Book of Abstracts

2011 Health Canada Science Forum

Integrating Information and Communication Technology (ICT) in Science, Research and Health Care Delivery

Foreword

Book of Abstracts

Integrating Information and Communication Technology (ICT) in Science, Research and Health Care Delivery

2011 Health Canada Science Forum
November 7-8, 2011

I am pleased to present the Book of Abstracts for the 2011 Science Forum. The annual Science Forum is a key milestone on the department's annual calendar and this year marks its 10th anniversary.

As in previous years, the Abstract Review Committee worked diligently to review 170+ abstracts submitted by employees under the Forum's theme of *Integrating Information and Communication Technology (ICT) in Science, Research and Health Care Delivery*. Committee members also played a key role in selecting the 30 abstracts featured in concurrent sessions. The Review Committee assessed the quality and relevance of the abstracts and ultimately chose 168 for presentation at the Forum. I wish to personally thank all authors who submitted abstracts.

Individuals wishing to reference or quote from these abstracts in whole or part should obtain permission from the author(s).

I would like to thank the members of the Abstract Review Committee – and its Chair, Dr. Azam Tayabali - for their significant help in ensuring that the Forum reflects the achievements of our scientific community.

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

FNIHB: First Nations and Inuit Health Branch
HECSB: Healthy Environments and Consumer Safety Branch
HPFB: Health Products and Food Branch
SPB: Strategic Policy Branch
RPB: Regions and Programs Branch
PACCB: Public Affairs, Consultation and Communications Branch
CSB: Corporate Services Branch

Other Acronyms:

PMRA: Pest Management Regulatory Agency
PHAC: Public Health Agency of Canada
CIHR: Canadian Institutes of Health Research

1.01 Mechanisms of Chemically Induced Obesity, Focussing on Dexamethasone and BPA

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SUMMARY: The rate of obesity and associated diseases is rising rapidly in Canada. While lifestyle factors (diet, lack of exercise, etc.) clearly contribute to this condition there is growing evidence that stress and pollution may also play a role. We have developed a cell culture assay that could be used to test chemicals to see if they can increase the formation of fat cells in exposed individuals. We used this assay to show that the common chemical bisphenol A can enhance fat cell formation. This assay will help Health Canada reduce exposure of Canadians to endocrine disrupting substances.

OBJECTIVES/BACKGROUND/ISSUES: The factors causing the obesity epidemic in North America are poorly understood. Excess caloric intake and lack of exercise are clearly important determinants. However, accumulating evidence suggests that stress and chemical toxicity may play a role. We investigated early gene markers and interaction between stress hormones and bisphenol A on fat cell formation. We investigated the early molecular mechanisms that mediate the effects of adipogenic factors (e.g., stress hormone, studied using dexamethasone; DEX) on adipogenesis to develop a screening assay for potential adipogenic chemicals.

DESIGN/METHODS/DESCRIPTION: We used 3T3 L1 preadipocytes for these studies. We established a serum-free model and identified the lowest dose of DEX necessary to induce differentiation. RNA was extracted at early time points following the initiation of differentiation. Fat cell formation under these conditions was confirmed in parallel incubations by examining fat accumulation and the expression of marker proteins (aP2 and adipsin). The ability of chemicals to act as potential obesogens was also examined using BPA as the model chemical. Cells were treated with BPA in the absence or presence of a low dose of dexamethasone.

OUTPUT/RESULTS: Several genes possibly responsible for the initiation of adipogenesis were identified and confirmed using RT-PCR. Low concentrations of BPA synergised with low doses of DEX in inducing differentiation. Co-treatment with BPA caused a significant increase on transcriptional activation of the glucocorticoid receptor (GR) over that of DEX alone.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: These studies have identified several target genes that could be early markers of differentiation. In addition these studies reinforce the notion that chemicals such as BPA should be investigated along with other environmental factors and in an appropriate hormonal environment to understand the full potential hazard. As fat cells contribute to metabolic syndrome our results support the hypothesis that chemicals may contribute to the obesity epidemic and the associated health risks.

1.02 Effect of Fermentation Rate of Dietary Fibre on Short-Term Food Intake and Gut Hormone Response in Male Rats

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SUMMARY: As obesity rates have increased worldwide, potential weight management tools have gained much attention. One such tool may be increased consumption of dietary fibre, which has been implicated in weight maintenance. Possible mechanisms include increased satiety and reduced food intake through influence on gut hormone levels. This study aims to measure the effect of two types of dietary fibre varying in fermentation rate on short-term food intake and gut hormone responses. Findings from the study would help in the evaluation of the scientific basis for foods with satiety health claims.

BACKGROUND/OBJECTIVE: Nutritional strategies that increase satiety and reduce food intake could be a potential tool for weight management. High consumption of dietary fibre is linked to gut hormone levels that play a role in food intake. However, the effect of dietary fibre appears to depend on both its extent and rate of fermentation in the large intestine. This study investigates the effect of fermentable material on short-term food intake with emphasis on the association between fermentation rate and gut hormones.

DESIGN/METHOD/DESCRIPTION: Male Fisher 344 rats (7 wks old) will be fed energy-matched diets containing either cellulose (control), 10% wheat bran (WB) or 5% fructooligosaccharides (FOS) for 3 weeks before the onset of testing. WB and FOS have the same total amount of fermentable material but differ in fermentation rates. After the adaptation period, rats will be given four nutrient preloads (glucose, whey protein, canola oil, and water) on different days by gavage. Short-term food intake (2 h), gut hormone response (GLP-1, PYY, ghrelin) at 30 minutes, and total daily food intake will be measured. Following an overnight fast, animals will be euthanized 30 minutes after receiving either glucose or water preloads, Blood and tissues (gastrointestinal tract and hypothalamus) will be collected for further analyses.

OUTPUT: Measurements of food intake, body weight and composition, and gut hormones will be performed during the summer and early Fall. We expect that rats fed the FOS diet will have a greater suppression of food intake after the nutrient preloads and that this effect is linked to gut hormone levels.

NEXT STEPS/IMPLICATIONS: Future studies might explore the direct involvement of gut hormones in food intake response through the administration of hormone receptor antagonists. Findings from the study would help in the evaluation of the scientific basis for foods with satiety health claims.

1.03 Glycidol Fatty Acid Esters in Foods: Analytical Method Development

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SUMMARY: Recent studies have identified the presence of glycidol (bound in form fatty acid esters) in many refined fats and oils and also products containing fats and oils, such as baby formulas. Glycidol fatty acid esters could in theory be hydrolysed to the parent glycidol in the gastrointestinal tract. Glycidol is an epoxide with a Group 2A designation by IARC - probably carcinogenic to humans.

OBJECTIVES/BACKGROUND/ISSUE(S): Glycidol fatty acid esters in processed oils were detected at levels up to 20 ppm and, in diglycerides rich oil, reached 250 ppm (Japan and EU data). Glycidol esters are forming during processing/refining of commercials oils. Glycidol is a direct acting mutagen and multisite carcinogen in rodents, but no epidemiological or clinical studies on glycidol have been reported for humans. There is no data on levels of glycidol esters in Canada. Current GC-MS methods for determination of bound glycidol require derivatization and are prone to artefact formation while current LC-MS methods suffer from the lack of robustness.

DESIGN/METHOD/DESCRIPTION: A new method based on LC-MS/MS was developed. The method incorporates isotope dilution method for quantifying the five target analytes: glycidol esters of palmitic, stearic, oleic, linoleic and linolenic acid. For analysis, 10 mg oil samples are spiked with deuterated analogs of glycidol esters and purified by a two-step chromatography on C18 and normal silica. A dried extract is redissolved in 250 µL of solvent and 15 µL is injected on a C18 column that is eluted in methanol. Detection of target glycidol fatty acid esters is by Multiple Reaction Monitoring in an APCI mode with 2 ion transitions for each analyte.

OUTPUTS/RESULTS: The methodology was tested on replicates of virgin olive oil, which was free of glycidol esters and the method detection limit is 0.125 ppm for each analyte.

The major advantage of our method is that spurious peaks present in LC-MS chromatograms are absent from MS/MS chromatograms.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The method will be applied to the survey of glycidol fatty acid esters in foods on the Canadian market. The method might be further refined by pre-concentration step, which would remove most of triglycerides and diglycerides from the sample.

1.04 Exposure of Male Gametes to Dietary Folic Acid Alters Folate Status of the Offspring

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SUMMARY: Since folic acid (FA) fortification of white flour was mandated in 1998, neural tube defect incidence has been reduced by 46%. However, a significant proportion of the general population have a folate status indicative of high FA intake. We aimed to determine the effect of FA deficiency and supplementation on the folate status of non-target populations, namely males and their offspring. We found that male offspring of male mice exposed to FA deficiency *in utero* or from weaning demonstrated increased folate in tissues, despite being fed a control diet, indicating that male gametes are sensitive to metabolic programming by FA.

OBJECTIVE: To examine the effect of folic acid deficiency and supplementation in pregnancy or post-weaning on folate metabolism and liver gene expression in male offspring.

METHOD: Inbred male BALB/c mice (F1) from dams fed a FA deficient (0 mg/kg) or supplemented (6 mg/kg) diet, were weaned on postnatal day 21 to a FA sufficient (2 mg/kg) diet. Inbred male BALB/c mice from dams fed a FA sufficient diet were weaned on postnatal day 21 to a FA deficient, sufficient or supplemented diet. F1 male mice were maintained on diet for 14 weeks, bred with one female and necropsied 3 weeks later. Male pups (F2) were weaned on postnatal day 21 to the sufficient diet, bred with one female at 8 weeks of age and necropsied. Male pups (F3) were weaned on postnatal day 21 to the sufficient diet and necropsied at 8 weeks of age. Blood, colon and liver tissue were collected. Q-PCR for Folr1, Slc19a1 and PCFT was performed. Tissue folate concentration was determined by microbiological assay.

RESULTS: Liver and RBC folate concentrations were increased by 10-15% and 15-20%, respectively in F2/F3 mice derived from F1 males exposed to deficiency *in utero* or at weaning compared to mice exposed to the sufficient diet *in utero* or at weaning. There was no effect of supplementation. Q-PCR results showed no difference in hepatic folate transporters.

CONCLUSIONS: The data indicate that male gametes exposed to folic acid deficiency during fetal/neonatal development or post weaning are programmed to produce offspring that sequester folate. Microarray analysis will shed light on mechanisms underlying this metabolic phenotype. ~70% of Canadians had a folate deficient or marginal status before mandatory fortification indicating that children born since 1998 to previously deficient men may be programmed to sequester folate.

1.05 Zinc Supplementation above the Tolerable Upper Intake Level does not Depress Copper Status in Boys

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SUMMARY: The Tolerable Upper Intake Level (UL) is the highest daily intake level that is likely to pose no risk of adverse health effects. As intake increases above the UL, the potential risk of adverse effects may increase. Many experts believe that the current ULs for zinc (Zn) for children are set too low. In this study boys were given Zn supplements and examined for adverse effects on copper (Cu) status. Zn supplementation increased weight gain and did not decrease Cu status. These results support the need for re-evaluation of the ULs for Zn for children.

OBJECTIVES: Zn is an essential nutrient that is important for growth and proper functioning of many physiological processes. Excessive Zn intake, however, can reduce absorption of Cu and lead to Cu deficiency. Depressed Cu status was the endpoint used by the Institute of Medicine (IOM) to establish the UL for Zn for adults. Nutrition surveys indicate that a large proportion of young children have Zn intakes exceeding the ULs indicating either a problem of Zn overload in children or that the ULs are currently set too low. In this study the effects of Zn supplementation on growth (height and weight) and biomarkers of Zn and Cu status in boys were examined.

DESIGN: Healthy boys living in Guelph, Ontario aged 6 to 8 years were given a placebo (n=10) or 5 (n=10), 10 (n=9) or 15 (n=8) mg Zn per day for 4 months in a double-blind, placebo-controlled clinical trial (ISRCTN77377400). The highest Zn dose was above the current UL for this age group (i.e., 12 mg/day). Blood and urine samples were obtained at the beginning (baseline), middle and end of the supplementation period for assessment of Zn and Cu status.

RESULTS: Boys that received a Zn supplement showed a larger change in urine Zn from baseline compared to boys that received the placebo ($P<0.05$). Zn supplemented boys also showed larger weight gains. Assessment of Cu nutriture with conventional (plasma Cu, plasma ceruloplasmin activity, erythrocyte Cu/Zn superoxide dismutase activity) and a novel (erythrocyte CCS/SOD1 protein ratio) biomarker of Cu status revealed no reduction in Cu status with Zn supplementation.

IMPLICATIONS: Together these data show that Zn supplementation of 5-15 mg/day increased weight gain in 6-8 year-old boys without negative effects on Cu status. These results provide evidence in support of the need for re-examining the current ULs for Zn for children established by the IOM.

1.06 Determination of Magnesium, Calcium, Sodium and Potassium Content in Bottled Waters Sold in Ottawa, Canada

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SUMMARY: Many Canadians do not consume enough magnesium (Mg) and calcium (Ca) and possibly potassium (K). In contrast, Canadians consume too much sodium (Na). In this study we measured the content of these minerals in a comprehensive set of bottled waters sold in Ottawa, Canada. Content of these minerals varied considerably in bottled waters suggesting that choice of drinking water might affect physiological status of these minerals in vulnerable individuals.

OBJECTIVES: The Canadian Community Health Survey 2.2 (CCHS 2.2) conducted in 2004 was a national nutrition survey that determined nutrient intakes of Canadians. This study showed that many Canadians have inadequate intakes of Mg and Ca and perhaps K. In contrast, Canadians have excessive intakes of Na. Drinking water can make a significant contribution to total dietary intakes of these minerals. To get a perspective of the supply of these minerals from bottled waters their content was measured in plain waters, flavoured waters and nutrient enriched waters sold in Ottawa, Canada.

METHODS: Bottled waters were bought from major supermarkets, convenience stores and bottled water distributors from November 5-30, 2010. Mineral concentrations were measured by flame atomic absorption spectrometry (Mg, Ca and K) or flame atomic emission spectrometry (Na). The percentage of reference values for Mg, Ca, Na and K that could be fulfilled by each water source was estimated. Percent errors of reported mineral concentrations on labels for bottled waters were also determined.

RESULTS: Mg, Ca and Na concentrations varied ranging from 0 to 103, 306 and 429 mg/L, respectively. All plain waters contained little K (< 28 mg/L), while a number of flavoured and nutrient enriched waters had higher concentrations (> 250 mg/L). Estimation of the percentage of reference values that could be supplied by each water source revealed that many waters could substantially contribute to recommended intakes of these minerals if habitually consumed. Comparison of analysed mineral concentrations with reported levels on the labels for bottled waters showed close agreement for most waters (less than 20% error), but large discrepancies for some waters.

IMPLICATIONS: These data show a large assortment of bottled waters available to Canadian consumers vary in Mg, Ca, Na and K content suggesting that choice of drinking water might influence physiological status of these minerals in individuals with low or high intakes of these minerals.

1.07 Characterization of Off-Label Use of Drugs in Newborn Infants by Anatomical Class and Indications

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SUMMARY: This study examines products listed in the SickKids Formulary to identify factors related to the benefit-risk profiles of off-label drug usage in newborn infants. A drug is considered off-label when it is prescribed for use, which has not been approved by Health Canada. Eighty-nine drugs were classified by two standard systems: ATC and AHFS. The top five prevalent drug classes in each system are then compared for the frequency of spontaneous reporting of adverse drug reactions (ADRs). This study is hypothesis-generating and findings could be used to explore policy issues for the off-label use of drugs in paediatric populations.

OBJECTIVE: To characterize the off-label use of drugs in neonates.

MATERIALS AND METHODS: Three standard sources of information about medications were used: the 2009-2010 SickKids Drug Handbook and Formulary's, which listed 89 drugs known to be used off-label in neonates; Health Canada's Drug Product Database, which provide links to product monographs; and the Compendium of Pharmaceutical Specialties, which is a published extract of product monographs. These sources allow for a comparison between the off-label, regulatory-approved, and the published extract of approved information commonly used for on-label. Drugs are classified using two systems: Anatomical Therapeutic Chemical (ATC), which groups drugs by anatomical class—according to the target organ or system, and the American hospital Formulary Service (AHFS), which groups by indication—valid reason for drug prescription. For each product, the frequency of spontaneous reporting of ADRs is obtained through the Canada Vigilance Adverse Reaction Online Database.

RESULTS: The formulary provides information which can be categorized into several distinct clusters such as: stratification of risk with regards to patient health status, class effects, toxicity, efficacy, discontinuation, drug interactions, restriction of prescription, and pharmacy compounding instruction. A preliminary examination has shown that the more prevalent drug classes—more populated classes such as anti-infective, cardiovascular, and nervous system—have a higher frequency of spontaneous reporting.

CONCLUSION: A list of 89 drugs used in neonates in the off-label context has been examined and organized by two different classification systems. Qualitative information, obtained through clinical practice, show several themes related to risk issues. There seems to be a correlation between the prevalence of drug classes and increased rates of ADR reporting. Future research into off-label drug use should focus on drugs used frequently and used with inadequate supporting evidence, particularly if further concerns are raised by known safety issues, high drug cost, recent market entry, and extensive marketing.

1.08 Metabolomic and Proteomic Analyses of Biomarkers in Individuals with Pathologies Known to Increase Vulnerability to Air Pollutants

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SUMMARY: Biomarkers are indicators of changes in biological systems caused by environmental contaminants or as a result of pathology. We aim to identify profiles of biomarkers in individuals with specific pathologies to gain insight into their potential vulnerability to environmental pollutants. Our analyses reveal that a number of biomarkers related to oxidative stress and inflammation are elevated in asthmatic and hypertensive individuals. These health conditions are known to increase risk from adverse effects of air pollutants. This work will help understand better the interaction of pollutants and disease processes in vulnerable populations, an important target of risk management strategies.

BACKGROUND: Exposure to ambient air pollutants is associated with adverse health effects (e.g., respiratory and cardiovascular morbidity and mortality), notably in individuals with hypertension, asthma, diabetes and obesity. We hypothesize that disease-related perturbation in biological mechanisms could explain the vulnerability of individuals to pollutants. In this study, our objective was to compare, using metabolomic and proteomic analyses, biological markers of pathways that are known to be implicated in adverse effects of contaminants, in the serum of individuals with defined pathologies that are also known to predispose to adverse responses to pollutants.

METHOD: Serum samples from healthy subjects and subjects with defined pathologies were obtained commercially (N=6/group/gender) and analyzed for nitrative and oxidative stress and inflammatory biomarkers using HPLC-coularray and protein array technologies. The lipid oxidation marker 8-isoprostane was measured by EIA. Main effects and interactions of disease and gender on biomarker levels were analysed by two-way ANOVA.

RESULTS: The oxidatively modified metabolite m-tyrosine and the nitratively modified metabolite N-tyrosine were elevated ($p<0.05$) in serum of subjects with asthma, hypertension and diabetes, by comparison to serum of healthy individuals. Similarly, matrix metalloproteinase-1 (MMP-1), monocyte chemotactic protein-1, and vascular endothelial growth factor were significantly higher ($p<0.05$) in asthmatics by comparison to healthy subjects. MMP-1 was also elevated in subjects with hypertension ($p<0.05$), while 8-isoprostane was elevated in asthmatic subjects ($p<0.05$).

IMPACTS/CONCLUSIONS: Biomarkers of oxidative stress, nitrative stress and inflammation are elevated in subjects with diseases that are known to increase vulnerability to air pollution, for example asthma, hypertension and diabetes. Interestingly, nitrative and oxidative stress and inflammation are known mechanisms of action of air contaminants, including NO_2, O_3 and particles. The findings will be applied to understand the biological bases of vulnerability of sub-populations and should be useful for interventions.

1.09 Towards New Estimated Daily Intakes for the Canadian Population

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SUMMARY: Health Canada's Contaminated Sites Division is involved in the revision and development of new human health soil quality guidelines (HHSQGs). Part of the HHSQG development process relies on Estimated Daily Intakes (EDIs) which estimate the typical concurrent background exposure to chemicals from all known or suspected sources (air, water, soil, dust, food and consumer products) via all known or suspected routes of exposure (inhalation, ingestion, dermal contact) for the average Canadian. Instead of using a deterministic approach to derive EDIs, a probabilistic one has been developed and will be presented as well as its strengths, limitations and recommended future improvements

OBJECTIVES: Assess new estimated daily intakes for the Canadian population needed to derive soil quality guidelines for human health.

METHODS: For each chemical under HHSQG revision or update, an extensive review of all the available Canadian databases covering air, water, soil, dust and food was performed through grey and scientific literature searches. The scientific validity of all available papers, grey reports and databases was assessed using a quality score tool developed for this purpose. Then, after final selection of key data, all the environmental concentration distribution parameters and all the physiological distribution parameters involved in the EDI equations were estimated. The Crystal Ball add-in software for excel was used for the Monte-Carlo simulations and probabilistic EDI distributions were obtained for each of the five Health Canada human receptor age groups.

RESULTS: Instead of deriving deterministic EDIs, EDI multimedia probabilistic distributions are obtained through a fully transparent and systematic approach. This has already been done for nine chemicals or species (Barium, Beryllium, Cadmium, Total and Hexavalent Chromium, Lead, Nickel, Vinyl chloride, Zinc).

CONCLUSION: This is a first step to derive Canadian EDIs integrating all the pertinent information available. However, through this systematic and transparent process a lot of limitations can be identified (data gaps, methodological limitations, no available correlations between media of exposure, etc.). This allows prioritization of future research projects to improve Health Canada EDIs.

1.10 Food Consumption Habits Between the US and Canada: Implications for Estimating Exposure from Pesticides

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SUMMARY: Recent studies indicate that Canadians may eat more fresh fruit and vegetables than Americans. Health Canada is therefore comparing eating habits between the two countries to determine if a Canadian version of the US Food Commodity Intake Database (FCID), presently used by both countries to assess pesticide exposure, should be developed. The results will test the common belief that the eating habits of Canadian and US populations should generally be the same since food commodities are available everywhere and to anyone in the North American market.

BACKGROUND: Recent studies indicate that Canadians may consume more fresh fruit and vegetables than Americans. Although disappearance rates in consumption of fresh fruits and vegetables also suggest a similar difference, the statistical validity of this trend is unknown. Health Canada is therefore evaluating food consumption habits between the two countries.

METHOD: This study examines the intake of food and ingredients common to the US National Health and Nutrition Examination Survey (NHANES) and the Canadian Community Health Survey (CCHS 2.2) to identify significant differences in ready-to-eat food between the two countries. The process involves creating a common list of food definitions that are the exact equivalent in the two databases and, from these, build a set of common food categories. The consumption of each food category is then calculated for pre-defined population subgroups classified by age and gender. Methods for estimating variance are essential to test for significance and must take into account the design of the respective surveys. We will use Bootstrap methods to estimate standard errors and confidence intervals in CCHS. The US NHANES uses masked variance units for a similar purpose.

RESULTS: We will test the common notion that the eating habits of Canadian and US populations should generally be the same, since food commodities are available everywhere and to anyone in the North American market.

IMPACTS AND CONCLUSION: Results will determine if differences between US and Canadian intake warrant generating a Canadian version of the US Food Commodity Intake Database (FCID) presently used by both countries for dietary exposure assessment of pesticides.

1.11 PMF-Derived Biologic Drugs - Navigating a Path to Market in Canada

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SUMMARY: Plant Molecular Farming (PMF) is a novel drug production platform where genetically engineered plants are used to produce therapeutic proteins. While the genetically modified plant is growing, it manufactures the proteins of the inserted gene, which are later extracted from the plant.

Health Canada is drafting a Guidance document that will explain how submission requirements are different for PMF-derived biologic drugs due to the risks particular to pre-harvest and harvest stages of the plants.

Health Canada is sharing the following for consultation: (1) proposed PMF-specific features of the regulatory framework in Canada; and (2) proposed PMF-specific submission requirements.

ISSUE: Health Canada recognizes PMF as an emergent manufacturing process, where therapeutic proteins are produced in plants using recombinant DNA technology. Health Canada provides a number of Guidance documents to assist sponsors in interpreting statutes and regulations for marketing biologic drugs in Canada. Currently, there is no Guidance document specifically addressing the differences in manufacturing processes required for biologic drugs derived from plants. Currently, product development for such biologic drugs in Canada is at the Phase II clinical trial stage.

METHOD: Health Canada is drafting a Guidance document that will interpret existing drug regulations, and will apply plant-specific safety/quality concepts in the regulatory evaluation of PMF-derived biologic drugs intended for the Canadian market. Health Canada is sharing these proposed concepts for preliminary consultation.

OUTPUT: Health Canada is proposing: (1) PMF-specific features of the regulatory framework in Canada, and (2) PMF-specific submission (Common Technical Document dossier) requirements, for both the upstream and downstream manufacturing practices. The concepts are intended to ensure that an appropriate quality management system is designed and implemented to incorporate basic Good Manufacturing Practices (GMP) and guidelines (Health Canada's GMP Guidelines – 2009 Edition). Product evaluation for PMF-derived biologic drugs is expected to specifically address the risks that are unique to this novel drug production platform, particularly product design, and the pre-harvest and harvest stages. The sponsor shall demonstrate how the PMF-specific submission requirements are met, in order to fulfill the general submission requirements of safety, efficacy, and quality. The proposed request for information is for New Drug Submissions. A subset of this information would be expected for Clinical Trial Applications.

NEXT STEPS: Health Canada will take into account all comments received during preliminary consultation. Health Canada is targeting external consultation of the draft Guidance document in 2012.

1.12 Bisphenol A in Human Placenta and Fetal Liver

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SUMMARY: Although information on exposure of the general population including infants to bisphenol A (BPA) is widely available, exposure of the human fetus to BPA during gestation is still relatively unknown. In this preliminary study, human placental and fetal liver tissue samples were analysed for both free and total BPA, generating information for the first time on human fetal exposure to BPA during early to mid-gestation and transfer of BPA from human placenta to fetuses.

OBJECTIVES: To investigate the presence of BPA in human placental and fetal liver tissues to provide information on *in utero* exposure to BPA.

DESIGN/METHOD/DESCRIPTION: Human placental (n = 21, 12.3 – 20 weeks fetal age (wFA)) and fetal liver (n = 21, 11.3 – 22 wFA) samples were obtained after elective pregnancy termination during 1998-2006 in the Greater Montreal area of Quebec with informed written consent. The tissues were placed in sterile vials and flash-frozen in a dry ice/acetone bath, initially stored at McGill University (-80 °C), and then transferred in dry ice to Health Canada for processing. Ethics approval for the collection and use of the human placental and fetal tissues was obtained from McGill University and Health Canada. Human tissues were analysed for free and total BPA by gas chromatography-mass spectrometry after treatment with β-glucuronidase (for total BPA measurement), solvent and solid phase extraction and derivatization with acetic anhydride.

OUTPUT/RESULTS: BPA was detected in 86% of the 21 placental samples; concentrations of free BPA in the positive samples ranged from 0.60 ng/g to as high as 64 ng/g with an average of 9.5 ng/g and a median of 3.0 ng/g, and conjugated BPA was as high as 7.8 ng/g. BPA was also detected in most of the 21 fetal liver samples; concentrations of free BPA in the positive samples ranged from 1.3 to 27 ng/g with an average of 8.5 ng/g and a median of 3.2 ng/g. Conjugated BPA was also detected in most of the liver samples analysed for total BPA, ranging from 0.64 to 20 ng/g with an average of 3.9 ng/g and a median of 1.5 ng/g.

IMPACT/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This study clearly demonstrates the presence of BPA in human fetal liver samples, providing the first direct evidence for human fetal exposure to BPA as early as the third month of fetal life.

1.13 Occurrence of Bisphenol A in Total Diet Food Samples and Dietary Intake Estimates

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SUMMARY: Total diet study is recommended by the World Health Organization as the most cost-effective approach to assess the actual dietary intakes of both toxic and nutritionally important chemicals. In this study, total diet food composite samples were analysed for bisphenol A (BPA) for the first time and the results were used to estimate the dietary intakes of BPA for different age and sex groups of Canadian populations.

OBJECTIVES: To investigate the presence of BPA in total diet food composite samples and to estimate dietary intakes of bisphenol A of Canadian populations.

DESIGN/METHOD/DESCRIPTION: Foods of various categories (dairy, meat, fish, poultry, fruit, vegetable, baby foods, etc.) were collected from Quebec City in 2008, and they were prepared as for consumption and combined into food composites. Food composites were analysed for BPA by gas-chromatography-mass spectrometry after solvent and solid phase extraction and derivatization with acetic anhydride.

OUTPUT/RESULTS: BPA was detected in 55 of the 154 samples tested. The highest BPA level was observed in canned fish (106 ng/g), followed by canned corn (83.7 ng/g), canned soups (22.2 – 44.4 ng/g), canned baked beans (23.5 ng/g), canned peas (16.8 ng/g), canned evaporated milk (15.3 ng/g), and canned luncheon meats (10.5 ng/g). BPA levels in baby food composite samples were low, with 2.75 ng/g in canned liquid infant formula, and 0.84 – 2.46 ng/g in jarred baby foods. BPA was also detected in some foods that are not canned or in jars, such as yeast (8.52 ng/g), baking powder (0.64 ng/g), some cheeses (0.68 – 2.24 ng/g), breads and some cereals (0.40 – 1.73 ng/g), and fast foods (1.1 – 10.9 ng/g). Dietary intakes of BPA were low for all age-sex groups, with 0.17 – 0.33 µg/kg body weight/day for infants, 0.082 – 0.23 µg/kg body weight/day for children aged from 1 to 19, and 0.052 – 0.081 µg/kg body weight/day for adults.

IMPACT/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Relatively high concentrations of BPA were found mostly in the composite samples containing canned foods. BPA was also found in some foods that are not canned or in jars. Dietary intakes of BPA were estimated to be well below the provisional tolerable daily intake of 25 µg/kg body weight/day established by Health Canada for all age-sex groups.

1.14 Effects of Long-Term Exposure to the Mycotoxin Ochratoxin A (OTA) on Kidney Gene Expression in Wild Type and Cancer-Prone Transgenic Mice

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SUMMARY: Cancer-prone mice have been developed to reduce costs, shorten study duration and provide mechanistic data when used to assess chemicals for carcinogenicity. The transgenic (TG) p53 heterozygous mouse has been tested in the Food Directorate. In TG mice and corresponding wild-type (WT) mice, OTA is nephrotoxic and a renal carcinogen causing lesions and changes in kidney, biochemistry. Physiologically, TG mice were slightly more sensitive to OTA than the WT mice. Data from this study will (1) contribute to decision-making on the future use of the TG mouse, and (2) provide molecular toxicity data for OTA for use in risk assessment.

OBJECTIVE: To provide molecular toxicity data for OTA mode of action using the TG p53 heterozygous mouse.

MATERIALS AND METHODS: Male TG and WT mice were exposed to OTA at levels of 0, 0.5, 2 or 10 mg/kg in diet for 26 weeks. Kidneys were harvested at necropsy and RNA was isolated from all dose groups of the WT and TG mice. The RNA from control and 2.0 mg/kg BW (WT and TG) were labelled and hybridized to Agilent 22k mouse microarrays. Arrays were normalized and changes in gene expression with a FDR adjusted p value <0.05 were determined. Pathway analysis of significantly changed genes between control and 2.0 mg/kg BW in WT and TG was performed using Ingenuity Pathway Analysis. Several genes markers of renal damage were specifically monitored via RT-PCR using TaqMan gene expression assays.

RESULTS: Microarray analysis of gene expression in kidneys of WT and TG mice identified 420 genes in WT mice and 371 genes in TG mice (filtering: $p \leq 0.05$; fold change ≥ 1.5) to be significantly affected when comparing OTA-treated vs. control mice.

Common genes between both mouse strains when comparing treated vs. control mice numbered 261. Pathway analysis indicates the primary network functions affected are cell death and cellular growth and proliferation. Genes specifically monitored as markers of renal damage were all affected at the medium dose.

CONCLUSIONS: Comparison of affected genes indicates possible pathways differences leading to nephrotoxicity with OTA between TG mice and WT mice. Further analyses are underway to determine whether these differences are indicative of the MOA for OTA.

1.15 Folic Acid in Canadian Breakfast Cereals

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SUMMARY: The top selling breakfast cereals were collected, processed into composites and analyzed for nutrient content through the Sampling and Nutrient Analysis Program of Canada (SNAP-CAN). The results unexpectedly show that the majority of breakfast cereals which are fortified with folic acid contain levels that exceed present regulations by as much as 700%.

BACKGROUND: Breakfast cereals were selected as a priority food because it is a commonly consumed food and a popular fortification vehicle. While fortification of these cereals is not mandatory, regulations stipulate which nutrients are permitted to be added and to what level. For added nutrients the amount measured should be within 10% of the regulated value. A proposed policy on discretionary fortification has also generated a need for baseline data on potential fortification vehicles to assist in future monitoring. Funding for the sample collection and processing was provided by targeted food fortification funds.

METHOD: Market share data revealed that 76 top selling breakfast cereal brands covered over 85% of the volume sold. Sample composites for these cereals (3 different lots) were collected and analyzed for naturally occurring folate and folic acid by an LC-MS/MS method, which can quantify both fractions simultaneously. Analyses were undertaken in Health Canada's regional lab in Longueuil.

OUTPUTS: Fortification of breakfast cereals is voluntary, but if folic acid is added the Food and Drug Regulations stipulate 60µg/100g cereal. Our folic acid results showed that of the 76 cereal brands tested, 60 contained added folic acid. Of these, 4 contained less than 90% of the required amount; 25 added folic acid to levels between 120-200% of the required amount and the remaining 28 ranged from 200-729% over the regulated value of 60µg/100g cereal.

IMPLICATIONS: The data seem to indicate a need for better control on the processes for adding folic acid to breakfast cereals. While folic acid is demonstrated to reduce the risk of neural tube defects, excessive folate consumption has been associated in some studies with risk of developing certain types of cancer in some population groups. These results confirm those of an academic study published in 2010. Further review of risk is warranted.

1.16 Chemical Tissue Accumulation Following Prenatal and/or Postnatal Exposure to Mixtures of Environmental Contaminants

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SUMMARY: Adverse effects of chemicals are often attributed to their accumulation in specific tissues. As part of a larger rat study investigating health effects in adulthood associated with developmental exposure to mixtures of environmental contaminants (prepared based on human blood profile), we report differences in the composition of administered mixtures, to mixture compositions measured in the blood, serum, and liver. Chemical partitioning between fluids was influenced by the fat and red blood cell content. The adult levels originated from prenatal, but mostly postnatal exposure. These results improve our understanding of chemical partitioning, mixture toxicity, and identification of critical period of exposure.

OBJECTIVE: Interpretation of toxicological investigations can be improved by knowing the internal dose resulting from the exposure protocol and partitioning of chemicals in various tissues. The objective is to report differences in the composition of the administered mixture, to the mixture composition in the blood, serum, and liver, following *in utero* and/or postnatal exposure.

METHOD: Rats were exposed *in utero* and/or lactationally to chemicals by feeding dams one of three chemical mixtures (prepared based on human blood profile of ubiquitous industrial legacy chemicals): (1) a mixture “M” including 14 PCBs, 12 organochlorine pesticides, and methylmercury, (2) "AhR", a mixture of 14 aryl hydrocarbon receptor agonists (non-ortho PCBs, PCDDs, PCDFs), and (3) “0.5MAhr”, containing mixture “M” at half-concentration plus “AhR”. Tissue concentrations were measured using gas chromatography. As biological effects, liver enzyme activities were assessed by three assays: EROD, BROD and PROD.

RESULTS: Some chemicals partitioned based on the red blood cell and fat content of the samples. For example, hexachlorobenzene and PCB153 in adult males represented 29% and 15% of the chemicals in the blood but 6% and 21% in the serum, respectively. Surprisingly, *in utero* exposure contributed to the accumulated chemicals measured in the blood at postnatal day 21. For example, the concentrations of PCB153 were 0.09, 1.7, and 53 ng/mL in controls, in rats exposed only *in utero*, and in those exposed during both the pre and postnatal period. The enrichment of the mixture 0.5M with AhR did not modify chemical persistence and liver enzyme activities, perhaps due to the unexpected content of PCB126 found as a contaminant of 0.5M.

CONCLUSIONS: These results on chemical tissue partitioning, internal dose, and identification of critical period of exposure during development, improve our understanding of mixture toxicity, and will permit a more accurate interpretation of the toxicity data and their policy implication.

1.17 Effects of Thyroid Hormone Disruption on Mouse Brain Development in Early Gestation

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SUMMARY: Thyroid hormone (TH) is critical for brain development, and many endocrine disrupting chemicals can alter TH levels and may lead to behavioural/cognitive impairments. Existing methods of detecting such effects are laborious and costly. We examined the effect of altered TH levels on genes expressed in the developing brain to identify markers of neurodevelopmental problems caused by disrupted TH. We identified 160 genes in the fetal mouse brain that were sensitive to changes in TH level. These findings increase our knowledge of how TH disruption impairs brain development. These results can be used to identify biological markers sensitive to thyroid hormone disrupters.

OBJECTIVE: The long-range objective is to understand the adverse effects of thyroid hormone (TH) disrupting chemicals on brain development, and identify biomarkers of TH disruption. In this experiment, we identify genes regulated by TH in the late fetal neurocortex.

METHODS: Timed-pregnant C57BL/6 mice were made transiently hypo- or hyperthyroid by treatment with antithyroid drugs for 3 days (starting from gestation day 13 (G13); “hypo”) or injection with TH (on G15, 12 hrs prior to sacrifice; “hyper”), respectively. A third group (hypo+) received both treatments while the control group received the vehicle only. The left cerebral cortex was collected to measure T4 levels, while the right one was used for RNA extraction. Global gene expression was analysed with Affymetrix microarrays, and miRNA analysis applied Agilent miRNA arrays. Statistical analysis was conducted in R software.

RESULTS: Cerebral TH levels were significantly decreased in hypo, and increased in hypo+ and hyper, relative to control. Cluster analysis indicated that gene expression patterns were similar between control and hypo, while hypo+ and hyper clustered separately. There were 29 genes that were significantly altered in common in hypo+ and hyper, relative to control. We confirmed 7 out of 8 of these genes with RT-PCR (88% confirmation of microarray results). miRNA expression was analyzed in the control and hyper groups. TH treatment increased the expression of the most miRNAs. The largest increases were observed for miR-693/30/302.

CONCLUSIONS: The genes affected by altered TH levels are involved in pathways critical to neurodevelopment, including thyroid hormone receptor activation, long-term potentiation, neurotrophin and reelin signaling. Altered miRNAs play a role in the release of neurotransmitters and neurobehaviour. These findings provide insight into impaired brain development induced by TH disrupting chemicals. Further investigation of these genes and miRNAs will identify potential biomarkers of TH disruption in the developing brain.

1.18 The Diabetes and Pregnancy TeleForm Project (DPTP)

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SUMMARY: Diabetes in pregnancy has been identified as an important issue among First Nations communities, stakeholders and the programs from the First Nations and Inuit Health Branch (FNIHB). In order to address information needs required for program and policy development, the Diabetes and Pregnancy TeleForm Project (DPTP) is exploring a novel process for collecting data on-reserve. Presently, data sharing agreements have been signed with six First Nations Communities, who have faxed single paged surveys containing data on 106 completed pregnancies. DPTP represents a community-based initiative that supports evidence-informed decision making at community and federal levels through the collection of client-level data.

BACKGROUND: Although recent evidence has suggested that the increased prevalence of diabetic pregnancies among First Nations women may play a key role in the risk for future type 2 diabetes and obesity, little information exists on related health status indicators and care processes on-reserve. A key priority for the First Nations and Inuit Health Branch (FNIHB) is to support First Nations and Inuit communities to collect and provide information to inform policy and programs at the community and federal level. The Diabetes and Pregnancy TeleForm Project (DPTP) is an initiative that is exploring a novel methodology for collecting data on-reserve.

METHODS: Six First Nations communities are participating in this project. The data collection period runs from January 2011 to December 2012 and the methodology includes five components: 1) a community workbook to provide baseline data; 2) a data sharing agreement; 3) a single-page, fax-based Teleform; 4) a process map which provides a graphical description of health care processes at the community-level; and, 5) ongoing meetings with communities and stakeholders to ensure a collaborative approach towards identifying needs, data collection and interpretation.

RESULTS: As of June 2011, data sharing agreements have been signed, community workbooks and Process Maps have been completed, and a total of 106 TeleForms have been faxed to FNIHB National Office. TeleForms include:

- client level information on modifiable risk factors including pre-pregnancy body mass index (BMI), multivitamin use, and maternal weight gain;
- health service data on service providers and gestational diabetes mellitus (GDM) screening practices; and
- pregnancy outcomes such as complications, birth weight, and breastfeeding.

IMPLICATIONS: Data collection is ongoing and preliminary data will be presented at the Health Canada Science Forum. DPTP is an example of a community-based, participatory project that has been met with strong participation and support by First Nations communities.

1.19 Development of a Bead Array for High Throughput Serum Screening in Non-Human Primates

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SUMMARY: Non-human primates are important animal models for AIDS-related research. The presence of indigenous viruses such as Herpes B poses a serious threat to animal handlers due to its high fatality rate. The simian foamy virus (SFV), another virus found in wild and captive macaques, presents serious problems with cell work as the virus degenerates cell cultures. Other retroviruses, such as SIV, STLV-1 and SRV-1 also belong to the list of targeted pathogens in colonies used in cancer and AIDS-related research. We developed a multiplex immunoassay to screen NHP serum as a tool to maintain a specific pathogen free colony.

OBJECTIVE: Develop a bead-based immunoassay for B virus, Simian foamy virus, STLV-1, SIV, and SRV-1 seroprevalence screening for the establishment and maintenance of specific virus free cynomolgus monkeys within the NHP colony.

METHODS: Antigenic preparations (viral or cell lysates) were covalently coupled to beads (Luminex) and used in a 9-plex assay to screen sera (n=163) from the 2010 HC NHP sampling. Beads coated with mock infected cell lysates and BSA were used as negative controls. Control sera reactive to each virus were used to develop the assay. The procedure was coupled to a robotic liquid dispenser and bead washer (Hudson Robotics). Results were compared to a commercial dot immunobinding assay (DIA) and an in-house Western Blot (WB).

RESULTS: Antibodies to B virus were detected in all positive reference samples (n=5) (titers 900 to >10,000) and none were detected in the negative reference samples (n=7). The bead assay performed as well as the WB for the detection of foamy virus antibodies (n=5 positive, n=2 negative). Concordance was observed between the results obtained using the multiplex bead assay and the DIA/WB. All sera carried antibodies to foamy virus except for a selected group of macaques established and maintained as SFV-free. Four specimens showed reactivity to HTLV-1 and one, for SIV-coated beads. No reactivity to SRV-1 was detected. Confirmatory testing of the antibody reactive specimens is ongoing.

CONCLUSION: This 9-plex-bead immunoassay showed excellent performance when compared to conventional assays. Antibody screening of the entire colony to all five viruses was done at a fraction of the time and cost of conventional assays. This multiplex immunoassay was a valuable tool for both the animal health and occupational safety of the HC NHP colony.

1.20 Health Effects of Northern Contaminants and Alcohol Consumption in a Rodent Model of Human Metabolic and Cardiovascular Diseases

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SUMMARY: The role of Northern Contaminants (NC) in the development of metabolic and cardiovascular disease and interaction with alcohol consumption were investigated using a rat model. Preliminary data suggest that NC may alter lipid metabolism and/or transportation and pancreatic insulin secretion, consistent with the development of metabolic and cardiovascular disease.

BACKGROUND/ISSUE(S): For the last few decades, Northern populations have been exposed to elevated levels of Northern contaminants (NC) such as polychlorinated biphenyls and heavy metals, mainly through consumption of contaminated fish and marine mammals. Along with this, there has also been an increased prevalence of obesity, hypertension, diabetes, and cardiovascular disease. Although changes in lifestyle and diets have been associated with this prevalence of chronic diseases, it remains to be clarified if exposure to NC also plays a part, and if lifestyles choices such as consumption of alcohol may modulate the effects of NC. This study explored these questions using a rodent model of human metabolic and cardiovascular diseases.

DESIGN/METHOD: Obese male JCR rats at eight weeks of age were acclimatized on a purified AIN93G diet. Animals were treated with 10% alcohol in drinking water or drinking water only for six weeks. From the third week of alcohol treatment, animals were orally dosed with a mixture of 23 NC (NCM) at 0 (corn oil), 1.6, or 16 mg/kg BW/day for four weeks. During the whole study, body weight and water/ alcohol consumption were measured daily. Food consumption was measured weekly. Blood and organs were collected and weighed at the end of the study. Tissue samples were analyzed for hematology and serum NCMs, glucose, insulin, and ethanol levels, as well as clinical biochemistry markers.

OUTPUTS/RESULTS: Serum NCM levels increased with NCM dose, and were mostly in the range of NCM levels found in Inuit blood. Serum NCM levels were generally lower in the rats treated with alcohol than those without. NCM at 16 mg/kg BW slightly decreased body weight gain and water and food consumption especially in the rats given no alcohol. Regardless of alcohol, this dose of NCM significantly increased absolute and relative liver weight, relative kidney weight, and serum ethanol levels. NCM also decreased serum insulin, cholesterol, triglyceride, HDL, and LDL levels.

CONCLUSIONS/IMPLICATIONS: Our data suggested that NCM at levels relevant to human exposure may alter lipid metabolism and/or transport in the liver and insulin secretion from the pancreas that are consistent with the development of metabolic and cardiovascular diseases.

1.21 Automated Identification of DNA Tandem Repeat Mutations by Image Analysis

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SUMMARY: Toxicological risk assessment of chemicals in the Canadian Environment (regulated under the *Canadian Environmental Protection Act*) requires determination of their potential to mutate DNA. Chemicals causing DNA mutations can be classified "genotoxic", potentially elevating the risk of genetic diseases in the exposed population. Mutations can be detected by separating different sizes of DNA through a gel matrix (gel electrophoresis). Traditionally, changes in DNA size have been measured manually from digitized images of the DNA in the gel, but this is time consuming, prone to human-error and impractical for thousands of chemicals. A computerized solution was developed, which increases speed and accuracy, making genotoxicity screening feasible for the thousands of chemicals in the Canadian environment.

BACKGROUND/OBJECTIVES: Agarose gels are used in the laboratory to detect DNA mutations at expanded simple tandem repeats (ESTRs) in single cells. Scoring ESTR mutations is labour-intensive and prone to measurement errors as result of the limited ability of the human human eye to measure spot intensity. Lanes on gels contain both single alleles and internal reference DNA size standards. Gels are Southern blotted, probed, and images are digitalized. Traditionally, mutations have been detected by physically measuring the distance between the reference and samples bands within each lane using a ruler. When digitalized, pixel intensity (0 to 255 in grey scale) is easily differentiated. Thus, distances between internal reference bands and analytical bands can be resolved with greater confidence and reproducibility.

METHOD: We used a combination of ImageJ from the NIH and R from CRAN to perform the analysis. A plug-in was written for ImageJ, which generated an R script to perform the statistical analysis. ImageJ was used to read the image, capture the lanes of interest, and identify the bands of interest. Part of the existing analytical tool from ImageJ (Analyse->gel) was used to produce a histogram of pixel intensities along the lanes. The program permits the user to select an R script that can run with minimal user intervention. In R, we used a smoothing algorithm followed by calculation of the 1st derivative. A Regular expression (RE) was then used to find peaks that correspond to the bands on the gels. REs are models that can quickly identify patterns in sequential data. Distance between bands (e.g., band of interest to internal size standards) was calculated from peak summits. Bootstrapping was utilized to estimate the variability and calculate the probability of a lane containing a mutant.

RESULTS: The program readily identified progenitor and mutant alleles, including both gains and losses in DNA repeat elements. The program produces a table containing the results generated by R, which summarizes the distances between bands and identifies mutant bands with a probability indicator.

IMPLICATIONS/NEXT STEPS: Comparison between results obtained by human scoring and this package will be conducted to determine the probability of false positives and false negatives. A package will be built and made available for other laboratories to use. The application saves a great deal of time by automating the scoring process in addition to improving reproducibility for ESTR mutation analysis.

1.22 Trace Analysis of Heavy Metals in Natural Health Products by ICP-MS

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SUMMARY: As natural health products (NHPs) are used by a large part of the Canadian population, the need for a fast and reliable method for analysing heavy metals at a trace level in these products was identified and an analytical method based on ICP-MS was developed. The method has been used for a few years and has proven its reliability and its advantages when compared to the method that was previously used. This new method supports the Natural Health Products Program in ensuring the safety of natural health products in Canada.

OBJECTIVES/BACKGROUND/ISSUE(S): Part of the work of the HC Inspectorate Laboratory Programme is to provide reliable results of chemical analyses in order to support inspections and investigations related to NHPs. The analysis of arsenic, cadmium, lead and mercury, is one of the tests that could be performed on NHPs using open vessel digestion and Flame Atomic Absorption (FAA). However, there can be significant losses of elements during the process of open vessel digestion and FAA allows for the detection of only one element at a time with a relatively high limit of detection when compared to other techniques like ICP-MS. There was a need for a more rapid cost-effective test capable of detecting and quantifying these contaminants at trace levels in NHPs. The potential of ICP-MS in trace element analysis is well known. It allows for low detection limits as well as short analysis time. It has great advantages when compared to older techniques such as FAA. The objective of this project was to improve the sample preparation step and to develop an ICP-MS method for the determination of heavy metals at trace level in NHPs.

DESIGN/METHOD/DESCRIPTION: The analysis can be separated into three steps: sample preparation, instrumental analysis and data analysis. Sample preparation is done using microwave oven digestion. Instrumental semi-quantitative and quantitative analyses were performed using ICP-MS. The semi-quantitative analysis takes only a few minutes and provides a fingerprint of the elements present in a sample with their approximate concentrations. The information gathered during that analysis is used to determine which elements will be quantified. In a typical quantitative analysis, the concentration of each element is determined by comparing the counts measured for a selected isotope to an external calibration curve that was generated for the particular element.

OUTPUTS/RESULTS: Many samples with FAA measurable concentrations of contaminants were analysed using both techniques. Results were compared and were deemed equivalent but were obtained more quickly by ICP-MS. Results from that study and the fact that ICP-MS allows for lower limits of detection lead us to adopt the new method. A wide variety of NHP products (tablets, capsules, herb mixtures, syrups, etc.) have been analyzed (about 140) and the reliability of the method is well established.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The method developed for the analysis of trace elements in NHPs is rapid, precise, accurate and sensitive. It enables us to provide reliable results to support the Natural Health Products Programme. Future work will focus on the development of speciation methods for arsenic and germanium in order to distinguish between organic and inorganic compounds containing these elements. The appeal of speciation is related to the toxicity of the various forms.

1.23 Determining the Reaction Rate and Interaction Potency of Chemicals with DNA as a Potential Tool to Screen for Chemical Hazards

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SUMMARY: It is important to develop rapid tools to evaluate the hazard to human health of those chemicals which are also present as environmental pollutants. We have developed a simple in-vitro method which uses DNA damage as a biomarker for measuring the relative interaction potency and reaction rates of chemicals. Potencies and rates were employed for evaluating the capability of the chemicals to damage DNA. This method has been demonstrated to be a rapid tool to screen environmental contaminants for potential hazard.

OBJECTIVES/BACKGROUND/ISSUE(S): The Canadian Government's categorization process identifies chemicals as priorities for risk assessment and risk management. It is important to develop rapid tools to detect and assess hazardous chemical pollutants. Hazard determination traditionally requires chemical identification and lengthy toxicity studies. In this study, we propose that DNA interaction can be used as a biomarker to estimate the relative hazard of test chemicals to DNA by determining the potency and reaction kinetics of chemical-DNA interaction. The objective is to develop a rapid tool to screen for potential chemical hazards.

DESIGN/METHOD/DESCRIPTION: Test chemicals benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE), phenyl glycidyl ether (PGE), tetrachlorohydroquinone (Cl_4HQ), methylmethane sulfonate (MMS), and styrene-7,8-oxide (SO) were chosen to represent hazardous chemicals capable of directly modifying DNA. DNA interaction was measured by HPLC and LC-MS following reaction of chemicals with 20mer oligodeoxynucleotides *in vitro*. Descriptive parameters were determined.

OUTPUTS/RESULTS: DNA modification was detected by BPDE, Cl_4HQ, MMS, PGE and SO. Potency equivalency quotients (PEQs) and kinetic rate equivalency (KEQ) were calculated to describe the extent of interaction. PEQs relative to BPDE were PGE > Cl_4HQ > MMS > SO for ds-DNA and PGE > SO > MMS > Cl_4HQ for ss-DNA. Kinetic rate equivalency (KEQ), defined as the ratio of first order kinetic rate constants of test chemical to BPDE, were found to be MMS > SO > PGE > Cl_4HQ for ds-DNA and SO ≈ Cl_4HQ ≈ MMS > PGE for ss-DNA.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The magnitude of interaction between direct-DNA-modifying chemicals was determined by HPLC and described by PEQ and KEQ. In addition to providing fundamental dose-response and kinetic data, results can potentially be employed to rapidly screen for the presence of potential chemical hazards in the environment without lengthy toxicity studies or prior knowledge of chemical composition. Next steps involve application with environmental samples for identification of chemical hazard.

1.24 Determination by Liquid Chromatography-Tandem Mass Spectrometry of Isoflavones in Rats Fed Soybean Proteins

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SUMMARY: Consumption and nutritional supplementation of soy and soy-based products have been linked to health benefits, such as lower cholesterol and triglyceride levels, and lower incidence of disease, such as cardiovascular disease and diabetes. More studies are needed, however, to identify and evaluate the bioactive components responsible for the health benefits. We have developed a method to determine isoflavone levels in the serum of rats whose diets were supplemented with soy. We were able to reproducibly detect low levels of isoflavones in rat serum. The measurements determined here will support studies on the safety and health effect of soy consumption.

OBJECTIVES/BACKGROUND/ISSUE(S): Genistein, glycitein and daidzen are the major phtyoestrogens found in soy. Additionally, daidzen is metabolized to equol, which can be detected in rat serum after soy consumption. We have therefore developed a sensitive and unbiased method to measure the concentration of these compounds in the serum of rats fed different diets.

DESIGN/METHOD/DESCRIPTION: Serum samples were deproteinated, enzymatically deconjugated, extracted by ethyl acetate, dried and reconstituted prior to analysis. Separation was conducted on an Agilent 1200 LC system (Kinetex 2.6µm PFP column; 50 x 2.1mm ID, 100A) coupled to an API 4000 QTRAP mass spectrometer in 0.1% formic acid in water (A) and acetonitrile (B) at 0.6 ml/min by gradient elution (0-3 min, 17.5-50% B; 3-4 min, 50-95% B, 4-5 min, isocratic at 95% B to wash column, 5-6 min, return to 17.5% B; 6-10 min, re-equilibrate at 17.5% B). Analytes were monitored by UV (258 nm) and by multiple-reaction monitoring (daidzein 255 → 65; glycitein 285 → 242; genestein 271→ 91; equol 243 → 123 m/z) at the same time. Deuterium labelled daidzein (-d3, 258→93), genestein (-d4 275→93) and equol (-d4 247→ 125 m/z) were used as internal standards. 4-methyl-umbelliferone was used as the internal standard for glycitein (177→ 77 m/z).

OUTPUTS/RESULTS: The developed method showed a linear dynamic calibration range of 10-5000 ng/mL with a detection limit ranging from 24-33 ng/mL for rat serum. Good accuracy was achieved with spiked rat serum, with recoveries ranging from 91-100%.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This method allows for sensitive, specific and reliable determination of serum isoflavones in rats fed soy proteins.

1.25 Development of an *In Vitro* Assay to Screen for Thyroid Hormone Transmembrane Transporter or Receptor Inhibitors

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SUMMARY: There is a global effort to develop rapid assays to screen chemicals for their ability to cause changes in hormonal function. One hormone that has a major role in proper brain development in the foetus is the thyroid hormone (TH). Substances that disrupt TH entry into target cells and/or the activation of the TH receptor can impair brain development. We have developed an assay using genetically-modified cells in culture to rapidly screen substances to test for both of these effects.

BACKGROUND/OBJECTIVES: Disruption of thyroid hormone (TH) signalling during sensitive periods of foetal development or infancy can cause impaired development of several organs including the brain. There is a global effort to develop methods to screen and test chemicals to identify those which may impact thyroid hormone signalling. We have developed a method to identify chemicals that impair uptake of TH into cells or impair interaction of TH with its receptor.

METHOD: We have implemented a reporter assay using transiently-transfected COS7 cells. These cells were co-transfected with the following plasmids: 1) expressing the human TH receptors (hTHR) alpha or beta; 2) a reporter plasmid expressing the firefly luciferase (pDR4-Luc) gene controlled by thyroid response elements; and, 3) and a plasmid that expresses the Renilla luciferase gene to control for transfection efficiency. Luciferase activities were measured after 24 hr exposure to test substances. Parallel incubations tested cell survival.

RESULTS: The active form of TH (T3) caused a dose dependant-increase in Luciferase activity when the cells were co-transfected with a hTHR-containing plasmid. Treatment with any of multiple substances previously reported to block T3 uptake by cells resulted in reduced T3-stimulated Luciferase activity. This assay was used to screen a variety of environmental contaminants.

CONCLUSION/NEXT STEPS: Our assay proved to be effective at identifying both substances that impair T3 transport into cells and substances that influence hTHR transactivation. The assay cannot distinguish between these functions as impairment of either will reduce T3-stimulated increase in luciferase activity. As these modalities are largely unstudied despite indications that disruption of either pathway can lead to impaired development, our assay will provide a valuable tool to identify substances with potentially hazardous properties. Future studies will further validate this assay and incorporate *in vitro* metabolism to provide better predictions of *in vivo* chemical action.

1.26 Gafchromic Film Calibration and Dosimetry: Initial Experience

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SUMMARY: Gafchromic film® presents an attractive solution for medical x-ray exposure assessment. It is a simple, flexible tool and can potentially show dose information with a high degree of detail over a given area. Recent studies [1,2] have proposed simple models that can describe the response of Gafchromic® film over a typical range of medical x-ray exposures. This work attempts to verify and assess these simple models prior to adapting film use as a means for quality assurance testing and research into two-dimensional dose mapping.

OBJECTIVE: To verify and develop a calibration process that will allow two-dimensional quantification and visualisation of radiological x-ray exposures.

MATERIALS AND METHODS: A calibration curve basis set was formed using 14 exposure points spanning 3-20 mGy, 4 points from 30 – 100 mGy and 1 unexposed control. Exposures were independently verified and measured using a calibrated 6cc ionisation chamber/electrometer combination. At each dose point, 3 film pieces (~3cmx3cm) were exposed and subsequently scanned using a commercial flatbed scanner. Images were saved in TIFF format (300ppi) and analysed using ImageJ (freeware) to obtain red-channel pixel data which is proportional to x-ray exposure. Two simple models (exponential and quadratic) describing the relationship between red channel pixel values and calibration points were found (Figure 1). The "predictability" of each model was also tested using 6-blinded exposures following calibration. Only the corresponding pixel values (PV) were given to the researcher for substitution into each model. Blinded control exposures and model calculated values are summarised in Table 1.

RESULTS: Figure 1 shows that both models fit very well (R^2 = 0.9977 and 0.9990 respectively) and show good agreement with calibration exposures (red circles). Analysis of blinded control exposures (Table 1) shows the exponential model has a strong trend of dose overestimation at low values and underestimation at high values (+17.6%, -13.4%), whereas the quadratic model is more consistent (+6.0%,-4.7%). Actual estimated error in dose, from low to high exposures was 8.4-1.7% (exponential) and 8.6-2.6% (quadratic), which agrees with previous results [2].

CONCLUSION: A Gafchromic, quadratic model shows good potential for general two-dimensional dosimetric applications. Further work formalising the calibration procedure and robust error estimation is in progress, prior to future research and quality assurance applications.

[1] Rampado et. al., Phys Med Biol, (51):2006. [2] Rampado et. al., Med Phys (37):2010.

FIGURE 1:

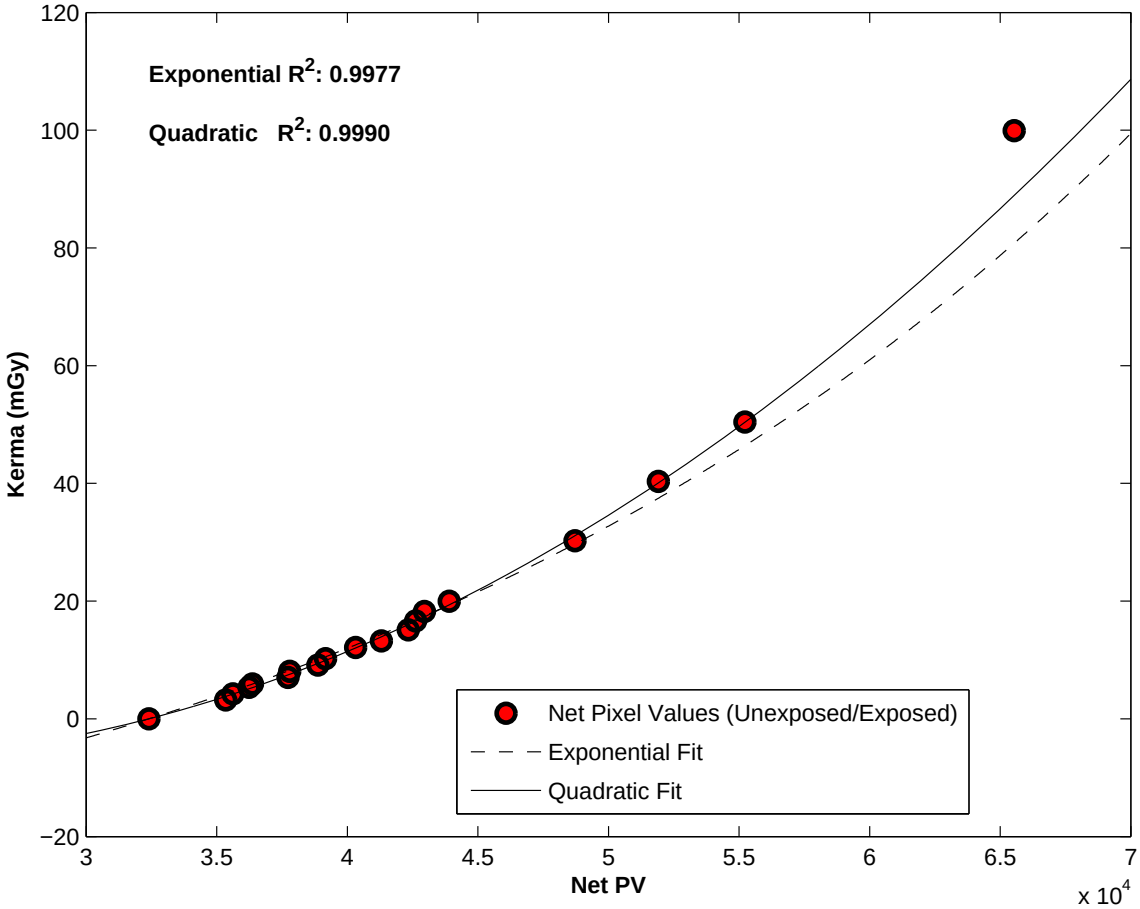

TABLE 1:

CONTROL (Ionisation Chamber)	EXPONENTIAL MODEL (Gafcrhomic® Film)		QUADRATIC MODEL (Gafchromic® Film)	
Dose, D (mGy) ± 5%	D_{calc} (mGy)	%Dev	D_{calc} (mGy)	%Dev
4.376	4.87 ± 0.41	11.3	4.31 ± 0.37	-1.5
5.360	6.30 ± 0.42	17.6	5.68 ± 0.43	6.0
9.103	9.96 ± 0.46	9.4	9.32 ± 0.51	2.4
22.62	23.2 ± 0.6	2.7	23.7 ± 0.8	4.9
43.93	41.8 ± 0.8	-4.8	45.1 ± 1.3	2.6
76.38	66.2 ± 1.1	-13.4	72.8 ± 1.9	-4.7

1.27 NMR Fingerprint Assay of Recombinant Glycoprotein Therapeutics

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SUMMARY: The assessment of subsequent entry biologics (SEB) (a SEB is a biologic drug that enters the market subsequent to a version previously authorized in Canada see "*Guidance for Sponsors: Information and Submission Requirements for Subsequent Entry Biologics*") is a very challenging task. For this purpose, our laboratory has developed a method that utilizes nuclear magnetic resonance spectroscopy to assess the structure of the active ingredient of SEB. The application of the method was limited to the assessment of non-glycosylated proteins. However, a number of products contain recombinant glycoproteins, such as erythropoietin and interferon beta, as active ingredients. These therapeutics present a higher level of difficulty in their characterization resulting from the presence of sugar chains of various lengths and compositions. Here we present the progress made toward the extension of this methodology to recombinant glycoprotein therapeutics.

BACKGROUND: A major focus of our laboratory is the development of methods using nuclear magnetic resonance (NMR) spectroscopy to characterize the structure of recombinant protein therapeutics. Among the latter, the assessment of glycosylated protein therapeutics is particularly challenging. This arises from the complexity of their glycan chains that makes the characterization of these products difficult to compare with a product already approved in the context of Subsequent Entry Biologics (SEB). The development of NMR-based methods requires the production of labelled glycoproteins with NMR-detectable isotopes such as carbon-13 and nitrogen-15. Labelled samples allow optimisation of NMR parameters for data acquisition used for the characterisation. It thus becomes possible to analyse the structure of the glycans (^{1}H,^{13}C-2D-HSQC experiments) and the polypeptide chain (^{1}H,^{15}N-2D-HSQC experiments) in a non-destructive manner. The yeast-based expression system *Pichia pastoris* can produce labelled glycosylated proteins in high yields and at affordable prices. It uses ^{13}C-labelled methanol and ^{15}N-ammonium salt as sole sources of carbon and nitrogen, respectively.

OBJECTIVE: The objective of the project is to develop a yeast expression protocol for the production of labelled GM-CSF, a recombinant protein used in part to increase the production of neutrophils (White Blood Cells) in immunosuppressed patients.

OUTPUTS/RESULTS: We have optimised the expression and purification of labelled *N*-glycosylated-GM-CSF with stable NMR active isotopes. Analysis of NMR spectra allows the comparison of the conformation of the protein in the presence and absence of glycans. For GM-CSF, the overall fold of the protein is maintained. However, the presence of glycans at residue N37 has modified the small one-turn helix preceding the glycosylation site, which adopted a random coil conformation.

IMPACT/OUTCOMES/CONCLUSION: The ability to produce labelled glycoprotein will greatly facilitate the development of NMR-based methods for the assessment of glycosylated SEB.

1.28 Salivary Endothelins as Non-Invasive Cardiovascular Biomarker of Air Pollution Effects

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SUMMARY: In this study, we show that saliva can be used as a surrogate matrix to plasma for measuring endothelin peptides, biomarkers of cardiovascular changes associated with air pollution. Series of saliva samples collected simultaneously with blood pressure measurements from healthy subjects at 30min intervals on two different days revealed a diurnal pattern for secretion of endothelins. In addition, salivary endothelin peptide bigET-1 levels correlated positively with systolic blood pressure and ambient air pollutants such as NO2, O3, PM2.5. This work identifies a practical non-invasive approach for biomarker monitoring in air pollution studies, notably with children.

BACKGROUND: Plasma endothelins (ET) have emerged as indicators of cardiovascular effects of air pollutants. Time-series analyses of cardiovascular physiology and blood biomarkers such as endothelins are limited by the invasiveness of blood sampling. We have shown that various isoforms of endothelin peptides in matched plasma and saliva samples of healthy subjects are positively correlated. The objective of this study was to investigate the relationship between different endothelin isoforms in saliva of healthy subjects, their blood pressure and the daily ambient air pollution levels.

METHODS: Experiments were conducted indoors at EHC building between September 2009 and March 2010. Ambient air pollution (National Air Pollution Surveillance) during the period was, mean (min, max): $PM_{2.5}$, 4ug/m^3 (0, 21); CO, 0.25ppm (0.10, 0.65); NO_2, 9ppb (0, 44); O_3, 15ppb (0, 48). Penetration of ambient pollutants indoors was 95-100%. Blood pressure and heart rate were measured and saliva was collected from ten healthy subjects (8 males, 2 females, 22-56y, 60-120kg) every 30min between 08:00 to 14:30 on two different days (exercise routine between 08:30-09:00) Saliva was analysed for endothelins (bigET-1, ET-1, ET-3) by ELISA.

RESULTS: Secretion of endothelin isoforms followed a diurnal pattern. BigET-1 was low in the morning and increased by 10:00, possibly in response to exercise. In contrast, ET-1 and ET-3 were higher in the morning, decreased progressively until 13:00, and increased thereafter. The daily average bigET-1 levels in saliva correlated positively with blood pressure and heart rate ($p<0.001$). Furthermore, there were significant positive correlations ($p<0.05$, Pearson) between between salivary bigET-1, systolic BP and pollutants such as NO_2, O_3, $PM_{2.5}$.

CONCLUSION: Our analyses show salivary endothelins as potential indicators air pollution effects. In contrast to blood sampling, the non-invasive collection of saliva should reduce anxiety and discomfort and simplify the procurement of repeated samples.

1.29 Development of a Mammalian Cystathionine β-Synthase (CBS)-Knockdown Cell Line to Characterize Genetic Polymorphisms of the CBS Enzyme

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SUMMARY: Human cystathionine β-synthase (CBS) is required for homocysteine catabolism and methionine, cystathionine and cysteine biosynthesis. CBS mutations are associated with high circulating homocysteine, a risk factor for cardiovascular disease and some cancers. This study examines the effects of common CBS gene mutations on homocysteine metabolism in human liver cells.

BACKGROUND: Human cystathionine β-synthase (CBS) catalyzes the first step in the reverse transulfuration pathway, converting homocysteine to cystathionine. CBS single nucleotide polymorphisms (SNPs) are associated with increased risk for cardiovascular disease, homocysteinuria and hyperhomocysteinemia. The characterization of disease-related CBS mutations has been restricted to recombinant proteins expressed in non-mammalian expression systems and cell-free systems.

OBJECTIVE: To develop a mammalian expression system in which the effect of CBS single nucleotide polymorphisms (SNPs) on homocysteine metabolism can be tested.

MATERIALS AND METHODS: The wild-type human CBS as well as four homocystinuria-related mutants (E176K, K384E/N and G307S) were made by site-directed mutagenesis and will be subcloned into the pcDNA3.1 vector which contains the Cytomegalovirus (CMV) promoter to drive expression in mammalian cells. Endogenous CBS expression in HEP G2 cells was transiently knocked down using CBS-specific small interfering RNA (siRNA) that targets the 3′ untranslated region of CBS mRNA. Simultaneous knockdown of endogenous CBS and over-expression of mutant CBS will be achieved by the double transfection of siRNA and expression constructs. Gene knockdown and mutant CBS over-expression will be determined by quantitative PCR and Western blotting.

RESULTS: A series of four homocystinuria-associated mutations (E176K, K384E/N and G307S) were created using site-directed mutagenesis and confirmed by sequencing. Expression of endogenous CBS in HEP G2 cells and transient CBS knockdown, have been confirmed by quantitative PCR and/or Western blotting. The double transfection protocol and overexpression of mutant CBS protein in HEP G2 cells is being optimized.

CONCLUSIONS: The development of a mammalian CBS expression model is essential to understanding the impact of CBS SNPs on altered homocysteine metabolism, disease risk and response to vitamin therapy in susceptible individuals in the Canadian population. The E176K, K384E/N and G307S mutants will be used for the characterization of the CBS active site that interacts with the homocysteine substrate and, indirectly with pyridoxal-phosphate (PLP). The G307S substitution is of particular interest as it is one of the most prevalent disease-associated mutations of the gene encoding CBS.

1.30 Analysis of Granulocyte Colony Stimulating Factor by the NMR Fingerprint Assay: Example of Comparability Study with the Innovator Product and Effects of Mutations

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SUMMARY: Filgrastim is a product that is used for treatment of neutropenia (low level white blood cell count) resulting mainly from chemotherapy. Since the only approved product for Filgrastim (Neupogen®) has lost patent protection, sponsors will apply for market authorization for their subsequent entry version of Filgrastim in Canada. According to the Guidance For Sponsors, they will need to demonstrate similarity with an approved product. Here we show that the NMR fingerprint assay can be used to assess the three-dimensional structure of the active ingredient of the approved product Neupogen®.

BACKGROUND: Filgrastim is the generic name for recombinant methionyl human granulocyte colony-stimulating factor (r-metHuG-CSF). It is produced in *Escherichia coli* (*E. coli*) in a non-glycosylated form. Filgrastim was marketed under the brand name Neupogen® by Amgen. Since this product has lost patent protection, many subsequent entry versions have been approved or are in the process of filing for market authorization throughout the world, including Canada. In order to be authorized as a subsequent entry product, the sponsors must demonstrate similarity with an approved product in Canada via an appropriate comparability exercise.

OBJECTIVE: Development of an NMR fingerprint assay for the assessment of the onformation of r-metHuG-CSF with the comparator Neupogen®. In addition, the study of the effects of solution conditions (pH, ionic strength, mutations, excipients) on the NMR spectra will be presented.

OUTPUTS/RESULTS: Recombinant metHuG-CSF was prepared in *E. coli* and isotopically enriched with ^{13}C and ^{15}N isotopes. Samples were analysed by NMR to study the effects of varying the pH, the concentration of excipients (sorbitol and polysorbate-80), the ionic strengths with several salts, and co-solutes. Spectra of mutants have been recorded to assess the sensitivity of the method to small structural changes. Finally, NMR spectra were recorded for Neupogen®, purchased at a local pharmacy, and a chemical reference standard from the European Directorate for Quality Medicine (EDQM).

IMPACT/OUTCOMES/CONCLUSION: The NMR fingerprint assay applied to Filgrastim provided residue specific information of the structure of the active ingredient of a product. In addition to current methods, the ability to assess the conformation with a high degree of resolution will greatly facilitate comparability exercises.

1.31 Estimated Dietary Exposures to Caffeine for Canada

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SUMMARY: Canadians consume caffeine either as a naturally occurring ingredient in some plants, as a food additive in some carbonated drinks and energy drinks, or as an ingredient in certain drug products such as cold and headache remedies. This study provides an assessment of dietary exposure to caffeine from all food sources for the Canadian population. This study informs current initiatives by HC to enhance the regulation of caffeine in the food supply.

OBJECTIVE: The study estimates key measures of the dietary intake of caffeine for the Canadian population. In particular, the study identifies the primary sources of caffeine in food, estimates average and 95th percentile daily caffeine intakes, and the percentage of individuals who exceed the recommended maximum daily intakes (RMDI), by age-sex category. This information is key in developing effective policies and regulations regarding the overall intake of caffeine.

MATERIALS AND METHODS: The estimation of various measures of dietary exposure to any chemical in food requires the combination of information on the dietary habits of consumers and the concentration of the chemical of interest in the foods consumed. The estimation of dietary exposure to caffeine is based, primarily, on the combination of two data sources – the 2004 CCHS2.2 survey results, and Canadian Nutrient File (CNF). Modern statistical methods were applied to combine information from the various data sources and estimate relevant population level measures of caffeine intake, including bootstrap estimates of standard errors for each estimated quantity.

RESULTS: The results indicate that in general about 90% adults both males and females consume caffeine. Children have less caffeine intakes than adults. Around 60% to 80% children consume caffeine. For adults, about 23% of male consume caffeine exceed the RMDI versus 16% female exceed RMDI. For children, it's around 15% for teenagers and 5% for others. The major contributors to caffeine exposure are coffee, tea and soft drinks. Energy drinks might be substantial contributor to young generation, which needs more study.

CONCLUSION/NEXT STEPS: Our next step is to catch the information on energy drink intakes in the future survey collection and update the results accordingly. The dietary exposure data to caffeine in Canadian population will provide scientific information for human health assessment of caffeine and policy-making.

1.32 An Assessment of Poison Control Data as a Novel Source of Information on the Safety of Natural Health Products

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SUMMARY: Natural health products (NHPs) are widely used by Canadians. Monitoring side effects to marketed health products is a key element in maintaining their safe use. Side effects to NHPs are not always reported to Health Canada, so additional sources of information on side effects are being sought. Poison control centre (PCC) data may complement the information currently submitted to Health Canada. This poster describes a study to investigate the nature and amount of data in two Canadian PCCs related to NHPs. Preliminary results show that information exists in poison control databases that may be useful in assessing the safety of NHPs.

BACKGROUND: The monitoring of adverse reactions (ARs) to health products, including natural health products (NHPs), is a key activity for improving their safe use. Under-reporting of ARs to regulators is a recognized problem that hinders the assessment of potential risks associated with the use of health products. The Marketed Health Products Directorate (MHPD) is investigating novel sources of AR information for NHPs, including poison control data from two Canadian Poison Control Centres (PCCs) which receive calls related to NHPs. The full extent and nature of this information is unknown.

METHODS: Based on Provincial population and the use of NHPs, the Ontario Poison Centre (OPC, supported and operated by The Hospital for Sick Children) and the British Columbia Drug and Poison Information Centre (DPIC) were selected for this project. A search strategy was developed to obtain high level information on the number and types of calls received by the PCCs between 2005-2009, related to single ingredient botanical products (e.g., ephedra), as well as multi-botanical products.

RESULTS: Currently, data on the number and type of calls received from the OPC are available. Preliminary data show that the OPC receives about 300 exposure calls per year related to botanical products. Although most of these calls are not related to ARs, AR information does exist in the OPC database.

CONCLUSIONS/NEXT STEPS: The OPC receives calls related to NHPs. AR information for botanical products do exist in their database. The next two to three years of the project will involve: (1) obtaining details on the exact nature of these data, to determine if information collected from calls can be used by Health Canada for the safety assessment of NHPs: and, (2) developing a prospective pilot for NHP AR collection which would capture relevant information in ‘real-time’ as calls are received by the OPC and DPIC.

1.33 An Approach to the Selective Extraction and Concentration of Erythropoietin by Immunochromatography and its Identification by Capillary Electrophoresis: Proof of Concept

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SUMMARY: To assess the presence of small amounts of the therapeutic protein, erythropoietin, in complex mixtures we studied its selective enrichment by the principle of immunoaffinity, that is, through an antibody – antigen complexation reaction. As a first step, we built an immunoaffinity chromatographic column, to which an anti-EPO antibody was attached through a chemical reaction. We then evaluated the ability of the column to selectively retain erythropoietin by performing complexation and decomplexation steps. While the results showed that this process was feasible better decomplexation conditions are needed in order to improve the yield of erythropoietin.

OBJECTIVES: To demonstrate the potential of immunochromatography for the selective extraction and concentration of erythropoietin (EPO) and to ascertain its identity by capillary electrophoresis.

METHODS: Immunoaffinity columns were prepared by binding anti-EPO antibodies to activated silica beads. The immunocolumns were placed on a high performance liquid chromatography system and evaluated for their ability to retain EPO. The optimization of the complexation and decomplexation conditions was carried out by varying physico-chemical parameters such as pH and flow rate. The non-retained and retained peaks were collected and the presence of EPO was ascertained by capillary electrophoresis (CE) using methods capable of separating EPO isoforms.

RESULTS: Two columns were prepared with different monoclonal antibodies: mouse anti-human EPO Clone 9C21D11 and Clone AE7A5. Using generic elution conditions and EPO solutions the two columns were tested and the clone 9C21D11 column was found to retain a significant amount of component. Detection of the retained component was increased significantly by using native fluorescence instead of ultraviolet detection. While the ratio of retained to non-retained components were increased by using a slower flow rate, changes in the buffer pH did not significantly increase the yield. CE analysis of fractions corresponding to non-retained and retained peaks demonstrated that EPO was present albeit at levels lower than anticipated. To determine the cause of the low yield the treatment of collected fractions was modified. Upon simply evaporating the samples CE analysis showed a large peak devoid of the usual isoform profile associated with EPO. Other treatments have also been examined.

CONCLUSION: Results have shown that it is possible to prepare immunoaffinity columns for the selective extraction of EPO. Generic elution conditions led to the decomplexation of EPO as observed by capillary electrophoresis. Further work is required to improve elution conditions to increase EPO recovery while preventing degradation.

1.34 Microarray Correlation Database (MACDB): A Database Model to Discover New Correlations between Genes Across Microarray Experiments

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SUMMARY: Microarray experiments (ME) are conducted to measure globally how genes change their expressions under a particular condition or treatment. To increase the ability to store and extract information across experiments, a relational database, the Microarray Correlation Database (MACDB), was created. MACDB allows scientists to search and look for trends and correlations across experiments to yield new information and maximize results from MEs. Such new information can be used to facilitate and provide new directions for further research.

BACKGROUND: DNA microarrays are widely used to measure changes in gene expression (GE). Datasets derived from MEs are large and are statistically analyzed to measure expression changes for each probe across conditions. In many laboratories, microarray datasets generated from an experiment are often stored independent of other experiments. With proper information management, one will be able to discover previously unidentified correlations between genes, or between genes and environment, by expanding analyses of ME data across experiments.

METHOD: A relational database, MACDB, which utilises the MySQL database engine as the core storage component and Java Server Page (JSP) to create the browser-based user interface, was designed to store and mine ME data. Data tables were designed to capture background information, such as conditions, controls and substances, of an experiment, as well as the customizable tags input by users to further describe the experiment. The database was designed to be compliant to the National Center for Biotechnology Information's (NCBI) Gene Expression Omnibus (GEO) platforms, making import and export data easy.

RESULTS: MACDB stores ME data, and allows users to search and correlate statistically significant genes across experiments. In addition to the probe annotation and experiment information, the ability for users to use customizable tags to describe an experiment provides flexibility and further possibility to mine GE data across analyses and experiments. Compliances with GEO platforms allow users to easily import public GE data with the same or different platforms, as well as output from MACDB to be submitted to GEO. Currently, 10 microarray datasets generated from benzo(α)pyrene experiments have been entered into the database.

CONCLUSIONS: MACDB not only stores data from MEs, but with customizable tags and flexibility to import and export GEO compliant data, it also provides a new dimension to facilitate the discovery of previously unknown correlations between genes across experiments, thus, improving the overall efficiency and depth of toxicogenomics research.

1.35 Development of a Green Fluorescent Protein (GFP) Reporter System to Detect Tandem Repeat Mutations in Murine Cell Lines

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SUMMARY: Exposure to environmental pollutants can cause DNA sequence alteration and structural changes. Such mutations in DNA can lead to cancer and various genetic diseases. Many assays have been developed to evaluate the toxicity of environmental contaminants; however, they are usually very time-consuming and/or lack sensitivity. In this study, we develop a green fluorescent protein (GFP) assay in mammalian cells, which can be used to quickly and effectively detect DNA mutations resulting from exposure to environmental mutagen or carcinogens.

OBJECTIVES/BACKGROUND: Tandem repeat sequences (TRS) are sensitive to environmental exposure and a wide range of stresses including classic mutagens as well as carcinogens. Instability in TRS has been associated with cancer and neurological disease. We sought to develop a quick and reliable *in vitro* green fluorescent protein reporter assay to detect frameshift mutation in TRS resulting from short interfering RNAs (siRNA) knockdown and chemical exposure.

DESIGN/METHOD: To build the reporter vector, a 79 base pair of oligonucleotides containing $(A)_{10}$ repeat sequence of the transforming growth factor beta receptor 2 (tumour suppressor) was inserted upstream of the enhanced green fluorescent protein (EGFP) coding DNA sequence of the pJ6-EGFP plasmid, which put the EGFP sequence out of frame. This reporter-construct was transfected into embryonic murine C3H10T1/2 cells to generate stable transfected populations. Clone 16, named pJ6 (A10)_c16, was chosen for further experiments. TRS instability in the transfected cell was assessed using various chemically synthesized siRNA knockdown technologies. Frameshift mutations resulted in green fluorescent revertants were determined by flow cytometry. mRNA levels of targeted genes with effect on TRS instability were measured by RT-PCR.

RESULTS: Mutation frequency was significantly increased after Fen1, Top2a, Top1, hek2, Recql, Recql4, Lig1 and Rpa2 gene knock down in pJ6(A10)_c16. The highest mutation frequencies were observed with Fen1 and Top2a treatment, more than 20-fold difference from negative control. The expressions of mRNA levels of these genes were decreased dramatically after knock-down.

IMPACTS/CONCLUSIONS: The TRS-GFP reporter system provides a quick and reliable method for detecting frameshift mutations in repeat sequences. It can be applied to characterizations/comparisons of potential carcinogens including agents without DNA reactivity (RNAi as a model). Generation of frameshift mutations by indirect means (with potential low-dose thresholds) has implications for default chemical risk assessment policies mandating linear low-dose extrapolation of cancer bioassay data.

1.36 A Method Based on Florisil Column Separation for Analysis of Non-ortho Co-planar PCB Congeners in Rat Adipose with Exposure to Aroclor 1254

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SUMMARY: Non-ortho substituted coplanar polychlorinated biphenyls (PCBs) are highly toxic and have extremely low concentrations in commercial PCB formulations such as Aroclor 1254. A new method was developed for analysis of the co-planar PCBs in rat adipose from a toxicological study with exposure to Aroclor 1254. A column packed with Florisil adsorbent was used to separate the lipid and ortho-substituted PCBs from the sample prior to gas chromatography analysis. The study was centered on conditions for separation of non-ortho co-plannar PCBs from other PCB components. The method was validated and applied for analysis of samples from toxicological study.

OBJECTIVES/BACKGROUND: To develop a new method for analysis of non-ortho substituted coplanar PCBs in rat adipose samples with exposure to Aroclor 1254.

DESIGN/METHODS: Retention of 35 representative non-planar and co-planar PCBs as well as a number of organochlorine compounds was tested on two sizes of Florisil column (8.0 g and 1.0 g) with hexane eluting, and the cut points of eluting were chosen based on the retention volumes. The non-planar PCBs were eluted out from the column first, and the co-planar PCBs were sequentially eluted with a stronger eluting solvent (30% dichloromethane in hexane). Minor carryover of non-planar PCBs was found with the Florisil column and could be solved with multiple times of column separation (one 8.0 g column and two 1.0 g columns). Oxychlordane, -BHC and cis-Nonachlor have similar retention on the Florisil column with the coplanar PCBs and were used as internal and surrogate standards, and were added to the sample before extraction.

OUTPUT/RESULTS: There is big difference of retention of the non-planar and co-planar PCBs on the Florisil column with hexane as the eluting solvent, and at least five non-ortho substituted co-planar PCBs (77, 81, 126, 127, 169) can be separated from other PCBs. The internal standard (Oxychlordane) was used for correcting the loss of sample during evaporating and transferring. Good recoveries of the surrogate standards (-BHC and cis-Nonachlor) indicate that the column separation is effective, while carryover of the non-planar PCBs is monitored by detection of PCB153. The method was validated and applied for analysis of rat tissue samples.

CONCLUSIONS: Florisil is widely used adsorbent for sample cleanup for organic analysis. In comparison with a method with graphite carbon material, the advantage of the Florisil column for separating the coplanar PCBs is that it can be used to separate the lipid from biological samples simultaneously.

1.37 Alcohol Consuming Reduced the Concentrations of Persistent Organic Pollutants in Laboratory Rat Serum

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SUMMARY: The effects of moderate alcohol consumption on serum retention and tissue distribution of environmental pollutants were examined using a rodent model. Preliminary data indicated that moderate alcohol consumption decreased serum levels of some organic pollutants as compared with no alcohol consumption. It remains to be determined if alcohol consumption also alters tissue distribution of these pollutants.

OBJECTIVES/BACKGROUND: Human beings are exposed to various environmental pollutants such as polychlorinated biphenols (PCBs), organochlorine pesticides (OCPs), brominated flam retardants (BFRs), and chlorinated and brominated phenols which may lead to multiple health problems depending on the levels of exposure. Some human populations are also exposed to alcohol. However, it remains to be a question if alcohol use alters tissue retention and distribution of these pollutants. To answer this question, an *in vivo* exposure study was conducted using a rodent model.

DESIGN/METHODS: Obese JCR rats at 8 weeks old were acclimatized on a purified AIN93G diet. Animals were treated with 10% alcohol in drinking water or drinking water only for 6 weeks. From the third week of alcohol treatment, animals were dosed orally with a mixture of 23 environmental pollutants at 0 (corn oil), 1.6, or 16 mg/kg BW/day for 4 weeks. At the end of treatments, the rats were sacrificed, and serum and a number of tissues were collected. Residue analysis of the organic pollutants in the tissues was carried out with gas chromatography (GC) following treatment of the samples with extraction, derivatization and cleanup. Serum lipid contents were measured gravimetrically.

OUTPUT/RESULTS: Daily consumption of alcohol significantly lowered the serum concentrations of organic pollutants including PCBs, OCPs, BFRs, and pentachlorophenol. The average serum concentrations in the rats were decreased by 29-40% for the high dose groups and 28-53% for the low dose group.

CONCLUSIONS: These results demonstrated that moderate alcohol use can decrease the residue concentrations of organic pollutants in serum, possibly due to altered absorption and/or metabolism of these chemicals. Further analysis is underway to determine if alcohol use also changes the tissue distribution of these pollutants.

1.38 Investigating the Utility of the Muta™Mouse Transgenic Rodent Assay for Regulatory Decision-Making: A Multi-Endpoint Comparison of Several *In Vivo* "False Negatives"

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SUMMARY: Several tests are routinely used to screen chemicals for their ability to damage DNA, an important step in the development of some cancers. These tests are generally effective at identifying hazardous chemicals; however, some animal carcinogens fail to induce positive responses in traditional animal tests for DNA damage. Such compounds are often referred to as "false negatives" for carcinogenicity. This project examines the ability of a recently recognized mouse test (Muta™Mouse assay), which permits DNA mutation scoring in most tissues, to resolve conflicts such as false negatives for carcinogenicity. Two other endpoints for DNA damage were also measured.

BACKGROUND/OBJECTIVE: Test batteries to screen chemical products for mutagenic hazard include several endpoints regarded as effective for detecting mutagens and, by extension, mutagenic carcinogens. However, there is increasing recognition of response inconsistency. *In vitro* "irrelevant positives" elicit significant responses *in vitro* that cannot be confirmed *in vivo*. *In vivo* "false negatives" are rodent carcinogens that fail to induce a positive response *in vivo*. This study is investigating the ability of the Muta™Mouse *lacZ* mutation assay to resolve the latter.

DESIGN/METHOD: Three carcinogenic compounds that elicit positive responses *in vitro* for gene mutation, but are negative for cytogenetic damage *in vivo* in heamatopoetic cells, were selected for evaluation (4,4-methylenebis(N,N'-dimethylaniline) (i.e., MBDA), 2,4-dinitrotoluene, and nitrofen). Muta™Mouse specimens were exposed to 3 doses for 28 days via oral gavage. Following a 3-day fixation period, mice were sacrificed and tissues (e.g., glandular stomach, small intestine, bone marrow, liver) were scored for *lacZ* mutant frequency. In addition, reticulocytes (RETs) and normochromatic erythrocytes (NCEs) were analyzed for *Pig-a* mutant phenotype and micronucleus (MN) frequency.

RESULTS: Results to date show a statistically significant effect of MBDA on the frequency of MN-NCEs and *Pig-a* mutant phenotype in NCEs. *LacZ* mutants did not show a treatment effect for liver and bone marrow; however, several other tissues are currently being examined. Nitrofen, and 2,4-dinitrotoluene exposures have been completed, and tissue analyses are underway.

IMPLICATIONS: The results obtained will contribute to the validation of transgenic rodent mutation assays, and moreover, an appreciation of their ability to resolve conflicts between older *in vivo* endpoints and the results of cancer bioassays.

1.39 Effects of Second-Hand Tobacco Smoke on Male Germ Cells

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SUMMARY: First- and second-hand tobacco smoke are known to cause detrimental health effects. It has been shown that first-hand smoking causes DNA damage and mutation in sperm that can be passed on to the offspring. Little is known about the reproductive consequences of second-hand smoke. This study demonstrates that second-hand smoke affects sperm function and induces DNA damage in a mouse model. These effects were measured at exposure levels that are comparable to those encountered by humans in proximity to smokers and support current Government of Canada regulations to prevent and reduce exposure to tobacco smoke.

OBJECTIVE: To determine whether exposure to first- and second-hand smoke affects the function and integrity of mouse sperm, and to determine whether the effects differ between the two types of smoke.

MATERIALS AND METHODS: Male mice were exposed to two doses of mainstream tobacco smoke (MTS), the smoke inhaled by active smokers, or sidestream smoke (STS), the main component of second-hand smoke. Animals were exposed in inhalation chambers to 3 or 16 cigarettes per day for two weeks using a cigarette smoking machine, alongside sham controls. Sperm function and integrity measures included: motility, DNA damage, and DNA mutations. Exposure to tobacco smoke was assessed by measuring cotinine levels in plasma.

RESULTS: Based on cotinine levels, exposures were comparable to light smokers (<5 cigarettes/day) and passive smokers for MTS and STS, respectively. Specific differences in sensitivity were noted for germ cells following MTS and STS exposure including: (1) only STS reduced sperm motility; (2) only MTS smoke induced DNA strand breaks in sperm; (3) both MTS and STS smoke increased sperm chromatin structure abnormalities; and (4) both MTS and STS smoke caused DNA mutations in sperm. Interestingly, for the majority of the endpoints investigated, there was little evidence for dose-related effects, as exposure to low doses of MTS and STS were as effective as high doses.

CONCLUSIONS: These results show that MTS and STS smoke have differential effects on the genetic integrity and function of sperm. Importantly, the results show that both MTS and STS can induce mutations in sperm that can be passed on to future generations with potentially detrimental effects on their health. These data provide further evidence that male exposure to second-hand smoke, as well as direct cigarette smoke, is likely to have reproductive consequences that go beyond the passive smoker.

1.40 *In Vitro* Dermal Penetration of Di(2-ethylhexyl)adipate (DEHA) in a Deodorant Cream on Human Skin

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SUMMARY: Di(2-ethylhexyl)adipate (DEHA) is widely used as a plasticizer and has its low short-term toxicity. *In vitro* dermal penetration of DEHA in a cream was conducted. The majority of DEHA (43%) was found in the skin and in the cream. Rapid metabolism of DEHA in the skin and its evaporative loss are likely responsible for the low recovery (<50%). The amount of DEHA penetrating skin into blood stream in a 24-hr period in humans is low, but the high amount remained in the skin may have an impact on long term dermal exposure to DEHA in the cream.

BACKGROUND/OBJECTIVE: Di(2-ethylhexyl)adipate (DEHA) has the characteristics of low short-term toxicity. The objective is to investigate *in vitro* dermal penetration of DEHA from a commercial deodorant cream through human skin.

MATERIALS AND METHODS: Cream was applied in triplicate at two dose levels to human breast surgical waste skins. The skin was placed (not grafted) in a Bronaugh flow-through Teflon diffusion cell at 32 °C that was connected with a receiver filled with flow-through solution for the 24-hour exposure experiment. The amounts of DEHA were determined by gas chromatography mass spectrometry or gas chromatography tandem mass spectrometry.

RESULTS: The total amount of DEHA in the receiver solution was less than 0.05% of the total applied DEHA for both the low and high dose studies. The majority of DEHA was found in the skin tissue depot (21% and 34% in the low and high dose experiments, respectively) and in the soap washing solution of the diffusion cell (21% and 8.6% in the low and high dose experiments, respectively). In both low and high dose experiments, DEHA remained in the cream and the skin was about 43% of the total amount applied. The total recovery of DEHA was less than 50% based on mass balance.

CONCLUSION: Molecular diffusion was the major limiting factor in DEHA penetrating the skin into the receiving solution. The low recovery of DEHA was likely caused by rapid metabolism of DEHA in the skin and evaporative loss of DEHA. Based on limited numbers of skin used in the experiments and 24-hr exposure time, it was concluded that DEHA penetrating skin into blood stream in a 24-hr period is likely low, 2.2 ng/cm^2/hr in both low and high doses. However, the remaining DEHA in the skin would likely continue migrating into blood stream over time, which needs further research.

1.41 *In Vitro* Exposure of A549 Cells to Ambient Air Particles and Investigation of Related Protein Changes by MALDI-TOF-MS

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SUMMARY: To gain insight into the acute effects of respirable particles on the lungs, we applied shot-gun mass spectrometric analyses after exposure of human cells to Ottawa particles. Comparison of peptide mass spectra identified integrin-alpha-2, cytoplasmic beta-actin, epithelial membrane protein-3, and immunoglobulin-lambda like-polypeptide-1 as potential markers of cellular response. Following this proof of concept, responses of epithelial cells and macrophages will now be studied following exposure to size-fractionated particles collected at different locations in Canada impacted by various sources of contaminants. The data should help link toxic potency of particles to sources in support of the Clean Air Regulatory Agenda.

OBJECTIVE/BACKGROUND/ISSUE: Breathing ambient particulate matter is associated with adverse health outcomes, but effects are complex, involving different organs, cellular targets and pathways. Simplified models are useful to investigate cellular and molecular mechanisms of toxicity. Proteomic analyses of cell cultures provide information on protein profile changes caused by stressors. For this, matrix assisted laser desorption-time of flight-mass spectroscopy (MALDI-TOF-MS) is particularly suitable for protein profiling of complex biological matrices. Our objective was to investigate proteome changes in lysates of cells exposed to ambient air particles in order to identify potential biomarkers of cytotoxicity.

DESIGN/METHOD/DESCRIPTION: Human lung epithelial cells (A549) were exposed to different doses (0, 30, 100 and 300ug/cm^2) of Ottawa EHC-6802 ambient particles for 24h. Freeze-thaw cell lysates were clarified at 12,000Xg, 10 min and digested with trypsin prior to MALDI-TOF-MS analyses. Mass spectra were mined for potential biomarkers of particle exposure by pattern recognition using k-nearest neighbor algorithm in ClinproTools software. Identity of candidate ions were determined using the MASCOT search engine through the SWISSPROT and MSDB databases.

OUTPUTS/RESULTS: Our analyses identified eighteen potential biomarkers of which four (m/z 1065.5, 1517.1, 2730.1 and 2015.3) were characterized further. These were identified as integrin-alpha-2 subunit fragment (integrins are involved in cell adhesion and cell-surface mediated signalling), cytoplasmic beta-actin (cytoskeleton and mediator of internal cell motility), epithelial membrane protein 3 (cell-cell interactions and negative regulation of cell proliferation), all three being up-regulated, and immunoglobulin-lambda like-polypeptide-1 (signal transduction), which was down-regulated.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS: MALDI-TOF-MS can be utilised for rapid screening of cells exposed to ambient air particles *in vitro*. This approach will be applied to screen a panel of size-fractionated particles collected at different locations in Canada and impacted by various sources of contaminants. The data should help link toxic potency of particles to sources in support of the Clean Air Regulatory Agenda.

1.42 Exploring DNA Adducts by Mass Spectrometry for the Application in Exposure Monitoring

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SUMMARY: Exposure to environmental contaminant results in the formation of DNA adducts. This in term can lead to negative health effects. Formation of these adducts can serve as biomarkers in contaminant exposure assessments. Here we describe two mass spectrometry methods, liquid chromatography-mass spectrometry (LCMS) and Matrix Assisted Laser Desorption Time of Flight-mass spectroscopy (MALDI-TOF-MS) for the identification of DNA adducts that can be used for exposure monitoring. The results exhibited that combination of both methods enhance the confidence in the analysis of these adducts in biological samples.

OBJECTIVES/BACKGROUND/ISSUE(S): Chemical components of environmental pollutants can bind to DNA resulting DNA adduct formation. DNA adducts are typically analysed by using radio labelled compounds, for instance ^{32}P post labelling method. Our intention here was to develop an alternative method that does not require use of radio labelled compounds. We therefore chose a mass spectrometry approach. In addition our goal was to tailor this method to be suitable for rapid exposure assessment analysis on environmental contaminants.

DESIGN/METHOD/DESCRIPTION: Here we chose to synthesize DNA adducts of adenosine with crotonaldehyde, a commonly occurring chemical contaminant present in the environment as a model. Adenosine was reacted with crotonaldehyde in the presence of rat liver S9 fraction for 48h at 37^0C followed by organic extraction. Samples were then subjected to LCMS and MALDI-TOF-MS/MS analyses. LCMS parameters were established as; Column: reversed-phase C18 (capillary), mobile phase: 10%ACN in aqueous solution (0.1%TFA) to 90%ACN in aqueous solution (0.1%TFA), flow rate: 10uL/min. Samples were spotted on MALDI target plates, dried, washed with cold aqueous solution (0.1%TFA) and analysed by tandem MALDI-TOF-MS/MS.

OUTPUTS/RESULTS: Our LCMS results revealed identification of two DNA adducts along with the starting DNA base. Both adducts were detected as m/z 337 which is most likely a Micheal addition product of adenosine (m/z 267) and crotonaldehyde (m/z 70). Our tandem mass analysis of MALDI-TOF-MS/MS also revealed the presence of the adduct at m/z 337 as a parent mass.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Preliminary results showed that both LCMS and MALDI-TOF-MS/MS can be utilised to detect formation of DNA adducts when biological tissues/cells are exposed to environmental contaminants. Use of these methods in combination provides greater confidence in identification of DNA adducts. These methods are amenable to high through-put analysis and can be applied in bio-monitoring studies and thus in hazard identification.

1.43 Altered Folate Metabolism and Placental Development

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SUMMARY: Pre-eclampsia (PE) is a disease of pregnancy, which develops during the third trimester. Folic acid supplementation in the second and third trimesters has been shown to decrease the risk for PE development. This study aims to determine the effect of MTR and MTHFD1 knockdown, two enzymes in the folate metabolic pathway, on placental cell proliferation and differentiation. Increased folic acid in the culture was associated with increased cell proliferation. Knockdown of the MTHFD1 or MTR genes, result in impaired cell proliferation. The response to folic acid supplementation in women at risk for PE may depend on their underlying gene profile.

BACKGROUND: Folate is an essential water-soluble B Vitamin required for purine, thymidylate, and, methionine synthesis. Pre-eclampsia (PE) is a two-stage disease of pregnancy characterized by hypertension and proteinurea, and risk for renal impairment, HELLP syndrome, and end-organ damage developing during the third trimester. Defective placental development is thought to contribute to PE risks. Folic acid (synthetic form of folate) supplementation in the second and third trimesters has been shown to decrease the risk for PE development.

OBJECTIVE: The objective was to determine the effect of MTR and MTHFD1 knockdown, two key enzymes in the folate metabolic pathway, on placental cell proliferation and differentiation.

METHOD: Human placental choriocarcinoma (JEG3) cell cultures were maintained in MEM Alpha media containing 10% fetal bovine serum and penicillin/streptomycin. Knockdown of MTR and MTHFD1 was achieved using gene-specific siRNA. Total RNA was extracted from siRNA-transfected JEG3 cells and cDNA was synthesized in a reverse transcriptase reaction. qPCR was used to determine gene expression knockdown. Cell proliferation was assessed by BrdU Immunohistochemistry.

RESULTS: 40% and 50% gene expression knockdown was achieved for MTHFD1 and MTR, respectively, 24 hours post-transfection. Increased folic acid in the culture was associated with increased cell proliferation. MTHFD1 and MTR gene knockdown significantly inhibited cell proliferation by approximately 12% and 20%, respectively. Folic acid supplementation corrected cell proliferation in MTHFD1, but not in MTR, knockdown cells.

CONCLUSIONS: These data indicate that JEG3 cell proliferation is dependent on folic acid. In addition, knockdown of the MTHFD1 or MTR genes result in impaired cell proliferation. Therefore, the response to folic acid supplementation in women at risk for PE may depend on their underlying gene profile.

1.44 From Micro to Nano: Instrumentation for Measurement of Airborne Particles in the Workplace

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SUMMARY: The rapid development of nanotechnology and the related increase of commercial nanotechnology consumer products have caused significant public and legislative concern about the health effects of exposure to nanoparticules (NPs) or ultrafine particles (UFPs) in size less than 100 nm. The assessment of NP's or UFP's potential exposure safety and health risks is, however, limited due to lack of suitable detection techniques. The purpose of this project is to assemble and test an instrument/equipment suite capable of detecting and characterizing NPs or UFPs in the workplace to address this knowledge gap.

OBJECTIVES/ISSUE(S): The objective is to establish a reliable sample collection, measurement and characterization strategy to address current data gaps related to workplace exposures to sub-micron and nano-scale airborne particles. The first goal is to develop standard operating procedures (SOPs) for a suite of direct-reading instruments and test the system in a federal laboratory environment. Key exposure parameters include exposure source and level, particle size distribution, surface area, and mass concentration.

DESIGN/DESCRIPTION: Determinations of particle size distributions and particle number concentrations were made using a water-based Scanning Mobility Particle Sizer (SMPS) an alcohol-based SMPS, Condensation Particle Counters (CPCs), an Aerodynamic Particle Sizer (APS), a Differential Mobility Analyzer (DMA) and a portable aerosol spectrometer. Real-time respirable mass estimates were obtained using the TSI DustTrak II and DRX models. Active surface area measurements were made using a EcoChem DC 2000 CE. This equipment suite was deployed in various Federal laboratory and office environments to establish typical background exposures.

RESULTS: The integrated instrument suite provided real-time simultaneous measurements of a wide range of particle sizes from 0.8 nm to 32 µm. Measurements of particle number concentrations ranged from 3.6 $x10^2$ to 6.6 x 10^6 #/cm^3 (at 3 nm) and from 3.3 $x10^1$ to 5.8 $x10^5$ #/cm^3 (at 100 nm); respirable mass concentration from < 2 to 30 µg/m^3; and active surface areas up to 64 mm^2/m^3. These measurements are particle size and environment dependent and vary widely over time.

IMPACTS/OUTCOMES: The direct-measurement suite of instruments will be used in combination with filter-based technologies to help address existing data gaps related to Canadians' exposures to NPs and UFPs that arise from their daily working and indoor activities.

1.45 De-Liver-Ing on Biomarkers: Hunting for Indicators of Thyroid Hormone Disruption

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SUMMARY: Thyroid hormone (TH), produced by the thyroid gland, is essential for growth and metabolism. This project examined the effects of changes in TH concentrations during liver development on gene expression, to understand how TH functions. We treated mice with chemicals causing low TH levels, and compared them to mice with normal and high TH levels. We found 28 genes in the liver that were directly responsive to changes in TH. We also identified small DNA sequences that appear to be involved in these responses. Results will help identify chemicals that affect TH action improving Health Canada's ability to regulate these substances.

BACKGROUND/OBJECTIVES: Toxicant-induced disruption of TH signalling during early life can alter growth, development and energy metabolism. THs exert their effects through interaction with specific receptors directly bound to DNA adjacent to genes whose transcription they control. To investigate the molecular consequences of thyroid hormone disruption during development, the effects of short-term TH perturbation on hepatic mRNA transcription in juvenile mice were evaluated. The objectives of this research are to further understand the mechanisms of TH action during liver development and to identify candidate biomarkers for the study of TH-disrupting chemicals.

METHOD: TH disruption was induced from postnatal day 12 -15 by adding goitrogens (substances that suppress the production of THs) methimazole and sodium perchlorate to dams' drinking water (hypothyroid). A subgroup of TH-disrupted pups received intraperitoneal injections of replacement THs 4 hr prior to sacrifice (replacement). An additional group received only THs prior to sacrifice (hyperthyroid). Hepatic mRNA was extracted and hybridized to Agilent mouse microarrays. A search of thyroid response elements (TREs) was performed by searching the regulatory regions of the genome adjacent to these genes for sequences that resemble known TREs.

RESULTS: Serum thyroxine levels in PND 15 pups were significantly altered after the three-day exposure. MAANOVA analysis identified about 400 significantly altered genes in male and/or female pups in at least one treatment condition. A bioinformatics search identified 33 TREs in the promoter regions of genes, thought to be directly regulated by THs. TREs found in the promoter regions of *tor1a*, *2310003H01Rik*, *hect3d* and *slc25a45* were further validated by confirming that the TH receptor is associated with these sequences *in vivo* and that it can bind directly to these sequences *in vitro*.

CONCLUSION/NEXT STEPS: These results provide insight into the TH-regulated hepatic transcriptome of juvenile mice and increase our understanding of the mechanism by which TH modulates liver development. Moreover, genes identified as directly responsive to thyroid hormone levels can be used to indicate chemical effects on thyroid hormone as part of toxicity studies.

1.46 Tumor Outcomes Following Dietary Acrylamide Exposure in Two Experimental Models of Human Colon Cancer

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SUMMARY: Acrylamide is a known rodent carcinogen, and has been classified as a "probable human carcinogen". Acrylamide is ubiquitously formed in certain foods cooked at high temperatures. Acrylamide reaches high levels in popular foods such as bread, French fries, potato chips and coffee; and thus requires health risk characterization for its exposure. In this study, we aimed to evaluate if dietary acrylamide acted as a colon carcinogen using experimental models. Acrylamide did not induce colon tumors, but in the presence of a known colon-specific carcinogen, it acted as a co-carcinogen. In addition, acrylamide did not exacerbate the progression of human colon tumors growing ectopically in an animal model.

OBJECTIVES/BACKGROUND/ISSUE(S): We earlier reported that acrylamide, when administered in the diet at doses known to cause rodent tumors, did not increase the formation of carcinogen-induced colon aberrant crypt foci (ACF), putative precancerous lesions. However, at the lowest dose of acrylamide tested (5mg/Kg diet), a trend to increase ACF was observed. In this study, we tested if dietary acrylamide, at levels reflecting those in popular human foods, modulated colon tumor formation and progression.

DESIGN/METHOD/DESCRIPTION: In Experiment-1, weanling male F344 rats were randomized into 4 groups to receive AIN-93G diets with acrylamide at 0.5, 1.0 or 2.0 mg/kg diet or without (0 mg/kg diet, control). After two weeks, rats in each diet group were further divided to receive two weekly sub-cuteaneous injections of azoxymethane (AOM; 15 mg/Kg body weight; n=24/group) or saline (0.2 mL/rat; n=8/group). All rats were on the experimental diets *ad libitum* for 20 weeks post AOM/saline injections, after which they were killed and their colons assessed for tumors. In Experiment-2, athymic nude mice (*nu/nu*) were subcutaneous injected with HT-29 colon adenocarcinoma cells and tumors were allowed to grow for three weeks, after which they were randomized to receive one of the four diets previously mentioned; tumor growth was monitored bi-weekly and the mice were killed four weeks after.

OUTPUTS/RESULTS: None of the saline-injected rats had tumors in their colon. 23 rats that received acrylamide in the diets had blood in their stool at 19 weeks post-AOM injections; of those, 10 rats were from the highest dose group tested, prompting us to end the study in a timely manner at 20 weeks post-AOM injections. The colon tumor incidence was 54.2% in the control group and 66.67% in the highest tested acrylamide group (2 mg/kg diet). Tumor multiplicity was similar across all diet groups. However, tumor size and burden were higher in the highest tested dose of acrylamide at 2 mg/kg diet by comparison to the control. In the nude mice study, there were no differences in the growth of ectopic human colon tumor growth between acrylamide-treated and control mice.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: These results suggest that acrylamide in the diet at the doses tested is not a “carcinogen”, but acts as a “co-carcinogen”, exacerbating the effects of a known colon-specific carcinogen AOM in increasing the risk of developing colon tumors. This co-carcinogenic effect of acrylamide is observed only at the highest tested dose of acrylamide (2 mg/kg diet) in this rodent model. Additionally, acrylamide did not increase the progression of ectopic tumor growth in the nude mice model. Our data implies that acrylamide by itself is not carcinogenic to the colon at upper exposure levels commonly seen in popular foods in this experimental model of human colon cancer. However, in association with other environmental tumor initiators/promoters, dietary acrylamide at similar doses may have a tumor-promoting role. Whether dietary acrylamide further impacts in a tumor-promoting capacity under conditions such as metabolic disorders, obesity or inflammation is unknown and provides impetus for future exploration. Our data provides additional information in understanding the health risks of acrylamide when exposed through the diet and would facilitate risk management strategies for policy and regulation of acrylamide by Health Canada. (This project is funded under the Chemicals Management Plan, Government of Canada)

1.47 Impacts of Mandatory Trans Fat Labelling Regulations and Nationwide Product Reformulations to Reduce *Trans* Fatty Acid Content in Foods on Trans Fat Levels in Canadian Women's Breast Milk

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SUMMARY: This study, measured *trans* fat in 282 Canadian human milk samples collected in 2009-11. The mean *trans* fat content found was 2.5% of total milk fat. This value is considerably lower than the value of 7.2% found in 1992. This data suggests that the *trans* fat labeling regulations implemented in 2005 and recommendations by Health Canada in 2007—instructing the food industry to limit *trans* fat in margarines to less than 2% of total fat and to 5% or less in all other foods—resulted in significant reductions of trans fat in human milk and the breast feeding mother's diet.

OBJECTIVE/BACKGROUND: Industrial *trans* fatty acids (TFA) are formed by partial hydrogenation of vegetable oils to produce semi-solid fats for use in food manufacturing. Consumption of TFA increases the risk of coronary heart disease. Recent efforts to reduce TFA in foods include mandated inclusion of TFA content on food labels and recommendations by Health Canada that encourage the food industry to voluntarily limit TFA content in margarines to 2% of total fat and in all other foods to 5% or less of total fat. To assess the impact of these efforts, the concentration of TFA in human breast milk samples was measured. The breast milk TFA reflects the TFA in the mother's previous day diet; therefore, breast milk is a convenient biological sample for establishing dietary levels of TFA.

METHODS: TFA content in 282 breast milk samples collected in 2009-11 across Canada, from Canadian mothers, was analysed by gas chromatography.

RESULTS: The mean TFA content was 2.5% of total milk fat. This value is considerably lower than the value of 7.2% found previously for Canadian human milk in 1992. Based on the current TFA mean value, the intake of TFA by Canadian breast-feeding mothers was estimated at 1.5 g per day. This value is lower than the maximum intake of 2 g TFA (or 1% of total food energy) for a healthy diet recommended by WHO. The average *trans* isomer profile of the human milk samples was similar to that of cow's milk fat, suggesting that dairy fats—not partially hydrogenated vegetable oils—are the primary source of TFA in the current Canadian diet.

CONCLUSIONS: Canadian *trans* fat labelling regulations and recommendations aimed at the food industry to limit trans fat in foods have resulted in significant reduction in industrial TFA content in the diet of Canadian breast-feeding mothers.

1.48 Mutagenic Activation of Bisphenol A in Inflammation-Stimulated RAW 264.7 Cells

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SUMMARY: Tissue inflammation, involving redness and swelling, as encountered during infection or arthritis, produces a number of reactive chemical species, which can contribute towards cancer development. The interaction of these reactive species with other components in the diet and effects on cancer risk are not usually included in chemical safety assessments. Using a cell line-based assay system, the cancer initiation potential of Bisphenol A, a food and water contaminant, was found enhanced under conditions where inflammation was induced. The inclusion of assays examining the impact of inflammation on chemical metabolism may warrant changes in policies regarding chemical health effects assessments.

OBJECTIVES/BACKGROUND/ISSUE(S): During the inflammatory response, expression for genes coding for nitric oxide synthase, prostaglandin H synthase 2 and myeloperoxidase is increased. These enzymes produce a variety of free radical species, which can participate in chemical mutagenic activation through corresponding oxidation, nitrosation and chlorination reactions. Thus, individuals fighting infection or afflicted by inflammatory disease might be considered a temporary member of a vulnerable population, moving in and out of higher cancer risk throughout life, according to inflammation status and extent. Test systems are therefore needed to examine the effect of inflammation on mutagenicity/ carcinogenicity as well as other health outcomes.

DESIGN/METHOD/DESCRIPTION: Mouse RAW 264.7 macrophage cells were stimulated with lipopolysaccharide and interferon γ. The toxicity of bisphenol A, a food contaminant of concern for endocrine disruption and carcinogenesis, was characterized using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay. The effect of inflammation on bisphenol A mutagenicity was then examined at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) gene locus by assessing formation of thioguanine resistant mutant colonies. Results were compared with unstimulated cells.

OUTPUTS/RESULTS: The toxicity of BPA, as measured by the MTT assay, was comparable in both unstimulated and stimulated RAW 264.7 cell cultures with an LD_{50}=55 μM found for unstimulated cells and LD_{50}=43 μM found for stimulated cultures. When examined for mutagenicity at the HGPRT gene locus, little evidence for BPA mutagenicity could be found in unstimulated cultures when tested to 10 μM, but BPA was found to be mutagenic in stimulated cultures, with a two-fold increase in mutagenicity found at 100 pM BPA, increasing to 6-fold increase at 1 μM.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The impact of inflammation on chemical metabolism may warrant changes in policies regarding chemical health effects assessments. The RAW 264.7 cell line provides a commercially available platform to examine the effects of inflammation induction on mutagenicity or other biological effects.

1.49 Differential Effects of Dietary Fructooligosaccharides and Wheat Bran on Expression of Genes Involved in Transport, Signaling, Apoptosis, Cell Proliferation and Oncogenesis in the Proximal Colon Epithelia of Healthy Fischer 344 Rats

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SUMMARY: Health Canada is investigating changing its dietary fibre (DF) definition to include “man-made” carbohydrates such as fructooligosaccharides (FOS), which are deemed DF in other countries. In order to be considered as DF in Canada, FOS must confer an “acceptable” physiological benefit. In addition, the list of acceptable physiological benefits may be expanded to include fermentation. This study examined links between fermentation and physiological outcomes by measuring gene expression in colonic epithelium of rats after feeding two different fermentable materials (FM): wheat bran (WB) or FOS. The results showed that similar amounts of FM from different sources elicited different effects. Therefore, both the source and amount of FM must be considered by Health Canada for the development of policies on dietary fibre.

OBJECTIVES/BACKGROUND/ISSUE(S): To investigate the differential effects of two sources of dietary FM (FOS and WB) on the expression of genes involved in specific functional pathways in the proximal colon of healthy rats.

DESIGN/METHOD/DESCRIPTION: Male Fischer 344 rats (10/group) were fed for six weeks either a control diet containing 10% (g/g) cellulose; or a 2%, 5%, or 10% (g/g) WB diet; or a 2%, 5% or 8% (g/g) FOS diet. RNA was extracted from proximal colon epithelia of each rat. Real-time quantitative PCR was used to assess the mRNA expression of genes known to be involved in monocarboxylate transport, G-protein coupled receptor signaling, cell proliferation, apoptosis and oncogenesis.

OUTPUTS/RESULTS: Compared to controls, rats fed rapidly fermentable FOS-based diets had higher mRNA levels of genes involved in SCFA transport such as monocarboxylate transporters, *Smct2*, *Mct1* and *Mct4* and lower mRNA levels of *Mct2* ($P \leq 0.05$). FOS-based diets also altered G-protein signaling genes by increasing *Gpr109a* mRNA expression, and decreasing mRNA levels of *Gpr120*, *Gpr43*, *Gprc5a*, *Rgs2* and *Rgs16* ($P \leq 0.05$). The FOS-based diets also increased mRNA levels of some known apoptosis-related genes including *Bcl2*, *Bcl2-like 1*, *Bak1*, *Caspase 3*, *Caspase 8* and *Caspase 9* ($P \leq 0.05$). FOS also decreased the mRNA levels of oncogenes and metastasis genes *Ros1*, *Fos*, *Cd44*, *Fn1* and *Plau*, as well as several genes involved in cellular proliferation including *Hbegf*, *Hoxb13*, *Cgref1*, *Wfdc1*, *Tgm3*, *Fgf7*, *Nov* and *Lumican* ($P \leq 0.05$). The diet containing slowly fermentable WB only increased mRNA levels of *Smct2*, *Rgs16*, *Gprc5a*, *Gpr109a*, *Bcl2-like 1*, *Caspase 8*, and *Fos* ($P \leq 0.05$).

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Similar amounts of dietary FM from FOS or WB differentially altered the expression of genes involved in SCFA transport, Gpr signaling, apoptosis, cell proliferation and oncogenesis in proximal colon epithelia of healthy rats indicating that fermentation alone is not the sole determinant of gene responses. Therefore, both the source and amount of FM may affect colon epithelial functions differently and both must be considered for the development of policies on dietary fibre.

1.50 Concurrent Measurement of Dechlorane Plus and Polybrominated Diphenylethers in Human Milk

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SUMMARY: One of the emerging flame retardant is Dechlorane Plus (DP). Its presence has been reported in various environmental matrices. The purpose of this study was to concurrently measure the levels of DP and polybominated diphenyl ethers (PBDEs) in human milk. DP levels in human milk were found to be lower than those of PBDEs and were not elevated when compared to those in other biological species. However, when compared to levels in house dust, DP seemed to have greater bioaccumulation potential than PBDEs, this shall be confirmed by further research.

BACKGROUND/OBJECTIVE: Polybrominated diphenyl ethers (PBDEs) were banned/restricted in various jurisdictions. Many other halogenated flame retardants (HFRs) are still in use. One such HFR is dechlorane plus (DP). DP is a chlorinated flame retardant identified by U.S. EPA as a High Volume Prodution (HVP) chemical. So far, there are no reports on the levels of DP in general population. The objective of this study was to determine the relative concentrations of DP and PBDEs in human milk.

METHOD: 87 milk samples from healthy women were collected. 2.0 g of milk was spiked with labelled surrogates. 12 g of sodium sulphate was added and the milk was extracted twice, each with 15 ml of DCM:hexane (1:1 v/v), followed by GPC cleanup. The GPC fraction of 12-22 min was collected and reduced to 500 µl. Internal standard was added prior to GC/MS (using DB-1MS column) analysis. The MS was operated in electron capture negative chemical ionization (ECNCI) mode using selected ion monitoring (SIM) program. Statistical analyses were done using SAS/STAT software, version 9.2.

RESULTS: Total DP concentrations varied from ND to 8.0 ng g^{-1} (mean ± SD: 0.98 ± 1.34 ng g^{-1}). The mean f*anti* (value of *anti*-DP divided by total DP) value (0.67 ± 0.10), was very similar to that in DP commercial products. BDE- 47 had the highest level (10 ± 12 ng g^{-1}), followed by BDE-153 (5.2 ± 10.1) and BDE-99 (4.1 ± 4.5), and BDE-100 had the lowest level (3.0 ± 3.7 ng g^{-1}). Three distinguished exposure sources: DP, deca-BDE, and penta- / octa-BDEs was seen.

CONCLUSION: DP levels in human milk were found to be lower than those of PBDEs and were not elevated when compared to those in other biological species. However, when compared to levels in dust, DP seemed to have greater bioaccumulation potential than PBDEs. This needs to be confirmed by further research.

1.51 Mainstream Smoke Emissions from "Super Slim" Cigarettes Sold in Canada Under Four Different Smoking Conditions

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SUMMARY: In 2007, several super slim cigarette (SSC) brands with much smaller circumference than the conventional cigarettes were introduced. The much slimmer cigarettes seemingly reduce the amount of smoke they produce and may give the impression that they are a reduced harm product. This study revealed that the SSC have less tobacco than the conventional cigarettes. However, due to the filter design of the SSC, the concentrations of many chemicals in the mainstream (MS) smoke of the SSC, including nicotine, were comparable to the conventional cigarettes. In addition, formaldehyde, the phenolic compounds, and ammonia were much higher in the MS smoke of the SSC than other Canadian cigarettes.

OBJECTIVE: To examine the super slim cigarette brands that are sold in Canada, and to analyse the effect of the smaller circumference cigarette design on smoke emissions.

MATERIALS AND METHODS: The study examined the effect of the smaller circumference cigarette design on mainstream smoke emission by comparing six Canadian brands of super slim cigarettes with conventional circumference cigarettes. Four different smoking conditions were used including the ISO, the Health Canada Intense and two other alternative smoking conditions. Mainstream smoke emission was analyzed according to the *Tobacco Reporting Regulations* (TRR) for analytes, which included for example tar, nicotine, carbon monoxide, ammonia, carbonyls and phenols, using the Health Canada Official Methods. To better assess how some of the cigarette designs could influence smoke composition, analyte yields per gram of tobacco in cigarette, i.e., analyte concentrations, were examined and were used for comparison.

RESULTS: The analyses of the Canadian super slim cigarette brands showed that while the super slim cigarettes had the same length and packing density as the conventional cigarettes, there was about half the amount of tobacco. When comparing the mainstream emissions of a super slim brand to a conventional brand with similar tip ventilation, all analyte concentrations of the super slim brand were either similar to or higher than those of the conventional brand under all four smoking conditions. The amounts of nicotine delivered by the super slim cigarettes were comparable to that of the conventional cigarettes, while formaldehyde, the phenolic compounds (phenol, o-cresol) and ammonia were predominantly higher in the super slim brands.

CONCLUSION: While it is important to examine the pattern of use as well as understand the interaction between product designs and smoking behaviour in humans when evaluating the risk of a tobacco product, the results from this study indicate that mainstream smoke emissions from Canadian super slim cigarettes are comparable to those from conventional cigarettes. Therefore, the Canadian super slim cigarettes should not be considered a reduced harm product.

1.52 The Classification of Foods in the Canadian Nutrient File (CNF) According to "Eating Well with Canada's Food Guide" (EWGFG)

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SUMMARY: The Canadian Nutrient File (CNF) is a database that lists nutrient values of over 5800 foods. It is often used to analyze the nutrient content of reported intakes of foods from food consumption surveys done at the national (Canadian Community Health Survey cycle 2.2 (CCHS 2.2) led by Statistics Canada) or provincial levels. The classification of foods within the CNF was not aligned with the recommendations of the most recent Canadian Food Guide (EWCFG). Therefore, assessment of the diets of Canadians according to EWCFG was not possible. A nutrient profile model was developed based on the EWCFG guidance to allow for such assessment.

BACKGROUND: In order to allow Health Canada to assess how well Canadians are following EWCFG, a classification of the foods in the CNF according to the EWCFG guidance was necessary. The objective of this work was to categorize (classify) foods in the CNF according to EWCFG and validate this classification.

MATERIAL AND METHODS: Based on EWCFG's guidance, a nutrient profiling model was developed. The key nutrients used to classify foods in the CNF were: total fat, sugars and sodium. Based on the Criteria for Nutrition Claims and the Nutrition Facts Education Campaign, upper and lower thresholds were established and foods were placed into one of three tiers within each food group. As such, Tier 1 foods follow the EWCFG guidance (low fat, low sugars, low sodium), Tier 2 foods mostly follow the guidance (little fat, and/or little sugars and/or little sodium), whereas Tier 3 foods do not follow the guidance (high fat or high sugars or high sodium).

RESULTS: Using the EWCFG food patterns, 500 simulated diets were created for the 16 age-sex Dietary Reference Intake (DRI) groups using foods from Tier 1-2. The nutrient content of these diets were assessed against their appropriate DRI values. This validation process will ensure that when eating foods from Tier 1 and Tier 2, energy requirement is not exceeded, while nutrient needs are met. The results of the validation exercise will ensure that the nutrient profile model developed classifies foods in alignment with the guidance in the EWCFG. The validation process in ongoing at the time this abstract was written.

CONCLUSION: The resulting nutrient profile model will allow the assessment of diets against EWCFG but could also serve other purposes and applications involving surveillance and/or research and/or policy within the Health Portfolio.

1.53 The Effects of Multi-Generational Deficient and Enriched Dietary Folic Acid on Chromosome Damage in Mice

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SUMMARY: The aim of this project was to determine whether high intakes of folic acid could lead to DNA damage in red blood cells of mice. This is important, as blood folate levels in the population have increased due to the mandatory fortification of grain products with folic acid in 1998. We found that a high intake of folic acid did not cause DNA damage or protect against DNA damage in mice. The data alleviate concerns relating to the DNA damaging potential of long-term exposure to high dietary folic acid.

BACKGROUND: Folate deficiency decreases *de novo* nucleotide synthesis and cellular methylation potential, which can lead to DNA damage. Folic acid supplementation has been hypothesized to inhibit folate-dependent one carbon metabolism, which could also lead to DNA damage. Since the introduction of fortification, a significant proportion of the general population has blood folate levels indicative of high folic acid intakes. The present work examines the effects of diets supplemented or deficient in folic acid on DNA damage to address concerns surrounding fortification.

METHODS: Micronuclei are pieces of damaged chromosomes that lag behind during anaphase, an indicator of DNA damage. The micronucleus assay was used to investigate micronucleus frequency in Balb/c mice maintained on folic acid deficient (0 mg/kg), regular (2 mg/kg) or supplemented diets (6 mg/kg) for: (a) three generations; or (b) from weaning for 15 weeks. Micronuclei were measured in reticulocytes (immature blood cells) and erythrocytes (mature blood cells), which were differentiated by reticulocyte-restricted transferrin receptor expression. Intracellular DNA was stained with propidium iodide. Micronucleus-containing cells were measured by flow cytometry.

RESULTS: *Weaning study:* The folic acid deficient diet resulted in a significant 1.24-fold increase ($P < 0.001$) in micronucleus frequency in the erythrocytes of mice. *Multiple generation study:* The deficient diet resulted in a statistically significant 3-fold and 2.7-fold increase ($P < 0.0001$) in micronucleus frequency in reticulocytes and erythrocytes, respectively. The supplemented diet did not cause changes in micronucleus frequencies.

CONCLUSIONS: Folic acid deficiency leads to an increase in DNA damage in red blood cells of mice from weaning to maturity and over multiple generations. Folic acid supplemented diets did not cause changes in micronucleus frequency, suggesting that excess folic acid is not detrimental nor provides protection against DNA damage. The data alleviate concerns relating to the clastogenic potential of chronic exposure to high dietary folic acid.

1.54 Weight of Evidence in Risk Assessments

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SUMMARY: A “weight of evidence approach” is frequently cited as the basis on which risk assessment conclusions are made. However, multiple interpretations and a lack of consensus about its meaning could potentially compromise communication between diverse stakeholders in the decision-making process. In response to this issue, a high level analysis of the Weight of Evidence approach was initiated by Health Canada's Science Policy Directorate in 2010, as a project under the workplan of the Task Force on Scientific Risk Assessment. By examining current interpretations and identifying potential best practices, the analysis aims to enhance the consistency and coherence of risk assessments.

BACKGROUND: Risk assessment of scientific evidence is a crucial component of evidence-informed decision making at Health Canada. Moreover, for many regulatory programs of the Department, risk assessment conclusions and recommendations must often be made in the absence of definitive proof of causation between a particular substance/activity and associated health effects. In this context, a “weight of evidence approach” is frequently cited as the basis on which risk assessment recommendations, and ultimately risk management decisions, are made.

However, use of such terminology is in many instances not accompanied by precise definitions or elaborations. In many cases, this results in multiple interpretations of the weight of evidence approach, and a lack of consensus about its meaning. In response to this issue, a high level analysis of the weight of evidence approach was initiated by Health Canada's Science Policy Directorate in 2010, as a project under the workplan of the Task Force on Scientific Risk Assessment.

METHOD: The weight of evidence approach as interpreted, practiced, and documented by various risk assessment programs at Health Canada was surveyed and compared with guidelines from key international partners.

RESULTS IMPACTS AND CONCLUSIONS: While specific tools and methodologies are often context specific to particular program areas, the underlying principles of the weight of evidence approach, in which multiple sources of information are gathered, assessed, and integrated into an overall conclusion, were commonly applied across the Department, and were judged to be consistent with international practice.

Potential areas for harmonization were identified in the form of checklists for transparent documentation. Additionally, graphically based evidence maps, profiles, or tables may be helpful as supplementary tools for communication from risk assessors to risk managers.

The explanatory document would serve as a value-added Departmental resource of high level contextual information and guiding principles to supplement program specific guidelines and tools.

1.55 Types, Quantity and Accuracy of Carbohydrate, Glycemic Index, Sugar and Fibre Messages in Canadian Magazines

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SUMMARY: Print media is a major source of nutrition information for Canadians. No known research has examined media messages related to carbohydrates, although it is important because carbohydrates are the most prominent source of energy in the diet. The objectives of this research were to examine the types, quantity and accuracy of carbohydrate-related messages in magazines. Samples of Chatelaine (CH) and Canadian Living (CL) magazines were examined for messages. The majority of messages related to carbohydrates provided information on general health and source of the nutrient. Messages found within these magazines were highly accurate and reflected scientific evidence and federal policy.

OBJECTIVES: Print media is a major source of nutrition information for Canadians. Carbohydrates, glycemic index (GI), sugar and fibre are topics of active nutrition research yet no known study has examined messages in Canadian magazines regarding these topics. The objectives of this research were to examine the types, quantity and accuracy of carbohydrate-related messages in magazines.

METHODS: The sample was a constructed year of 12 Chatelaine (CH) and 12 Canadian Living (CL) magazines from 2009-2010. Deductive content analysis was conducted using pre-established criteria (i.e., message purpose, message format, etc). Message accuracy and policy congruency were assessed against scientific evidence and Canadian nutrition policies.

RESULTS: 358 total messages were coded in CL and CH with 43, 5, 130 and 197 pertaining to carbohydrates, GI, sugar and fibre, respectively. Most message formats were articles (n = 191, 53%) followed by advertisements (n = 163, 46%). The majority of messages informed readers on general health (39%), source of a nutrient (27%) and a nutrient content claim (21%), respectively. 91% of all messages were accurate when compared to nutrition literature and scientific evidence. Messages relating to Canadian nutrition policies were all congruent.

IMPLICATIONS AND CONCLUSIONS: Messages were found mainly in articles and advertisements. Although much research attention is being placed on GI, this was a topic minimally covered in these magazines. Fibre messages were most prevalent which was predictable since dietary fibre has been a nutritional science and policy issue of importance for decades. Messages found within these magazines were highly accurate and reflected scientific evidence and federal policy.

1.56 Validation of High Resolution Melt-Curve Analysis for Detection of Changes in DNA Methylation

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SUMMARY: Methylation of DNA is a form of epigenetic marker that plays a critical role in the maintenance of cellular function through control of gene expression. Exposure to environmental chemicals can alter DNA methylation, resulting in changes in gene expression that can contribute to disease processes. Measuring changes in DNA methylation and gene expression may therefore serve as early indicators of effects of contaminant exposure. We have validated a method for the sensitive detection of changes in DNA methylation to complement gene expression data in toxicological screens, expanding the capacity of these studies to identify risk from environmental exposures.

BACKGROUND: Epigenetic markers such as DNA methylation play a critical role in human health and disease through their regulation of gene expression. Environmental contaminants, including air pollutants, can alter DNA methylation, making it an important endpoint for toxicological study. Chemically treating DNA with sodium bisulfite allows for differentiation of methylation state by converting unmethylated cytosines to thymine while methylated cytosines remain protected, essentially creating a polymorphism. This study presents a high-throughput screen for changes in DNA methylation using high-resolution melt-curve analysis (HRM).

METHOD: Serial dilutions of methylated and unmethylated-genomic DNA and synthetic oligonucleotides of known sequence were used to precisely control methylation profiles for assay validation. Primers were designed to amplify bisulfite-converted DNA, regardless of methylation status. By changing the primer annealing temperature, the range and resolution of the assay could be optimized. Following PCR-amplification, DNA was assessed by HRM, and samples were grouped according to melt profile. In order to demonstrate the utility of the approach, human alveolar epithelial cells (A549) were treated with the hypomethylating agent 5-aza-2'-deoxycytidine (DAC; 0, 0.1, 5 M) for 24 and 72h, and examined for changes in DNA methylation.

RESULTS: Altering primer annealing temperature allowed detection of 1% methylation in a population of unmethylated sequences. As little as one differentially methylated site was sufficient for discrimination between homogeneous populations. Treatment of A549 cells with 0.1µM DAC decreased methylation of the long interspersed element (LINE-1) repetitive DNA region, with no change observed at higher dose, consistent with published pyrosequencing data. Methylation state of specific gene promoters did not necessarily follow the trend observed for LINE-1, suggesting changes in methylation may be pathway dependant.

CONCLUSIONS: HRM analysis enabled sensitive detection of expected changes in both 'global' (as assessed by LINE-1) and gene-specific DNA methylation. Future work will focus on using this approach to complement gene expression data for toxicity screening.

1.57 Aptamers: DNA-Based Sensors for Bioanalysis and Hazard Detection

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SUMMARY: With the ever-increasing interest in monitoring agents in our environment, there is greater demand for fast, cost effective and field amenable methods of detection. Aptamers are a class of DNA molecules capable of recognizing specific target compounds, and offer advantages over antibodies in terms of stability, synthesis and ease of manipulation. The Exposure and Biomonitoring Division (EBD) initiated a project to delevop a quick and inexpensive aptamer-based sensor to detect algal toxins in water.

OBJECTIVES: The objective of this project is to develop DNA aptamers with specific affinity for algal toxins. These aptamers would then be used in a cost effective sensor to monitor the presence of these toxins in Canadian drinking water and freshwater in real time.

METHODS: Aptamers are generated by a process of *in vitro* selection (termed SELEX), where a random library of 10^{15} DNA sequences are passed through a solid-support to which a target molecule is tethered. Only the DNA molecules with affinity for the target are retained, then amplified by the polymerase chain reaction (PCR), and reintroduced to the column for further rounds of selection. Generally, up to 20 rounds of selection are needed to obtain aptamers with high affinity and specificity for a target molecule. The binding affinity of the aptamer pool for the target can be evaluated by surface plasmon resonance (SPR). Once a pool of aptamers with good binding affinity is established, their sequences are determined by molecular cloning so that the best aptamers can be integrated into an SPR sensor for water analysis.

RESULTS: The first generation of aptamers obtained at Health Canada demonstrated promise after 6 rounds of SELEX. Various methods to determine their binding affinities gave inconclusive results (fluorescence polarization, equilibrium dialysis with LC-MS/MS). Finally, comparing these aptamers to commercially available antibodies suggested binding affinities in the μM range for the free target. HC is currently developing a second generation of aptamers using more counter-selection strategies and introducing competitive molecules to saturate non-specific interactions, which should improve binding affinity. In addition, preliminary results using SPR suggest that this technique will be fundamental for binding affinity determination and sensor development.

CONCLUSIONS: Aptamer development presents many challenges for small molecules. Changing the SELEX protocols and implementing SPR promises to provide real-time determination of cyanotoxins in the field. Although laboratory techniques to detect algal toxins are available, the analysis is costly and in places with high demand, the time to report is lengthy (days). The development of cyanotoxin sensors will be an invaluable tool to respond quickly when a cyanobacteria bloom appears, in both drinking water sources and recreational water bodies.

1.58 Do Deamidated Product-Related Impurities Reduce Shelf-Life for Therapeutic Protein Drugs Like Interferon?

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SUMMARY: Therapeutic protein drugs have much more complex structures than traditional, small-molecule drugs and thus tend to have shorter product shelf-life. In this study, we have examined the effect of deamidation of one specific residue in the drug substance Interferon alpha 2a (IFNa-2a) on the protein stability, on IFNa-2a performance in accelerated stability studies and on the ability of IFNa-2a to be stored in solution at -20oC. Preliminary results indicate that not only does this product-related impurity reduce thermal stability, it may also have an increased degradation rate on storage.

OBJECTIVES/BACKGROUND: Protein-based therapeutic products almost invariably contain small amounts of product-related impurities (e.g., oxidized, deamidated or clipped variants of the active ingredient) and these impurities are known to increase with storage time of the drug product. Because these product-related impurities may be less potent than the authentic protein product or have an increased tendency to aggregate, levels of oxidized and/or deamidated products are limited by release specifications and can, in part at least, determine product expiry dates. Earlier work has shown that chemical modification of proteins like oxidation and deamidation can also affect conformation and protein stability and, as a result, may in fact be promoting product degradation by other pathways. In this study we examine one such product-related impurity, deamidation at asparagine 65, in IFN-2a to see if it affects the inherent protein stability (as measured by thermal denaturation), the performance of the product in accelerated stability studies (in liquid, buffered solutions at 42°C) and the real-time ability to store frozen (-20°C) solutions of IFN-2a for a number of months.

DESIGN/METHODS: An N65D IFN-2a variant was prepared by site-directed mutagenesis of the IFN-2a gene, expression of the rDNA and purification of the resultant protein. Thermal stability of the protein was followed by circular dichroism for the freshly prepared protein, and after storage for a various number of weeks/months at -20°C or after incubation at 42°C and compared to similarly-treated EDQM standard.

OUTPUT/RESULTS: N65D IFN-2a showed significantly less thermal stability than the EDQM standard (Tm ~ 52 °C vs. Tm ~ 70 °C) and this stability appears to decrease with storage time. Therefore, deamidation at N65 may have a direct, deleterious effect on the shelf-life of IFN-2a preparations.

IMPACTS/OUTCOMES: Deamidation at N65 not only reduces thermal stability, it may also decrease product shelf-life and thus must be monitored closely.

1.59 Soy Isoflavones Prevented Lipid Accumulation in Both Rat and Human Liver Cells

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SUMMARY: Fatty liver disease is the most common chronic liver condition in the Western world. Our recent study in rats showed that feeding soy protein diets prevented the formation of lipid droplets in liver, a typical feature of fatty liver disease. However, which components (proteins or associated isoflavones) in soy are bioactive and whether they are effective in humans remain unclear. This study demonstrated that soy-derived isoflavones (ISF) and their major components, genistein and daidzein, significantly suppressed oleic acid (OA)-induced lipid accumulation in both cultured rat and human liver cells.

OBJECTIVE(S): To examine the preventive effect of soy ISF, genistein and daidzein on fatty liver disease using cultured rat and human liver cells as models.

METHOD: A rat liver cell line, H4IIE, was plated in 96-well plates at a density of 1 x 10^4 cell/well. Cells were treated with increasing concentrations of OA (0, 50, 100, 200 µM) or 200 µM OA in the absence or presence of 0.1, 1, 10, 50 µM genistein, or daidzein or 0.1, 1, 10 or 50 µg/ml ISF for 24 hr. Lipid droplet formation was examined using immunocytochemical staining. Lipid accumulation was quantified by a steatosis colorimetric assay. The same experiment was conducted in HepG2, a human liver cell line, to determine if soy ISF has similar effects in human cells.

RESULTS: OA dose-dependently increased lipid accumulation and formation of lipid droplets in H4IIE cells. Addition of soy ISF significantly suppressed OA-induced lipid accumulation and formation of lipid droplets. Supplemental ISF at a concentration of 50 µg/ml completely blocked the effects of OA (200 µM). Presence of genistein or daidzein, the major soy ISF, inhibited OA-induced lipid accumulation and formation of lipid droplets in H4IIE cells. Furthermore, similar effects of soy ISF, genistein and daidzein have also been observed in HepG2 cells.

CONCLUSIONS: Soy ISF can effectively suppress lipid accumulation and formation of lipid droplets in both rat and human liver cells, suggesting that ingestion of ISF through consumption of soy foods or supplementation may reduce the incidence of fatty liver diseases. This information is useful for Health Canada in evaluating soy-related health claims.

1.60 Effects of Dietary Soy Proteins and Isoflavones on Growth, Serum Lipid and Amino Acid Levels in Young Female Rats

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SUMMARY: The effects of soy protein-associated isoflavones (ISF) on amino acid metabolism and growth are not fully understood. This study showed that intake of large amounts of soy ISF resulted in decreased body weight, increased serum citrulline and methionine, and elevated kidney and ovary weights in young female rats, suggesting that excess ISF may adversely affect growth, alter protein metabolism and certain organ weights in young consumers.

OBJECTIVE(S): Consumption of soy proteins has been associated with many health benefits; however there are also concerns over their nutritional quality and safety of the protein-associated ISF. This study examined the effects of soy proteins and increasing amounts of supplemental ISF on growth, serum amino acid profiles and development of reproductive organs in young female rats.

METHOD: Sprague-Dawley female rats at 28 days old were randomly divided into four groups and fed diets containing either 20% casein or 20% alcohol-washed soy protein isolate (SPI) with 50, 250 or 1000 mg/kg diet of supplemental soy ISF. After six wks, all rats were necropsied, and serum lipid, amino acid, genistein, daidzein, glycetin and equol levels were determined.

RESULTS: The contents of serum genistein, daidzein, glycetin and equol increased with the amounts of dietary soy ISF. Body weights of the rats fed 20% SPI with 250 or 1000 mg ISF/kg diet were significantly lower than those fed the casein diet ($p<0.05$). However, the relative weights of kidney and ovaries (percentage of their body weight) of the rats fed 1000 mg ISF were higher than those of rats fed casein diet. The relative weights of uterus and liver were not affected by dietary proteins and ISF. The rats fed SPI diets had lower serum glutamic acid, but higher glycine compared to the rats fed casein diet. Dietary ISF at 1000 mg/kg diet remarkably increased serum citrulline and methionine, and decreased serum LDL, HDL, total and free cholesterol, and triglycerides compared to casein.

CONCLUSIONS: Consumption of large amounts of soy ISF, equivalent to intake of ISF supplements in humans, appears to adversely affect growth and alter amino acid levels in the blood and increase relative weights of kidney and ovaries in young female rats. Whether humans taking soy ISF supplements are affected similarly warrants further investigation. This information is important for Health Canada in evaluating soy-related health claims and safety management of soy-based products such as soy-based infant formulas.

1.61 Combination Therapy with Purine Analogues and Tumor Necrosis Factor (TNF) Blockers for Inflammatory Bowel Disease (IBD) May Further Increase the Risk of Hepatosplenic T-cell Lymphoma Post-Market Analysis

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SUMMARY: The risk of lymphoma is a significant safety issue pertaining to the management of patients with Inflammatory Bowel Disease (IBD). IBD patients are at an increased risk of developing several cancers, including lymphomas. Sponsor and academia initiated study data has shown a particular increased risk of a rare form of lymphoma, hepatosplenic T-cell lymphoma (HSTCL), primarily in adolescents and young adults with Crohn's disease or ulcerative colitis (UC) in patients treated with purine analogues and TNF blockers either in combination or in close succession (consecutively). The question is whether these data impact current risk mitigation strategies for these products.

OBJECTIVE: To ascertain whether there is a need for additional risk mitigation strategies for HSTCL in patients receiving purine analogues and TNF blockers, concurently or consecutively.

DESCRIPTION(S): The two main types of IBD, Crohn's disease and UC, are chronic conditions with high morbidity rates but only a slightly increased rate of mortality compared with the general population. The results of a meta-analysis suggest that patients receiving purine analogues for the treatment of IBD have a 4-fold higher lymphoma risk than expected in this patient population. Examination of lymphoma risk in patients receiving TNF blockers are confounded by concomitant use of immunosuppressive agents in most of these patients.

OUTPUT(S): Recently, a French observational cohort study demonstrated an increased incidence of lymphoma in IBD patients receiving thiopurines. While worldwide case reports have shown a particular increased risk of a rare form of lymphoma, hepatosplenic T-cell lymphoma (HSTCL), primarily in adolescents and young adults with Crohn's disease or UC in patients treated with purine analogues and TNF blockers either in combination (concurrently) or in close succession (consecutively). This further raises concerns about the risk benefit profile of such therapies for the management of IBD.

OUTCOME(S)/ NEXT STEPS: Currently in North America, safety information for TNF blockers used in the management of IBD indicates the risks and benefits of using TNF blockers, together with or following azathioprine, and/or mercaptopurine therapy. Such use should be carefully weighed when prescribing these drugs to children and young adults, especially for the treatment of IBD where there may be other alternative treatment means. A warning in the product monographs (a document that specifies the directions for the drug's use) highlights the risk of HSTCL in patients with the TNF blocker class receiving concomitant treatment of a TNF blocker and purine analogue(s). Safety information for the pertinent purine analogues have been similarly updated in several regulatory jurisdictions to reflect this increased risk. Whether the current risk management strategies are sufficient to minimise this risk or if there is a need for more stringent measures remains to be seen. By delivering on these risk management strategies, HC will ensure that all Canadians receive safe use of these products.

2.01 Composition of Fluorinated Surfactants in Aqueous Film-Forming Foams

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SUMMARY: Aqueous film-forming foam (AFFF) is an industrial product widely used for extinguishing hydrocarbon fuel fires, with significant usage at military installations and airports. AFFFs are primarily made up of water, but usually contain between one and six percent of a fluorosurfactant. Since the compositions of AFFFs are proprietary, the contribution of these fluorosurfactants, potentially relevant to both human and ecological health, to AFFF mixtures is largely unknown. In this study, we analyzed AFFFs to determine the concentrations of several classes of fluorosurfactants in the products, as a means of predicting what compounds may be present at AFFF-contaminated sites.

BACKGROUND: The frequent use of AFFF products has resulted in numerous sites contaminated with fluorinated surfactants across Canada and around the world. Knowledge of the composition of these prevalent products can guide the assessment, management, and remediation of these contaminated sites. This knowledge can also aid in directing the future development of environmental quality guidelines.

METHOD: Four unique fluorinated and three non-fluorinated AFFF products were sampled from two Canadian Air Force Bases in Winnipeg, MB and Greenwood, NS, as well as a municipal fire hall and an airport in Winnipeg, MB. Samples were collected directly from product pails and diluted. Liquid chromatography tandem mass spectrometry (LC-MS-MS) was employed to quantify several commonly analyzed flourosurfactants, including perfluoroalkylcarboxylates, perfluoroalkylsulfonates, fluorotelomer sulfonates, and perfluoroalkyl sulfonamides.

RESULTS: Perfluoroalkylsulfonates (including perfluorooctane sulfonate; PFOS) and the PFOS precursors perfluorooctane sulphonamide were absent from current-use AFFF formulations. Fluorotelomer sulfonates represent the dominant class of fluorosurfactants detected in the AFFF mixtures, with a predominance of 8:2 and 6:2 fluorotelomer sulfonate. Several perfluoroalkyl carboxylates, including perfluorooctanoic acid (PFOA) were also present in significant quantities in several formulations. These results are consistent with the limited environmental occurrence data that exists for AFFF-impacted sites.

CONCLUSIONS AND FUTURE WORK: The prevalence of fluorotelomer sulfonates in these commonly used products highlights the need for more investigation of the impacts of these compounds on human health and the environment. While these compounds were found to be in significant concentrations in AFFFs, other unknown fluorinated compounds may still predominate. Future work will focus on more detailed characterization analysis, including the use of high resolution (time of flight) mass spectrometric techniques as well as a mass balance via total organic fluorine analysis.

2.02 Expression of Peroxisome Proliferator Activated Receptors-α, -δ/β, and – γ in Perfluorooctane Sulfonate (PFOS) Treated Rat Livers: An Immunohistochemical Investigation

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SUMMARY: Determining the mechanism(s) of toxicity of chemical food contaminants is essential to characterize, manage and mitigate the health risks that may be posed by their occurrence in foods for Canadians. PFOS, an industrial chemical, bioaccumulates in the food chain and human blood, suggesting widespread human exposure. Our recent rat studies suggested perturbations in lipid metabolism as a major contributor to liver toxicity of PFOS. Such changes in lipid homeostasis may be attributed to liver peroxisome proliferation (PP) via PFOS interaction with peroxisome proliferator-activated receptors (PPARs). To date, three PPAR subtypes (PPAR- á, -ä/â, and – ã) with a distinct, tissue-specific expression pattern and role(s) have been identified in many species including humans. In this study, we have determined the relative expression of these three forms of PPARs in untreated and PFOS-exposed rat livers in order to relate their potential role in PFOS hepatotoxicity.

OBJECTIVES/BACKGROUND/ISSUE(S): To study the expression of PPAR sub-types and their potential contribution to rat hepatotoxicity induced by PFOS exposure.

DESIGN/METHOD/DESCRIPTION: Formalin fixed livers from rats exposed to PFOS in feed (0 - 100 mg /kg diet) for 28 days were processed and stained for PPARs using the immunohistochemical (IHC) method. PPARs, visualized as immuno-stained protein-antibody cellular complexes, were quantified as numbers of positively stained cells per unit area of liver section using microscopic image analysis.

OUTPUTS/RESULTS: IHC indicated that compared to untreated livers, PFOS treatment significantly increased PPAR-á (p=0.019) and PPAR-ã ((p=0.022) protein expression in both male and female livers. PPAR- ä/â protein expression increased in a dose dependent fashion in both male & female livers, but with no significant differences between PFOS exposed and untreated rats (p=0.516).

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The increase in the liver-specific PP-induced PPAR-á in PFOS exposed rat livers correlates with our previously observed altered fatty acid profiles and elevated acyl-coenzymeA oxidase 1 in livers from the same study, thus confirming a PP-type mode of action for PFOS. PPAR- ã, shown by others to be expressed more highly than PPAR-á in human tissues and predominantly in adipose tissues, was also significantly increased in PFOS treated rat livers. Current evidence suggests that in general, humans are refractory towards PPAR-á -dependent hepatic peroxisome proliferation, and therefore, perhaps to PFOS hepatotoxicity. However, future studies are necessary to assess the toxicological and human health significance of the observed PFOS induced PPAR- ã activation in the rat model in relation to PFOS levels detected in human blood.

2.03 Examining Human Lymphocytes for γ-H2AX Response and Chromosome Damage as Potential Biomarkers of Radiation Sensitivity

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SUMMARY: Cancer patients who have undergone radiation therapy may develop severe side effects after treatment. This project examines blood samples from patients who have shown radiation-induced side effects as well as those who did not have any radiation-induced toxicity. The goal is to determine a method of identifying patients who are more sensitive to radiation. Preliminary results showed that after being exposed to a high dose of radiation, blood cells from sensitive patients showed more damage compared to the blood of non-radiosensitive patients. By comparing the levels of damage after irradiation, sensitive patients could be identified and considered for a reduced or alternative therapy. Understanding the range of patient response to radiation is essential when assessing the health risks from exposure to ionizing radiation and will assist in setting guidelines for exposure to radiation emitting devices.

OBJECTIVES: Some patients who have undergone radiation therapy develop severe side effects. Phosphorylated H2AX (γ-H2AX) is a rapid response signal of DNA double strand break damage, which occurs when cells are exposed to damaging agents such as ionizing radiation. The goal of this project is to examine the *in vitro* γ-H2AX response in lymphocytes, as well as cytogenetic endpoints, in patients who have shown a radiosensitive response to radiation to determine a specific marker for radiosensitivity.

EXPERIMENTAL DESIGN: This project is part of a phase III clinical trial involving 430 prostate cancer patients in which 3% of the patients (radiosensitive (RS)) developed grade 3 proctitis. Peripheral blood samples were taken from 10 RS patients, as well as from 12 normal responding patients (control). The samples were examined for *in vitro* γ-H2AX response in both lymphocytes and lymphocyte subsets, as well as for cytogenetic endpoints.

To examine the γ-H2AX response a dose course was run with samples exposed to doses between 0-10 Gy, and as wel as a time course with samples exposed to 2 Gy and incubated for times varying from 0-24 h between insult and processing. After incubation, the samples were fixed and stained with γ-H2AX-FITC, CD4-PE, CD8-APC and CD19-PC7, and analyzed by flow cytometry.

The dicentric chromosome assay was used to analyze 0 and 6 Gy irradiated samples for chromosome aberrations and excess fragments per cell.

RESULTS: There was no noticeable difference between the RS and the control for the dose course. The time course results showed a trend for the RS γ-H2AX response to be slightly higher as compared to the control. At 0 Gy, there was no significant difference between aberrations per cell or excess fragments per cell, while at 6 Gy, the mean number of excess fragments per cell in the RS group was significantly higher than in the control with mean values of 2.1 ± 0.4 and 1.7 ± 0.3 respectively ($p = 0.005$).

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: These preliminary results suggest the existence of potential for markers for predicting radiosensitivity, which could be useful for tailoring radiotherapy treatments. Further work is being done to validate the results.

2.04 Carbon Black Nanoparticle Instillation Induces Persistent Pulmonary DNA Damage, Inflammation, Oxidative Stress and Hepatic Genotoxicity in Mice

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SUMMARY: Carbon black nanoparticles (CBNPs) are combustion-derived particles used in the fabrication of rubber products and pigments. They have unique properties due to their small size. For example, when inhaled CBNPs can penetrate into deep regions of the lungs and even reach other tissues via the blood. In this study, increased DNA damage and inflammation was found in mice after a single exposure to CBNPs. Damaged DNA was observed in both lung and liver, and persisted up to 28 days after the exposure. This finding is important because damaged DNA can lead to mutation and cancer. Thus, the results provide important information on potential health effects of nanoparticles exposures inhalation for regulatory toxicology.

OBJECTIVE: 1. To determine dose-responsive genotoxic outcomes in lung and liver following inhalation of carbon black nanoparticles. 2. To relate genotoxicity to oxidative stress and inflammation across doses and post-exposure recovery time points.

MATERIALS AND METHODS: We investigated bronchial alveolar lavage (BAL) cell composition, DNA strand breaks (SBs) and oxidatively damaged DNA in C57BL/6 mice 1, 3 and 28 days post-instillation of 0.018, 0.054 or 0.162 mg Printex 90 CBNPs, alongside controls. The comet assay was used to measure SBs in BAL cells, lung and liver as well as formamidopyrimidine DNA glycosylase sensitive sites (FPG-SS) in lung.

RESULTS: BAL cell counts remained elevated for the two highest doses up to 28 days post-exposure ($p<0.001$). Pulmonary SBs occurred at all doses on post-exposure day 1 ($p<0.001$) and remained elevated at the two highest doses until day 28 ($p<0.05$). BAL cell DNA SBs were elevated relative to controls at the highest dose on all post-exposure days ($p<0.05$). Pulmonary FPG-SS was increased at all doses and time-points with significant increases occurring on post-exposure days 1 and 3, in comparison to controls ($p<0.001$-0.05). Hepatic SBs were detected on post-exposure days 1 ($p<0.001$) and 28 ($p<0.001$). BAL cells correlated strongly with pulmonary FPG-SS ($r=0.88$, $p<0.001$), whereas the correlation with SBs were more modest ($r=0.52$, $p=0.08$).

CONCLUSION: Deposition of CBNPs in lung induces inflammatory and genotoxic effects in mouse lung that persist for a considerable period of time after exposure. CBNPs cause oxidative stress and genotoxicity in both the primary exposed tissue (lung and BAL cells), and in a secondary tissue (liver).

2.05 Dieting Increases Serum and Tissue Levels of the PCBs and Organochlorine Pesticides that are Stored in Fat

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SUMMARY: Indigenous populations have had higher exposure to PCBs and organochlorine pesticides and higher obesity rates than other populations. These chemicals are stored in fat tissues so dieting to reduce fat mass may increase release of these chemicals into blood and other organs. This study examined this using rodents exposed to a chemical mixture of these chemicals. Dieting to reduce fat mass increased levels of PCBs and pesticides 4 fold in blood and 2-4 fold in muscle, liver and brain. Dieting has clear health benefits but also increases organ exposure to these chemicals but the health effects of this are unknown

BACKGROUND: Indigenous populations consuming country foods have historically higher exposure to lipophilic contaminants (PCBs and pesticides) and have higher levels of obesity than the general Canadian population so weight loss to reduce obesity is advocated in these populations. Because lipophilic contaminants are stored in fat tissue dieting may increase the release of these contaminants into circulation. Limited human dieting studies show that dieting increases serum contaminant levels. The current study determined whether weight loss increased serum and tissue levels of fat-stored contaminants in rodents under controlled laboratory conditions.

DESIGN/METHODS: Rats were exposed to a chemical mixture based on Inuit blood profiles at 2.5 mg/kg/day for 28 days and then placed on a weight loss regimen that reduced body weight by 20% over 14 or 28 days. Separate animals received the same dose but no weight reduction. Tissues were collected 0, 14 or 28 days after dosing and residue levels were measured in serum, adipose, liver, muscle and brain.

RESULTS: In animals not subjected to weight reduction contaminant levels in serum, liver, muscle and brain decreased by about 25-50% over 28 days after dosing but increased in adipose over the same period. Weight reduction increased serum levels (up to 4-fold) 14 and 28 days after dosing and increased levels in liver, muscle and brain (2-4 fold) as well as in adipose.

CONCLUSIONS/IMPLICATIONS: The increase in liver, muscle and brain concentrations after weight reduction indicates that weight loss sequesters lipophilic contaminants from adipose stores into circulation and that adipose stores of chemicals are re-distributed to other organs (liver, muscle and brain). Dieting to reduce weight in populations with past exposures to PCBs would be expected to produce increased circulating contaminant concentrations and organ tissue levels. Because contaminant loads in in lipophilic tisues in human populations is not well characterized, the health effects of dieting-induced increases in circulation and organ levels are also not known. While dieting can be expected to reduce some health risks, the associated impact of the re-exposure to contaminants is unknown.

2.06 Development of the OECD Extended One-Generation Reproduction Study Test Guideline

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SUMMARY: A new test has been developed by a group of international scientists to study the effects of chemicals on development, both before and after birth. This new design enhances the study that is now used to test for these effects, while making more efficient use of animals. The test results will improve regulatory decisions pertaining to the health of Canadians and will now be used as the global standard to determine if chemicals pose a hazard to reproduction and development.

BACKGROUND: The Organization for Economic Co-operation and Development (OECD) developed an Extended One-Generation Reproduction Toxicity Study (EOGRTS) as an alternative to the existing two-generation reproduction toxicity study (OECD Test Guideline (TG) 416) to provide additional guidance for testing the effects of chemicals on: 1) the integrity and performance of reproductive systems; 2) pre- and postnatal development; and, 3) systemic toxicity throughout the life stages.

DESCRIPTION: An OECD working group, comprised of scientists from government, academia, and industry (with expertise in reproductive, developmental, neurodevelopmental, and immune toxicology), was convened to determine the structure, content, and feasibility of an EOGRTS.

OUTPUTS: The EOGRTS will provide and/or confirm information about the effects of chemicals on the development, integrity and performance of the adult male and female reproductive systems. This new design includes additional reproductive and developmental parameters in comparison to the existing TG 416 (e.g., ano-genital distance, toxicokinetics, nipple retention, additional histopathological examinations, clinical chemistry and hematological measurements). Although the EOGRTS requires the same number of pregnant animals as TG 416, several parameters are assessed in more animals within the study, resulting in a more efficient use of animals. The decision to assess the second generation will be guided by internal and external triggers (OECD Guidance Document (GD) 117). Furthermore, the EOGRTS includes the option to assess developmental neurotoxicity and/or developmental immunotoxicity, which could result in a significant reduction in animal use when those stand alone studies are not conducted.

IMPACT: The EOGRTS, with examination of additional endpoints and animals per litter, has increased power and sensitivity to detect reproductive and developmental effects compared to TG 416. Furthermore, as the second generation will not be routinely required, this assay will ultimately use substantially fewer animals than TG 416. The results of the EOGRTS will improve regulatory decisions pertaining to the health of Canadians.

2.07 Do Air Pollutants Modify the Effect of Aeroallergens on Hospitalisation for Asthma? Results from a Time Series Study in 11 Canadian Cities

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SUMMARY: There is evidence that the asthmatic response to airborne allergens can be increased by exposure to air pollution. To further investigate this phenomenon we analysed thirteen years of hospital admission and air pollution data from Ten Canadian Cities. We found that air pollutants modify the association between short-term exposures to ambient aeroallergens and asthma hospitalizations suggesting that allergic predisposition confers increased susceptibility to poor air quality. The aeroallergen effect was enhanced on days of higher air pollution. Minimizing exposure to air pollution may reduce allergic exacerbations of asthma.

PURPOSE: There is experimental evidence that the asthmatic response to an aeroallergen can be enhanced by prior exposure to an air pollutant. To determine the significance of this in the general population we compared the effect of ambient aeroallergens on hospitalization for asthma between high and low air pollution days in ten large Canadian cities.

METHODS: Daily time-series analysis was employed and results were adjusted for day-of-the week, temperature, barometric pressure, relative humidity.

RESULTS: The combined population of the cities was 12 155 739 in the year 2000, the midpoint of the study. The allergen season is generally between March and October. The average number of daily admissions for asthma during the thirteen years of observation during the allergen season was 28.67. Mean daily air pollutants did not exceed U.S. National Ambient Air Quality Guidelines concentrations (http://www.epa.gov/air/criteria.html). Variations in pollution levels were seen between cities with the largest for PM_{10}, which ranged from about 12 to 27 ug/m^3 between March and October.

Daily pollen counts averaged fifteen per cubic meter for grass and weeds and about thirty for trees. Spore counts (Ascomycetes- the cup or flask-like fungi, Basidiomycetes- Truffle-like fungi, Deuteromycetes- fungi imperfecti) ranged from three hundred to twenty-two hundred per cubic meter.

Significant ($p = 0.05$) changes in the relative risk of admission for an interquartile increase in tree pollen were observed on days of lower versus higher PM2.5. In addition, significant ($p = 0.05$) relative risks of admission for lower versus higher PM10 were observed for Ascomycetes, Basidiomycetes, Deuteromycetes, and for weeds.

CONCLUSIONS: The influence of basidiomycetes and deuteromycetes appears to be enhanced on days of high air pollution. Reducing levels of ambient air pollution may reduce the frequency of allergic asthma exacerbations requiring hospitalization.

2.08 Equivalent Radon Concentration for Practical Consideration of Exposure to Thoron

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SUMMARY: Radon exposure has been identified as the second leading cause of lung cancer after tobacco smoking. Thoron is another isotope of radon. To consider the total exposure to radon and thoron, a concept of equivalent radon concentration for thoron is introduced. It is defined as the radon concentration that delivers the same annual effective dose as that resulting from the thoron concentration. The total exposure is the sum of the radon concentration and the equivalent radon concentration for thoron, and should be compared to the radon guideline value. With this concept, a separate guideline for indoor thoron exposure is not necessary.

OBJECTIVE/BACKGROUND/ISSUE: Radon is a naturally occurring radioactive gas generated by the decay of uranium and thorium bearing minerals in rocks and soils. Radon and its decay products are the major contributors to human exposure from natural radiation sources and have been identified as the second leading cause of lung cancer after tobacco smoking. Radon-222 and radon-220 are the most common isotopes of radon, with the term “radon” typically referring to radon-222 and “thoron” to radon-220. Studies have shown that thoron contributes about 10% of radiation dose due to indoor radon exposure in Canada. To consider the total exposure to indoor radon and thoron, a concept of equivalent radon concentration for thoron is introduced.

DESIGN/METHOD/DESCRIPTION: Equivalent radon concentration for thoron is defined as the radon concentration that delivers the same annual effective dose as that resulting from the thoron concentration. The total indoor exposure to radon and thoron is then the sum of the radon concentration and the equivalent radon concentration for thoron.

OUTPUTS/RESULTS: The total exposure to radon and thoron should be compared to the radon guideline value. If the total exposure exceeds the guideline value, appropriate remedial action is required. For homes already tested for radon with radon detectors, Health Canada's recommendation of a 3-month radon test performed during the fall/winter heating season not only ensures a conservative estimate of the annual average radon concentration but also covers well any potentially missing contribution from thoron exposure.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: With this concept, a separate guideline for indoor thoron exposure is not necessary. Because thoron concentration is much lower than the radon concentration in most homes in Canada, there is no real need to re-test homes for thoron if a radon test has already been done.

2.09 Trends and Analysis of General Population Exposure – High Priority Substances Assessed during the "Challenge" under Canada's Chemicals Management Plan

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SUMMARY: Since 2007, Health Canada and Environment Canada have conducted risk assessments on high priority substances as part of the "Challenge" initiative. To identify drivers of human exposure, we examined the different parameters and exposure metrics used in estimating general population exposure. Based on this analysis, major routes of exposure were identified from various sources, mainly via the inhalation and dermal routes. More results from this analysis will be presented, including exposure-based trends that were observed across these high priority assessments. Understanding the drivers of exposure will assist in developing and refining approaches moving forward in Canada's Chemicals Management Plan.

BACKGROUND: Assessing risk as part of Canada's global commitment to address legacy chemicals by 2020 is implemented through the Chemicals Management Plan. Following categorization of approximately 23 000 existing commercial substances, Health Canada and Environment Canada addressed the highest priority substances by conducting screening assessments as part of its "Challenge" initiative; this included over 50 assessments on substances that were categorised as high priority with respect to human health. In these assessments, general population exposure to substances in the environment was characterised by using available monitoring data and exposure models such as ChemCAN to estimate environmental concentrations. Exposure to substances via the usage of consumer products was characterised using exposure models, algorithms, exposure-based studies, and concentration data from various sources.

DESCRIPTION: An analysis of human exposure characterisations from approximately 200 risk assessments completed as part of the Challenge initiative was conducted by collectively examining data inputs, exposure models, sources and routes of exposure, exposure metrics and key exposures.

RESULTS: In general, the major routes of exposure when considering consumer products and environmental media were via the dermal and inhalation routes, respectively. Exposure from consumer products were identified and estimated in approximately 70% of the assessments. Exposures from environmental media (air, drinking water, food, soil or dust) were identified and estimated in nearly 70% of the assessments, of which approximately 50% were based on Canadian monitoring data. More results from the analysis will be presented in this poster, including exposure-based trends that were observed across these assessments.

IMPLICATIONS: Understanding the drivers of exposure will assist in developing exposure tools for future assessments, in addition to refining approaches moving forward in Canada's Chemicals Management Plan. Data and exposure tool needs will be identified within the context of risk assessment for chemical regulation.

2.10 Review and Evaluation on the Use of Metabolic Activation Mixtures Containing Human Liver Post-Mitochondrial Supernatant for *In Vitro* Genetic Toxicity Assessment

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SUMMARY: Health Canada routinely uses toxicological information generated by exposing cultured cells to chemical agents (i.e., *in vitro* assays). Preparations derived from rodents are used to simulate mammalian liver metabolism. By comparing results obtained using conventional rodent-derived material to those obtained using human-derived material, this study sought to determine whether the use of human-derived material could increase the relevance of toxicological assays. The results indicate that rodent-derived materials often overestimate toxicity. However, certain groups of compounds show the opposite trend. Further research is required to definitively assess the relative utility of human-derived metabolism systems for *in vitro* toxicity assessment.

OBJECTIVES/BACKGROUND/ISSUES: *In vitro* genetic toxicity assessment routinely employs an exogenous metabolic activation mixture to simulate mammalian metabolism. Activation mixtures commonly contain post-mitochondrial supernatant (i.e., microsomes or S9) derived from rodent liver. The predominant activation system contains S9 from Aroclor-1254 induced male Sprague Dawley rat liver. Although OECD guidelines permit the use of other S9 preparations, assessments rarely employ human-derived S9. The objective of this study is to review and evaluate the use of human-derived S9 for genetic toxicity assessment.

DESIGN/METHOD DESCRIPTION: The scientific literature was extensively reviewed and all published genetic toxicity assessments employing human liver S9 were assembled in a MS Excel workbook. In total, 1178 observations (concentration-response functions) were collected. Of these, 904 observations were generated using the Salmonella reverse mutation test (i.e., Ames assay). The collected data were analysed by analysis of variance using SAS v. 9.2.

OUTPUT/RESULTS: Human liver S9 activation often yields significantly lower Salmonella mutagenic potency, especially for compounds requiring cyp1A1 (e.g., BaP, 3MC), as well as aflatoxin B1, and imadazo azaarenes like Trp-P-2 and IQ (1 to 2.5 orders of magnitude). Conversely, assessment with human liver S9 activation yields higher potency for aromatic amines such 2AA, 2AF and AAF (0.5 to 1.5 orders of magnitude). Although the results did not show significant differences between pooled versus individual human S9 samples, there are noteworthy human outliers with substantially elevated enzymatic activity.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The *Salmonella* mutagenic potency of compounds that require exogenous S9 metabolic activation is generally lower when assessed using preparations that include human hepatic S9. However, certain substances, such as aromatic amines, can elicit higher responses when tested with human hepatic S9. Inter-individual variations in the enzymatic profiles of human hepatic S9 necessitates the use of pooled material. Further research should evaluate the relative effect of human hepatic S9 on mutagenic activity assessed using mammalian cell assays.

2.11 Methylation of DNA Repeated Elements in the Liver of Rats Exposed Perinatally to Persistent Organic Pollutants

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SUMMARY: The levels of chemical induced DNA modifications were measured in the liver of rats exposed to mixtures of environmental pollutants before and after birth. While human studies have demonstrated that DNA methylation in blood cells were associated with blood pollutant levels, rats displayed no DNA changes in the liver following chemical exposure. DNA from liver was measured since it is the major detoxification organ in the body, however white blood cells may prove to be a more sensitive indicator of chemical exposure.

OBJECTIVES/BACKGROUND/ISSUE(S): Previous human studies have negatively associated plasma concentrations of persistent organic pollutants (POPs) with global DNA methylation levels in blood. Global hypomethylation of repetitive genomic sequences, such as long and short interspersed nuclear elements and satellite alpha (LINEs, SINEs, and satα), have been linked to chromosome instability in cancer.This study's objective is to determine whether perinatal POPs exposure perturbs DNA methylation in the liver; the major detoxification organ of POPs.

DESIGN/METHOD/DESCRIPTION: Rats were exposed *in utero* and/or lactationally to POPs by feeding dams from gestation day 0 until postnatal day (PND) 20 one of three chemical mixtures: 1- "M" at 1mg/kg/day (PCBs, organochlorine pesticides, and methylmercury), 2- a mixture of aryl hydrocarbon receptor "AhR" agonists at 1.7ng/kg/day (non-ortho PCBs, PCDDs, PCDFs) or 3 - a 0.5M-Ahr mixture. DNA methylation was measured from the livers of offspring rats at PND0, 21, and 85 using pyrosequencing assays that target the rat repetitive elements, LINE-1, Identifier Elements (ID; a member of SINE family), and satα.

OUTPUTS/RESULTS: All assays measured methylation of individual cytosine positions with Pearson correlation coefficient values ranging from 0.910-0.941 for LINE1, 0.997 for ID elements, 0.982-0.983 for satα. All assays detected significant ($p<0.0001$,ANOVA) age related increases in methylation of 2 to 4%. The LINE1 assay also detected 1.5% higher methylation in the livers of female versus male rats ($p<0.0001$,ANOVA). No chemical induced changes in global DNA methylation were detected in offspring liver.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Methylation of DNA repeated elements in the liver were not a sensitive indicator of perinatal POP exposure. Methylation of gene promoters is currently being investigated. Also, assaying rat blood DNA could better mirror studies performed on human blood. This project will help clarify the chemical origin for DNA methylation changes observed in human and will contribute critical perspective to evaluators and policy analysts.

2.12 Maternal Contaminant Concentrations in Northern Canada: Sampling Considerations

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SUMMARY: The Northern Contaminants Program has analyzed a large number of maternal blood samples for environmental contaminants in the Canadian Arctic. Regional datasets permit trend monitoring to evaluate whether contaminant levels are increasing or decreasing. Data analysis also allows an assessment of study design and provides information on appropriate sample sizes, collection frequency, and parity status of mothers. Current sampling strategies have provided data that suggest increased exposures to some organochlorines and heavy metals for mothers in Northern regions compared with women of childbearing age in Southern Canada; however, in general, maternal blood concentrations are decreasing over time in the North.

OBJECTIVES / BACKGROUND: Since 1991, the Northern Contaminants Program (NCP) has analyzed a wide range of metals and organochlorines in maternal blood over one or more time points in the Canadian Arctic. Challenges inherent to biomonitoring studies include participant recruitment, cost, and data quality. The objectives of the study are to analyze existing NCP data and identify sampling considerations for future biomonitoring to facilitate follow-up study design, and, second, to compare Northern data to women of childbearing age (WCBA) from the Canadian Health Measures Survey (CHMS) in Southern Canada to determine if factors such as parity status or region influence contaminant levels.

METHODS: NCP studies have collected convenience maternal blood samples from the Arctic as the result of small population sizes in most regions. Conversely, the CHMS recently examined over 5000 individuals in a stratified random sample and measured concentrations of contaminants in a subsample of WCBA (n=285, 2007-2009). Two contaminants (PCB 153, and total mercury) common to Northern and Southern samples are chosen for this analysis.

RESULTS: Initial analysis indicates that the collection of current sample sizes in the North permits the detection of significant changes in contaminant levels between populations. Sampling over several time points can clarify temporal trends and enable the detection of smaller changes in contaminant concentrations. The inclusion of all mothers in our assessment has positive implications for future sampling and will help to maintain current sample sizes

CONCLUSIONS: The analysis of both PCB 153 and total mercury in maternal blood samples indicate that, in general, contaminant concentrations in maternal blood are decreasing over time in the Canadian Arctic, but remain higher for Inuit / Inuvialuit mothers compared with WCBA from Southern Canada. These trends can be verified during subsequent studies by increasing the number of time periods.

2.13 Organophosphate Ester Flame Retardants in Canadian House Dust: Analytical Methodology and Occurrence

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SUMMARY: Organophosphate esters (OPEs) are widely utilized as flame-retardants, plasticizers, hydraulic fluids, solvents, extraction agents, antifoam agents, adhesives, and coatings for electronic devices. Worldwide consumption of OPEs as flame-retardants increased from 108 000 tons in 1995 to 186 000 tons in 2001. Recent studies found that some OPEs could have adverse health effects. However there is limited information about human exposure to these chemicals. We developed a simple and robust method for the measurement of selected OPEs in indoor dust, since indoor dust is a major exposure route to indoor pollutants.

OBJECTIVES/BACKGROUND/ISSUE(S): Organophosphate esters (OPEs) are widely utilized as flame-retardants, plasticizers, hydraulic fluids, solvents, extraction agents, antifoam agents, adhesives, and coatings for electronic devices. Recent studies found there are indications that some OPEs could have adverse health effects. The objective of this study is to develop a rapid and sensitive method for the simultaneous measurement of 13 OPEs in indoor house in vacuum dust samples collected under the Canadian House Dust Study. The results of this study could enable a better understanding on human exposure to these compounds from indoor house dust.

DESIGN/METHOD/DESCRIPTION: Dust samples were obtained from vacuum systems used by study participants as part of their regular house cleaning routine. Samples were air-dried and sieved. The fine fraction (<80 µm, 0.05 g) was solvent extracted and cleaned up by solid phase extraction prior to GC/MS/MS analysis in positive chemical ionization (PCI) mode.

OUTPUTS/RESULTS: Sample cleanup by solid phase extraction (SPE) coupled with GC/MS/MS detection provided clean chromatograms for target analytes in dust samples. The method provided good sensitivity and recovery for each target analyte. The method detection limits (MDL) ranged from 0.01 µg/g to 0.12 µg/g and recoveries from 58% to 118%. The following chemicals TCEP, TCPP, TBEP, EHDPP, TPhP, TDCPP, and TCrP were detected in all dust samples (n=20), with median concentrations (µg/g) of 1.46, 0.46, 11.4, 0.77, 0.88, 0.85, and 0.06, respectively. Other OPEs were detected at very low concentrations or below their MDL.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Widely scattered concentration levels were observed for target analytes, suggesting a broad variability in Canadian household exposure to these chemicals. High detection frequency of major OPEs suggests that indoor dust may represent a potentially significant exposure pathway to these chemicals. Data from this study will provide information for risk assessment of this class of emerging contaminants.

2.14 Variables that Influence MP3 Player User Listening Habits in Children and Adolescents

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SUMMARY: This study of 247 children/adolescents evaluated whether mp3 players are used in a way that could harm hearing. Measured sound levels together with self-reported duration of use, were high enough to cause hearing damage in 3% to 9% of subjects. Self-reported symptoms of hearing loss were found in one-quarter of subjects, while hearing tests showed that 23% had some measurable hearing loss. Some subjects who listened to their devices at maximum volume were unaware of the associated risks and already showed measured hearing loss. Educational programs to raise awareness of noise-induced hearing loss in children/adolescents and re-testing may be beneficial.

BACKGROUND AND OBJECTIVES: Health Canada research has established that the maximum sound pressure levels from MP3 devices can exceed the most stringent occupational noise limit (85 dBA over 8 hours) in as little as three minutes. The objective of this study was to evaluate whether subjects are using their MP3 players for sufficient time that their sound levels could increase their risk of hearing loss.

METHOD: Children/adolescents (n=247, aged 10 to 17 years) recruited from 15 schools completed a questionnaire that assessed patterns of device usage, headphone type/fit and subjective hearing health. Two MP3 player sound level measurements were taken; one at a volume setting subjects normally listen to (typical) and a second at the volume setting subjects indicated was their maximum listening level (high). Audiometric evaluation (0.5 to 8 kHz) was carried out in a sound booth.

RESULTS: For typical listening conditions, 3% of subjects exceeded the occupational limit. This increased to 9% if we assumed all listening was at the high volume settings. The magnitude of uncertainty around specific parameters (e.g., fit, background sound levels and listening time) could affect study results. Prevalence of hearing loss above 15dBA, when averaged over low (0.5, 1 and 2 kHz) and high (4 and 8 kHz) frequencies was 22.7%. Self-reported symptoms of hearing loss were reported by one-quarter of respondents.

IMPACTS AND CONCLUSIONS: Some subjects in our study listened at hazardous volume levels placing them at risk of hearing loss. Due to the cumulative nature of acquiring noise-induced hearing loss, it is relevant that we found subjects also reported engaging in other high noise exposure activities (i.e. >85 dBA) including motorcycling, gardening equipment usage, music band member, ski-doing, go-karting). Targeted educational efforts to increase awareness of noise-induced hearing loss aimed at this age group and longitudinal studies that minimize uncertainty would be valuable.

2.15 Evaluation of the Impact of Personalised Medicine: A Health Portfolio Perspective

Health Portfolio Personalised Medicine Working Group[1] and Secretariat[2] and Chair H. Yu[2] , and L. Gémar[2]

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SUMMARY: Advances in computing power are increasing abilities to identify molecular markers of disease, and creating new tools and strategies whereby health professionals can more specifically tailor medical treatment and prevention. This project examines how these rapid advances impact the activities of the Health Portfolio. A working group has made recommendations on identified issues within the Portfolio's mandate where additional or new evidence is needed. Their analysis revealed key areas for proposed actions designed to inform Health Portfolio regulatory, policy and research program activities.

OBJECTIVE: To examine advances in science and technology, including bioinformatics, which drive the science of personalised medicine, in order to identify potential gaps and impacts on the Health Portfolio, and to make recommendations to inform policies, research priorities and regulatory actions.

DESIGN/METHOD/DESCRIPTION: The tailoring of preventive, diagnostic and treatment measures based on a patient's genomic profile is touted as personalised medicine's potential benefit. Key scientific drivers for this emerging field include increasing capacity for sequencing genes, decreasing costs for these activities, and increasing computational power. The Personalised Medicine Working Group, a multidisciplinary team of Health Portfolio scientists, regulators and policy analysts developed an integrated approach to personalised medicine, using published literature, analysis of current projects, expert opinion and professional networks to synthesize and analyze relevant information. The results were honed through discussions and peer review.

OUTPUTS: The Working Group identified several areas where personalised medicine may implicate the current or the future work of the Health Portfolio. These include gaps related to knowledge translation in biomarker research and the availability of robust data to demonstrate costs and benefits to the system, as well as issues such as public and professional education and ethical considerations (e.g., privacy and genetic discrimination). Gaps were also identified in the regulatory framework (e.g., guidance on biomarker and companion diagnostic evaluation) and access policies (e.g., orphan drugs, pricing of medication and diagnostic tests). Results of the evaluation led to recommendations to address the issues and challenges identified as a result of the gap analysis.

CONCLUSIONS AND NEXT STEPS: The analysis and recommendations form the basis of an action plan to guide Health Portfolio regulatory, policy and research activities. The action plan will be presented to a multi-stakeholder meeting jointly organised by Health Canada and CIHR, in January 2012.

2.16 MetaPath: A Global Path to Modernizing Chemical Risk Assessment

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SUMMARY: A project involving international scientists is underway to further develop, and expand the content, of a database of pesticide breakdown products and pathways. This database, called MetaPath, also contains modern evaluation tools and will enhance our ability to share and review scientific data on a global scale. MetaPath will also contribute to developing new methods to predict how chemicals are broken down in animals and in the environment, and will allow better use of all available data for assessing risks to human and environmental health.

OBJECTIVE: An Organization for Economic Cooperation and Development (OECD) project was initiated to develop and populate MetaPath as a globally accessible structure-searchable database of pesticide chemical transformation pathways. While the database itself is not yet freely available, a version of MetaPath's software tools is among the suite of OECD tools currently available for structure-based hazard prediction (i.e., QSAR Toolbox).

DESCRIPTION: MetaPath, a computational tool and database, combines versatile information retrieval with sophisticated chemical structure evaluation. This platform, and its supporting data-flow software (DER Composers), were initiated and developed by the US EPA and Bourgas University. The EPA initially populated MetaPath with rat metabolism data. Data from other biological and environmental matrices can also be coded. Now advancing as an OECD project, a global community of risk assessors is collectively developing and expanding MetaPath to modernize chemical risk assessment.

OUTPUTS: A MetaPath Users Group, involving North American, European and Australian risk assessors, guides and contributes to MetaPath's development and expansion, and is engaged in training and piloting efforts co-ordinated by the EPA. A successful Pest Management Regulatory Agency pilot resulted in software development, expansion of the database, and the first coded livestock residue data. Similar pilot work is under development in Europe. Currently 218 chemicals and 391 pathways have been coded. Approximately 65% of these have been quality assured and transferred into MetaPath.

IMPLICATIONS: MetaPath integrates chemical information across biological and environmental matrices. This contributes to modernizing chemical risk assessment and expedites the pesticide residue definition process. MetaPath is also a model for streamlining information flow between industry and regulators. Applicant-coded information can be "painted" according to agency-specific summary data requirements. This information-flow paradigm reduces the regulatory effort required to generate study summaries. The database also facilitates the development and refinement of predictive methods, which will ultimately reduce animal testing and guideline study requirements.

2.17 Oral Health Results of the Canadian Health Measures Survey for Adults aged 60-79

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SUMMARY: This presentation will provide information about the oral health of Canadians aged 60-79. This information comes from the Canadian Health Measures Survey, which examined the mouths of 6000 people from across Canada. Before this survey was undertaken, little was known about Canadian's oral health, including the fact that seniors have poorer oral health and less access to dentists than other Canadians. Governments and those concerned with dental health will be able to use this information if they wish to develop programs and policies that can improve the oral health of Canadians, particularly those who are, or soon will be, seniors.

OBJECTIVES/ BACKGROUND/ISSUE(S): The presentation is an overview of the oral health results from the Canadian Health Measures Survey (CHMS). The presentation will highlight the results from both the qualitative questionnaire and from the clinical examination with a focus on Canadians aged 60-79.

DESIGN/METHOD/DESCRIPTION: The CHMS was led by Statistics Canada in partnership with Health Canada and the Public Health Agency of Canada. The data collection occurred in 2007-2009 on approximately 6000 people representing 97% of the population aged 60-79. Data was collected from a team of physical measurement specialists who travelled to the 15 collection sites across Canada in mobile clinics.

Health Canada partnered with the Department of National Defence to obtain the dentists who conducted the clinical examinations. Health Canada implemented the training of the dentists and calibrated them to World Health Organization standards to ensure each dentist recorded conditions in the same manner.

The data were analyzed by Statistics Canada in collaboration with Health Canada.

OUTPUTS/RESULTS: Overall, Canadians were found to have good oral health although areas were found where improvement is needed:

- 62% of Canadians have private dental insurance compared to only 39% of those 60+;
- 20% of Canadians have a history of root decay compared to almost half (43%) of those 60+;
- 21% of Canadians have problem periodontal conditions (loss of attachment), whereas 47% of those 60+ have problem conditions; and
- 6% of Canadians have no teeth compared to almost 22% of those 60+.

CONCLUSIONS: The results from the oral health component will support continued discussions at a national level on oral health disparities and on issues related access to care. The information can also be used to guide the development of oral health public policies and programs designed to improve the oral health, and thus the overall health, of older Canadians.

2.18 Preliminary Comparison of Two QSAR Methods for Toxicity Prediction

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SUMMARY: The New Substances Bureau conducts risk assessment of new chemicals and polymers (substances) that are proposed for use in Canada. When a low amount of a new substance is notified, the submitter is not required to provide toxicity studies, such as those for DNA changes (mutagenicity). During evaluation for health safety, the new substance is compared to similar substances (read-across) and/or evaluated using Quantitative Structure-Activity Relationship programs (QSARs). In this project, the accuracy of two QSARs were compared for several chemicals with known positive or negative mutagenicity data. One (QSAR Toolbox) was more accurate than the other program, TOPKAT.

OBJECTIVES/BACKGROUND: This project was conceived as a short-term (6-week) investigational exercise to compare two QSARs which are currently available for use by our Bureau. The objective was to compare their accuracy to predict of mutagenic activity in the bacterial reverse mutation (Ames) test, then using the programs to predict mutagenicity on other chemicals with no Ames data. A secondary objective was to familiarise ourselves with the QSAR Toolbox.

DESIGN: Toxicity Prediction by Komputer Assisted Technology (TOPKAT) results were compared to those from QSAR Toolbox 2.1. Our in-house Integrated Science Information System (ISIS) database, of substances notified to the Bureau, was mined for chemicals positive in the Ames assay (positive controls), chemicals with a functional group sometimes associated with mutagenicity (azo colourants) which had a positive result, and chemicals which were negative (negative controls). Finally a group of 30 azo chemicals with no Ames data was chosen.

RESULTS: For chemicals which were positive in the Ames assay, but did not contain an azo group, accuracy (agreement between the prediction and the actual data) was low: TOPKAT accuracy was 0% and QSAR Toolbox accuracy was 27%. For azo chemicals which were positive in the Ames assay, TOPKAT accuracy was 27%, compared to QSAR Toolbox at 60% accuracy. For negative controls, TOPKAT predicted 13% correctly, whereas QSAR Toolbox predicted 87% to be negative in the Ames, in agreement with the data. For 30 azo compounds with no data, TOPKAT validation (criteria for determination of structural similarity) failed for most substances; the compounds which passed validation were all predicted to be positive. QSAR Toolbox predicted 14/30 of the compounds would show positive results on the Ames test, including those predicted by TOPKAT.

CONCLUSIONS/NEXT STEPS: QSAR Toolbox may be more useful than TOPKAT. QSAR Toolbox will be evaluated for other functional groups of concern.

2.19 Elevation of Systolic Blood Pressure in Healthy Adults Exposed to Low Levels of Ambient Air Contaminants in a Single Blind, Randomized, Crossover Study

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SUMMARY: We introduce a novel approach to investigate physiological changes in unrestricted subjects breathing air pollutants, but blinded to their exposure. Subjects were breathing through filters for organic vapours and particles (clean air), or dummy cassettes from which filter media had been removed (ambient air). An increase in mean daily systolic blood pressure was observed in the subjects when breathing ambient air through dummy filters, but not when breathing filtered air. Effects were observed for pollutant levels well below current standards. Our experimental data provide direct support to the concept of a no-threshold association between ambient air contaminants and cardiovascular responses.

INTRODUCTION: The current Canada Wide Standards for $PM_{2.5}$ is 30ug/m^3 (24h avg), and for O_3 is 65ppb (8h avg). The National Air Quality Objective for CO is 13ppm (8h avg), and for NO_2 is 106ppb (24h avg). It remains unclear whether there are threshold concentrations for health effects of air pollutants. To gain insight into low-dose effects, we studied the physiological responses in healthy subjects breathing ambient or filtered air.

METHOD: Experiments were conducted indoors at EHC building between September 2009 and March 2010. Ambient air pollution (National Air Pollution Surveillance) were, mean (min, max): $PM_{2.5}$, 4ug/m^3 (0, 21); CO, 0.25ppm (0.10, 0.65); NO_2, 9ppb (0, 44); O_3, 15ppb (0, 48). Penetration of ambient pollutants indoors was 95-100%. Subjects (8 males, 2 females, 24-55y, 68-118kg) were provided a positive air pressure respiratory protection system fitted on two days with organic vapours and HEPA filters (clean air) or on two days with dummy cassettes from which filter media was removed (ambient air). Blood pressure measurements and saliva samples were obtained every 30min, from 08:00-14:30 (exercise routine from 08:30-09:00). From 14:00-14:30 all subjects were switched to dummy filters. Saliva was analysed for endothelins by ELISA.

RESULTS: When breathing ambient air, systolic blood pressure increased during the day (8am, 131mmHg; 2:30pm, 138mmHg). When breathing clean air, systolic BP remained stable (8am, 130mmHg; 2pm, 130mmHg; full vs dummy at 2pm, $p=0.0098$), but increased after switching to dummy filters (2:30pm, 138mmHg; 2pm vs 2:30pm, $p<0.0001$). Changes in salivary endothelins (daily mean, full vs dummy filter) were in line with BP data (bigET-1, 74 vs 84pg/ml; ET-1, 0.86 vs 1.22pg/ml).

CONCLUSION: The study shows significant cardiovascular effects of air pollutants in healthy subjects at concentrations one order of magnitude below the current air quality standards. Further analyses are required to determine the impact of individual pollutants on the observed effects.

2.20 The U.S.-Canada Grower Priority Database

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SUMMARY: The U.S.-Canada Grower Priority database, established in 2008, was developed to address concerns raised by farmers over differences in pesticide-registered uses and/or maximum residue limits in food between countries. In order to better understand the needs of the agricultural community, Health Canada's Pest Management Regulatory Agency (PMRA) actively assisted Canadian stakeholders to prioritize their pest control product needs. It is expected that the database will become a valuable tool for US and Canadian farmers to identify their pesticide priorities to both registrants and regulatory agencies.

OBJECTIVES/BACKGROUND/ISSUE(S): In response to grower concerns about pesticide harmonization across NAFTA countries, officials from the NAFTA Technical Working Group on Pesticides began working with key stakeholders to develop a broad approach for grower priority needs on an active ingredient basis, leading to the development of the U.S.-Canada Grower Priority Database. Intended for use by growers, pesticide registrants and regulators, the goal of the database is to provide a single point of access for growers on both sides of the border to identify and prioritize their pest control product needs.

DESIGN/METHOD/DESCRIPTION: In late 2008, under guidance of the U.S. Minor Crop Farmer's Alliance and funded by a grant from the United States Department of Agriculture, the U.S. portion of the database became operational while a Canadian Screening Committee, facilitated by the PMRA (Health Canada), was formed to compile Canadian grower priorities. The original list was based on grower-identified priorities from existing programs, including the minor use priority setting list (2008), the joint Agriculture and Agri-Food Canada (AAFC)/PMRA Pesticide Risk Reduction Program, and NAFTA commodity-based projects.

OUTPUTS/RESULTS: In March 2010, the PMRA facilitated the addition of over 5000 grower-identified priorities spanning more than 100 commodities to the joint US-Canada Grower Priority Database. The PMRA also contributed to making Canadian priorities available in both official languages on the Canadian Federation of Agriculture web site. The database has already been used in existing regulatory programs at the PMRA, including the identification of candidate active ingredients for shorter review times and ongoing risk reduction strategies in the identification of potential alternatives to products being lost through the re-evaluation of older pesticides. Industry representatives have indicated that they have used the Grower Priority Database to identify opportunities for business development. Discussions are ongoing to develop a process to manage the information contained in the database to ensure it remains current.

2.21 Pollinators and Pesticides: Revising Approaches to Environmental Risk Assessments

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SUMMARY: The past few years have seen increased scientific and public concern on declines in pollinator populations. A number of factors, including pesticides, have been proposed as potential contributors to pollinator declines. HC-PMRA has been proactively working with national and international partners to ensure relevant science is available and used to inform regulatory decisions. Based on this work, the PMRA is developing a revised approach for the assessment of risk to pollinators to better characterize risks from foliar spray applications, seed coatings and soil applications of both systemic and non-systemic pesticides.

BACKGROUND: Pesticides, among a number of other factors, are proposed to have a potential contribution to the world-wide pollinator declines. Traditional pesticide risk assessment approaches for pollinators are focused almost exclusively on adult effects resulting from direct exposure. Recent information suggests that these risk assessment approaches may need to be updated to include additional exposure routes and toxicity profiles.

METHOD: HC-PMRA has been co-leading or actively participating in several national and international activities related to the issue of pollinator declines, including: OECD Survey on Pesticide-Pollinator Interactions, OECD Expert Working Group on Pesticide Effects on Insect Pollinators, SETAC Pellston Workshop on pesticide risk assessment for pollinators, collaboration between federal departments which have responsibilities related to pollinator issue, and an NSERC funded research initiative on pollinators (CANPOLIN). Using outcomes of these activities, PMRA is developing a revised approach to update the assessment of risk to pollinators.

The updated assessment approach further examines: (1) potential exposure routes related to the pesticide use information (crops, space, and timing), pesticide characteristics (systemic and non-systemic), and application methods (foliar spray application, seed coating or soil treatment); (2) the level of pesticide residues in various environmental compartments (air, water source, soil and plants including pollen and nectar) where bees may contact or feed on pesticides; and, (3) potential effects on either pollinator adults or brood. A tiered approach for data (laboratory, semi-field, and field studies) required to address either exposure or toxicity concerns is described. The honeybee is used as surrogate species while other insect pollinators are considered.

RESULTS/IMPACTS/CONCLUSIONS: Revised problem formulations, conceptual models and approaches for risk assessments are developed for foliar spray applications, seed coatings and soil applications using either systemic or non-systemic pesticides. Potential risk mitigation measures are also considered.

2.22 Measurement of Fine Particle Exposure ($PM_{2.5}$) in Residential Buildings in Five Canadian Cities

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SUMMARY: Canadians spend most of their time indoors. However, there is little data on their exposure to various air pollutants in their homes. Health Canada recently conducted a series of exposure studies in different urban centres across Canada. The studies were designed to document levels of air pollutants and produce an increased understanding of their sources. Fine particle levels ($PM_{2.5}$) are of particular interest due to their documented effects on cardiovascular and respiratory health. These studies will support the development of risk communication and management tools.

BACKGROUND AND OBJECTIVES: Health Canada recently conducted a series of exposure studies in different Canadian urban centres. The studies were carried out to produce an increased understanding of indoor air pollution sources and the levels to which Canadians are exposed in their residential buildings. The contribution of outdoor air pollution to indoor levels and the variability of concentrations by season were also examined.

MATERIAL AND METHOD: Health Canada measured exposure to $PM_{2.5}$ inside and outside of homes in Windsor, Toronto, Hamilton, Regina and Halifax between 2005 and 2009. Comparable homes with smokers were also included in the study conducted in Regina. In each residence, standardized questionnaires on the characteristics of the homes and the activities of participants during the sampling were administered.

RESULTS: A total of 369 homes participated in the studies. The majority of these residences were single-family homes. The average $PM_{2.5}$ concentrations ranged from 4.1 to 8.5 $\mu g/m^3$ for smoke-free homes and from 17 to 22 $\mu g/m^3$ for homes with smokers. The average ratio between indoor and outdoor concentrations (I/O ratio) ranged from 0.47 to 1.2 for smoke-free homes and from 3.6 to 4.4 for homes with smokers.

DISCUSSION AND CONCLUSIONS: The fine particle concentrations measured were generally higher outdoors than indoors (I/O < 1). In the specific case of the Halifax study, the I/O ratios higher than 1 can be attributed to particularly low outdoor particle concentrations. In homes with significant indoor particle sources, such as those with smokers in Regina, the I/O ratios were much higher than 1. In that case, controlling the indoor sources would be an effective means of reducing the fine particle exposure of residents.

2.23 Guidance Documents Outlining Health Canada's Role and Expertise Provided under the *Canadian Environmental Assessment Act*

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SUMMARY: Environmental Assessment (EA) is a planning tool used to predict the significant adverse environmental effects of development projects in order to minimize and monitor those effects. Health Canada, in response to requests from other departments, is developing guidance documents and fact sheets for the evaluation of human health information for EA reports. Six guidance documents outlining Health Canada's expertise in the areas of air quality, water quality, contamination of country foods, noise, radiological impacts and human health risk assessment are being developed. These documents will be available to federal departments and other stakeholders.

BACKGROUND: EAs are carried out under the *Canadian Environmental Assessment Act (CEAA)* for projects that involve the federal government (i.e., provision of land, funds or permits, or if the federal government is a proponent for the project). "Projects" may consist of physical works (e.g. construction of road), or physical activities (e.g., clear-cutting) as defined under *CEAA*. Guidance documents are being created in response to requests from other federal departments wishing to inform proponents/consultants of Health Canada's specific information needs for the review of project-related human health effects presented for EAs. Providing this information promotes better assessment of effects on human health, and increases efficiency in the provision of Health Canada's advice and allows for targeted mitigation measures and follow-up programs.

DESCRIPTION: Six guidance documents outlining Health Canada's expertise and role in the EA process are being developed for the areas listed above. They identify key elements, procedures, data requirements and collection methods, and other suggestions related to the evaluation of project-related human health information for EA reports. Industry-specific fact sheets (e.g., hydroelectric, wind farms, mining, and nuclear power plant projects) are also being developed to provide federal departments and other stakeholders with a short overview of the areas of Health Canada expertise that may apply to a specific industry sector.

OUTPUT: Health Canada's Environmental Assessment Division is planning to publish a series of guidance documents and fact sheets online within the next year. Their purpose is to contribute to meeting our department's obligations under *CEAA* by providing detailed guidance on the assessment of human health impacts to other federal departments and consultants involved in conducting EAs for development projects.

NEXT STEPS: Webinars are being planned to familiarize federal departments and other stakeholders with these guidance documents and fact sheets. The guidance documents will be updated periodically or as specific needs arise.

2.24 Effects of Alpha Particle Radiation on MicroRNA Responses in Human Cell-Lines

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SUMMARY: A variety of alpha (α)-particle emitters (a radiation type) are found in the environment, in commercial products and are a potential threat in the form of devices of malicious intent. Our understanding of the biological mechanisms and associated long-term health effects resulting from α-particle exposure is limited. Exposure to radiation may affect gene networks that involve MicroRNAs (miRNAs). In this study cells exposed to α-particle radiation were assessed for changes in miRNAs. Our data shows that α-particle cause changes in miRNAs that have been shown to result in diseases such as cancer.

OBJECTIVE: With the prevalence of α-particles in the form of radon gas and their current use in cancer treatment therapies, understanding long-term biological effects are of importance. No studies to date have assessed the biological impacts induced by α-particle radiation at the level of miRNA regulated gene networks. The aim of the present study was to identify miRNAs responding to low to moderate doses of α-particle radiation in a dose-dependent manner, as they would represent reliable radiation responsive targets. These responding miRNAs would provide insight into regulatory networks induced by α-particle exposure. For this purpose, three human-derived cell-lines (A549, THP-1 and HLF) were exposed to 0, 0.5, 1.0 and 1.5 Gy of α-particle radiation and analyzed 24 h post-exposure for differential changes in miRNA expression patterns relative to a control group.

MATERIALS AND METHODS: High quality RNA was extracted from samples exposed to á-particle radiation and converted to fluorescently labelled RNA to be hybridized onto bead array chips for miRNA expression profiling. Alterations in expression level of spots between control- and exposed- treatment groups were determined using stringent statistical methods. Selected miRNA responses were subjected to target prediction analysis (DIANA-mirPath) to identify molecular pathways potentially altered by the expression of the multiple miRNAs.

RESULTS: The screening of 1145 miRNA across three human cell-lines resulted in unique, cell-specific responses with no overlap in miRNA expression observed in the three cell-lines. A549 cells showed the highest number of responding miRNAs, a total of 7 miRNA, were expressed at the three doses test. Highest expression was observed with miR-486-5p and the lowest expression was observed with miR-424*. Human lung fibroblasts exposed to α-particle radiation expressed 5 responding miRNAs all of which were upregulated. Among the 5 miRNAs, three (miR-663, miR-560:9.1 and miR-675) were shown to have 2 fold or higher levels of expression. Screening of THP-1 cells following radiation insult resulted in no statistically significant responses 24 h post-exposure. However, the induction of miR-708 was observed three days post-exposure. Prediction analysis suggests these á-particle induced miRNA to target genes related to ribosomal assembly, lung carcinoma development, cell communication and keratin sulfate biosynthesis.

CONCLUSION: Taken together, the data suggest that alterations in expression levels of specific miRNA's may be indicative of radiation exposure and associated with adverse biological effects related to carcinogenesis. However, further validation would be required to identify the specific pathways that are involved in these regulatory responses.

2.25 The Development of the Public Health Agency of Canada's (PHAC) National Cells Tissues and Organs Surveillance System (CTOSS) for the Monitoring of Transplantation Adverse Events (TAE): A Background

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SUMMARY: Within Canada, a need exists to improve the safety of tissue and organ transplantations. No focused monitoring or traceability type surveillance system of transplantation adverse events (TAEs) exists within Canada. There remains a lack of robust and accurate adverse event data (on the 'what', 'where', 'why', and 'how corrected' aspects) that could lead to improved safety for those who opt for transplantation. The PHAC has developed the first such monitoring system referred to as the cells, tissues, and organs surveillance system (CTOSS). CTOSS data could help improve transplantation safety locally and internationally, by revealing where in the transplantation chain corrective actions are needed. CTOSS adverse event information can be shared/disseminated and used as a basis to generate benchmarks and incidence rates for comparisons.

BACKGROUND: Transplantations carry risks yet also yield life-saving and life-enhancing benefits. Each year in Canada, approximately 2000 organs are transplanted. Moreover, over 90 000 tissue allografts are distributed for transplantation. PHAC recognizes that improving transplantation quality and safety to Canadians also means improving surveillance and reporting of adverse events. At a national level, PHAC thus initiated the development (with province/territory and stakeholder involvement) of the first dedicated monitoring system for TAEs known as CTOSS. CTOSS does not track cells, tissues, or organs as well as their transplantation, but rather focuses on adverse events arising from transplants. A brief overview of CTOSS development follows.

DESIGN: The CTOSS system presently functions as a pilot involving four transplantation sites (one additional transplantation site added for 2011/2012). CTOSS's initial focus is on surveillance of recipients of tissue allografts and tissue products.

Two distinct patient groups in Canada receive tissue allografts: 1) receipt of surgical or dental grafts in hospital settings; or 2) in private clinic settings. Transplant recipients of surgical bone, cadaver bone, soft tissue, skin tissue, cardiac tissue, vascular tissue, ocular tissue and amniotic membrane are also under surveillance. Principle TAE data includes type of adverse reaction, clinical signs/symptoms, investigations initiated, and findings. Anonymous TAE data will be transmitted electronically to PHAC each quarter with data collection beginning by the second quarter of 2011. To initiate the adverse event surveillance, PHAC worked with the NWG, stakeholders, and experts to formalize: 1) a dedicated CTOSS adverse event reporting form; 2) an instructions and definitions manual; 3) a minimum data elements manual; and, 4) data transfer agreements. The aim is to unify and integrate the CTOSS data collection in line with reporting to Health Canada yet CTOSS reporting does not negate the regulatory reporting requirements to Health Canada (MHPD, BGTD, Inspectorate) under the existing CTO regulations.

RESULTS: CTOSS can be informative in the provision of much needed data to develop TAE incidence rates (for comparisons) and benchmarks. Such adverse event data can serve as the basis for general and targeted process improvements along the transplantation chain.

CONCLUSIONS: The transplantation of cells, tissues, and organs is becoming more commonplace in Canada as the treatment of choice for a host of illnesses, yet carries risks. These risks and adverse events must be monitored and characterized. CTOSS provides this opportunity. CTOSS information dissemination and reporting can result in corrective actions.

2.26 Blood Transfusion Errors Reported to the Public Health Agency of Canada's (PHAC) Transfusion Error Surveillance System (TESS) for 2005 to 2009: A Brief Epidemiology

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SUMMARY: Blood transfusions are commonplace within Canada. However, such procedures carry risk to the recipient (e.g., errors in transfusion chain procedures, infectious disease transmission, adverse reactions, etc.). The understanding of what the challenges are that contribute to such risks and potentially adverse outcomes, is vital for process improvements and corrections. The PHAC has developed a transfusion error surveillance system (TESS) to meet this need. TESS surveillance collects information on Canadian blood transfusion errors and collaborates/ exchanges data findings with hospital sites so as to ensure corrective actions are implemented. The result is reduced risk to the transfusion recipient.

BACKGROUND: The Transfusion Error Surveillance System (TESS) is a national surveillance system for transfusion errors developed by the PHAC.

METHODS: TESS uses standardized coding with ongoing training to participating hospitals/sites located in four provinces (small, medium, and large hospitals based on annual RBC transfusion volume). Data is web-based via a secured server, sent quarterly to PHAC for its annual reports. Reported errors for 2005/07-09 are described.

RESULTS: From January 2005 to December 2009, 60 705 errors were reported (10 273, 9918, 11 798, 15 193, and 13 523, respectively). Of those, 5781 (9.5%) were high severity errors (HS) that could potentially result in patient harm (7.2% to 10.1% 2005/07-2009). Sample collection (SC) was the main clinical error (29.3 vs 29.6% 2005/07-2009). Sample testing was the main laboratory errors (12.6 vs 8.3% 2005/07-2009). SC, sample handling, and product request were the main HS errors.

Most errors occurred from 8 am to 8 pm (72.6%), and 82.2% were on weekdays. Same day detection was 56.9%. Principle persons involved were nurses (47.7%), technologists (37.9%), and doctors (7.2%) for 2009 (46.6%, 39.7%, and 6.5% reported for the 2005/07). The primary error occurrence locations were transfusion service, medical/surgical wards, and emergency rooms (2005/07-2009). There were 2.7% actual and 97.2% near-miss errors, with 0.05% resulting in patient harm. SC and unit transfusion (clinical stage) and sample receipt and unit selection (laboratory stage) revealed the highest rates for the 05/07-09 period.

CONCLUSION: High SC rates continue to present a major threat to patient safety as it has the potential to cause grave ABO-blood group incompatible transfusions. Serious HS i.e., ordered for the wrong patient and administration of product to the wrong patient, continue. Automation (bar coding) may increase transfusion safety. TESS is continually being expanded and training is ongoing. Monthly data quality teleconferences are led by PHAC to standardize coding and to share error mitigation strategies. Ongoing education and periodic reviews of transfusion safety by personnel remain important to improved outcomes.

2.27 Estimating Daily Ambient Air Pollution at the Household-Level using Coupled Land-Use Regression Models and Continuous Monitoring Data in a Panel Study of Lung Function Among Asthmatic Children

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SUMMARY: Accurate assessment of both short- and long term exposure is needed to evaluate the health effects of air pollution. Pollution measurements can be expensive and burdensome; however, models predicting air pollution provide a cost-effective alternative. This study used information from annual pollution models at the household level and daily measurements at the community level to predict daily concentrations at the homes of asthmatic children in Windsor, Ontario. The model was able to accurately predict both air pollution levels measured at the home and adverse health effects related to air pollution, suggesting that daily models may improve short-term air pollution studies.

BACKGROUND: Land-use regression (LUR) models have emerged as a widely-used methodology for characterizing ambient pollution in health studies. However, they lack the fine-scale temporal resolution critical for predicting acute exposure. Conversely, fixed-site monitoring data provide daily concentrations, but fail to capture spatial variability within urban areas.

METHODS: This study coupled LUR models with continuous monitoring from the National Air Pollution Surveillance (NAPS) Network to predict daily ambient nitrogen dioxide (NO2) and particulate matter (PM2.5) at 40 homes in Windsor, Ontario. To evaluate the model, predicted concentrations were compared with measurements collected at each home for five days each in winter and summer. Associations between air pollution and lung function were also examined for children residing in those homes using both predicted and observed concentrations.

RESULTS: Predicted and measured pollutant concentrations were highly correlated (Rho-NO2=0.75; Rho-PM2.5=0.86). Mixed effect analysis suggested that coupled NO2 models explained a greater proportion of the spatial and temporal variance in measured pollutants compared with NAPS or LUR alone. For example, coupled models captured 27% of spatial and 39% of temporal variance in summer NO2 compared with LUR (23% spatial, < 1% temporal) and NAPS (< 5% spatial, 40% temporal). Ambient PM2.5 showed little spatial variation; therefore daily PM2.5 models were similar to NAPS in the proportion of variance explained.

Daily residential NO2 concentrations (measured and predicted) were associated with 6-7% decrements in FEV1 per IQR adjusting for age, sex, height, weight, and exposure to smoking ($p<0.05$). Daily NAPS-NO2 was not significantly associated with lung function ($p>0.10$). None of the PM2.5 measures were associated with decreased lung function.

IMPACTS AND CONCLUSIONS: These results suggest that LUR models can be combined with continuous monitoring to predict short-term (daily) household-level ambient pollution in epidemiologic studies.

2.28 Air Pollution and Air Exchange in an Urban High-Rise Apartment Building

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SUMMARY: Approximately 80% of Canadians live in cities, and many city-dwellers reside in high-rise apartments; however there is limited information available about air pollution in high-rise apartment buildings due to the challenges of conducting air pollution studies in high-rise homes. This study measured pollutant concentrations throughout a high-rise building, airflow to each apartment from outdoor and indoor spaces (including other apartments), and indoor sources such as cooking and smoking. The results of this study will support regulatory policy and guidelines to protect the Canadian population.

BACKGROUND: More than half of the global population currently resides in cities and many urban dwellers live in high-rise apartments. This study examined indoor and outdoor air pollution and air exchange in a residential high-rise building in downtown Ottawa, Ontario.

METHODS: We recruited apartments on lower, middle, and upper levels of the building. Neighboring units were included to assess vertical and horizontal infiltration. The study measured volatile organic compounds, particulate matter (e.g., $PM_{2.5}$), nitrogen dioxide, and carbon dioxide, as well as air exchange, temperature and relative humidity.

Continuous and integrated daily air pollution and air exchange measurements were collected at approximately 30 sites including apartments (indoor and balconies), hallways, elevators, stairwells and the rooftop. Perfluorocarbon tracers (PFT) were deployed to provide air exchange rates between indoor micro-environments as well as outdoor air exchange rate. Eight different PFTs were used in each of the three building levels. Building and occupant information were obtained through baseline and daily questionnaires.

RESULTS: Preliminary air exchange results for the summer suggest that the greatest contribution to air flow was from the outside, which explained a mean of 53% of total air flow to each apartment compared with a mean contribution of 41% from the central intake system and 1% from adjacent units. However, percent contribution from outside, central system, and adjacent apartments varied significantly across participating units. Preliminary analyses of DustTrak data suggest that continuous $PM_{2.5}$ trends were reflected between indoor and outdoor micro-environments.

IMPACTS AND CONCLUSIONS: The presentation will discuss factors influencing air exchange and air pollution concentrations in the building including occupant behavior, vertical dispersion, and wind direction. Findings from this study will characterize air pollution in high-rise buildings and identifying factors that may be modified to reduce exposures.

2.29 Biological Effects of Inhaled Diesel Exhaust after Removal of Diesel Particles

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SUMMARY: We assessed the toxicity of gas phase and particulate components of diesel exhaust. Acute exposure of rats to direct diesel exhaust produced lung injury characterized by neutrophilia and inflammatory cytokines. To determine relative contributions of nitrogen dioxide (NO2) and particles to the effects, the exhaust was treated through a diesel oxidation catalyst to reduce NO2 and a HEPA filter to remove particles. Surprisingly, no frank effects were observed in animal breathing exhaust after removing the NO2, with or without the particulate phase. Our data suggest that toxicity of inhaled diesel emission particles may be conditionned by NO2.

BACKGROUND: Automotive emission particles and gases such as NO_2 and carbon monoxide (CO) are important components of traffic-related air pollution and are implicated in adverse health impacts. We aimed at establishing a model to delineate biological responses attributable to gas and particulate phases of diesel exhaust emissions. To determine relative contributions of NO_2 and particles, the exhaust was treated through a diesel oxidation catalyst (DOC) to remove NO_2 and a HEPA filter to remove particles.

METHODS: Fisher rats were exposed by inhalation for 4h to clean air or the following diesel exhaust matrix. Untreated exhaust: particles, 0.25mg/m^3; CO, 6ppm; NO, 44ppm; NO_2, 4ppm. DOC exhaust: particles, 2mg/m^3; CO, 0.5ppm; NO, 18ppm; NO_2, <0.1ppm. DOC/HEPA exhaust: particles, <0.0001mg/m^3; CO, NO and NO_2 as DOC exhaust. Animals were euthanized 2h or 24h post-exposure. A number of biochemical endpoints relevant to the inflammatory and cardiovascular status of exposed animals were measured.

RESULTS: Exposure of the animals to the untreated exhaust resulted in acute lung injury characterized by increased lung neutrophilia 24h post-exposure and elevated expression of interleukin-6, prostaglandin synthase and metallothionein 2h post-exposure. Surprisingly, exposure to DOC exhaust did not cause any statistically-signicant biological changes in a wide panel of clinical (hematology, transaminases, creatine kinases, lactate dehydrogenase etc), biochemical (lung lavage and plasma cytokines, endothelins), lung and heart genes expression (inflammation, xenobiotic metabolism, oxidative stress and vasoregulation) endpoints. Delivery of particles was confirmed by dark lung macrophages in animals inhaling DOC exhaust. Macrophages from animals, inhalting DOC/HEPA exhaust were indistinguishable from clean air controls.

CONCLUSION: Inhalation of a diesel particle concentration almost two orders of magnitude higher than the Canada Wide Standard for $PM_{2.5}$ is not sufficient to cause frank biological effects in healthy rats. Toxic potency of particles may be modulated by the presence of reactive gases such as NO_2.

2.30

WITHDRAWN

2.31 Subchronic Oral Exposure to Benzo[a]pyrene Induces Gene Expression Changes in Mouse Lungs Associated with Cellular Transformation

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SUMMARY: Benzo(a)pyrene, BaP, is a combustion by-product commonly found in cigarette smoke and charred meats. It is primarily broken down in the liver, however, tumours develop in the lung, not the liver. This study examines both the working state of all genes (toxicogenomics) and traditional toxicology endpoints to explore the biological mechanisms that specifically make lungs prone to cancer development. We show that lungs of mice exposed to BaP have increased frequency of DNA damage and mutation, the first steps in cancer development. Gene analysis highlighted genes associated with cancer development. The use of toxicogenomics data in risk assessment of environmental chemicals, such as BaP, is discussed.

BACKGROUND: BaP is an environmental mutagen that alters the expression of genes involved in several biological processes. The primary route of BaP exposure is oral ingestion, however oral exposure does not result in hepatic tumours, but does elicit lung tumours. We examined pulmonary mRNA expression changes following subchronic exposure to BaP in an effort to understand specific molecular mechanisms that selectively contribute to the development of tumours in the lung.

METHODS: Adult male Muta™Mice were exposed to BaP (25, 50 or 75 mg/kg bodyweight/day) or vehicle control daily by oral gavage for 28 days and sacrificed 3 days post-exposure. DNA adduct and *lacZ* transgene mutant frequencies were measured in the lung tissue. Using whole-genome DNA microarrays, the pulmonary mRNA expression was assessed. The results were validated using pathway-specific PCR arrays. Expression profiles in the lungs were compared to the liver profiles from the same mice.

RESULTS: Dose dependent 240 to 600-fold increases in DNA-adducts, and 5 to 18-fold increases in mutant frequency were observed in the lungs relative to matched controls. 423 genes were significantly differentially expressed (fold-change>1.5 and False-Discovery-Rate-adjusted p-value<0.05) in lungs, and were associated with molecular processes linked to cancer development such as: DNA repair, cellular proliferation, apoptosis, angiogenesis, and calcium homeostasis. Compared to the liver, the lung showed greater than 2-fold increase in DNA adducts and a larger response in the number of altered genes following BaP exposure; 134 genes mainly involved in metabolism, inflammation and immune-response pathways were differentially expressed.

IMPACTS AND CONCLUSIONS: Here we demonstrate that lung is more responsive than liver to oral BaP exposure. Moreover, we observe altered expression of genes associated with carcinogenic processes only in the lung. Implication of such selective alterations following subchronic BaP exposure will be discussed.

2.32 Controlled Human Exposure to Fine and Coarse Ambient Particles and Effects on Systemic Biomarkers

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SUMMARY: We studied human biological effects of particulate air pollutants with different sizes and components. The information is required for conducting human health risk assessment on air pollution. The clinical exposure facility draws air from downtown Toronto. Healthy non-smoking adults were exposed to fine and coarse particles and particle-free air on separate days. We observed adverse changes in some proteins that mediate blood vessel function, and biomarkers of oxidative stress in blood and urine following exposure to the pollutants. The potency of fine and coarse particles appear to be different, which may be due to their different chemical and biological contents.

OBJECTIVE: Limited research has been reported in literature on adverse health effects of coarse air particulates on human subjects. This information is needed to develop air quality policy. We investigated biological changes induced by fine and coarse particulate air pollutants in a clinical setting.

METHODS: Controlled exposure was conducted in a facility that drew air from a Toronto street. Nineteen healthy non-smoking volunteers, 18-50 years old, were exposed to fine and coarse particles, and particle-free air in random order for two hours, separated by ≥two weeks. Mean concentrations of fine and coarse particles were 244 and 206 $\mu g/m^3$, respectively. Systemic biomarkers for inflammation, vascular function and oxidative stress were examined in urine and peripheral blood pre-exposure, and 3-hour and 24-hour post exposure.

RESULTS: In urine, exposure to fine particles was significantly associated with elevated vascular endothelial growth factor concentration, a vascular permeability-inducing agent and angiogenesis stimulator. Thiobarbituric acid reactive substances (TBARS, a biomarker of oxidative stress) also increased post exposure to fine particles. Exposure to coarse particles did not result in significant change in urine biomarkers. In blood, exposure to coarse but not fine particles resulted in a significant increase in endothelin-1, a vasoconstrictor. Exposure to both fine and coarse particles resulted in a reduction in C-reactive protein (an acute-phase reactant biomarker for systemic inflammation), while exposure to fine but not coarse particles resulted in a reduction in TABARS.

CONCLUSIONS: Fine and coarse particles appear to exert adverse effects in a different manner. Fine and coarse particles originate from different sources, with fine particles mainly from combustion of fossil fuels and coarse particles from construction, road dusts and biological sources (pollen, bacteria, fungi). Information from this study is useful for Health Canada to evaluate health risks of particulate pollutants and their source and component, and develop air quality guidelines.

2.33 Evaluating Human Biomarkers of Exposure to Wood Smoke Resulting from Traditional Temazcal Use

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SUMMARY: Indigenous populations in Central America use temazcals (wood-fired sweat lodges) for bathing and healing purposes. The wood-burning process generates particles that, when inhaled, are known to cause adverse health effects. Urine samples were collected from individuals before and after temazcal use, and were tested for their ability to induce mutations in bacteria. Urine from those who use temazcals was found to induce mutations, and the level of activity was associated with other measures of exposure (e.g., length of time in the temazcal). This shows that urine mutagenicity is a good measure of exposure to wood smoke particles.

BACKGROUND/OBJECTIVES: Traditional temazcals (i.e., sweat lodges) are commonly used by native populations in Central America. Temazcals are heated via biomass combustion, and exposure to particulate emissions has been associated with numerous adverse human health effects (IARC 2A). Additionally, biomass is a popular choice for home heating in developed countries, and even electric power generation. Urinary mutagenicity is a known biomarker of human exposure to combustion emissions. This study evaluates urinary mutagenicity as a biomarker of exposure to biomass combustion emissions in individuals who regularly use traditional temazcals.

DESIGN/METHODS: Study subjects are indigenous Mayan families from Guatemala who regularly use traditional temazcals, as well as control individuals from the same population who do not engage in this practice. Urine samples collected before and after temazcal exposure were enzymatically hydrolyzed overnight, and de-conjugated urinary metabolites were concentrated by solid-phase extraction. Mutagenic potency (MP) of concentrated extracts was assessed using the standard plate-incorporation version of the Ames/ *Salmonella* assay (strain YG1041 with S9 activation). Exhaled carbon monoxide (ppm) and blood CO levels (%COHb) were also measured. This study was conducted under approval by Health Canada's Research Ethics Board.

RESULTS: Both exhaled and blood CO levels were significantly increased following exposure. MP also appears to be modified by temazcal use. A general trend towards increasing mutagenicity following temazcal exposure was observed (2.1-fold increase over controls). Blood and exhaled CO levels were significantly correlated with the MP of the urine concentrates, and the MP of urine concentrates from exposed individuals was significantly correlated with time spent in the temazcal.

CONCLUSIONS/IMPACTS: The use of temazcals results in the production and urinary excretion of mutagenic metabolites. Temazcal use contributed to an increase in urinary MP, which is empirically related to other measures of exposure, such as %COHb and duration of temazcal use, demonstrating that urinary mutagenicity can be used as a biomarker of exposure to wood smoke in traditional temazcals.

2.34 Past, Present and Future - Human Health and Ecological Exposure Assessment under Canada's Chemical Management Plan

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SUMMARY: The Ministers of Health and the Environment jointly conduct screening assessments of existing chemicals under the *Canadian Environmental Protection Act*, 1999. Since 2007, Health Canada and Environment Canada, through the Chemicals Management Plan (CMP), have addressed approximately 200 high priority substances under the "Challenge" initiative. This poster will highlight strengths and limitations from past exposure-based prioritization exercises, exposure-based lessons learned from the 'Challenge' initiative, and current data and tool needs moving forward with the remaining 3000 prioritized substances.

BACKGROUND: Assessing risk as part of Canada's global commitment to address legacy chemicals by 2020 is implemented through the CMP. The CMP integrates all existing, relevant federal programs together to ensure that chemicals are assessed and managed appropriately to prevent harm to Canadians and their environment. A key part of the activities is the environmental and human health exposure characterization.

OBJECTIVE: The poster will highlight the strengths and limitations from past exposure-based prioritization exercises, exposure-based lessons learned from the "Challenge" initiative, and current data and exposure tool needs moving forward within the context of chemicals management.

DESCRIPTION: In 2006, Canada categorized approximately 23 000 existing commercial substances through a priority-setting exercise using available data, quantitative structure-activity relationship (QSAR) modelling, and simple tools that ranked substances based on a number of parameters including their potential for human exposure. Since 2007, Health Canada and Environment Canada have addressed the highest priorities from this exercise by jointly conducting screening assessments on approximately 200 substances as part of its 'Challenge' initiative. Exposure was characterized using empirical data and exposure models to estimate environmental concentrations in air, water, soil, sediments, food and dust, as well as exposure resulting from use of consumer products. Rapid screening approaches were also developed for lower priorities. An overview is presented of both human and ecological exposure tools and methods used to estimate environmental releases and assess ambient environmental exposure, and approaches to assess exposures in indoor environments.

NEXT STEPS: Moving forward, it is recognized that novel approaches to complete the assessment of approximately 3000 remaining prioritised substances and that enhancements to exposure tools available to regulators are needed. Understanding where and how chemicals are used throughout their life cycle is crucial in exposure assessment and continued efforts, be it through international initiatives or stakeholder engagement, are key to improving the quality of exposure assessments.

2.35 The Validity of Self-reported Smoking Status among Canadians

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SUMMARY: Cigarette smoking is associated with numerous adverse health effects, including cancer, respiratory illness and heart disease. In Canada, self-reported data are generally used to produce and monitor trends in cigarette smoking. However, cotinine measurements in urine may also be used and are widely accepted as an objective measure of exposure to tobacco smoke. Therefore, the purpose of this study was to examine the validity of self-reported smoking status among Canadians by comparing estimates of smoking prevalence based on self-report vs. urinary cotinine concentrations. These data suggest that smoking prevalence based on self-report provides a valid estimate of smoking prevalence in Canada.

OBJECTIVE: To examine the validity of self-reported smoking status among Canadians by comparing estimates of smoking prevalence based on self-report vs. urinary cotinine concentrations.

MATERIALS AND METHODS: Data were from the 2007-2009 Canadian Health Measures Survey, a nationally representative cross-sectional survey, which included self-reported smoking status and the first nationally representative measures of urinary cotinine in Canada for 4530 Canadians aged 12-79. The prevalence of cigarette smoking calculated based on self-report was compared to the one calculated based on urinary cotinine concentrations.

RESULTS: Compared to smoking prevalence based on urinary cotinine concentrations, smoking prevalence based on self-report was lower by 0.3 percentage points. There were no significant differences between prevalence based on self-report versus cotinine concentrations for any of the sex or age groups. Correlation results indicated strong agreement between smoking status based on self-report and cotinine ($r=0.90$, $p<0.001$). Sensitivity estimates (i.e., the percentage of respondents who reported being smokers among those who were classified as smokers based on cotinine concentrations) were similar for males and females. Although sensitivity tended to be lower for respondents aged 12-19 yr compared to those aged 20-79 yr, the difference did not attain statistical significance.

CONCLUSION: Results from this study based on nationally representative data from the Canadian population showed no significant difference between national estimates of smoking prevalence based on self-report vs. urinary cotinine concentrations, which suggest that smoking prevalence based on self-report provides a valid estimate of smoking prevalence in Canada.

2.36 Scientific Advancements in Human Health Risk Assessment and the Derivation Process of Soil Quality Guidelines for the Protection of Human Health

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SUMMARY: Health Canada's Contaminated Sites Division develops Canadian Soil Quality Guidelines for the Protection of Human Health (SQG_{HH}) in collaboration with the Canadian Council of Ministers of the Environment (CCME). SQG_{HH} provide levels of chemicals in soil below which no human health risks are expected. The process to derive SQG_{HH} and human health risk assessment (HHRA) are similar. Scientific advancements in HHRA such as improved exposure, toxicological and environmental data, are being applied to the SQG_{HH} derivation process to decrease uncertainty and avoid over conservatism. This enables Health Canada to derive defensible SQG_{HH} and to better address emerging environmental chemicals of concern.

OBJECTIVE: SQG_{HH} are developed for use at contaminated sites to provide levels in soil for which no human health risks are expected. It is our objective to incorporate scientific advancements in HHRA into the SQG_{HH} derivation process to improve management of contaminated sites in Canada through the development of soil quality guidelines based on improved exposure, toxicological and environmental data.

DESCRIPTION: The CCME has published *A Protocol for the Derivation of Environmental and Human Health Soil Quality Guidelines* (CCME 2006) which outlines the SQG_{HH} derivation process. In support of HHRA and deriving SQG_{HH}, research has been completed to refine the following areas: environmental fate/behaviour of chemicals, chemical toxicology, background exposure, human physiology (soil and dust ingestion and bioavailability) and anthropometric parameters (body weight).

OUTCOMES:
1) Toxicological profiles were recently developed (new SQG_{HH}) and updated (existing SQG_{HH}) for polycyclic aromatic hydrocarbons, n-hexane, and several metals (i.e., Pb, Ni, Zn and Ba) and are in progress for vinyl chloride, perfluorinated compounds and a number of other metals (i.e., Cr).
2) New exposure media (dust, indoor air, breast milk) were included in the assessment of environmental sources of chemicals.
3) Estimated daily intake (EDI) methodology was updated to include a probabilistic approach.
4) Exposure factors were updated to reflect current data collected from the Canadian population (i.e., body weight harmonization project) (ongoing).
5) Updated soil and dust ingestion rates (ongoing).
6) Collaboration with Geological Society of Canada to obtain background soil concentrations relevant to human health (ongoing).

IMPACTS/IMPLICATIONS: Health Canada derives SQG_{HH} which are published by the CCME and are used by site managers across Canada. SQG_{HH} are used to screen potentially contaminated sites for further management activities. Additionally, SQG_{HH} scientific supporting documents, which describe the methods and data used to derive $SQG_{HH,}$ present information site managers can use to interpret HHRAs and develop site-specific objectives.

Implementation of new research allows site managers to have increased confidence in SQG_{HH} values such that they protect human health, and allows Health Canada to be consistent with regulatory organisations in other provinces and countries. It also allows Health Canada to better address emerging environmental chemicals of concern. SQG_{HH} development draws on data and activities used by other Health Canada departments within Water Air and Climate Change Bureau and Existing Substances and Risk Assessment Bureau with whom the Contaminated Sites Department collaborates.

2.37 Deployment of the Vitrocell System for *In Vitro* Toxicity Assessment of Aerosols and Vehicular Emissions at an Air-Liquid Interface

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SUMMARY: The Vitrocell exposure device permits exposures of cultured animal cells to aerosols, such as vehicle exhaust, at an air-liquid interface. Prior to deploying the instrument for examination of diesel exhaust (DE), the performance was evaluated using a reference gas (i.e., nitrogen dioxide). Subsequently, the Vitrocell was used to examine the toxicological activity of dilute diesel exhaust. Significant reduction in cell viability was observed when cells were exposed to both NO2 and dilute diesel exhaust. The results of these exposures confirm that the Vitrocell system is a useful tool for routine toxicological assessment of vehicular emissions.

BACKGROUND: *In vitro* toxicity assessment of aerosols is problematic since cultured cells are ordinarily exposed in liquid medium, creating difficulty in establishing contact between the aerosol and the cells. The Vitrocell offers a practical exposure scenario that closely resembles *in vivo* conditions. Specifically, cultured cells are grown on porous membranes and exposed to aerosols (i.e., diluted vehicular emissions), in real-time, under controlled experimental conditions. The device offers the possibility of routine toxicological assessments of vehicular emissions without the inherent complexity of animal inhalation exposures.

METHODS: A549 adenocarcinoma lung epithelial cells were grown on porous membrane inserts and placed in the Vitrocell test chamber. Cells were exposed to 5ppm and 20ppm NO_2 and dilute diesel exhaust (1:8), as well as clean air controls, at a previously established flow rate of 8.3 ml/min for one hour. Cell viability was examined using cleavage of the tretrazolium salt WST-1 by mitochondrial dehydrogenases, and relative ATP activity was assessed using the ATPlite luciferase system. All results were compared to incubator controls.

RESULTS: A significant reduction in mitochondrial activity and ATP activity were observed following exposure to NO_2 (5ppm, 20ppm) as compared to incubator controls. Synthetic air-exposed cells were slightly less viable than incubator controls. Cells exposed to dilute diesel exhaust showed a significant reduction (42.6 ± 3.4%) in ATP activity and a significant reduction (44.0 ± 1.0%) in mitochondrial activity compared to incubator controls.

CONCLUSIONS/FUTURE DIRECTIVES: The results from the NO_2 and vehicular emissions confirm that the Vitrocell system with A549 cells is suitable for toxicity assessment of aerosols. Future work includes toxicological assessment of diesel exhaust representing a host of fuel formulations and after-treatment conditions. Other endpoints (lipid peroxidation, DNA damage) will also be employed. The use of primary human airway epithelial cells will be used to assess inter-individual responses to vehicular emissions.

2.38 How Much do we Know About the Fate of Airborne D4 and D5?: Full Scale Chamber Study Under Various Environmental Conditions

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SUMMARY: Octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), two typical organic cyclic siloxanes, are volatile and toxic. They are ubiquitously present in the environment including indoor air due to their many consumer and industrial applications. Therefore, there is increasing concern of their effects on human health. This study investigated the possible transformation of D4 and D5 under various environmental conditions. While D4 and D5 were stable under ozone or UV light alone, they appear to be unstable and transformed to new compounds in the presence of UV and ozone together.

OBJECTIVES/BACKGROUND/ISSUE(S): D4 and D5 are ubiquitously present in the environment due to their many consumer and industrial applications such as cosmetics. They are classified as toxic for reproduction category 3 and raise concerns on health effects. However, their fates under environmental factors including ozone and UV light are not clear. This information is important for accurate risk assessment of these substances. The objective of this study is to conduct investigations of stability of airborne D4/D5 under ozone and UV light in a full-scale environmental chamber system.

DESIGN/METHOD/DESCRIPTION: The tests were conducted in a full-scale environmental chamber (interior volume 56.4 m^3). D4 or D5 was spiked into the chamber at a concentration of 1 mg/m^3 followed by introduction of ozone gas or/and UVA/B irradiation. The intensities of UVA and UVB in the chamber were around 1mW/ cm^2. Ozone was monitored continuously by an ozone analyzer. D4, D5 and other VOCs were analyzed by GC/MS.

OUTPUTS/RESULTS: Results showed that D4 and D5 were stable in the presence of either ozone or UV light alone. However, they were unstable in the presence of both ozone and UV light due to likely breakage of the D4 or D5 ring. It appears that UV light promoted the production of ozone and promoted reduction of airborne siloxanes likely by combination of photo-oxidation and ozonation.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This study demonstrated that airborne siloxanes of CMP priority such as D4 and D5 are unstable in the presence of ozone and UV light together. The potential degradation of siloxanes indicates the complex nature of contaminants in the environment and importance of considering their environmental fate in risk assessment. The identification and quantification of products, as well as the mechanism need to be further investigated.

2.39 Zinc Bioaccessibility in House Dust: Speciation and Transformation

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SUMMARY: Zinc (Zn) and its compounds have been identified as a priority group of substances under the Chemicals Management Plan. In indoor environments, exposures arise from a wide variety of products containing Zn (e.g., paint, pharmaceuticals, cosmetics, plastic), and through ingestion of settled dust which contains metals from outdoor and indoor sources. This study investigates the forms of Zn in dust, and shows that highly humid conditions may favour an increase in soluble forms of Zn. The pathways of exposure to Zn vary within a home depending on Zn sources, amount of settled dust, particle characteristics, and environmental conditions (e.g., humidity).

OBJECTIVES/BACKGROUND: This study investigates the speciation and transformations of zinc (Zn) in house dust. Analysis of residential dust and soil sampled from non-industrial neighbourhoods showed that Zn bioaccessibility tends to be higher in the indoor environment (65%) compared to corresponding garden soil (29%). Possible reasons for this trend include differences in Zn sources, potential influences of the dust matrix chemistry, and transformations in the indoor environment. Experimental work was undertaken to determine the impact of transformations in the indoor environment on the change in Zn bioaccessibility in house dust.

METHOD: X-ray absorption fine-structure (XAFS) spectroscopy was used to characterize the main Zn species in a control house dust sample containing 500 mg/kg bioaccessible Zn. The impact of humidity on Zn speciation was evaluated on a set of three dust samples showing elevated Zn bioaccessible concentrations (> 1100 mg/kg) by subjecting the samples to an oxygenated, 100% humid atmosphere in a closed chamber for three months. Zn bioaccessibility before and after the experiment was determined using a simulated gastric acid digestion.

RESULTS: While house dust is rich in organic matter (mean organic carbon conc. = 27 wt. %), Zn was dominantly associated with the inorganic mineral fraction as carbonates, sulfides and bound to iron-oxyhydroxides. Exposure of house dust to humid conditions led to a significant increase in Zn bioaccessibility, from 20 to 60% of the original values depending on the sample.

CONCLUSIONS/ NEXT STEPS: These results suggest that elevated humidity in indoor microenvironments may sustain higher Zn bioaccessibility in settled dust compared to drier conditions. The next stage will focus on elucidating the chemical transformation of various Zn species during weathering in a humid, organic-rich environment.

2.40 Pesticides in Canadian Groundwater: A Preliminary Examination

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SUMMARY: Health Canada's Pest Management Regulatory Agency (PMRA) is attempting to gain insight into the current state of pesticides in Canadian groundwater by compiling monitoring data from various sources. Although only some of the existing data on this subject has been critically reviewed, preliminary analyses suggest that detection of most pesticides in groundwater occur at low frequencies and at concentrations below available Drinking Water Guidelines.

BACKGROUND/OBJECTIVES: The presence and levels of pesticides in groundwater across Canada is not well understood. Various studies and monitoring programs have, and continue to take place throughout Canada, but the results are not always easily accessible. The objective of this project is to compile data from a variety of sources in order to gain insight into the current state of pesticides in groundwater in Canada.

DESCRIPTION: The initial data collection was limited to pesticides identified by the PMRA as having a high potential to leach into groundwater, and pesticides for which the PMRA has received water monitoring information through the PMRA's re-evaluation program. Other readily available information such as monitoring data from prominent U.S. databases (NAQWA, STORET) was also considered. Environment Canada and Federal Provincial and Territorial Committees were contacted to access any additional available monitoring data.

RESULTS: A wide array of studies were examined ranging from very focused, short-term experiments to province-wide, ongoing monitoring programs. A summary chart including over 80 pesticide active ingredients has been created containing information regarding detection frequencies, concentrations detected, and the date and authors of the research. A preliminary analysis of the data suggests that overall, detection frequencies of most pesticides are fairly low and concentrations detected tend to be below Drinking Water Guidelines.

CONCLUSIONS/NEXT STEPS: Since this project only focuses on a subset of the available data on this topic, it is difficult to draw significant conclusions at this time. The next step may involve examining additional publicly available data as well as the more extensive American data to assess trends in pesticide detection patterns in both countries. Once a sufficient amount of data has been analysed, the findings of the project may be used to support the development of risk assessment and mitigation approaches or policies for groundwater contamination by pesticides.

2.41 Does Your Health Risk from Ozone Exposure Depend on When and Where you Live in Canada?

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SUMMARY: Canadians are regularly exposed to ground-level ozone from outdoor sources, transportation and industrial activities. This exposure can lead to chronic disease or death caused by heart or lungs (cardio-pulmonary (CP) mortality). As a partner with Environment Canada, Health Canada developed an indicator to monitor the impacts of ozone over time on the health of Canadians (http://www.ec.gc.ca/indicateurs-indicators/default.asp?lang=en&n=CB7B92BA-1). Based on 24 cities that were classified into four regions, we examined CP mortality attributable to ground-level ozone for warm season for 1984-2006 and investigated changes in annual regional and national CP mortality risks, respectively. It was found CP mortality risk associated with ozone is overall constant across Canada for the study period.

OBJECTIVES/BACKGROUND/ISSUES: In Canada ground-level ozone has been monitored as a main air pollutant that is linked to health outcomes such as mortality and morbidity. We wish to examine regional and temporal differences in mortality risks.

DESIGN/METHOD/DESCRIPTION: We have previously developed a dynamic model for annual public health risk at national and regional levels applying a Bayesian hierarchical 2-level model. Applying the dynamic model, we estimated annual association between daily ground level ozone concentrations and daily mortality counts during warm season (April to September) for 23 years (1984 to 2006) at 24 Canadian cities classified into 4 regions (Eastern Canada, Golden Horseshoe, Western Ontario and Western Canada). To identify regional differences, we tracked heterogeneities within and between regions over time. Estimates of risk were compared by cause of mortality (cardio-pulmonary (CP) versus non-CP).

OUTPUT/RESULTS: For the annual national risks, CP mortality, in comparison to non-CP mortality, was found to have overall higher risk and less variation over time. An 0.8% increase in CP mortality and an 0.5% increase in non-CP mortality were associated with a daily 10 ppb increase in daily ozone through the whole 23 years. The four regional risk estimates showed overall similar patterns being positively correlated (0.02-0.60). The CP mortality did not show any time trend in national risks even if a decreasing time trend in region 3 (Western Ontario region) was detected (p=0.04). The heterogeneity between the four regions was usually larger than that between cities within regions. The between region heterogeneities displayed an increasing time trend marginally (p=0.05), while no time trend in within region heterogeneities was detected.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: We observed overall no time trend in the 23 annual regional and national CP mortality risks but a slightly increasing time trend in the regional differences. Further studies are necessary to understand differences between regions.

Region classification

Region 1 (Eastern Canada): Halifax, Saint John, Quebec, Montreal, and Ottawa.
Region 2 (Golden Horseshoe): Oshawa, York, Toronto, Peel, Oakville, Hamilton, and Niagara.
Region 3 (Western Ontario): Waterloo, Windsor, Lambton, London, Sudbury, and Sault Ste. Marie.
Region 4 (Western Canada): Winnipeg, Regina, Saskatoon, Calgary, Edmonton, and Vancouver.

2.42 Combined Retrospective Analysis of 498 Rat Multi-Generation Reproductive Toxicity Studies: The Impact of Parameters Related to F1 Mating and F2 Offspring

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SUMMARY: The multi-generation reproductive toxicity study [Organization for Economic Cooperation and Development (OECD) Test Guideline 416 and USEPA 870.3800] has been extensively used internationally to assess the adverse effects of substances on reproduction. With fewer resources and calls for decreased animal testing, the necessity of producing a second generation to assess the potential for human health risks has been questioned. Working together, the Netherlands, the United States, Canada, Germany and the United Kingdom analysed the results from 498 rat reproduction studies and found that a second generation rarely provided additional information for risk assessments, regarding effects of chemicals on reproduction. This information has lead to the development of a new, more efficient test design (see EOGRTS poster).

DESCRIPTION: This standardized retrospective analysis of the impact of the second generation on overall study outcome combines earlier analyses and includes 498 rat multi-generation studies representing 438 different tested substances.

OUTPUTS: Detailed assessment of study reports revealed no critical differences in sensitivities between the generations on the basis of a consideration of all endpoints evaluated. This analysis indicates that the second generation mating and offspring will very rarely provide critical information.

IMPACT: These findings are consistent with the conclusions of previous retrospective analyses conducted by RIVM, USEPA and PMRA and support adoption of the proposed OECD extended one-generation reproductive toxicity study protocol in regulatory risk assessment testing strategies (see EOGRTS poster).

2.43 Reference Methodologies for Radioactive Controlled Discharges: An Activity within the IAEA's program "Environmental Modeling for Radiation Safety II" (EMRAS II)

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14

SUMMARY: The International Atomic Energy Agency (IAEA) organizes an Environmental Modeling for Radiation Safety II program, in which Working Group 1 (WG1) is comparing different dose assessment methods for regular discharges of radioactivity into the environment from nuclear facilities. This comparison is being performed by modeling two different release scenarios; one for a nuclear power reactor located on the coast and one nuclear power reactor located on a river bank. The outcome of this modeling exercise is better insight in the procedures for evaluation of the radiological impact of nuclear installations taking into account all possible exposure pathways.

OBJECTIVES: In January 2009, the IAEA launched the EMRAS II program to develop and compare models for the assessment of radiological impacts to the public and the environment due to anthropogenic sources of radioactivity. By providing a forum for the exchange of experience the program aims to support countries to build and to harmonize their capabilities. Within EMRAS II, nine working groups are active; this paper will focus on WG1: Reference Methodologies for Controlling Discharges of Routine Releases. Within this working group a comparison of different environmental transfer and dose assessment models for different release scenarios was carried out. This could allow each participating country to refine their methods/models of estimating the impact of radionuclide releases into the environment and to provide guidance in performing environmental modelling.

DESIGN: In the first phase, the group worked on a scenario where a nuclear power plant located at the coast routinely discharges ^{60}Co, ^{85}Kr, ^{131}I, and ^{137}Cs into the atmosphere and ^{60}Co, ^{137}Cs, and ^{90}Sr to the marine environment. To effectively compare the results, the model parameters, and the characteristics of the representative group were pre-defined. Various models have been used in this inter-comparison, namely PC-CREAM, CROM, IMPACT, CLRP, and POSEIDON.

OUTPUTS/RESULTS: The results of the first scenario demonstrate that the atmospheric model outputs are within a factor of 4 for the total radiation dose to the public. However the individual pathways (inhalation, ingestion, external exposure, etc) tend to vary significantly between the different models.

IMPACTS/CONCLUSIONS: The goal of this exercise is to provide guidance in performing environmental modelling through the comparison of state-of-the art dose assessment models. To conclude, the various radionuclide environmental transfer models used by different modellers from different countries were inter-compared. Considering the complexity of modelling the physical and chemical processes governing the fate and behaviour of radioactivity in the environment, the results are reasonable.

2.44 Mapping Biological Effects of Inhaled Pollutants: The Brain and Pituitary as Targets

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SUMMARY: Population studies indicate that air pollution may impact the brain. This implies that reducing air pollution levels would have positive societal benefits that extend beyond improving cardiopulmonary health. Because the underlying processes are not understood, we used an animal model to screen for effects of exposure to the common pollutants ozone and particulate matter. We found that several biological pathways were activated in the brain and pituitary in response to pollutant exposure, and effects throughout the body were consistent with hormonal signalling. Further studies are warranted to investigate the physiological consequences of these effects.

BACKGROUND: Air pollution is recognised as a risk factor for respiratory and cardiovascular disease. Recent data suggest that short-term variation in air pollution is also associated with headache, irritability, aggression, increased psychiatric admissions and emergency calls, and suicide, but the biological mechanisms are not understood. We investigated impacts of pollutant exposure on a variety of biological pathways in the brain.

METHOD: Fisher-344 rats were exposed for 4h to particulate matter and ozone, and euthanized immediately or 24h after exposure. Genes involved in inflammation, oxidative stress, xenobiotic metabolism, metal-response, and endothelial dysfunction were screened by real-time polymerase chain reaction in a number of organs, including the brain and pituitary. Potential impacts on the neuroendocrine system were also explored.

RESULTS: The cerebral hemisphere and pituitary exhibited transient increases in the mRNA expression of redox sensitive genes (e.g. hypoxia-inducible factor-3α, metallothioneins). Expression of cyclooxygenase-2, an important activator of the hypothalamic-pituitary-adrenal (HPA) axis, was increased by both pollutants in the pituitary but not the cerebral hemisphere. Expression of pituitary proopiomelanocortin, the precursor of the glucocorticoid-signalling adrenocorticotropic hormone, was increased following particle exposure. In line with impacts on glucocorticoid production, there was a systemic (e.g., spleen, heart, liver) decrease in the mRNA expression of inflammatory mediators (e.g., tumour necrosis factor-α, interleukin-1β, monocyte chemotactic protein-1) and increased expression of glucocorticoid-responsive genes (e.g., glucocorticoid-induced leucine zipper, serum/glucocorticoid-inducible kinase-1).

CONCLUSIONS: Dysregulation of the HPA axis is known to be associated with neurological disorders. Our results indicate that both particulate and gaseous pollutants can activate transcriptional pathways in the brain and pituitary. The neuroendocrine perturbations can have systemic consequences, as revealed by the depression of immune response genes. Further studies are warranted to investigate the health significance of our observations.

2.45 Seasonal and Diurnal Variation of Ultrafine Particle Exposure Levels in Private Vehicles in Toronto, Canada

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SUMMARY: The Urban Transportation Exposure Study (UTES) was conducted to characterize traffic pollution exposures in buses, subways, and private vehicles in three major Canadian cities. As part of this study, ultrafine particle (UFP) counts were monitored inside and outside of private vehicles using TSI Model 3007 Condensation Particle Counters for 10-days in September 2011 and 5-days in March 2011. Innercity, suburban and highway routes were monitored during morning (7-10 am) and afternoon (3-6 pm) commutes. In-vehicle UFP levels in Toronto tended to be higher on highway routes during winter morning commutes.

BACKGROUND: The Urban Transportation Exposure Study (UTES) was conducted to characterize traffic pollution exposures in buses, subways, and private vehicles in three major Canadian cities. Here we present findings for private vehicles in Toronto.

METHODS: Ultrafine particle (UFP) counts were monitored inside and outside of private vehicles using TSI Model 3007 Condensation Particle Counters for 10-days in summer (September) and 5-days in winter (March) along inner-city routes, suburban routes, and highway routes. Morning (7-10 am) and afternoon (3-6 pm) routes were completed in three vehicles (Dodge Minivans 2008-2010) travelling simultaneously along each individual route. Ventilation settings were set to closed windows, low fan speed, and recirculation off.

RESULTS: During the summer mornings, mean in-vehicle UFP levels were 26 600 cm^{-3}, 21 000 cm^{-3}, and 40 000cm^{-3} for the inner city, sub-urban, and highway routes, respectively. Mean in-vehicle UFP levels during summer afternoon commutes were similar to morning levels for the inner city (21 300 cm^{-3}) and sub-urban (21 500 cm^{-3}) routes but were lower for the highway routes (31 300 cm^{-3}). For all routes combined, in-vehicle UFP levels tended to be slightly higher during the winter session (Mean Difference=8800 cm^{-3}, 95% Confidence Interval (CI): -40, 17 626) and during the morning time-period (Mean Difference=6100 cm^{-3}, 95% CI: -600, 12 800). Maximum single-route, 3-hour mean UFP levels occurred on the highway routes in the morning during winter sampling (133 400 cm^{-3}). On average, the indoor:outdoor ratio for UFPs was 0.86 (Range: 0.26-1.51), suggesting slightly reduced in-vehicle exposures relative to outdoors levels with windows closed, low fan speed, and recirculation off.

CONCLUSIONS: In-vehicle UFP levels in Toronto were similar along urban and sub-urban routes but tended to be higher on highway routes. In addition, in vehicle UFP levels tended to be higher during the winter morning commutes.

2.46 The Challenges Associated with the Use of Water Monitoring Data in Making Regulatory Decisions for Pesticides

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SUMMARY: The Pest Management Regulatory Agency (PMRA) considers available water monitoring data to enhance regulatory decisions on the risk of pesticides to human health and the aquatic environment. Ideally, monitoring data are most useful when spatial and temporal information are available to correlate sampling with pesticide use along with adequate information on study design and ancillary data. However, in reality the level of variability and uncertainty associated with existing monitoring data can make their use to estimate exposure concentrations a challenge. This presentation discusses possible options for dealing with this variable data, as well as emphasising continued cooperation with stakeholders generating the monitoring data.

BACKGROUND: The purpose of this presentation is to summarise the current strategy for use of water monitoring data for pesticide regulation, to highlight challenges in using this data, and to invite recommendations for improving the process. Available water monitoring data on pesticides are considered by the PMRA during human health and environmental risk assessments. These data come from sources such as other federal government departments, provinces and territories, municipalities, registrants and public literature. If the data are of appropriate quality and quantity the PMRA uses them to estimate exposure concentrations to assess the potential risk of exposure to humans through drinking water and to aquatic organisms.

DESCRIPTION: The current challenges for use of this data stem from varying quality and quantity of available monitoring data. Challenges in the analysis and the interpretation of the monitoring data relate to a general lack of spatial and temporal information, insufficient sampling frequency and dealing with left-censored datasets (i.e., a large number of samples with no detections).

OUTPUT: The proposed strategy to deal with these uncertainties for risk assessment, basic statistical analyses will be conducted to generate estimated acute and chronic exposure concentrations. All monitoring data will be considered as part of a weight of evidence approach in human health and environmental risk assessments, but the weight given to these data will vary depending on their overall quality and the availability of temporal and spatial information. The statistically weighted data will be used to more accurately estimate pesticide exposure for humans via drinking water and to aquatic organisms.

IMPACT AND NEXT STEPS: The PMRA will be re-examining the process of determining exposure concentrations based on monitoring data. The utility of the available monitoring data within a regulatory context would be strengthened with the inclusion of additional information on study design, as well as adequate temporal and spatial ancillary data. The Agency is working to fill in these gaps and acquire more high-quality data on pesticide concentrations in Canadian water sources. Continued cooperation with other jurisdictions within the federal, provincial, territorial and municipal levels of government as well as with registrants is important in this process.

2.47 Toxicity of an Environmentally-Relevant Mixture of Flame Retardants, Based on Levels Found in House Dust, to Adult Male Rats

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SUMMARY: Recently, Health Canada restricted the use of polybrominated diphenyl ethers (PBDE) flame retardants in new products. However, PBDEs are still present in many existing products resulting in continuing exposure. We examined the effects resulting from exposures to an environmentally-relevant mixture of flame retardants (mainly PBDEs). There were no effects of this mixture in adult male rats at doses estimated to occur from current house dust exposures. At a 1000X higher exposure, some toxicity was observed. These results suggest that current house dust PBDE exposures do not influence the overall health, reproductive system or thyroid function in adult males.

OBJECTIVES/BACKGROUND/ISSUE(S): Recently, all forms of Polybrominated Diphenyl Ethers (PBDE) have been removed from commerce in Canada. As PBDEs are still present in high amounts in existing home furnishings and electronics, human and environmental exposures will continue for many years. Understanding the potential risks of this continued exposure is necessary to determine if this risk is sufficiently great to warrant the active removal of contaminated materials from Canadian homes.

DESIGN/METHODS: The relative proportions of PBDE congeners measured in a comprehensive study of house dust samples from an Eastern North American city (Boston) were used to formulate a dosing mixture. A small proportion of another brominated flame retardant, HBCD, was also included in the mixture based on its presence in house dust. Dose levels were confirmed by measuring levels of all flame retardants in samples of the diets. Sexually mature male rats were exposed to the mixture through the diet at levels estimated to mimic exposures based on the 95% percentile of each congener (lowest dose) and to 3 doses at order of magnitude increments. After 70 days of treatment males were sacrificed and indicators of general toxicity, reproductive function, and thyroid hormone were measured.

OUTPUTS/RESULTS: At the highest dose (1000 times higher than likely exposure from house dust) the PBDE mixture caused liver and kidney enlargement, induction of hepatic drug metabolizing enzymes, reduced serum glucose, uric acid and thyroxine. Analyses of a broad spectrum of indicators of male reproductive health failed to reveal any effects on these parameters at any dose tested. Further, multiple analyses of target tissue responses to thyroid hormone revealed a similar lack of response to PBDE exposure.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: These data show that sub-chronic exposure to an environmentally-relevant mixture of flame retardants, composed mainly of PBDEs and based on North Americans, has very little effect on adult male animals. Notably, no effects were observed on reproductive function which fails to confirm epidemiological evidence linking PBDE exposure to reduced semen quality. Moreover, there were no effects on most measures indicating thyroid hormone action in target tissues, suggesting a lack of effect on thyroid function. However, ongoing studies examining effects of this mixture on fetal development are necessary to determine if further risk management action is needed to reduce exposure to PBDEs.

2.48 Environmental Transformation of PBDEs under UV Radiation: Formation of Secondary Pollutants

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SUMMARY: Decabromodiphenyl ether (BDE-209) and the commercial pentaBDE (a major mixture of tetrabromobiphenyl (BDE-47) and pentabromobiphenyl ethers (BDE-99)) are most commonly used as flame retardants in flexible polyurethane foam and other applications. They are found everywhere in the environment including indoor air and dusts, and usually considered as persistent organic pollutants. However, we found that all three testing BDEs can be degraded under UV radiation and transformed to BDEs containing less number of bromine in the molecules in this study. Recent studies indicated that the bioaccumulation and toxicity of BDEs increase with less number of bromine in the molecule.

OBJECTIVES/BACKGROUND/ISSUE(S): In the list of CMP priority chemicals, BDE-209 and the commercial pentaBDE are most commonly used as flame retardants in flexible polyurethane foam and other applications. They are ubiquitously found in the environment including indoor air and dusts. However, their airborne transformation under environmental factors such as UV light is not completely understood. The objective of this study is to investigate the fate of these three BDEs and their transformation products under UV photo-oxidation.

DESIGN/METHOD/DESCRIPTION: 2.5 µg of BDE-209, BDE-47 or BDE-99 solution prepared in various solvents was first injected to a sealed quartz chamber (~200 ml in volume). The chamber was then exposed to UVA/B light from 0 hour to 4 days. After the UV radiation, the starting chemical and products in the chamber were washed out with a mixture of DCM/hexanes (50/50, v/v). The washing extracts were then collected and analysed with Agilent GC/MS for the degradation of test BDEs and identification of new transformation products.

OUTPUTS/RESULTS: Under UVA/B radiation, BDE-209, BDE-47 and BDE-99 degraded and transformed to BDEs with less number of bromine in molecules through debromination. For BDE-99 and BDE-47, the degradation behaved differently between the solutions of BDEs dissolved in isooctane and nonane, indicating likely different fate pathways of airborne BDEs. The results showed degradation rate of BDEs in the order of BDE-209 > BDE-99 > BDE-47.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The results have demonstrated that BDEs are unstable under UVA/B light, a typical beam of sunlight. They can be easily transformed to lighter, more volatile BDEs, which could form so-called secondary pollutants. Such transformation under environmental conditions should be considered in risk assessment of BDEs and other CMP chemicals.

2.49 Traffic-Related Air Pollution and Acute Changes in Heart Rate Variability and Respiratory Function in Urban Cyclists

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SUMMARY: The short term health effects of air pollution exposures experienced while cycling are not well understood. During summer of 2010, Health Canada conducted a study to examine the impact of air pollution exposures on short-term changes in lung function and heart rate variability among healthy cyclists. Forty-two healthy adults cycled for 1-hour on high and low-traffic routes, as well as indoors. Traffic-related air pollutants were measured along each cycling route and health measures were collected before and after cycling. Air pollution exposures were significantly increased when cycling on the high traffic route compared to the low traffic route. While air pollution did not have an important impact on lung function, nitrogen dioxide, ozone, and ultrafine particles appeared to have an important impact on the biological systems that regulate heart rate. The results of this research suggest that, when possible, cyclists should select routes that minimize exposure to traffic in order to reduce their risks of the negative health effects of traffic pollution.

BACKGROUND: Few studies have examined the acute health effects of air pollution exposures experienced while cycling in traffic.

OBJECTIVES: A cross-over study was conducted to examine the relationship between traffic pollution and acute changes in heart rate variability (HRV). HRV provides important information related to autonomic regulation of the heart an is important for healthy heart function.

METHODS: Forty-two healthy adults cycled for 1-hour on high and low-traffic routes as well as indoors. Health measures were collected prior to cycling as well as 1-4 hours after the start of cycling. Ultrafine particles (UFPs) (<0.1 um), $PM_{2.5}$, black carbon, and volatile organic compounds were measured along each cycling route and ambient NO_2 and O_3 levels were recorded from a fixed-site monitor. Mixed-effects models were used to estimate associations between air pollutants and changes in health outcome measures relative to pre-cycling baseline values.

RESULTS: An inter-quartile range (IQR) increase in UFP levels (18,200/cm^3) was associated with a significant decrease in high-frequency power 4-hours after the start of cycling (Beta=-224 ms^2, 95% CI: -386, -63). Ambient NO_2 levels were inversely associated with the standard deviation of NN intervals (Beta=-10 ms, 95% CI: -20, -0.34) and positively associated with the ratio of low-frequency to high-frequency power (Beta= 1.4, 95% CI: 0.35, 2.5) 2-hours after the start of cycling. Significant inverse associations were also observed between ambient O_3 levels and the root mean square of successive differences in adjacent NN intervals 3-hours after the start of cycling. Exposure to traffic-related air pollution was not associated with changes in respiratory function or exhaled NO.

CONCLUSIONS: Short-term exposures to traffic pollution may contribute to altered autonomic modulation of the heart in the hours immediately following cycling.

2.50 Developing Canada's First Human Health-Based Sediment Quality Guidelines

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SUMMARY: The Contaminated Sites Division develops health-based Soil Quality Guidelines (SQGs) for the Canadian Council of Ministers of the Environment (CCME). Currently, CCME sediment guidelines are based solely on the protection of plants and animals, and human health is not considered. Risk assessors often use SQGs to stand-in for sediment guidelines, which may underestimate risk because of differences between sediments and soils. A Working Group was formed whose ultimate goal is to develop sediment guidelines that protect human health. To date, a workshop was held to identify information gaps and next steps; three contracts were let to address some gaps; and a Technical Advisory Group was formed.

BACKGROUND: Health Canada's Contaminated Sites Division (CSD) supports the Canadian Council of Ministers of the Environment (CCME) in the development of human health based Environmental Quality Guidelines for soil and drinking water, etc.

Currently, CCME quality guidelines for sediment are based solely on the protection of ecological health because there are no human health based guidelines. Typically, risk assessors adopt or modify soil human health based quality guidelines as surrogates for sediment guidelines and exposure. Such an approach under- or overestimates risk based on physio-chemical differences between soil and sediment (Table 1) and different exposure scenarios (Table 2).

METHODS: CSD formed a Sediment Working Group to develop: 1) guidance for risk assessment of contaminated sediments; and, 2) a protocol for health based sediment guidelines.

RESULTS:

1. 2010 Initial Scoping project:
   - Searched world-wide to determine that no health-based sediment quality guidelines exist, other than for fish/shellfish consumption pathway.
   - developed Conceptual Exposure Models (Figures 1 -3) of human activities in sediment environments, and
   - proposed an approach for risk assessment of contaminated sediments.

2. A Sediment workshop followed that solicited feedback from external experts. The Workshop identified the following gaps:
   - Sediment ingestion rates
   - Relative importance of suspended sediment as an ingestion pathway
   - Estimates of parameters for dermal contact with sediments
   - Relevance of floc and implications for sampling sediment contaminant concentrations
   - Regional variability and availability of background sediment data

3. In 2011, contracts on contaminated sediments developed:

- draft interim risk assessment guidance;
- draft guidance on the fish/shellfish consumption pathway; and
- *de novo* sediment ingestion rates for sediment and suspended sediment.

IMPACTS OF THE PROJECT: Over the next few years, SedWG will:

- publish risk assessment guidance for contaminated sediments; and

produce the first human health based sediment quality guidelines for direct contact (ingestion and dermal) and fish/shellfish consumption pathways.

2.51 Core Characteristics of Cultural Safety: An Evidence Review to Help Inform Decision-Making for First Nations and Inuit Community Health Policies and Programs

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SUMMARY: Mainstream evidence may not be directly applicable to the diversity of First Nations and Inuit community contexts. Valid evidence sources include cultural and traditional approaches. It would be helpful for a tool to be created to assist First Nations and Inuit Health Branch (FNIHB) employees to make culturally safe evidence-informed decisions.

This study consists of an evidence review undertaken with First Nations and Inuit partners to help inform the potential development of a tool. Results highlighted the relational nature of cultural safety, including the importance of working in partnership with First Nations and Inuit when engaging in a process such as developing a tool to guide decision-making.

OBJECTIVE: The concept of cultural safety evolved in clinical settings, where it means "…that the educator/practitioner/professional, whether Indigenous or not, can communicate competently with a patient in that patient's social, political, linguistic, economic, and spiritual realm... Cultural safety involves recognizing the health care provider as bringing his or her own culture and attitudes to the relationship" (National Aboriginal Health Organization, 2008).

This study focused on a key initial step toward ensuring that the evidence-informing processes of First Nations and Inuit Health Branch are culturally safe: an evidence review to identify core characteristics of cultural safety that are relevant to decision making in FNIHB's organizational context.

METHODS: Three approaches were used to identify potentially pertinent references. Aboriginal and other partners, professional contacts, and networks were contacted to ask for sources that might help inform this review. Searches were performed using a number of electronic search engines. The third approach used to identify potentially useful resources focused on grey literature. Project design was also informed by ongoing dialogue with First Nations and Inuit partners, and reflected the identified characteristics of cultural safety.

RESULTS: Five core characteristics of cultural safety were identified as relevant to decision making in FNIHB's organizational context. These include: 1) ongoing personal and organizational growth, based on principles rather than procedures; 2) reflection, including self-reflection, toward understanding the way cultural backgrounds, historic and structural contexts, social inequality and positions within power relationships may influence personal and organizational perspectives, interests, values, priorities and behaviours and actions; 3) recognition of diversity among and within Aboriginal communities; 4) refraining from stereotyping; and 5) building and maintaining relationships of trust through open communication and working in partnership with Aboriginal communities, including at the local level, and recognizing community members as experts on their communities.

CONCLUSIONS: While further work in partnership to validate these results will be required, the review of evidence on cultural safety appears to provide a series of key cultural safety principles that have the potential to help create an ethical space for program and policy-related processes. The intent of the evidence review was to help inform the process of tool creation, rather than to create a tool. However, feedback thus far suggests that these identified principles may be more broadly applicable, given the importance of bridging the various sources of evidence in FNIHB decision making contexts.

2.52 Effects of Environmental Contaminants on Expression of Drug Metabolism Phase I Enzymes, Metabolic Activity, and DNA Methylation of DNA Repeated Elements in Two Human Liver Cell Lines

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SUMMARY: DNA methylation is a normal chemical modification of our genetic material. It occurs in various regions of the DNA, including promoters (regions regulating our genes) and various DNA segments that are repeated numerous times throughout the DNA. Abnormal methylation in these DNA repeated elements and in promoters is associated with numerous diseases, including cancers. Environmental contaminants can modify DNA methylation, but in our 72h cell culture experiments, contaminants induced many effects without inducing changes in methylation of five DNA repeated elements. The latter endpoints derived under our experimental conditions are not useful for the risk assessment of the tested chemicals.

OBJECTIVES: In the context of short term 72h cell culture experiments that can be used in strategies to screen chemicals for prioritization for further testing, or for chemical classification based on mechanism of action, our objective was to investigate if changes in DNA methylation of various DNA repeated elements are sensitive indicators of chemical exposure.

METHOD: The HC04 and HepG2 liver cell lines were characterized based on differences in DNA methylation relative to a normal human liver biopsy, and based on dose-response effects of chemicals. Vanadium, nickel, and three mixtures of polybrominated diphenyl ethers (PBDE-71, -79, and 83) were tested, in addition to activators of the AhR and CAR nuclear receptor pathways (polychlorinated biphenyls126 and 153, respectively), and a demethylating standard, 5-aza-2'-deoxycytidine (5aCdR). DNA methylation of five DNA repeated elements (Line-1, AluYb8, NBL, Sat-alpha and D4Z4) was measured using pyrosequencing assays. These endpoints were compared to changes in metabolic activity (AlamarBlue assay as surrogate for toxicity) and expression of 84 drug metabolism phase-I enzymes.

RESULTS: The methylation level differs between the biopsy DNA, the HC04 and HepG2 cells (e.g., 81%, 64% and 63% methylation in Line-1, respectively). Both cell lines responded to the demethylating agent 5aCdR, with decreases in DNA methylation but the effects differed between cell lines and among the repeated elements. Effects of the other chemicals on DNA methylation were minor despite significant inductions of numerous genes and decreases in metabolic activity. Vanadium was more toxic than nickel and PBDE 71 was the most toxic PBDE.

CONCLUSION: DNA methylation of the repeated elements differed among the cell types, but these endpoints were not sensitive indicators of exposure to environmental chemicals. The cell lines responded differently and therefore using both cell lines, instead of one, provided better coverage of potential effects. Methylation of gene promoters is being investigated.

2.53 Global Gene Expression in Human Villous Mesenchymal Fibroblasts (HVMF) Exposed to Bisphenol A (BPA) under Different Culture Conditions

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SUMMARY: Human placenta is essential for normal fetal development. To explain the reported effects of bisphenol A (BPA) on fetal development, human placenta cells were exposed to varying concentrations of BPA under three culture conditions. Global gene expression was compared using microarray analysis. BPA altered gene expression only at a high concentration used. Culture conditions influenced these effects of BPA.

ISSUES/OBJECTIVES: There exists a large discrepancy in the reported "Low Dose ($<10^{-7}$M) Effects" of BPA on cellular responses. It was unclear if this discrepancy was due to use of cells grown in media with different serum conditions. Human placenta, which is essential for normal fetal development, was recently found to accumulate BPA. To identify target genes and pathways of BPA in human placenta and to explain the discrepancy in the reported *in vitro* "Low Dose Effects" of BPA, we compared global gene expressions in HVMF exposed to BPA under three serum conditions.

METHODS: HVMF were cultured in medium containing 10% fetal bovine serum (FBS), 10% charcoal stripped FBS (CSFBS), or no FBS (NFBS), and exposed to 0, 0.01, 1, or 100 µM BPA for 24 hrs. Global gene expressions were examined using Affymetrix Human Gene 1.0 ST array. Expression values were analyzed using the Microarray Analysis of Variance (MAANOVA) library in R (a statistical analysis).
Gene function and pathway networks were identified using the "Core Analysis" of Ingenuity Pathways Analysis (IPA).

RESULTS: Only at 100 µM, BPA significantly altered (>1.5 fold) expression of 533, 800, or 712 genes in HVMF cultured under FBS, CSFBS, and NFBS conditions, respectively. Among these, 188 genes, mostly involving in cell cycle control, cellular assembly and organization, and DNA replication, recombination, and repair, were altered in all three conditions, whereas 21, 242, or 452 genes were uniquely affected in FBS, CSFBS, and NFBS conditions, respectively. IPA analysis revealed downregulation of cell cycle control pathways and upregulation of interferon signalling pathways by BPA under all three serum conditions. However, upregulation of antioxidant/stress response pathway by BPA was only found in NFBS condition. Examples of BPA-altered genes include growth differentiation factor 15, hemeoxygenase-1, endothelin-1, and fibroblast growth factor 7 for all conditions, phosphoprotein associated with glycosphingolipid microdomains 1 and coronin for FBS condition only, epithelial mitogen homolog and interleukin 1 receptor 1 for CSFBS condition only, and interleukin 6 and insulin-like growth factor binding protein 5 for NFBS condition only.

CONCLUSIONS: BPA expressed no low dose effects on gene expression in HVMF regardless of the serum conditions used. Pathways of cell cycle control were primary targets of high dose of BPA. Other cellular processes were differentially affected by BPA depending on serum conditions. These data provided mechanistic insights for some of the developmental effects of BPA observed in experimental animals, and may contribute to the health risk assessment of BPA in humans.

3.01 Health Canada's Response for Protection of Canadians at Home and Abroad Following the Events at the Fukushima-Daiichi Nuclear Power Plant Accident in Japan

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SUMMARY: Following the March 2011 Great East Japan earthquake and tsunami, problems at the Fukushima-Daiichi nuclear power complex in Japan lead to failure of containment at several reactor units and release of radioactivity. In support of the Government of Canada's response to this event, Health Canada convened a multi-departmental expert group under the Federal Nuclear Emergency Plan, and used its monitoring networks, radiological impact expertise, decision support tools and information products to assess and manage risks, support decision-makers and implement protective measures. This multi-disciplinary science-based approach was critical for formulating appropriate advice for protecting Canadians in Canada and Japan.

BACKGROUND: The March 2011 Great East Japan earthquake/tsunami and concurrent emergency at the Fukushima-Daiichi nuclear power complex presented Canadian authorities with the challenge to protect more than 10 000 foreign nationals in Japan. Additionally, action was also required to address concerns about possible exposure to radioactive contamination as raised by workers at customs, ports and postal facilities, by air carriers and airports, travellers to and from Asia, and those living in Canada, particularly along the west coast.

While Canada's nuclear management arrangements have been developed and tested over many years, this event was the first major nuclear emergency since Chernobyl requiring a broad, coordinated response across multiple departments and jurisdictions.

METHOD: Nuclear emergency response requires the integration and assessment of a large set of technological parameters in order to take appropriate interventions for reducing risks. In parallel with the government's management of the natural disaster, Health Canada convened scientists from key federal organisations under the Federal Nuclear Emergency Plan to provide rapid and ongoing technical assessment of the nuclear emergency situation and its potential evolution.

Health Canada's Radiation Protection Bureau also established teams for radiation monitoring, radiological impact assessment, health advice, communications and international assistance. Analyses and recommendations for protective measures were founded on the operation and assessment of specialized Canadian and global radiation monitoring networks, as well as communications with international counterparts.

RESULTS: Close collaboration between key federal partners including Health Canada (radiological protection, radiation surveillance), Canadian Nuclear Safety Commission (reactor analysis), Environment Canada (atmospheric modelling) and Natural Resources Canada (radiation surveillance) provided the capability for predicting the possible radiological consequences of the evolving situation, and monitoring and evaluating impacts. From this, daily updates and recommendations on appropriate protective measures were provided to decision-makers over the course of the emergency.

IMPACTS AND CONCLUSIONS: The well-developed, multi-disciplinary science-based approach used to respond to the Fukushima emergency was critical for formulating timely advice for protecting Canadians in Canada and Japan. Lessons learned nationally and internationally are being used to improve arrangements for nuclear emergency management.

3.02 Using a Global Airborne Radionuclide Monitoring Network and Health Canada's National Data Centre (NDC) to Assess the Risks and Impacts on Canadians During the Fukushima Reactor Accident

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SUMMARY: Can a globally distributed, 80 station CTBT radioactivity monitoring network contribute to risk assessments from reactor accidents like Fukushima, whose direct hazards perhaps extend no further than a few tens to a few hundreds of kilometres from the accident? Yes, using a highly automated science network for analysis and state-of-the art atmospheric transport models, we related the remote stations observations to emissions from the Fukushima site. This system provided independent, accurate estimates of releases from the Fukushima reactor within 30 hours of the initial accident. The dual use of the CTBT NDC for accidents is recommended.

BACKGROUND: Can the global, but sparsely distributed 80 stations of the Comprehensive Nuclear Test-Ban Treaty (CTBT) radioactivity monitoring network contribute to risk assessment in event of releases from reactor accidents like Fukushima, whose direct risks extend perhaps no further than a few tens to a few hundreds of km from the accident? Yes, by using a highly automated science network for analysis and state-of-the art atmospheric transport models to relate the remote stations observations to emissions from the source site. As scientific support to the Canadian Government in monitoring compliance to the CTBT, HC has access to raw data and analysed products from a global network of eighty stations of unsurpassed detection capability. These stations are operated to monitor airborne radioactivity, both aerosol and noble gas. This study demonstrates the value of interpretation of data from a network with an inter-station distance on the order of 2000km in support of the risk assessment within a much smaller reactor environ, provided one can assimilate the station data and interpret the source magnitude on an accident crisis management timeframe.

METHOD: The Canadian NDC uses an automated gamma spectroscopy processing pipeline and database storage system co-developed with STUK, the Finnish Radiation Protection Authority (http://linssi.hut.fi/). All stations automatic detections were interactively analysed along with relevant noble gas results. Unique feature of this system include the ability to export analysis result reports and database to database full results sharing. The latter allowed 24/7 analysis of new spectra as they arrived, sharing work with STUK scientists. The former allowed significant peer review of results on the fly with CTBT colleagues worldwide. Atmospheric Transport models provided by Environment Canada allowed estimates of the reactor releases based upon the station observations.

RESULTS AND IMPACT: This system provided independent, accurate release estimates from the Fukushima reactor within 30 hours of the initial accident using the Takasaki CTBT station 200km from Fukushima, Within 48 hours, the Canadian Nuclear Safety Commission used this information to decide the most realistic reactor failure mode and for all subsequent risk assessments in the reactor environ. Within a week, several other CTBT sites confirmed the magnitude of the reactor release and failure still in use by Canadian emergency managers. This included significant evidence of fuel rod failure and partial core melting only recently confirmed by Japanese authorities.

CONCLUSION: In short, The CTBT network can be of substantial value in nuclear emergency management providing one has the proper analysis system and can integrate efficiently into an expert community of practice. The dual use of HC's NDC for accidents is recommended.

3.03 Fixed Point Surveillance Network Monitoring in Canada and Abroad of the Radiological Releases from the Fukishima Nuclear Power Plant Emergency

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SUMMARY: For the past several years Health Canada has installed and operated a network of 75 Fixed Point Surveillance (FPS) spectroscopic NaI detectors measuring, in real-time, airborne radiation around Canadian nuclear power plants (NPPs) and regionally.

In response to the nuclear emergency in Japan, Health Canada deployed several additional detectors to supplement the FPS network in the western part of Canada and the Canadian Embassy in Tokyo. Radiation dose levels were monitored on these systems to assist decision-making during the emergency, and ensure the health and safety of Canadians at home and abroad.

BACKGROUND: Health Canada operates a network of 75 Fixed Point Surveillance (FPS) spectroscopic NaI(Tl) detectors measuring radiation in the air around Canadian nuclear power plants (NPPs) and regionally. These detectors monitor and report airborne radiation in real-time. These detectors monitor the daily emissions from the NPPs and also would be used in the case of an emergency to determine the dose rate to the public and help assess the types of emergency measures required. Health Canada has also developed a set of similar detectors that can be rapidly deployed for special events.

METHOD: In March of 2011, an emergency at the Fukishima NPP in Japan prompted enhanced radiological monitoring across the world. In response, Health Canada supplemented the 8 FPS detectors in the western part of Canada with an additional 9 of the rapidly deployable detectors. Two detectors were also sent to the Canadian Embassy in Tokyo upon request of DFAIT to monitor the situation for Canadians abroad and formulate protective action decisions.

RESULTS: The airborne nobles gases (Xe-133) were measured and these activity concentrations were fed into Environment Canada's air transport models to determine the quantity of radioactive material released from the Fukishima NPP. Daily assessments of the dose rates were made and posted to the Health Canada website for the public. Isotope specific dose assessments were done to ensure that there was no health risk to Canadians.

Monitoring in Tokyo was reported to the embassy daily with assessment of health risk. The real-time monitoring of the situation included 24/7 alarming in case levels exceeded safety guidelines. This type of monitoring is indicative of the response Health Canada is prepared for in the case of a radiological emergency on Canadian soil.

CONCLUSION: This environmental monitoring of dose rates to the population and enhanced situational awareness helps to ensure the health and safety of Canadians at home and abroad.

3.04 Radiological Surveillance in Support of the Federal Nuclear Emergency Plan Following the Japanese Fukushima Nuclear Accident

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SUMMARY: On March 11, 2011 Japan experienced a major earthquake and tsunami and subsequent accident at the Fukushima nuclear facility. Under Canada's Federal Nuclear Emergency Plan, Health Canada's environmental radioactivity monitoring networks were looked upon to provide assurance that fallout arriving from Japan presented no danger to Canada. The National Monitoring Section analyzed many samples from Canada and food imports from Japan over the weeks that followed the accident, providing assurance that Canadians living in Canada were not subjected to any significant radiation risks as a result of the emergency.

OBJECTIVE: To determine if radiological risk to Canadians from the Japanese Fukushima nuclear accident was very low.

MATERIALS AND METHODS: High-volume air sampler filters and precipitation samples from the Canadian Radiological Monitoring Network were analyzed by high purity germanium gamma spectroscopy. Food samples imported from Japan and milk samples produced in B.C. were also analyzed by gamma spectrometry. Foods imported from Japan were analyzed as a co-operative effort between the Canadian Food Inspection Agency (CFIA) who collected samples, Health Canada's Food Directorate who then processed and delivered suitable samples to the Radiation Protection Bureau's National Monitoring Section (NMS) who performed the gamma spectrometric analyses. The NMS reported results to CFIA within 24 h of sample receipt. Swipe samples from container ships arriving to the Port of Vancouver were also analyzed by gamma spectroscopy as a co-operative effort between Canada Border Services Agency, the Canadian Nuclear Safety Commission, and Radiation Protection Bureau.

RESULTS: Air filters from stations in Western Canada were the first to show very low levels of I-131, Cs-134, and Cs-137. These nuclides were found at concentrations lower than 6 mBq/m^3. Several hundred air filters have been analyzed since March. In addition, 180 food samples imported from Japan have been analyzed to-date and found to be safe. 34 British Columbia milk samples produced during April, May, and June were all measured for gamma ray radioactivity, and some were analyzed for Sr-90, and were found to contain normal levels of radioactivity. 39 container ship swipes were also found to have low levels of I-131, Cs-134, and Cs-137.

CONCLUSION: Results from radiological surveillance were used to support science-based decision-making, to demonstrate that the Fukushima nuclear accident posed no risk to Canada, and that multi-agency co-operation can facilitate the types of surveillance that are required to support the Federal Nuclear Emergency Plan.

3.05 Monitoring of Fukushima Nuclear Accident Related Aerosol Radioactivity at the CTBT Radinulide Station in Sidney of Canada: A New High-Volume Aerosol Sampler Design and Development

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SUMMARY: A high volume aerosol sampler, namely Grey Owl, has been designed and developed at Radiation Protection Bureau (RPB) of Health Canada. Its design guidance incorporates the following key principles: a low operational cost and reliable sampler in parallel with Snow White to provide daily aerosol monitoring samples that can be used as reference aerosol samples for Canadian radiological study. Most recently the Grey Owl II has been developed to provide a constant air flow rate at low pressure drops (~3 kPa for a day sampling) with variations of less than ±1% of the full scale flow rate. Its energy consumption is only about 1.5 kW for a filter sampling over 22 000 standard cubic meter of air, which is 10 times lower than that of Snow White. The sampler installed at monitoring station of the Sydney BC detected the first radioactive isotopes landed in Canada from Fukushima nuclear crisis releases.

OBJECTIVES/BACKGROUND/ISSUE: Since 1998, Health Canada has been contributing to the International Monitoring System (IMS) associated with the Verification Regime overseen by the Comprehensive Nuclear-Test-Ban Treaty (CTBT). Monitoring the airborne radionuclides released from a nuclear weapon tests has been thought is the most certain way to ensure that the treaty is not violated. For this purpose, a radionuclide monitoring system, which consists of a network of 80 globally distributed radionuclide monitoring stations, has been being implemented. Under the CTBT global network, Health Canada operates four certified IMS particulate monitoring stations (Sidney, BC; Yellowknife, NWT; Resolute Bay, NU; and St John's, NL). The stations are equipped with Snow White aerosol samplers produced by Senya Oy., Finland. The gamma spectra collected from these stations will be sent over secure data links to the International Data Centre (IDC) in Vienna for radionuclide identification and activity analysis. It is also mandatory to ship all collected aerosol filters to IDC for a historic archive. In order to have a parallel sampler at each station for Canadian radiological study purpose, a new high volume air sampler, namely Grey Owl, was design and developed at RPB. It has been installed in all Canadian CTBT stations and some of stations of Canadian radiological monitoring network to improve its aerosol sampling capabilities.

DESIGN/METHOD/DESCRIPTION: The Grey Owl sampler draw air through about 2500 cm^2 filters at a flow rate of approximately 700 m^3 per hour, with a daily sample volume around 16 000 m^3. These filter samples are shipped to RPB laboratory in Ottawa for gamma-emitting artificial and natural radionuclides analysis by a high pure germanium detector.

OUTPUTS/RESULTS: On March 17, seven days after the Fukushima nuclear accident, the radioactive cloud had crossed Pacific Ocean; and traces of fission products were picked up both by the Grey Owl and Snow White samplers running at IMS Canadian particulate monitoring station of Sidney BC. Since the first analysis results of the monitoring data became available, several radioisotopes, such as ^{131}I, ^{132}I, ^{132}Te, ^{134}Cs, ^{136}Cs and ^{137}Cs, have been detected in a range of radioactive concentrations. A linear regression was used to modeling the relationship between the results by two samplers. The results indicate that that the agreement between the two monitoring systems is very well with a correlation coefficient factor (r2) of 0.993.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The results demonstrated an excellent linear correlation between two samplers, which indicated that the Grey Owl can provide an accurate flow rate measurement and control. The operational cost of Grey Owl sampler is very low, which consume only about 1 kW of power to filter over 22 000 SCM of air per day through a 2500 cm2 area of the selected high-efficiency and low pressure drop filter media.

4.01 Pathogenesis of *Cronobacter* Species: Adhesion and Invasion of the Blood Brain Barrier

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SUMMARY: *Cronobacter* species cause serious infections such as meningitis and enteritis in newborns and neonates, the major vehicle being contaminated powdered infant formula. It remains unknown what factor(s) play a role in the pathogenicity or its ability to cross the blood-brain-barrier. A novel protein was identified that may play an important role in the survival of the organism in food processing environments.

OBJECTIVE: To identify virulence factors required *Cronobacter* species to adhere or invade human brain microvascular endothelial cells (HBMEC); and determine whether strains from clinical, food, and environmental sources differ in their ability to produce biofilms.

METHODS: Adhesion to and invasion of human blood-brain-barrier cells (BBB) was tested using a modified gentamicin protection assay. Random transposon mutagenesis on high invasion and high adhesion strains are being screened. HPB strain 3267 (highest invasion of HBMEC), and 3404 (greatest adherence to HBMEC) are used as positive controls. The isogenic mutant library will be screened to identify virulence factors implicated in BBB pathogenicity. Characterization of biofilms of a 30-strain diversity set is currently being conducted using the minimum biofilm eradication (MBEC) assay.

RESULTS: Screening a transposon library revealed that one isogenic mutant of strain HPB3231 lost the ability to adhere to BBB cells. The transposon rescue was combined with DNA sequencing to reveal the insertion site to be within a diguanylate cyclase (DGC) gene. An isogenic mutant of 3787, a food isolate, significantly reduced the ability to produce biofilms, is currently being investigated. The absence of diguanylate cyclase in *Cronobacter* strain 3231 due to the insertion of the transposon has shown to affect adhesion to HBMEC.

CONCLUSION: A major role of DGC in many Gram-negative bacteria is to synthesize cyclic diguanylate (c-di-GMP). C-di-GMP is a bacterial second messenger, which is known for regulating biofilm formation, motility, and virulence or aspects of microbial pathogenicity. High levels of c-di-GMP stimulate various biofilms associated functions, such as the formation of fimbriae and other adhesins and various matrix expolysaccharides; whereas, low levels promotes motility and virulence. For successful adhesion to the BBB and biofilm production in *Cronobacter* species, DGC needs to be triggered. The findings of the study could assist in unraveling the mechanism(s) of *Cronobacter* pathogenesis and potentially lead to intervention strategies aimed at inactivating the function of DGC.

4.02 Comparative Gene Expression Analyses of *Campylobacter jejuni* Strains Isolated from Clinical, Environmental and Animal Sources

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SUMMARY: *Campylobacter jejuni* (*C. jejuni*)is the primary cause of bacterial food-borne diarrhoea in Canada. We analyzed 70 strains collected from clinical, environmental and animal sources to gain a broader understanding of their ability to cause disease. We found that strains from clinical cases were more likely to have properties linked with propensity to cause disease than strains collected from water sources. Results will be used to identify markers associated with strains of *Campylobacter* that are most likely to cause illness.

OBJECTIVE: Comparative genomic analyses of *Campylobacter* strains reveal genome plasticity that provides insight into the evolution of this bacterium's virulence traits. Survival in different environments depends on gene content and adaption of gene expression in response to environmental signals. To identify genes important for virulence of the bacterium, transcriptome analysis was conducted on strains acquired from clinical, environmental and animal sources.

DESIGN: Based on molecular typing by Comparative Genomic Fingerprinting (CGF), we selected a subset of 70 *C. jejuni* strains (26 clinical, 23 water and 21 animal) collected between 2004 and 2007 from across Canada. We performed assays for growth rate, adhesion and invasion, motility, and biofilm formation to establish phenotypic properties of each strain. Assay results for strains from different sources and clusters of genetically related strains were compared using the Mann-Whitney test. A subset of 10 strains in each group was selected and transcript profiles were generated using a pan-genomic *Campylobacter* DNA microarray.

RESULTS: A number of phenotypic differences were observed among *C. jejuni* isolates from different sources. Clusters of water isolates had significantly lower invasion rates than clinical and animal clusters combined ($p=0.01$). Clinical strains showed higher adhesion than water strains ($p=0.05$). Gene expression profiling of a subset of these strains also revealed significant differential expression in clinical and animal isolates compared to environmental. Among these differences was increased expression of virulence-associated genes.

OUTCOMES/CONCLUSIONS: We have used phenotype and transcript profile data to identify naturally occurring variability among *C. jejuni* isolates that is associated with differential gene expression. Our analyses reveal a decreased expression of pathogenic properties in water isolates when compared to clinical strains. The identification of genetic and phenotypic markers of clinically important *C. jejuni* strains will be essential in the development of regulations for detection and control of this pervasive organism in the food supply.

4.03 Detection of Diverse *Vibrio parahaemolyticus* Strains Sourced to Canadian Estuaries: Microevolution and Potential Hazard

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SUMMARY: Various types of bacteria are commonly found in estuaries around the world, with regional variations influenced by temperature and available organic matter. These environmental parameters are likely to impact members of the genus *Vibrio*, which accumulate inside filter-feeding shellfish, a popular seafood. These bacteria are prone to adaptation and evolution by environmental challenges. *V. parahaemolyticus* (*Vp*) is a clinically significant species of this group that have been detected in molluscan shellfish sourced to Canadian estuaries, and are capable of causing illness to consumers of raw or partially cooked seafood.

OBJECTIVE: To monitor the prevalence of *Vp* in molluscan shellfish harvested in Canada, with special reference to targeting any risk-prone biotypes/ecotypes emerging as potential human pathogens.

METHODS: Bivalve molluscs (clams, mussels and oysters), which are filter-feeders of planktons and algal-derived organic pollutants, were sampled from the coastal waters of Canada for the presence of *Vp*. Molluscs were sampled from various harvest sites within the same regions from the east and west coasts of Canada, during May to October of each year from 2002 to 2010. In-house procedures based on standard biochemical, immunological and polymerase chain reaction (PCR) techniques were used to isolate and characterize the strains.

RESULTS: During the 9-year period, 360 mollusc samples were tested for the presence of Vp and 177 (49%) turned out to be positive, yielding 58% of the 200 samples from the west and 39% of the 160 samples from the east, respectively, as presumptive *Vp* isolates. Overall, when the study was divided up into two time periods and analysed, a notable trend was observed. Potentially pathogenic *Vp* strains were non-detectable during 2002 to 2006 (P1), but were isolated during the years from 2007 to 2010 (P2). Combining strains from both regions, all of the 75 *Vp* isolates from 157 samples were negative for the thermostable direct hemolysin encoded by *tdh* (during P1). In contrast, 102 *Vp* strains isolated from 203 samples during P2, yielded 14 potentially pathogenic strains which showed significant genetic diversity.

CONCLUSION: Global warming and eutrophication are two potential driving forces altering the risk for *Vp*-related disease in the temperate regions. Significance of the emerging traits observed in *Vp* may be to sustain the species in the northern hemisphere at the cost of microevolution. However, further investigation and evidence are required to understand the true significance.

4.04 The Invisible War in the World of Foodborne Pathogens: A Case Study Enteric Bacteria Against *Shigella* spp.

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SUMMARY: The inhibition of *Shigella* by *E. coli* in human gut was demonstrated by Alfred Nissle in 1917. In 1925, André Gratia was the first to demonstrate the antagonism between two *E. coli* strains on agar plate. In 1948, Halbert working on the field in South Texas, USA was the first to demonstrate the inhibition of *Shigella* by *E. coli* in humans gut and in culture media. By 1967 and 1968, Hentges demonstrated the mechanisms of inhibition between coliforms and Klebsiella against *Shigella*. The presence of the above gut flora in outbreak food samples explains the difficulties to isolate *Shigella* from foods involved in outbreaks. Even if detection of *Shigella* by molecular methods, e.g., PCR, yields mostly positive results, the need for a live *Shigella* isolate still obligatory in case of lawsuit following an outbreak. So the development of methods for the isolation lives *Shigella* cells stills a priority for food microbiologists.

OBJECTIVES: To demonstrate the occurring of inhibition of *Shigella* by foodborne background microflora leading to false negative results.

METHODS: CBK-Campos antagonistic test, a simplified Halbert (1948) antagonistic test, was used in this study. First, 94 *E. coli* were challenged to *Shigella sonnei* clinical (HCSL (Health Canada *Shigella* Laboratory)-83 and HCSL-103) isolates involved in outbreaks and four *Shigella* spp. reference strains (Table 1). Second, 63 enteric bacteria from food were challenged against the two clinical isolates (Table 2). Third, the antagonism effect of positive isolates from Table 2 against 111 Enterobacteriaceae isolates from our collection (Table 3). Finally, we demonstrate the inhibition effect of an *E. coli* strain (Fd (Food) - 18) isolated from an outbreak the carrots sample (Table 2) against HCSL-103 using MFLP (Method for Food Laboratory Procedure) -25 (isolation) and MFLP-26 (PCR) methods.

RESULTS: Rates of 56% (53/94) and 62 % (58/94) *E. coli* strains inhibited *S. sonnei* HCSL-103 and HCSL-83, respectively. *Shigella* spp. reference strains were inhibited by 57% for *S. sonnei*, 38% for *S. flexneri*, 6% for *S. boydii* and 0% for *S. dysenteriae*, respectively (Table 1). From the 63 foods enteric bacteria 23/63 (37%) inhibited *S. sonnei* HCSL-83 at 37 ˚C and 18/63 (29%) at 42˚C with 5% CO_2. Also 24/63 (38%) of the foods enteric bacteria were able to inhibit *S. sonnei* HCSL-103 at 37 ˚C but only 19/63 (30%) at 42˚C with 5% CO_2. The incubation at 42˚C with 5% CO_2 shows its selective effect against *Acinetobacter* spp., *Hafnia alvei* and *Pantoae* spp. (Table 1). *P. aeruginosa* isolated from spinach was the inhibitoriest to Enterobacteriaceae 102/111(92%). The culture test MFLP-25 yielded a negative result, no *Shigella* colony could be isolated from the spiked carrots but the PCR-MFLP-26 tested positive (Figure 1). The CBK-Campos test showed that *E. coli* (Fd-18) was inhibitory to *S. sonnei* HCSL-103 (Figure 1).

CONCLUSIONS: We demonstrate for the first time the impact of certain background microflora on the isolation of foodborne pathogens. Considering the present lack of appropriate selective culture media for *Shigella*, we highly recommend the use of a PCR method (e.g., MFLP-26) and CBK-Campos antagonistic screening test to demonstrate the presence of antagonistic bacteria in implicated food sample.

4.05 Effects of Long-Term Exposure to the Mycotoxin Fumonisin B1 (FB1) on Wild Type and Cancer-Prone Transgenic Mice

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SUMMARY: Transgenic mice have been developed to improve the animal studies used to determine if chemicals are carcinogenic. The p53+/- (TG) mouse was developed to be more susceptible to cancer than its wild-type (WT) counterpart. This mouse is being tested to predict and study the carcinogenicity of naturally occurring toxins produced by moulds that grow on crops. The fungal toxin FB1 caused liver damage and tumours in TG and WT mice. Overall, the effects of FB1 were slightly more pronounced in TG than in WT mice. This study will: (1) contribute to decision-making on the future use of the TG mouse; and, (2) provide chronic toxicity data for FB1.

OBJECTIVE: To compare TG and WT p53+/- mice and to provide chronic toxicity data for the fungal toxin FB1.

METHODS: Male TG mice heterozygous for the p53 tumour suppressor gene and corresponding WT mice homozygous for the p53 gene were exposed to FB1 at 0, 5, 50 or 150 mg/kg in diet for 26 weeks. Body weight and food consumption were monitored throughout the exposure period. Changes in toxicological and biochemical parameters were assessed in blood and tissues post-necropsy.

RESULTS: Body weight gain was lower in high dose TG mice than in TG controls; there were no changes in weight gain in WT mice. Leucocyte numbers and plasma immunoglobulins were increased in WT and TG mice, indicating altered immune responses. Increased severity of hepatic proliferative lesions and apoptosis was observed in WT and TG mice. Hepatic cholangiomas and adenomas were observed at the highest dose, with equal numbers of tumours in WT and TG mice. FB1 is known to disrupt sphingolipid metabolism by inhibiting ceramide synthase, resulting in increased sphinganine and sphinganine-1-phosphate in liver and kidneys. These analytes were significantly increased in WT and TG mice. Furthermore, a new class of bioactive sphingolipid metabolites were identified and found to be increased, in both WT and TG mice due to FB1 exposure.

CONCLUSIONS: FB1 was hepatocarcinogenic to TG and WT mice. The effects of FB1 on TG mice were more significant, but the difference between WT and TG mice was not pronounced. This study will contribute to the weight of evidence on the sensitivity of the TG p53+/- mouse for carcinogenicity studies. In addition, the data generated by this study has been used to support the toxicological evaluation of FB1 by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA).

4.06 Optimization and Validation of an Integrated *In Vitro* Bioassay for Cytotoxicity Screening of Nanomaterials

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SUMMARY: We have evaluated a cell culture-based assay platform for screening nanomaterials. Availability of small amounts of surface-modified nanoparticles limits the number of assays that can be performed. Nanomaterials have unique physico-chemical properties, which can cause interference with assays, preventing correct interpretation of results. We have combined several endpoints within a single exposure assay, and we show that the assay can be adapted to eliminate or reduce interference of the materials. The platform makes use of robotics to investigate nanomaterials of regulatory concern to Health Canada and as part of an Organisation for Economic Co-operation and Development (OECD) international initiative.

BACKGROUND: Nanomaterials have unique structural and chemical properties with promises of substantial societal benefits. Nevertheless, nanomaterials need to be assessed for potential health and environmental impacts. Significant efforts and resources have been dedicated worldwide to address the safety of nanomaterials. However, due to the unique properties of nanomaterials, difficulties have arisen with the use of some of the current bioassays for toxicity assessment.

METHODS: A549 lung epithelial cells and J774A.1 macrophages were exposed for 24h to suspensions of nanomaterials and reference materials (carbon nanotubes, SiO_2, TiO_2) at various doses in 96-well plates. Multiple endpoints were assessed including mitochondrial function (Cell-Titer Blue), cell proliferation (BrdU incorporation), energy metabolism (ATP levels) and cell membrane damage (LDH release). The assays possess distinct chemistries and utilize a variety of detection/quantification methods (absorbance, fluorescence, and luminescence).

RESULTS: Leakage of cytoplasmic LDH was measured by colorimetry in the 24h supernatant after clarification by centrifugation to remove any particulate material. Cell-Titer Blue was then added to assess the cellular redox potential. Carbon nanotubes prevented direct reading of fluorescence because of physical quench. The problem was circumvented by transferring an aliquot of cell culture supernatant at t_{0h} and t_{2h} to reading plates, followed by centrifugation prior to reading fluorescence. Cells were then lysed with dilute Triton X-100 in saline with magnesium to maintain protein and mRNA integrity (for proteomic and genomic analyses) and enzymatic activities (e.g., LDH). To ensure stability of ATP, an aliquot of the lysate was transferred directly to the luciferase reaction mix (luminescence is stable for 4h). All endpoints were easily adaptable by simply removing nanomaterials by centrifugation prior to analyses.

CONCLUSION: The platform is being deployed to investigate nanomaterials of regulatory concern to Health Canada and as part of an Organisation for Economic Co-operation and Development (OECD) international initiative.

4.07 *Campylobacter* spp. in Farm Environmental Water Sources in Eastern Ontario, Canada

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SUMMARY: Each year, an estimated 1% of the population is afflicted with *Campylobacter*-induced gastroenteritis, mainly due to poultry consumption, but other foods (i.e., meats, raw milk, and water) have been associated with infection. We have analyzed water collected in rural areas near poultry farms and found it to be contaminated throughout the year. Intervention measures aimed at preventing exposure of the poultry to farm water sources may reduce broiler contamination with this organism.

BACKGROUND/OBJECTIVES: Exposure to contaminated poultry products is likely to account for a large proportion of the Canadian cases of *Campylobacter*-induced gastroenteritis. Chickens frequently become commensally colonized with this organism on the farm, and studies indicate that water sources near barns may be a source of bacteria for flock colonization. The purpose of this study was to investigate contamination of on-farm water sources with *Campylobacter* spp.

DESIGN: To determine levels of *Campylobacter* in natural water sources in the farm environment and to investigate how this fastidious organism survives in this niche, we collected water, sediment and biofilms from four sites along a ditch close to a crop farm, a cattle farm, and two chicken farms (North Lancaster, Ontario). Samples were collected monthly over a one year period (July 2009-June 2010) and *Campylobacter* spp. were isolated, quantified by Most Probable Number (MPN) method, speciated by multiplex PCR (mPCR) and typed by comparative genomic fingerprinting (CGF).

RESULTS: *C. jejuni* was detected in all water samples collected throughout the year at concentrations ranging from 0.003-4.6 cfu/ml with highest levels observed at warmer temperatures in the summer and fall. During the winter months, with water temperatures at 0°C, counts were lower, but *C. jejuni* was detected in all samples. No *C. coli* or *C. lari* was isolated from any of the samples, and mPCR done directly on enrichment broths confirmed that these species were not present in the samples collected. CGF typing data of the strains isolated identified a small number of genotypes persistent in the water throughout the year.

CONCLUSIONS: *Campylobacter* is highly prevalent in natural water sources in the farm environment, even in conditions (high temperatures, high oxygen) that are normally detrimental to the organism. Open water close to poultry barns may act as a reservoir for campylobacters in the poultry farm environment. Results of this work will inform the development of interventions to prevent on-farm colonization of poultry with *campylobacters*.

4.08 Human Health Risk Assessment of Nanomaterials: Understanding Relevant Exposure Scenarios Throughout the Life Cycle of Nano-Enhanced Consumer Products: A Case Study on Nanosilica

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SUMMARY: Advances in nanotechnology have resulted in the commercialization of many nano-enhanced consumer products, leading to the development of novel applications as well as providing improved performance and durability to existing products. In particular, nanomaterials are increasingly being used as additives in polymeric materials in food packaging, textiles, paints and coatings, cement, construction materials, sporting goods and personal care products. In the absence of information on the toxicological effects specific to nanomaterials, identifying relevant exposure pathways during the life cycle of the products is crucial to mitigate potential health risks. Through a case study on nanosilica, critical exposure pathways will be identified for a variety of consumer applications at all stages of their life cycle, and realistic exposure scenarios will be discussed. Data gaps and opportunities for research and collaboration will be identified.

OBJECTIVES: Develop an integrated approach for the assessment and management of nanomaterials in consumer products throughout their life cycle in order to protect human health and the environment from potential adverse effects of nanoparticles.

DESIGN: The scientific and regulatory literature on nanosilica was reviewed to identify current manufacturing, use and disposal practices, current knowledge on release of nanoparticles from nanocomposite materials under different scenarios, and available methodologies for the detection, quantification and characterization of released materials. A life cycle analysis of representative applications of nanosilica in consumer products was conducted, critical exposure pathways were identified, and gaps in the information needed to derive exposure estimates were identified. Research needs were highlighted and prioritized.

RESULTS: The available information indicates that silica nanoparticles embedded in a polymer matrix have the potential to migratate within the matrix and be released from the products. The scientific knowledge on the release of nanoparticles from consumer products and nanocomposites is still very limited, but potential mechanisms that have been identified include leaching, migration, abrasion, weathering, and polymer degradation. Sources of particle release could include sanding, washing, sweating and incineration. The degree of nanoparticle release would be affected by the characteristics of the polymeric matrix, the physico-chemical properties of the nanomaterial, the interactions between the nanomaterial and the matrix, and the properties of the media into which it would be released. Life cycle analysis therefore must be conducted for each application and polymer-nanomaterial matrix.

CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This preliminary analysis has identified many data gaps at all stages of the life cycle of nano-enabled consumer products. Research needs have been identified, and partnerships to develop standarized methodology for characterization and quantification of nanoparticle release have been initiated. Data on the availability of nanomaterials in the Canadian marketplace should be collected and analysed. This information is critical for establishing sources of human exposure to nanomaterials that can be used in the health risk assessment of new substances.

4.09 Challenges Around Interpreting Experimental Data on the Characterization of Select Domestic Substance List (DSL) Microorganisms in the Bioinformatic Age

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SUMMARY: One of Health Canada's mandates is to assess whether a substance is toxic or capable of becoming toxic as described in the *Canadian Environmental Protection Act* (CEPA). In our laboratory, we develop and evaluate toxicity tests for this purpose and use bacteria listed in the DSL of CEPA as test substances. These bacteria are significant in the context of industry, research and medicine. During our investigation, we noted that some strains yielded colonies that differed when using several microbiological tests. Our follow-up observations suggest that starting with a single colony prior to experimentation can generate contradictory data.

OBJECTIVE: To compare distinct colony morphology variants derived from select microbial starter-cultures by assays for antibiotic resistance, colony morphology, fatty acid composition, selective and differential media, hemolytic activity, microscope examination, and cytotoxicity against two mammalian cell lines.

MATERIAL AND METHODS: Purchased lots of several *Bacillus* species and strains were scaled-up aseptically in trypticase soy broth (TSB) with an oxygen gradient at room temperature, until a substantial amount of growth was visible. Bacterial cultures were aliquoted and stored at -20C in 20% glycerol. The cultures were then spread onto TSB and sheep blood agar plates to obtain single colony isolates. Visually distinct colonies were isolated and re-cultured as described above. The taxonomic identities of isolates were screened using the Sherlock™ microbial identification system. Different production lots were also used to validate reproducibility and discount any possibility of contamination. Isolates were further screened using various well-established microbiological assays and techniques.

RESULTS: Comparison of distinct bacterial isolates derived from repository starter-cultures revealed that some colonies have unique morphological, biochemical and physiological properties. This observation was most notable when culturing strains from the closely related species *Bacillus amyloliquefaciens*, *B. licheniformis* and *B. subtilis*. Colony morphology signatures were complex and may not represent a distinct variant but rather the outcome of two or more variants growing as a single colony.

CONCLUSION: Scale-up cultures derived from certain repository starter-cultures are complex and can yield colony variants with different morphological, biochemical and physiological traits that are stable and reproducible. The observation has significance for production of scale-up cultures from single colony isolates, which is widely used for genomic, microbiological and taxonomic research. The heterogeneity in the complexity of the strain is not observed when only one isolate/variant is chosen for scale-up and testing. This could result in ambiguous and/or contradictory information submitted to microbial repositories or publicly accessed databases.

4.10 Flow Cytometry Method for Identification and Viability Assessment of *Giardia* and *Cryptosporidium*

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SUMMARY: Up to a quarter of the reported gastrointestinal illnesses in Canada are associated with infection with the food and waterborne parasites, *Cryptosporidium* and *Giardia*. As these organisms can't be enriched in the laboratory, methods for enumeration often use microscopy, are time consuming and require expertise for interpretation. We have developed a flow-cytometry-based method for quick and accurate detection of live *Cryptosporidium* oocysts and *Giardia* cysts. This method will enable the accurate analysis of many samples in a much shorter time period.

OBJECTIVES: Current methods for detection of *Cryptosporidium* oocysts and *Giardia* cysts involve microscopy of samples. Microscopic analysis is limited as only small numbers of cells in limited volumes can be investigated at one time. Flow cytometry is a fast and efficient method of quantifying particles in large volumes of samples. This technology has been employed previously for detection of *Cryptosporidium* and *Giardia* in water samples. The purpose of this study was to develop a method for rapid enumeration and viability assessment of oocysts/cysts in manure samples using flow cytometry.

METHODS: The viability dyes SytoX Red Dead, propidium iodide (PI) and DAPI were compared in conjunction with different monoclonal antibody fluorochromes (FITC, R-phycoerythrin, ALexa647) at multiple incubation concentrations and times (at 37°C). Samples were analyzed using a BD LSRFortessa flow cytometer equipped with three lasers. Oocysts/cysts were extracted via flotation from spiked manure samples and analysed using flow cytometry with parameters set by the preliminary experiments.

RESULTS: The combination of monoclonal antibodies conjugated with R-phycoerythrin and FITC for *Cryptosporidium* and *Giardia* (respectively) with SytoX Red Dead to determine viability provided accurate results with less spectral overlap compared to P.I. and Alexa647. The use of Alexa647 conjugated antibodies resulted in oocyst clumping which skewed events, forcing changes in gating. This flow cytometry method is advantageous over current microscopy-based methods allowing sampling of larger volumes in a short time. Use of SytoX Red Dead dye in place of P.I. is valuable for future investigations, as SytoX has minimal spectral overlap and this series of cell impermeant stains is available in multiple colours, allowing for use of different laser-fluorochrome combinations.

CONCLUSION: Identification and viability determination of *Cryptosporidium* and *Giardia* using flow cytometry enables rapid, sensitive and accurate identification and quantitation of these parasites in manure samples. This method will be evaluated for use with other environmental samples and for foods.

4.11 Building Rapid Response Capability Through a Novel Microfluidic Platform for the Detection of Foodborne Pathogens

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SUMMARY: The national listeriosis outbreak that occurred in Canada in 2008, is a prime example that foodborne pathogens are a constant threat. Current methods for detecting bacterial pathogens in foods rely on lengthy culture-based approaches. Traditional bench techniques can, for example, take up to 10 days for Listeria monocytogenes. We are implementing microfluidic technologies to improve detection efficiency of foodborne pathogens. The platforms developed in this project will enable the deployment of a sensitive sample preparation device able to separate pathogens from food debris, a capture platform and a molecular identification allowing rapid detection of viable foodborne pathogens.

OBJECTIVES/BACKGROUND/ISSUE(S): Recent advances in microfluidics technology indicate that both conventional and molecular based approaches may be used together to provide a rapid and portable solution for pathogen detection. The objectives of this project are to integrate into a lab-on-a-chip platform sample preparation for the physical recovery and isolation of viable bacterial pathogens with molecular characterisation within a few hours.

DESIGN/METHOD/DESCRIPTION: We are developing, integrating and validating a high-throughput, non-clogging microfluidics-based platform (inertial separator) for the rapid isolation of *L. monocytogenes* from food debris to improve the speed, sensitivity and specificity of food testing. A hydrodynamic method is applied for high-throughput filtration in microfluidic channels to separate pathogens from food debris. The filtrate is then entering a fine filtration unit (bump array) to further clean and concentrate pathogens in hundreds of microliters. The C-Chip platform then allows for rapid capture of the target exiting the sample preparation unit. Capture is then followed by on-chip culture and a sample is deposited onto agar for further forensic investigation. *In situ* lysis is used for molecular identification through multiplex PCR.

OUTPUTS/RESULTS: The inertial focussing chip successfully separates *L. monocytogenes* from ground beef debris of ≥ 8 um. Prototypes of the bump array have been produced and tested with *Listeria* cells achieving fine filtration down to 3um. This innovative sample preparation platform handles rapidly and efficiently large volumes of food (15 min, 10 ml). The C-Chip was thoroughly tested with different surface chemistries, flow speeds and pillar sizes to improve *L. monocytogenes* capture. Currently, capture-culture platforms have shown good sensitivity (100 CFU in PBS and ground beef) and sustained bacterial growth. Within 24h, foods with low bacterial counts can be detected following on-chip culture and molecular characterisation.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS: Microfluidic platforms would give CFIA and regulatory agencies a rapid diagnostic tool to apply towards screening.

4.12 Benefit/Risk Considerations in Integrating Safety Pharmacology into Toxicity Test Strategies

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SUMMARY: Safety pharmacology (SP) studies are designed to investigate how vital organs, e.g., central nervous, cardiovascular and respiratory, react to drugs, with a view to detect potential adverse effects. Safety pharmacologists conduct stand-alone core-battery experiments in conscious animals while making optimal use of non-invasive techniques, which focus on vital structures/functions. The International Conference on Harmonisation (ICH) has published guidelines M3(R2), S6(R1), S7A and S7B that welcome the integration of SP into non-clinical toxicity test guidelines (TTGs). Laboratory toxicologists offer resistance to this original industry proposal (SP proposal).

OBJECTIVES: Assess the benefits/disadvantages of planning/performing meaningful SP tests within the same context/program as toxicity testing (TT), and as part of conventional TTGs.

MATERIALS AND METHODS: Current testing strategies were weighed against SP proposal scenarios, based on post-market experiences, e.g., NSAIDS, TNFα inhibitors, and the scientific/regulatory literature reviewed, including: 1) OECD's TTGs; 2) ICH guidelines; and, 3) guidance for Industry documents from various Agencies' on non-clinical studies and risk management/pharmacovigilance plans of Health Canada, the US Food and Drug Administration, the European Medicines Agency.

RESULTS: Under the SP proposal, safety pharmacology data are made available from the same animals as used for collecting pharmacodynamic/toxicological/ toxicokinetic data. This reduces laboratory effort while optimizing animal use, maximizes hazard identification, and facilitates regulatory approval of and passage to clinical trials by offering evidence necessary for protecting subjects recruited in first-in-human drug testing. In contrast, SP integration into TT breaks TT routines as standardized by OECD TTGs, adds testing time, and changes strategies/formats of data collection/presentation. These drawbacks are of concern to toxicologists involved in planning/implementing laboratory testing and data analysis. Additional cost will seem minimal or null if the SP proposal only entails: 1) meaningful commonality/collaboration with TT environments, of existing human and material SP resources; and, 2) same data presented under adequately modified reviewer-friendly formats. Amongst challenges associated with this proposal, are: 1) how much SP information shall merge with TT data; and, 2) what's in it for post-market surveillance.

CONCLUSION: The diversity of SP proposal benefits contrasts with the unique dependence of its disadvantages on TT environment. Added to favorable ICH guidelines and SP Society's support, the advent of new non-invasive technologies could facilitate toxicologists' acceptance of the SP proposal. The impact of this proposal on post-market surveillance remains to be weighted against its benefits in the context of drug life cycle management approach.

4.13 Identification and Enumeration of Microorganisms in Admixture Products

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SUMMARY: Biotechnology products containing microorganisms are regulated by a variety of governmental departments and agencies, including Health Canada. One category of microbial products includes admixtures of different microorganisms that work together to produce a function, such as plant growth promotion. This project involves an analysis of different techniques for identifying the components of a microbial admixture product. Partial identifications of each microbe were obtained by comparing genomic sequences and cell membrane components to laboratory databases. The results of this study can be used as basis for further research and are an important step in the validation and optimization of future protocols for analyzing more complex microbial mixtures, such as consortia.

OBJECTIVES: A range of biotechnology products used in Canada, consist of mixtures of microbes, and the development and validation of methods for identification and enumeration may facilitate product evaluation. Here we present the comparison of microbiology methods, 16S ribosomal DNA (rDNA) genomic sequencing and fatty acid methyl ester (FAME) analysis applied to a proprietary product containing two bacterial species as an admixture in an organic matrix, as well as in separate liquid cultures of the strains.

DESIGN: Each product was plated and grown on selective media for enumeration. 16S rDNA was sequenced from liquid cultures, or from metagenomic DNA extracted from the admixture and compared against a proprietary 16S rDNA database. Liquid cultures were saponified by a standard FAME protocol and extracts analyzed by gas chromatography and then compared to a proprietary FAME library.

RESULTS: 16S rDNA from the liquid cultures best-matched entries for *Bradyrhizobium* sp (ATCC 10319; (Bsp)) and *Bacillus* sp (ATCC 23350) *B. amyloliquefaciens*. By FAME, the best matches were for a *Bradyrhizobium japonicum* sub-group and those of a *Bacillus* subgroup respectively. The colony counts were within experimental error of supplier values. At high *Bacillus* plating densities, bacteriophage plaques were observed. Admixture metagenomic 16S rDNA was observed in a 9:1 ratio of Bsp and *Bacillus sp* (ATCC 6633).

OUTCOMES/CONCLUSIONS: Microbiology, genetic and fatty acid data, generated from microbial admixture constituents in liquid and in organic matrix, was generally consistent with microbial identities and quantities. Direct analysis of liquid cultures allowed protocol optimization for FAME and the unexpected observation of bacteriophage plaques. Indirect analysis of the admixture, carried out by extraction of metagenomic DNA followed by cloning and sequencing of 16S rDNA showed promise as a means of yielding the proportion of admixture constituents. Next steps will involve sequencing of an additional bacterial phylogenetic marker (gyrB) and validation and identification of the bacteriophage. The results of this study will enable the optimization of future assessments of more complex microbial mixtures, including those listed on the Domestic Substances List.

4.14 Quantification of the Viral Antigens Hemagglutinin and Neuraminidase in Influenza Vaccines by Mass Spectrometry

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SUMMARY: Vaccines provide protection from infectious diseases through generation of antibodies to critical microbial proteins. For influenza, this is achieved by injection of viral proteins, or antigens, that mimic exposure to an actual infection. To be protected against the most prevalent circulating virus strain, the population should be revaccinated annually. The quantification of the key antigens in influenza vaccine is usually carried out using an antibody-based method that relies on an international reference standard. An independent mass spectrometric method has been developed that accurately identifies and quantifies antigens in vaccines and reference standards.

OBJECTIVES: To develop a rapid method to identify and quantify the hemagglutinin and neuraminidase antigens in influenza vaccines using a combination of enzymatic proteolysis, peptide separation and tandem mass spectrometry. The method(s) must be able to identify specific virus strains in all products and quantify critical antigens. To be applicable in emergency situations (recent H1N1 pandemic), the method should not depend on the time consuming generation then standardisation of antibodies.

METHODS: A two-step procedure was developed that first identifies then quantifies hemagglutinin (HA) and neuraminidase (NA) in annual trivalent or pandemic monovalent influenza vaccines. The identification step relies on two independent proteolytic enzymes (trypsin and chymotrypsin) followed by triplicate HPLC/MS/MS analyses. The HPLC/MS/MS analyses were optimised to maximise the peptide coverage of the HA and NA to ensure unambiguous database search results. This is necessary because many strains differ by substitution of only a single amino acid. To reduce the variability of the quantification method, the sample manipulation was kept to a bare minimum. The vaccine sample was simply treated with excess trypsin and an acid labile surfactant, acidified, then the tryptic peptides were analysed in triplicate by tandem mass spectrometry and the results searched against a customised database produced from the previously identified strains and the chicken genome.

OUTCOMES: These methods show excellent correlation with current methodology (single radial immunodiffusion, SRID) for influenza A strains (HA1 and HA3); however, there is markedly disagreement regarding the amount of HA from influenza B. Currently, there is no NA quantitation method required.

IMPACTS: The SRID methodology has been a valuable method for standardising HA concentrations in annual influenza vaccines. The issues addressed with this new method include: standardisation of reference antigens, rapid alternative method of HA quantification without antibodies, method for quantitation of NA and egg proteins, and strain identification.

4.15 Toxicity Evaluation of Carbon Nanotubes: A Proteomic Approach

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SUMMARY: Properties of manufactured nanomaterials, unlike the bulk materials, depend on shape, size and surface chemistry. Nanomaterials with varying physico-chemical properties can induce different types of biochemical changes at cellular and molecular (protein) levels. In this study, we exposed lung macrophage cells to carbon nanotubes with different physico-chemical properties and levels of impurities to investigate risk associated with their exposure. This project is part of Health Canada's mandated research initiative (CMP-Nano) and international obligation (OECD-WPN Initiative) to fill in the knowledge gaps for reliable risk assessment of nanomaterials.

BACKGROUND AND OBJECTIVES: *In vitro* cytotoxicity endpoints can reveal overall toxicity of nanomaterials; however, it is difficult to predict in-depth analysis of mechanistic pathways. Thus, analyzing proteomic profile changes in cells exposed to nanomaterials can lead to the understanding of toxicity pathway and corresponding dose response analysis. The objectives of this study are to: 1) study the protein profiles in lysates of J774 cells after exposure to single and multiwalled CNTs (both pristine and oxidized forms); and, 2) study the role of surface chemistry and metal impurities on proteomic profiles that can in turn be related to cellular toxicity.

METHODS: J774 cells were exposed to variants of CNTs in 96 well plates at a dose of 30 g/cm^2 for 24 hours. Cell lysates were passed through 100kDa molecular weight cut off filter, trypsin digested and analyzed using nano-chip-cube LCMS. The protein identities were obtained from peptide mass fingerprint of each exposure using SpectraMill search engine (Agilent LTD).

OUTPUTS/RESULTS/CONCLUSIONS: Peptide mass fingerprint analysis of global proteome by 2D LCMS revealed nearly fifty proteins in cell lysate of J774 exposed to CNTs. The identified proteins including heat shock proteins, M2-type pyruvate kinase, prothymosin alpha, etc. Oxidized CNTs, with hydrophilic surface and lower metal impurities, were found to induce more proteomic changes than as-synthesized CNTs, indicating the significance of nanoparticle surface chemistry on biological responses. The association of physicochemical properties with induced proteomic changes can provide new insights into mechanisms of CNT toxicity.

IMPACTS: The proteomics approach for toxicity assessment will help address knowledge gaps in understanding the role of physico-chemical properties on toxicity of nanomaterials and pathway analysis. Results will reveal mechanistic information on cell-CNT interaction and possible health outcomes. This information will be useful for proper risk assessment and regulation of carbon nanotubes.

4.16 Comparing and Contrasting the Utility of Risk Management Plans and Periodic Safety Update Reports in Pharmacovigilance: A Regulatory Viewpoint

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SUMMARY: A Risk Management Plan (RMP) is a description of the risk management and mitigation measures put in place by the sponsor to deal with the anticipated risks associated with their product and should be submitted during the pre and post-authorization phases of the product's life cycle. The Periodic Safety Update Report (PSUR), a risk management tool for regulated marketed health products, is designed as a stand-alone document. Signals of new or increased risks associated with the marketed health products may be identified from these assessments and appropriate risk mitigation or management measures put in place to mitigate these risks.

OBJECTIVE: To compare and contrast the utility of RMPs and PSURs in pharmacovigilance.

BACKGROUND/ISSUES: The RMP may be defined as a set of pharmacovigilance activities designed to identify, characterize, prevent, or minimize risks related to the medicinal product; assess the effectiveness of those interventions and to communicate those risks to patients and health care providers. Further pharmacovigilance activities after marketing use the PSUR, an important post-marketing evaluation tool of the benefits and risks of a medicinal product and should support ongoing risk management initiatives, as well as a tracking mechanism monitoring the effectiveness of such initiatives.

DESIGN/METHOD/DESCRIPTION: Regulatory requirements regarding submission frequency and content of PSURs and RMPs, are not the same for regulators in the different regions worldwide (EU, Japan, US, Australia and Asia i.e., Singapore). While both PSURs and RMPs provide important information about the safety of regulated health products, in PSURs the aggregate data is used in the identification of new safety signals especially of rare adverse events. The RMP should include identified anticipated risks and additional information on the planned mitigation actions for each safety concern.

OUTPUT/RESULTS: Both RMPs and PSURs are important information tools in pharmacovigilance. They allow for the continuous surveillance of any public health risk associated with a health product and are vehicles intended to identify and manage the risk in question. Both PSURs and RMPs need to be submitted regularly to regulators in order to allow a proper evaluation of the benefit-to-risk profile for a medicinal drug that may have many uses and many user profiles.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The PSUR helps identify the risk, while the RMP provides a means to minimise and/or mitigate the risks, anticipated or newly identified. Both play a significant but complementary role in the continued monitoring and management of the safety of health products throughout their life cycle and are not mutually exclusive.

4.17 Health Canada's Contribution to the Strategic Emergency Management (EM) Plan: Proposing an Evidence-Based, Coordinated Approach to EM

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SUMMARY: Since WWII, emergency management (EM) planning within the Canadian government has become increasing fragmented and hazard-specific. At Health Canada (HC), the Office of Emergency Preparedness (OEP) conducted an inventory of existing EM plans confirming that the department is implicated in at least 98 EM planning documents. HC is moving towards a more evidence-based approach to integrate and coordinate these hazard-specific plans. OEP, in consultation with the Public Health Agency of Canada (PHAC) and HC programs and regions, is drafting a Strategic Emergency Management Plan (SEMP) for the Health Portfolio (HP), which could serve as the "core reflex" and outline the department's EM activities from preparedness and mitigation through preparation, response and recovery.

OBJECTIVE: To develop, in collaboration with PHAC and HC programs and regions, a consistent, integrated and science-based approach to EM beginning with the development of HP SEMP.

METHODS: The OEP in the Regions and Programs Branch (RAPB) conducted an environmental scan on the science of EM and the history of federal EM planning in Canada; a review of past lessons learned; and an inventory of existing HC EM plans.

RESULTS: Preliminary findings suggest that the Government of Canada has come a long way in advancing EM planning within departments; however, its approach since WW II has become increasingly hazard-specific. This has led to a multicity of plans and procedures, contributing to a fragmentation of emergency management planning.

At HC, an inventory of existing EM plans found that the department is implicated in at least 98 EM planning documents and nearly all are hazard-specific with no integration between them. HC needs an overarching EM plan to provide the "core reflex" for the department regardless of the hazard at hand.

NEXT STEPS: Currently, HC is seeking to better coordinate its EM plans so that they work together. In taking a more evidence-based approach to EM planning, OEP is working closely with PHAC and HC programs and regions to develop a consistent and integrated approach to EM, founded on education, training, experience and continuous improvement. One of the key components of this change is expected to be HP's SEMP, which will be an evergreen document to serve as the "core reflex" and outline EM activities from preparedness and mitigation through preparation, response and recovery. HC will be one of the first departments to fulfil its obligation in establishing a SEMP and in promoting a more integrated and coordinated approach.

4.18 New Microbiological Methods Committee Guidelines for the Relative Validation of Indirect Qualitative Food Microbiological Methods

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SUMMARY: The Microbiological Methods Committee (MMC) provides methodology in support of Health Canada and Canadian Food Inspection Agency mandates in overseeing the safety of the Canadian food supply. Food microbiological methods published in the Compendium of Analytical Methods can be used for regulatory purposes to determine compliance with various standards and guidelines, and as such must have undergone appropriate validation and testing. New method validation guidelines were established to provide a focus on data in each food commodity, permit additional types of method validations, and to harmonize as much as possible with method validation guidelines of international standardization organizations.

OBJECTIVES: The new method validation guidelines can evaluate unpaired sample data stemming from initial enrichments not common to the two methods. Unpaired samples are increasingly encountered with rapid methods that use proprietary components. The new guidelines require data in each food commodity, not an overall number of data points. The selection and type of food samples, and statistical evaluation of the data set were also modified. Finally, there was a need to harmonize method validation guidelines with international standardization organizations as much as possible.

DESIGN: A consensus document outlining the design and evaluation of relative method validation studies was prepared.

OUTPUT: A key requirement for method validation is comparison to an accepted reference method, with the results demonstrating sensitivity > 98%, specificity ≥ 90.4%, false positive rate < 9.6%, false negative rate < 2%, and efficacy ≥ 94%. To achieve “all-foods” status, validation data must be accepted for five food categories, with three food types represented in each food category. A table provides the classification of food categories and suggested food type-pathogen combinations. Studies using artificially contaminated samples should have 20 samples spiked at a low level, 20 samples at a high level, and five uninoculated samples for each food type. At least five relevant bacterial strains should be used for inoculation. Cultures are to be stressed and equilibrated in foods, with consideration given to background and interference organisms. Additional requirements include inclusivity/exclusivity studies, a limit of detection study, and a transfer study.

OUTCOMES: The Guidelines for the Relative Validation of Indirect Qualitative Food Microbiological Methods (Part 4 of the Development of Methods in the *Compendium of Analytical Methods*) are posted on Health Canada’s website. This document will aid method developers in designing validation experiments to allow for inclusion of their methods into the *Compendium of Analytical Methods*.

4.19 Thermostability, Potency and Seasonal Influenza Vaccines

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SUMMARY: Influenza viruses spread rapidly and cause significant annual morbidity and mortality worldwide. Currently, the most effective protection against infection is annual vaccination. Although a number of developments in vaccine production, safety and immunogenicity have been reported advances in stability are limited. We develop a model to explain vaccine potency loss and use a bioinformatics approach to screen possible influenza vaccine stabilizers. We take several identified compounds and test their effect on the thermostability of trivalent influenza vaccines. These results suggest a method for the development of thermostable vaccines and alternative biophysical methods for potency testing influenza vaccines.

BACKGROUND: The standard influenza vaccine currently recommended by the WHO is a trivalent vaccine derived from two A/influenza sub-types and one B/influenza type viruses. To maintain full protection yearly vaccination is recommended, and the long-term shelf-life of an influenza vaccine is not usually of concern. Soon after the release of 2009 monovalent H1N1 vaccines, however, manufacturers and regulators began to report decreases in the hemagglutinin (HA) content leading to revisions to the expiry date of many of these vaccines. We noted the correlation between specific vaccine strains and the monovalent products whose shelf life had been revised. We suggested that the conformational stability of hemagglutin in the virus may be a primary determinant of influenza vaccine stability and applied this to examining potency loss and thermostability in the 2010-2011 trivalent seasonal vaccine.

METHOD: We analysed different hemagglutinins using crystal structure and sequence information and selected compounds, which should interact with either the H1- or H3-subtype contained in the 2010-2011 influenza vaccine. We tested these compounds for their dose-dependent effect on the fusogenic activity of several vaccine viruses, using plaque inhibition assays. These compounds were then tested using the standard lot-release test, the single radial immunodiffusion (SRID) assay, for their dose-dependant effect on the thermostability of monovalent bulk HAs from one single manufacturer. Finally we used SRID assays to screen the effect of these compounds separately or in combination on different final vaccine lots made by multiple manufacturers.

RESULTS: Our results are consistent with the conformational stability of HA in the virus being a primary determinant of vaccine stability. These data suggest:

- The pathway of potency loss in influenza vaccines.
- An approach to develop thermostable influenza vaccines.
- The antigenic HA structure detected by SRID analysis.

IMPACTS/NEXT STEPS: This study will allow the development of methods to:

- Use biophysical approaches to evaluate potency
- Select candidate vaccine strains, which will yield more thermostable vaccines

4.20 From Research to Regulation: New *In Vitro* Assays to Quantify Pertussis Toxin Activity in Acellular Pertussis Vaccines

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SUMMARY: The Biologics and Genetic Therapies Directorate (BGTD) evaluates and releases vaccine lots for all Canadian immunization programs. Presently, there is strong interest in developing *in vitro* methods to replace animal-based tests currently used for testing vaccines. Recently, BGTD research and Quality Control (QC) laboratories participated in an international collaborative study organized by the National Institute for Biological Standards and Control (NIBSC), to investigate new *in vitro* assays, either provided by NIBSC or developed in-house for QC testing of acellular pertussis vaccines. The results from both BGTD laboratory groups are comparable and our in-house methods offer several key advantages.

BACKGROUND/OBJECTIVES: Vaccination against pertussis (whooping cough) is an essential part of immunization programs worldwide. A key component in acellular pertussis vaccines is the toxoided (detoxified) form of pertussis toxin (PTx) and testing for residual non-toxoided PTx activity in vaccines is necessary to ensure their safety. The animal-based histamine sensitization test (HIST) is currently a key QC test, but like many *in vivo* assays the results are highly variable. The potential advantages of the *in vitro* methods include better quantitation, greater reproducibility and a reduction in animal testing. Once validated, use of *in vitro* methods would mark a change in regulatory approach from animal-based “safety” assays, to measures of manufacturing consistency for products with appropriate safety profiles.

MATERIALS AND METHODS: BGTD tested an array of nine vaccine samples using HPLC and ELISA protocols provided by NIBSC, as well as analogous in-house methods developed in BGTD research laboratories. The HPLC assays determine PTx enzymatic activity by monitoring changes in levels of a fluorescent peptide substrate. The ELISAs quantitatively determine PTx binding to a model glycoprotein (fetuin) using either polyclonal (NIBSC) or monoclonal (BGTD) antibodies raised against PTx.

RESULTS: Using NIBSC and in-house HPLC methods, BGTD laboratories identified the same samples as, having either high (4-10 µg/mL), medium (0.1-1 µg/mL) or low (trace) PTx activity. When measured by ELISA, the PTx levels in the vaccine samples were ~200 fold higher using the NIBSC protocol (mean=58.6 ng/mL, 0-300 ng/mL) than with the in-house method (mean=0.09 ng/mL, 0-1.5 ng/mL), suggesting that the NIBSC polyclonal antibodies are less specific for PTx.

CONCLUSIONS: This work demonstrates the potential for *in vitro* assays to set specifications of residual PTx in pertussis vaccines and replace the animal HIST test. Additionally, the BGTD assays were transferable, robust and offer several important advantages.

4.21 Improved Stability of Proteins Through the Utilization of Nanoparticles

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SUMMARY: Maintaining the refrigeration of vaccines and protein drugs is crucial as they are heat sensitive and high temperatures can damage them, reducing their potency and efficacy. Here we present evidence that nanoparticles made of lipids (LNPs) can reduce heat-induced damage of albumin, a protein used in a number of important drug formulations.

BACKGROUND: Correct structure is critical for the proper function of protein therapeutics. Numerous factors can affect protein structure such as denaturing agents (i.e., urea) or thermal stress. Thermal stress is of particular concern as breakages in the cold chain can lead to detrimental changes (i.e., loss of efficacy) to the product. Maintenance of the cold chain is a particular challenge in regions with inadequate infrastructure, and the use of nanotechnology may allow for improved thermal stability of therapeutic proteins. In the studies presented here we utilize charged lipid nanoparticles to increase the thermal stability of albumin.

METHODS: The thermal stability of three albumins (bovine serum albumin (BSA), recombinant human serum albumin (rHSA) produced in yeast (*P. pastoris*) or rice. (*O. sativa*)) in the presence of various LNP formulations were assessed through monitoring alpha helical content at 222nm with far U/V-circular dichroism spectropolarimetry.

RESULTS: The presence of negatively charged LNPs improved the thermal stability of BSA and rHSA (*P. pastoris*) by 10 and 15°C respectively. Neutrally charged LNPs had little or no effect on the thermal stability of these two albumins. Conversely; negatively charged LNPs had little to no effect on rHSA derived from *O. sativa* and neutrally charged LNPs decreased the thermal stability of this protein by approximately 10°C.

CONCLUSION: We demonstrate that LNPs are an effective means of improving thermal stability of BSA and rHSA from *P. pastoris* but not rHSA derived from *O. sativa*. Improving the thermal stability of albumin is critical as it is used as an excipient for many pharmaceutical formulations and a delivery system for small molecules. Studies have suggested that it has the potential for therapeutic use.

4.22 New Methodology for Isolation of *Listeria* Species from Food: Addressing the 2008 Listeriosis Outbreak in Canada

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SUMMARY: The 2008 Canadian listeriosis outbreak in ready to eat meat raised many questions over the safety and reliability of the national food supply. Three recommendations of the Weatherill report specifically focused on the improvement and validation of methods in order to better detect and thus enhance responsiveness in foodborne emergencies. The purpose of this study was to generate a new method for isolation of *Listeria* species that was faster and more sensitive than the current "gold standard" culture method (MFHPB-30).

OBJECTIVES: To develop a culture-based method designed to isolate *Listeria* species in a shorter timeframe to allow for faster regulatory or compliance activities.

DESIGN: The new method consists of UVM1, the primary enrichment broth, incubated for 48 h. A doubling of the transfer of UVM1 to secondary Fraser broth (200 µL) and the inclusion of a chromogenic (Rapid'L'mono) agar was added to increase sensitivity. Oxford agar was replaced with Palcam agar, allowing for experienced workers increased odds of picking a presumptive *Listeria monocytogenes*. Blue colonies from the chromogenic agar were confirmed using MFLP-78 and OBIS, a PCR and chromogenic test, respectively; whereas colonies from Palcam agar were tested for sugar fermentation (rhamnose, mannose, xylose), hemolysis, and motility. Experimental validation consisted of using 20 low (~0.2-1 MPN/25 g) 20 high (~2-5 MPN/25 g) and five uninoculated samples of cooked chicken product, smoked fish, raw and pasteurized milk commodities, and fermented meat products. Prior to inoculating the food matrix, bacterial strains (major serotypes of *Listeria*, including the outbreak strain) were stressed accordingly, depending on the commodity tested. The MFHPB-30 method, in contrast, has 48 h incubation in UVM1, with only 100 µl transfers being made at 24 and 48 h to Fraser broth. The Fraser broth is further incubated up to 48 h prior to streaking on agar plates.

OUTPUTS/RESULTS: Statistical analyses revealed that omission of secondary enrichment in Palcam broth would not affect the sensitivity of the method. The new method was designed to be able to detect *Listeria* species within 48 h in highly contaminated food matrices. Overall, the time to results has been reduced from 7-10 days (MFHPB-30) to 3-5 days. The method was further validated in an Association of Analytical Communities (AOAC) approved, government validation (GovVal) study.

IMPACTS/OUTCOMES/CONCLUSIONS: While the new method will continue to be validated against high-risk food commodities known to be contaminated with *Listeria* species, reduction of time to results without loss of sensitivity will increase confidence in the food supply, while permitting investigators to test more foods during an outbreak investigation. Inclusion of the method in the Canadian Compendium of Analytical Methods will aid the Canadian industry.

4.23 Risk of Activation or Reactivation of Latent Tuberculosis (TB) Associated with Monoclonal Antibody Therapy

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SUMMARY: Monoclonal antibody therapy has advantages over conventional therapy in terms of potency, specificity and safety, as they are engineered to bind specific molecular targets. Working by selectively blocking mechanisms involved in inflammatory and immune response, they interfere with the body's immunity often causing increased rates of infections among treated patients. Tuberculosis (TB) is among the common infections reported in patients treated with immune modulating monoclonal therapies (IMT). While most monoclonal agents are generally safe, working knowledge of the infectious complications associated with these is essential to inform patients of the risks as well as maintaining vigilance for these adverse events.

OBJECTIVE: To highlight the risk of TB infection associated with monoclonal antibody (MAB) immune moderating therapies, and the importance of screening of patients before and close monitoring following initiation of MAB therapy.

DESCRIPTION: MAB therapy is a type of immunotherapy for the treatment of various serious diseases such as rheumatoid arthritis. The advantages for this therapy are potency, specificity, and safe, as the product is engineered to bind to a specific molecular target. However, since they interfere with the body's natural immunity, this often causes increased common and unusual infections among treated patients. TB has been reported in patients treated with immune suppressive MAB therapies. Adalimumab (Tumour Necrosis Inhibitor (TNF)-α inhibitor) is one of MAB used for the treatment of rheumatoid arthritis. The TNF-α is a cytokine that plays a central role in establishing and maintaining the inflammatory response against infection. TNF-α blockade results in the interruption of TNF-α mediated functions, which include cell activation, proliferation, formation and maintenance of granulomas. Therefore TNF-α inhibitors have been shown to increase the risk of granulomatous infections, most importantly tuberculosis (TB).

RESULTS: Tuberculin skin testing, baseline chest X-ray performed prior to the initiation of therapy are important in the management of risk for TB. Whole blood Interferon-gamma assay is a promising new test for latent TB, which can be used to complement the tuberculin skin test. Monitoring for active TB should also be done while patients are on MAB immunotherapy, including those who tested negative for latent TB.

CONCLUSION: As a result of the increased awareness of the risk of infections associated with MAB immune moderating therapies, TB has been labelled in the Canadian Product Monograph for the entire class of immune moderating MAB therapies. It is essential to inform patients with the infectious complications associated with this therapy as well as maintaining vigilance for these adverse events.

4.24 Assessing the Impact of a National Guideline to Improve Transfusion Safety in Canada

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SUMMARY: The *Guideline for Investigation of Suspected Transfusion Transmitted Bacterial Contamination*, published in 2008, was developed by a working group formed by the Public Health Agency of Canada (PHAC) to provide standardized instructions for investigation of suspected bacterial contamination related to transfusion of blood components. A survey was developed and administered by PHAC in 2010 to assess the impact of the *Guideline* on hospital blood bank staff. Compared to a 2006 PHAC survey, results showed increased compliance with best practices, encouraging for the production of other guidelines to increase transfusion safety in Canada.

OBJECTIVE: To assess the impact of the *Guideline for Investigation of Suspected Transfusion Transmitted Bacterial Contamination,* published by PHAC in 2008 to provide standardized instructions for investigation of suspected bacterial contamination related to transfusion of blood components.

METHODS: A web-based survey in English and French was administered to blood bank staff in hospitals sampled from each province. The survey asked about awareness of the *Guideline,* its usefulness and hospital practices used to investigate suspected transfusion-transmitted bacterial contamination cases. Responses were anonymous. To compare pre- and post-*Guideline* practices, a set of questions was identical to those in a prior 2006 survey.

RESULTS: Twenty-four (53%) respondents completed the survey. Ninety-two percent indicated the *Guideline* had been useful and 63% reported their facility's investigation protocol had been changed following publication of the *Guideline*. The most frequent change was the addition of the direct examination (Gram stain) to facilitate rapid diagnosis of bacteria and interpretation of culture results, representing 40% of reported protocol changes. Protocol changes were more frequently reported among respondents from community hospitals (77%) than teaching hospitals (50%). Compared to the 2006 survey, noted changes in practice included a decrease in the proportion of hospitals using segments of bags for culture when no residual product remains in the blood bag, from 35% to 4%, and, correspondingly, an increase from 22% to 83% of hospitals that inject culture media or saline into the blood bag in such situations. The proportion of hospitals that immediately dispose of blood bags following transfusion decreased from 51% to 21% and those that perform a direct examination as a component of a laboratory investigation protocol increased from 53% to 79%.

CONCLUSION: The survey results indicated that the *Guideline* was useful for hospitals and resulted in changes that improved compliance with best practices.

4.25 Optimization of Comparative Genomic Fingerprinting (CGF) Multiplex PCR for Molecular Typing of *Campylobacter jejuni (C. jejuni)*

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SUMMARY: Each year, an estimated 1% of the population is afflicted with *Campylobacter*-induced gastroenteritis. Currently, few food or clinical isolates of this organism are characterized, and sources of this mainly sporadic infection are rarely identified. The high cost and expertise required for the current typing methods may be partially responsible for the scarcity of strain information. This study was done to optimize Comparative Genomic Fingerprinting (CGF), a new method for typing these bacteria. The low cost and increased speed of this method will lead to more widespread use of typing data for monitoring human epidemiology of *campylobacteriosis* in Canada.

OBJECTIVE: Comparative genomic fingerprinting (CGF) is a binary typing method involving the amplification of a set of 40 genes (Eight five-plex PCR reactions) to determine their presence in an isolate of *C.jejuni*. The purpose of this study was to optimize CGF to reduce cost and time of this method for typing strains of *C.jejuni*.

MATERIALS AND METHODS: Strains of *C. jejuni* were grown microaerobically for 48 hours in Brucella broth. Cells from one mL of sample were pelleted by centrifugation and genomic DNA was extracted using phenol: chloroform, alkaline lysis, and Chelex® methods. We also evaluated commercial PCR reaction mixtures (Qiagen® multiplex, Promega GoTaq®Clear, Qiagen®TopTaq , MP Taq®), varying concentrations of reaction mix components ($MgCl_2$, dNTPs), PCR cycling conditions, and additives (BSA, betaine, DMSO, Qiagen®Q solution) to determine how each component impacted the multiplex amplification. Following optimization, a 16s universal eubacterial control was incorporated into each of the multiplex reactions.

RESULTS: High quality nucleic acid template is necessary for optimal results with this method. We found that incorporation of Chelex® into a boiling lysis method gave results that were comparable to the use of high quality gDNA isolated by standard methods. Use of the microwave for cell lysis produced equivalent results to boiling, and reduced time required and condensation in the tubes, a factor that may be important for preventing cross contamination when dealing with multiple isolates. Results also varied with different commercial PCR systems. We found the Qiagen® TopTaq mixture performed the best as assessed by measurement of band intensities in all 8 multiplex PCR reactions. The most important addition to the PCR reaction mixture was the incorporation of a final concentration of 3.0 uM of MgCl2 and 400 uM of dNTPs. The added $MgCl_2$ led to increased PCR product concentrations, and the addition of nucleotides reduced the nonspecific amplification of small fragments. Using these reaction conditions, increased denaturation time in the PCR cycling conditions was found to improve the consistency of the assay. Finally, a 16s rRNA control was incorporated into each 5-plex reaction to improve the reliability of scoring of these assays.

4.26 Weight of Evidence: Factors to Consider for Appropriate and Timely Action in a Foodborne Illness Investigation

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SUMMARY: A foodborne outbreak investigation is complex, multi-disciplinary, non-linear and dynamic. Data collection from laboratory, food safety and epidemiological investigations is critical. As part of the lessons-learned exercises resulting from the 2008 Canadian deli-meat listeriosis outbreak, a guidance document was developed to provide information on factors to consider in determining the weight of evidence to ensure timely and appropriate actions. This is a general guidance document primarily for federal level decision-makers during foodborne outbreak investigations. The document describes factors to consider and provides guidance on how much weight to assign when assessing evidence obtained

OBJECTIVE/BACKGROUND/ISSUE: This is a federal guidance document for potential use during a foodborne outbreak investigation to ensure the implementation of timely and appropriate risk mitigation strategies.

DESIGN/METHOD/DESCRIPTION: A team of representatives from Canadian federal government Departments involved in foodborne outbreak investigations including Health Canada, the Public Health Agency of Canada and the Canadian Food Inspection Agency, were assembled. Through discussions and consensus building, a guidance document was developed to examine and determine the type and weight of evidence necessary and/or sufficient to take action. National and international food safety experts were also consulted.

OUTPUTS/RESULTS: Decision-diagrams stipulating what information should be gathered during an investigation, as well as guidance to assign “strength” to the evidence gathered, e.g., weak or strong, were developed. A framework was developed to determine the strength of all the evidence gathered. For the strength of the microbiological evidence, criteria, such as, does the organism show suitable Pulsed-Field-Gel-Electrophoresis clustering are considered, while for epidemiological evidence factors such as plausibility, consistency, specificity or the strength of statistical association are examined. For the strength of traceback/traceforward information, criteria such as the ability to identify the manufacturer, the point of purchase, distribution channels etc., are included.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The document provides a systematic approach to decision-making using the overall weight of scientific evidence to decide whether to proceed with a health risk assessment and appropriate risk management actions. It is hoped that this type of systematic approach will prove to be useful by all those involved in foodborne outbreak investigations in Canada.

4.27 Subcomponent Analysis of Meningococcal Polysaccharide and Polysaccharide-Conjugate Vaccines from China

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SUMMARY: An ongoing and unique international collaboration between the Centre for Vaccine Evaluation of Health Canada and the National Institutes for Food and Drug Control of the State Food and Drug Administration (People's Republic of China) allows for unprecedented access to Chinese biological therapeutics and vaccines. Under this reciprocal agreement a large number of different Chinese meningococcal vaccines were obtained. Methods for subcomponent analysis of the vaccines, which had been developed and qualified "in house" with a narrow range of available vaccines, were challenged with a much wider assortment of previously inaccessible vaccines.

OBJECTIVES: The active components of most meningococcal (*Neisseria meningitidis*) vaccines are four antigenic serogroup polysaccharides (ACYW135) derived from the bacterial capsule. The vaccines, monovalent or multivalent mixtures of either free polysaccharides or polysaccharides conjugated to antigenic carrier peptides, may be in liquid or lyophilized formulations with or without excipients. The presence of saccharide excipients such as sucrose or lactose may interfere with the determination of some serogroup polysaccharides. The goal of this project was to apply and challenge established methods to measure the serogroup polysaccharide subcomponents present in a broad collection of multivalent polysaccharide-based meningococcal vaccines provided by the NIFDC.

MATERIALS AND METHODS: Previously optimized hydrolysis and chromatographic methods were used to determine the serogroup subcomponents in 23 lots from 8 different polysaccharide-based meningococcal vaccine products for interlot and interproduct comparisons. Replicate operations by three different analysts further challenged the methods. Analysis of Y and W135 serogroups required the removal of lactose excipient prior to hydrolysis. Several dialysis and centrifugal filtration systems were tested and one particular brand of centrifugal filter proved far superior to all other options for this purpose.

RESULTS: Centrifugal filtration successfully removed lactose excipient without loss of polysaccharides to allow for the determination of Y and W135 serogroups. Statistical analysis indicated that the methods were remarkably reproducible when challenged by a greater variety of vaccines and multiple analysts (<5% CV). Results indicated some interlot and interproduct variations. However, all vaccines were within acceptable specifications for each serogroup polysaccharide, with the exception of all lots of one vaccine – which were deficient in the serogroup A polysaccharide subcomponent.

CONCLUSIONS: The previously established meningococcal vaccine analysis methods performed extremely well, when applied to a wider range of vaccine samples. Continued cooperation and exchange of expertise between CVE/Health Canada and NIFDC/China in this research area and others is anticipated.

4.28 Optimization, Qualification and Application of Methods for the Quantitative Analysis of Commercial Meningococcal Polysaccharide-Based Vaccines

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SUMMARY: Preventative vaccines are used to protect against life-threatening bacterial infections of the brain (meningitis) and blood (septicemia). These vaccines are based on the outer sugar shells of meningococcal bacteria, which are distinct for different strains. Commercial vaccines are available against up to four different strains in one shot. To approve and release these vaccines, methods were devised to measure the characteristic sugar component of each strain in multiple lots of several vaccine products.

OBJECTIVES: The active components of most meningococcal vaccines are four antigenic serogroup polysaccharides (ACYW135) in monovalent or multivalent mixtures. The polysaccharides contain repeating units from which galactose, glucose, and *N*-acetylmannosamine-6-phosphate are unique to serogroups W135, Y and A, respectively, while *N*-acetylneuraminic acid is common to C, Y and W135 serogroups. The goal of this project was to optimize, qualify and apply techniques to measure the four polysaccharide serogroups present in a variety of meningococcal vaccines to ensure these vaccines contain effective amounts of each component.

MATERIALS AND METHODS: Polysaccharide-based standards were subjected to a range of different depolymerizing hydrolysis conditions. The resultant monosaccharide mixtures were separated by chromatography and quantitated using a set of calibration standards. A multivariate survey of these conditions determined optimal yields of each monosaccharide from the individual polysaccharide standards and in an imitation multivalent vaccine. The optimized methods were qualified in the analysis of different lots of a variety of meningococcal vaccine products.

RESULTS: For serogroups W and Y, the maximum yields of galactose and glucose were achieved using a single hydrolysis condition. The maximum yield of neuraminic acid from serogroup C was obtained with a much milder condition with minimal contributions from serogroups W and Y. For serogroup A, the maximum yield of mannosamine-6-phosphate was achieved using an intermediate condition. Statistical validation demonstrated that the methods are remarkably reproducible (<5% CV). Results indicated some interlot and interproduct variations, but all vaccines were within the approved specifications for each serogroup polysaccharide.

CONCLUSIONS: These techniques are extremely useful for interlot/interproduct comparisons and for the evaluation of efficacy and safety of meningococcal vaccines. This regulatory research was instrumental in the approval of a commercial vaccine product and is now routinely used for lot release of that product. These hydrolysis and chromatographic methods may be adapted for evaluation of other polysaccharide-based vaccines.

4.29 A New ELISA for the Detection of *Clostridium botulinum* Toxin Surrogate Biomarkers

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SUMMARY: *Clostridium botulinum* (*C. botulinum*) toxins, the agents causing botulism, are lethal at extremely low dose and are present at very low levels making their detection in contaminated foods difficult during an outbreak or possible bio-terrorist attacks. We designed an assay to detect toxin surrogate biomarkers, which are present at higher concentrations than botulinum toxin in bacterial cultures. We determined the limits of detection, specificity, and robustness of the assay. This new method of detection will lead to faster food recalls and early identification of potential bioterrorism attacks.

BACKGROUND: While botulism outbreaks are rare in Canada, they are too often lethal. *C. botulinum* produces the most potent toxin known, with a lethal dose of 1-3ng/kg of bodyweight. This potency makes botulinum toxin an ideal weapon for bioterrorists. Detection of very dilute chemicals can be technically challenging and time-consuming. We designed an assay for the detection of toxin surrogates which allows us to infer the presence of toxin in foods.

METHOD: In order to isolate our target biomarkers, we first grew 20 strains of toxin-producing *C. botulinum* from groups I (at 35 ºC), II (at 25 ºC), and III (at 35 ºC) representing all toxinotypes (A, B, C, D, E, and F) and 15 additional clostridial species (including five strains of *C. sporogenes*) (all grown at 35 ºC) on McClung-Toabe or Brain Heart Infusion Yeast agar. We then inoculated 100mL of TPGY (special peptone, peptone, glucose and yeast) broth and cultured for 22h before harvesting cells and isolating biomarkers by centrifugation. We performed ELISA tests using monoclonal antibodies (mAbs) specific to the biomarkers developed at the NML.

RESULTS: During the course of the initial characterization of the ELISA, we determined the limit of detection for each toxin-producing *C. botulinum* strain, and demonstrated that the assay is specific to these strains as mAbs do not react with biomarkers from any other near-neighbours species tested. We are currently testing various foods and beverages to better determine the robustness of the assay.

CONCLUSION/FUTURE PLANS: Once thoroughly tested, this unique method of detection will constitute a faster tool for the early detection of potential botulism outbreaks or bioterrorism event in Canada. We are also exploring the possibility of extending this method to include other *C. botulinum* biomarkers.

4.30 Molecular Determinants Correlated with Loss of Potency in 2009 H1N1Monovalent Vaccines

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SUMMARY: The emergence of a novel influenza virus in 2009 sparked both a pandemic and a rapid world-wide response. Initially derived vaccine strains grew poorly and yielded low amounts of antigen. Additional strains were generated and subsequently used for the generation of influenza vaccines. Soon after many monovalent pandemic vaccines were released, however, their shelf-life was revised downward due to unexpected losses in HA potency. In analysing the various vaccines manufactured internationally we noted that they differed markedly in terms of antigenic content and stability. We find that these differences correlated with the strain used to derive the original vaccine.

OBJECTIVES/BACKGROUND/ISSUE(S): In response to the influenza pandemic in 2009, vaccine strains were derived by multiple centres in order to provide manufacturers the necessary tools to create an effective vaccine. Initially derived strains grew poorly and subsequent strains were selected for better growth. Following reports of potency loss by different regulators we analysed multiple vaccines and found differences in glycosylation, antigenic content and protein stability. We correlated these differences with the specific vaccine viruses used to derive them. We re-engineered the various mutations into novel viruses in order to identify individual contributions to the respective phenotypes

DESIGN/METHOD/DESCRIPTION: We analysed multiple monovalent pandemic vaccines using Mass Spectrometry as well as thermal or low-pH treatment followed by hemagglutination or trypsin-sensitivity assays. We analysed different vaccine strains using PNGaseF treatment and thermal or low pH treatment followed by hemagglutination or trypsin-sensitivity assays as well as plaque morphology, growth and neuraminidase activity. We modeled the specific sites in the HA molecule from the vaccine strain correlated with decreased stability using Deep View Swiss PDB viewer to understand potential contributions to the observed phenotypes. Finally we re-engineered the mutations found in various A/California/7/2009-like viruses individually and in combination. We evaluated these re-engineered strains to determine the relative contributions to the observed phenotypes.

OUTPUTS/RESULTS: We show the correlation of antigenic content and conformational stability in the influenza virus to the antigenic content and stability in the final product. We demonstrate that the Q223R mutation, present in some A/California/7/2009-like strains used to generate pandemic vaccines had the largest effect on conformational stability.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This study adds to our knowledge of the role of individual mutations in the replication and growth of influenza viruses and how these contribute to the properties of the final vaccine product.

4.31 Finding Lower-Risk Alternatives for Azinphos-Methyl-Containing Products: A Transition Strategy

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SUMMARY: Under the Pest Management Regulatory Agency's (PMRA) pesticide re-evaluation program, pesticide products are re-evaluated according to modern standards to determine whether, and under what conditions, their continued registration is acceptable. For certain critical pesticide uses being phased-out, the PMRA is committed to assisting affected commodity groups to gain access to lower risk pesticides and management practices by providing discussion forums for affected commodity groups, termed Transition Strategies. Currently, the PMRA is working with stakeholders to address various products, including those that contain the insecticides azinphos-methyl and diazinon.

BACKGROUND: Insecticides containing the active ingredient azinphos-methyl (AZM) are widely used by the fruit industry. In 2004, as part of its re-evaluation program, the PMRA concluded that risks to workers did not meet current standards, and announced the complete phase-out of AZM-containing products by the end of 2012. The United States Environment Protection Agency (EPA) announced a similar phase-out schedule in 2006. Both the EPA and PMRA created and collaborated on a pilot project termed the AZM Transition Strategy, to minimize the impact on growers and to facilitate risk reduction through the use of alternatives to AZM. The objectives of the Canadian transition strategy were to collaborate with stakeholders to: 1) develop a work plan outlining what was needed to achieve the registration of lower risk alternative products; and, 2) provide assistance throughout the regulatory process for identified alternatives.

METHODS: Working groups consisting of growers, researchers and regulators, were created for the major affected crops (cranberry and tree fruits). Each was consulted on numerous occasions and prioritised alternative products for registration. Through this process, the PMRA communicated data requirements, the working groups established research needs and a work plan was created. As regulatory requirements were addressed by working group members, completed product packages were submitted to the PMRA for review.

RESULTS: To date, the AZM Transition Strategy has succeeded in either submitting or registering over 50 percent of the alternative products prioritised in the work plans. More regulatory packages are in preparation and are expected to be submitted in the near future.

CONCLUSION: The PMRA is committed to working with stakeholders to find alternative lower risk products by the end of the AZM phase-out period.

4.32 How the Superiority and Non-Inferiority Trial Objectives Got In The Way of Discourse Concerning the Ethics of Placebo and Active Controlled Clinical Trials

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SUMMARY: The ethics of using placebo control in clinical trials has been hotly debated for 15 years. Canada embarked on a process to reconcile different ethical perspectives, but that proved to be a long and difficult process, lasting over 10 years, and delayed the adoption of a regulatory guideline on clinical trial design. The crux of the impasse has to do with lack of appreciation of the implications of science and applied research methodology on validity of trial results for different trial designs. Ultimately, if a trial cannot reliably be expected to produce valid results, its ethics must be questioned. This presentation will be useful in exploring the next steps towards developing regulatory guidelines on clinical trial design.

OBJECTIVE: To explain the science behind the 10-year impasse in the national consultation concerning the use of placebo control in randomised controlled clinical trials.

HISTORY AND BACKGROUND: Since the inclusion of an article concerning the use of placebo control in the Declaration of Helsinki (DOH) in 1996, a sometimes heated debate concerning the ethics of placebo control has been on-going. Canada embarked on a process of reconciling the different positions represented by the Canadian Tri-Council Policy Statement on Ethical Conduct for Research Involving Humans (TCPS) and the International Conference on Harmonization (ICH). This process ultimately lasted over 10 years, ending in the 2010 update of the TCPS and official the adoption of the 11-year-old ICH E10 document on choice of control groups for clinical trials.

FINDINGS: The real debate concerning the ethics of use of placebo control is not simply between active vs. placebo control, but involves the science behind the difference between a superiority trial design and a non-inferiority trial design. A superiority trial is differentiated from a non-inferiority trial based on the statistical tests employed. A superiority trial is designed to demonstrate that one treatment is more effective than another. A non-inferiority trial is designed to demonstrate that a treatment is at least not appreciably worse than another.

There is a built-in, unblinded component in non-inferiority trials, which does not exist in placebo controlled trials. The need to appeal to external historical information for validity relegates active controlled non-inferiority trials to a status resembling that of historical controlled trials. Because of the methodological implications, a finding of non-inferiority is no guarantee of its validity. Parties involved in the ethical discussions did not all recognise, understand, or accept this deficiency of the non-inferiority trial design as fact, resulting in repeated impasse and failed negotiations for years.

CONCLUSION: Results of a clinical trial are valid and useful when the employed research instrument is well-articulated, scientifically sound and ethically sensitive. A methodologically weak clinical trial could be labelled a waste of resources and unethical since research subjects are put through unnecessary risks. It is extremely difficult to engage in discussions concerning research ethics when the interplay of science and ethics is not recognised and appreciated. As this lack of appreciation of the science behind the non-inferiority trial design persists, the debate will go on.

4.33 Addressing the Most Critical Step in Food Microbiology Testing: The Sample Prep

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SUMMARY: Foods are complex and contain many inhibitors that create unique challenges for microbiology testing laboratories that use rapid, molecular-based diagnostic methods. Food routinely tested contain low (or no) concentrations of suspect pathogens and so the challenge is how to isolate the proverbial "needle in a haystack". This study describes a novel sample preparation method that allows for the removal of inhibitory material while permitting the concentration of foodborne pathogens. It has the potential to be applied to current approaches to sample preparation used in Canadian laboratories prior to isolating bacterial, viral and parasitic pathogens.

OBJECTIVES: To develop a novel sample preparation method applicable to food matrices that removes particulate matter that interferes with downstream applications such as detection or characterization, using *Listeria monocytogenes* as a paradigm to test the method.

DESIGN: Samples of ready-to-eat (RTE) deli meat or soft cheese were added to the primary *Listeria* enrichment broth and homogenized for 5 minutes in a membrane-containing stomacher bag. Contents were filtered through a "rock" column for gross size-exclusion, followed by filtering through a 75 µm filter prior to centrifugation for 10 minutes at 5000 × g. The pellet was resuspended in 1-2 ml of phosphate buffered saline (PBS) and plated on selective media for isolation of viable *L. monocytogenes*. Experiments using 1-10 CFU/g with injured cells of *L. monocytogenes* will determine the limit of detection and (if any) need for a short enrichment period. The 1-2 ml PBS suspension used in direct plating will be divided into three aliquots: 100 µl for a rapid diagnostic PCR assay (MFLP-78) targeting the *hlyA* gene to detect presence/ absence; 500 µl for direct plating; and 400 µl to be added to 4 ml *Listeria* enrichment broth (LEB), to allow for growth, should the sample contain less than 5 cells per
25 gram sample.

OUTPUTS/RESULTS: Deli turkey or cheese was artificially contaminated with *L. monocytogenes* and stored at 4 ºC for 48 hours. Duplicate samples were enumerated to reveal spiking levels of 25 to 1500 colony forming units per gram (CFUs/g). Even a low inoculation levels, the method allowed for detection of pathogen load, without loss of sensitivity. Isolation of *L. monocytogenes* was possible in two days.

IMPACTS/OUTCOMES/CONCLUSIONS: Emerging technologies based on nanotechnology or microfluidics requires a small sample input that is free of interfering debris. The method described here will aid the transfer of such technologies to the food industry and will be a useful complement to the traditional method of isolation of foodborne pathogens from complex food matrices.

4.34 Social Media and Emergency Management

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SUMMARY: The use of Social Media (SM) by first responders and government agencies to monitor, respond to and report on emergencies represents a growing trend. This study examines the value of SM in emergency management (EM) monitoring and response, and if that value outweighs the communication risks involved with SM. Such communication risks include: 1) information updates that may be incorrect, unapproved (by senior management) or contain misleading information due to the real time nature of social media; and, 2) information monitoring that is incorrect due to false intelligence gathered from SM platforms. Based on preliminary reviews of available literature and case studies of recent emergencies (including: H1N1, Haiti Earthquake and Cholera outbreak, and other more recent major emergencies or natural disasters), there are benefits of adopting a broader SM policy, which can help save the lives of Canadians in the event of an emergency.

OBJECTIVE: This study aims to highlight the benefits and risks involved with Social Media (SM) use in Emergency Management (EM) and determine if a broader Government of Canada (GoC) SM policy, outweighs the potential communication risks.

METHOD: The research will include a comprehensive environmental scan, including: reviews of existing studies on SM use in EM, as well as further reviews on SM use during planned events, including: the V2010 Winter Olympic Games, and the G-8 and G-20 Summits. Methodology will also include a review of the existing GoC SM policy. Additionally, it will also review existing SM platforms (i.e., Twitter, Facebook) and the software available to tailor them to EM (i.e., ArcGIS, a geographic information system).

RESULTS: Initial findings suggest that the advent of SM use during emergencies presents many communication risks as first responders and government agencies increase their use of SM for monitoring, responding and reporting on emergencies. Initial findings also revealed the benefits of SM use during emergencies, which can result in the conservation of life. Such benefits include:

- Locating people during an emergency, through the use of Twitter or the use of SMS to identify their locations.
- Collection of information and intelligence, such as: lack of resources; infrastructure issues (i.e., road closures), information updates, etc.
- Opening lines of communication with the public. This allows for not only the sharing of information with the public, but also a means to connect with the public.

IMPLEMENTATION: To harness the full potential of SM, the GoC SM policy would have to set the stage for a more open SM landscape. If that were to become a reality, Health Canada could establish a dedicated EM presence on various SM platforms, in conjunction with software designed to better monitor such platforms, and use such tools to inform the public during, and prior to, emergencies. When a solid SM network has been realised by the Department, engaging the public via SM dialogue could inform them as to EM initiatives and strategies that could directly impact them.

CONCLUSION: As already discussed, there are associated communication risks with the use of SM for EM. There are also further risks involved with such use of SM, including: hacking; inability for human resources to keep up with SM driven dialogue; ability for certain members of the public to post negative or false information. Though such risks may present themselves when using an engaged and transparent SM strategy, the potential benefits to the safety of the Canadian public may outweigh such risks. If this is the case, a broader GoC SM policy may be warranted, in order for the GoC to move towards a more program area specific policy.

4.35 Characterization of Fine and Nano-Sized Polystyrene Particles by Flow Cytometry

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SUMMARY: Nanoparticles (NPs) are particles that are less than or equal to 100 nm in any one dimension. With the growing use of NPs in environmental applications, therapeutics, packaging and consumer products, there comes an urgent need for research into methods for analyzing their characteristics and any possible health effects from exposure. Towards this goal, this study was aimed at developing and implementing methods for the detection and characterization of NPs in liquid suspension and within mammalian cells. The methodology developed here will add to the analytical repertoire available for studying NPs, and will enable evaluators to acquire timely data on specific NPs of interest.

OBJECTIVE: Using fine- and nanoparticle-sized polystyrene particles (PSPs) as a model, this study was designed to determine the lower limit of detection for particle size and concentration. This was done for diluted particles and also for those taken up by mammalian cells. Another objective was to study whether it was possible to measure and manipulate the degree of agglomeration of these particles.

MATERIALS AND METHODS: PSPs (20-2000 nm) were used either in dilution series, or in mouse macrophage cell line (J774A.1) exposures. A BD FACSCalibur™ cytometer was used for characterization, and a Nikon C1 confocal microscope was used to confirm the data. For the dilution study, differently sized PSPs were added to various diluents, with the goal of minimizing the amount of agglomeration. Different concentrations of diluted PSPs were analysed to determine the cytometer detection limit. For experiments with macrophage, cells were dosed with varying PSP sizes and concentrations before being analysed for uptake and agglomeration.

RESULTS: One percent BSA was the most effective diluent for reducing agglomeration, since it showed the lowest coefficient of variation. Diluted PSPs can be discriminated by size and concentration ($\geq$200 nm and 10^1 PSPs/mL) using the cytometer. It was possible to determine the degree of agglomeration using the number of fluorescence peaks. Differently sized beads appeared in discrete regions of the forward scatter (FSC) versus side scatter (SSC) plots and fluorescence histograms. With PSP-treated macrophage, a linear correlation exists between bead size $\geq$20 nm and cell complexity/granularity. Changes in PSP concentration could not be detected by changes in SSC.

CONCLUSION: Using the cytometer, PSPs could be detected down to 200nm when diluted, and down to 20nm when taken up by cells. The results shown here demonstrate that flow cytometry can be a valuable method for detection and characterization of PSPs. Further work will be done to study the effects of PSPs on different cell types, and whether this work can be extended to different types of NPs.

4.36 Regulatory Capacity Building: The Canadian HIV Vaccine Initiative

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SUMMARY: Under the Canadian HIV Vaccine Initiative (CHVI), Health Canada is responsible for CHVI's regulatory capacity building program as part of global efforts to develop a safe, affordable, effective and globally accessible HIV vaccine. Health Canada aims to strengthen the regulatory capacity of developing national regulatory authorities (NRAs) in order to protect the ethical and scientific integrity of HIV/AIDS vaccine clinical trials occurring in these countries. Health Canada has successfully implemented capacity building initiatives in countries with developing NRAs to strengthen their capacity in the regulation of HIV/AIDS vaccine clinical trials, through the development of innovative and evidence-based capacity building training solutions.

OBJECTIVES: To strengthen the regulatory capacity of developing NRAs to help protect the ethical and scientific integrity of HIV/AIDS vaccine clinical trials occurring in these countries.

DESCRIPTION: Policy and regulatory personnel capitalized on the science-policy interface by working in close collaboration with scientific reviewers and subject-matter experts in order to develop and deliver evidence-based regulatory capacity building activities. The successful implementation of regulatory capacity building training solutions was achieved by fulfilling two important steps under the science-policy interface. First, a needs-assessment was performed to ensure that solutions were tailored to meet the specific regulatory needs of the developing NRAs; and second, the expertise of scientific reviewers was harnessed and strategically translated into practical learning experiences that the NRAs could effectively apply in their country-specific situations.

RESULTS: Since 2010, when the CHVI was renewed to include its key regulatory capacity building program, Health Canada has achieved the following:

- The HPFB International Regulatory Forum has modified its structure and course content to specifically meet the regulatory needs identified by the developing NRAs.
- Preparation and delivery of regulatory training sessions via vaccine and clinical trial forums, and the sponsorship of applicable NRAs to attend these forums to provide learning opportunities and encourage the exchange of best regulatory practices.
- Developed and provided assisted review mentoring programs as well as vaccine- and clinical trial-related courses in collaboration with the World Health Organization.

OUTCOMES: Developing NRAs and the World Health Organization have expressed their appreciation to Health Canada for assistance in strengthening their regulatory capacity. A reflection of lessons learned as well as positive achievements from this past year will be used to improve on and create more innovative regulatory capacity building initiatives so that Health Canada continues to successfully deliver on the regulatory capacity building program under the CHVI.

4.37 Mitochondrial Toxicity of Cadmium Telluride Quantum Dot Nanoparticles in Mammalian Cells

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SUMMARY: Health Canada is responsible for developing a regulatory framework for nanomaterials. Studying the mechanisms of toxicity of a given nanomaterial will provide a basis for classifying materials for regulatory purposes and prescribing strategies for risk management. Cadmium Telluride quantum dots (CdTe-QDs) are widely used in microelectronics and biomedical research and offer great potential in medical imaging, diagnosis, and therapeutic targeting. Even though previous studies have reported the toxicity of the nanoparticles, the mechanisms related to their toxic effects are still unclear. In this study, mechanisms of CdTe-QD toxicity in mammalian cells were investigated. The results revealed that exposure to CdTe-QDs caused mitochondrial toxicity and dysfunction in test cells.

OBJECTIVE: The aim of this study is to investigate the mechanisms of CdTe-QD toxicity by examining the effects of the nanoparticles on mitochondria in mammalian cells.

DESIGN: Human hepatocellular carcinoma HEPG2 cells were exposed to different concentrations of CdTe-QDs for 24h. Confocal microscopy and transmission electron microscopy (TEM) were used to study mitochondrial morphology. Enzymatic and Enzyme-linked Immunosorbant Assays (ELISA) assays were employed to examine oxidative stress in exposed cells. Isolated mitochondria were used to study the changes in components of the mitochondrial electron transport chain (ETC) and cellular respiration.

OUTPUTS/RESULTS: Confocal microscopy and TEM results showed the enlargement of mitochondria and change in mitochondrial membrane potential caused by CdTe-QD treatment. CdTe-QD treated cells also exhibited an increase in reactive oxygen species production, a decrease in glutathione level, a decrease in reduced glutathione/oxidized glutathione ratio, and an increase in superoxide dismutase activity suggesting that cells were undergoing oxidative stress. CdTe-QDs also caused changes in levels and activities of ETC enzymes and decreased cell respiration, indicating mitochondrial dysfunction.

IMPACTS/OUTCOMES/CONCLUSIONS: The results reveal that CdTe-QDs induced mitochondrial toxicity by causing changes in mitochondrial morphology and function. This study provides much needed mechanistic details for understanding the toxicity of CdTe-QD nanoparticles that might be important for screening potential risks of nanomaterials conducted by evaluators at Health Canada and the scientific community at large.

* This research is conducted under the co-supervision of Drs. Willmore (Carleton University) and Tayabali (Health Canada).

4.38 Effects of Ultraviolet Illumination on Cytotoxicity of Cadmium Telluride Quantum Dot Nanoparticles

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SUMMARY: Cadmium Telluride quantum dots (CdTe-QDs), as fluorescent probes, have great potential in medical imaging and diagnosis. These applications are usually associated with ultraviolet (UV) radiation. Understanding the mechanisms of CdTe-QD-induced cell damage under UV illumination is meaningful for nanoparticle risk assessment and clinical application promotion. This study examines UV radiation effects on CdTe-QD toxicity in human liver cells as the liver appears to be one of the relevant target organs for QD toxicity when the nanoparticles are used in clinical applications. Cells were exposed to CdTe-QDs under different illumination conditions. The results revealed that UV radiation caused a significant increase in CdTe-QD toxicity. These findings provide mechanistic insights on CdTe-QD toxicity which will help regulatory personnel to define conditions for its safe use.

OBJECTIVE: The aim of this study was to investigate the effects of UV radiation on CdTe-QD cytotoxicity in a human liver cell line.

DESIGN: Different concentrations (10^{-5}-10ug/ml) of CdTe-QDs were used for human hepatocellular carcinoma HEPG2 cell exposures in different lighting conditions. In one set of experiments, cells were exposed to CdTe-QDs for 24h in the dark, illumination under an ambient fluorescent room lightning, or irradiated with UVA (365nm, 3mW/cm^2) for varying lengths of time (2-24h). In another set of experiments, CdTe-QDs alone were pre-illuminated with either UVA (365nm, 3mW/cm^2) or UVB (312nm, 3mW/cm^2) for 2h, then added to the cell culture, and incubated for 24h. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays were used to measure viability/cytotoxicity in test cells and to optimize exposure conditions.

OUTPUTS/RESULTS: Exposure of cells to non-illuminated CdTe-QDs in the dark caused 10-20% less cytotoxicity compared to exposure of cells to CdTe-QDs handled under ambient room lighting conditions. In contrast, exposure of cells to CdTe-QDs under UVA illumination caused 15-60% greater cytotoxicity in treated cells. For the selected time and dose of UV illumination, there was no observable change in viability of control cells that were treated with UV light alone. Exposures of cells to CdTe-QDs that were previously illuminated with UVA or UVB showed a decrease (10-15%) in cytotoxicity, compared to exposures to untreated CdTe-QDs.

IMPACTS/OUTCOMES/CONCLUSIONS: The results showed that UV radiation significantly enhances toxicity of CdTe-QDs to mammalian cells when being exposed to the nanoparticles. The study has confirmed a correlation between CdTe-QD toxicity and the photolysis reaction of the nanoparticles that produces toxic free cadmium and reactive oxygen species. This study provides information that should be considered by evaluators for toxicity associated with radiation-activated CdTe-QDs which may be introduced into future clinical use.

* This research is conducted under the co-supervision of Drs Willmore (Carleton University) and Tayabali (Health Canada)

4.39 An Application of Isotope Dilution Mass Spectrometry: Determination of Ochratoxin A in the Canadian Total Diet Study

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SUMMARY: This study measures the levels Ochratoxin A (OTA) in a typical Canadian diet. OTA is a mycotoxin that has been associated with human and animal kidney disease and has been classified as possibly carcinogenic to humans by the International Agency for Research on Cancer. One hundred and forty samples were analyzed for OTA as part of the Canadian Total Diet Study. The results show that 73% of the foods tested had detectable levels of OTA with some of the highest levels of OTA contamination found in bread samples.

OBJECTIVE: The objective of this study was two-fold: To develop a sensitive method to quantify the levels of OTA in a TDS and to identify the major contaminated food products in order to more fully characterize the health risk.

DESCRIPTION: The Canadian Total Diet Study (TDS), representing typical food products “as consumed” by Canadians is an important government surveillance program. The data is used to monitor nutrient and contaminant levels and trends in our food supply and to inform planning and policy. A total of 140 samples were analysed for OTA as part of the Canadian Total Diet Study. Samples were collected at retail level from two Canadian cities, Quebec City and Calgary, in 2008 and 2009, respectively. Total Diet Studies pose an ananalytical challenge since every food product is different. A new analytical method was developed that used an acidified solvent extraction, an immunoaffinity column for cleanup, liquid chromatography-tandem mass spectrometry for identification and quantification, and a uniformly stable isotope-labeled OTA as an internal recovery standard.

RESULTS: The method is accurate (101% average recovery) and precise (5.5% RSD) based on 17 duplicate analyses of various food products over two years. One hundred and forty food composites were analyzed for OTA as part of the TDS. Samples were collected by the Canadian Food Inspection Agency at retail level from two cities, Quebec City and Calgary, in 2008 and 2009, respectively. The results indicate that 73% (102/140) of the samples had detectable levels of OTA.

CONCLUSIONS/NEXT STEPS: Bread is a high-consumption staple for both adults and children. The results indicate that in Canada, the bread supply and more generally, cereal-containing food products, appear to be a primary source of OTA exposure for the 2008 and 2009 sampling years. Health Canada is currently considering the adoption of Maximum Levels for OTA as a health risk management tool.

4.40 Good Clinical Practices Compliance and Enforcement: A Proactive Approach through Compliance Promotion

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SUMMARY: Regulatory requirements are frequently subject to interpretation. Stakeholders involved in clinical research of drugs in humans in Canada, in particular, find this a challenge in their efforts to be in compliance with the Regulations. The Inspectorate has implemented compliance promotion initiatives to address this issue, such as the GCP Information Sessions held in six Canadian cities in November 2010. These sessions provided information on regulatory requirements and Health Canada's expectations for compliance, as well as providing an opportunity to collect input from stakeholders on how Health Canada can promote compliance in this sector.

BACKGROUND: Regulations regarding clinical trials, "Drugs for Clinical Trials Involving Human Subjects", came into force in September 2001. Regulatory activities including inspections, compliance verifications and investigations of clinical trials have been conducted since. The main objectives for these activities, conducted mainly at Qualified Investigator sites, Sponsors and Contract Research Organizations, are to ensure that the generally accepted principles of good clinical practices are met, validate the data generated, and verify compliance to Division 5 of the Regulations. The Regulations are subject to considerable interpretation which leads to many inquiries from stakeholders involved in clinical trials and challenges in applying regulatory requirements.

METHOD: Several compliance promotion initiatives have been put in place; the main recent activity being a series of information sessions given across Canada in November 2010. These information sessions were conducted by Inspectorate Program staff in HPFB and RAPB, with support from TPD and BGTD, as well as OCAPI.

RESULTS: Through this forum, key components of the Regulations that pose implementation challenges were identified by stakeholders as potential areas for delivery of further guidance. It was demonstrated that clear and open communications between stakeholders and Health Canada are crucial to achieve compliance.

IMPACTS: Stakeholders expressed enthusiastic appreciation for the chance to learn about Canadian GCP requirements, to discuss their thoughts on compliance issues, and the opportunity to meet with Health Canada staff. Health Canada's GCP Compliance Program will use their feedback to shape future guidance and policies. A report on the evaluations of the sessions and feedback received is currently being prepared to analyze the results of the sessions and develop a plan for future compliance promotion initiatives.

CONCLUSIONS: It was concluded that more compliance promotion initiatives and more guidance on specific aspects of the Regulations are beneficial to promoting compliance of clinical trials in Canada.

4.41 Canadian Biotechnology Regulatory Roadmap for Living Animals and Derived Products

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SUMMARY: This project's aim was to develop a biotechnology regulatory guide or "Roadmap", which focuses on unifying existing approaches to the regulation of living animals and their derived products. The roadmap consists of a comprehensive overview of who is responsible for regulating various applications of animals derived through biotechnology, the legislative instruments that provide the authority for their assessment, and key contacts in each of the regulatory responsibility centres. A similar guide has been produced for micro-organisms .

BACKGROUND: Canada regulates biotechnology-derived animals, derived products, and by-products based on their intended uses. If you intend to manufacture, import, sell, distribute, or use living animals, their derived products and by-products, the Government of Canada recommends that you consult with appropriate departments and agencies, as shown in this regulatory guide. The *Canadian Environmental Protection Act*, 1999 (CEPA 1999) plays a major role in the regulation of new biotechnology-derived animals. However, if these animals are subject to an Act or regulation listed in Schedule 4 of CEPA1999, it is considered CEPA 1999 equivalent, and are thus exempt from the New Substance Notification Requirements of CEPA, 1999. This provides for an effective process as regulatory duplication is minimized.

OBJECTIVE: The purpose of this project is to increase awareness and eliminate possible confusion to notifiers and regulators alike on how Canada regulates living animals and their derived products.

METHOD: This information was compiled as a result of numerous correspondences with regulatory bodies of the Government of Canada (GOC), as well as with members of the Interdepartmental Working Group on Animal Biotechnology (IWGAB). In addition, extensive research was conducted on the Acts and Regulations responsible for living animals and their derived products in Canada.

OUTPUT: The Living Animals and Derived products Roadmap is a one page guide that clearly outlines all legislative instruments that provide the authority for assessment of such products and key contacts in each of the regulatory responsibility centres.

CONCLUSIONS: The main purpose of this guide is to compile in one place the most up-to-date comprehensive information on the regulatory landscape for new living animals in Canada. It is hoped that this will save time and effort in trying to identify the appropriate regulatory bodies for animal products. The Roadmap is currently available for internal use only, however, we move forward with the hopes of making such information available publicly in the near future.

4.42 Rapid and Accurate Determination of the Potency of Varicella Vaccine by Quantitative Polymerase Chain Reaction

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SUMMARY: All human vaccines must be tested before they are authorized for sale in Canada, and this critical step for vaccine quality control is the responsibility of Health Canada. Varicella vaccines are designed to protect children against chicken pox. There is now an increasing demand for this vaccine, and also an urgent need to develop faster and more accurate quality control assays. The work presented here describes the development of a new method that allows rapid and accurate measurement of varicella vaccine. Future application of the method to routine vaccine quality control could improve the availability of the chicken pox vaccine.

OBJECTIVES/BACKGROUND: The potency of varicella vaccines is currently determined by the plaque assay technique. This technique requires the virus be incubated with the cells for seven days, allowing the viral plaques to develop. The plaques are then counted with the naked eye. Limitations of this technique are: 1) assay duration; 2) labour intensity; and, 3) ambiguous results, leading to variation within and between labs. Recently, the National Advisory Committee on Immunization has recommended to the Public Health Agency of Canada (PHAC) that a two dose schedule be implemented in routine immunization of children against varicella, rather than the existing single dose. With increasing demand for this vaccine, a new assay is needed that would expedite the ability of manufacturing and regulatory authorities to release an increased number of vaccine lots while maintaining quality requirements.

DESIGN/METHOD/DESCRIPTION: We have developed a new assay to evaluate the potency of varicella vaccines using quantitative PCR in combination with a step resulting in a more efficient infection of the cells. Using this technique potency results are obtained 24 hours after infection. We have not only reduced the length of assay time, but have also modified it into a 96-well plate format providing for high-throughput. Using the PCR technique we have also automated the data acquisition reducing variation between and within labs.

OUTPUTS/RESULTS: This assay demonstrated acceptable reproducibility and accuracy as compared to the traditional plaque method which relies on the manual counting of plaques seven days after infection.

IMPACTS/ OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEP: Compared with the conventional plaque assay, our assay provides a high-throughput method with reduced variation and the desired results being obtained in 24 hours with acceptable accuracy and reproducibility. With the increased demand for varicella vaccines it will allow manufacturing and regulatory authorities to release an increased number of vaccine lots while assuring vaccine quality.

4.43 Fragment A of CRM197 Shows Striking Structural Differences Compared to the Wild-Type Catalytic Domain of Diphtheria Toxin as Revealed by NMR Spectroscopy

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SUMMARY: Vaccines against Meningococcal-C or Haemophilus-influenzae-type-b (Hib) consist in chemically connecting the active ingredient, one or several polysaccharides isolated from the bacteria, to a carrier protein such as cross-reacting-material 197 (CRM197). The latter is a natural mutant of diphtheria toxin resulting from a single modification of the gene that produces a non-toxic molecule. These vaccines utilize the ability of CRM197 to stimulate the immune system (to act as an adjuvant) in order to enhance the production of neutralizing antibodies against the target bacteria. Using NMR spectroscopy, we show that the fragment A of CRM197 has a very different structure from the corresponding domain of diphtheria toxin.

BACKGROUND: CRM_{197} is a naturally occurring non-toxic mutant of Diphtheria toxin (DT). A single nucleotide change results in glycine 52 change to a glutamate in the catalytic domain (fragment A) of Diphtheria toxin (DTA). The lack of toxicity of CRM_{197} most likely results from a change in structure of the catalytic domain that disrupts the catalytic activity. Our main objective is to compare the structure of DTA and CRM_{197} using NMR.

METHOD: Labelling the protein of interest with NMR detectable isotopes (^{13}C and ^{15}N) is required for the application of NMR techniques. This is achieved by expressing DTA, CRM_{197} and G52X mutants (where X=A, S, Q, N) in *Escherichia coli*. Bacteria are incubated in growth minimal media where the sole source carbon and nitrogen are ^{13}C-labelled glucose and ^{15}N-ammonium chloride, respectively. After protein purification, NMR spectroscopy is then applied to record and assign resonances of all atoms and to measure structurally dependent parameters.

OUTPUT: Here, we present the resonance assignment of DTA and the initial structural analysis of DTA, CRM_{197} and other G52X mutants. Our results show that the catalytic domain is well folded in solution. However, NMR spectra of CRM_{197} and mutants show all features of an unfolded domain. This suggests that all mutations of residue G52 of DT prevents folding of the catalytic domain.

IMPACT: CRM_{197} and its formaldehyde-treated analogue are widely used as carrier protein and adjuvant in vaccines. To our knowledge, this is the first study that provides insights at the structure of this protein. Our findings fill an important knowledge gap in the field of glycoconjugate vaccines.

4.44 Analysis of Gene Content in Pseudomonas Biotechnology Strains by Microarray

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SUMMARY: Health Canada carries out screening assessments of microorganisms in support of the *Canadian Environmental Protection Act* (1999). *Pseudomonas aeruginosa* (Pa) is an environmental bacterium and some strains are used for biotechnology, while others cause animal and human infection. The genes of three biotechnology strains of Pa were compared to those of a clinical strain in order to determine similarity and how many of the harmful genes may be present. A small proportion of the genes differ from biotechnology to clinical strain, but that includes results that were borderline positive. Some of these results were clarified by additional genetic experiments.

OBJECTIVES/BACKGROUND/ISSUE(S): Comparative genomic hybridization (CGH) studies involving microarrays designed from fully sequenced microbes may enable comparisons between closely related strains at the level of genes that are in common. Genes that contribute to virulence can be scored and help predict genotypes for uncharacterized strains. However the confidence in the results relies on meaningful parameters for signal calls and for the compared genomes to feature similar genes. This poster summarizes data from a CGH study involving Pa biotechnology strains and a validation of microarray results by examining select genes by PCR, southern hybridization and sequence analysis.

DESIGN/METHOD/DESCRIPTION: Microarrays designed from the sequenced strain PaO1 were hybridized with chromosomal DNA extracted from PaO1 and three DSL (Domestic Substance List) Pa strains with uncharacterized genomes. Hybridization intensity data was normalized and signal presence and absence calls were determined by comparison of hybridization signals with values from the PaO1 hybridizations used as a baseline. Chromosomal genes and virulence genes were scored across the strains. Four genes that varied in presence or absence across the strains were tested by PCR Southern hybridization and partial nucleotide sequencing for validation.

OUTPUT/RESULTS: Each DSL Pa strain hybridized to the PaO1 genes with similarity indexes of over 95%, however, a small fraction of genes (less than 3%) were scored as ambiguous amongst each strain. A variable small number of PaO1 genes were absent in each of the DSL Pa strains and these included several virulence genes. For the small gene set tested, PCR and Southern results matched microarray results for three of the genes. The fourth gene, imm2, featured microarray results that were discordant with PCR and Southern results.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: By CGH, the biotechnology strains appear to contain many of the genes of the PaO1 strain, however, the content of virulence genes in DSL strains appears to be lower than that of PaO1. There appear to be false calls amongst the results as one gene has been observed that scores oppositely by microrarray to PCR and Southern blot results. Presently, several virulence genes that varied in microarray score across the strains are being validated. Clarification of the genetic potential of the DSL strains will also require further analysis of the ambiguously called genes for each strain.

4.45 Mapping Science Forum Abstracts 2002-2010

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SUMMARY: All the abstracts from the Health Canada Science Forum 2002-2010 are being characterized for their contribution to the departmental mandate to help Canadians maintain and improve their health. The abstracts represent research and development (R&D) and related scientific activity (RSA), in both the natural and social sciences. This 'mapping' aims to: 1) identify areas of expertise, in order to help staff at all levels to better understand, manage and take advantage of the work being done and the skills that are available; 2) characterise research nodes or themes; and, 3) show the relationship of Health Canada's science and other research activities to departmental priorities, in a way that is both visual and communicative.

OBJECTIVE: To improve understanding, management, and use of research skills, directions and products at Health Canada through an evidence-based visual representation of the work done and being done across the department, that is represented by the work presented at the Science Forum.

METHODOLOGY: The abstracts presented at each Science Forum from 2002 to 2010 were entered on an *Excel* spreadsheet under conventional headings Author, Affiliation, Summary, Methods, Results and Conclusions. This information was characterised by how, in the judgement of one author (NS), it relates to one or more *Mission Critical Themes*, *Potential Scientific Functional Areas*, or *Activities Requiring Science*, that were identified in the 2010 Science Priority exercise. Five (5) additional *Potential Scientific Functional Areas* (Modelling/Mapping; Survey construction/Use; Guidelines; Knowledge Translation/Integration/Management; and Database creation/Use) were added to the seven (7) described in the Science Priority Exercise. Each characterisation was represented by a number, to allow simple, computer-generated, visualizations of research directions, themes, skill sets, trends, and other features of Health Canada's R&D, and RSA work. All the categories used in the analysis, together with interpretation, are shown as part of the Poster material.

RESULTS: Results are shown for 2002-2010. Alternative and complementary visualization tools are used. The spreadsheet of abstracts (including the characterisations of the contents), and the vizualizations. are freely available from Science Policy Directorate (contact Nigel Skipper).

CONCLUSIONS:

1) The analysis represents a first attempt to characterise the department's research based on a categorisation of the 'works-in-progress' that are represented by Science Forum abstracts. Because the results are captured in numbers, they can be displayed in different ways according to the need and viewer preference.

2) A practical challenge has been to maintain an appropriate specificity or resolution. For example it would not have been difficult to characterise practically all the abstracts as pertaining to the *Activities Requiring Science* that are 'Risk Assessment/Risk Management/Risk Communication,' and this is no surprise since these activities are at the core of the department's mandate. But this low level of resolution is not very helpful in uncovering potential trends, strengths etc. Considerable judgement had to be used to improve the resolution and subsequent observers may not agree in all cases with the conclusions of the current analysis.

3) To maximize the value going forward of the information contained in Science Forum abstracts, consideration could be given to incorporating a fixed vocabulary of key words, including an improved set of categories or ‘collectors’ with which authors would categorise their work.

4) Other indicators of science focus and direction, with potential to provide complementary insights, are the publications produced by the department, and the research proposals intended to involve human subjects which are provided to the Research Ethics Board. Taken together, analysis of Research Proposals, Science Forum Abstracts, and Publications, could provide a useful window on the nature and the 'ecology' of the department's science.

4.46 The Canadian Biotechnology Regulatory Roadmap for Micro-Organisms and Derived Products

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SUMMARY: Before a new "substance" can be manufactured, imported or sold for use in Canada it must be evaluated for potential effects on human or environmental health by the appropriate Government regulatory authority. The *Canadian Environmental Protection Act*, 1999 (CEPA) is the primary legislative vehicle by which new substances are regulated; however, it is one of a number of laws designed to regulate substances and protect human health and the environment. As a result, the "regulatory landscape" for these substances is complex and can create confusion for notifiers and regulators alike.

The project consolidates the existing regulatory approaches for one category of new substance (micro-organisms) in Canada into a single graphical representation. It also highlights the appropriate authority a specific product and provides an overview of the key departments, agencies, and units involved in their regulation. Products are matched with the most applicable regulatory legislation to permit identification of the most appropriate regulatory unit based on consideration of the intended use of the product.

BACKGROUND: Micro-organisms considered "new" to Canada are regulated under Part 6 of CEPA; however, they can also be subject to a number of other Acts and regulations. In order to avoid regulatory duplication, a substance that is regulated by an equivalent Act is exempt from the new substance notification requirements of CEPA. As such, CEPA represents a legislative safety net for new products to Canada.

OBJECTIVE: The regulatory landscape for new substances is complex and often results in confusion among notifiers and regulators alike regarding the appropriate regulatory stream for a given substance. The objective of this study was to develop a regulatory "roadmap" for one category of substance regulated under CEPA.

METHOD: The roadmap was developed following an extensive review of these Acts and regulations. The Roadmap consolidates the results of this review into a single document that includes a graphical depiction of the regulatory landscape for micro-organisms. It also provides an overview of the key departments, agencies, and regulatory units that are involved in the regulation of micro-organisms and highlights the appropriate authority for a specific product.

OUTPUT: The Roadmap consolidates the results of this review into a single document that describes the regulatory landscape for micro-organisms. It also provides an overview of the key departments, agencies, and regulatory units that are involved in the regulation of micro-organisms and highlights the appropriate authority for a specific product.

CONCLUSIONS: In its current iteration, this roadmap represents an internal guidance document for regulators and evaluators in the New Substances Assessment and Control Bureau (NSACB) at Health Canada; however, there is considerable value in developing a version that could be made publicly available. A public version would serve to decrease confusion among the regulated community and would increase the Government of Canada's transparency among external stakeholders.

5.01 Representative Sampling Adjustments and Modeling of Food Habits: Significant Impacts on Acrylamide Daily Intakes?

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SUMMARY: Health Canada - Quebec Region coordinated an exposure assessment study of acrylamide in teens consuming acrylamide-rich foods in collaboration with the Institut de Santé publique du Québec and Université de Montréal. Chronic exposure to acrylamide is known probable human carcinogen by IARC but little is known in terms of exposure assessment. Because of recruitment issues, the initial sampling frame was changed resulting in a “non-representative” sample of teens. Using probabilistic modelling of exposure scenarios and demographic data, representative sampling adjustments were used to estimate acrylamide daily intake in µg/kg.bw and better characterize its statistical distribution and uncertainties in teens.

BACKGROUND/AIMS: Human health quality guidelines depend on estimated daily intakes, but exposure data are lacking for numerous priority chemicals. Health Canada (HC) relies on exposure assessment studies, but in such population-based studies, it is often hard to get a representative sample. Generalization and probability density function (PDF) estimation then becomes challenging. In this paper, original acrylamide data were used to assess quantitative impacts, of representative sampling adjustments, on daily food intake estimates of acrylamide in teens. Results, strengths and limitations of statistical analyses will be compared.

METHODS: An exposure assessment study pre-designed for teens socio-economically representative of the Montreal population was conducted in 2009-2010. Approximately two hundred teens aged 10-17 years were recruited by phone. They were asked to fill detailed dietary questionnaires and to provide urine and blood samples for acrylamide, acrylamide by-products and DNA adduct analytical measurements (out of scope here). Acrylamide contents of food, most consumed by teens during the study, were also measured while some contents were inferred from previous analyses made by HC. Daily acrylamide intakes were then assessed according to a usual approach used in nutritional epidemiology (consumed food x frequency x serving size).

RESULTS: Because of recruitment issues, the initial sampling frame was changed for a complex one, based on three different sub-samples, resulting in a “non-representative” sample of teens. Three data processing methods will be applied: i) representative sample adjustments; ii) probabilistic exposure scenarios; and, iii) a combination of i) and ii). For these three approaches, acrylamide daily intakes in µg/kg.bw will be derived and compared with the ones obtained without any adjustment (A = 0.58 ± 0.80; median = 0.29).

CONCLUSION: This study allows drawing methodological recommendations for exposure assessment studies where representative sample is a challenge, and to derive an internationally missing PDF for acrylamide in teens.

5.02 Tracking Plasma Proteomic Changes Relevant to Pregnancy Outcomes

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SUMMARY: In this work we describe the use of proteomic tools to investigate biological changes in plasma of pregnant women. These changes were probed to find biomarkers specific to pregnancy outcomes. Our results exhibited specific protein changes in plasma of mothers that were related to low infant birth weight. These changes are known to be relevant to oxidative stress and endothelial dysfunction scenarios.

BACKGROUND AND OBJECTIVES: Exposure to elevated levels of environmental chemicals may be associated with negative impacts on reproductive health. The Maternal-Infant Research on Environmental Chemicals (MIREC) Study was initiated to establish a national profile of *in utero* and lactational exposure to environmental contaminants and to investigate potential impacts on pregnancy outcomes. Maternal health determinants can modify the outcome of pregnancy. The premise of this study is that the physiology of mothers when modified by factors such as contaminant exposures can lead to potential alteration of utero-placental perfusion, and affect fetal development. Low birth weight is an indicator of the general health of newborns, and a key determinant of infant survival, health and development. The objective of this investigation was to follow proteomic changes in maternal plasma that can be related to birth outcome.

METHODS: We report here on analyses of third trimester maternal plasma samples for proteomic changes followed by targeted and global proteomic approaches. A subset of plasma samples obtained from pregnancies in ten medical centres across Canada were stabilized at recovery and analyzed for proteomic changes by mass spectrometry (MALDI-TOF-TOF-MS) using shot-gun proteomics followed by MS/MS analyses and affinity-based protein array analysis.

OUTPUTS/RESULTS/CONCLUSIONS: Although, ca. 30 proteins were identified by the global shot-gun mass spectrometry approach, some proteins such as ubiquitin ligase activator of NFKB1, transforming growth factor β1 and T cell receptor β chain appeared to be characteristic of birth weight outcomes. Meanwhile, MCP-1, MMP-9, and VCAM analyzed by affinity-based protein array analyses were negatively associated with infant birth weight. Interestingly, MCP-1 and MMP-9 are implicated in endothelial dysfunction as well as cardiovascular diseases, and are linked to oxidative stress scenarios.

IMPACTS: Our results support the view that both these approaches generate complementary information in determination of proteomic biomarkers relevant to mechanistic pathways such as oxidative stress and endothelial dysfunction. This information is vital in understanding maternal determinants of fetal development that result in low infant birth weight.

5.03 Emerging DBPs in Canadian Drinking Water- Results from the National Survey of Disinfection by-Products and Selected Emerging Contaminants (2009-2010)

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SUMMARY: This National Survey investigated levels of contaminants, including regulated and emerging disinfection by-products (DBPs) in Canadian drinking water. Previous results show progress in protecting Canadians against potential health risks from regulated DBPs by lowering the exposure to these contaminants. The current study focuses on emerging, un-regulated, DBPs. This addresses concerns that, by concentrating on reducing exposure to regulated DBPs, exposure to other classes of DBPs may increase. Data show that efforts towards regulatory compliance lower population exposure to some emerging DBPs but also demonstrate the necessity of sustained research to keep pace with changes in the industry.

OBJECTIVES/BACKGROUND/ISSUES: To reduce formation of regulated DBPs, water facilities often switch from chlorine to chloramines as secondary disinfectants. Under certain conditions, this change leads to increased formation of other DBPs with potentially higher toxicity than the regulated ones. One of our objectives was to evaluate un-regulated DBPs formation in water systems using a variety of disinfection processes.

DESIGN/METHOD/DESCRIPTION: Sixty-five municipal systems across Canada were selected based on water source, treatment process and population size, and sampled twice in the same year (winter/summer). Water was stabilized by quenching with ascorbic acid and pH adjustment. N-Nitrosodimethylamine (NDMA) and substituted nitrosamines were determined by adsorption on Ambersorb 572, followed by desorption in dichloromethane and GC-MS-MS analysis. Other N-containing DBPs were extracted in methyl-t-butylether and quantified using GC-ECD. Bromide, iodide, total bromine, total iodine concentrations were also determined.

OUTPUTS/RESULTS: Haloacetonitriles (HAN) concentrations were well correlated with regulated total trihalomethane (THM) concentrations. Cyanogen chloride concentrations were < 3.4 ug/L, with higher values found in systems using chloramines.

Chloropicrin concentrations were typically < 1 ug/L. Only three water systems had detectable levels of NDMA, one being over the proposed MAC (40 ng/L). Mutagen X (MX) concentrations varied over two orders of magnitude and were correlated with pH. Iodo-THMs were found at about 50% of locations, three locations being in the 1-8 ug/L range. In many instances, halogenated DBPs speciation correlated with the presence of halogen precursors and increased along distribution systems.

IMPACT/ OUTCOMES/ CONCLUSIONS/ IMPLICATIONS/NEXT STEPS: The correlation between total HAN and total THM suggests that existing regulation, aimed at reducing total THMs concentrations, has a concurrent effect on HAN concentrations. Data confirm that cyanogen chloride formation is higher in systems using chloramination, a factor in decisions about the disinfection process. NDMA formation is challenging to predict. Data presented support the HC proposed guideline.

Considering the suspected high toxicity of N-containing and iodo-DBPs, it is important to determine water characteristics that enhance their formation. A targeted study has been started.

5.04 Metal Sources, Speciation, and Bioaccessibility in Residential Environments

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SUMMARY: In home environments, metals accumulate in settled dust from a wide variety of sources, including consumer products containing metals, hobbies and renovation activities, tracking in dirt from outdoor sources, and penetration of airborne particles. This research used synchrotron-based techniques to investigate residential dust and soil samples, and elucidate sources and pathways of exposure to metals within urban Canadian homes.

OBJECTIVES: Metal speciation was investigated at the molecular scale in samples of house dust and garden soil of Canadian urban homes, to: 1) clarify metal bioaccessibility; and, 2) distinguish metals associated with soil particles from metals originating from products in the indoor environment.

METHODS: X-ray absorption fine-structure (XAFS) spectroscopy was used to characterize Pb compounds in dust samples containing elevated Pb (>750 mg/kg). Other metal compounds were identified using micro-XRD and micro-XRF. Metal bioaccessibility in the reference compounds and dust samples was determined using a simulated gastric acid digestion followed by inductively-coupled plasma mass spectroscopy.

RESULTS: A detailed study of one old home (built before 1960) demonstrated that Pb compounds in contemporary settled dust were derived from subsurface layers of old paint. In the living room dust of another old home, Pb was found to be associated with Mn and Fe hydroxide and phosphate minerals, similar to particles identified in the garden soil. Dust particles in the bedrooms of this same home showed a contrasting signature, arising from localized renovation activity. Pb-based and non-Pb paint pigments as well as gypsum, bassanite and portland cement were identified in the bedroom dust.

CONCLUSIONS: The results demonstrated that Pb bioaccessibility in dust can be predicted from its speciation. The results also showed that house dust from younger homes (<10 yr) may provide a pathway for elevated Pb exposure, which brings an important clarification to the perception that indoor Pb contamination is an issue only related to older homes. This research underscores the importance of taking precautions to minimize exposures to Pb in housedust, especially during renovation activities.

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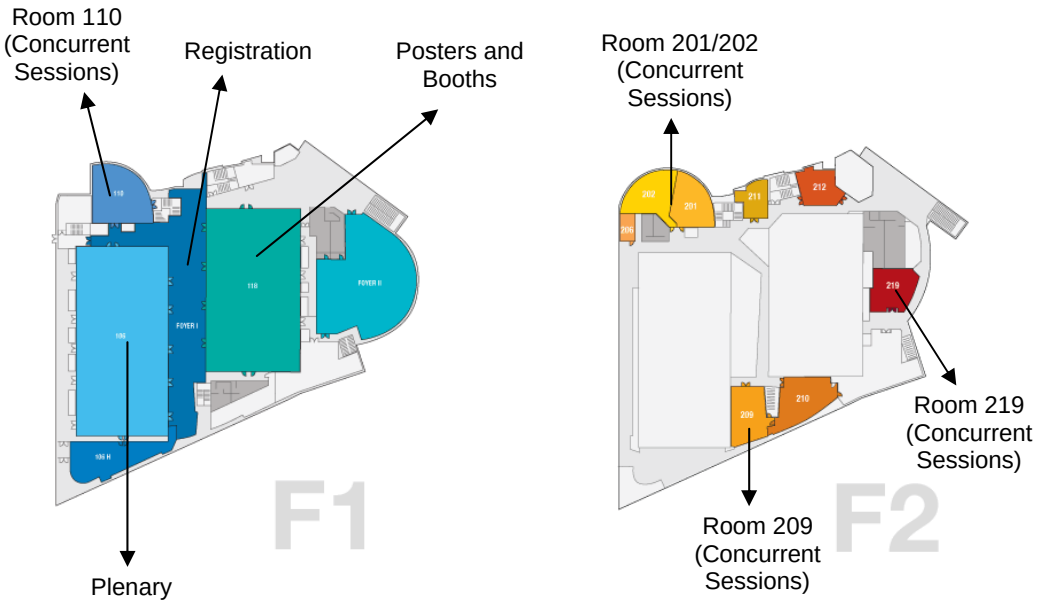
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