresorufin-O-deethylase (PROD) activities were measured in liver S9 fraction. Total liver RNA was isolated and hybridized to the custom oligonucleotide microarray (TOXARRAYTM), containing 1300 genes known to respond to a wide range of toxic stressors. Selected genes showing expression differences using microarrays were confirmed by real time RT-PCR.

RESULTS: PCB126 treatment resulted in a significant ($p < 0.05$) increase in liver-body weight ratios at 15 and 30 µg/kg/day. Significant treatment-related effects were found in serum cholesterol (increased by 15% at 30 µg/kg/day), indicating effect on cholesterol metabolism. EROD activity was significantly elevated at all doses, reaching a 45-fold increase at the highest dose; PROD activity was not induced by PCB126. Consistent with these results, microarray analysis showed significant dose-related induction of both Cyp1a1 and Cyp1a2, which was confirmed using real time RT-PCR. These two genes are known to respond to PCBs. In addition, other novel genes correlated with clinical chemistry endpoints were identified. Some of these genes are not previously identified as responsive to PCB126, but involved in xenobiotic metabolism, stress response and inflammation, etc.

CONCLUSIONS/IMPLICATIONS: Our results showed a strong correlation between clinical chemistry and gene expression changes induced by PCB126, suggesting that gene expression measured using microarrays may provide a sensitive and predictable method for the classification of environmental toxins. Further validation using other chemicals with known pathology is currently under investigation.

2.47   Internet Use by the Public to Find Health Information

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OBJECTIVE: The Internet gives the public access to an abundance of information, including on health. Despite the growing use of the Internet to find health information, studies have suggested that people with a higher socio-economic status are more likely to use the Internet. The objective of this study was to determine the factors influencing the choice of Canadians to use the Internet, particularly to acquire health information.

DATA AND METHODOLOGY: Statistics Canada's Survey of Household Spending, General Social Survey (Cycle 14) and Household Internet Use Survey were used. Logistical regression models were applied.

RESULTS: Preliminary analyses revealed that people in one of the four age groups between ages 25 and 64, those whose mother tongue was English, those with a high level of education or income, and those who were in poor health were significantly more likely to use the Internet to find health information. The Internet sites visited were mainly sites on diseases, symptoms and lifestyle.

CONCLUSION: The Internet is a new information and communication tool that can help provide health information, for example, information on drug side effects, and promote new government programs. Identification of the determinants of Internet use is essential for targeting the population groups likely to use this technology, in order that they may adopt a behaviour beneficial to their health.

2.48   Health Capacity, A New Perspective

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OBJECTIVES: To provide a functional definition of health; to create a didactic model for devising policies and determining forms of intervention; to aid health professionals to strategize and prioritize policy objectives via cost benefit analysis; and to prompt readers to view health in terms of capacity measures, not as a status.

DESIGN: We initially surveyed other models on this topic, and extrapolated key aspects into our conceptual framework. We then devised a theoretical framework that also parallels simple theories of physical energy where health was viewed in terms of personal/societal health capacities (resilience), and effort components. After setting up a theoretical underpinning, we then created a graphical representation of our model using self-rated health status statistics, and life expectancy measures. Lastly, we formulated a new definition of health, and a rudimentary method of conducting cost benefit analysis on policy initiatives.

OUTPUTS/RESULTS: This paper provides both a conceptual and theoretical model that outlines how one can conduct a cost-benefit analysis when implementing a program. We also devise a new definition of health based on our concept of individual and societal capacity.

IMPACTS/OUTCOMES/CONCLUSIONS: By devising a definition for health that links with a conceptual and theoretical framework, decisions implementing certain cost saving programs can be more logically constructed, where the repercussions on the general population are minimized. Equally important, our model also sets itself up nicely for future microsimulation modeling and analysis.

3.01 Occurrence of *Vibrio parahaemolyticus* on Molluscan Shellfish at Harvest in Canada: A Monitoring Program in Support of Health Policies and Regulations

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OBJECTIVES: Develop a reliable and cost-effective approach for the detection and enumeration of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in molluscan shellfish; develop a reliable database to assess the potential health hazard from coastal waters in Canada.

DESIGN: Several harvest sites from the coastal waters of British Columbia, and the Gaspé peninsula and Îles-de-la-Madeleine in Québec were sampled for molluscan shellfish from May to October (2002 to 2004). Molluscs were shucked in laboratories near the harvest sites and shipped overnight under refrigeration to the analytical laboratory in Ottawa. Biochemical (standard diagnostic kits) and molecular (Polymerase Chain Reaction) assays were used to identify the *Vibrio* isolates.

OUTPUTS/RESULTS: During the three-year period 35/59 (59%) and 9/42 (21%) of the samples from western and eastern coastal waters, respectively, yielded *V. parahaemolyticus*. Levels of *V. parahaemolyticus* ranged from 100 to 10000 CFU/g in oysters from the west coast and less than 1000 CFU/g in clams and mussels from the Gaspé region. The occurrence of *V. parahaemolyticus* was highest in mid-summer months. *V. vulnificus* was not detected in any of the samples.

IMPACTS/OUTCOMES/CONCLUSIONS: The widespread occurrence of *V. parahaemolyticus* in molluscs from Canadian coastal waters indicate that aquacultural and feral molluscs present a potential human health risk if consumed raw or lightly cooked. A sustainable monitoring program will facilitate the development of policies and guidelines pertaining to the safety of molluscan shellfish.

3.02   Methanol Exposure to Car Occupants from Windshield Washing Fluid: A Pilot Study

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We set out to determine the possibility of a widespread exposure of automobile occupants to methanol through inhalation of vapours of windshield washing fluid. After application, some of windshield washing fluid could drain through the front air intake duct(s) of a car, as this geometry is very common in modern car design.

Air samples were collected in passenger cars during simulated operating conditions and analysed for methanol via headspace Gas Chromatography Mass Spectrometry (GC/MS), Electron Impact, Selected Ion Monitoring (EI SIM). The method was linear in the 2 to 2000 ppm range. Our data indicate that owing to the common use of winter-grade windshield washing fluid in colder climates, and because of considerable quantities of methanol usually present in such fluid, exposure to substantial levels of methanol vapour is likely widespread within the general population. The peak levels found in the pilot study exceed 1000 ppm. In view of the widespread use of this product, more studies are necessary to elucidate, in depth, the concentrations of methanol vapours, which could be encountered in various weather and driving conditions as well as the concomitant contributing influences of car design. These studies are necessary to properly assess the hazards associated with use of the fluid and possible mitigation approaches, which might include substitution of methanol by less toxic formulations.

3.03 Health Impacts of Greenhouse Gas Mitigation Technologies and Measures

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OBJECTIVE: To review international and domestic research that examines the health impacts of greenhouse gas mitigation technologies and measures, and highlights implications for climate change policies and programs in Canada.

DESIGN: An international literature review on greenhouse gas mitigation technologies and measures such as ethanol, fuel cells, electric vehicles, carbon sequestration, solar energy technologies, wind power, and nuclear power, was undertaken and potential health risks and benefits were investigated.

OUTPUTS/RESULTS: The review identified some of the potential health risks and benefits of certain greenhouse gas mitigation technologies and measures. The investigation indicated that some authorities in other countries have recognized certain risks and are taking action to further understand these risks and manage them.

IMPACTS/ OUTCOMES/CONCLUSIONS: The world is responding to climate change by trying to reduce greenhouse gas emissions. The implementation of new technologies or measures (e.g., ethanol, bio-diesel, domestic emissions trading, hybrid cars, etc.) to reduce greenhouse gas emissions in Canada may pose unintended threats to human health. The 2005 Budget allocates $5 billion, over the next five years, to reduce greenhouse gas emissions. The new climate change plan for Canada, *Moving Forward on Climate Change,* calls for a framework to assess the health risks of new greenhouse gas mitigation technologies and measures. This literature review provides important information that will help inform development and implementation of this framework.

3.04   A Multiplex PCR Test for the Detection of Enterotoxin Genes in *Aeromonas* spp. Isolated from Foods

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OBJECTIVES: Development of a multiplex PCR for the specific detection of enterotoxin genes of *Aeromonas* spp.

DESIGN: Three pairs of unique primers were designed for the detection of enterotoxin genes in reference (49) and foodborne (537) *Aeromonas* spp. Isolates were identified and characterized using traditional biochemical tests. Primers AHCF1 and AHCR1 were designed for the detection of *act* gene, AHLF and AHLR for *alt* gene, AHSF and AHSR for *ast* gene and yielded amplicons of 232, 361 and 536 bp, respectively. PCR conditions were optimized using reference and 22 non-*Aeromonas* strains.

OUTPUTS/RESULTS: Multiplex PCR assay of reference strains showed that the *alt* gene was more widely distributed and occurred singly in 13/49 (26.5%) strains. The frequency of gene combinations in reference strains was 13/49 (26.5%) for *act/alt*, 2/49 (4.1%) for *alt/ast*, and 2/49 (4.1%) for *act/alt/ast*. The presence of an unexpected amplicon (approx. 150 bp) was detected in 10/13 (77%) of reference strains. Four strains belonging to HG1 yielded *ast* amplicon in combination with *alt* or *act/alt* amplicons. Multiplex PCR of foodborne isolates detected the presence of *act* and *alt* in 30/537(5.6%) and 128/537 (24.0%) of strains, respectively. The frequency of occurrence of the *act/alt* gene combination was highest and occurred in 315/537 (57.0%) food isolates. The *ast* gene was detected only in the *act/alt/ast* (0.7%) and *alt/ast* (0.9%) combinations. The most frequent toxin genes in *Aeromonas* spp. were *act* 349/537(65.0%) and *alt* 452/537(84.0%), which occurred either singly or in combination with other enterotoxin genes.

IMPACTS/OUTCOMES/CONCLUSIONS: This novel multiplex PCR can detect three enterotoxin genes of *Aeromonas* spp. in a single PCR assay. The optimization of this assay for the detection of the three genes from enrichment cultures is in progress.

3.05   Is the Price of Cancer Treatment Falling?

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OBJECTIVES: Cancer care represents one of the most costly sectors of the medical care. Rapidly rising costs from medical care pose a crisis of "sustainability" of publicly funded health care systems. This paper provides evidence to distinguish price changes from quantity and quality changes for health care costs related to the treatments of cancer.

DESIGN: We estimate a cost of living index (COL) and a service price index (SPI), for the period 1995/96 and 2002/03, combining data on cancer patients admitted to hospitals participating in the Ontario Case Costing Initiative (OCCI) with death data from the Vital Statistic Death database (VSD). We focus first on the effects over time of changing prices and technology, including pharmaceuticals unlike most studies, and second on the health gain in cancer treatment.

RESULTS: Our results for cancer are compared to recent cost analyses of Acute Myocardial infarction treatments, which suggest that, the expenditures are mostly driven by quantity/quality changes, and that there is little or no profit taken by health care sector in form of price increases. However, the prices index for pharmaceuticals show an increase over time.

CONCLUSIONS: Recognition of the cost drivers in the total medical cost is important, as policy implications would vary. If most of the increase comes from pure price changes, resources are being allocated inefficiently to the medical sector, while if it is due to changes in utilization, then a net social benefit is associated to the total expenditure increase.

3.06 Primary Cause of Death in the 22nd Century: Diseases of Comfort

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OBJECTIVE: The world has started to feel the impact of a global chronic disease epidemic, which is putting pressure on our health care systems. If uncurbed, a new generation of "diseases of comfort" (such as those chronic diseases caused by obesity and physical inactivity) will become a major public health problem in this and the next century. The objective of this study was to describe the concept, causes, and prevention and control strategies of diseases of comfort.

DESIGN: Brokered by a senior research scientist specialised in knowledge translation, a chair, a president and a past-president of three national public health associations (UK, USA and Australia) contributed their views on the subject. The study used an innovative approach to collect data from the public health officials. The chair and presidents of the three national public health associations were introduced to each other, and invited to contribute ideas on the topic of "diseases of comfort". Based on their inputs, the senior research scientist drafted the first version of the paper. Over the course of more than a year, the authors exchanged ideas. The paper underwent 7 major revisions, until a consensus was finally reached.

RESULTS: Diseases of comfort have emerged as a price of living in a modern society. It is inevitable that these diseases will become more common and more disabling if human "progress" and civilization continue toward better (more comfortable) living, without necessarily considering their effects on health. Modern technology must be combined with education, legislation, intersectoral action and community involvement to create built and social environments that encourage, and make easy, walking, physical activity and nutritious food choices, to reduce the health damaging effects of the modern society for all citizens and not only the few.

CONCLUSIONS: Public health needs to be more passionate about the health issues caused by human progress and adopt a health promotion stance, challenging the assumptions behind the notion of social "progress" that is giving rise to the burden of chronic disease.

3.07   A Ranking of Pesticides for their Potential to Enter Surface and Ground Water

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OBJECTIVES: Drinking water guidelines, environmental quality guidelines and monitoring pesticide residues in water are important for protecting and safeguarding water resources. PMRA developed a comprehensive ranking of 250 pesticides to assist priority setting of several federal departments by identifying pesticides with the greatest potential to contaminate surface and ground waters.

DESIGN: Rankings were developed using data on physical/chemical properties, environmental fate and ecotoxicological data. For surface water, we used a modified version of APPLES (A Pesticide Priority List Evaluation Scheme) rating system originally developed by Environment Canada. For groundwater, we used three models: Groundwater Ubiquity Score (GUS)(Gustafson 1989), the Leaching Potential (LP), and Leaching Index of Laskowski et al. (1982). Data were primarily derived from studies submitted by pesticide registrants.

OUTPUTS/RESULTS: 250 of 286 active ingredients with uses likely to result in releases into the environment had sufficient information to run the four ranking models. Overall rankings are presented as well as rankings for major categories (e.g., insecticides, fungicides, and herbicides) of pesticides.

IMPACTS/OUTCOMES/CONCLUSIONS: Results of the ranking are being shared with Health Canada, Environment Canada, Department of Fisheries and Oceans and Agriculture/Agri-Food Canada. HECS used our preliminary results to prioritize re-evaluation of existing pesticides drinking water guidelines. Final ranking will be used to prioritize the development of future for drinking water and water quality guidelines for the protection of aquatic life. Other departments will consider ranking results for pesticide monitoring programs and pesticide research.

3.08 *Cryptosporidium parvum.* Development of a Novel Technique for the Production of Large Quantities of Oocysts *In Vitro*

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Cryptosporidium parvum is prominent among those parasitic protozoa that are now recognized as major public health problems. While most human cases result in self-limiting diarrhea, infections can be severe or even fatal in the immunocompromised. Development of tools for the rapid and efficient detection and recovery of *C. parvum* oocysts in foods, water, and faecal samples depends heavily on access to large quantities of the oocysts from experimentally infected livestock. With the advent of newer policies to search for alternatives to the use of animals in research, attempts are now underway to employ cell cultures for producing *C. parvum* oocysts.

While *C. parvum* can grow in various sites in the host, its natural habitat is the epithelial cells of the small intestine. The main focus of this investigation is to mimic *in vitro* this natural habitat. Therefore, with the Madin-Darby canine kidney (MDCK) cell line as host, four culture conditions were tested using Eagle minimal essential medium (MEM) supplemented with vitamins.

The total number of developmental stages of the parasite showed a 3.5-fold increase in Medium 4 compared to the other three media. As far as we are aware, this is the first successful attempt at culturing *C. parvum* in such a medium. Further work is now underway to optimize this novel and promising approach for producing large quantities of the parasite's oocysts in the laboratory. Related studies currently underway include the development of a *Cryptosporidium* plaque assay to determine the viability of oocysts and their resistance to various control measures, the development of a cryopreservation/recuperation method for oocysts, and a modification of this cell culture method for use with another notable foodborne protozoan parasite, *Cyclospora cayetanensis,* which has no known animal models. This project supports Health Canada policies regarding increasing the microbiological safety of food and water, and minimizing the usage of animals in research.

3.09   Literature Review of the Effects of Cross-Linking on the Mechanical Properties, Wear Characteristics, and Biocompatibility of Ultra-high Molecular Weight Polyethylene Used in Total Joint Replacements

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OBJECTIVES: Review cross-linking effect on mechanical properties, wear characteristics, and biocompatability of UHMWPE in total joint replacements (TJR). Also, review effectiveness of current *in vitro* testing to produce clinically relevant data.

DESIGN: Detailed, scientific review of available literature pertaining to the above topics.

OUTPUTS/RESULTS: Cross-linking reduces certain mechanical properties, such as yield and tensile strength, toughness, and elongation to failure. Moderate cross-linking improves the wear performance of UHMWPE. Total volumetric wear rates, as well as linear wear rates decrease. Particles of cross-linked UHMWPE are smaller (sub-micrometer range) than those of conventional UHMWPE. Particles produced by cross-linked UHMWPE are more numerous in the biologically active range of 0.1-10 μm and are phagocytosed by macrophages; larger particles are surrounded by macrophages to form giant cells. These macrophages give off pro-inflammatory cytokines, causing inflammation and activating osteoclasts, resulting in bone resorption and subsequent loosening of the joint fixture. *In vitro*, TJR wear simulators provide clinically relevant results concerning the size, distribution, and morphology of cross-linked UHMWPE particles. Lubrication of wear simulators with bovine serum simulates best the synovial fluid found around hip and knee joints.

IMPACTS/OUTCOMES/CONCLUSIONS: Benefits of cross-linking UHMWPE include reduced wear. Risks include reduction of large strain mechanical behavior, embrittlement, and increased bioactivity of wear particles. Additional evidence is required for the clinical performance of cross-linked UHMWPE. A proper amount of cross-linking is able to reduce the risk of osteolysis and improve the lifespan of TJRs. This is important, especially for younger, more active patients.

3.10   Rapid and Sensitive Procedure for the Analysis of the Nonapeptide, ALX40-4C, in Human Plasma by Ion-Pairing Reversed-Phase HPLC

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OBJECTIVE: To develop a rapid and sensitive alternative to ELISA for the monitoring of the highly charged peptide, ALX40-4C in human plasma.

DESIGN: 1) Fractionation of plasma to obtain a concentrated fraction containing the substrate; and, 2) use of high-resolution chromatography in the presence of mobile phase additives to augment substrate retention.

RESULTS: A rapid and sensitive procedure has been developed which involves fractionation of human plasma through ultrafiltration and detection and quantitation of the substrate by ion-pairing reversed-phase high performance liquid chromatography (RP-HPLC). Plasma samples spiked with the nonapeptide were acidified and ultrafiltrated through a 10 000 MW cut-off membrane. The filtrate was recovered, concentrated and then analyzed by RP-HPLC using sodium heptanesulfonate (SHS) as ion-pairing agent with monitoring at 200 nm. The nonapeptide eluted at around 28 minutes on a C4 stationary phase using a gradient of 0.035M SHS, pH 3.0 / acetonitrile. The recovery of the drug using this procedure was between 40-45%. Usual test parameters were carried out to validate the method. The lower limit of detection was 0.1 μg/mL while the lower limit of quantitation was assessed at 0.25 μg/ml (18 ng on column). The method was linear in the range of concentration tested (0.1 – 8.0 μg/mL). The overall precision of the method was found to be very good.

CONCLUSION: This methodology represents a more specific alternative to ELISA methods and allows monitoring of ALX40-4C serum levels at therapeutic doses. The developed procedure provides further evidence of the capability of physico-chemical separation techniques to enable the separation and detection of small therapeutic peptides in a complex matrix. In addition such methods can be readily validated and incorporated in new drug submissions.

3.11 Safety Evaluation of Adenovirus as Vaccine Delivery Systems: Intranasal Administration Causes Inadvertent Infection of the Central Nervous System

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OBJECTIVES: To address the efficacy and safety issues concerning recombinant adenovirus (Adv), one of the most explored vaccine and gene therapy delivery approaches.

DESIGN: Wild-type (wt) and genetically modified adenoviruses were analyzed for biodistribution and immune responses in experimental animal models.

RESULTS: Intravenous administration of the vaccine using wild type adenovirus resulted in predominant deposition of the recombinant viruses in the liver and spleen, while subcutaneous or intradermal delivery saw antigen processing mostly in the local draining lymph nodes. Remarkably, 20-30% of wt viruses inadvertently infected the brain following intranasal administration. To minimize neurotoxicity of the recombinant viruses, chimeric adaptor proteins were used to target the recombinant viruses to dendritic cells instead of the CAR (Adv receptor) positive cells. Such modification not only resulted in a twenty-fold increase in the uptake of the viruses by the dendritic cells but also successfully abolished viral invasion of the brain. The genetic modification approach is versatile as the recombinant viruses can be selectively re-targeted to other tissues by swapping the ligands. Furthermore, interaction of the chimeric ligand with the adenoviruses is specific as the biodistribution of vaccinia virus, another commonly used vaccine and gene vectors, was not altered by the ligands. Additional data with respect to mucosal and systemic immune responses following various immunization schedules will be presented.

IMPACTS/OUTCOMES/CONCLUSIONS: Intranasal administration of recombinant live virus is the most effective way of inducing mucosal immunity against respiratory viral infection, but inadvertent infection of the brain is of great concern. Our data suggest that alteration of the viral tropism can minimize neurological adverse reaction while enhance immune response.

3.12　Health Canada's Math Table: Policy Outputs and Lessons Learned from an Intersectoral Forum for Technical Exchange

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OBJECTIVE: A forum of Health Canada specialists in mathematics, epidemiological, clinical medicine and and health policy has been formed in order to discuss the policy issues in controlling infectious disease outbreaks and nosocomial infections. We have informally named the group, The Math Table. Our objective is to prepare and offer information and theoretical models for policy-making in the management of infectious disease outbreaks.

DESIGN: The "Table" is made of policy and technical experts including epidemiologists, mathematicians, economists and physicians. It also involves end users of the models and information from groups dealing directly with policy making and emergency preparedness.

The group attempts to model outbreak events to address important operational and policy issues--frequently that require rapid decisions--that arise as outbreaks develop.

OUTPUTS TO DATE: Provided advice and helped to develop Policy Research Briefing Notes on SARS, nosocomial infections; technical consensus advice concerning vaccination strategies, including withdrawal of vaccination for TB.

PLANNED OUTPUT: Preparing a draft protocol to guide officials faced with challenging situations involving infectious disease.

CONCLUSION: Planners demand a toolbox of models to assist in outbreak management and a clear and accepted consensus regarding first data requirements at the beginning of outbreak development. This need is addressed by a network of experts in different fields who can interact effectively. The "Table" is continually at pains to remain coordinated and to reach out to the elements of Health Canada that will need to make these decisions if and when infectious disease outbreaks develop and the "balloon goes up" again.

We summarize the forum activities. Interested new participants from the Health portfolio are welcome.

We summarize the forum activities. Interested new participants from the Health portfolio are welcome.

3.13 The Effects of Workplace Exposure on the Prevalence of Asthma and Chronic Obstructive Pulmonary Disease (COPD) in Canada

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OBJECTIVE: The aim of this study is to identify the factors encountered in the workplace that can affect the prevalence of COPD and asthma in Canada. This is a first step in providing the evidence that government needs to decide on interventions to reduce the prevalence of occupational asthma and COPD.

DESIGN: A review of the literature from Canada and other countries was conducted. Main occupational factors were identified, and their applicability to the Canadian situation was examined by assessing whether or not these factors were likely to be present in the Canadian work environment.

RESULTS: A few Canadian studies of the links between asthma and workplace exposures were reviewed, while no equivalent for COPD could be found. However, results from other countries' studies and international studies were applicable to the Canadian situation. For example, Canadian workers in restaurants and bars are still exposed to tobacco smoke in some provinces. In the case of occupational COPD, mineral or biological dusts, some gases and fumes, as well as tobacco smoke are the main risk factors. For asthma, the main risk factors are: chemicals, isocyanates, wood dust, laboratory animal gases, other dusts, as well as gases and fumes. For COPD, there is a significant dose-response relationship.

CONCLUSION: Better understanding of factors causing occupational asthma and COPD provides a basis for actions to create safer working environments and reduce the prevalence of these diseases. Potential interventions include: reductions of exposures to specific factors and complete removal from exposure of workers who developed immunological asthma.

3.14 Advertising, Physician Prescribing and Drug Pricing with Varying Patient Compliance

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OBJECTIVE: This paper provides an economic analysis of the relationships between drug advertising aimed at doctors, physician prescribing practices and drug pricing, and the impact of drug advertising on patients' access to appropriate products and on social welfare.

DESIGN: In a two-stage theoretical model, two firms produce different drugs to treat the same condition. Reasonable assumptions are made on the behaviours of the firms, physicians and patients. First, drug advertising influences doctors' perception of the therapeutic properties and thus their prescribing. Patients are assumed to have a positive probability of non-compliance to their doctors' prescription, which decreases with their severity of illness. Firms take physician prescribing and patient non-compliance into account, and maximize their profit by making strategic choices over their levels of drug advertising in the first stage, and their drug prices in the second stage.

RESULTS: Theoretical results show that the price of the product that matches better with severe conditions increases with both firms' level of advertising, while the price of the other drug decreases. In addition, the firm that produces this drug chooses to spend less on advertising than the other firm. Calculation of the consumer surplus shows that advertising may be welfare-decreasing in some cases.

CONCLUSION: In such cases, adopting appropriate limiting regulation regarding drug advertising may be welfare-improving. Results highlight the impact of drug advertising aimed at physicians on patients' access to therapeutic products that match their needs, and provide a basis for action in this area.

3.15   Is Methylmercury Exposure a Risk Factor for Cardiovascular Disease? Evidence from an Animal Study

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OBJECTIVES: Emerging evidence from epidemiological studies suggests that methylmercury (MeHg) exposure may increase the risk of cardiovascular disease. However, underlying mechanisms are unknown. An animal study was thus conducted to seek evidence of possible mechanistic links between MeHg, dietary fats and the risk of cardiovascular disease.

DESIGN: Male Sprague Dawley rats were maintained on a starch-based basal diet containing lard, fish oil, soy oil, seal oil, or docosahexaenoic acid (DHA) oil for 28 days, and gavaged with 0, 1 or 3 mg MeHg/kg BW for 14 consecutive days. Blood and urine samples were collected before full necropsy, and analyzed for hematology, biochemistry, and oxidative stress related parameters. Abdominal fat was analyzed for fatty acids.

OUTPUTS/RESULTS: At 3 mg/kg BW, MeHg significantly increased neutrophil and monocyte counts in all except seal oil groups; platelet counts in fish oil group; serum cholesterol levels in all dietary groups; and systemic lipid peroxidation in fish oil, seal oil, and DHA groups. MeHg decreased levels of DHA and eicosapentaenoic acid in abdominal fat in fish oil, seal oil, and DHA groups.

IMPACTS/OUTCOMES/CONCLUSIONS: Depending on the dietary fats used, MeHg stimulated systemic inflammatory response, increased serum cholesterol and oxidative damage to lipids, and/or altered transport/metabolism of omega-3 fatty acids in rats, which are known risk factors for cardiovascular disease in humans. Additional cardiovascular system specific biomarkers are currently being analyzed to confirm these observations. Results of this study will contribute towards characterizing the beneficial effects of dietary constituents in managing health risks associated with mercury exposure.

3.16 Quantiative Measurement of Cu, Hg, Ag and Sn Released from Dental Amalgam during Brushing and Mastication
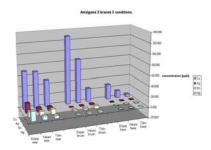
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OBJECTIVES: To measure the increase in concentrations of mercury (Hg), copper (Cu), silver (Ag) and tin (Sn) in an artificial saliva, during the corrosion of dental amalgams under simulated brushing and mastication conditions.

DESIGN: Nine amalgam samples were prepared from three different brands of amalgams. Each sample was polished to 1μ surface roughness and immersed in artificial saliva at 37°C for two hours during which the potential and corrosion current were measured using polarization resistance method. Experiments were conducted under 'no wear', brushing and sliding wear (mastication). After 2 hours of each experiment, a 50 mL aliquot of the artificial saliva was sampled for ICP-MS determination of Cu, Hg, Ag and Sn. This process was repeated in two subsequent experiments to simulate "brushing" and "sliding wear" (mastication) conditions *in vivo.* Accuracy, precision, and detection limits of the ICP-MS measurements were quantified using certified reference material (NIST 1640), procedural blanks, and spiked blanks of artificial saliva. All ICP-MS measurements were conducted in triplicate.

RESULTS: Brushing increased the release of Cu, Ag, Sn and Hg compared to "no wear" conditions. Sliding wear conditions increased ion release even further (except for Cu). Under the base and brushing conditions Ag, Sn and Hg ions were well below 10 ppb. Under sliding wear conditions ion concentrations were near or above 10 ppb, significantly above the background levels of the blanks. Copper was the most dominant ion present in the electrolyte and its concentrations ranged between 5 (base conditions) to 120 ppb (brushing conditions).

IMPACTS: Amalgam corrosion releases Mercury, Copper, Tin and Silver and may cause serious adverse effects. Quantification of ion release will permit us to carry out more accurate health hazard evaluations of dental amalgams based on the measured levels of dissolved mercury, tin, copper and silver.

3.17 Acute Exposure to Air Contaminants and Effects on the Reproductory System of Male Fisher 344 Rats: A Preliminary Analysis

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OBJECTIVE: Even though air contaminants are mainly associated with amplification of cardiorespiratory diseases, they are also implicated in male infertility. For example, reports on occupational exposure to traffic pollution and reduced semen quality; inhibition of spermatogenesis by diesel exhaust; occupational exposure to Pb, an urban air pollutant and reduced sperm production. Our objective therefore, was to expose animals to air pollutants and to investigate biological changes that are relevant to adverse reproductive effects.

DESIGN: Fisher 344 rats were exposed to either clean air, ozone (0.8 ppm) or urban air particles (EHC-93, 50 mg/m3) or a mixture of ozone (0.8 ppm) and EHC-93 (50 mg/m3), for 4 hours, by inhalation. Animals were sacrificed either immediately after exposure or 24 hours post-exposure. Broncho-alveolar lavage (BAL), plasma and sperms from both testes were collected. Endpoints measured were, alveolar macrophage counts, total lavage protein, sperm counts, and oxidative stress markers in plasma and sperms.

OUTPUT/RESULTS: Total BAL protein significantly increased (main *ozone* effect, $p < 0.001$, 3- way ANOVA) in ozone-exposed rats compared to controls. A time X ozone interaction was observed and was significant ($p < 0.001$, 3-way ANOVA). EHC exposed animals had higher total BAL protein levels compared to controls (not significant). Plasma isoprostane increased in pollutant-exposed animals compared to controls (not significant). Sperm counts were reduced in ozone-exposed animals (not significant). Tyrosines (m- + o-), oxidative hydroxylation markers were increased (main *ozone* effect, $p < 0.017$) in sperms of ozone-exposed animals.

IMPACTS/OUTCOME/CONCLUSIONS: These results demonstrate that air pollutants can elicit/potentiate biological changes such as oxidative stress reactions even in distant organs as reflected by plasma and sperm oxidative stress markers. Increased formation of m- and o-tyrosines in sperms suggest enhanced production of reactive oxygen species (ROS). Since redox-regulated signal transduction events are associated with sperm capacitation, and H2O2, one of the ROS is associated with antisteroidogenic actions in sperm, environmental pollutant-induced ROS enhancement can potentially impact on these biological pathways and therefore, on sperm quality.

3.18   A Sheep in Wolf's Clothing: Phenotypic and Genotypic Characterization of *Enterobacter* sakazakii

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OBJECTIVES: *Enterobacter sakazakii* (Es), an emerging foodborne pathogen, is linked to a number of outbreaks involving consumption of powdered infant formula (PIF). The purpose of this work is to perform comprehensive phenotypic and genotypic characterization of Es.

DESIGN: Very little research has focused on identification or molecular characterization of Es. Current methods involve observation of yellow colony pigmentation for positive alpha-glucosidase activity. In this study, antibiotic resistance, API20E/API32E, chromogenic media (DFI, ESPM), pigment/coloration/ morphology on TSA/VRBG/DFI/ESPM, ribotyping, BAX PCR, pulsed-field gel electrophoresis (PFGE), and 16s rDNA sequencing were performed on 220 environmental, clinical and food isolates.

OUTPUTS/RESULTS: To date, 16s rDNA analysis has shown 96% identity among 81 isolates tested. One of 4 BAX PCR negative strains was identified as Es by 16s rDNA sequencing, although it was not identified by API20E/32E. However, 14/81 and 1/81 strains were not correctly identified by API20E and API32E as Es, respectively. PFGE patterns of 220 strains tested showed 176 clusters, with unrelated strains having identical Pulsotypes. Automated ribotyping clustered the same 220 strains into 163 ribogroups, appearing less discriminatory than PFGE. PFGE and ribotyping successfully clustered related strains. Congruence was higher between the API32E and chromogenic (versus API20E and chromogenic) media.

IMPACTS/OUTCOMES/CONCLUSIONS: For regulatory agencies to make decisions with respect to the presence of Es in PIF and other foods, it is necessary to have in place accurate methods for confirming the presence and identity of the pathogen. Correct identification is crucial in epidemiological investigations and removal of contaminated food supply from the Canadian diet.

3.19   The Ecology of *Enterobacter sakazakii* in Human Breat Milk, Fortified Breast Milk and Powdered Infant Formula

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OBJECTIVES: *Enterobacter sakazakii* (Es) is linked to outbreaks involving debilitated neonates consuming reconstituted powdered infant formula (PIF). The only investigation of human breast milk (BM) against Es indicated positive antimicrobial activity. The growth of Es in BM, human breast milk with fortifiers (BMF) and PFI is assessed herein.

DESIGN: BM and BMF were inoculated in triplicate with clinical, environmental and food isolates and growth was monitored at 10°C, 23°C and 37°C. PIF served as a control for evaluating the divergent ability of different sources of Es to grow and survive.

OUTPUTS/RESULTS: Generation times (GT) were obtained for clinical, food and environmental isolates, respectively, in BM at 37°C (44.4, 29.4 and 46.3 minutes), 23°C (72.8, 67.9 and 88.0 minutes) and 10°C (3.3, 2.2 and 4.5 days). Interestingly, in BMF $GT_{37°C}$ (43.2, 43.9 and 40.2 minutes), and $GT_{23°C}$ (102.3, 75.4 and 100.8 minutes) indicated that human milk fortifiers, designed to increase nutrient content, may be inhibitory to Es. While Es did not survive more than six days in BMF at 10°C, it increased 2 logs in number over 18 days in BM without fortifiers. Experiments using PIF, BM or BMF individually showed no significant differences amongst sources of Es.

IMPACTS/OUTCOMES/CONCLUSIONS: To our knowledge, this is the first report demonstrating that Es has the potential to grow in human BM and BMF. While all BM and BM fortifiers were negative for Es, putative antimicrobial properties of BM do not appear to be effective against this foodborne pathogen.

3.20 Exposure to Particulate Air Pollution is associated with a Reduction in Vascular Reactivity: The Bus Stop Study

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OBJECTIVES: This study was designed to determine the effects of exposure to ambient fine particulate matter (PM2.5 and PM1.0), nitrogen dioxide (NO2) and traffic density in a Canadian urban centre on the vascular function of healthy adults.

DESIGN: Many epidemiological studies have reported that exposure to fine particulate air pollution is associated with cardiovascular morbidity and mortality, without clear biological mechanisms. To test the hypothesis of vascular effects by air pollution, we recruited 39 healthy nonsmoking volunteers, aged 18-50 years, who sat outside for two hours at a busy downtown Ottawa bus stop, and on a separate occasion, at a bus stop outside downtown with less traffic. PM2.5 mass, number of PM1.0, concentration of NO2 and traffic counts were measured. We measured flow-mediated vasodilation (FMD) of the brachial artery using an ultrasound machine, heart rate and blood pressure. Blood samples were collected to determine the serum concentration of endothelin-1, an endogenous vasoconstrictor.

RESULTS: An increase in PM2.5 was associated with a reduction in FMD. There were also changes in blood pressure associated with increased NO2 or traffic density. PM2.5 was not significantly associated with heart rate, blood pressure and serum endothelin-1.

CONCLUSIONS: Particulate air pollution may adversely influence vascular function, an effect previously only observed in a controlled human exposure study. This could be one potential pathway explaining the adverse effects of air pollution on cardiovascular morbidity and mortality. The information on biological mechanisms of air pollution-induced health effects is essential for developing scientifically sound air quality policies.

3.21 Investigation of Gene Targets for Epidemiologic and Phylogenetic Analysis of *Campylobacter jejuni*

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OBJECTIVES: To develop a simple, low cost, effective alternative for defining genetic relationships in *Campylobacter jejuni (C. jejuni)*, that will provide results comparable to those derived from complex and expensive methods such as Comparative genomic hybridization (CGH), and multilocus sequence typing (MLST).

DESIGN: CGH data on 60 *C. jejuni* isolates was generated using the Campychip Version 3.0, an amplicon-based, DNA microarray developed at the National Research Council of Canada. Based on results of these 60 strains and previous CGH studies, a subset of 120 highly divergent and highly variable genes were identified. These hypervariable genes, which include putative virulence genes, were targeted to develop four multiplex PCR assays for rapid screening and typing of *C. jejuni* strains. The same 60 *C. jejuni* isolates were characterized by multilocus sequence typing (MLST).

RESULTS: A functional set of target genes were identified from the large set of hypervariable genes and used to develop four multiplex PCRs. UPGMA (Unweighted Pair Group Method with Arithmetic Mean) analyses of the Multiplex PCR, CGH, and MLST data were compared to validate the usefulness of this method.

CONCLUSIONS/IMPACTS: This easy and accessible Multiplex PCR typing method can be used for epidemiologic and phylogenetic analysis of large strain sets of *C. jejuni*. Genomic comparisons of many strains are essential to understanding the as yet poorly understood factors that make *C. jejuni* pathogenic for humans. The application of new genomics tools for assessment of *C. jejuni* virulence and the concomitant risk to human health will strengthen surveillance, risk assessment and risk management of foodborne illness related to *campylobacter*.

3.22   The Infectivity Risk of Porcine Cytomegalovirus: Will Xetransplantation Be the Answer to Donor Shortages?

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BACKGROUND: Xenotransplantation raises concerns of potential zoonotic transfer of porcine viral agents. Accordingly, porcine cytomegalovirus (PCMV), which is endemic in pigs, warrants further investigation. CMV infection in humans, although widespread and non-pathogenic in healthy individuals, can cause severe disease under immunocompromised conditions. Although CMVs are considered species specific, this has not been shown in terms of xenotransplantation.

OBJECTIVES: The major goal of this study is to explore interaction of PCMV with human cells *in vitro*, and to evaluate how permissive these human cells are to PCMV. Here, we follow PCMV infection of human fibroblasts over time.

METHODS: Confluent human fibroblasts were incubated with either live PCMV or heat-killed PCMV (negative control), over 30 days. At time points throughout this period, CPE was evaluated and total RNA was extracted and analyzed by RT-PCR.

RESULTS: RNA extracted at 10 time points throughout a 30 day period showed a decrease in total RNA over time for cells incubated with live PCMV and an increase for cells incubated with heat-killed PCMV. This trend indicates cell death and cell growth, respectively. RT-PCR with primers specific for PCMV polymerase resulted in products that were identified on an agarose gel as the same molecular weight as PCMV polymerase. By DNA sequencing of these amplified products, samples derived from cells incubated with live PCMV were identified definitively as PCMV polymerase.

CONCLUSIONS/IMPLICATIONS: Our results confirm our demonstration of PCMV infection of human fibroblasts, *in vitro*. The infection is first identified at 9 days post-infection – approximately mimicking the progression of infection expected from infection of these same human cells with human CMV. This suggests that, *in vitro*, human CMV and PCMV have similar patterns of infection in human fibroblasts. The data have significant implications for human infection with PCMV subsequent to human xenotransplantation with porcine-derived tissues/organs.

3.23 Waking Levels of Salivary Biomarkers are Altered Following Sleep in a Lab With No Further Increase Associated with Nighttime Noise Exposure

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The goals of this study were twofold. First, we assessed if waking salivary hormone profiles are altered by nighttime noise exposure in a laboratory environment. Second, we evaluated the potential influence that sleeping in the lab *in itself* may have had on salivary biomarkers, by comparing results obtained following sleep at home.

Twelve adults (7 males, and 5 females) between 19-25 years old who slept at home and in a sleep laboratory. Subjects provided six saliva samples during waking hours on the day prior to sleep in the lab, on both days after sleeping in the lab and on the day following the resumption of sleep at home. Following one night of adaptation, subjects were exposed throughout the 2nd night to simulated backup alarms that consisted of trains of five consecutive 500 ms duration audible tones. The time between the onset of each tone was 1 s and the time between trains (offset to onset) was 15 to 20 s. When compared to home conditions, cortisol and melatonin levels were higher following sleep in the laboratory 30 minutes after awakening. However, no significant differences were noted for any salivary biomarker between the 1st and 2nd night in the sleep lab, suggesting that these endpoints were not influenced by exposure to noise on the 2nd night. Waking profiles of alpha-amylase were not influenced by where the subjects slept. Subjective reports of sleep disturbance following sleep in the lab were also obtained. For most of the day there was no apparent influence of the laboratory noise exposure. However, subjects did report more sleepiness during the evening (8 pm) following the 2nd night in the laboratory. In general, overall sleep quality was rated slightly higher upon awakening from sleep at home, compared to awakening from sleep in the lab. Factors that might have contributed to these observations are discussed.

3.24   *In Vitro* Dermal Absorption of Soil Contaminants: Bioavailability of Benzo[a]pyrene

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OBJECTIVES: To investigate percutaneous absorption of benzo[a]pyrene (B[a]P) in order to predict bioavailability of soil contaminants at hazardous waste sites.

DESIGN: The absorption of 14C-B[a]P was measured using a standard *in vitro* Bronaugh system with fresh dermatomed human skin.

Tests were conducted with triplicate skin punches from each of at least four different skin donors both with 14C-B[a]P applied alone in acetone vehicle or spiked in sieved garden soil.

The 14C-activity remaining in skin tissue after soap washing was measured to determine the skin 'depot' and the potential depot bioavailability was assessed by extended 42 hr receiver collections.

OUTPUTS/RESULTS: Surprisingly, the dermal absorption as assessed by receiver solution analyses with 14C-B[a]P applied either alone or in spiked soil was similar with 6.7% and 7.8% of the applied dose detected with and without soil, respectively in the 24 hour tests and 11.2% and 16.6%, respectively, in the extended 42 hour tests. However, the skin depot levels demonstrated a major effect of soil with much lower skin depots, observed percutaneously with soil present. Including skin depots the % absorbed was 19.9% versus 54.3% with and without soil, respectively by 42 hours. Only a small decrease in depot size was observed by 42 hours.

IMPACTS/OUTCOMES/CONCLUSIONS: The data suggest that significant absorption of B[a]P a potential carcinogen, may occur following exposure to contaminated soil even following skin cleansing with soap. Although the apparent persistence of the skin depot suggests this non-washable residue would not be bioavailable, further tests are needed to examine this issue in order to provide guidance on dermal risk assessment for contaminated sites and for health policies concerning skin cleansing/decontamination.

3.25 *In Vitro* Dermal Absorption of Chemical Warfare Simulants: First Responder Safety and Skin Decontamination

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DESIGN: The standard *in vitro* Bronaugh cell system was used to determine percutaneous absorption after a brief 30 minute exposure of fresh dermatomed human skin to three chemical warfare (CWA) simulants, Methyl Salicylate, Parathion and Malathion.

The effect of three dose levels of each of the three simulants was tested with 14C-radiochemical mixed with increasing amounts of unlabeled chemical added to provide 2, 20 and 200 mM dose concentrations.

To mimic 'worst case scenarios' skin decontamination was conducted at 30 minute post-exposure by washing skin with soapy water.

OUTPUTS/RESULTS: There was no apparent simple QSPR correlation of soap wash % recovery between test chemicals nor dose levels. As the amount of chemical washed-off skin is a factor of both the efficacy of the washing procedure and the amount no longer available due to factors such as skin storage, absorption, and evaporative loss, the data will be cross-compared and further evaluated for this conference.

IMPACTS/OUTCOMES/CONCLUSIONS: Our data impact generally on human dermal exposure to chemicals as common skin hygiene/cleansing practices involve washing skin with soaps/surfactants. Children are at particular risk of dermal contact with chemicals given their recreational preferences. It is recommended that further tests be conducted with other chemicals in order to provide a more comprehensive skin 'decon' database for QSPR modelling.

3.26 Skin Viability at Issue: An Alternative to Animal Testing Method for Assesing Dermal Absorption of Environmental Contaminants

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OBJECTIVES: To present and interpret data concerning the effect of formaldehyde fixative on human skin permeability to tritiated water as per the validation of our Automated *In vitro* Dermal Absorption (AIDA) method.

DESIGN: Our test protocol for assessing percutaneous absorption has evolved from its early origins of testing rhesus monkeys *in vivo* to its present use of fresh viable human skin specimens *in vitro*. The refinement of this alternative method to animal testing involves the importance of ensuring skin tissue viability. A series of *in vitro* tests were conducted following the standard 'Bronaugh method' to determine effects of formaldehyde fixative on the permeability of human skin to tritiated-water.

OUTPUTS/RESULTS: Tests conducted to date suggest there was no major effect of formaldehyde fixation on tritiated-water permeability. This presentation will explore the basic principles of percutaneous absorption to better evaluate the impact of present data on the need for such alternative methods.

IMPACTS/OUTCOMES/CONCLUSIONS: The OECD provides guidelines for use of viable skin in such *in vitro* tests. As the data suggest no impact of tissue viability on skin permeability and since the test permeant water is the international calibrating standard for such *in vitro* tests, it is clear that this issue needs further study.

3.27 Neurochemical Effects of Development Exposure Guideline to a Complex Mixture of Pollutants or its Major Components

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OBJECTIVES: Determine whether the neurochemical effects of developmental exposure to an environmentally-relevant complex mixture of pollutants can be predicted by exposure to its major constituents – polychlorinated biphenyls (PCBs), persistent organochlorines (OCs) and methylmercury (MeHg). The mixture profile was based upon blood levels of people living in the Canadian Arctic.

DESIGN: Pregnant rats were exposed to low and high doses (0.05 and 5 mg/kg/day) of the Arctic Mixture, or each of its major components, throughout gestation and lactation; pups were never exposed directly. The rats were dosed with the PCBs, OCs or MeHg at the same levels as in the mixture. One male and one female from each litter were sacrificed at 29 days of age. Neurotransmitters from select brain regions were examined (this work) along with a battery of developmental, behavioural, biochemical, molecular and pathological parameters (reported separately).

OUTPUTS/RESULTS: The dopamine and/or serotonin neurotransmitter systems in multiple brain regions were affected by developmental exposure to the Arctic Mixture or its components. These neurotransmitter systems play a role in muscular coordination, mood and cognition and imbalances in their levels have been implicated in disorders such as Parkinson's and Alzheimer's. The high dose of MeHg (2 mg/kg/day) had the widest range of effects but these were often attenuated by exposure to the complete mixture.

IMPACTS/OUTCOMES/CONCLUSIONS: These results indicate that effects of exposure to environmentally-relevant complex mixtures may not be accurately predicted from exposures to their components and that mixture effects of environmental contaminants should be considered for regulatory purposes.

3.28 An Animal Model for Aiding Human Exposure Guideline Development for Protection of the Fetus from the Consumption of Mercury in Fish

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OBJECTIVES: Explore use of stable mercury isotope tracers to track transplacental transfer of methylmercury to the fetal brain, in a mouse (C57BL/6) model.

DESIGN: In a pilot phase, pregnant mice were administered daily doses of methylmercury chloride containing either 'native' mercury or formulated enriched stable isotopes of mercury to provide a distinct isotopic label or 'fingerprint'. One dosing regimen also included simulated 'bolus' (spike) doses on two occasions, but the total quantity administered during pregnancy was the same.

After harvesting of tissues at term, fetal and maternal brain samples were analyzed using gas chromatography and high-resolution mass spectrometry.

OUTPUTS/RESULTS: Methylmercury was found to readily accumulate in fetal brain and maternal brain tissue. Tissue concentrations of methylmercury were substantially higher in fetal brain (1 ½ to 2 times) than in the maternal brain, which may be a function of the development process occurring as the growing fetal tissue assimilates methylmercury.

Data transformation of isotope-specific measurements allowed attribution of the quantities of methylmercury present in the tissues to the different isotopic mixtures administered during pregnancy.

IMPACTS/OUTCOMES/CONCLUSIONS: The results demonstrate that the method is capable of distinguishing between the methylmercury doses given in a 'chronic' manner and those doses administered as a bolus.

The study will explore the relationship between such tissue concentrations and observations of subtle effects on the pups as determined by a range of neurobehavioural tests, in a similarly dosed group of animals.

The outcome of this research has potential relevance to communities, particularly in the Canadian North, where consumption of country foods containing methylmercury during pregnancy might include periodic high intake of fish containing methylmercury, and where there may be limited opportunities for alternative food sources.

3.29   Development of a DNA-Based *Salmonella* Typing Microarray

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OBJECTIVES: The objective was to construct a DNA-based microarray to identify the *Salmonella* somatic (O) and flagellar (H) antigen sequences to serotype the 100 most commonly isolated serotypes from humans in Canada and the United States.

DESIGN: Established sequence information was utilized to design 20 to 26 bp oligonucleotide probes based on the unique regions of the O and H antigens. O and H targets were prepared by PCR amplification of phase 1 (*fliC*) and phase 2 (*fljB*) H antigen genes as well as targeted somatic genes within the *rfb* cluster. Signal was detected by tyramide signal amplification.

RESULTS: Two separate microarrays were developed, one to detect the somatic antigens and one for the detection of flagellar antigens. To date, thirty percent of the phase 1 H antigens (b, c, d, eh, f, g, s, l, w, i, r, z10) and 56% of the phase 2 H antigens (1,2, 1,5, 1,6, e, n, z15, z6) on the flagellar array can be detected. Thus far, serogroups A, B, C1, C2, and D can be detected using the somatic array without false positives. The specificity of the remaining probes will be verified by testing known *Salmonella* serotypes.

IMPACTS: DNA-based typing microarrays have the potential to be a faster and automated alternative to the traditional antigen-antibody-based Kauffmann-White serotyping scheme currently used for *Salmonella*. Microarrays lend themselves to automation, which would facilitate establishment of a standardized global surveillance system. Elimination of *Salmonella* from the food chain through more effective surveillance would significantly reduce the burden of human disease.

3.30   Investigation of the Relationship between Virulence and Antimicrobial Resistance in *Salmonella* Using a DNA Microarray Based Strategy

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OBJECTIVES: To investigate the putative relationship between virulence and antimicrobial resistance in *Salmonella* using a DNA microarray to assess gene expression patterns of strains exposed to antimicrobials.

DESIGN: Gene-specific microarray probes, 304 in total, were prepared by PCR, sequenced, purified, and spotted onto glass slides. Antimicrobial resistant *Salmonella* isolates in log phase growth were exposed to florfenicol (n = 4), tetracycline (n = 5) or were not treated (n =4). cDNA targets were prepared from total RNA using an indirect labelling strategy. Samples were hybridized against the common reference, *S.* Typhimurium LT2. Microarrays were scanned using the ProScanArray (Perkin Elmer) and the results were analysed using Multiple Array Viewer (TIGR).

RESULTS: Strains exposed to florfenicol or tetracycline revealed similar patterns of gene regulation. The overall comparison between antimicrobial-treated versus untreated cells revealed a highly significant up-regulation ($p < 0.00005$) in expression of approximately 2% (6/304) of genes. When the antimicrobial treatments were analyzed separately, 8 and 10% (25 and 30 out of 304) genes were up-regulated ($p > 0.05$), respectively, for tetracycline and florfenicol treatment. Significantly up-regulated genes included flagellar genes (fliC, fliD, motA, motB), LPS and outer membrane-associated genes (rfbB, ompC, ompX), virulence plasmid-associated genes (parA, mig5, traT) and a positive regulator of pathogenicity island genes (hilD).

CONCLUSIONS: These findings support the occurrence of a complex relationship between virulence and drug resistance *in vitro*. Developing an understanding of this relationship will aid the development of improved antimicrobial treatment programs and of policy for appropriate use of antibiotics.

3.31 Functional Genomic Analysis of Vaccine-Induced Protection in a Nonhuman Primate Model for HIV/AIDS

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OBJECTIVES: Despite 20 years of effort, an effective HIV vaccine remains elusive. Live attenuated viral vaccines are currently the most effective vaccines and have shown great promise in macaque models of HIV/AIDS. To identify genes regulating vaccine-induced protection, we vaccinated macaques with live, attenuated simian immunodeficiency viruses (SIVs) and challenged them with a highly pathogenic SIV/HIV hybrid virus (SHIV89.6P).

DESIGN: Three groups of four cynomolgus macaques (WT SIVmacJ5-infected, Attenuated _nef_-deficient SIVmacC8-infected or uninfected controls) were challenged via the _intravenous_ route with SHIV89.6P. Plasma associated viral load, SHIV provirus as detected by nested-set PCR and CD4+ T-cell levels were monitored in whole blood for 5-weeks after challenge. Alteration in host gene expression was analyzed using cDNA microarray and gene specific quantitative real-time PCR. Only genes found to be differentially expressed at a significance of $p<0.02$, and a fold-change of >2 (modified t-test) were used for downstream modeling.

RESULTS: Acute infection of the unvaccinated control macaques with SHIV89.6P induced viraemia, a rapid loss of CD4+ T cells and expression of 16 type I interferon-stimulated genes (ISGs) and downregulation of several molecules regulating innate immunity, including CD14, toll-like receptor 4 and several interleukin 1 family members. Macaques previously infected (vaccinated) with SIVmacJ5 or the attenuated SIVmacC8 maintained stable CD4 T-cell levels and SHIV was not detected in 4/4 SIVmacJ5 infected nor 2/4 SIVmacC8 infected macaques. Interestingly, increases in ISG expression were not observed in the vaccinated animals.

CONCLUSIONS: Though we did not identify gene expression changes that correlated to vaccine-induced protection, the increased expression of type I ISGs, which occurred in naïve SHIV89.6P challenged macaques, were absent in the protected macaques. The implication of these differences will be discussed in the presentation.

3.32 Transcriptional Responses of J774A.1 Macrphage During Phagocytosis and Infection by *Bacillus cereus* Group Organisms

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OBJECTIVES: Our focus is on developing genomic-based methods and data to establish a baseline for toxicity/pathogencity testing of *Bacillus* biotech-organisms covered by *Canadian Environmental Protection Act* (Domestic Substance List) and *Pest Control Products Act*. Here we studied the dose-dependent effects of *B.cereus* (Bc) group members (Bc, Bt.israelensis and Bt.kurstaki) on mouse macrophage in order to clarify the timing of events (toxicity or clearance) after spore phagocytosis.

DESIGN: Comparative mapping of Bc, Bti and Btk was done using custom oligo-arrays. Mouse macrophage cells were exposed to spores and the resulting expression of 364 automimmune/inflammatory response genes was monitored during phagocytosis and infection by Bc, Bti, Btk (up to 3 h) by using cDNA microarrays. Controls consisted of unexposed J774A.1 tested with/without gentamicin and also J774A.1 exposed to Bs or 1.0 µm latex spheres.

OUTPUT/RESULTS: Comparative analysis of macrophage gene expression data for each exposure allowed identification of gene groups that featured similar expression responses to bacterial spores, irrespective of type, and not latex spheres. Expression patterns of some genes were verified by RT-PCR. The most significant gene groups included members involved in cellular homeostasis, chemokines, receptors and pro-inflammatory response.

IMPACTS/OUTCOMES/CONCLUSIONS: This analysis is consistent with the events of bacterial exposure effects, as monitored using metabolic (bio-reduction) activity assays and fluorescent microscopy. The similarity of expression of certain genes in Bs and Bc exposures indicate a common early gene response to spores during their uptake, but late Bc cytotoxic effects are so rapid that host gene expression changes are not well detected.

3.33   Measurements of Radioxenon in Yellowknife as a Possible Tracer of Pollution of the Arctic

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OBJECTIVES: Pollutant transport to the Arctic is studied via radioxenon monitoring. Radioxenon in the Arctic could indicate air transported from industrialized regions, since radioxenon results from weapons testing, reactor operations, nuclear fuel reprocessing, and medical isotope production. These activities do not occur at Yellowknife. Pollution transport knowledge increases the understanding of emerging threats to public health.

METHODS: Health Canada operates a radioxenon monitoring station at Yellowknife in support of the Comprehensive Nuclear-Test-Ban Treaty and the Canadian Federal Nuclear Emergency Plan. 131mXe and 133Xe have been observed in Yellowknife. 133Xe was observed each day from March 17-26, 2005.

Environment Canada Meteorological models were employed to trace the radioxenon's origin.

At the Yellowknife site, Health Canada also operates a particulate air sampler station for radioactive aerosols. Samples corresponding to the radioxenon observation were measured in a High Resolution Inductively Coupled Plasma Mass Spectrometer (HR-ICP-MS) for 113Cd and in a semi-quantitative measurement of the full spectrum of elements.

RESULTS: Atmospheric modelling shows that the radioxenon in Yellowknife is consistent with air originating from North American reactor areas. Preliminary analysis of the 113Cd measured on the particulate filters shows that Cd tended to track with radioxenon during the March 22 – 26 episode. Analysis of the stable elements by ICP-MS is continuing.

CONCLUSION: The relatively large amount of radioxenon observed in Yellowknife tends to come from the reactor belt in the North Eastern United States according to Environment Canada's atmospheric transport and dispersion models.

IMPLICATIONS: Improved understanding of Arctic pollution pathways.

3.34 Effects of Electromagnetic Fields on Medical Devices Implications for Setting Hospital Policies

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OBJECTIVES: Determine whether electromagnetic fields generated from wireless communication systems can interfere with medical devices and how the results affect the current hospital policies.

DESIGN: Seven wireless communication technology systems (five cellular phones and two Blackberries), two unlicenced two-way General Mobile Radio and Family Radio Services (GMRS/FRS) systems, and one wireless local area network (WLAN) system was used to test 41 medical devices from 13 device classes. These tests were carried out, using the ANSI C63.18 test method, to determine whether any medical device malfunctions were observed when a communication system was placed at a given distance during testing.

OUTPUTS/RESULTS: Cellular phones and Blackberry digital communication systems affected the operations of six medical devices at close distances (< 10 cm). GMRS/FRS systems caused 10 medical devices to malfunction at distances from 5 to 80 cm. WLAN system did not cause any interference. The degree of malfunctions varied from false output readings, device manual reset to the cease of operation. These malfunctions might lead to medical intervention and were considered significant. Results from these tests reveal that these electromagnetic fields can interfere with medical devices at close distances.

IMPACTS/OUTCOMES/CONCLUSIONS: These wireless systems can be essential in hospitals for good patient management. However, the electromagnetic interference on medical devices has raised concerns when these systems are brought into hospitals by patients, visitors and staff in an uncontrolled manner. Many hospitals have total ban policies on the use of these wireless systems. Based on the risk analysis, results of this study support that hospital policies need to be revised to allow the safe use of wireless communication devices in hospitals, such as a 1m and 3 m rules for the use of cellular phones and Blackberries, and two-way systems respectively. Wireless LAN systems with power < 100 mW present minimum risk and are allowed to be used in hospitals.

3.35   Non-Invasive Thermal Imaging Screening for Detection of SARS

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OBJECTIVES: To assess the non-invasive thermal imaging screening of airport passengers in the detection of SARS patients in Canadian airports.

DESIGN: Four models of non-invasive infrared thermographic screening camera systems were assessed based on their performance characteristics in obtaining high sensitivity rather than high specificity. Three of them that met these criteria were recommended to screen airport passengers. As a pilot project, six systems were set up to monitor inbound passengers, while another six for outbound passengers at Toronto and Vancouver airports, respectively (Report of the National Advisory Committee on SARS and Public Health, October 2003). Passengers with forehead temperatures greater than 38.2°C were referred to for further assessment by nurses or quarantine officers from Health Canada, stationed at these airports. A statistical analysis of the temperature records of these passengers was carried out.

OUTPUTS/RESULTS: During the four months, about 25 thousands inbound passengers were screened in Toronto daily, among them four passengers were further assessed, compared to about five thousands outbound passengers screened in Vancouver with only one suspected subject. A total of about 2.4 millions inbound and outbound passengers were screened by mid-September. Among them, only 832 were required for further assessment. No SARS cases were found. Results obtained from this pilot study indicate that the thermographic system can provide a cost-effective screening of passengers whose forehead temperatures exceed the set temperature.

IMPACTS/OUTCOMES/CONCLUSIONS: During the SARS outbreak in May 2003, the World Health Organization (WHO) suggested that the use of thermal scanners be considered. Health Canada responded to the suggestion and installed a total of twelve thermographic screening systems at two Canadian airports. There were no SARS cases found from May to September 2003, using this screening method.

3.36 Murine Intra-Nasal Exposures to Commercial and Domestic Substances List *Bacillus* Strains

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OBJECTIVES: According to the *Canadian Environmental Protection Act* (CEPA 1999), animate products of biotechnology must be assessed for harmful effects prior to import, or manufacture in Canada. Towards this requirement, we developed a multi-indicator *in vivo* approach using commercially-produced and Domestic Substances List (DSL) bacteria from the *Bacillus* genus as models to scan for potentially harmful effects. This abstract describes the testing regime at the screening level for these bacteria.

DESIGN: Spores were derived from sporulation-phase cultures of four DSL bacteria (American Type Culture Collection), or by dilution of two commercial bioproducts, and delivered by intra-nasal instillation to BALB/c mice. After exposure (2 hours, 24 hours, 48 hours, 4 days and 1 week), several indicators were evaluated: bacterial clearance/persistence; tissue architecture, leukocyte infiltration and cytokines (expression arrays); serum levels of acute phase response markers, inflammatory cytokines and immunoglobulin isotypes; and haematology.

RESULTS: Several quality assurance procedures (e.g., use of fluorescent tags and confocal imagery) were developed to ensure authenticity and purity of spores, and efficiency of dose delivered to lung and other tissues. Overall, pulmonary deposition ranged from 0.1 to 1% of the applied dose (10^6 bacteria/mouse). Severity of response varied from no observed tissue or serum changes, and clearance within one week, to shock-like symptoms such as behavioural modifications, pulmonary neutrophil infiltration and elevated circulatory interleukin-6, as observed in 2 hour exposures involving spores and vegetative cells.

CONCLUSION: The dosing strategies and multi-indicator assays described here were effective in discriminating *Bacillus* spp for potential short-term adverse effects. This testing regime enables work on other bacterial genera included in the DSL, and also studies on potential chronic adverse effects.

3.37   The Use of Beryllium Isotope Ratios to Assess Climate Change Effects in the Far North

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OBJECTIVE: Health Canada operates a network of air samplers throughout Canada for emergency response purposes. This network can detect airborne radionuclides at minute concentrations. Our objective was to determine whether the measured ratios of beryllium-10 (Be-10) to beryllium-7 (Be-7) could be used as tracers of atmospheric circulation.

DESIGN: The naturally-occurring radionuclides Be-10 (half life = 1.6 million years) and Be-7 (half life = 53 days) are produced by cosmic ray bombardment of air molecules at high altitudes. Through atmospheric exchanges and circulation these radionuclides eventually reach ground level. Because the shorter-lived Be-7 decays away, the ratio Be-10/Be-7 should indicate the elapsed time since the air left the upper atmosphere. Be-7 is routinely measured by gamma ray spectrometry on daily air filters from the network. The measurement of Be=10 requires the specialized technique of accelerator mass spectrometry, which was developed in collaboration with IsoTrace Laboratory. The Canadian Meteorological Centre supported this project with atmospheric transport calculations.

RESULTS: The Be-10/Be-7 atom ratios were found to vary from about 0.5 to 3. Beryllium levels in Ottawa were generally found to be higher than at the more northerly location of Yellowknife, NWT. On at least one occasion there was clear evidence of a fresh intrusion of air from the stratosphere at Yellowknife.

CONCLUSIONS/IMPLICATIONS: The Be-10/Be-7 ratio is a valid indicator of air circulation. This tool will aid in understanding climate change effects and ozone depletion. The latter leads to increased in UV radiation which can cause skin cancer.

Index by Author and Abstract Number

H

Hall, K., 1.36, 1.42
Hassan, N., 1.32
Hatt, S., 1.17, 1.28
Hatt, S.R., 1.08
Hayward, S., 1.18, 2.23
He, R., 3.11
Healy, C., 1.19, 1.31
Hébert, K., 3.18, 3.19
Hefford, M., 3.11

Hickey, R., 2.38
Hierlihy, A., 1.49
Ho, S.C., 1.07
Hofmann, L., 3.04
Holtslander, B., 2.31
Hosiawa, K.A., 3.31
Houston, B., 1.20
Huang, D., 3.11
Huang, W., 1.39
Hunter, D.J., 3.06

I

Inskip, M.J., 3.28

Iskandar, M., 2.22

J

Jacobson, Z., 1.20, 2.34, 3.12
Jaentschke, B., 3.11
Jaros-Denis, A., 3.13, 3.14
Jean, M., 3.33, 3.37
Jin, D., 1.24
Jin, X., 1.25, 3.15

Johnson, R., 2.31, 3.21
Johnston, D., 3.36
Johnston, J., 1.44, 2.23
Joncas, J., 3.24
Junkins, B., 1.44, 2.43, 2.44

K

Kaiserman, M.J., 2.24, 2.27
Kapal, K., 1.24, 1.25, 3.15
Karov, J., 3.09, 3.16
Karthikeyan, K., 1.29
Keen, P., 1.36, 1.42
Keith, S., 3.23
Kelvin, D.J., 3.31
Kennedy, I., 1.02
Kiser, S., 2.09, 2.10, 2.30

Kittle, K., 3.36
Konkle, A., 3.23
Korokhov, N., 3.11
Kou, Y., 3.18
Kovalchuk, O., 1.01
Krantis, A., 1.16
Kulka, R., 3.20
Kulkarni, S., 1.22
Kumarathasan, P., 2.20, 3.17

L

L'Abbé, M.R., 1.39, 2.11, 2.15, 2.22, 2.45
Laffey, P., 2.44
Lambert, I.B., 1.23, 1.40
Langlois, I., 2.37
Lanouette, M., 1.32, 1.33, 3.16

Lanthier, R., 1.28
Larivière, D., 2.08, 2.09, 2.10, 2.29, 2.30, 3.33
Larocque, L., 3.11
Lau, B.P.-Y., 1.38, 3.28
Lee, A., 2.12
Lee, N., 2.23

P

Packer, A.P., 2.09, 2.10, 2.29, 2.30, 3.33
Paddock, K., 2.28
Padhi, B.K., 1.48, 1.49
Pagotto, F., 1.30, 3.18, 3.19, 3.21
Pang, T., 1.07
Pardhan, S., 2.48
Parenteau, M., 3.31
Parfett, C.L.J., 1.19, 1.26, 1.31, 2.46
Parks, D., 3.36
Parrington, L., 3.08
Pelletier, G., 1.27, 1.48, 1.49
Pelletier, L., 2.38
Pepin, L., 3.07
Pereboev, A., 3.11

Perets, A., 2.31
Perry, A., 2.38
Persad, D., 3.31
Phenauf, M., 3.17
Pilon, R., 1.26
Plante, M., 1.29, 1.30
Plouffe, L.J., 1.08, 1.34
Plouffe, L.A., 2.28
Polyzos, A., 1.01, 1.31
Poon, R., 2.32
Popovic, S., 3.36
Poppe, C., 3.30
Potter, I., 2.48
Pozzobon, S., 1.11
Puska, P., 1.07

Q

Quinn, J., 1.01

Quon, T., 2.34

R

Racine, S., 2.35, 2.36
Rahn, K., 3.21, 3.29, 3.30
Rajbhandary, S., 2.03, 2.04
Ran, L., 3.31
Rasmussen, P.E., 1.32, 1.33, 3.16
Ratnayake, W.M.N., 1.08, 1.34, 1.35, 2.23, 2.45
Richardson, M., 1.33, 3.24
Robertson, P., 1.38

Rocheleau, H., 2.45
Rodney, W., 2.38
Rondeau, I., 2.43, 2.44
Rondeau, I.M., 2.11
Ross, W.H., 1.37
Rowan-Carroll, A., 1.01
Rud, E.W., 3.31
Ruddy, T.D., 3.20

S

Sainsbury, P., 1.07, 3.06
Salvadori, 2.31
Sara, A., 3.17
Saravanamuthu, A., 2.20
Sattar. S., 3.08
Schrader, T., 2.37
Scoggan, K.A., 2.45
Scott, G., 2.38
Scott, I., 1.16
Seligy, V.L., 3.32, 3.36
Sepehr, E., 1.38
Shang, D., 1.36, 1.42

Sherman, G., 1.07
Shwed, P., 3.32
Sinclair, S.E., 2.13
Singer, T.M., 1.40
Sit, D., 1.36, 1.42
Skinner, H., 1.07
Smith, D., 1.17, 1.28
Snider, J., 2.39
Somers, C., 1.01
Song, D., 3.12, 3.35
Soto, C., 3.37
Sparling, M., 3.28