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2015 Health Canada ScienceFORUM

Book of Plain Language Summaries

February 23–24, 2015

Canada

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

AAB: Audit and Accountability Bureau
CFOB: Chief Financial Officer Branch
CPAB: Communications and Public Affairs Branch
CSB: Corporate Services Branch
FNIHB: First Nations and Inuit Health Branch
HECSB: Healthy Environments and Consumer Safety Branch
HPFB: Health Products and Food Branch
PMRA: Pest Management Regulatory Agency
RPB: Regions and Programs Bureau
SPB: Strategic Policy Branch

Portfolio:

AHRC: Assisted Human Reproduction Canada
CIHR: Canadian Institutes of Health Research
HMIR: Hazardous Materials Information Review
PHAC: Public Health Agency of Canada
PMPRB: Patented Medicine Products Review Board

Other commonly used acronyms:

BPA – Bisphenol A
CARA – Clean Air Regulatory Agenda
CDC – Centre for Disease Control
CEPA – Canadian Environmental Protection Act
CMA – Canadian Medical Association
CMP - Chemicals Management Plan
DNA - Deoxyribonucleic Acid
E. coli – *Escherichia coli*
EPA – Environmental Protection Agency (United States)
FDA – Food and Drug Administration (United States)
F/P/T – Federal/Provincial/Territorial
GDP – Gross Domestic Product
GRDI – Genomics Research and Development Initiative
MIREC – Maternal-Infant Research on Environmental Chemicals
NCR – National Capital Region
OECD – Organisation for Economic Co-operation and Development

Environmental Pollution and Epidemiology

1.01 Bisphenol A β-D-Glucuronide Induces Adipogenesis in Murine 3T3L1 Preadipocytes

J.G. Boucher[1], A. Boudreau[1], and E. Atlas[1]

[1] Environmental and Radiation Health Sciences Directorate, HECSB, Health Canada, Ottawa, ON

OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada is responsible for the assessment and management of health risks to Canadians associated with exposure to products and chemicals in the environment. Bisphenol A (BPA) is a chemical used in the production of plastics found in a number of commercial products. BPA has certain biological activities analogous to hormones and in experimental animals and has been shown to cause weight gain as well as fat accumulation in specialized fat cells. Following exposure to BPA it is quickly converted in our bodies to a modified form called BPA-Glucuronide. For many years, it was believed that this modified form of BPA was inactive and simply got excreted in the urine

DESIGN/METHOD/DESCRIPTION: We used mouse and human fat cells and exposed them to different amounts of the modified version of BPA called BPA-Glucuronide. Human and mouse fat cells exposed to BPA-Glucuronide were then assessed for the degree to which they became mature fat cells and started accumulating fat by measuring the expression of certain specific fat-type genes and the actual amount of fat (triglycerides) that accumulated within these cells.

OUTPUTS/RESULTS: Our study showed that both human and mouse cells exposed to BPA-Glucuronide (previously believed to be inactive) did in fact cause an accumulation of fat within these cells and converted them to mature fat cells which could be an early step in the development of obesity.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The next step will be to determine whether BPA-Glucuronide exposure can have an effect in an animal model.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The results will help increase knowledge about the effects of BPA and will provide a better understanding of how chemicals may be impacting the development and accumulation of fat.

1.02 Identification of Mechanisms of Action of Bisphenol A-Induced Human Preadipocyte Differentiation by Transcriptional Profiling

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada is responsible for the assessment and management of health risks to Canadians associated with exposure to products and chemicals in the environment. Bisphenol A (BPA) is a chemical used in the production of plastics found in a number of commercial products. BPA has certain biological activities analogous to hormones and in experimental animals it has been shown to cause weight gain and fat accumulation in specialised fat cells.

DESIGN/METHOD/DESCRIPTION: This study used human fat cells exposed to BPA to identify genes that might be important for fat accumulation in these cells. To better understand the biological mechanisms underlying chemical impacts on metabolic processes that could affect fat development and accumulation, we investigated how BPA causes fat accumulation in human tissues by studying how genes respond in human fat cells exposed to this chemical *in vitro*.

OUTPUTS/RESULTS: The results revealed that human fat cells exposed to BPA show changes to specific genes that are involved in how cells make and accumulate fat, as well as how fat cells regulate cholesterol levels within the cell.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This study suggests that BPA does affect how human cells control fat and cholesterol levels at the cellular level and that it is biologically plausible that BPA could be contributing in some manner to weight gain and obesity. This knowledge will be useful in developing test methods to screen for these effects.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The results will help increase knowledge about the effects of BPA and will provide a better understanding of how chemicals may be impacting the development and accumulation of fat.

1.03 Using New DNA Sequencing Technologies to Compare Mutations Induced in Different Tissues

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada protects Canadians by assessing and managing the health risks associated with chemical exposures. Certain chemicals can cause mutations (i.e., changes in the DNA sequence) that can potentially lead to the progression of diseases. For effective human health risk assessment, it is important to understand the mechanisms by which chemicals damage DNA. This is often done by determining the types of mutations that are induced. The objective of this work was to develop a cost-effective and time-saving method to rapidly characterize mutations induced by chemical exposure.

DESIGN/METHOD/DESCRIPTION: We have integrated a new DNA sequencing technology, known as next-generation sequencing (NGS), with the Muta™Mouse system, a mouse model that contains a bacterial DNA sequence that can be recovered from every tissue in the body and analyzed for mutation induction. NGS allows us to sequence thousands of mutant sequences simultaneously. We used this approach to identify and characterize mutations in germ cells (sperm) and somatic cells (all the other bodily cells).

Muta™Mouse males were exposed to benzo(a)pyrene (BaP), a common environmental pollutant. Over 5500 mutations were collected from the bone marrow and sperm of control and treated animals. We sequenced these mutations to investigate the types of mutations induced by BaP relative to control animals, and how they differ between cell types.

OUTPUTS/RESULTS: We sequenced thousands of mutants by NGS in a short period of time, making this method twenty five times more efficient than the existing low-throughput sequencing method (Sanger sequencing). We found that the frequency of specific types of mutations induced by BaP were different in sperm and bone marrow.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: These results demonstrate that tissues can have different mutation profiles in response to chemical exposures, potentially due to differences in cell division, replication, DNA repair as well as chemical metabolism and excretion. Specifically, this work emphasizes the importance of examining the effects of chemicals on germ cells as they may respond differently to chemical exposures and mutations in germ cells can impact future generations.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: We demonstrated the utility of NGS in rapidly establishing the mutation profiles in multiple tissues. This is useful for elucidating tissue-specificities and the mechanisms by which a chemical causes mutations. This new method will ultimately allow for better assessment of the genotoxic effects of chemicals.

1.04 Factors Influencing Volatile Organic Compounds in Canadian Homes

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OBJECTIVES/BACKGROUND/ISSUE(S): Under the Clean Air Regulatory Agenda (CARA) and Chemicals Management Plan (CMP), Health Canada conducts research into indoor air quality, including the levels and effects of chemical contaminants. We investigated potential sources of volatile organic compounds (VOCs) in Canadian homes.

DESIGN/METHOD/DESCRIPTION: We examined the determinants of indoor VOC concentrations in Canadian homes, including activities such as renovations and pesticide use as well as home characteristics such as the existence of an attached garage. Identifying possible sources of VOCs in homes is important for developing risk management strategies to reduce exposure.

During the second cycle of the Canadian Health Measures Survey (CHMS-2) (August 2009 through November 2011), we tested 84 VOC concentrations in indoor air in 3857 homes located within 50 km (or up to 100 km for rural areas) of 18 collection sites in 7 provinces including Newfoundland and Labrador, Nova Scotia, Quebec, Ontario, Manitoba, Alberta and British Columbia. Forty seven VOCs were detected in at least half of the homes. To identify potential factors affecting indoor concentrations of these 47 VOCs, we used linear regression models to link home characteristics obtained from questionnaires and measured indoor VOC levels.

OUTPUTS/RESULTS: We found that activities in the homes that involved the use of chemicals increased VOC levels. For example we found that recent renovation increased the level of most measured VOCs, specifically the levels of all measured alcohols were increased. Increasing age of the house tended to be associated with decreased levels of VOCs.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The results suggest that different housing characteristics and activities contribute to indoor VOC concentrations. This study provides useful insight for designing prospective epidemiological studies on health effects of indoor VOCs.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The results of this study, contribute to the scientific basis for supporting indoor air guidelines for VOCs in Canadian homes, and provide further information to enhance communication with the public about sources of VOCs in homes.

1.05 Carcinogenic Potency Database (CPDB) Version 5: Distribution of Tumorigenic Doses for 804 Substances

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[1] New Substances Assessment and Control Bureau, SED, HECSB, Health Canada, Ottawa, ON

OBJECTIVES/BACKGROUND/ISSUE(S): The Carcinogenic Potency Database (CPDB) was developed between 1980 and 2005 by Lois Gold and colleagues as a repository of data on substances which have been tested for their potential to cause cancer and measured for their ability to give rise to tumours in live animal experiments (tumorogenicity). By 2008 the CPDB contained data on the proposed tumorigenic dose 50% (TD50) of 804 substances found tumorigenic, as well as the dose estimated to produce an extra lifetime cancer risk of 10% (LTD10). The LTD10 is the dose recommended in EPA guidelines as a benchmark dose for low-dose cancer risk assessment. Determinations of thresholds of toxicological concern (TTC) have also been derived from information for substances in the Carcinogenic Potency Database (CPDB), however, the database was historically one third of this size when most of these studies were conducted. This current analysis is an examination of the expanded data set to describe the range and distribution of 804 substances which have tested positive for their ability to give rise to tumours.

DESIGN/METHOD/DESCRIPTION: The data analysed in this study is public data which can be found in the Carcinogenic Potency Database (CPDB) version 5 which is current as of November 2008. Data was manipulated as little as possible, and there was no selective sorting according to substance, or test species, or route of administration.

When TD50 and LTD10 values for the 804 substances with positive test results were graphed, a highly skewed curve resulted, however, a simple mathematical transformation in which the value is raised to the power of a constant eliminated the skew and successfully created a mathematically normal distribution. From these normal distributions, ranges were estimated from the standard deviations representing 68%, 95% and 99.7% away from the average.

OUTPUTS/RESULTS: For 804 substances shown to cause tumours in test animals, the distribution of carcinogenic potency for 99.7% of substances started with doses as low as 0.000242 and went up to doses as high as 52176.015 mg/kg –d when comparing substances that cause cancer in 50% of test animals. The average value of the dose that caused cancer in 50% of test animals was 18.14 mg/kg –d, while 95% of the doses causing cancer in 50% of the animals lay within the dose range of 0.0172 to 4687.948 mg/kg -d.

The distribution of carcinogenic potency for 99.7% of substances started with doses as low as 0.0000210 and rose to doses as high as 4479.273 mg/kg –d when comparing doses that cause an extra lifetime cancer risk of 10% in test animals. The average value of the dose estimated to produce an extra lifetime cancer risk of 10% in test animals was 1.77 mg/kg –d, while 95% of the doses causing an extra 10% lifetime risk of cancer lay within the range of 0.00164 to 424.684 mg/kg -d.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The CPDB was started in 1980, and the volume of data in this repository has increased dramatically over the years; it is possible that this increase in data has resulted in a shift in the average and range of tumorigenic doses for known cancer causing substances. In this analysis, the distribution of tumorigenic doses was shown to range by at least eight magnitudes for 804 positive tumorigenic substances. These distributions serve as a benchmark for relative carcinogenic potency for substances not already included in the database. Consideration could be given to updating benchmarks or validating current thresholds of toxicological concern derived from the range of data in earlier versions of this database.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Results of this analysis can be used to provide the department with an up to date context for comparison of the relative potency of tumorigenic substances.

1.06 Transplacental Mutagenicity of Benzo[a]pyrene in the Mouse

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OBJECTIVES/BACKGROUND/ISSUE(S): Many chemicals to which humans are exposed through the environment are associated with cancer and other genetic diseases; thus, Health Canada is evaluating the health risk associated with exposure to environmental pollutants. In particular, pregnant mothers are a high-risk group because developing fetuses may be negatively impacted by *in utero* exposure (*i.e.*, in the womb) to environmental pollutants; however, the effects of such chemicals on the fetus are poorly characterized.

DESIGN/METHOD/DESCRIPTION: In this study, we asked whether benzo[a]pyrene (BaP) causes mutations (changes in DNA sequence) in mice exposed *in utero*. BaP was chosen because it is a ubiquitous environmental pollutant that is found in a wide variety of sources, including vehicle emissions, industrial effluents, and grilled food.

We used the Muta™Mouse system to measure mutations. This mouse model contains a viral DNA sequence that can be used to detect mutations in any tissue of the body. Pregnant female mice were exposed to BaP and their offspring were examined for induced mutations.

OUTPUTS/RESULTS: The results showed that *in utero* exposure to BaP significantly increased mutations in bone marrow, liver, brain, and sperm of the offspring. Additionally, male offspring had significant reductions in sperm count and motility.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: These findings suggest that *in utero* development is a highly sensitive window for the induction of mutations by environmental chemicals. Elevated mutation levels are associated with increased risk of cancer and other genetic diseases. Moreover, because we observed increased mutations in the sperm of the offspring, there exists potential to impact not only the exposed individuals, but also their future progeny.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This project examines the transplacental mutagenicity of BaP and advances our understanding of the danger to future generations caused by parental exposure to toxic chemicals. The end-users of this project are regulators from Health Canada and other agencies. The data generated in this project can be used by regulators for the risk assessment of fetal exposures to mutagenic/ carcinogenic chemicals. In addition, the work provides a strategy for the use of the Muta™Mouse model in assessment of *in utero* genotoxic hazards for Chemicals Management Plan (CMP) chemicals.

1.07 An Environmentally-Relevant Mixture of Polybrominated Diphenyl Ethers (PBDEs) Induces Adipocyte Differentiation in 3T3-L1 Cells

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada is responsible for the assessment and management of health risks to Canadians associated with exposure to environmental contaminants. Several studies have shown that exposure to a number of different environmental pollutants may result in the manifestation of metabolic conditions including obesity. As such, development of cell culture models to assess the ability of chemicals to induce the differentiation of immature fat cells to mature fat cells will be useful in assessing the potential of these compounds to cause obesity.

DESIGN/METHOD/DESCRIPTION: The purpose of this study was to examine the ability of an environmentally-relevant mixture of polybrominated diphenyl ethers (PBDEs) to induce the formation of fat cells in a mouse cell line (3T3-L1).

3T3-L1 cells were exposed to increasing concentrations of the PBDE mixture. Fat cell formation was assessed by measuring markers for fat cells, transcription factors involved in fat cells formation, and fluorescent staining for lipid droplets.

OUTPUTS/RESULTS: It was found that PBDEs induce the formation of fat cells from immature fat cells by activating various genes important in fat cell development.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The results will be used to help design testing methods for the identification of chemicals that have effects on fat cell formation.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Screening of chemicals in this system may allow for identification of compounds that contribute to obesity, and aid in the development of a more effective regulatory program.

1.08 Market Survey of Aromatic Amines in Textiles and Leathers to Support Health Canada's Chemicals Management Plan

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OBJECTIVES/BACKGROUND/ISSUE(S): The Chemicals Management Plan (CMP) was launched in 2006, aiming to reduce the risks posed by chemical substances to Canadians and their environment. CMP2 began in 2011, including an assessment over 300 dyes and pigments that contain azo and benzidine components. Some of these substances have been identified by other countries as a concern because they can release cancer-causing substances (aromatic amines and benzidines). Since 2003, Europe has regulated the presence of certain aromatic amines ("EU22") and routinely tests consumer products to ensure compliance with their regulation (DIRECTIVE 2002/61/EC).

DESIGN/METHOD/DESCRIPTION: In the EU, non-compliant products are usually textiles and leather products. However, the exposure levels in Canada are unknown. A preliminary market survey was conducted that focused on products considered to have potential for mouthing by young children.

Many textile and leather products were purchased from retail stores in Ottawa and analyzed by the Health Canada Consumer Product Safety Division's Laboratory. Products were selected in many different colours, fabric types, and countries of origin and represented four categories: children's toys, leather infant/toddler slippers, children's clothing, and woollen items.

From each sample, 24 aromatic amines, including the EU22 and 2 benzidine congeners, were quantified using a UHPLC-MS test method according to both procedures from the EU Regulation (EN 14362-1:2012 standard).

OUTPUTS/RESULTS: Only one orange polyester toy was found to have a targeted analyte above the LOQ; (2,4-diaminotoluene, CASRN 95-80-7) at 4.2±2.5 ppm from the EU standard's method B. None of the other products tested contained quantifiable levels of the EU22 or benzidine congeners.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: While the presence of EU22 aromatic amines are routinely identified in textiles and leather during compliance testing in Europe and other jurisdictions, azo dyes based on the EU22 aromatic amines have mostly been phased out internationally.

This preliminary market survey of the Canadian market demonstrated that the children's products sampled contained negligible levels of the 24 aromatic amines tested. The information generated supported the CMP assessments on Aromatic Azo and Benzidine-based Substances.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The Government of Canada is proposing that exposure to some aromatic amines and associated azo dyes from textiles and leathers in the Canadian marketplace, is not harmful to human health at current levels of exposure.

1.09 Development of an *in vitro* Assay to Detect Toxic Phorbol Esters in Biodiesel Feedstock

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OBJECTIVES/BACKGROUND/ISSUE(S): The oil extracted from the seeds of the tropical shrub *Jatropha curcas* is a promising biodiesel feedstock, but it also contains toxic and pro-inflammatory phorbol esters. New health risks associated with the increased production and use of Jatropha oil will therefore need to be properly assessed. Unfortunately, available methods to measure the toxicity of phorbol esters present important shortcomings.

DESIGN/METHOD/DESCRIPTION: The goal of this project was to take advantage of the high sensitivity of a dog kidney cell line to develop a simple, sensitive and quantitative bioassay to routinely detect and quantify the biological activity of phorbol esters in Jatropha oil.

Phorbol ester is a large family of compounds that can present different activities. Hence, it was first confirmed that exposure to Jatropha oil's phorbol esters can trigger the characteristic changes in dog kidney cell morphology observed following exposure to a well-known and very potent pro-inflammatory phorbol ester (12-O-tetradecanoylphorbol-13-acetate, or TPA). As assessment of cellular morphology is labour-intensive, prone to artefacts and difficult to quantify, it was decided to monitor the expression of *Cox-2*, a gene involved in response to inflammation. The expression of this gene, which can vary by a hundred fold, was well correlated to changes in cellular morphology. Induction of *Cox-2* expression resulting from exposure to Jatropha oil was compared to a standard dose-response curve prepared with TPA. The pro-inflammatory potential of Jatropha oil was finally expressed as the concentration of TPA required to achieve the same effect using a toxic equivalency (TEQ) approach.

OUTPUTS/RESULTS: The robust and sensitive bioassay developed in this project can quickly and efficiently detect the biological activity of phorbol esters directly in Jatropha oil and express it quantitatively using a toxic equivalency approach.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: In addition to the routine testing of phorbol ester activity in Jatropha oil, this bioassay may also be adapted for the detection of phorbol esters in other materials and for additional research applications.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: There are no guidelines, regulations or labelling requirements concerning the presence of phorbol esters in biodiesel. The described method provides a useful tool for risk assessment and management of such fuels.

1.10 Determination of Flame Retardants in NIST Standard Reference Material (SRM 2585 – Organic Contaminants in House Dust)

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OBJECTIVES/BACKGROUND/ISSUE(S): Many commercial products have been treated for several decades with brominated flame retardants (FRs) and polybrominated biphenyl ethers (PBDEs) were the most used. However, due to their potential toxicity, two major PBDE formulations (i.e., Penta-BDE and Octa-BDE mixtures) were banned in North America and European Union counties in 2004. The production and usage of Deca-BDE was expected to be phased out by 2013 in Canada and the USA. Therefore, various other compounds have been developed as alternatives to PBDEs and are currently used in many applications. Those alternatives are mainly organophosphate esters (OPEs) and non-BDE halogenated FRs. Recent studies have shown some of them may have adverse health effects. The objective of the current study was to measure PBDEs, OPE and non-BDE flame retardants in an NIST standard reference material, the SRM 2585- Organic Contaminants in House Dust.

DESIGN/METHOD/DESCRIPTION: The analysis of certified reference materials is one of the requirements for the validation of newly developed analytical methods. Appropriate amounts on SRM 2585 (0.06-0.10g) were spiked with internal standards prior to solvent extraction using a mixture of hexane/acetone (1:1, v/v). The sample extracts were subjected to cleanup by solid phase extraction and analysis by gas chromatography (GC) coupled with mass spectrometry (MS) operated in electron capture negative ion chemical ionization (ECNI) mode for PBDEs and non-BDE halogenated FRs, and in positive chemical ionization (PCI) for OPEs.

We have developed and validated three analytical methods to for the analysis of selected FRs in house dust: 1) 13 OPE FRs, 2) 18 non-BDE halogenated FRs, and 3) 13 PBDE congeners. The new methods were used for the determination of selected FRs in NIST SRM 2585.

OUTPUTS/RESULTS: The developed methods demonstrated good recovery for each individual compound and low detection limits as low as 0.03 ng/g of dust. The measurements of several PBDEs were close to their certified values in SRM 2585 with relative errors less than 20%. The concentrations of two major non-BDE FRs (EHTBB and BTBPE) in SRM 2585 were 30.7 ng/g and 25.8 ng/g, respectively.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The developed methods are being use for the analysis of dust samples collected under the Canadian House Dust Study (CHDS), in order to provide national baseline data of selected flame retardants.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The FR data generated from Canadian House Dust Study (CHDS) samples will support Health Canada's risk assessment/ management activities under the Chemicals Management Plan (CMP).

1.11 Polybrominated Diphenyl Ethers and Novel Flame Retardants in the Canadian House Dust Study

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OBJECTIVES/BACKGROUND/ISSUE(S): The objective of this study was to develop analytical methods for three groups of flame retardants (FRs) in house dust: polybrominated diphenyl ethers (PBDEs), organophosphate esters (OPEs), and non-BDE halogenated flame retardants, and then to apply these methods to house dust samples collected under the Canadian House Dust Study. The large datasets generated by this study will contribute to the risk assessment and/or risk management of the selected FRs under investigation.

DESIGN/METHOD/DESCRIPTION: Prior to solvent extraction, sieved dust (<80 µm) was spiked with a solution of internal standards. Sample extracts were cleaned up using solid phase extraction. OPEs were analyzed by GC/MS/MS in positive chemical ionization (PCI) mode, while PBDEs and non-BDE halogenated flame retardants were analyzed by GC/MS in electron capture negative ion (ECNI) mode.

We have developed analytical methods for the analysis of 13 organophosphate esters, 18 non-BDE halogenated flame retardants and 13 PBDEs in house dust. OPEs were analyzed in a complete set of 818 dust samples, while PBDEs and non-BDE halogenated FRs were determined in a subset of 478 dust samples.

OUTPUTS/RESULTS: Widely scattered concentration levels were observed for target FRs detected in dust samples. Among the OPEs detected, the most dominant was tri(butoxyethyl) phosphate (TBEP) with a median concentration of 34.1 µg/g and a maximum of 275 µg/g (n = 818). Five out of 18 non-BDE FRs were not detected in any dust samples (n = 478), and four others were detected at concentrations close to or below their respective method detection limits (MDLs). High detection frequencies (≥ 93%) were observed for hexabromobenzene (HBB), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), and Dechlorane Plus. The most dominant non-BDE halogenated FRs was EHTBB with a median concentration of 101 ng/g and a maximum of 15600 ng/g. Thirteen PBDE congeners were detected with frequencies higher than 93%, out of which BDE-209 had the highest median concentration of 1300 ng/g (n = 478).

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This research has produced the largest datasets available internationally for these three classes of compounds, and will enable a better understanding of potential human exposures to flame retardants from house dust.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Information from this study will be used in the Chemicals Management Plan screening assessments of selected non-BDE and OPE flame retardants. Information about occurrences of PBDE compounds in house dust is relevant for Health Canada risk management activities.

1.12 Modified QuEChERS Approach for the Analysis of Pesticides in Honeybees and Honeybee Products

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OBJECTIVES/BACKGROUND/ISSUE(S): Honeybees are routinely exposed to pesticides applied to agricultural crops. Foragers encounter pesticide-containing nectar and pollen, which are subsequently transported to the hive, resulting in contamination of the colony and accumulation of pesticides in beeswax, honeycomb, and honey. While field pesticides are largely regulated at non-lethal levels for honeybees, sub-lethal levels of insecticides, particularly neonicotinoids, may be detrimental neurologically to foragers and thus the viability of colonies. To date, a limited number of analytical methods for the determination of honeybee contaminants have been reported with varying degree of success, as the primary challenge resides in the high abundance of interfering lipids, proteins, and sugars within these matrices.

DESIGN/METHOD/DESCRIPTION: This study involves the adaptation of a previously developed QuEChERS-based approach for the targeted simultaneous analysis of 44 unique pesticide residues in honeybees and honeybee products by high performance liquid chromatography-tandem mass spectrometry.

A comprehensive and elaborate pesticide residue extraction technique was developed that combines a rapid and robust QuEChERS approach in addition to a solid-phase extraction cleanup step for the simultaneous analysis of 44 different pesticide residues. The method was validated for a number of bee and bee-related matrices including beeswax, honeycomb, and honey.

OUTPUTS/RESULTS: Samples analyzed from a previous study were re-analyzed with the newly developed methodology for validation. In addition, previously un-analyzed samples were also screened to showcase the method's robustness over a wide range of matrices with high sensitivity and precision. All 44 pesticides residues targeted were successfully detected simultaneously in beeswax, honeycomb, and honey matrices.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The method was found to be effective for detecting all 44 different pesticide residues targeted with high sensitivity, precision and accuracy. Future goals will include further expanding the list of pesticide residues analyzed to keep PMRA ahead in the enforcement of compliance in new pesticide technology.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The availability of a rapid and comprehensive method for the screening of neonicotinoids and other pesticides in bee and bee-related matrices will allow policy makers to assess the risk of these pesticides and will therefore be essential in PMRA's enforcement of compliance regarding the use of pesticides screened therein.

1.13 Development of an Analytical Method for the Determination of Pyrethroid Pesticides in Salmon Gills Using GC/MC-NCI

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OBJECTIVES/BACKGROUND/ISSUE(S): With the salmon farming industry growing, there has been an increasing problem with the sea louse, a marine fish parasite. The crowded environment of commercial fish farming provides excellent conditions for the spread of this parasite. Two effective pyrethroid pesticides (Cypermethrin and Deltamethrin) used to treat salmon against sea lice are extremely toxic to crustaceans, such as lobster larvae, shrimp and crab. Consequently, their use is banned. However the continuing prohibited use of these pyrethroids in the fish farming industry demonstrates the need for regular monitoring and enforcement to ensure a safe and healthy environment for Canadians.

DESIGN/METHOD/DESCRIPTION: Salmon gills concentrate the two noted pesticides from the environment and consequently are the tissue of choice for this study. Analytical challenges include the small size of the salmon gill samples and the requirement that the methodology must be robust and sensitive enough to consistently detect the pesticides in the desired 1 – 5 ppb (parts per billion) range.

A new method was developed by adapting an existing PMRA method that determined cypermethrin concentrations in antifouling material and salmon flesh. Extensive methodology changes were required to allow for the small size of the salmon gill samples. Sample extraction and clean-up techniques were both modified. Furthermore, implementation of a change in chemical instrumentation technology resulted in a five times increase in the sensitivity of detection of the pesticides.

OUTPUTS/RESULTS: The method was extensively validated and found to be effective for detecting residues of the pesticides with high sensitivity, precision and accuracy. The methodology was successfully used to screen pesticides in samples collected from salmon farms in Canada's Atlantic region.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Successful use of this methodology helped protect the health and environment of Canadians as part of a coordinated monitoring of salmon farms. Future goals include the implementation of new sample extraction technology and expansion of the list of pesticide residues analyzed.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The availability of a robust, sensitive and precise method for the detection of pesticides in salmon allows policy makers to assess the use of banned pesticides in the salmon farming industry, and helps ensure the PMRA remains ahead in the enforcement of compliance with the Pest Control Products Act.

1.14 Reducing Exposure to House Dust Mites in Canadian Homes: Technical Document

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OBJECTIVES/BACKGROUND/ISSUE(S): Under the Clean Air Regulatory Agenda (CARA), Health Canada evaluates the health effects of biological and chemical contaminants in indoor air and the strategies for reducing exposure to these contaminants. House dust mites (HDM) are present in most Canadian homes and exposure can contribute to the development of asthma and allergies and an aggravation of symptoms in sensitive individuals.

DESIGN/METHOD/DESCRIPTION: We reviewed the literature investigating frequently recommended HDM reduction strategies. These documents included published reports examining exposure routes, health effects and methods employed to eliminate HDMs. The major systematic reviews and summary documents we consulted were published by the World Health Organization, the U.S. Department of Health, the U.S. Institute of Medicine, the Public Health Agency of Canada, and the Cochrane Collaboration.

Many of these reports make recommendations for eliminating HDMs, but there is debate regarding the effectiveness of both single and multiple interventions. We found that reducing moisture, using bedding encasements, improved cleaning methods and removing carpets can lower exposure to HDMs and their allergens. Individual interventions, however, were not found to consistently provide significant health improvements when implemented alone. Multiple, concurrent interventions often had greater success in reducing exposure and improving health.

OUTPUTS/RESULTS: Based on the literature review, we developed a technical document synthesizing information on the health issues from HDM exposure and methods for reducing exposure in Canadian homes. We reviewed the evidence for the effectiveness of different strategies to (i) reduce exposure to HDM, and (ii) improve health, as evaluated in the major systematic reviews.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This knowledge is important for accurate and appropriate recommendations on risk management measures for reducing HDM exposure. This document also indicates that there is a requirement for further research examining the effectiveness of multiple interventions in improving health outcomes.

1.15 Adaptation of a Fast High Throughput Assay to Screen CMP3 Chemicals for DNA Damaging Effects

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OBJECTIVES/BACKGROUND/ISSUE(S): While Health Canada has completed many health risk assessments on substances that were identified as priorities, there remain a number of “data-poor” chemicals for which more information is required to evaluate their potential health hazard. This poster describes the development of a rapid assay that can screen many doses simultaneously for their potential to damage cellular DNA and to consequently create predisposition to cancer development.

DESIGN/METHOD/DESCRIPTION: A series of substances incorporating copper or zinc and of varying structural complexity, need to be tested, within a short period of time, for their potential to induce early DNA damage. Several *in vitro* DNA damage assays were considered, and the Microfast 96-well format DNA unwinding assay was adopted based on its reported reliability, sensitivity, and rapidity. This Microfast method provides an index of DNA damage by measuring the rate of DNA unwinding, which accelerates with accumulating DNA breakage.

Chemicals are often transformed in the liver to facilitate their elimination from the body, but this process sometimes creates DNA reactive compounds. Therefore, given the nature of “data-poor” chemicals, assay performances were tested using two human liver cell lines with different chemical-transformation capability, HepG2 and HepaRG. Two chemicals that are known to induce DNA damage were used to optimise experimental conditions; 4-nitroquinoline-N1-oxide induces DNA damage directly, while Aflatoxin B1 must be transformed by the cells to induce effects.

OUTPUTS/RESULTS: DNA damage detection sensitivity using the Microfast method was comparable to the well established comet assay and sensitivity could be further enhanced by optimising cell density. Both cell lines exhibited DNA breakage after exposure to either prototypical chemical, with HepaRG cells proving to be the more sensitive to damage induced by these substances.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The Microfast method, conducted in a 96 well plate, is a rapid, inexpensive assay without the operator bias that can be introduced while conducting comet assays. It generates reproducible quantitative (continuous) data that are easy to analyse and interpret.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This rapid, high throughput assay is an emerging technique that can enhance DNA damage screening capacity for "data-poor" chemicals and will assist in chemical testing, risk assessment, and prioritisation strategies.

1.16 Mutations in Sperm: A Novel Detection Method

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada is responsible for evaluating the potential health risks that chemical exposures may pose. Genotoxic chemicals can cause damage to DNA that may result in cell death, but they may also cause mutations (a change in the DNA sequence). If mutations occur in sperm, they can be inherited by that individual's offspring. Understanding the potentially heritable consequences of chemical exposure is of the utmost importance to population health. However, mutation detection in offspring is difficult because mutations are quite rare. In this work, we developed a new method to study mutations in the DNA from mouse sperm.

DESIGN/METHOD/DESCRIPTION: We developed a method to identify and count the number of mutations occurring in highly unstable regions of repetitive DNA by rapidly screening these DNA regions in thousands of individual sperm. These unstable regions represent on a small scale what the whole genome may be experiencing after exposure to an insult.

We demonstrate how we can use this approach to detect mutations in two regions of DNA in mice. We compared mutation frequencies in mice exposed to a highly genotoxic chemical and untreated control mice for proof of principle.

OUTPUTS/RESULTS: We found similar increases in mutation frequencies in both DNA regions following exposure to a genotoxic chemical (BaP) compared to untreated control mice. We show that the approach is much more rapid and precise, and is significantly less expensive than existing methods used to study germ cell mutagenesis. In addition, we demonstrate that the two genetic regions can be analyzed simultaneously within a single reaction, further reducing the time and cost of the assay.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Although further validation is required using other genotoxic chemicals, preliminary data suggest that through the use of multiple genetic sites and sperm-based analyses, this approach is a dramatic improvement over existing methods to screen repetitive DNA regions that are 3x the cost, require the use of radioactive reagents and take 3x as long. In addition, similar genetic regions to the ones screened are also found in humans; thus, the method can readily be adapted for human studies.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Health Canada is responsible for helping Canadians maintain and improve their health. Assessing the effects of chemicals on the next generation is an important gap in existing tests. Health Canada is playing a leading role in international efforts to improve testing strategies in this area. Tools such as the one described here are an important step in this direction.

1.17 Experience from Field Deployments in 2014 with respect to a Nuclear Emergency

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OBJECTIVES/BACKGROUND/ISSUE(S): In the event of a domestic nuclear emergency, Health Canada has a field response team which can be deployed to measure radioactive contamination in areas where the public could be impacted. These measurements would help public authorities to make informed decisions for radiation protection recommendations.

DESIGN/METHOD/DESCRIPTION: Health Canada's field response team has a number of deployable assets. One of which is the Mobile Nuclear Laboratory (MNL), which has been outfitted with a High Purity Germanium (HPGe) detector within a lead castle. The HPGe detector can be used for identification as well as quantification of gamma emitting radioisotopes in contaminated soil, water, air, and other samples. The MNL equipped with the HPGe detector has been successfully deployed in the field for various exercises.

The field response team also has a number of handheld detectors which can be used to survey areas, samples and people for nuclear contamination. Vehicles have been outfitted with Radiation Data Collection and Mapping System (RaDCAMS) technology which allows the field team to rapidly survey areas for increased levels of radioactivity. In-situ gamma ray spectrometry measurements can also be performed to a) provide ground validation of the results of airborne surveys and b) to determine the amount and type of radioactivity deposited on the ground from the accident.

OUTPUTS/RESULTS: The experience and results of two field exercises will be presented here. Field exercises consist of deploying a communications trailer, tents and equipment for a decontamination line, sample reception which includes chain of custody, and finally Mobile Nuclear Laboratories for analysis. One exercise, completed in May of 2014, included the field deployment for the multi-jurisdictional exercise: Exercise Unified Response in which a nuclear accident scenario was simulated at the Darlington Nuclear Power Plant. In this exercise no live sources of radioactivity where used, however a mock release and resulting plume was simulated and the data was sent to the field team's ground survey vehicles which were outfitted with radiation detectors able to determine instantaneous dose rate (RaDCAMS). This information was used to inform monitoring locations for the field team and mock in-situ measurements were performed. This led to a number of interesting questions which would need to be addressed in the event of a real emergency. Mock decontamination of personnel was also performed. Results from the exercise in Borden in November 2014 are very interesting because the field team tested their capabilities in winter condition. For both exercises, the field team has been successfully deployed. The lessons learned from these experiences will be used to further improve future deployments.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The field team has been successfully deployed at various exercises. The lessons learned from these experiences will be discussed and used to further improve future deployments.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Under the Federal Nuclear Emergency Plan, Health Canada shares primary responsibility for the conducting and coordinating radiological monitoring and surveying, which is critical information for decision makers. The HC field team is an important asset for delivering on this responsibility.

1.18 A Preliminary Investigation of the OPSIS SM200 System for Monitoring Airborne Particulate Matter and Metals

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada recently evaluated a new air quality monitoring system (the OPSIS SM200) which provides two types of measurement: a direct reading of airborne particulate matter (PM), plus collection of PM on a filter for gravimetric analysis and chemical characterization. The OPSIS has been approved by several countries (USA, Germany, UK and Russia) for regulatory monitoring of ambient PM. However, there is a lack of information on comparability between the OPSIS and existing approaches used for exposure monitoring in Canada.

DESIGN/METHOD/DESCRIPTION: The new OPSIS MS200 air quality monitor has the advantage that it can operate unattended for weeks to months and provides both direct-reading and filter-based measurements of airborne PM. This study investigates the OPSIS to evaluate consistency and comparability with air quality datasets obtained using traditional instrumentation and approaches.

Experiments were conducted indoors to compare OPSIS direct reading measurements of airborne PM with traditional direct-reading instruments (DustTrak II and DustTrak DRX), and with traditional filter-based gravimetric measurements. Gravimetric analysis was performed using Health Canada's patented weighing facility (Archimedes M3^{TM}) which controls temperature and humidity and minimizes analytical errors. Simultaneous particle size distributions were monitored using a scanning mobility particle sizer (SMPS). Metals were determined on the OPSIS filter PM samples using acid digestion and inductively coupled plasma mass spectrometry (ICP-MS).

OUTPUTS/RESULTS: In preliminary testing, direct reading measurements of PM obtained with the OPSIS correlated well with filter-based gravimetric measurements of PM (R^2 = 0.996; 24 hr averages). The correlation was acceptable between direct-reading measurements obtained with the OPSIS and those obtained with the DustTrak (R^2 = 0.83; 24 hr averages). Analysis of metals on the OPSIS filter PM samples yielded indoor concentrations ranging from pg m^{-3} to ng m^{-3} for a wide variety of airborne metals.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The OPSIS appears to provide direct reading measurements that are consistent and comparable with traditional gravimetric approaches. A key advantage of the OPSIS is its ability to monitor variations in air quality over long time periods (weeks to months). Also it is easily transported to different monitoring stations or source locations using a small cart. Future testing of the OPSIS in different indoor and outdoor environments will be conducted to further evaluate performance of the OPSIS under different temperature and humidity conditions.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The acquisition of the OPSIS expands departmental capacity for monitoring air quality, and particularly enhances our ability to assess long-term exposures to airborne metals and particulate matter under the Clean Air Regulatory Agenda.

1.19 Bioaccessibility of Trace Metals in Household Dust: Method Comparison

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OBJECTIVES/BACKGROUND/ISSUE(S): The accidental ingestion of settled dust and soil is a key source of childhood exposure to metals. The oral bioaccessibility of a metal in dust or soil is defined as the fraction that is soluble in the simulated gastrointestinal environment and has the potential for uptake in the human bloodstream.

DESIGN/METHOD/DESCRIPTION: The purpose of this study is to evaluate the relative advantages and disadvantages of two approaches for estimating oral bioaccessibility of metals in dust: a simple gastric phase simulation and a two-phase gastrointestinal simulation. The gastric phase alone has been validated for lead in soil and dust, but there is a lack of comparison data for other metals.

A physiologically-based extraction technique recommended for contaminated soils by the United States Environmental Protection Agency, consisting of two phases (gastric and intestinal), was selected for this study. Bioaccessibility estimates of six metals prevalent in Canadian contaminated sites (cadmium, chromium, copper, lead, nickel, and zinc) were compared using the gastric phase simulation alone and the complete gastrointestinal simulation. Samples included vacuum dust samples from 33 homes, certified dust and soil reference materials, and a house dust control sample.

OUTPUTS/RESULTS: Bioaccessibility measurements using the gastric phase simulation were greater than or equal to measurements obtained using the gastrointestinal simulation for the six studied metals. Simulation of the gastric phase alone was also simpler, less time-consuming and less costly than the gastrointestinal simulation. As pH is a key controlling parameter for metal solubility, it is expected that the selection of a gastric simulation or gastrointestinal simulation will depend on the metal being considered, as different metals respond differently to changing pH conditions.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: A number of bioaccessibility extraction tests reported in the literature simulate the gastric and intestinal phases of digestion for young children (≤ 3 yr), which is the age group thought to be at most risk from accidental ingestion of dust and soil. This research found that for the studied metals, a simple simulation of the gastric phase provides the most conservative and cost-effective approach for estimating oral bioaccessibility of ingested metals. Future research will investigate whether these conclusions apply to other metals beyond those considered in this study.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Departmental risk assessments consider that total metal concentration does not necessarily reflect the amount of metal that will be available for biological uptake. This research addressed the need for a practical methodology for improving estimates of the fraction of metal contaminant in dust and soil that, through oral ingestion, is available for uptake in the human body.

1.20 Cadmium in Dust: How Different Sampling Metrics Inform Exposure Assessments

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OBJECTIVES/BACKGROUND/ISSUE(S): House dust contains a wide variety of compounds from both indoor and outdoor sources, and provides a useful medium for assessing exposures in residential environments. The Canadian House Dust Study (CHDS) was designed to provide nationally representative baseline information about inorganic and organic substances in urban house dust.

DESIGN/METHOD/DESCRIPTION: The metal load (e.g., ng m^{-2}) is widely considered the most appropriate index of potential childhood exposure to metals in settled house dust. In this study, metal loading (ng m^{-2} day^{-1}) is examined as a function of the dust loading (mg m^{-2} day^{-1}) and the concentration of metal (µg g^{-1}) in the settled dust, using cadmium (Cd) as an example.

“Active” or fresh dust was collected in 1025 randomly selected homes from 13 Canadian cities, according to sampling protocols developed to obtain three different types of measurements of house dust: metal concentration, metal load, and dust load. These measurements provide different but complementary types of information for exposure and risk assessments.

OUTPUTS/RESULTS: Multivariate analysis indicates that the Cd loading rate in the CHDS is influenced more by the dust loading rate than by the Cd concentration of the dust (68% vs 38%). The overriding influence of dust mass can be observed in homes of smokers versus non-smokers. The Cd loading rate is significantly higher in homes occupied by smokers than in homes occupied by non-smokers, yet there is no significant difference in the Cd concentration of dust from homes of smokers versus non-smokers. The key factor is the significantly higher dust loading rate in homes of smokers compared to homes of non-smokers. Analysis of the influence of urban setting shows the same trend: homes in urban industrial zones are characterized by significantly higher dust loading rates and Cd loading rates but display no significant difference in dust metal concentrations, compared to homes in residential zones.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The results of the CHDS show that, while concentration information is useful for identifying the presence of metal sources in the home and for comparing indoor dust with outdoor dust and soil, dust levels within the home and the dustiness of the external environment are important drivers of potential metal exposure.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This research addressed the need for nationally representative baseline data on metals in typical urban housedust. Complete CHDS datasets have been published for nine metals (Pb, Zn, Co, Cd, Cu, Cr, Ni, As, and Se). These baseline data provide a point of comparison for risk assessments on or near contaminated sites. These data also feed into Canadian estimates of total daily intake and guideline development for metals in environmental media.

1.21 Rodent Developmental Neurotoxicity (DNT) Study Paradigm: Establishing Additional Guidance for Regulatory Reviewers

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OBJECTIVES/BACKGROUND/ISSUE(S):): The rodent Developmental Neurotoxicity (DNT) study paradigm has evolved over time with the most recent test guidelines updated in 2007 with the introduction of Organizations for Economic Co-operation and Development (OECD) guideline 426. Over the past 4 years a joint USEPA-PMRA intergovernmental group has been working to create a document to serve as internal guidance for regulatory reviewers in both countries. Previously, we discussed the origin of the project; the consultative process, highlighting the key stakeholders; outlining the key outputs; and gave a small sample of what the guidance would entail.

Over the past year, efforts have focused providing the content for the guidance document; this is a breakdown of the regulatory definitions of what constitutes the major themes of the document in order to inform both our Health Canada colleagues as well as external stakeholders. As a NAFTA-inspired multi-governmental initiative that involved consultation with both governmental and non-governmental stakeholders, Health Canada has undertaken this initiative to provide better context to key parameters necessary for the review of a DNT study, not only for the individual behavioural tests, but for their integration into the weight of evidence for the entire study and for the ultimate assessment of hazard and risk. The internal guidance will be the net result of this process.

DESIGN/METHOD/DESCRIPTION: Through 2011-2013 the PMRA engaged in an extensive consultative process with both governmental and non-governmental stakeholders to identify issues that presented challenges to both the study conduct and regulatory review. In 2013, a PMRA-USEPA intergovernmental technical group was formed to look at the issues identified in these consultations with an underlying objective for creating a document that would serve as internal guidance.

OUTPUTS/RESULTS: In a joint effort between the Health Canada and the USEPA, internal guidance for regulatory reviewers will be created to consolidate all the issues identified in the consultation process and combine them with the information provided in the test guidelines to be used by reviewers in both Canada and the United States, and ultimately, any other national review agencies that choose to utilize the guidance. The work is currently ongoing, with hopeful completion in 2015.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: While the initial goal is to have this DNT evaluation guidance available to regulatory reviewers under the NAFTA umbrella, much of the guidance can be extended to other studies such as the OECD Extended 1-Generation Reproduction study. The guidance may also be useful to those conducting DNT studies or pursuing DNT research.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The development of additional guidance for reviewers who are responsible for evaluating DNT studies will improve the robustness and efficiency of assessment and ultimately ensure the protection of human health from chemical risks.

1.22 Immunomodulation by Carbon Black Nanoparticles in a Mouse Model of Food Allergy

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OBJECTIVES/BACKGROUND/ISSUE(S): Humans may be exposed to carbon black nanoparticles (CBNPs) through contaminants in foods, cosmetics, drinking water, or other industrial processes. Acute respiratory, subcutaneous and direct immune cell exposure to 50 µg CBNPs has been shown to enhance allergy to chicken egg ovalbumin (OVA) protein in animal models. However, little is known about the effects of CBNPs on gastrointestinal (GI) immunological responses and oral tolerance to food antigens. We hypothesized that GI exposure would similarly enhance the development of food allergy.

DESIGN/METHOD/DESCRIPTION: Allergy prone DO11.10 mice were orally administered CBNPs every second day for 2 weeks (22nm particles in aggregates smaller than 220 nm; total cumulative dose 10.8 µg [LOW] or 108 µg [HI]), with and without OVA, and then sacrificed. Systemic immune parameters and transcriptome analysis reflective of allergy were measured at sacrifice.

OUTPUTS/RESULTS: Exposure to OVA+CBNPs resulted in a significant increase in sensitization associated serum anti-OVA IgG1 antibodies compared to Phosphate Buffered Saline +/- CBNP controls but not relative to OVA alone. No anti-OVA IgE was detected in any group. Immunophenotyping revealed that the relative and absolute numbers of OVA specific CD4 T cells were reduced in the OVA and OVA+CBNPs treatment groups relative to controls in the spleen but not in the mesenteric lymph nodes. However, secretion of the allergy associated Th2 cytokines IL-4, IL-9 and IL-13 was higher in OVA peptide pulsed splenocytes from OVA+CBNPs treated mice compared to controls. Immunology-associated transcriptome analysis of splenocytes revealed a statistical changes in genes for allergy associated enzymes, transcription factors, chemokines, cytokines and the IgE receptor in mice treated with OVA+CBNP compared to PBS control or OVA alone.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Oral administration of CBNPs with OVA allergen to allergy-prone mice enhanced the expression of systemic allergy-associated biomarkers. However, an increase in OVA-specific antibodies that drive the biological allergic response was not observed. The immune transcriptome was sensitive to CBNP exposure; its relationship to immune phenotype will be explored further in order to determine functional relevance.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This immunotoxicology information can be used by risk assessors when making decisions about hazards associated with exposure to CBNPs.

1.23 Assessing Exposure of Canadians to Household Cleaning Products

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OBJECTIVES/BACKGROUND/ISSUE(S): Canadians use a variety of household cleaning products (HCPs) every day. The way people use these products, including how much and how often, are important when estimating exposure to substances that may be present in HCPs. These factors are utilized in exposure assessments conducted under Canada's Chemicals Management Plan (CMP).

DESIGN/METHOD/DESCRIPTION: This project aims to standardize and streamline exposure algorithms for each route of exposure, and to recommend exposure factors that are reasonable and relevant to the Canadian population for use in regulatory risk assessment.

Over 38 algorithms and 30 exposure factors were reviewed and synthesized for 35 different types of cleaning products as part of this project. HCPs were grouped into 3 categories: laundry and dishwashing products; non-spray surface cleaning products; and spray cleaning products. Algorithms and exposure factors pertaining to assessing human exposure to these products were first reviewed and analyzed based on available information from various regulatory authorities, industrial associations, surveys, and the open literature. A systemic rating scheme was developed for recommending the default values based on the relevance and comprehensiveness of the data.

OUTPUTS/RESULTS: Exposure scenarios, including pre, during and post-application, were considered for laundry detergents, laundry pre-treatment products, fabric conditioners, and dishwashing detergents for both chronic and non-chronic exposure scenarios. Tiered algorithms for inhalation, dermal and/or oral routes of exposure will be presented. The default values that were selected from the study with the highest ranking score for the product-specific factors will also be discussed, including use amount, exposure duration, use frequency, dermal contact area, and transfer fraction.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This is the first time exposure factors specific to HCPs have been compiled across a wide range of product types and subpopulations including adults, adolescents, children and infants for regulatory use in Canada. This project provides an up-to-date source of information on exposure factors and estimation approaches, which further supports and strengthens risk assessment related work under the CMP as well as other programs assessing or managing risks associated with HCPs. By 2014-2015, an additional 30 different types of HCPs will be examined, and an *in silico* exposure software tool will be developed for estimating exposure to HCPs.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This work is conducted by a working group with evaluators from two different bureaus. The guidance for exposure assessment on HCPs and the software tool generated from this work will be distributed across the bureau and branch for consultation. This will help to establish consistency within and across the branch for exposure assessments conducted under the CMP. In addition, this work will contribute to information sharing and potential collaboration across international regulatory agencies.

1.24 Protecting Canadians from the Health Impacts of Climate Change: New Science for Effective Adaptation

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OBJECTIVES/BACKGROUND/ISSUE(S): The World Health Organization (WHO) estimates that for the year 2030, 241,000 additional deaths will occur globally due to climate change. A number of climate-related hazards such as floods, wildfires, droughts, extreme heat events and ice storms continue to impact communities in Canada and many of these hazards are expected to increase in frequency and severity due to climate change. Events like the 2013 flood in Calgary can severely impact communities through the destruction of infrastructure and the displacement of populations.

DESIGN/METHOD/DESCRIPTION: A mixed methods approach was used to identify new knowledge about health risks and vulnerabilities to Canadians from climate change. A literature review was conducted to identify new information on key risks to the health of Canadians from climate change for the period 2007-2013. In addition, research results related to projections of future health impacts from climate change were analyzed and presented in the report. A review of climate change and health adaptation efforts at federal, provincial, territorial and local levels related to assessing vulnerabilities, and preparing for the impacts and communicating health risks to Canadians was also conducted.

OUTPUTS/RESULTS: The assessment has been completed and the final report is available at http://www.nrcan.gc.ca/environment/resources/publications/impacts-adaptation/reports/assessments/2014/16309

There is stronger evidence that health risks related to weather variability and climate change are increasing in Canada. Recent research suggests that climate change could affect ambient air pollution by increasing aeroallergens (e.g. pollens), ground level ozone (O_3), and particulate matter (PM).

Surveillance data indicates that, because of climate warming, the tick vector that causes Lyme Disease is expanding rapidly into Canada and human cases are on the rise. Risks from food-borne diseases will increase as climate change leads to higher temperatures and more extreme precipitation events. Aboriginal populations in the North are currently being impacted by the effects of a changing climate on the distribution and abundance of traditional foods. Efforts to prepare Canadians for the health risks of climate change need to be strengthened to prevent future impacts on health and potentially catastrophic events that lead to significant loss of lives and dislocation of populations.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The vulnerability of Canadians and their communities to the health impacts of climate change is significant and risks will continue to grow over the coming decades. Health decision makers at all levels of government can use the results of this assessment to develop tailor made adaptations to protect the health of populations at highest risk.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The results of this study are being used to inform the completion of a *Climate Change Risk Assessment for Health Canada* which is scheduled to be complete by June, 2015. Information from that assessment will help the department reduce future risks from climate change to operations and programs, thereby helping to protect Canadians.

1.25 Top-Down Analysis of Intact Proteins with Q-T of Mass Spectrometry Operating in Data-Independent Analysis (Mse) Mode

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OBJECTIVES/BACKGROUND/ISSUE(S): Proteomics is the large-scale study of proteins, with the goal of identification and characterization of the entire set of proteins produced by a biological system under specific conditions. Mass spectrometry has become the dominant tool in proteomics, with most of the technological developments to date focused on “bottom-up proteomics”, where the protein or protein mixture is cleaved with an enzyme prior to analysis.

By analysing these smaller pieces of proteins (termed peptides) the presence of the protein is inferred. However, proteins exist as multiple proteoforms, which are different molecular forms of a single gene including changes due to genetic variations, alternatively spliced RNA transcripts and post-translational modifications (PTMs). As these characteristics are not evident at the peptide level, important information is lost during bottom-up analysis. Much of the biological function of a protein depends on the specific proteoform present so an alternate proteomics strategy is required. Over the last few years the approach known as “top-down proteomics” has emerged to overcome the limitations inherent in bottom-up proteomics. Intact proteins are analysed without prior digestion allowing proteoform characterization.

DESIGN/METHOD/DESCRIPTION: This project aims to develop a workflow to analyse intact proteins and increase our capabilities using our existing instrumentation. Here we present an analytical strategy using the Synapt Q-Tof mass spectrometer where intact proteins are injected directly to the instrument and fragmented in the gas phase to generate structural information.

We have optimized a protocol that includes sample preparation, protein separation, data acquisition and data analysis, and applied this protocol to a mixture of test proteins.

OUTPUTS/RESULTS: Our method was able to confidently identify most proteins from the fragmentation data, and provide further information regarding modifications and proteoforms.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This method is effective in determining which protein species is present in a sample, and allows us to compare between biological samples.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This project enhances the analytical capabilities of the department and in turn will allow us to address deeper biological questions.

1.26 Characterizing Virulence and Pathogenecity Potential of Industrial *Saccharomyces cerevisiae* Strains for the Development of a Microbial Risk Assessment Strategy

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OBJECTIVES/BACKGROUND/ISSUE(S): *S. cerevisiae* has a long history of safe use in the fermentation of foods/beverages. Recently, industrial derivatives of *S. cerevisiae* are used in the production of feed, biotherapeutics (probiotics), bioremediation and biofuels. Lately, reports of *S. cerevisiae* infections, mainly among immunocompromised individuals, highlight the need for a strategy to identify potentially harmful *S. cerevisiae* strains. In Canada, the import and/or manufacture of micro-organisms for uses such as bioremediation and biofuel production, are regulated under the *New Substance Notification Regulations* (Organisms) [NSNR (Organisms)], where information on pathogenicity/toxicity is assessed to determine its potential adverse effects on humans.

DESIGN/METHOD/DESCRIPTION: We conducted a comprehensive review of current methods described in the scientific literature that characterized the pathogenic potential of various *S. cerevisiae* strains. We evaluated their relevance towards the human health risk/safety assessment of *S. cerevisiae* industrial strains.

OUTPUTS/RESULTS: The analysis shows the extent to which various virulence factors and fitness characteristics of *S. cerevisiae* contribute to disease in the host are not well-understood. Since industrial strains may have arisen by mating/selection/mutation or genetic engineering, their genotypes/phenotypes are diverse. Each strain is likely to feature a unique virulence potential. Putative virulence determinants that may prove useful for screening *S. cerevisiae* pathogenicity are: growth temperature, ability to form pseudohyphae, adhesive and invasive growth, enzyme production, and activate innate immunity and oxidative stress.

Relevance of murine models studies for pathogenicity screening is also discussed. We propose a comprehensive tiered research approach that combines multiple lines of evidence (genome analysis, in vitro and in vivo animal testing) to predict the pathogenic potential of *S. cerevisiae* strains.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Risk assessment of *S. cerevisiae* strains will benefit from more research that involves genome analysis of virulent strains, identification of virulence-specific DNA sequences and definition of relevant toxicity endpoints, which are currently being investigated at Health Canada. Future studies employing the tiered approach will be applied in the determination of risk potential of *S. cerevisiae* strains.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This analysis and the tiered research approach will help the department develop a risk assessment strategy to identify potentially harmful strains of *S. cerevisiae* and analyze information on pathogenicity/toxicity tests for other opportunistic pathogens and novel organisms.

1.27 Hyperspectral Characterization of Nanomaterials in Consumer Products, Canadian Cosmetics and Personal Care Products

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OBJECTIVES/BACKGROUND/ISSUE(S): Consumer products, personal care products, and cosmetics containing nanomaterials (NM) are increasing on the Canadian market, with the global impact of these products projected to reach $3-trillion by 2020. Health Canada is responsible for regulating these novel NM-containing products. Canada, like many OECD countries, applies regulatory frameworks available for chemicals and nanomaterials. However, given the lack of validated methodologies to detect NM in consumer products, the presence of NM in products will be difficult to confirm and they may be poorly characterized.

DESIGN/METHOD/DESCRIPTION: This study employs a novel microscopic method - darkfield enhanced nanoscale hyperspectral microscopy (HS) - to specifically examine several products claiming to contain nano-gold, silver, titanium dioxide and zinc oxide, and to clearly define the NM content.

Several hyperspectral reference libraries from different sizes of raw NMs were built. Using image classification algorithms such as spectral angle mapping (SAM), reference spectral profiles were mapped to the spectral images of products. NM in these products were precisely identified, and further characterised for size distribution using Nanoparticle Tracking Analysis methods.

OUTPUTS/RESULTS: Using HS and SAM, we have confirmed the presence of gold, silver, titanium dioxide and zinc oxide, all within 1-100nm range, in a wide variety of personal care products, such as foundations, body lotions, mascara, and moisturizers, and in consumer products, such as paints. This novel method allows facile confirmation of the presence of NM in cosmetics and personal care products for routine screening of these products.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The accurate assessment of NM exposure from products requires detailed knowledge of products that contain NM ingredients, and the precise detection and characterization of NM in these products. This study establishes the applicability of a novel microscopic method to confirm the presence of NM in cosmetics and personal care products. HS addresses current methodological limitations in detecting low concentrations of NM and detection of functionalized or liposome coated NM in these products. Using this method we are examining the potential of NM in products to cross the skin barrier during normal use in an *ex vivo* skin equivalent model.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The methodologies established as part of this study may potentially be used by the Consumer Product Safety Directorate for routine screening of cosmetics and consumer products containing NM and to investigate the potential of NM in products to penetrate skin.

1.28 A Method to Assess RNA Quality for DNA Contamination in Rat

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada is increasingly facing gene expression data applied to the assessment of chemical toxicity in laboratory animal exposure studies. RNA, a nucleic acid used by the cells to convey genetic information, constitutes the starting material for gene expression studies. However, DNA found in cell nucleus (referred to as genomic DNA or gDNA) often comes along with RNA during the extraction process from biological samples. The contamination of RNA by gDNA can cause measurement errors and improper interpretation of gene expression data. Although assessing RNA quality for DNA contamination is often necessary, there is currently no easily adoptable method for gene expression analysis in toxicological studies in rats.

DESIGN/METHOD/DESCRIPTION: The goal of this study was to develop an inexpensive and reliable method to detect genomic DNA contamination in rat RNA samples to be used for gene expression analyses.

Sadh, a gene abundantly expressed in all tissue types, was used as the molecular target to identify gDNA contamination in RNA samples by applying polymerase chain reaction (PCR) method. Two sets of gene primers (A and B), which are short synthetic pieces of DNA, were designed by computational tools for PCR analyses. While primer set A would multiply the proper RNA target, primer set B would amplify gDNA impurities present in RNA samples. Thus, a RNA sample would be considered contaminated with gDNA, if PCR analyses using the primer set B can generate a detectable amplification product.

OUTPUTS/RESULTS: A variety of samples isolated by Health Canada and acquired from external sources were tested along with positive controls for genomic DNA contamination. The described method successfully discriminated RNA samples contaminated with gDNA.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Details of the experimental protocols and reagents used in this study will be made available to the scientific and regulatory communities through publication in a peer-reviewed international journal. This method to assess gDNA contamination in rat RNA samples was designed to be easily adoptable in gene expression analysis studies.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Health Canada is responsible for assessing and managing thousands of existing substances under the Chemicals Management Plan. The researchers are increasingly relying on gene expression studies to assess the toxic potentials of chemicals. The greater adoption of RNA quality control measures should improve the reproducibility and reliability of toxicogenomic data, which in turn would lead to more informed human health risk assessment.

1.29 Comparison of Ethanol and Chloropropanol Toxicities in Human Cell Lines Containing Introduced Alcohol Metabolizing Systems

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OBJECTIVES/BACKGROUND/ISSUE(S): Chloropropanols (CP's) are small chemical compounds formed from fatty acid components during the manufacture of hydrolyzed vegetable protein and soy sauces, and have been detected as contaminants in many foodstuffs. Within this class of compounds, 3-monochloropropane-1,2-diol (3-MCPD) has been most studied. In the rat, 3-MCPD is associated with infertility, immune system suppression and increased kidney cancer incidence. However, studies examining 3-MCPD for a cancer-related mode-of-action in cell culture models have been inconsistent between bacterial and mammalian culture systems.

DESIGN/METHOD/DESCRIPTION: The lack of 3-MCPD effects in human or mammalian cell cultures may indicate the need for some form of chemical transformation, which could be generally missing from cultured cell lines or found only in specific cell-types. For instance, the vast majority of cell lines lack the ability to metabolize alcohols, and therefore would also be unable to metabolize the chloropropanol family of chlorinated alcohols. Techniques are available to re-introduce these metabolic activities into the cells, thus allowing more representative toxicological assessments.

Genes coding for enzymes involved in alcohol metabolism were introduced into human liver and kidney cell lines for comparison and the effects on the toxicity of ethanol and chloropropanol-related chemicals measured. Effects on purified enzyme activity were also determined.

OUTPUTS/RESULTS: Introduction of the alcohol metabolizing systems decreased toxicity, although not dramatically. In general, increased toxicity was found with increased exposure time, and kidney cells were more sensitive to test chemicals compared to liver cells. Ethanol was the least toxic of the chemicals tested, while the chloropropanol metabolite glycidol was the most toxic. The effects of alcohol metabolism on cancer-related endpoints will be examined next.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Ultimately, these studies aim to provide the regulatory community with in vitro systems better able to supply mechanistic understanding of alcohol toxicities.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: One application of these *in vitro* systems should be the better identification of cancer risk factors involved in CP exposure which should assist in determining the relative risk of each of the major CP's found in foodstuffs.

1.30 Determination of Polonium-210 in Fish Samples from Canadian Lakes, Rivers and Oceans: An In-House Method Development

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OBJECTIVES/BACKGROUND/ISSUE(S): A large fraction of the natural background radiation experienced by individuals through ingestion of food is from the radionuclide polonium-210 (^{210}Po). ^{210}Po is considered to be one of the most toxic naturally occurring radionuclides. The affinity of ^{210}Po for protein enables it to pass through the food chain, and increased body burdens of ^{210}Po have been found where diets include protein-rich meat and seafood. The Radiation Protection Bureau (RPB) of Health Canada has undertaken a study for the measurement of ^{210}Po in fish samples collected from Canadian lakes, rivers and oceans in order to determine a baseline level for this radionuclide. An accurate knowledge of the baseline concentration of a naturally occurring radionuclide such as ^{210}Po is important in order to compare its radiological impact on human health with that of exposure from other radionuclides.

DESIGN/METHOD/DESCRIPTION: This presentation describes a radio-analytical method that was developed for the measurement of ^{210}Po in fish samples.

The method involves (a) homogenisation of a fish sample to obtain a representative aliquot, (b) wet digestion to dissolve the sample and to mineralise the target radionuclide, (c) matrix removal, pre-concentration, and separation of ^{210}Po, (d) deposition of ^{210}Po on silver disks, and (e) quantitative determination of ^{210}Po by alpha spectrometry using ^{209}Po as a tracer.

OUTPUTS/RESULTS: A number of fish samples were analysed for ^{210}Po and the results were compared with the measurements carried out by an ISO certified commercial laboratory. The results from the two methods were in good agreement. The method was also validated by measuring a fish standard reference material (SRM). The agreement between the measured and the certified concentration for ^{210}Po in the SRM was evaluated to be satisfactory.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The new method has been shown to be effective for the determination of ^{210}Po in fish samples. This method will allow comparison of the risk to human health from naturally occurring radionuclides versus radionuclides released in case of a radiological/nuclear accident.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Health Canada will be able to better evaluate and communicate the risk from radionuclides released as a result of a nuclear accident/incident by comparison with the risk from naturally occurring radionuclides such as ^{210}Po.

1.31 Investigating the Validity of the Use of Benzo(a)pyrene as a Point of Reference in Polycyclic Aromatic Hydrocarbon Mixture Risk Assessment

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OBJECTIVES/BACKGROUND/ISSUE(S): The human cancer risk assessment of environmental chemical mixtures containing cancer-causing polycyclic aromatic hydrocarbons (PAHs) relies on a set of 8 structurally similar PAHs. Health Canada's approach uses the most well-studied PAH, benzo(*a*)pyrene (BaP), as a reference PAH in understanding the toxicity induced by the other PAHs and in calculating the cancer risk associated with environmental mixtures containing PAHs. This approach is based on the assumption that the 8 structurally similar PAHs induce cancer by altering similar biological events (mode/mechanism-of-action).

DESIGN/METHOD/DESCRIPTION: This project investigates the accuracy of this assumption by comparing changes in the regulation of genes (gene expression) and associated biological pathways primarily responsible for cancer promotion following exposure to BaP with those occurring following exposure to the other 7 PAHs.

We employed gene expression profiling tools, which measure the effects of chemical exposures on all genes by examining which genes are turned on or off, to characterize the expression changes in the lungs of mice exposed to PAHs. In addition, we analysed routinely assessed toxicological outcomes of exposures to PAHs: DNA damage levels and consequent DNA mutations in mouse lungs.

OUTPUTS/RESULTS: Exposure to BaP and other PAHs resulted in increased levels of DNA damage and enhanced frequency of DNA mutations. However, the extent of the DNA damage or number of mutations varied between the PAHs. Additionally, the gene expression profiles and altered biological pathways indicative of cancer following exposure to each PAH were notably different from those observed following exposure to BaP, implying that all structurally similar PAHs do not cause cancer *via* similar biological events.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Mixtures containing PAHs make up a large portion of the complex mixtures Canadians are regularly exposed to. Our analysis revealed significant differences in the cancer-causing mechanisms-of-action of these PAHs, challenging the assumption that all PAHs induce cancer by altering similar biological events. The results of our study call for careful reconsideration of Health Canada's approach to understanding the toxicity induced by other PAHs and of the assumption that all cancer-causing PAHs act *via* common mechanisms-of-action. The mechanistic information generated by the study can be used by Health Canada to minimise uncertainties involved in the risk assessment of PAH mixtures.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The results of the study can be used to adjust the existing risk assessment methodologies for complex mixtures and to enhance the accuracy of the regulatory decisions.

1.32 Validation of a Predictive Gene Expression Signature in the Presence of Metabolic Activation to Classify Chemicals as Genotoxic or Non-Genotoxic in Human TK6 Cells

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada is responsible for evaluating the health risks posed by chemicals to the Canadian population. Chemicals that are genotoxic (i.e., damage DNA) can cause adverse outcomes such as cancer and inherited genetic diseases. As such, genotoxicity testing is used to determine whether or not a chemical is able to damage DNA and is a critical component of chemical safety testing.

DESIGN/METHOD/DESCRIPTION: In this study, Health Canada collaborated with the Health and Environmental Sciences Institute (HESI) to determine whether changes in the regulation of genes (gene expression) can be used to predict genotoxicity, as these changes provide an indication of the cellular response to a chemical.

We exposed human cells in culture to various genotoxic and non-genotoxic chemicals. Cell survival, DNA damage and gene expression changes were measured in the exposed cells.

OUTPUTS/RESULTS: We confirm that human cells in culture exposed to genotoxic and non-genotoxic agents exhibit changes in gene expression that accurately predict genotoxicity. We demonstrate that these changes are highly correlated with measures of DNA damage and cell survival. In addition, we confirm that the strength of the gene expression response can be used to predict the concentration at which genotoxicity begins to occur.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: While further testing and refinement of this method are necessary, preliminary data suggest that this approach has the potential to add significant value to the existing genotoxicity testing system. This approach provides mechanistic insight into a chemical's mode of action (i.e., how a chemical exerts its effects) and can be used to classify chemicals as genotoxic or non-genotoxic. This additional test may help to address the high rate of false positive results obtained in the standard genotoxicity testing approach.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The intent of this study is to evaluate a metabolically competent *in vitro* biomarker that provides rich mechanistic data to complement the standard genotoxicity testing strategy. This will allow for more effective regulation of genotoxic chemicals. This genomic biomarker is currently under formal evaluation by the US Food and Drug Administration as a first step in accomplishing a more integrated genotoxicity testing strategy to better inform human health risk assessment.

1.33 Toxicogenomic Analysis of Formalin-Fixed Paraffin-Embedded (FFPE) Samples: A Case Study of the Liver Carcinogen Furan

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OBJECTIVES/BACKGROUND/ISSUE(S): Toxicogenomics is an approach to toxicology in which chemically-induced gene changes are measured; its use in chemical risk assessment is increasing because it produces large amounts of data quickly, using fewer animals, and at a lower cost than standard toxicological approaches. Toxicogenomics uses modern genomics technologies that typically require high-quality tissue samples that are stored in -80°C freezers. A more common method for tissue preservation is formalin-fixing and paraffin-embedding (FFPE), which allows samples to be stored at room temperature. FFPE samples are ideally used for looking at tissue structures through a microscope; however, they are thought to be less suited to genomics approaches because formalin damages important molecules (DNA, RNA and protein). Globally, there exist thousands of FFPE samples archived from previous toxicology studies. Toward the goal of reducing experimental animal usage, we propose that retrospective toxicogenomic studies could be conducted using these archival FFPE tissue blocks (instead of repeating chemical exposures using new cohorts of animals).

DESIGN/METHOD/DESCRIPTION: Our goal was to demonstrate that accurate and informative biological information can be obtained through toxicogenomic analysis of FFPE-preserved livers, which had been exposed to the carcinogen furan.

Livers from furan-treated or control mice were divided into pieces that were either preserved by freezing or fixing in formalin. Gene changes were measured using two technologies and differences between furan treated and control mice were compared for the high-quality frozen and degraded FFPE samples.

OUTPUTS/RESULTS: Gene changes induced by furan were similar for frozen and FFPE samples for both technologies. These changes were also consistent with the molecular changes that are known to occur following furan treatment.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: We have demonstrated that FFPE tissue samples can be used for toxicogenomics. We suggest that data from these analyses could be used to: (1) increase the level of confidence for the use of toxicogenomics in chemical risk assessment, and (2) produce a database of gene changes against which new substances could be compared.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This study demonstrates that toxicogenomic analysis of FFPE preserved tissue samples is feasible and can potentially reduce experimental animal usage. This method may contribute towards efficient human health risk assessment.

1.34 Development of Short Path Thermal Desorption GC/MS Method for Determining Airborne Polycyclic Aromatic Hydrocarbons

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OBJECTIVES/BACKGROUND/ISSUE(S): Chemicals Management Plan (CMP) is a Government of Canada initiative aimed at reducing the risks posed by chemicals to Canadians and their environment. Some of CMP priority chemicals are semi-volatile organic compounds (SVOCs). Data on indoor air SVOC levels are needed to provide inhalation exposure information for human health risk assessment. A sensitive analytical method is critical to achieve this goal. We selected polycyclic aromatic hydrocarbons (PAHs), which are also a group of homologs that span large vapour pressure values (10^{-1} – 10^{-10} mmHg), as model compounds to develop a thermal desorption based analytical method for airborne SVOCs.

DESIGN/METHOD/DESCRIPTION: Currently available methods for indoor air SVOCs are passive sampling based and require several weeks of sampling time as samples need to be solvent extracted and only a small portion of extract (<1%) is utilised for analysis. Thermal desorption based methods use a much larger portion (usually >10%) of collected samples, thereby reducing required sampling volumes. Smaller sampling volume is more suitable for indoor air studies, especially for large scale surveys. Our goal is to develop Short Path Thermal Desorption (SPTD) GC/MS method for airborne SVOCs in indoor environments. Samples were collected in-house packed multi-sorbent tubes (glass beads, Carbopack C and Carbopack B) using active sampling.

Analysis conditions for the SPTD-GC-MS were optimized. This included selection of the sorbent material for trapping the SVOCs, optimized temperature conditions for thermal desorption of the SVOCs, along with the conditions for the desorption duration and desorption flow and split ratios as well as GC/MS conditions.

OUTPUTS/RESULTS: Desorption efficiencies of this method for detecting PAHs was from 97.8 to 100%. The power regression response of R^2 was > 0.9924, the analysis repeatability (n=7), expressed as RSD was 0.85-10.38% at the spiking levels of 10ng/tube, and the limit detection was 0.01 – 0.05 ng/sample. Eleven indoor air samples were analyzed using this method. The mean concentration was in the range of 2.0 – 41.1 ng/m^3 for PAHs with 2 to 3 rings (excluding naphthalene), and 0.04 to 1.4 ng/m^3 for PAHs with 4 to 6 rings.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Using PAHs as model compounds, we demonstrated that SPTD-GC/MS is a good alternative method to analyse SVOCs with several advantages. The method is more sensitive, requires less air volume to be collected and cost effective. Compared to traditional solvent extraction, our method provided a better analytical sensitivity for airborne SVOCs. This method also eliminated tedious sample preparation associated with solvent extraction based method for SVOCs.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This method can be expanded to include airborne SVOCs within the vapour pressure range of measured PAHs, and therefore has the potential to be used in human inhalation exposure studies such as large scale surveys of indoor air SVOCs to support the department's regulatory work including risk assessment of CMP priority chemicals.

1.35 BMDExpress Data Viewer: A Visualization Tool to Analyze BMDExpress Datasets

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada scientists use statistical methods to evaluate scientific data to determine significant changes in biological processes occurring following chemical exposures in animal experiments. One method involves mathematical modelling of dose-response relationships to determine a change in response relative to control animals; the dose where effects begin to occur is known as the 'Benchmark Dose' (BMD). The BMD is being increasingly applied in regulatory toxicology to estimate acceptable exposure levels in humans.

DESIGN/METHOD/DESCRIPTION: An open-source tool called BMDExpress is used to establish BMDs for changes in gene expression following chemical exposure. This tool mathematically models thousands of genes simultaneously, resulting in spreadsheets that contain tens of thousands of rows and over 60 columns. Analyzing these data can be time-consuming and difficult.

In this project we explored the use of publicly available Google Developer tools to develop a web-based application to facilitate interpretation and presentation of BMDExpress data. We applied the tool to an in-house dataset to determine how it can be used to graphically display data, assess data quality, and rapidly identify the most sensitive biological processes in response to chemical exposure.

OUTPUTS/RESULTS: We developed a tool that we have named 'BMDExpress Data Viewer'. We demonstrate how this tool can be used to perform three major functions: 1) summarize dataset quality and statistics, 2) identify the most affected biological processes and the lowest BMD values, and 3) compare multiple datasets to reveal important trends (e.g. how toxicity of a chemical changes over exposure time or in different tissues).

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: BMDExpress Data Viewer will expedite evaluation and interpretation of data produced by BMDExpress, which will be useful to both HC research and regulatory scientists. Important biological processes can be selected and compared easily across experiments using built-in statistical and graphical tools.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: BMDExpress Data Viewer provides a complementary visualization component to BMDExpress, which is a software commonly used for high-throughput computation of Benchmark Dose (BMD). This tool will facilitate and expedite the interpretation of gene expression data, which has been identified as a critical need to improve assessment of toxic chemicals.

1.36 Northern Contaminants Disrupt Insulin Secretion in Rat Pancreas and Min6 Insulinoma Cells

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OBJECTIVES/BACKGROUND/ISSUE(S): The Arctic population is experiencing an increased rate of obesity and diabetes while also exposed to elevated levels of environmental contaminants. However, it remains unclear if and how contaminants may interplay with other factors such as lifestyle and genetic background to modulate the pathogenesis and outcome of metabolic diseases.

DESIGN/METHOD/DESCRIPTION: Therefore we conducted an animal study to investigate this issue.

Lean and genetically obese JCR rats are given 10% ethanol in drinking water for six weeks, starting at two weeks before a four-week oral treatment with a Northern contaminant mixture (NCM) containing 22 different chemicals commonly found in Inuit blood. At the end of treatment, pancreas and blood were collected and analyzed for levels of glucose, glucagon, and insulin and pathological changes associated to diabetes. To confirm the effects of contaminants on insulin secretion, human insulin-secreting cells were dosed and examined.

OUTPUTS/RESULTS: NCM treatment reduced pancreatic insulin levels as a result of direct pancreatic injury in both lean and obese rats with or without ethanol treatment. Studies conducted with cultured insulin-secreting cells showed that the NCM inhibited insulin release and induced cell death through oxidative stress and mitochondrial dysfunction.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The results strongly suggest that pancreas may be a vulnerable target of contaminants and thus exposure to high levels of contaminants may contribute to pathogenesis of diabetes, although human studies are warranted to confirm this.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This project has no immediate impact on policies and regulations, however, suggests that more studies should be supported to investigate the role of contaminants in metabolic diseases that has become a major health problem and a burden to health care system in Canada.

1.37 Bishphenol A (BPA) Exposure Alters Release of Immune and Developmental Modulators and Expression of Estrogen Receptors (ERs) in Human Fetal Lung Fibroblasts (hFLF)

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OBJECTIVES/BACKGROUND/ISSUE(S): Bisphenol A (BPA) is used primarily in the production of polycarbonate plastics and epoxy resins which are used in food contacting materials. Maternal exposure to BPA can elevates fetus exposure to BPA through blood transport or direct contact in amniotic fluid. Fetal lung may be exposed to BPA at a greater level than other tissues due to its high surface to volume ratio. Recent animal and human studies suggest that prenatal exposure to BPA may increase the airway sensitivity to allergenic stimuli in postnatal time, resulting in increased risk of childhood asthma. However the underlying mechanisms remain unclear.

DESIGN/METHOD/DESCRIPTION: BPA is known to exert effects through both estrogen receptor (ER)-dependent and independent mechanisms. Fetal lung fibroblasts are known to secrete various molecules to modulate lung development and immune response. Therefore we conducted a cell culture study to determine how BPA may affect ERs expression in and secretory activities of human fetal lung fibroblasts (hFLF).

hFLF was dosed with a range of concentrations (0-100 μM) of BPA for 3, 6, or 24 h. Cell viability, cellular content and localization of BPA, ERs localization and expression, and release of growth and immune response modulators were determined.

OUTPUTS/RESULTS: BPA was located in perinuclear regions of the cells, and its content increased dose-dependently. BPA had no effect on cell viability at the doses used, while increased protein expression of two of three ERs examined, and altered release of growth and immune modulators. The effects of BPA were not blocked by antagonists of any of the ERs.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Although the connection between prenatal exposure to BPA and increased airway responsiveness and childhood asthma remains to be established through human studies, our data suggest that at certain dose levels, BPA may affect immune response by altering secretory activities of fetal lung cells possibly by ERs-independent mechanisms. Biomonitoring data on human fetal lung exposure to BPA will help to clarify the implication of our findings.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This project has no direct impact on policies and regulations, however, provides guidance to future research activities.

1.38 Assessment of Rat Primary Neural Cells Culture System for Developmental Neurotoxicity Testing

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OBJECTIVES/BACKGROUND/ISSUE(S): Early brain development is extremely sensitive to the effects of some substances. However, current animal-based *in vivo* protocols for the assessment of developmental neurotoxicity are very expensive and time consuming and hence, only a limited number of chemicals in commerce have been thoroughly assessed. Thus, there is a need for faster and cheaper alternative methods to screen and prioritize chemicals.

DESIGN/METHOD/DESCRIPTION: The goal of this study was to first characterize the differentiation and maturation of primary cerebellar granular cells (CGCs) isolated from 8 day-old rat brain, as these cells *in vitro* mimic critical biological processes occurring in the developing brain *in vivo*. The expression of key genes associated with these processes was then assessed under normal conditions and following exposure to chlorpyrifos (a well-known pesticide used as a positive control for neurotoxicity).

Microscopic observation of CGCs revealed the appearance of neuronal outgrowth on the fourth day of culture *in vitro* and the formation of a network of synapses by the eighth day in culture. The temporal expression patterns of key genes associated with these processes were assessed by polymerase chain reaction (PCR). CGC's response to chlorpyrifos at or below toxic concentrations (as assessed by conventional cell integrity assays) was then evaluated.

OUTPUTS/RESULTS: The *in vitro* differentiation and maturation of CGCs was confirmed by microscopy and gene expression analyses. Quantitative assessment of gene expression after exposure to sub-lethal concentrations of chlorpyrifos revealed altered expression of key genes involved in the formation of synapses.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The observed changes in the expression patterns of key genes associated with the formation of synapses in rat brain cells following exposure to a known neurotoxicant suggest that this approach may be suitable for the rapid screening of the neurotoxicity potential of untested chemicals.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The development and validation of this *in vitro* neurotoxicity assay will allow the high-throughput identification of substances that may affect brain development and hence may require further testing. Such a screening and prioritization tool will inform and assist developmental neurotoxicity hazard identification.

1.39 Natural Radioactivity Accumulated in the Arctic from Long-Range Atmospheric Transport: Observations in Canadian Monitoring Stations

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OBJECTIVES/BACKGROUND/ISSUE(S): The Fukushima-Daiichi nuclear accident in Japan on March 11th, 2011 released large amounts of radioactive material into the air. Increased levels of airborne radionuclide concentrations detected in Canada corresponded to the arrival of air masses from Japan to Canada after traveling over the Pacific Ocean. The arrival of radionuclides from the Fukushima accident was first detected at the Canadian CTBT (Comprehensive Nuclear-Test-Ban Treaty) radionuclide monitoring station in Sidney, BC on March 17th 2011. The possible health effects from the radioactivity transported by the radioactive plumes from Japan were of concern to many Canadians.

DESIGN/METHOD/DESCRIPTION: The aims of this study were to (i) report the airborne radionuclides and their activity concentrations measured at Canadian CTBT stations located in the north west coast of Canada (i.e. Sidney BC and Yellowknife NWT) before, after and during the Fukushima-Daiichi nuclear power plant accident, (ii) estimate committed effective doses due to both natural radionuclides and Fukushima-Daiichi nuclear accident related contaminants in the air, (iii) compare the estimated effective dose due to Fukushima contaminants with those from the natural radiation background.

OUTPUTS/RESULTS: The committed effective dose for an adult through inhalation and cloudshine pathways was calculated based on the daily observed activity concentrations (Bq/m3) of the radionuclide contaminants from the Fukushima-Daiichi nuclear accident and natural radionuclides in air. The dose due to the natural radionuclide and Fukushima contaminants from March 17 to June 5 of 2011 are calculated, which shows that the dose due to inhalation of airborne radioactive particulate is orders of magnitude higher than that due to the cloudshine or external radiation exposure from immersion in a cloud of radioactivity.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Human exposure to ^{210}Pb and ^{212}Pb, which are constantly present in the environment, are much higher than those caused by Fukushima contaminants. The increased radiation dose associated with Fukushima contaminants during the accident period was indistinguishable from background radiation dose attributable to natural sources at the Yellowknife and Sidney stations. This explains why the elevated level of airborne fission products from the Fukushima-Daiichi nuclear accident did not cause any noticeable changes in the effective dose to human.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The intent of this study is to develop a method that can provide committed doses through aerosol inhalation and cloudshine pathways. This will allow for more effective regulation of dosiemtry resulted from nuclear accident and natural radiation background to better inform human health risk assessment.

1.40 Interaction of Carbon Nanotubes with the Resazurin Assay: Impact on Cellular Toxicity Assessment of Nanoparticles

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OBJECTIVES/BACKGROUND/ISSUE(S): A clearer understanding of the toxicological behavior of nanomaterials (NM) is emerging with an increasing number of studies using cell-based methodologies for toxicological assessments. Many of the assays use color/fluorescence-based detection methods. One such assay, the resazurin assay is a cell viability indicator, based on the chemical reduction of blue, non-fluorescent resazurin to pink, fluorescent resorufin by metabolically active cells. However, as recently documented, numerous assays are susceptible to interference from the test compounds themselves (including particles, such as NMs), impacting the ability of the assay to detect compound related effects.

DESIGN/METHOD/DESCRIPTION: This work specifically focuses on the effects of carbon nanotubes (CNTs) on fluorescence and identifies a simple approach to eliminate both chemical and physical interference of CNTs with the resazurin assay, so that reliable and consistent assessment of CNT toxicity can be achieved.

A fluorescence-based resazurin assay was conducted in the presence of lung epithelial cells and mouse macrophages and in their absence (i.e., cell-free), with multiple doses of CNT variants added to the wells, to examine potential for interaction of the CNTs with the resazurin assay.

OUTPUTS/RESULTS: There was no indication of direct reaction of resazurin with the CNTs, however, a reduction of the fluorescence signal due to the physical presence of the CNTs was observed. The stability of resorufin, the fluorescent product of the resazurin reduction was then assessed. It was observed that charged CNTs could decrease the fluorescence signal for resorufin, possibly through chemical oxidation to resazurin or reduction to hydroxyresorufin.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: It was shown that physical interference of CNTs with the fluorescence signal can be eliminated by removing the CNTs from the cell culture supernatants. It was further shown that the additional observed chemical interference with fluorescence by charged CNTs can be determined and simply subtracted for elimination of the bias. Careful consideration must be given to color/fluorescence-based toxicological assessments of NMs in cells in order to eliminate any potential artefacts due to the NMs themselves, and to ensure that valid scientific methodologies are applied in the toxicological testing of NMs currently found in consumer products.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The project addresses priority knowledge gaps under the Chemicals Management Plan of Health Canada, on the relationship between NM properties and toxicity and contributes towards the development of alternative methods for toxicity testing of NMs, by providing improved methodologies and data required for evidence-based risk assessment of NMs.

1.41 Health Canada's Contribution to the Integrated Fukushima Ocean Radionuclide Monitoring (InFORM) Project

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OBJECTIVES/BACKGROUND/ISSUE(S): The Fukushima accident in 2011 caused increased public concern related to radionuclides. While the radiation level associated with the Fukushima accident has been shown to be low and not of health concern, it is still important to fully assess the environmental impact of the accident and subsequently address any public concerns.

The Radiation Protection Bureau (RPB) of Health Canada joined the Integrated Fukushima Ocean Radionuclide Monitoring (InFORM) project. The InFORM is a collaborative radiation monitoring network aimed at assessing and communicating potential environmental impacts on Canada's Pacific and Arctic Oceans stemming from the Fukushima-Daiichi nuclear accident (http://fukushimainform.wordpress.com). InFORM includes government agencies (Health Canada and Fisheries and Oceans Canada), academics (University of Victoria, University of Ottawa), the private sector (Woods Hole Oceanographic Institution), and citizens.

DESIGN/METHOD/DESCRIPTION: The goal of the collaboration is to assess and communicate the potential environmental impact on Canada's Pacific and Arctic Oceans stemming from the Fukushima-Daiichi nuclear accident.

The collaboration will focus on three aspects: 1) To perform direct measurements of radiation in sea water and on biota; 2) To assess the environmental impact and potential health risks to Canadians that are associated with the radioactivity levels measured; and, 3) To actively communicate the scientific results, assessment and conclusions to the public. The project is unique in that it will involve citizen scientists in the gathering of samples. Health Canada will be involved in the aspects of conducting radiological measurements in biota (fishes), providing a health impact assessment and supporting communication of this information through the Health Canada website and other departmental mechanisms.

OUTPUTS/RESULTS: Health Canada's role in the project is to conduct radiological measurements in biota (fish samples) and provide a health impact assessment while also supporting communication of this information through the Health Canada website and other departmental mechanisms. The biota samples will come from a number of sources, including west coast First Nations groups.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The project is still in its primary phase.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The project could become a model for collaboration involving unaffiliated citizens (citizen scientists) and community activists (NGO, concerned citizen).

1.42 Iodo-trihalomethanes Formation during Chlorination of Source Waters with Naturally Occurring Ammonium and High Sodium Content

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada is responsible for the assessment and management of health risks to Canadians associated with exposure to products and chemicals in the environment. Various by-products formed during drinking water disinfection have been found to be carcinogenic in animal studies or associated with adverse reproductive or developmental effects in laboratory animals. Efforts are made by regulators and industry to minimize human exposure to disinfection by-products. Data from the National Survey of Disinfection By-Products in Canadian Drinking Water suggest there are particular challenges in the treatment of waters with high natural salinity and naturally- occurring ammonium. Conditions in drinking water systems using these water supplies appear to favour the formation of emerging disinfection by-products, like iodine-containing trihalomethanes .

DESIGN/METHOD/DESCRIPTION: This study looked at the levels of iodo-trihalomethanes formed when treatment plants used high salinity source waters with naturally occurring ammonium. The study attempted to correlate the iodo-trihalomethane levels formed to relevant water quality and treatment process parameters.

16 treatment plants with elevated sodium content (50mg/L to over 900 mg/L) and naturally occurring ammonium source waters were included in this study. Of these, 14 water treatment plants used chlorine for water disinfection. Source, treated and distribution water samples were collected under winter/summer conditions. Samples were stabilized, shipped cold, and analysed within 72 hours for iodo-trihalomethanes, using solid phase microextraction gas chromatography with electron-capture detection. Data for 35 other disinfection by-products, water quality parameters, free/total chlorine and bromide concentrations, were also collected. Detailed information about the treatment plant processes was collected using questionnaires.

OUTPUTS/RESULTS: Elevated total iodo-trihalomethane concentrations (up to 39µg/L), well within the usual range of regulated THMs, were found in some of the systems using source waters with high salinity (over 250 mg/L sodium). The total concentrations and the speciation of iodo-trihalomethanes were strongly influenced by the bromide content of the source water and by the free chlorine residual.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: As most of the systems studied already face challenges related to high trihalomethane concentrations, the results of the present study could provide guidance for decisions relating to disinfection and treatment strategies for these drinking water treatment systems.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: These data provide drinking water-related exposure information for the iodo-trihalomethanes produced in water treatment systems using particularly challenging water sources. Data will be used by the drinking water group in WAQB for risk assessment and risk management decisions relating to iodine-based disinfection by-products. They will also be used in prioritizing potential contaminants for drinking water guideline development and in assessing the need for additional toxicity studies for these compounds.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Genomic sequence data will aid in understanding the population structure of *Cryptosporidium* and provide new tools for determining the sources and improved risk assessments.

1.43 Comparison of Persistent Organic Pollutants (POPs) and Metals in Primiparous Women from Canada and Mexico

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OBJECTIVES/BACKGROUND/ISSUE(S): Under the North American Commission for Environmental Cooperation (CEC) and its Sound Management of Chemicals (SMOC) program, a tri-national human contaminant monitoring initiative was completed. This pilot biomonitoring study collected blood samples from primiparous women in Canada and Mexico, to produce baseline exposure information for several environmental contaminants.

The purpose of this analysis was to determine differences in the concentrations of environmental contaminants among the Canadian and Mexican participants in the biomonitoring study after adjusting for several variables such as age, ever smoking status, pre-pregnancy BMI, years at current residence, and monthly family income.

DESIGN/METHOD/DESCRIPTION: Blood samples were collected from primiparous women in Canada and Mexico, and were analysed for a suite of environmental contaminants including polychlorinated biphenyls (PCBs), dichlorodiphenyldichloroethylene (p,p'-DDE), beta-hexachlorocyclohexane (β-HCH), mercury and lead. Study participants completed a questionnaire providing information on demographic variables. Multiple linear regression analyses were then conducted using data from Canadian and Mexican primiparous mothers to assess differences in concentrations after adjusting for demographic covariates.

OUTPUTS/RESULTS: Stepwise multiple regression models found ethnicity group, ever smoked status, age and pre-pregnancy BMI were significant covariates for several contaminants measured (for which at least 70% of samples were detected). Scheffé multiple comparisons were performed and found differences between ethnicity groups (i.e., Canadian-born, Canadian-foreign born, and Mexican), when other factors were held constant, for cadmium, cobalt, lead, nickel, selenium, p,p'-DDE, trans-nonachlor, and B-HCH.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Concentrations of p,p'-DDE, β-HCH, and lead were found to be higher among Mexican participants; however, concentrations of most PCBs (except PCB 180) among Mexican participants were similar to Canadian primiparous women after adjusting for covariates. Concentrations of total mercury were generally higher among Mexican primiparous women although the difference between Mexican and Canadian mothers was smaller as age increased.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: While these results are not nationally representative due to the non-probability sampling strategy used, it does provide an initial dataset of regional data and analysis of POPs and metals concentrations in first-birth mothers from these countries. This can be used to inform priorities for future activities and to track progress in the management of the selected chemicals, both domestically and on a broader cooperative basis within North America.

1.44 Muta™Mouse Primary Hepatocytes as a Promising New Tool for *In Vitro* Mutagenicity Assessment

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada routinely uses animal cells grown in the laboratory to assess chemical toxicity. The increasing use of cells grown in the laboratory reflects a global shift away from toxicity testing in experimental animals. Induction of genetic (DNA) damage is correlated with a chemical's ability to cause cancer, and assessment of genetic toxicity is an important component of regulatory evaluations. Unfortunately, genetic toxicity assessment in cultured cells is technically challenging. One problem is a lack of physiologic similarity between cells grown in the laboratory and healthy animal tissue. Additionally, some chemicals that cause genetic damage must be processed in organs like the liver, and this metabolic processing is difficult to simulate.

DESIGN/METHOD/DESCRIPTION: The goal of this ongoing project is to characterize liver cells (i.e., hepatocytes) isolated from a strain of mouse known as the Muta™Mouse, and use them to develop an improved system for genetic toxicity assessment.

The hepatocytes were isolated from the Muta™Mouse, grown under optimal conditions in the laboratory, and characterized to assess their utility for the detection of chemically-induced genetic toxicity.

OUTPUTS/RESULTS: We confirmed that the isolated hepatocytes are capable of dividing in culture, are metabolically active, and retain the traits of typical hepatocytes. In addition, the hepatocytes were shown to be capable of detecting known genetic mutation-causing chemicals using the Muta™Mouse mutation scoring system.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: We have developed a novel test for the regulatory assessment of chemically-induced genetic toxicity using primary liver cells from a strain of mouse known as the Muta™Mouse. This test was designed to address the drawbacks associated with existing cell-based assays. The cells behave like normal liver cells, are capable of metabolizing chemicals, and permit the use of the reliable Muta™Mouse mutation scoring system. This test has been shown to be capable of detecting known mutagens from different chemical classes, and it is anticipated that this novel assay will be a valuable complement to the in vitro test battery currently employed by regulatory agencies.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Once completed, this project will provide Health Canada, and the regulatory toxicology community in general, with a valuable tool for efficiently assessing the hazards posed by new and existing chemicals.

1.45 Systematic Characterization of Lung Toxicity Induced by Chemically Coated or Uncoated Carbon Nanotubes of Two Different Sizes in Mice

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OBJECTIVES/BACKGROUND/ISSUE(S): Carbon nanotubes (CNTs) are the third most extensively produced and used nanomaterials globally, making human exposure to CNTs in the environment inevitable. While their nano-size associated properties make them commercially desirable, the fibrous nature of CNTs mimics that of asbestos fibres (widely known cancer causing environmental chemical) suggesting that CNTs behave like asbestos toxicologically. The lung responses to CNTs are characterized in rodent models; however, the gaps in the data related to exposure, hazard, the biological mechanisms of CNT-induced toxicity and link between physical-chemical properties and toxicity have hindered the regulatory decision-making process.

DESIGN/METHOD/DESCRIPTION: Gene expression profiling tools were used to investigate the lung toxicity mechanisms in mice exposed to small or large CNTs unmodified or surface-modified with three different chemical groups.

An analysis of the whole mouse lung was performed to identify genes with increased or decreased expression after CNT exposure. Statistical and bioinformatics analyses were conducted to determine the biological processes activated or repressed by CNTs. The results were compared to the published data on known lung diseases to determine the ability of CNTs to induce lung fibrosis (scarring of tissue) and cancer.

OUTPUTS/RESULTS: Preliminary analysis revealed that all CNTs are capable of inducing lung diseases. Smaller CNTs induce greater biological responses and the magnitude or the severity of the response is influenced by the chemical modifications. However, the underlying biological mechanisms of CNT-induced lung response leading to lung inflammation or fibrosis were the same.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Although all CNT types are known to be harmful in rodents, the results of the study identify specific properties of CNT that are more important to their toxic potential. Future experiments will focus on examining the gene signatures and altered biological functions to better understand and predict the harmful effects of CNT exposure.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Understanding the toxic effects of CNTs of varying physical-chemical properties will be essential and urgently needed to predict the hazards posed by the uses of this diverse group of nanomaterials. As part of the Chemical Management Plan nano initiative, the results of the study have enhanced our understanding of the influence of CNT length and chemical modification in relation to their toxicity, which can be used for the risk assessment of CNTs.

1.46 Protein Markers of Vascular Changes in Healthy Volunteers Exposed to Source Emission

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OBJECTIVES/BACKGROUND/ISSUE(S): Through the federal Clean Air Regulatory Agenda (CARA), Health Canada is responsible for assessing and helping to manage the health risks to Canadians from exposure to air pollution produced from various industrial sectors including the steel industry. The objective of this study was to assess whether exposure to air pollutants emitted from a steel plant can lead to cardiovascular changes in healthy humans.

DESIGN/METHOD/DESCRIPTION: The goal of this study was to determine if changes in target protein markers of cardiovascular function and inflammation could predict changes in air pollution exposure levels and physiological changes, such as blood pressure, in healthy individuals to identify source emission-related cardiovascular effects.

We used biochemical assays to measure levels of protein markers such as big endothelin-1 from the blood and saliva of healthy volunteers at a site near a steel plant with or without a personal air filter system as well as those at a site several kilometers away from the plant. Statistical analyses were conducted to evaluate potential associations of these biomarkers with air pollutant levels at the different sites of exposures.

OUTPUTS/RESULTS: Air pollutant levels of nitrogen dioxide, sulfur dioxide, ozone and particulate matter (particle size ≤2.5 um) were measured using a fixed-site ambient air quality monitor. Ultrafine particles (particle size 0.01–0.1 um) were measured using a TSI®Model 3007 ultrafine particle counter. Air pollutant levels were higher near the steel plant than away from the plant. Changes in protein levels provided an indication of biological responses to air pollutant exposures. In general, protein marker levels were positively associated with blood pressure levels. Furthermore, big Endothelin-1 levels were correlated with air pollutant levels at the site and blood pressure measurements. This data suggests that changes in protein levels (in particular big Endothelin-1) could be markers of events in the pathways of air pollution induced cardiovascular effects.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Observations from this study suggest that source emission exposure can impact negatively on cardiovascular performance. This information contributes to the mechanistic understanding of source emission-induced adverse health effects.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Such mechanistic information is helpful in characterizing risk associated with specific source emissions and thus contributes to decision making in regulatory processes under CARA.

1.47 Association of Plasma Markers of Vascular Performance and Infant Birth Weights

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OBJECTIVES/BACKGROUND/ISSUE(S): During pregnancy, environmental pollutants may affect the health of both mother and child. Health Canada scientists, in collaboration with other researchers, are involved in a Canada-wide mother-infant cohort study (Maternal-Infant Research on Environmental Chemicals-MIREC) to better understand how the above factors affect maternal and perinatal health.

DESIGN/METHOD/DESCRIPTION: The goal of this work was to assess the levels of biological markers relevant to vascular integrity in plasma of women in their third trimesters from the MIREC study to test for associations with adverse pregnancy outcomes.

In order to investigate the biological mechanisms involved in adverse pregnancy outcomes (such as low/high infant birth weight), third trimester maternal blood samples from the MIREC cohort were analysed for changes in multiple target biomarkers of vascular function namely matrix metalloproteinases (e.g. MMP-2, MMP-9), endothelin isoforms, C-reactive protein and cellular adhesion molecules. Statistical analyses were conducted to determine association between maternal biomarkers and infant birth weights or gestational age.

OUTPUTS/RESULTS: Changes in the levels of these markers especially markers of endothelial function such as endothelins and MMPs seem to influence alteration in infant birth weights and time of gestation. Our findings also revealed a relationship between maternal oxidative stress and vascular parameters.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: These findings will help us to focus future in-depth investigations in the larger MIREC population to understand the specific maternal risk factors namely, chemical exposures that lead to adverse pregnancy outcomes.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Information generated from this project will support the Department's biomonitoring efforts and the risk assessment process relevant to a vulnerable population.

1.48 The Role of Maternal Biomarkers in Identifying Associations Between Prenatal Chemical Exposures and Pregnancy Outcomes

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OBJECTIVES/BACKGROUND/ISSUE(S): Exposure to environmental chemicals (e.g. metals and phthalates) during pregnancy can affect the health of both mother and child and may lead to adverse pregnancy outcomes, such as low infant birth weight and preterm birth. Health Canada scientists, in collaboration with other researchers, are involved in a Canada-wide mother-infant cohort study (Maternal-Infant Research on Environmental Chemicals-MIREC) to better understand how these exposures affect maternal and perinatal health. Understanding chemical exposure-related alteration of maternal biological pathways and pregnancy outcomes is critical for risk estimation.

DESIGN/METHOD/DESCRIPTION: The goals were to study the associations between biomarkers and maternal physiological changes/pregnancy outcomes, and between maternal biomarkers and levels of chemical exposures. This exercise will help us to identify risk associated with the different chemical exposures.

In this study, we analyzed multiple biomarkers relevant to vascular performance, inflammation and oxidative stress in the third trimester maternal blood samples from the MIREC study by using both targeted method and high-content global proteomic approach. Some of these biomarkers are reported to be affected by maternal chemical exposures. Statistical analyses were conducted to test the associations between maternal biomarkers and maternal physiological changes/pregnancy outcomes. This multiple biomarker strategy will be used to understand chemical exposure-induced toxicity mechanisms related to adverse birth outcomes.

OUTPUTS/RESULTS: Our results to date indicate that infant birth weight is related to levels of specific maternal blood biomarkers. Also, maternal oxidative stress and inflammation appeared to influence the pregnancy outcomes by altering vascular performance in mothers.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: These findings will advance our understanding on maternal adverse outcome pathways related to chemical exposures. Biological plausibility of associations between prenatal chemical exposure and adverse birth outcomes will be established using this biomarker approach permitting the determination of point of departure values for the corresponding chemical exposures.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Information generated in this work will address some of the data gaps and enable chemical exposure-related risk analysis with minimal uncertainties, as these data are from a human cohort study. This work will also support the development of early preventive intervention, and can have positive impact on science and policy decision making.

1.49 A High Content Proteomic Screening Approach to Investigation of Air Particle Toxicity Using the J774 Cell Line

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada is responsible for evaluating the health risks posed by chemicals to the Canadian population. Airborne particles found in air pollution represent an important source of chemicals. When inhaled and deposited in the lungs, these particles interact directly with cells, and studies have shown that some particles pose health risks. This study investigated the response of cells to particles to generate information on toxicity mechanisms that will be useful in assessing the risk posed by their inhalation.

DESIGN/METHOD/DESCRIPTION: The composition of airborne particles is complex and source-dependent. A systematic approach is needed that will quickly analyze particles to identify the components which can be traced to specific sources that may be harmful. Our approach is to identify differences the protein profile of cells exposed to specific types of particles, and correlate these differences to toxic responses in cells. At present, such a high through-put method of screening air particles does not exist.

We exposed macrophage cells to air particles collected across Canada, from key locations associated with air pollution (steel mills, refineries etc). We measured the health of these cells using established testing strategies such as indicators of cellular metabolism, cell membrane integrity and cell proliferation, and collected the protein content from these cells, which was further analyzed for identification of proteins affected by particle exposure.

OUTPUTS/RESULTS: Our method is able to identify protein changes that are characteristic for different particles. In order to improve the ability to detect potentially toxic particles, we are optimizing the method to identify the greatest number of protein signatures for these particle exposures. This information will also permit us to explore associations with the particles' source of origin.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: While further testing and refinement of this method are necessary, preliminary data suggest that this high-content/high-throughput approach has the potential to add significant value in terms of assessing risk due to air particle exposure.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The intent of this study is to assess protein changes as a result of source-specific particle exposures using a method that can provide a wealth of information; such an approach can complement existing cytotoxicity testing strategies. Information from this study will help inform assessment of the risk posed by air particles.

1.50 Validation of a two-color fluorescent cytosine extension assay (TCF-CEA) for rapid determination of global DNA methylation

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OBJECTIVES/BACKGROUND/ISSUE(S): Methylation of cytosine bases is a natural modification of DNA, defined as epigenetic because in controls aspects of DNA structure and the expression of many genes, but not the DNA base sequence information. Abnormal global DNA methylation is associated with cancers, infertility, developmental, neurological, immunological and age-related disorders. Rapid, safe and sensitive measurements of DNA methylation changes are needed to screen chemicals for toxicity and help to determine their mechanisms of action. We previously reported a quantitative measurement procedure (assay) that measured methylation-sensitive DNA restriction enzyme cutting by adding a single fluorescent marker nucleotide (cytosine) at the cut sites. The assay was normalized by reference to DNA cut with a methylation-insensitive enzyme, plus a spiked-in pre-labelled DNA to control for any losses during purification.

DESIGN/METHOD/DESCRIPTION: Our objective was to make the assay more operationally efficient and to improve the accuracy and reliability of data normalization by adapting it to a Two-Color Fluorescent Cytosine Extension Assay (TCF-CEA) using a single reference DNA. Serial dilutions of synthetically methylated DNA and DNA samples from cells treated with a demethylating drug (5-Azdc) were used for validation and to determine TCF-CEA detection sensitivity. Global DNA methylation levels in human HepG2, HC-04, HepRG cell lines and liver biopsy cells were compared. TCF-CEA results were also compared with data from two other global DNA methylation assays: LUMA (LUminometric Methylation Assay) and AluYB8 DNA repeat element pyrosequencing assay).

OUTPUTS/RESULTS: TCF-CEA was more efficient and less costly than the single color approach, since only one purification column was used. 100 ng of DNA could be used to detect DNA methylation levels in a few hours. The standard curve generated by TCF-CEA with serially diluted methylated DNA (R^2=0.9945) was superior to the single color method. Methylation differences of 7.5% (+/-) were measurable. Significant hypomethylation was detected in the samples from 0.05 uM to 50 uM of 5-Azdc treated HepG2 cells ($p<0.01$). Significant DNA hypomethylation was detected in human liver cell lines compared with liver tissue cells, as expected. TCF-CEA and LUMA assays similarly detected relative differences in DNA methylation.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Validation exercises showed that TCF-CEA is a sensitive, rapid, non-radio-isotopic and low cost assay for detection of DNA methylation changes. It provides a medium through-put means to screen for effects among chemical classes of interest to the Chemical Management Plan or among diverse other environmental agents in order to detect disruptions to epigenetic states with known linkages to the development of cancer and other diseases.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Improved assays that measure epigenetic mechanisms contributing to human toxicities will enhance Health Canada's ability to effectively regulate chemical agents in consumer products and the environment.

1.51 Low Dose Genetic Toxicity of Benzo(a)pyrene in the Muta™Mouse

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OBJECTIVES/BACKGROUND/ISSUE(S): Humans are frequently exposed to chemicals that can damage genetic material (i.e., they are genotoxic). If genetic damage is not repaired, the consequence can be permanent DNA sequence changes (i.e., mutations) or chromosome breakage. Genetic damage is recognized as an *enabler* of cancer, and genotoxin exposure is a human health concern. Chemical screening generally assumes that the relationships between exposure and genotoxic effects are linear to zero (i.e., there is no safe level of exposure). Consequently, genotoxicity tests are generally interpreted using a "screen and bin" approach (i.e., yes or no), with an accompanying assumption that it is not possible to identify a level below which effects are expected to be negligible. This assumption is increasingly being questioned; however, most existing data relate to effects observed only at high doses, and determination of an accurate point-of-departure (PoD) (i.e., exposure level associated with a pre-defined level of response) is rarely possible.

DESIGN/METHOD/DESCRIPTION: Using benzo(*a*)pyrene (BaP) as a model genotoxin, we examined the dose-response relationships for four genetic damage endpoints over an extended dose range (i.e., 10 doses), and scrutinised the assumption that the responses are linear to zero. Effects were measured in bone marrow, stomach, liver, small intestine, lung, and blood.

Various types of mathematical models were fit to each dose-response data set, and PoD values were calculated for each endpoint.

OUTPUTS/RESULTS: With the exception of DNA damage frequency, the best model for all datasets was not linear. The lowest PoD values were obtained for DNA damage, followed by induced mutation in bone marrow and blood, and chromosome damage in blood. The observed PoD pattern is consistent with the sequence of key events leading to mutations and/or cancer. The PoD pattern for induced mutations across various tissues is consistent with the PoD pattern for cancer in experimental animals.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The observation of non-linear responses at low levels of exposure is consistent with the existence of known mechanisms that can prevent mutation (i.e., DNA repair).

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Evidence of non-linear responses for genotoxic substances may influence the procedure employed for chemical risk assessment and management.

1.52 Differential Toxicity of Fractionated of Ambient Air Particles Investigated with Proteomics

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OBJECTIVES/BACKGROUND/ISSUE(S): Levels of respirable particulate matter (PM) in ambient urban air are positively correlated with non-accidental hospital admissions. Urban PM is a complex mixture of particles of a continuous range of sizes and chemical compositions. The adverse health effects depend on the concentration as well as the chemical and physical properties of the inhaled particles.

DESIGN/METHOD/DESCRIPTION: It is a challenge to identify which components are responsible for inducing the numerous types of adverse health effects attributed to PM. The goal of this study was to apply proteomic analyses to human cells exposed to PM in a cell culture model to assess the toxic potency of urban particles.

Human lung epithelial cells grown in the laboratory were incubated in the presence of whole PM, and separately to the water-soluble and insoluble fractions. The next day, a range of cytotoxicity tests were applied, including measurement of cell membrane integrity, energy production, cellular division and cell death. The changes in the relative abundance of proteins in the cells following exposure to particles were then examined by gel electrophoresis, image analysis and mass spectrometry to obtain more detailed information on biological effects at the molecular level.

OUTPUTS/RESULTS: The soluble and insoluble components of urban particles were found to have independent effects on human lung cells, resulting in noticeable differences in the types and quantity of proteins synthesized by cells. Interestingly, changes noted with the whole particles were more complex than simply adding the effects of its soluble and insoluble components. For example, the proteins INA, CSTF1, HSPD1 were increased with all treatments; TUBB4B, SEPT2 and UBXN1 were decrease with all treatments; ANXA7, PGK1, ACTN4 were decreased with total PM but increased with the soluble alone. Cellular effects included damage to the mitochondria and activation of cell suicide mechanisms.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Our experiments show that the biological effects in human lung cells depend on the chemical and physical properties of the PM, and that there is likely a complex interaction among the components of the PM, which are known to vary with the sources of the pollutants and their residence or aging in the atmosphere. The methods developed in this study will be useful to compare the toxicity of PM from different cities in Canada.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This work contributes to the *in vitro* toxicoproteomic and toxicogenomic approach for toxicity testing of urban particles to support assessment and management of risks associated with air pollution in Canada.

1.53 Analysis of p30 Deleted in Breast Cancer (DBC) Gene as a Potential Biomarker in Rat Mammary Gland Tumours

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OBJECTIVES/BACKGROUND/ISSUE(S): Breast cancer is an important health concern in Canada. We routinely use a rodent mammary gland cancer bioassay to assess potential cancer causing effects of chemical food contaminants. Resulting tumours in this bioassay are further analysed for specific gene products using the immunohistochemistry technique in order to develop a battery of markers for comparing tumour types generated in response to different chemical exposures. This study examined DBC gene presence in mammary gland tumours from female rats exposed to hormonal agents following cancer initiation with N-nitrosomethylurea. The DBC gene, originally believed to be deleted in breast cancer, produces a protein with multiple functions in cell survival, cell death signaling and estrogen receptor-α modulation.

DESIGN/METHOD/DESCRIPTION: To assess the value of DBC gene product as a potential immunohistochemical biomarker for rat mammary tumours. Following chemical treatments, rat mammary tumours were removed, fixed in formalin and diagnosed by a pathologist. Tumour sections were stained with DBC polyclonal antibody followed by detection of the antigen-antibody complexes with diaminobenzidine and Harris hematoxylin as the counter stain. Percentage of DBC-positive nuclear staining in tumours was determined using microscopic image analysis.

OUTPUTS/RESULTS: Two types of tumours were observed by histology, namely, ductal carcinoma cribriform (DCC) type and ductal carcinoma papillary (DCP) type. Of the 508 tumours tested, 86.4% were DBC-positive. Significantly ($P = 0.010$) greater DBC was present in DCC tumours. Test chemical related differences in DBC levels were not observed.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The greater abundance of DBC in DCC tumours suggests that DBC may be valuable in distinguishing DCC from DCP type tumours. Next steps include comparison of DBC expression with gene products that regulate cell proliferation/death and tumour suppression for further insight into characteristics of these tumours.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: While histology is necessary for tumour diagnosis, immunohistochemistry allows analysis of the presence of additional biomarkers in the form of affected gene products in specific cell types *in situ* and in single cells. Such character analysis of DCC and DCP type tumours may aid in establishing a battery of biomarkers for future applications to discriminate benign lesions from tumours, and also lesions/tumours induced by different chemical contaminants.

1.54 Cheminformatics Applied to Data Curation, Data-Gap Filling and Assessment Prioritization

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OBJECTIVES/BACKGROUND/ISSUE(S): The Safe Environments Directorate (SED) conducts assessments on chemicals under Canada's Chemicals Management Plan (CMP, an initiative aimed at reducing the risks posed by chemicals to Canadians and their environment). Moving forward, one of the key challenges that the CMP faces is assessing the potential for risk to human health of substances that have markedly poor data availability. For those that do have data, it is often scattered across multiple, non-curated sources, and needs to be normalized and standardized.

DESIGN/METHOD/DESCRIPTION: The goals of this work were to identify tools for and perform: data extraction, data processing, data-gap filling, translation of data into knowledge relevant to risk assessment, and, prioritization of chemicals for risk assessment.

To meet these goals, evaluators have used the (Q)SAR Toolbox, free software developed by the OECD, the IUCLID international standard for database management of chemical information, and a set of purpose-built programs created in-house. For data-poor chemicals, data gaps were filled using experimental data for similar chemicals in conjunction with computer modeling techniques.

OUTPUTS/RESULTS: With the tools mentioned above, evaluators were able to (1) propose groups of chemicals to be considered for risk assessment based on molecular similarity, (2) inform these groups by cross-referencing data-poor chemicals with similar chemicals having experimentally measured toxicity, and (3) model the chemicals' physicochemical properties and toxicity.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This work has helped to provide a more robust prioritization of the CMP remaining priorities, for which many have little or no data available, into chemical groupings for future assessment. Incorporation of information management, knowledge extraction, and computer modeling has played a key role in our ability to carry out this work in an improved and efficient manner.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Increasing the use of modeling and data analytic methods will help support informed decision making in the face of limited data-sets. In addition, the further refinement provided by these methods, aids in the identification of priorities for research under the CMP.

1.55 Toxicity of Silver Nanoparticles in a Mouse Model

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OBJECTIVES/BACKGROUND/ISSUE(S): Nanomaterials possess novel properties that lead to their widespread applications in biomedical, industrial and commercial fields. Health Canada is responsible for regulating the products of nanotechnology to protect Canadians from health risks. Owing to their antimicrobial properties, silver nanoparticles have been widely used in consumer and medical products; yet, knowledge about their impacts on human health is limited. Thus, there is an urgent need to assess human health risk of these nanoparticles.

DESIGN/METHOD/DESCRIPTION: The hazard of silver nanoparticles was evaluated in animal models to obtain toxicological data for silver nanoparticle safety testing. Representative silver nanoparticles (Organisation for Economic Cooperation and Development (OECD) reference materials) were introduced by instillation into the trachea of mice. The toxic effects of silver nanoparticles from single-dose and repeated-dose treatments to the animals were investigated.

The physical appearance and activity of treated animals were monitored during exposures. Tissue distribution of silver nanoparticles in the animals was examined. The pulmonary and systemic effects of these nanoparticles were assessed using multiple toxicological assays, including those for blood cell counts, tissue structure, inflammation and oxidative stress.

OUTPUTS/RESULTS: Single dose exposures of silver nanoparticles induced lung inflammation in a dose dependent manner. The effects appeared transient and the inflammation subsided by one week. Repeated exposures (weekly for four weeks) of silver nanoparticles, however, caused substantial damage in the lungs and adverse systemic effects in treated animals.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This study provides information on possible adverse effects of silver nanoparticles. Further work is required to define key mechanisms for the effects observed following distribution of these nanoparticles.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This research contributes to our understanding of the effects after single and repeated exposure to silver nanoparticles. Our study provides initial data and knowledge that will be helpful for human risk assessment. It addresses concerns on the widespread human exposure to silver nanoparticles from consumer products. Similar studies using animal models are required to better understand and estimate the potential human health hazard of these nanoparticles for regulatory purposes at Health Canada.

1.56 *In Vitro* and *In Vivo* Assessment of Potential Pathogenicity of *Bacillus subtilis* Group Strains Used in Biotechnology

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OBJECTIVES/BACKGROUND/ISSUE(S): The Domestic Substances List (DSL) of the *Canadian Environmental Protection Act, 1999* (CEPA 1999) is a compilation of substances used, imported, or manufactured in Canada for commercial purposes. DSL details living microorganisms as well as chemical substances. Many microorganisms on this list such as *Bacillus* strains are widely used in industrial and biotechnological applications including fermentation, biodegradation, and biopesticides, but have not been tested for their potential adverse effects to human health. Experimental methods are being developed to screen for the potential harmful effects of various DSL microorganisms in order to provide data for input into the screening assessments conducted by Health Canada evaluators.

DESIGN/METHOD/DESCRIPTION: This study investigated the potential harmful effects of various bacterial strains belonging to the *Bacillus subtilis* group. Characterization of their potential pathogenic attributes was performed using a number of biochemical and microbiological tests. Toxicity of these bacterial strains towards cultured mammalian cells and mice was examined with an immunology-based testing strategy.

Bacterial effects were first assessed by examining changes in viability of mammalian cell cultures. As well, the responses of mice were examined 24 h or 1 week following treatment with a single dose of bacteria introduced by instillation into the trachea. The animals were monitored for alterations in behaviour and physical activity during the exposures. Blood and lung tissue were collected for various toxicological and immunological assays.

OUTPUTS/RESULTS: The results demonstrated low toxic effects of test bacteria towards cultured cells. No changes in the activity or behaviour of treated mice were observed. All bacteria were cleared from lungs, trachea and esophagus by 1 week. Although minor inflammation in the lungs resulted from exposure to some test strains, no systemic effects were induced by any test strain.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The results suggest relatively low pathogenicity potential of the bacterial test strains. Higher doses or repeated exposures could be tested in the future to confirm the low-level hazard effects.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The study provides data to clarify pathogenicity potential of various bacteria from the *Bacillus subtilis* group which are currently used in biotechnology. The findings from this study have been provided to Health Canada evaluators so that strain-specific data can be incorporated into screening assessments reports published in the Canada Gazette.

1.57 A Model to Assess the Role of Glucocorticoids in Mediating Effects of Ozone Inhalation

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OBJECTIVES/BACKGROUND/ISSUE(S): There is increasing evidence that adverse health effects of air pollutants extend beyond respiratory and cardiovascular disease, including effects on metabolic (metabolic syndrome, type II diabetes) and neurobehavioural (cognitive decline, depression, suicide) disorders. Although seemingly distinct, these disease states are characterised by several common underlying processes, including metabolic, inflammatory, and hormonal dysfunction. We have shown previously that inhalation of the air pollutants ozone and particulate matter increases levels of stress hormones (glucocorticoids) in rats. Glucocorticoids (corticosterone in rodents, cortisol in humans) are well known for their role in the regulation of stress, but dysregulation of these hormones is also implicated in metabolic, neurological, and cardiovascular disorders.

DESIGN/METHOD/DESCRIPTION: To better understand the processes that link exposure to air pollutants and disease, in the present study we tested the hypothesis that glucocorticoid signalling is involved in mediating adverse effects of ozone exposure. Rats were exposed by inhalation to air or ozone for 4 h with or without drugs that block production or signalling of corticosterone. Corticosterone levels were measured in the blood, and effects on inflammatory, antioxidant, and metabolic pathways were assessed in the lungs and liver immediately after exposure.

OUTPUTS/RESULTS: Ozone significantly increased corticosterone in the blood, and this increase was blocked entirely by a drug that inhibits corticosterone synthesis. Ozone-induced inflammatory and antioxidant responses in the lungs were not blocked by this drug, reflecting direct responses to pollutant exposure. Inhibition of corticosterone did, however, prevent effects of ozone on specific immune system and metabolic genes in the lungs and liver, consistent with a role for glucocorticoids in mediating these effects. The impact of ozone on several other genes was unaffected by drug treatment, indicating involvement of additional signalling pathways.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The results show that we have established a model that can distinguish between effects of ozone inhalation that are mediated directly by glucocorticoids and those resulting from ozone signalling through other pathways. Further research will focus on defining the role of ozone-induced changes in glucocorticoid signalling on metabolic, inflammatory, and central nervous system outcomes.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This study provides mechanistic data to assist interpretation of population studies and inform future research aimed at assessing the risk posed by air pollutants.

1.58 Source-Dependent Differences in the Toxicity of Particulate Matter Collected Repeatedly within a Small Geographical Area

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OBJECTIVES/BACKGROUND/ISSUE(S): Airborne particulate matter levels are linked to adverse health effects such as heart and lung disease. As a result, regulations have been implemented that aim to control how much inhalable particulate matter is in the air. Although the regulations target the quantity of particulate matter, the composition of the particles can also impact toxicity. Emissions from a variety of sources contribute to the amount and composition of airborne particles, and there is limited information on the extent to which the toxicity of the material changes in relation to source contributions at any given place and time.

DESIGN/METHOD/DESCRIPTION: This project, funded under the Border Air Quality Strategy and the Clean Air Regulatory Agenda, examines whether particulate matter collected close to specific sources displays differences in toxicity, and to what extent this toxicity varies with time.

Size-fractionated particulate matter samples collected repeatedly near industrial, high-traffic, and residential sites within Windsor, Ontario, were screened in two cell lines for impacts on measures of cell toxicity and inflammation.

OUTPUTS/RESULTS: Results suggest site- and size-dependent differences in particle toxicity: larger particles from the industrial site tended to exhibit greater toxicity, while smaller particles from the traffic site generated a heightened inflammatory response. Particle toxicity at a single site varied considerably across time. Chemical analysis of collected particles displayed variation in chemical composition in relation to prevailing winds, consistent with enrichment of source contributions (e.g. higher metal and polycyclic aromatic hydrocarbon content downwind of the industrial site).

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The data show that the composition and toxicity of particles can vary considerably between sites and across time within a small geographical area in relation to source contributions. This is in line with the notion that health effects of particles depend not only on mass concentration but also on source-dependent changes in composition.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: A better understanding of source contributions to particulate matter toxicity will help guide regulatory efforts aimed at reducing the health burden due to particulate air pollution.

1.59 Comparison of Phthalate Biomonitoring Data and High Throughput Screening Dose-Response Using Pharmacokinetic Modeling

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OBJECTIVES/BACKGROUND/ISSUE(S): Advances in toxicity testing approaches have brought new ways to measure for a large numbers of biological endpoints. The leading challenge is now interpreting these results in terms that are applicable for dose exposure relevant in human or animals. This study shows how computer models of basic pharmacokinetic relationships with phthalate exposure and toxicity data can:

- Translate toxic levels seen in animal studies into equivalent blood or urine levels in humans;
- Estimate equivalent tissue doses from high throughput screening results of laboratory tests;
- Compare the internal dose estimates of potential toxicity against biomonitoring survey results.

DESIGN/METHOD/DESCRIPTION: High throughput screening (HTS) of chemicals is a rapid method to help prioritize research targets for health evaluation. This study used pharmacokinetic models and relationships to estimate these biological responses from HTS into blood or urine levels of phthalates. These model results can then be compared and related to population biomonitoring surveys.

Screening data was collected from the ToxCast™ database, now accessible through the US EPA Interactive Chemical Safety for Sustainability (iCSS) Dashboard. The results were filtered for phthalates with available metabolite data: mono-2(ethylhexyl) phthalate (MEHP), mono-n-butyl phthalate (MnBP), mono-benzyl phthalate (MBzP) and mono-methyl phthalate (MMP). A simple steady-state pharmacokinetic relationship was used to extrapolate the adverse concentration (AC_{50}) from a series of assays used for screening. A similar pharmacokinetic relationship was also used to estimate urine concentrations of their respective major mono-ester metabolites in urine.

OUTPUTS/RESULTS: The AC_{50} ranged from 5.22-62.5 µM, 1.48-53.9 µM and 5.12-34.9 µM for MEHP, MnBP, and MMP respectively. MBzP had no significant response. Pharmacokinetic parameters were obtained from published models and chemical structure-properties equations. Once extrapolated as urine concentrations, the AC_{50} assay results overlapped the range of different Biomonitoring Equivalents based on animal point-of-departure. The *in-vitro* and *in-vivo* generated screening values were greater than the population urine levels from the Canadian Health Measures Survey (CHMS); thus, indicating that levels of these phthalates are below guidance values.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The pharmacokinetic relationships and simple models in this work are scientific tools to provide the necessary weight-of-evidence for the evaluation and prioritization of chemicals. The models allow use of these biological relationships to extrapolate in vitro assays potency (AC_{50}) into in vivo tissue concentrations or point-of-departure (mg/kg/day). The modeling results can then be used for prioritization of the chemicals based on relative HTS tissue concentrations or relative potency similar to a read-across analysis. Using phthalates as an example, this research shows how to translate HTS concentrations into tissue concentrations and reduce interpretation uncertainty with biomonitoring data.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This research provides a case study of a scientific tool that can help interpret and integrate different types of biological data (ex. high throughput toxicity screen and biomonitoring surveys) to support the evaluation of chemicals in the Chemicals Management Plan.

Food Contaminants and Nutrition

2.01 A Pilot Survey of 2- and 3-MCPD and Glycidol Fatty Acid Esters in Baby Formula on the Canadian Market 2012-2013

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OBJECTIVES/BACKGROUND/ISSUE(S): Studies have identified the presence of 2- and 3-monochloropropanediol and glycidol bound in the form of fatty acid esters - (2- and 3-MCPDEs and GEs) - in many refined fats and oils. Most commercial baby formulae are fortified with refined fats / oils to obtain a required nutritional profile. Fatty acid esters of 2- and 3-MCPD and glycidol can be hydrolysed in vivo to their respective parent compounds, glycidol and 2- and 3-MCPDs. Glycidol has designation of being probably carcinogenic to humans while 3-MCPD is classified as a non-genotoxic carcinogen. The toxicological significance of 2-MCPD is largely unknown due to a lack of comprehensive data.

DESIGN/METHOD/DESCRIPTION: This pilot study probed the levels of MCPDs and GEs in baby formulas on the Canadian market because, until now, there were no data.

Occurrence of 2- and 3-monochloropropanediol esters and glycidol esters in 34 baby formulae on the Canadian market was examined. Products were purchased in 2012 and again in 2013.

OUTPUTS/RESULTS: The sum of MCPDs are somewhat lower in 2013 as compared to 2012, with respective means of 26 and 43 and maxima of 108 and 135 ng/g. The levels of GEs detected are lower in 2013 as compared to 2012, with respective maxima of glycidol equivalents of 40 and 70 ng/g and respective means of 21 ng/g and 20 ng/g.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The variations in levels of GEs and MCPDEs between different products (by a factor of ~ 20) point to a possibility of future mitigation strategies.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Health Canada's Food Directorate will use the data to update its exposure estimations and risk assessment for 2- and 3-MCPD and glycidol esters in food.

2.02 Multi-residue analysis of 328 different pesticides in various plant extracts

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada's Pest Management Regulatory Agency (PMRA) is responsible for verifying compliance of pesticide use in Canada to protect the health and environment of Canadians. The PMRA laboratory analyzes plant samples and other matrices to determine non-compliant pesticide use, i.e., the use of an unregistered product or improper use of a registered product. It is sometimes impossible to know what type of pesticides have been applied when an incident is reported to authorities.

DESIGN/METHOD/DESCRIPTION: A robust, sensitive analytical method capable of simultaneously identifying several types of pesticides in various plant extracts is being developed at the PMRA laboratory following the acquisition of a new GC/MS/MS instrument (TSQ Quantum XLS by Thermo Fisher). The method consists of a single extraction of a ground leaf sample with acetonitrile followed by purification by solid-phase extraction (SPE) columns coupled with dispersive SPE.

OUPUT/RESULTS: This improved method can identify 328 different pesticide residues simultaneously. The technology used in the new GC-MS/MS instrument (TSQ Quantum XLS by Thermo Fisher) delivers increased sensitivity and reduces not only the required sample quantity, but also the number of GC/MS and LC/MS analyses from five to two.

IMPACTS/EFFECTS/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Multi-residue analysis of pesticides by GC-MS/MS and LC-MS/MS will deliver increased sensitivity and reduce the analysis time and required starting sample size. New pesticide residues that are not currently analyzed will be added to this new method to bring it more up to date.

IMPACTS ON THE DEPARTMENT/POLICY/REGULATION: This new method will enable the PMRA to be at the forefront of new pesticide analysis technologies. With the addition of new compounds, the modification of the method and the instrumental capacities available to it, the PMRA laboratory will be better able to fulfil its mandate to protect the health and environment of Canadians.

2.03 Magnesium Bioavailability from Common Inorganic and Organic Compounds Does Not Differ in Rats Fed a High Phytic Acid Diet

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OBJECTIVES/BACKGROUND/ISSUE(S): In North America a large proportion of the population is not meeting recommended intakes for magnesium (Mg). Mg supplementation and consumption of Mg-fortified foods are strategies for increasing intakes. Various Mg compounds can be added to foods or supplements but there is limited information on the bioavailability of Mg from different compounds and efficacy in improving Mg status. Information on the bioavailability of Mg sources will improve Health Canada's ability to develop regulations on the addition of Mg to foods or supplements. For example, should some sources not be allowed because of low bioavailability? This work was performed to get a better understanding of the bioavailability of magnesium from different compounds that can be added to foods or supplements since this information is scant.

DESIGN/METHOD/DESCRIPTION: In this study we compared the bioavailability of 8 Mg compounds in a rat feeding study.

Male Sprague-Dawley rats (n=12/diet group) were fed 1 of 8 test diets supplemented with low (inadequate) levels of Mg (155 mg elemental Mg/kg diet) from Mg oxide, Mg sulphate, Mg chloride, Mg citrate, Mg gluconate, Mg orotate, Mg malate or ethylenediaminetetraacetic acid disodium Mg salt for 5 weeks. Test diets were supplemented with phytic acid (5 g/kg diet), a component found in many foods that impairs absorption of minerals. Three control diet groups were included in the experimental design. Control diets did not contain added phytic acid and were supplemented with 500 (NMgO, normal), 155 (LMgO, low) or 80 (DMgO, deficient) mg Mg/kg diet as Mg oxide.

OUTPUTS/RESULTS: Rats fed the LMgO control diet had lower ($P<0.05$) Mg concentrations in serum, bone (femur) and urine compared to rats fed the NMgO control diet with normal amounts of Mg demonstrating depressed Mg status in rats fed low Mg. Mg concentrations in serum, femur and urine did not differ ($P\geq0.05$) between rats fed the test diets indicating that Mg status of rats fed the different Mg compounds was comparable. Addition of phytic acid to the diet did not affect Mg status of the rats.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The 8 compounds tested showed similar ability to maintain Mg status of rats when fed at inadequate amounts. These results indicate that any differences in bioavailability between compounds were small and physiologically irrelevant. Comparable Mg bioavailability from these compounds means that greater emphasis can be placed on other factors when choosing the most appropriate Mg source for formulation of supplements or addition to foods such as cost, molecular weight (size) and organoleptic properties.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This research supports Health Canada's capacity to develop regulations on the addition of Mg to foods or supplements.

2.04 Whole Genome Sequence Analysis of Food and Waterborne, Zoonotic *Cryptosporidium*

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OBJECTIVES/BACKGROUND/ISSUE(S): Public Health Agency of Canada surveillance programs, including FoodNet Canada and the National Enteric Surveillance Program (NESP), monitor emerging and priority enteric bacteria, viruses and parasites. *Cryptosporidium* is one of the protozoan parasites that are monitored. Very little is known about the sources and transmission of *Cryptosporidium* in Canada. *C. parvum*, one of the two major species causing human infections, can be transmitted between humans and animals, with cattle being a major reservoir of infection. However, certain subtypes of *C. parvum* are transmitted only from human to human. Current tools for genetic typing of *Cryptosporidium* are limited and new tools are required to better understand the sources of infection and risks to humans. With the advent of new lower cost sequencing platforms it has become more affordable to perform whole genome sequencing on *Cryptosporidium* to gain a better understanding of its genetic diversity.

DESIGN/METHOD/DESCRIPTION: The objective of this study was to compare the genome of a clinical isolate of *C. parvum* with a reference isolate from cattle, to identify regions of the genome that can be used as diagnostic markers. Currently only one genome of *C. parvum,* a cattle isolate, is publicly available, thus, as part of the study, we sequenced the genome of a clinical isolate.

OUTPUTS/RESULTS: A comparison of the clinical isolate to the reference isolate of *C. parvum,* showed a high degree of sequence similarity. The isolates were identical at 20 markers currently in use for genetic typing. An examination of single nucleotide polymorphisms (SNPs) revealed less than 1,000 SNPs between the two organisms.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: We found a very high degree of similarity between the two isolates and current typing tools would not enable us to identify sources of infection, whether from humans, cattle, or other animals. This high similarity requires that more isolates be sequenced for development of improved typing tools in order to develop methods with higher sensitivity.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Genomic sequence data will aid in understanding the population structure of *Cryptosporidium* and provide the necessary information to aid in accurately determining the risks to humans of infection with this important food and waterborne pathogen.

2.05 Core Genome Sequence Typing (CGST) of *Listeria monocytogenes*: Molecular Characterization Using Genome Sequence Data

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OBJECTIVES/BACKGROUND/ISSUE(S): Studying the relatedness of foodborne pathogens (known as molecular characterization, genotyping, or simply typing) is useful for estimating variability within species, defining pathogenic lineages, and comparing isolates from different sources. Typing of pathogenic lineages helps to inform persistent sources of contamination within food processing facilities and to perform trace-back investigations in which agents responsible for sporadic or outbreak cases of foodborne illness are identified. Thus, the continual development of novel typing methods using the most current technology remains an important activity of the Food Directorate's scientific community in order to be better able to track where pathogens come from, thereby making regulatory decisions such as recalls and/or novel policies better to ultimately protect Canadians.

DESIGN/METHOD/DESCRIPTION: Using a rigorous computational approach, we set out to identify a set of genes within the entire genomes of a wide range of *Listeria monocytogenes* strains from clinical, environmental and food origins that can be used as reliable markers for fast, accurate, and highly-sensitive molecular characterization methods.

We analysed over 165 high-quality *Listeria monocytogenes* whole-genome sequence datasets with bioinformatics in order to develop completely novel typing methods. We have studied all genes within genomes and the proteins that they encode to identify suitable targets for typing methods.

OUTPUTS/RESULTS: We identified a set of 1013 *Listeria monocytogenes* genes that, when analyzed together, are useful for molecular characterization. Furthermore, we wrote software that will allow researchers and regulators to use whole-genome sequence data to better characterize *L. monocytogenes*. Finally, we developed a database for storing information about different sequence types that will provide useful information for researchers and regulators.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The CGST method developed here provides improved accuracy and resolving power relative to currently used typing methods such as pulsed-field gel electrophoresis ("gold standard"), multi-locus sequence typing, and ribotyping. Furthermore, the interpretation of results is more accessible and standardized to a wide range of investigators than either whole-genome sequence or single-nucleotide polymorphism analyses.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The intent of this study is to develop a highly reproducible, accessible, standardized and transparent molecular typing method with results that are easily interpreted. This will allow more effective regulatory and research activities by providing a level of accuracy in the identification of *Listeria monocytogenes* that is unrivaled.

2.06 Surveillance of Hepatitis E Virus (HEV) in Canadian Retail Foods

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OBJECTIVES/BACKGROUND/ISSUE(S): Hepatitis E virus (HEV), which can cause liver inflammation, is a potential emerging pathogen in Canada. While HEV is endemic in developing countries, zoonotic transmission of HEV genotypes 3 and 4 are thought to occur mostly through food in industrialized countries. Genotype 3 is responsible for most sporadic infections in Europe and North America. In fact, studies show that nearly 21% of U.S. blood donors have been exposed to HEV. Most genotype 3 infections appear to be asymptomatic. Recently, HEV genotype 3 has been shown to be able to chronically infect populations with weakened immune systems, such as organ transplant recipients, causing liver inflammation and leading to cirrhosis and potential death. This study was conducted to generate data on the prevalence of HEV in Canadian retail foods.

DESIGN/METHOD/DESCRIPTION: Three types of retail foods were tested for the presence of HEV: 100 pork products (pâté and raw sausages), 284 fresh-cut fruit and 36 leafy green samples (such as lettuce, spinach, mixed salads, etc). Most products (93%) were labelled “ready-to-eat” on the packaging which means that the consumer would eat the product right out of the package without cooking it first. To extract HEV from pork samples, a modified OPFLP-01 method from Health Canada’s Compendium of Methods was used. For fresh-cut fruit and leafy green samples, a 25g sample was rinsed with buffer and then filtered through a Zeta-Plus filter as per OPFLP-04. Viruses were concentrated using 100kDa filters and total nucleic acids were extracted using the Nuclisens EasyMag system (Biomerieux). HEV was detected using a one-step RT-PCR kit (Qiagen) targeting the ORF2 region of the HEV genome. Presumptive positive samples were confirmed by DNA sequencing and quantified using real-time RT-PCR.

OUTPUTS/RESULTS: All retail fresh-cut fruit and leafy green samples were negative for HEV. However, approximately 40% of the pork samples labelled “ready-to-eat” were positive for HEV genotype 3.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This study provides data to evaluate the current source of exposure of the Canadian consumers to HEV. Since HEV was detected by molecular approaches, their viability is unknown. Health Canada will use the results of this study to support the need for further research into this area.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: In Canada, HEV is not a nationally notifiable disease and information on illness incidence is lacking. Further research in this area is needed to fully assess potential sources of HEV exposure and to estimate any potential risk to human health.

2.07 Development of a Dry Reagent-Based Multiplex PCR Assay Incorporated in a Lab-on-a-Chip Platform for the Detection of Foodborne Pathogens VTEC and *Listeria monocytogenes*

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OBJECTIVES/BACKGROUND/ISSUE(S): Foodborne pathogens represent a significant threat to the health of Canadians. More technologically advanced systems are needed for the rapid and more efficient detection of these pathogens in foods. To this effect, key elements of a microfluidic platform were developed to detect *Listeria monocytogenes* and Vero toxigenic *E. coli* (VTEC) in food products. The Lab-on-a-Chip (LOC) platform consists of sample preparation, pathogen-specific antibody capture, PCR amplification and DNA sequence confirmation by hybridization. The objective of this study is to develop and assess the performance of LOC-integrated dried PCR reagents for easy to use one-step PCR assay.

DESIGN/METHOD/DESCRIPTION: Drying PCR reagents on a microfluidic PCR chip offers a simple device with an adequate shelf life, and provides an increased template testing capacity in the reaction. The volume of template added to a classical in-tube PCR mix is usually 1-5µl. When the PCR reagents are dried in the PCR chamber, 50 µl of bacterial suspension are added to rehydrate them, thus increasing genomic DNA (gDNA) input. The PCR reagents were dried on a piece of 125µm nylon filter and deposited inside the PCR chamber upon fabrication.

PCR reagents (without gDNA) were traditionally prepared. The drying process was optimized using a VACUFUGE apparatus (Eppendorf Inc). The shelf life of the dried PCR reagent was assessed by incubating them up to 70 days at 4°C. PCR was then performed and results visualized by gel electrophoresis and DNA hybridization. Serial dilutions of *E. coli* O157:H7 cells were prepared, and the detection limit of the PCR assay was estimated.

OUTPUTS/RESULTS: PCR and microarray hybridization with dried PCR reagents in a PCR tube and on chip was possible with *E. coli* O157:H7 gDNA after 30 days of storage in the fridge (4°C). The threshold of detection was estimated at 100 cells per reaction with *E. coli* O157:H7.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Drying the PCR reagents allows for stable, consistent and user-friendly detection approach to detect foodborne pathogens. In addition, the risks of PCR contamination are dramatically reduced due to minimal manipulation of reagents.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: On-chip amplification will offer a rapid, easy-to-use portable device for the detection of foodborne pathogens providing faster results for regulatory action.

2.08 Sulfite Analysis by Isotope Dilution Liquid Chromatography/Tandem Mass Spectrometry

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OBJECTIVES/BACKGROUND/ISSUE(S): Food safety labeling has been identified as one of the 2014-2015 operational priority of Health Canada's Health Product and Food Branch. Sulfite is a food additive that has been widely used in the food industry as a preservative and antioxidant. Because of its allergy-like effect on those hypersensitive individuals, sulfite is usually considered as a food "allergen". Even trace levels of these constituents present in food may cause severe reactions in highly sensitized individuals. Therefore, it is important for the food industry to include the accurate sulfite labelling on food commodities. The Monier-Williams (W-M) method, which is the classic method and the AOAC official method used for sulfite analysis, is not sensitive enough (method detection limit is 10 mg/kg or 10 parts per million (ppm), expressed as sulfur dioxide (SO_2) equivalent), and often introduce biased high results. Thus, when determining residual sulfite in natural sulfur-containing foods, such as garlic, onion and broccoli, a more sensitive and selective method is demanded. However, there are significant analytical challenges when using instrumental techniques for sulfite analysis because it is highly reactive and able to interact with a wide variety of other compounds and functional groups in food.

DESIGN/METHOD/DESCRIPTION: We report herein a highly sensitive and selective method for sulfite analysis by using isotope dilution liquid chromatography/tandem mass spectrometry (ID LC-MS/MS). This method integrates the selective extraction and stabilization of sulfite, the use of a stable isotope internal standard, and the application of multiple reaction monitoring (MRM) mode of detection by LC-MS/MS.

Method validation and sample analysis were conducted.

OUTPUTS/RESULTS: The method detection limits (MDL) in food samples are at sub-ppm level (sulfite expressed as sulfur dioxide (SO_2) equivalent). The practical application was demonstrated by the analyses of dehydrated garlic (MDL, 0.2 ppm), onion (MDL, 0.1ppm) and other food samples.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The current study provided detection limits meeting Health Canada labelling requirements (10ppm) and will be used to provide occurrence datasets of sulfite/sulfate in foods in support of Health Canada's new allergen labeling regulation and Canadian food agency's food compliance activity.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This project was initiated in assisting the regulating of enhanced labeling of sulfite in food; provide limits of quantitation below the Health Canada labeling requirements set to take force August 4, 2012. The method development will support data gathering required to assist in risk assessment for the presence of sulfite in foods at concentration lower than 10 mg/L. This method will also support policy development, health promotion and health safety regarding the presence of undeclared sulfite in Canadian Food Supply.

2.09 Determination of Arsenic Species in Water Samples using Ion Pair Chromatography Hyphenated to Inductively Coupled Plasma Mass Spectrometry Analysis

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OBJECTIVES/BACKGROUND/ISSUE(S): Recently released human biomonitoring data as part of the second cycle (2009-2011) of the Canadian Health Measure survey (CHMS) indicated that inorganic arsenic levels measured in urine of Canadians are higher than the internal reference dose BE (biomonitoring equivalent, calculated from existing risk assessment values (i.e. tolerable daily intakes (TDIs), cancer risk specific doses) that was calculated by Health Canada. Therefore, there is a need to estimate Canadian exposure to inorganic arsenic through various sources. Inorganic arsenic is known to be a human carcinogen, and As^{III} (arsenite) is more toxic than As^{V} (arsenate).The accurate exposure assessment of arsenic depends on its oxidation state and chemical forms (speciation), so it is important to measure species of arsenic instead of total arsenic in water systems. For these reason, there is a need to implement a rapid and accurate method for routine analysis of arsenic species. The current presentation reported a rapid analytical technique for determination of arsenic species (As^{III} (arsenite) and As^{V}(arsenate)) in water samples. This current study is part of a Chemical management plan (CMP) project (*Surveillance of arsenic speciation in various food samples)* for measuring the baseline level of arsenic species in total diet study foods and drinking water samples in Canada.

DESIGN/METHOD/DESCRIPTION: An analytical method previously used for arsenic speciation in juice matrix was modified and validated for analyzing water samples. A high performance liquid chromatography hyphenated to inductively coupled plasma mass spectrometry (HPLC-ICP-MS) was used to separate and detect arsenic speciation in water samples. An analytical column (Prodigy 3µ ODS (3) 4µm × 2.0 × 150mm) and a mobile phase consisting of 5 mM malonic acid, 3.7ml of Tetrabutylammonium hydroxide solution and 5% methanol was used to achieve arsenic speciation separation. The arsenic species were detected at m/z 75 with an Agilent 7500cx ICP-MS. The possible conversion of As^{III} to As^{V} in water samples is prevented using 2.0M acetic acid and 0.1M EDTA as preservatives.

OUTPUTS/RESULTS: The method limit of detection (LOD) was 0.1 $\mu g \cdot L^{-1}$and limit of quantification (LOQ) was 0.3 $\mu g \cdot L^{-1}$. A total of 9 samples were tested so far, which included 7 tap water, 1 spring water and 1 mineral water sample. The results showed that the levels of As^{III} were all below the LOD, while As^{V} ranged from <LOQ to 1.27 $\mu g \cdot L^{-1}$, with an average of 0.42 $\mu g \cdot L^{-1}$. Our results also found that under unpreserved condition, spiked As^{III} would fully convert into As^{V} within 6-7 hrs.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The fast, reliable and simple method used in this research was a success in the speciation of arsenic in different types of water samples. Preliminary results indicate that the levels of arsenic in samples analysed are very low compared to the Guideline for Canadian Drinking Water Quality for arsenic of 10$\mu g \cdot L^{-1}$. Further analysis will focus on using this method to complete the arsenic speciation analysis in drinking water and groundwater samples collected from provinces as proposed in our CMP project.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This study will provide information on arsenic speciation in various water samples from participating municipalities across Canada, and provide scientific data to Health Canada. These results will better inform human health risk assessments and assist in risk management, which will help to protect Canadians from potential risk related to arsenic exposure.

2.10 A Longitudinal Study of Trend and Dynamics of Molluscan *Vibrio* Species in Canada's Coastal Waters

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OBJECTIVES/BACKGROUND/ISSUE(S): Environmental factors and human activity in the estuaries are potential contributors of adaptation and evolution amongst marine bacteria, including *Vibrio* spp. Pathogenic *Vibrio* spp. have been detected in molluscan shellfish of Canadian origin, and, if present in high enough numbers, these strains are likely to cause illnesses to consumers of contaminated seafood.

DESIGN/METHOD/DESCRIPTION: Bivalve molluscs (clams, mussels, oysters) were sampled from harvest sites in Canada, for the presence of *Vibrio* species, such as *V. parahaemolyticus* (Vp), *V. vulnificus* (Vv) and *V. cholerae* (Vc), during May to October of each year from 2002 to 2013. Same methods of biochemical and molecular assays were used to identify and characterize the isolates throughout the study period. Other *Vibrio* spp., such as *V. alginolyticus* (Va) and *V. fluvialis* (Vf), were included in the study from 2007.

We investigated the trend and dynamics of molluscan *Vibrio* community detected in samples from the estuaries of Canada.

OUTPUTS/RESULTS: Out of 531 molluscan samples tested during the 12-year period, a trend was observed in the composition of the *Vibrio* community. During the first four years, Vp was detected in 45% of the 126 samples tested. From 2007 to 2013, the spectrum of *Vibrio* population appeared broader with the detection of Va (93%), Vp (49%), Vv (16%), Vf (10%), Vc (2%) and unidentified *Vibrio* spp. of marine origin (10%). Overall, 13% of the samples tested positive for pathogenic Vp with regional and periodic variations. If the study period was divided into three 4-year segments, the frequency of samples with pathogenic Vp changed from 16% to 8% to 9% in the West, and 2% to 14% to 28% in the East coast molluscs, respectively. Interestingly, one isolate of Va isolated in 2007 from the West coast, tested positive for a pathogenic marker.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: A significant outcome of this study was the increasing frequency of detection of potentially pathogenic Vp from the East coast molluscs over time. Trend and dynamics of the *Vibrio* species may be influenced by climate change.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The acquired data will be useful to the Food Directorate for establishing policies, setting standards and providing authoritative advice and information on the safety of seafood, for consumers to follow safe seafood-eating practices.

2.11 What Should I Make for Dinner this Week? An In-Depth Analysis of Supermarket Store Flyers and what is Being Promoted

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada is involved in the development and dissemination of nutrition messages related to healthy eating. Retail supermarkets often use sales flyers to promote advertised specials while increasingly providing additional food and nutrition information to help the consumer make purchase decisions. The objective of this research was to examine the quantity, types, and nature of foods advertised in supermarket flyers.

DESIGN/METHOD/DESCRIPTION: Over a 6-week period in 2014, flyers from seven of the largest supermarkets in Ontario (Loblaws, No Frills, Metro, Food Basics, Sobey's, FreshCo, Walmart) were reviewed. For each flyer, the proportion of food products within food groups, level of processing and special highlights e.g. nutrition information were recorded.

OUTPUTS/RESULTS: Over the six-week period, 6015 food and beverage products were advertised in the seven flyers. The percentage of foods advertised from each food group were 22.0% as meat and alternative, 19.1% as fruits and vegetables, 14.3% as snacks, 12.0% as grain products, 9.6% as combination foods, 8.6% as milk and alternatives, 8% as beverages, and 5.6% as other foods. Over 68% of the products advertised were processed foods. Within the flyers, internal industry advertisements with some nutrition information, rewards programs and 'holidays' were highlighted features

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Processed foods represented a large proportion of foods advertised within the flyers. Supermarket flyers have become an additional vehicle by which food and nutrition messages are now provided to the consumer. This research provided a unique evaluation of the grocery store flyers as a reflection of the food marketing environment in Canada.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This research provides further insight into the food retail environment, while providing an understanding of how and what foods are being marketed to consumers. These results provide meaningful insights into how some nutrition policy and regulatory messages might be provided to consumers and aids in our understanding of the types of consumer messages we are faced with as a government.

2.12 Foodbook: An Update on the Canadian Food Exposure Study to Strengthen Outbreak Response

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OBJECTIVES/BACKGROUND/ISSUE(S): The primary objective is to establish Canadian food exposure data at the population level. These data are compared to foods eaten by people with foodborne illness during anoutbreak, identifying foods in need of further investigation as possible outbreak sources. These data will inform timely and effective response to foodborne illness outbreaks in Canada. Other key data gaps identified by federal/provincial/territorial (FPT) stakeholders are also being addressed by the study.

DESIGN/METHOD/DESCRIPTION: This is a population-based telephone survey employing landline and cell phone sampling frames to randomly interview 11,000 Canadians from all provinces and territories over 12 calendar months. The sample will be distributed evenly over time and four age groups (0-9, 10-19, 20-64, 65+). Data collection began April 11, 2014. The survey asks about specific food, water, and animal exposures over a seven-day period, acute gastrointestinal illnesses over a one-month period, consumer food safety knowledge and practices, the incidence and burden of acute gastrointestinal illness, obesity indicators and demographic factors.

OUTPUTS/RESULTS: The Canadian food exposure and risk factor database to inform foodborne illness outbreak response will be accessible in 2015 to all stakeholders per data sharing agreements. Preliminary data that has been shared with FPT public health officials for use during outbreak investigations will be presented.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Foodbook will provide data to inform: outbreak investigations; microbial risk assessments; retail sampling components of integrated enteric disease surveillance programs; estimates of the burden of gastrointestinal illness in Canada; the relationships between eating patterns, obesity and socioeconomic status and development of disease prevention and control measures.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Foodbook data will enhance technical capacity to respond to foodborne illness outbreaks. This is crucial for timely and effective response, reducing the impact of outbreak events on the health of Canadians. Foodbook will provide critical data to inform multi-disciplinary efforts to prevent and control foodborne illness across Canada.

2.13 Scientific and Regulatory Framework for Supplemented Foods

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OBJECTIVES/BACKGROUND/ISSUE(S): A supplemented food is a product that is manufactured, sold or represented as a food that has been modified or has added substances, such as vitamins, minerals, amino acids, herbals or bioactives. Some supplemented foods are non-compliant with current food regulations; Health Canada is collecting data on these products to inform the development of a new regulatory framework. The objective of this project is to facilitate the development of evidence-based amendments that will modernize the food regulations so that currently non-compliant but safe supplemented foods will have a legal route to market.

DESIGN/METHOD/DESCRIPTION: Phase I of this project is a risk-based Temporary Marketing Authorization (TMA) process for noncompliant supplemented foods. Stakeholder TMA submissions must meet criteria set out in the *Food and Drug Regulations* B.01.054. A TMA is granted provided there is sufficient evidence of the safety of the supplement food and provided the applicant agrees to a specified risk mitigation strategy and to complete specified research needed to address data gaps and to provide marketing data. Phase II involves analysis by the Food Directorate of the research provided by TMA holders, which will inform Phase III, amendments to modernize the regulations regarding supplemented foods.

OUTPUTS/RESULTS: Several hundred supplemented food TMAs have been issued. As part of Phase I, expanding on earlier guidance published in 2012 for caffeinated energy drinks, the Food Directorate consulted with stakeholders in summer 2014 on additional draft guidance for supplemented food TMAs with respect to the definition, scope, application process, maximum levels for certain ingredients posing potential risks to health, labelling, advertising and claims.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Based on stakeholder input the supplemented food guidance is being revised for a target publication date in the winter quarter of 2015, while evaluations and decisions on TMA applications continue to be made. Initial TMA holder-generated research received to date is being assessed to improve our knowledge of these innovative foods and their marketing.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The enabling regulations for TMAs were created in order to generate information in support of amendments to the regulations. This project is allowing stakeholders to sell safe but otherwise non-compliant supplemented foods temporarily while Health Canada gathers the data needed to make informed decisions on regulatory modernization that will facilitate their long-term market access.

2.14 Concurrent Isolation of the "Top Seven" and other VTEC Serogroups from Ground Beef

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OBJECTIVES/BACKGROUND/ISSUE(S): Verotoxigenic *E. coli* (VTEC) can cause severe illness in humans when transmitted in undercooked ground beef and other foods. VTEC O157 and now six non-O157 serogroups (O26, O45, O103, O111, O121 and O145) require prompt regulatory action when found in ground beef. These "Top 6" non-O157 VTEC are more difficult to detect than O157 VTEC, and rapid screening tests often give equivocal results. Consequently methods are needed to definitively detect and isolate the Top 6 non-O157 VTEC plus O157 VTEC (the Top 7 VTEC).

DESIGN/METHOD/DESCRIPTION: We are investigating a double immunoblot method for concurrent detection of the Top 7 and any other VTEC serotypes in ground beef. Our current VT immunoblot method for all VTEC growing on a Hydrophobic Grid Membrane Filter (HGMF) is combined with immunoblot detection of the O antigens of the Top 7 serogroups on a second membrane under the same HGMF. Colonies can be identified as the Top 7 VTEC, as VTEC of other serotypes, and non-VTEC of the Top 7 serogroups.

VTEC-negative ground beef enrichment broths inoculated with individual or mixed strains of the Top 7 VTEC and other VTEC were filtered onto HGMFs. Each HGMF was laid over a VT and an O antigen capture membrane on an agar plate. After incubation, the two capture membranes were probed separately with VT antibodies or antibodies to the Top 7 O antigens. Colonies on the HGMF corresponding to stained dots on the immunoblot membranes were picked and tested for VT and O antigens.

OUTPUTS/RESULTS: In each case, isolates were recovered and confirmed as the Top 7 VTEC, other VTEC serotypes or non-VTEC of the Top 7 serogroups.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The double membrane immunoblot has potential to detect, differentiate and isolate the Top 7 VTEC, other VTEC and non-VTEC of the Top 7 serogroups. This potential is being evaluated in on-going testing of retail ground beef, with comparison to the BAX real-time PCR screening tests for the Top 7 VTEC.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Favourable results will provide the Public Health Agency of Canada, Health Canada, the Canadian Food Inspection Agency and others with reliable methods for concurrent and definitive detection and isolation of the Top7 VTEC and other VTEC, as are needed for outbreak detection, surveillance and source attribution.

2.15 The Effect of Paternal Dietary Folic Acid on Male Fertility and Embryo Development

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OBJECTIVES/BACKGROUND/ISSUE(S): Folate deficiency is associated with increased risk for neural tube defects (NTDs) such as spina bifida. In 1998, Canada made the fortification of white flour with folic acid (FA) mandatory to reduce the incidence of NTDs in women of childbearing age. However, FA fortification of a staple food like flour also increased FA intake in the general population, including men and boys. Approximately 73% and 65% of men and boys, respectively, have a folate status indicative of FA intake above the recommended Upper Tolerable level, which may have unknown benefits or harms. For example, folate deficiency has been associated with decreased sperm counts and increased sperm DNA damage, but data in male mice suggest that FA supplementation may potentially reduce the number of viable embryos.

DESIGN/METHOD/DESCRIPTION: Our objective was to determine the effect of paternal FA intake on male fertility and embryo development.

Male Balb/c mice were fed a FA deficient, sufficient, or supplemented diet and were bred with FA sufficient-fed female mice. Sperm counts, motility, and morphology, paternal organ weights, litter rates and sizes, and corpus luteum: litter size ratios were assessed as measures of male fertility. Developmental delay and congenital birth defects in embryos, as well as resorption rates (embryo death during development), sex ratios, and crown-rump lengths were assessed as measures of embryo development.

OUTPUTS/RESULTS: No significant differences among the diets were observed in sperm motility, testes weight, gestational day (GD) 16.5 embryo weight, crown-rump length, or placenta weight and diameter. However, 50% of litters from FA deficient fathers had embryos with congenital anomalies, including omphalocele (abdominal contents protrude in an enveloped sac) and gastroschisis (abdominal contents flow freely from the abdomen), or delayed development, compared to 10% of sufficient fathers. No significant differences were observed between supplemented and sufficient mice.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: No significant differences were observed in male fertility outcomes. However, the presence of embryo congenital anomalies was dependent on paternal folate status, where folate deficiency was associated with increased numbers of embryos affected by congenital anomalies or developmental delay. Our data suggest FA deficiency in males can result in an increased risk for congenital anomalies and/or developmental delay in the offspring. No significant differences in congenital anomalies were observed between supplemented and sufficient mice.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The results of this study will allow Health Canada to assess additional benefits or potential risks associated with current FA intake recommendations, including supplement use and food fortification.

2.16 Modelling the Effect of Temperature and Time on Time-to-Toxin Production of *Clostridium botulinum* in Preserved Garlic

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OBJECTIVES/BACKGROUND/ISSUE(S): Garlic-in-oil has caused several botulism outbreaks worldwide, including the largest recorded botulism outbreak in Canada. Botulism is a type of food poisoning caused by toxins produced by the bacteria *Clostridium botulinum*. The Food Directorate develops guidance for the public on reducing the risk of illness caused by microbial pathogens, including *C. botulinum*. Currently, the Food Directorate would like to investigate storage conditions (i.e., time and temperature) for home-made garlic-in-oil products to ensure that current recommended practices for consumers are effective.

DESIGN/METHOD/DESCRIPTION: Experimental data were collected from challenge studies of garlic-in-oil inoculated with known concentrations of *C. botulinum* type B spores followed by incubation at refrigeration temperatures. Samples of 1 g of chopped garlic were inoculated with 40, 400, and 4000 spores, covered with 10 ml of olive oil and stored at 4°C and 8°C for a period of 8 weeks. For each combination of temperature and inoculum level, 6 replicate samples were examined every 7 days for the growth of *C. botulinum* and toxin production. The resulting data were used within a waiting time modelling approach to assess the combination effects of temperatures and *C. botulinum* inoculum levels on the time to toxin production.

OUTPUTS/RESULTS: Experimental data from the challenge study and the waiting time modelling indicate that home-made garlic-in-oil could become toxic within one week at refrigeration temperature. In general, an increase in temperature and/or concentration of *C. botulinum* in garlic-in-oil will lead to a significant decrease in time to toxicity. Time to toxicity was affected more by temperature, than by spore inoculum level. The effects of spore inoculum level on time to toxicity showed a threshold, where concentration level increases beyond 850 spores/g, resulted in only small additional reductions of the time to toxicity.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This study indicates that effects of storage temperature and level of *C. botulinum* in chopped garlic-in-oil can be used to identify safe storage conditions for these products.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This data and model can be used to inform guidance on reducing the risk of illness caused by *C. botulinum* in home-made garlic-in-oil.

2.17 Rapid Identification and Classification of Pathogenic *Escherichia coli* by a Portable Infrared Analyzer

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OBJECTIVES/BACKGROUND/ISSUE(S): The emergence of pathogenic *Escherichia coli* has created the need for rapid and reliable methods for its sub-typing in food, clinical, and environmental samples. The sub-typing of pathogenic *E. coli* involves the further categorization of the species based on different serotypes and symptoms (pathotypes). This need may potentially be met by Fourier transform infrared (FTIR) spectroscopy, as this technique allows whole-organism fingerprinting of *E. coli* isolates in a relatively short period of time.

DESIGN/METHOD/DESCRIPTION: The infrared spectra of bacteria include absorptions from all the biochemical constituents within the cell. Accordingly, differences in the biochemical composition of different bacterial species will result in distinct infrared spectral profiles. However, the discriminatory capability of infrared spectroscopy at the sub-species level has only been investigated for a limited number of species, and most studies have employed FTIR instrumentation that is much more sophisticated and costly than the portable infrared analyzer evaluated in the present study.

An infrared spectral library of pathogenic *Escherichia coli* was compiled by recording the infrared spectra of 98 clinical isolates of *E. coli* belonging to 4 pathotypes [EHEC (serotype O157:H7), EPEC (16 serotypes), STEC (20 serotypes), and UPEC (19 serotypes)] using a portable infrared analyzer. The spectra of 20 unknowns were recorded in the same manner in a subsequent blind validation study.

OUTPUTS/RESULTS: In order to evaluate the potential of this approach for pathotype identification, each of the spectra of 20 unknown pathogenic *E. coli* acquired in the blind validation study was classified based on its similarity to the spectra in the spectral library. All 20 unknowns were classified correctly.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: These preliminary results indicate that a compact portable infrared analyzer with inexpensive single element detector can be employed for the rapid identification of pathogenic *E. coli* at the pathotype level and warrant further testing of this methodology with larger numbers of isolates.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: It has been estimated that foodborne diseases cause approximately 90 million illnesses in North America each year. In view of the prevalence of foodborne illnesses, there is a critical need for rapid methods of identification of pathogenic bacteria isolated from food matrices and/or from patients. Pathogenic *E. coli* strains are among the major foodborne pathogens causing gastrointestinal disease. The infrared analyzer employed in this study would allow for rapid pathotype identification at much lower cost than any other methodology.

2.18 Listeria Detection and Surveillance Using Next Generation Genomics

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[10] Alberta Innovates Technology Futures

OBJECTIVES/BACKGROUND/ISSUE(S): *Listeria monocytogenes* (Lm) is a major concern for human health and food safety in Canada. Recent outbreaks linked to ready-to-eat meats, cheeses, fruits and vegetables highlight the need for better detection methods including intervention strategies. To address these important issues, Health Canada's Listeriosis Reference Service collaborated with leading academic and government experts in *Listeria* and in pathogen detection in order to develop a novel test for food and food processing surfaces. The goal was to develop a quick and inexpensive assay to rapidly pre-screen and identify potential contamination prior to testing with the traditional gold standard culture-based lab test. Partners included public health (PHAC) and food regulatory (CFIA) agencies as well as Maple Leaf Foods. The work described was funded by Genome Canada, Canadian Food Inspection Agency and Alberta Innovates BioSolutions.

DESIGN/METHOD/DESCRIPTION: Whole genome sequencing (WGS) was conducted on over 200 strains strains of Lm and Listeria species associated with human illness, food and food processing environments in Canada over the past 15 years to identify biomarkers (targets unique to Listeria). The biomarkers were incorporated in a technique called loop-mediated, isothermal amplification (LAMP) as it has many advantages over traditional testing in that it can yield a result within 10-15 minutes. Preliminary assessment of LAMP assay performance and feasibility was done on artificially contaminated stainless steel and food samples. The specificity and sensitivity of the LAMP assays were independently validated using blind panels (samples where analyst not aware if LAMP assay should test positive or negative).

OUTPUTS/RESULTS: Assays were developed and validated for three biomarkers. Results were obtained within 20 minutes and were congruent with laboratory-based culture-based results. The project generated a comprehensive database of Listeria genome sequences analysed by two team-developed bioinformatics pipelines. LAMP assays were incorporated into the new Listeria culture testing method developed at the Listeriosis Reference Service.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Rapidity of compliance and regulatory testing is advantageous as ready-to-eat foods are expected to be pathogen-free. Globally, the "gold standard" lab test is culture-based isolation of Listeria species. LAMP assays can therefore be used to rapidly pre-screen and identify potentially contaminated food commodities.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Detection assays, standardization of testing protocols and guidelines arising from this collaborative endeavour will be disseminated via Health Canada's Compendium of Analytical Methods, and are expected to be used by the CFIA's food testing laboratories and the food industry.

2.19 Effects of High Fat and Sugar Diet on Tissue Retention and Distribution of Organic Contaminants in Rats with Different Genetic Background

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OBJECTIVES/BACKGROUND/ISSUE(S): Humans are exposed to various chemical contaminants such as pesticides, automotive exhaust, and flame retardants through ingestion/inhalation of contaminated food, water and air. The tissue retention and distribution of chemicals in the human body depends on the property of the chemical and the metabolic status of the human individual. While both dietary components and genetic background are known to affect energy metabolism, their effects on tissue retention and distribution of chemical contaminants ingested with food remain to be investigated.

DESIGN/METHOD/DESCRIPTION: To address this issue, we determined tissue retention and distribution of organic chemical contaminants in rats with different genetic background and given different diets.

Obese (genetically predisposed) and lean (normal) rats were given either normal nutritional or high fat/sugar diet, while also dosed with a vehicle solvent as control or a mixture of 22 chemical contaminants found in human blood. The treatment lasted for a total of six weeks, with the dietary treatment starting two weeks ahead of the chemical treatment. Different tissues/organs were collected at the end of treatments, and extracted and analyzed for levels of chemicals.

OUTPUTS/RESULTS: Genetic background had significant influence on the retention of organic chemicals in kidney, heart, spleen, brain, and adipose, which cannot be explained by differences in lipid contents. High fat/sugar diet had much greater influence on chemical retention in the liver, but much less in the serum, in the lean than the obese rats. The tissue retention profile of chemicals and its influence by genetic background also differed between chemical groups.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Genetic background, diet, and chemical property all have significant influences on tissue retention and distribution of chemical contaminants. The chemical profile of one tissue/organ may not represent the chemical profile of another tissue/organ. Tissue lipid content may not reflect tissue retention of organic chemicals. Assessment of human exposure to chemicals should consider multiple organs.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The results will have an impact on the proper use of human monitoring data for risk assessment of chemicals, which is mainly conducted based chemical levels in blood and urine, which takes little consideration of the differences in genetic background, diet, and exposure of other tissues/organs.

2.20 Consumption of Active Soybean Trypsin Inhibitors Remaining in Soy Beverages Altered the Content and Function of Estrogen and Androgen Receptors in Rat Pancreas

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OBJECTIVES/BACKGROUND/ISSUE(S): Our recent studies showed that commercial soy beverages can contain high levels of active soybean trypsin inhibitors (SBTI) if they are not properly processed. Ingestion of active SBTI markedly increased pancreatic weight, enzymatic secretion and cell enlargement in rats. However, the other health impacts of consumption of active SBTI remain to be determined.

DESIGN/METHOD/DESCRIPTION: Estrogen (ER) and androgen (AR) receptors in pancreas play important roles in mediating insulin secretion and death of insulin producing cells which can affect the onset of diabetes or cancer. The present study aimed to investigate the effects of dietary SBTI on the content and functions of ER and AR in pancreatic tissue in rats.

Weanling Sprague Dawley rats (8 males and 8 females/group) were fed diets containing either 20% casein, or soy protein with either the same amount of active or inactive SBTI for 8 weeks. At the end of the feeding, rats were necropsied for collection of pancreases. Pancreatic total and nuclear proteins were extracted. The protein content and DNA binding ability of ER (α, β) and AR were determined by western blotting and filter plate assay, respectively.

OUTPUTS/RESULTS: The results showed that the pancreatic content of AR in both male and female rats fed diets containing active SBTI were reduced by 99.1% and 83.8%, while ERβ content were attenuated by 99.3% and 92%, respectively, compared to those fed inactive SBTI ($p < 0.01$), Active SBTI remarkably reduced ER's and increased AR's DNA binding abilities to their target genes.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Overall, these results indicate that active SBTI affected pancreatic ER and AR expression and function. Long term consumption of high levels of active SBTI remaining in soy foods may adversely affect pancreatic functions.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This information is useful in the development of guidelines for the manufacturing processes of soy products and the establishment of upper safe levels of anti-nutritional factors, which are consistent with Health Canada's mission of ensuring the Canadian food supply is safe.

2.21 Comparison of Detection Methods for *Cronobacter* spp in Powdered Infant Formula

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OBJECTIVES/BACKGROUND/ISSUE(S): *Cronobacter* spp, is a bacteria commonly found in a wide variety of foods as well as onto various environmental surfaces such as those in homes, hospitals and food plants. *Cronobacter* illness is rare but can be life-threatening, especially among infants and neonates. Newborns are exposed to *Cronobacter* through contaminated powdered infant formula (PIF) although the exact contamination routes of the product are unknown. Microbiological testing is an important tool to ensure the overall safety of PIF but a fully validated method is lacking in Health Canada's Compendium of Analytical Methods.

DESIGN/METHOD/DESCRIPTION: The objective was to generate validation data for three detection methods. Those were ISO 22964:2006E, US FDA BAM 29 and the Dupont BAX detection system (a commercially available kit).

Multiple sets of artificially inoculated and blind-coded PIF samples were prepared by the organizing laboratory and sent to two other participating laboratories. The sample PIF sets comprised whey-based, whey-based with probiotics and soy-based formulations. The results were used to calculate performance characteristics for each method. Comments from the analysts concerning the ease of use, costs and general applicability were also collected.

OUTPUTS/RESULTS: The proportion of positive samples correctly identified by each method (sensitivity) were very close to each other for ISO 22964, BAM 29 and BAX (93.7%, 96.6% and 94.6%, respectively). BAM 29 required significantly more hands-on time and materials than the other two methods while ISO 22964 was the least labor- and cost-intensive. BAX gave negative screening results the fastest and was also quite sensitive, but with more work than ISO 22964. It also exhibited a high level of false positives.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Microbiological screening is a compromise between the number of samples in a sampling plan (often can be high) and the number of samples that can be tested given human and financial resources of laboratories (which tend to be low). This study has shown that beyond the apparent similarities in performance characteristics between FDA BAM 29 and ISO 22964, the latter may allow the screening of many more samples than FDA BAM 29 in a shorter time-frame and for similar expenditures. The usefulness of the BAX system was hampered by its high rate of false positives.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Following the completion of this project, a recommendation will be made to use ISO 22964 as the reference method for the detection of *Cronobacter* spp. in powdered infant formula in Canada. The reference method will be used for outbreak investigations and data collection from surveys of PIF at retail level.

2.22 Comparison of Four Methods for Detection of Listeria monocytogenes Isolates in Porcine Slaughterhouse and Cutting Facilities in Quebec

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada's "Policy on *Listeria monocytogenes* in Ready-To-Eat Foods", implemented in 2011, highlights environmental verification and control of meat processing facilities as important risk-reduction tools. To promote effective implementation of the Policy by the industry, the University of Montreal's Research Chair in Meat Safety (CRSV) aims to provide an accurate description of residual *L. monocytogenes* contamination in pork meat production facilities.

DESIGN/METHOD/DESCRIPTION: In the present study Health Canada has cooperated with the CRSV for the purpose of comparing the performance of detection methods for *L. monocytogenes* at early pork-processing steps, and to collect multiple *L. monocytogenes* isolates for further genetic characterization of bacterial populations in processing plants.

A total of 71 swabs were taken at a meat-processing facility. Samples covered various areas such as lairage, post-evisceration area, cold room and cutting surfaces. The samples were tested in parallel with 3 classical microbiological (cultural) methods (MFHPB-30, a modification of MFHPB-30, and the CRSV internal method) and a commercial DNA-based screening kit (Dupont BAX for *Listeria monocytogenes*).

OUTPUTS/RESULTS: *L. monocytogenes* was detected in 18% of the samples, which is concordant with results obtained previously in other similar facilities. Most of the positives originated from cutting surfaces, which showed the highest proportion of contaminated samples (77% of all positives). The sensitivity of all cultural methods was the same (92.9%) while that of BAX was slightly lower (85.7%). All cultural methods each missed one positive sample while the BAX system missed two. The internal CRSV method was revealed to be the most efficient cultural method in terms of hands-on-time and costs.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This study confirms the validity of results obtained with the CRSV method in prior studies and supports its use in the context of large-scale monitoring of industrial facilities. A number of *L. monocytogenes* isolates have also been collected and will be genotyped in order to better describe the variability of strains in a single plant.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This work will help provide better monitoring tools and a more thorough understanding of the distribution and variability of *L. monocytogenes* in processing plants. This, in turn, will enable the industry to better implement the Listeria Policy by targeting environmental surveillance efforts in the most efficient manner.

2.23 Genetic Modifiers of Folate, Vitamin B12 and Homocysteine Status in the Canadian Population

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OBJECTIVES/BACKGROUND/ISSUE(S): Food fortification in Canada has become an important step in the prevention of chronic diseases caused by malnutrition. For example, folic acid fortification of flour has resulted in a significant decrease in neural tube defects (NTDs) in Canada. Here we hypothesize that genetic variants, single nucleotide polymorphisms (SNPs), of genes involved in folate (vitamin B_9) and vitamin B_{12} metabolism modify B-vitamin status making those individuals more or less responsive to vitamin intake.

DESIGN/METHOD/DESCRIPTION: A panel of 116 candidate SNPs was identified through a literature screen. The SNPs were sequenced using the Sequenom iPLEX Gold platform in a sample of 3114 adults aged 20-79 years from the Canadian Health Measures Survey (CHMS), cycle 1. Red blood cell (RBC) folate, serum vitamin B_{12} and plasma homocysteine (Hcy), markers of B-vitamin status, were determined for each individual. Associations between SNPs and status were identified using logistic and linear statistical analyses.

OUTPUTS/RESULTS: We identified suite of genetic variants in 16 genes that were significantly associated with RBC folate, serum vitamin B_{12} and/or plasma Hcy concentrations. Most SNPs associated with vitamin status were in genes involved in vitamin absorption/uptake, vitamin B_{12} transport or metabolism. Other SNPs included those genes indirectly related to B-vitamin metabolism, chronic diseases association with B-vitamin status (NTDs, cardiovascular diseases and cancers) or not known to be associated with any specific physiological process.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: We have confirmed the association of a number of SNPs with B-vitamin status in this large, cross-sectional study. In addition, we show that SNPs previously associated with disease risk are also associated with B-vitamin status. We have shown that genes previously associated with NTD or colorectal cancer and cardiovascular risk, are also associated with vitamin B_{12} and folate status, respectively, providing a plausible explanation for their relationship with B-vitamin related chronic disease. Future analyses will assess the modifying effect of these SNPs on the relationship between nutrient intake from food and supplements, and nutrient status.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The outcome of this and future studies can provide information for assessing the nutritional status of the Canadian population, in particular in response to food fortification and supplement use. The information derived from this and future studies can be used to better assess the nutritional status of Canadians, to refine our recommendations for supplement use and to identify populations that have and increased or decreased susceptibility to population interventions, such as food fortification.

2.24 Rapid Identification of *Salmonella enterica* serovars using Single Nucleotide Polymorphisms

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OBJECTIVES/BACKGROUND/ISSUE(S): *Salmonella* bacteria are major causes of foodborne illness requiring prompt public health responses. Current initial responses include traditional serotyping of *Salmonella* isolates, which uses agglutination tests with antibodies to specific markers (O and H antigens) on the surface of *Salmonella* bacteria to classify the isolates into one of over 2600 recognized "serovars" (or "serotypes") of *Salmonella*. However, traditional serotyping is costly, labour-intensive and time consuming (5-7 days). Advances in analysis of pathogen genomes potentially can increase capacity and reduce the time and cost of serotyping by identifying *Salmonella* serovars at the DNA level, thereby enhancing public health responses.

DESIGN/METHOD/DESCRIPTION: We explored the potential of DNA-based serotyping of Salmonella by evaluating the ability of small differences known as single nucleotide polymorphisms (SNPs) in the Salmonella genome to classify *Salmonella* strains into their respective serovars, as determined by traditional serotyping. This initial study included 35 common serovars that together represent over 80% of reported *Salmonella* infections.

In-depth analysis of numerous *Salmonella* genomes identified 10 candidate SNPs located in a small hypervariable genome fragment present in all *Salmonella* genomes so far surveyed. Evaluation comprised targeted PCR amplification and sequencing of this fragment in three strains of 20 commonly reported serovars, and *in silico* (computer-based) targeted PCR of publicly available genomes of 15 additional serovars. The amplicon sequence data were analysed phylogenetically to determine if these 10 SNPs reliably grouped the strains into their traditionally established serovars.

OUTPUTS/RESULTS: The 35 serovars were resolved into distinct, serovar-specific clusters with 100% correlation to traditional serotyping. The tested isolates included closely related pairs of serovars such as Pullorum & Gallinarum and Saintpaul & Newport that are not differentiated accurately by other molecular serotyping methods.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Such promising results indicate this simple SNP-based approach has potential as a faster, less costly alternative to traditional serotyping. On-going efforts include testing a broader range of serovars and development of an even faster and simpler analytical platform for this assay. Importantly, this SNP-based serotyping is directly applicable to the increasing use of whole genome sequence analysis.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: SNP-based serotyping potentially will enhance the capacity, speed and effectiveness of regulatory and other public health responses of HC, CFIA, PHAC and provincial counterparts to outbreaks and sporadic cases of *salmonellosis*.

2.25 Reconciliation of Bacterial Genome Sequences with Antibiotic Resistance Profiling for *Bacillus metagerium*

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada carries out the risk assessment of microorganisms that are used for biotechnology applications. Whole genome sequence (WGS) data can be collected for microorganisms and used to show if particular genes for harmful traits are present. However, the utility of strain specific WGS data may be limited if harmful traits have not been defined at a genetic level.

DESIGN/METHOD/DESCRIPTION: Antimicrobial susceptibility is not well studied in particular *Bacillus* species. In order to develop methodology for risk assessment, we collected antimicrobial susceptibility test (AST) and WGS datasets for a range of *Bacillus megaterium* (Bm) strains. We sought to reconcile the two datasets by looking for antimicrobial resistance determinants (genes and mutations) revealed from the WGS data.

A selection of Bm biotechnology, environmental and clinical strains were subjected to both antimicrobial susceptibility testing (AST) against 17 antibiotics commonly used in clinical settings, and WGS determination. The WGS data were searched using an in-house database created for antibiotic resistance sequences.

OUTPUTS/RESULTS: The AST results showed all the Bm strains in the current study were resistant to three antibiotics. The WGS data of each Bm strain featured a gene similar to one that provides this pattern of resistance in clinically relevant Gram-positive organisms.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Bm is ubiquitously present in environment and is categorized as a low risk microorganism, however, in rare cases; it has been reported to be involved in human infections (e.g. eye, skin). The observation and knowledge of Bm strain resistance to particular antibiotics signifies that a wider choice of newer antimicrobials can be useful for effective treatment of the infection. The identification of a potential antibiotic resistance gene requires further testing.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The data generated from this project will contribute towards the risk assessment of a Domestic Substance List microbial strain assessed under CEPA 1999.

2.26 Whole Genome Based Identification of a Biotechnology Microbe Strain

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada carries out the risk assessment of biotechnology microorganisms and one component involves identification by molecular and microbiology tests. However, the results cannot always suggest species identity, especially in cases where data is conflicting, or of low resolution. Microbial whole genome sequence (WGS) data can yield predictions for marker and gene content and further analysis can lead to more precise identification.

DESIGN/METHOD/DESCRIPTION: A microbe under risk assessment featured a marker gene that provided poor resolution of species identity. Also, the microbe did not feature molecular characteristics of claimed microbial identity. We sought to identify this microbe by WGS analysis.

The WGS of a microbe under risk assessment was determined by Illumina technology and genes were predicted and annotated. Particular marker genes were compared to a public database to identify other isolates.

OUTPUTS/RESULTS: Comparison of marker genes of the assessed microbe showed a close, but non-exact match to another identified isolate from the *Bacillus cereus* (Bc) group. In order to determine relatedness to other whole genome sequenced Bc genomes, a relationship model was constructed, based on all identified genes from all Bc group whole genomes.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: While an exact match has not been established, it is probable the microbe belongs to the Bc group, that has several members of concern. This information will be incorporated into the risk assessment of the strain and be communicated to the supplier of the microbe.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The data generated from this project will contribute towards the risk assessment of a Domestic Substance List microbial strain assessed under CEPA 1999. In general, whole genome data analysis can make a significant contribution towards the risk assessment of microbes that would otherwise yield conflicting data using conventional methodologies (i.e. marker gene sequencing and microbiology tests).

2.27 Evidence for a Health Claim about Soy Products and Cholesterol Lowering

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OBJECTIVES/BACKGROUND/ISSUE(S): A health claim for a food is any representation in labelling or advertising that states, suggests or implies that a relationship exists between the consumption of a food and health. The Food Directorate assessed a submission for a health claim about protein-rich soy foods and cholesterol lowering.

DESIGN/METHOD/DESCRIPTION: The claim was evaluated against the standards of evidence set out in Health Canada's *Guidance Document for Preparing a Submission for Food Health Claims* to ensure that it is truthful and not misleading.

A systematic review was undertaken and bibliographic databases were searched for clinical or observational studies reporting on changes in serum lipids such as total and low-density lipoprotein (LDL) cholesterol following consumption of foods containing soy proteins. Studies were excluded if there was no control or comparison group and if subjects were taking any medications known to alter lipid levels. A meta-analysis was conducted with the included studies to estimate the effect of soy foods on cholesterol levels.

OUTPUTS/RESULTS: Statistically significant reductions in total and LDL cholesterol levels were observed with soy protein consumption (-0.15 mmol/L, $p<0.00001$ for both total and LDL cholesterol), representing a reduction of 2.6% and 4% for total and LDL cholesterol levels, respectively. Statistical significance was lost for LDL cholesterol levels when only studies using isoflavone-depleted isolated soy proteins were considered but there are plausible mechanisms for soy protein contributing to the food's cholesterol-lowering effect. The cholesterol lowering effect of soy protein does not appear to be modulated by gender, source of soy protein, study design the level of baseline total cholesterol.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Elevated LDL cholesterol is recognised as an important modifiable risk factor for cardiovascular disease (CVD), the second leading cause of death in Canada. The scientific evidence available supports a cholesterol-lowering effect for foods containing soy protein with their associated soy isoflavones. The intake of soy protein needed to obtain the cholesterol lowering effect is 25 g per day. Reducing elevated LDL cholesterol levels is a significant public health goal. Epidemiological and intervention data suggest that for every 1% reduction in LDL cholesterol there is a corresponding 1-2% reduction in cardiovascular events.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: By making a summary of this assessment available to stakeholders, manufacturers and sellers of any foods meeting the prescribed conditions will have the opportunity to use the claim. Although a premarket authorization is not required for this cholesterol-lowering claim, the publication of the Food Directorate's opinion will increase predictability for the food industry and facilitate enforcement for the Canadian Food Inspection Agency.

2.28 Scientific Rationale Underpining Health Canada's Proposed Daily Value for Total Sugars

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OBJECTIVES/BACKGROUND/ISSUE(S): Consultations on ways to improve nutrition labelling indicated that Canadians want to be able to appraise whether their foods contain a little or a lot of sugars. To this end, Health Canada proposed a Daily Value (DV) for total sugars that will be the basis for the declaration of the % DV in the Nutrition Facts table. The purpose of this work is to describe the scientific rationale underpinning this proposal.

DESIGN/METHOD/DESCRIPTION: DVs for use in nutrition labelling are based on reference values established by authoritative bodies. Since there are no such values for sugars, Health Canada developed a DV for total sugars that is as low as possible and compatible with an achievable, health promoting diet.

In setting the proposed DV for sugars, Health Canada considered the following: 1) sugars intake of the Canadian population; 2) sources of sugars intake by Canadians; 3) ability of the proposed DV to identify the sources of sugars that are a concern; and 4) consistency with Canada's Food Guide (CFG) recommendations.

OUTPUTS/RESULTS: The average total sugars intake among Canadians 4 years or older was 20.8% of total energy. Among the top contributors to sugars intake, all but two categories of foods (fruits and unsweetened milk) are sources of free sugars (sugars added to foods or naturally present in juices, syrups and honey). A DV for total sugars set at 20% of total energy, which corresponds to 100 grams based on a 2000-calorie diet, flagged many of these foods as containing "a lot" of sugars using the 15% DV threshold. Analysis of simulated diets compatible with CFG recommendations revealed that the total sugars content of approximately half of those diets exceeded 20% of total energy; however, the sugars were from sources that are consistent with a healthy eating pattern, such as fruits and unsweetened milk.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The proposed DV is an ambitious, yet achievable target. It could help Canadians reduce their sugars consumption; however, policy options might need to be considered to accommodate nutritious foods containing naturally-occurring sugars.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Health Canada is in the process of finalizing the proposed changes to nutrition labels. The results of this work could help inform decisions related to the proposals.

2.29 Fermentable Carbohydrates Fed at Similar Total Levels of Fermentation Exert Different Effects on Colon Tumour Formation in Azoxymethne-Induced Male Fischer 344 Rats

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OBJECTIVES/BACKGROUND/ISSUE(S): The Health Canada dietary fibre (DF) definition acknowledges the role of microbial fermentation in providing absorbable energy through the production of fermentation end products. In addition to providing energy, one of these products, butyrate, has been postulated to have beneficial effects in humans by promoting colonocyte apoptosis and differentiation. This is thought to potentially reduce the risk of colon cancer. The present study was designed to test the hypothesis that butyrate production by itself is the important factor in a model of chemically induced colon cancer in the rat. While the chemical-rat model does not translate well to humans, it does provide a readily testable environment for probing relationships between types of DF and outcomes.

DESIGN/METHOD/DESCRIPTION: The design relied on feeding rats a constant amount of total fermentable material to see what effect the source material had on physiological outcome: in this case tumor load/number and gene response.

Six diets were used: one without fermentable material (control) and the others with 3% fermentable material from 5 different sources: fructooligosaccharides (FOS), polydextrose (PD), wheat bran (WB), oat bran (OB), or high-amylose maize starch (HAMS; a form of resistant starch). After 2 wk on the diets, rats were injected with AOM and sacrificed after an additional 24 wk. Tumor numbers and characteristics were recorded. An additional group of rats was fed diets containing either FOS or WB at different concentrations and gene expression in the colon epithelium was measured.

OUTPUTS/RESULTS: Different fermentable materials had different impacts on colonocyte gene expression, colon tumor formation, and tumor growth.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Although a defining characteristic of DF, fermentation alone is not predictive of the physiological effect fermentable materials have in the large intestine and colon. The source of the material appears to have an important effect on outcomes.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This study has implications for Health Canada messaging around healthy diets and policies on package labelling. The colonic health effects of dietary patterns associated with high fruit/vegetable/whole grain intake have long been hypothesized to be a result of increased intakes of phytochemicals and DF. These include a lower risk of colon cancer and other colonic disease. Part of this hypothesis includes the idea that the principal causative agent is butyrate, formed through fermentation of DF by the resident colonic microbiota. While it is not possible to directly test this hypothesis, it is possible to assess whether different fermentable materials exert the same physiological outcomes. Our results show that not all DF are created equal, and that proposals to make diets healthier simply by increasing the amount of fermentable material (regardless of source) are misinformed.

2.30 Cardio-Metabolic Disease Risks and their Associations with Circulating 25-Hydroxyvitamin D and Omega-3 Levels in South Asian and White Canadians

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OBJECTIVES/BACKGROUND/ISSUE(S): Vitamin D and omega-3 fatty acids status are associated with many health outcomes such as cardiovascular (CVD) and diabetic diseases. However, the omega-3 status in the Canadian population has not been assessed. The association of omega-3, vitamin D status and cardio-metabolic disease risks in different ethnic groups has not been investigated.

DESIGN/METHOD/DESCRIPTION: This study was aimed to determine the serum 25-hydroxyvitamin D [25(OH)D], red blood cell omega-3 status and cardio-metabolic disease risk factors and their association in South Asian (SAC) and white Canadians (WC) living in Ottawa, Canada. In total, 308 SAC and 341 WC aged 20 to 79 years living in Ottawa were recruited. Fasting blood samples were taken and analysed for 22 risk factors for type 2 diabetes and CVD.

OUTPUTS/RESULTS: SAC had significantly higher omega-3, but lower 25(OH)D levels than WC ($p < 0.05$). Many of the risk factors for cardio-metabolic diseases measured in SAC were markedly higher than in WC ($p < 0.05$). Body mass index (BMI), serum 25(OH)D and omega-3 indices were correlated with 14, 12, and 4 of the remaining 21 risk factors measured, respectively. Adequate or optimal levels of 25(OH)D were associated with lower BMI, insulin and leptin levels in WC, but not in SAC. Intermediate or high levels of omega-3 indices were related to lower risk factors for CVD in WC, but to none of the risk factors measured in SAC. Normal BMIs were favorably related to 16 of the remaining 21 risk factors in WC, but only 9 in SAC.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The associations of vitamin D, omega-3 status, BMI and risk factors were more profound in the WC than SAC. Compared to WC, vitamin D status and omega-3 index may not be good predictive risk factors for the prevalence of CVD in SAC.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This information is important for Health Canada in the development of future nutritional policy related to prevention of chronic diseases and dietary recommendation.

Toxicology

3.01 Expert Elicitation to Estimate Source Attribution for 28 Enteric Pathogens via Foodborne, Waterborne, Animal Contact and Person-to-Person Transmission

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OBJECTIVES/BACKGROUND/ISSUE(S): Enteric illness (e.g. "foodborne" illness) is a significant contributor to the burden of illness in Canada and globally. Understanding sources of enteric illness is key to reducing that burden; however, there is a paucity of available data for exploring sources of transmission of enteric pathogens. This work originated within PHAC to address the broad issue of enteric disease transmission, and the complexity associated with many of these diseases that are transmitted by multiple routes, to inform the development of Canadian Burden of Illness estimates for enteric pathogens transmitted by food, water and animal contact.

DESIGN/METHOD/DESCRIPTION: This project aims to estimate sources of transmission for 28 enteric pathogens using expert elicitation to support PHAC priorities to improve understanding of sources of illness to inform prevention, promotion and protection efforts. Expert elicitation is the surveying of a group of subject matter experts on a particular risk and synthesising the collected opinions. It is a useful tool to answer scientific questions about risk where data are difficult or expensive to collect.

A six stage expert elicitation was implemented. Thirty-one Canadian experts in food safety, water safety, public health, veterinary medicine, etc. were recruited for the study. In the survey, they were asked to estimate what proportion of cases of infection with each of 28 enteric pathogens were transmitted via foodborne, waterborne, animal contact, person-to-person and other transmission pathways, based on contamination (e.g. of food, water) at the point of consumption. In addition, they were asked to rank their uncertainty in their estimates, to inform confidence levels for each estimate.

OUTPUTS/RESULTS: Experts produced estimates of enteric illness transmission. Foodborne transmission was the dominant transmission pathway identified for single and multi-source pathogens followed by waterborne transmission.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This research provided an important first look at transmission of 28 enteric pathogens over all possible transmission routes. This study identified knowledge gaps for some pathogens.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This work is being used to inform the development of Canadian Burden of Illness estimates for enteric pathogens transmitted by food, water and animal contact, and to highlight vulnerabilities in transmission pathways which could inform future policy and intervention activities.

3.02 Do Socioeconomics Influence Susceptibility to the Physiological Effects of Air Pollution

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OBJECTIVES/BACKGROUND/ISSUE(S): The volume and type of traffic and exposure to air pollution have been found to be associated with respiratory health, but few studies have considered the interaction with socioeconomic status.

We investigated the relationships of respiratory health related to traffic type, and traffic volume, stratifying by socioeconomic status (education and income level) in 1570 schoolchildren.

DESIGN/METHOD/DESCRIPTION: Roadways near subjects' residences was identified by using land use regression models. Interquartile range changes in traffic exposure were linked to respiratory health symptoms and objective measures of lung function using generalised linear mixed models for three levels of income and education.

All data related to socio-economic status is for the year 2005, which is when the Windsor Health study with pulmonary lung functions testing occurred. From this data, students are classified into three categories of family income (low, medium and high), and three categories of education. First, we have identified significant factors impacting respiratory health outcomes; we then generate the percent change in health outcomes and the accompanying standard errors in relative risks, for each strata of social economic status.

OUTPUTS/RESULTS: In a few of the lower and mid-range educational attainment groups, the prevalence of reported wheezing was positively associated with traffic density measured by turning movement counts between 0700HR and 1800HR No significant effects of traffic density on respiratory symptoms were seen for any of the groups stratified by total household income.

In the group with family incomes under $35,000 yearly, a decrease in 1-second forced expiratory volume (FEV1) was associated with an increase in traffic density measured by simple traffic counts or turning movement counts. The largest observed effect was a -3.75% (95% CI -5.57, -1.92) change in FEV1 for an interquartile increase in turning movement counts between 0700HR and 1800HR. In the mid-range and higher income groups, no significant effect was found. No significant effects of traffic density on FEV1 were seen for any of the groups stratified by greatest household educational achievement.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: There were significant increases in wheezing and reductions in FEV1. However, no significant effects were seen in the highest education and income groups. This provides some evidence that lower social status may increase susceptibility to traffic related air pollution.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This project strengthens the Clean Air Regulatory Agenda (CARA) mission in terms of supporting clean air regulations for the transportation sector. Risk estimates from this study may also be used in Air Quality Benefits Assessment Tool (AQBAT) to evaluate the health impacts of traffic-related air quality more accurately and estimate the total cost of traffic pollution, or to develop strategies to control or mitigate traffic pollution costs. It also contributes a better understanding of who is the most affected by traffic pollution. From this, better air quality standards and transportation guidelines can be put in place.

3.03 Maternal and Infant Exposure to Environmental Phenols as Measured in Multiple Biological Matrices: The Plastics and Personal-care Products use in Pregnancy (P4) Study

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada is responsible for the assessment and management of health risks to Canadians associated with exposure to products and chemicals in the environment. Results of recent national surveys have shown the high prevalence of exposure to bisphenol A (BPA) and triclosan among the general population; however, biomonitoring data for pregnant women and infants are limited. BPA is an industrial chemical that has been used in the manufacture of polycarbonate plastics and in paperboard packaging, adhesives, and thermal receipts, and is also found in epoxy resins used to line metal food and beverage cans. Triclosan is used to preserve materials such as textiles, leather, paper, plastic, and rubber, and as an anti-bacterial and anti-fungal agent in a number of cosmetics and personal-care consumer products, including non-prescription drugs and natural health products. Given the high prevalence of exposure to these chemicals and the uncertainty raised by some animal studies relating to their potential health effects, it is important to measure exposure in susceptible populations such as pregnant women and infants.

DESIGN/METHOD/DESCRIPTION: We conducted this study to measure exposure of pregnant women and their infants to these chemicals. Women (n = 80) were recruited from early prenatal clinics and asked to collect urine samples multiple times during pregnancy and once 2–3 months post-partum. Samples of infant urine and meconium (baby's first stools) as well as breast milk and infant formula were also collected. These samples were analysed for BPA and triclosan.

Our partner lab at INSPQ developed sensitive methods for measuring these chemicals in unique biological materials such as meconium and breast milk, as well as urine.

OUTPUTS/RESULTS: Triclosan was detected in over 80% of the maternal urine samples (mean 21.61 µg/L), 60 % of the infant urine samples, 46% of the breast milk and 80% of the meconium samples. BPA was detected in about 90% of the maternal urine samples (mean 1.21 µg/L) and only 40% of the infant urine samples.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: As expected, given the widespread use of the chemicals, this study found that most of the women had some exposure to these chemicals. Large differences in the concentrations of triclosan measured were observed among women in the study. The results will be used to fill gaps in our knowledge of exposure to these chemicals that will help to more fully assess their potential health risks.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Data from this work are being used in the final screening assessment for triclosan, as this study is providing the only available Canadian data on infants' exposure to this chemical.

3.04 Neighbourhood and City-Level Contrasts in Air Pollution and Mortality in 10 Canadian Cities

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OBJECTIVES/BACKGROUND/ISSUE(S): Previous studies have found that long-term exposure to outdoor air pollution leads to increased mortality rates. In this context, we wanted to see which was more important: the level of pollution in someone's neighbourhood, or the level of pollution in their city. We investigated the link between survival and exposure to local and citywide estimates of nitrogen dioxide (NO_2) – a marker for traffic-related pollution – among 735,600 adults in 10 large Canadian cities.

DESIGN/METHOD/DESCRIPTION: Our subjects were drawn from a national-level cohort of Canadians who completed the 1991 long-form census and who were followed for 16 years. We created models that partitioned estimates of exposure into: residential (i.e., local neighbourhood; city-wide mean; and, the sum of these (i.e., total exposures) for each year of follow-up. We used historical data to adjust the estimates according to historical levels, and residential histories to track subjects' mobility patterns. We then calculated "moving windows of exposure" that considered changes in levels of pollution over time and residential mobility patterns.

OUTPUTS/RESULTS: Increases in exposure of 5 parts per billion were associated with increased risk of mortality of 6% from non-accidental causes (95% confidence interval (CI): 4-7%), 5% from cardiovascular disease (95% CI: 2-7%), and 4% from respiratory disease (95% CI: -0.1-9%). Most of the increased risk was determined by the local, residential contrasts in exposure, as opposed to by the citywide mean contrasts.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: These findings suggest that where you live in a given city may be more important than in which city you live, in terms of air pollution exposure risk. Our results also suggest that the mixture of pollutants across a single city may be more variable than that between cities.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: We found that Canadians who live in neighbourhoods with low air pollution lived longer than those who lived in close proximity to sources of pollution, such as a busy highway, even if they were in a city with relatively high overall pollution levels. This information will help Health Canada understand how different sources and scales of air pollution relate to health outcomes.

3.05 Bisphenol A and Phthalate Metabolite Urinary Concentrations: Daily and Across Pregnancy Variability

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada is responsible for the assessment and management of the health risks associated with exposure to chemicals in the environment. Phthalates and bisphenol A (BPA) are chemicals that are used in a variety of plastic consumer products. Because these chemicals can interact with the endocrine system, they may have harmful effects on developmental and reproductive functions, which can be of particular concern for pregnant women. Little is known about how the levels of these chemicals may vary throughout the day or over a prolonged period because they are quickly metabolized and excreted from the body. This is a significant question because biomonitoring studies typically assess exposure from a urine sample collected at a single point during the day.

DESIGN/METHOD/DESCRIPTION: This study looked at urine levels of phthalates and BPA over an entire day (24-hour period) and throughout pregnancy by collecting and analyzing numerous urine samples from a group of 80 pregnant women. Women who were recruited at prenatal obstetrical clinics in Ottawa before their 20th week of pregnancy collected multiple urine samples throughout a day and then a single-spot urine sample at 3 other times points in pregnancy and when the baby was up to 3 months old.

The collected urine samples were shipped to our partner lab in Quebec, INSPQ, and analyzed for BPA and phthalate metabolites.

OUTPUTS/RESULTS: The results showed that BPA and phthalate levels varied a lot throughout pregnancy for each woman. However, within a day the levels were much more consistent. We found the highest levels in the evening for BPA and several of the phthalate metabolites. The variability in levels was higher for metabolites for chemicals for which diet is likely to be the main source (e.g., BPA, DEHP metabolites).

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: For those conducting or interpreting biomonitoring studies, repeated measurements are required over an extended period, especially when diet is the main source of chemical exposure, in order to get the most accurate picture of exposure levels.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Data from this study are being used by the Existing Substances Risk Assessment Bureau for its phthalate assessments.

3.06 Air Pollution and Emergency Department Visits for Depression in Ontario, Canada: A Multi-City Case Crossover Analysis

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OBJECTIVES/BACKGROUND/ISSUE(S): Depression is among the most common mental health problems: 1 in 10 Canadians will experience major depression at some point in their lives. Depression interferes with daily functioning and quality of life and represents a significant cost for the health care system. Many factors can contribute to depression: life stresses, some medicines and other (non-psychiatric) illnesses. In addition, air pollution has been shown to initiate or intensify symptoms of depression.

DESIGN/METHOD/DESCRIPTION: Health data were retrieved from the National Ambulatory Care Reporting System (NACRS). Daily emergency department visits for depression were retrieved from the NACRS using the International Classification of Diseases (ICD-10) codes. Associations were evaluated using a case-crossover design and conditional logistic regression models, adjusting for temperature and relative humidity. We have developed and ran several statistical models using data from nine cities in Ontario. The present results were obtained by pooling estimations across all cities.

OUTPUTS/RESULTS: We found that patients presenting to hospital emergency departments with symptoms of depression was associated with high ambient ozone and sulphur dioxide levels; as well as with dust levels and the time of year.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Knowing which air pollutants affect which diseases allows hospital emergency departments to plan and provide for specialized staff on high pollution days. In addition, people susceptible to depression can be informed of which air pollutants can adversely affect symptoms of depression; and use this information to minimize their exposure.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The results support the premis that certain air pollutants may adversely affect symptoms of depression.

3.07 Air Pollution and Emergency Department Visits for Hypertension in Edmonton and Calgary, Canada: A Case Crossover Study

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OBJECTIVES/BACKGROUND/ISSUE(S): Ambient air pollutant exposures have been associated with a wide variety of cardiovascular events; however, few studies have evaluated their impact upon acute emergency department (ED) visits for hypertension. We examine the associations between ED visits for hypertension and ambient air pollution concentrations in Edmonton and Calgary.

DESIGN/METHOD/DESCRIPTION: The purpose of this study was to examine the associations between ED visits for hypertension and ambient air pollution concentrations (adjusted for time-varying weather factors - temperature and relative humidity) among 6532 individuals during the period of January 2010 to December 2011 in Edmonton and Calgary, Alberta, Canada. Health data were retrieved from the National Ambulatory Care Reporting System (NACRS).The associations were evaluated using a case-crossover design. Conditional logistic regression models were used in statistical modelling.

OUTPUTS/RESULTS: We found statistically significant positive results for nitrogen dioxide (NO_2), sulphur dioxide (SO_2), ozone (O_3), and fine particulate matters (PM_{25}). Odds ratios (ORs) and their 95% confidence interval (CI) have been calculated for one unit increase in their interquartile range (IQR). The IQR values were 10.66 ppb, 13.42 ppb, 5.37 µg per cubic meter and 0.78 ppb, respectively for daily means of NO2, O3, PM2.5, and SO2. For example: during the cold season (October – March), statistically significant positive results were observed for SO_2 among lag days 4 to 6 and 8, OR=1.108 (95% CI: 1.040, 1.177), OR=1.077 (1.009, 1.144), OR=1.061 (1.000, 1.126) and OR=1.068 (1.0050, 1.131) respectively for females. The findings support the hypothesis that recent exposures to ambient levels of several ambient air pollutants can be capable of elevating blood pressure to a clinically significant extent such that it leads to ED visits for hypertension.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: These findings support the hypothesis that recent exposures to ambient levels of several air pollutants can be capable of elevating blood pressure to a clinically significant extent such that it leads to ED visits for hypertension.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Given the worldwide epidemics of hypertension and elevated levels of air pollution, this relationship is not any important to residents of Calgary and Edmonton, but also maybe of global importance.

3.08 UNMIX Methods Applied to Characterizing Sources of Volatile Compounds in Toronto, Ontario

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OBJECTIVES/BACKGROUND/ISSUE(S): Man-made volatile organic compounds (VOCs) contribute significantly to the urbane air pollution. Widely occurring VOCs, as e.g. Benzene, Ethylbenzene, Xylenes, 1,1,1-Trichloroethane, Trichloroethylene, Tetrachloroethylene, Carbontetrachloride, Chloroform, are toxics and/or carcinogens. In large cities the harmful effects of increasing ambient concentrations of pollutants are made even more dangerous to the public health by the extent of exposure in the densely populated areas. The main objective was to identify chief sources of the volatile organic compounds that have been measured by the NAPS stations (air pollution monitors) in Toronto, Ontario.

DESIGN/METHOD/DESCRIPTION: UNMIX–sensor modeling method from U.S. Environment Protection Agency (EPA)–was used to build a robust routine for modeling the receptors of the VOCs. The routine was employed in processing the ambient VOC concentration data acquired in years 2000–2009 for 175 VOC species in four air quality monitoring stations in Toronto.

OUTPUTS/RESULTS: UNMIX, by performing multiple modeling attempts with varying input VOC menus–and rejecting the allegedly unreliable results–allowed for discriminating sources by their most consistent chemical characteristics. The method enabled assessing occurrences of VOCs in the typical distributed sources existing in urban environment (traffic, evaporative emissions, banks of fugitive inert gases), in the industrial sources (plastic, polymer, metalworking manufactures) and, in secondary sources (evaporation from water, sediments, and contaminated urban soil).

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The algorithm for building chemical profiles of putative pollution sources produced robust VOC apportionment data. The data, combined with known environmental fates of the VOCs and known sources, can be efficiently used to assign the detected ambient emissions to physical sources. This in turn provides means of assessing the bearing of the implemented environmental policies on VOC occurrences. The distributed sources are represented by the hydrocarbons from exhaust, heavier hydrocarbons from contaminated urban soil, fugitive evaporations of gasoline and liquefied petroleum gases (LPG), leakage from the industrial and commercial use of solvents, and the inert, ozone depleting gases permeating the urban atmosphere.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The paper presents possible uses of UNMIX (EPA software) in classifying and characterizing the sources of volatile organic compound, specifically in the city of Toronto, where, therefore, the major sources and their characteristics are described in detail. The results provide means of assessing the impacts on VOC air pollution of the environmental policies.

3.09 Free and Total Bisphenol A in Human Milk Samples from Canadian Women

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OBJECTIVES/BACKGROUND/ISSUE(S): The presence of bisphenol A (BPA) in various foods, including infant formula, has been investigated recently due to its hormone mimicking properties and possible effects on human health. However, information on the presence of BPA in human milk, which is the recommended sole diet for infants up to 6 months of age and thus critical for dietary exposure assessment of this age group, is very limited, and is not currently available in Canada.

DESIGN/METHOD/DESCRIPTION: To develop a sensitive method to determine both free (unbound) and total BPA (the sum of both free/unbound BPA and bound/conjugated BPA) in human milk and generate occurrence data of BPA in human milk for exposure assessment of the infant population.

A sensitive and selective method was developed and used to analyse both free and total BPA in 278 human milk samples from the Maternal-Infant Research on Environmental Chemicals (MIREC) Study. MIREC Study was one of the initiatives on human monitoring under the Chemicals Management Plan launched by the government of Canada. One of the goals of this study was to measure the concentrations of various environmental chemicals in human milk.

OUTPUTS/RESULTS: Total BPA was detected in 72 of the 278 human milk samples (25.9%) at concentrations from <0.036 to 2.5 ng/g with an average of 0.13 ng/g, while free BPA was detected in fewer samples, 46 of the 278 samples (16.5%) at concentrations ranging from <0.036 ng/g to 2.3 ng/g with an average of 0.11 ng/g. Concentrations of free and total BPA in most samples were low (< 1 ng/g), and they are also lower than those reported in other countries.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Based on the low frequency of detection of free BPA in human milk samples, in general, dietary exposure to BPA for Canadian breast-fed infants is expected to be somewhat lower compared to exposure among formula-fed infants.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The results from this study will be used to update the dietary exposure assessment of BPA for the infant population.

3.10 Di-(2-ethylhexyl) Adipate and Phthalate Plasticizers in Human Milk Samples from Canadian Women

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OBJECTIVES/BACKGROUND/ISSUE(S): Di-(2-ethylhexyl) adipate (DEHA) and some phthalates are used as plasticizers in food packaging such as polyvinyl chloride (PVC) wrapping film and PVC gaskets of lids for glass jars. They are of increasing concern to humans due to the potential for exposure and the findings of adverse effects from toxicology studies in animals. Both DEHA and certain phthalates have been identified as priority chemicals for assessment of risk under the Government of Canada's Chemicals Management Plan. Since food is expected to be a major source of exposure for both DEHA and several phthalates, current occurrence data of DEHA and phthalates in foods including human milk are critical for accurate dietary exposure assessment.

DESIGN/METHOD/DESCRIPTION: To develop a sensitive method to determine both DEHA and selected phthalates in human milk and generate their occurrence data in human milk for exposure assessment of the infant population.

A sensitive and selective method was developed and used to analyse DEHA and eight phthalates in 305 human milk samples from the Maternal-Infant Research on Environmental Chemicals (MIREC) Study . MIREC Study was one of the initiatives on human monitoring under the Chemicals Management Plan launched by the Government of Canada. One of the goals of this study was to measure the concentrations of various environmental chemicals in human milk.

OUTPUTS/RESULTS: In general, phthalates were not detected in most of the samples, and the levels in the positive samples are low. DEHA was detected in only one human milk sample at 31.4 ng/g. Four of the eight phthalates were not detected in any of the 305 human milk samples, while the other four phthalates were detected in 31 samples or less, with the highest level of less than 240 ng/g.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Although DEHA and the eight phthalates were detected at low frequency in human milk samples in this study, and dietary exposure to these plasticizers for Canadian breast-fed infants is expected to be low, this needs to be confirmed in the follow-up dietary exposure assessment based on the occurrence data.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The results from this study could be used to support exposure assessment of DEHA and phthalates for the infant population.

3.11 Trend in Health Risks Attributable to Short-Term Exposure to Ambient Nitrogen Dioxide: Analysis of 24 Canadian Cities, 1984-2009

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OBJECTIVES/BACKGROUND/ISSUE(S): This study addresses two themes of the Clean Air Regulatory Agenda, "Public Information Tools" and "Air Quality Management System". Previous studies have established the health risk associated with air pollutants from short-term exposure within one to several days. The Air Health Indicator (AHI) was developed to track changes in this relationship over a long time period and demonstrated no linear trend based on annual analysis of the association between ground-level ozone and heart or lung related (cardio-pulmonary) deaths. The objective is to investigate trends in population health risks attributable to nitrogen dioxide (NO_2).

DESIGN/METHOD/DESCRIPTION: A time series analysis was conducted of NO2 and cardio-pulmonary deaths for 24 cities for the period 1984-2009, where annual city-specific risks were estimated adjusting for seasonal variations and temperature. Other models were used to pool the 24 city-specific risk estimates to generate a Canada-wide annual risk, and non-parametric linear trend tests were applied to detect trend in the national annual risks. A sensitivity analysis was conducted to assess impact of overtime for both NO_2 and temperature.

OUTPUTS/RESULTS: For warm season (April to September) the population-weighted annual averages of daily NO_2 concentrations and daily cardio-pulmonary deaths decreased by 53% and 14%, respectively. Overall, from all 26 years combined, the national risk was consistently higher for 1-day lagged NO_2 but sensitive to the lag of temperature: 1% with 95% posterior range (-0.5%, 2.8%) and 2% (0.3%, 3.6%) for the same day and 1-day lagged temperature, respectively, per 10 ppb NO2. However, the annual national risks did not show any linear trends either increasing or decreasing. This implies the association between NO_2 short-term exposures and cardio-pulmonary deaths has not changed over time.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The study results can be used to verify the effectiveness of measures to improve air quality and thus have potential implications for standard setting, air zone management, and international air quality agreements. The next step is to account for the correlation between ozone and NO_2 through a new risk model for two pollutants together.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Findings from the study provide evidences for consideration in preparing risk assessments, air quality standard recommendations and strategies, in updating Health Canada and Environment Canada's Air Quality Health Index (AQHI), and in conducting Health Canada's benefits assessments using the Air Quality Benefits Assessment Tool (AQBAT).

3.12 Health Impacts of the Closure of the Oakville Oil Refinery

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OBJECTIVES/BACKGROUND/ISSUE(S): Under the Clean Air Regulatory Agenda, Health Canada studies the effect of air pollution from various industrial sources on Canadian population health. The Oakville, Ontario Oil Refinery closed in 2005, providing an opportunity for a natural experiment, comprising analysis of the effects of changes in oil refinery-related air pollution on human health.

DESIGN/METHOD/DESCRIPTION: In order to evaluate the impact of the oil refinery-related air pollution on public health, we compared air pollution levels, hospitalization and mortality before and after the refinery closed. The refinery was located between two census subdivisions, Oakville and Burlington, which were considered the primary impacted areas. The next closest census divisions, the Greater Toronto Area and Hamilton, were used as reference population.

We used data from the National Air Pollution Surveillance network, hospital admission and mortality databases. We studied the associations between air pollution and health outcomes using Generalized Additive Models adjusting for temperature, weekly patterns, and seasonal variations.

OUTPUTS/RESULTS: About 6000 tonnes of sulphur dioxide (SO2) were emitted annually by the refinery prior to its closure. Following the refinery closure, ambient SO2 concentrations dropped on average 13% in Oakville and 21% in Burlington. Cold season heart-related circulatory mortality rate decreased significantly after refinery closure (in Oakville) as did respiratory hospitalization rate (Influenza and Pneumonia in Oakville and Burlington) among others. We also found a statistically significant association between SO2 and respiratory hospitalizations.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The refinery closure was associated with an immediate and measurable reduction in circulatory mortality and respiratory hospitalizations. This natural experiment provides evidence on association between refinery emissions and adverse health effects and demonstrates the health benefits of reduced exposure. Further analysis is required to identify precisely which constituents of refinery emissions are most toxic.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: These findings strengthen the evidence base specifically associating refinery emissions and adverse health effects, which provides scientific evidence to inform decisions about whether mitigation strategies may need to be recommended to reduce human exposure.

3.13 Population Exposure to Tobacco: Results of the Canadian Health Measures Survey Cycle 1 (2007-2009)

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OBJECTIVES/BACKGROUND/ISSUE(S): The Canadian Health Measures Survey (CHMS) is a population survey that consists of household interviews and direct measurements of blood and urine for environmental exposures, chronic diseases, infectious diseases, fitness and nutritional status. In CHMS-Cycle 1, urinary measures of cotinine (a metabolite of nicotine) were collected for ~5000 participants aged 6-79. Further, biomarkers of exposure to tobacco were also measured for a sub-set of ~2500 participants aged 12-79, along with NNALs (nicotine-derived nitrosamine alcohols), the biomarkers of exposure to the tobacco-specific lung carcinogen, NNK (nicotine-derived nitrosamine ketone), also known as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

DESIGN/METHOD/DESCRIPTION: This study is to estimate the prevalence of tobacco use and exposure to second hand smoke (SHS) in the Canadian population by sex and age using urine levels of tobacco biomarkers reported by the CHMS-cycle 1. Population exposure to tobacco and NNK were estimated using the sub-set of urinary biomarker data and household interviews. Cotinine was also compared against a panel of tobacco biomarkers to study population exposure to SHS.

OUTPUTS/RESULTS: The statistical analysis of data from the household interviews showed that 20.5% of Canadians are smokers, compared to 19.9% when cotinine biomarker data was used. The self-report data also found that 23.4% of non-smokers are exposed to SHS but when cotinine data alone was used SHS exposure was estimated at only 8.8%. SHS exposure estimates based on the other individual biomarkers were also substantially lower than self-report data, at 8.9%, 7.0%, 3.6% and 15.6% for cotinine-total, 3'-Hydroxy Cotinine-total (3'-HC-total), nicotine-total and NNAL-total, respectively. In contrast, the use of a panel of tobacco biomarkers to estimate SHS exposure found 17.3% of the population to have detectable levels of at least one of the four major biomarkers i.e. nicotine-total, cotinine-total, 3-HC-total and NNAL-total.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Our results suggest that cotinine is a reliable measure for estimating the prevalence of tobacco use among the Canadian population, but that assessment of cotinine only results in under-estimation of SHS exposure. A panel of tobacco biomarkers, however, may provide more accurate estimates of SHS exposure.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The research highlights the importance of choosing the appropriate tobacco biomarkers when developing monitoring population exposure to SHS. Accurate measures of exposure are required to inform future policy development for tobacco control.

3.14 Toolkit to Assess Health Care Facility Resiliency to Climate Change Impacts

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OBJECTIVES/BACKGROUND/ISSUE(S): In Canada, climate change is expected to pose greater health risks to Canadians from increases in the frequency and magnitude of extreme weather events, poor air quality, impacts on drinking and recreational water quality, food-borne diseases and zoonotic and vector-borne diseases. A changing climate threatens the quality and continuity of care provided at health care facilities that will become increasingly vulnerable to impacts from climate change without adaptation. More extreme heat events and generally warmer summers are expected to increase heat-related illnesses and exacerbate chronic diseases in the country. Furthermore, health care facilities are highly dependent on critical community services that are vulnerable to disruption during extreme weather events as evidenced by recent events in Canada and internationally. Health care facilities need a comprehensive tool to support climate change resiliency. The goal of this study was to develop a health care facility resiliency tool for use by health care facility officials across Canada to reduce climate related risks.

DESIGN/METHOD/DESCRIPTION: The Climate Change and Health Office in partnership with the Canadian Coalition for Green Health Care used a mixed-methods approach to develop, test and validate a health care facility resiliency toolkit for health care facilities in Canada. A literature review was conducted in 2012 to inform development of a health care facility resiliency tool. Indicators were identified to capture key climate change resiliency metrics to describe actions to prepare for, cope with and respond to climate change hazards. The indicators were then used to develop a resiliency assessment checklist. The checklist was then piloted in six health care facilities representing three provinces in Canada (Nova Scotia, Ontario and Manitoba). Senior level officials were invited to review the checklist and provide feedback during qualitative interviews and at a stakeholder workshop.

OUTPUTS/RESULTS: The health care facility resiliency toolkit includes an assessment checklist, a facilitator's guide and a resources guidebook. The checklist has 82 questions in 4 broad areas: general information (n=4), assessing climate-related risks (n=19), risk management (n=45) and building capacity to adapt to climate change (n=14). The facilitator's guide provides background information on climate change and instructions for officials on how to complete the checklist. The resource guide includes information highlighting effective measures that can be taken to reduce risks to the facility from climate change. The toolkit is available at http://greenhealth care.ca/climateresilienthealth care/.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This study contributes to a growing evidence base of practical information to help health care facilities adapt to climate change. Application of the health care facility resiliency assessment toolkit can supplement existing hazard identification and assessment processes, support the development and enhancement of emergency management and health care services programs and support engagement of partners in efforts to address climate change risks and build community resilience. The toolkit also highlights the importance of investing in environmentally sustainable measures to help mitigate greenhouse gas emissions, improve the state of the environment and human health. Future efforts should be made to develop a more comprehensive best practices guidebook and incorporate a scoring component to enable facility officials to track progress overtime. Similar tools could also be developed for smaller or more specialized health care facilities as needed.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The toolkit may be used by health partners to enhance the operations and sustainability of health care facilities and the health care system in general. These goals are of direct interest to the mandate and activities of Health Canada.

3.15 Associations between Air Pollution in an Industrial Area and Urinary Markers of Oxidative Stress: Results from the Sault Ste Marie Crossover Study

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OBJECTIVES/BACKGROUND/ISSUE(S): Under the federal Clean Air Regulatory Agenda, Health Canada conducts health risk assessments on air pollution emitted from various industrial sectors. Outdoor air pollution has been linked to alterations in lung function and cardiovascular physiology accompanied by the production of detrimental reactive oxygen species, a condition known as oxidative stress.

DESIGN/METHOD/DESCRIPTION: The goal of this study was to determine if human exposure to air pollution close to a steel plant can cause changes in biomarkers of oxidative stress measured in urine.

Healthy volunteers spent 5 consecutive days, 8 hours per day, outdoors either at a site close to a steel plant or at a site several kilometers from the plant. Urine was collected at the end of each on-site day and frozen for later testing. Urine samples were tested for several different markers of oxidative stress: a DNA damage product, 8-OHdG; a fatty acid oxidation marker, MDA; a stress induced growth factor, VEGF; and a stress-related inflammatory mediator, 8-Isoprostane. Biomarker concentrations were then linked to air pollution measurements, and mathematical models were created in order to look for associations between exposure to air pollution and biomarker concentrations.

OUTPUTS/RESULTS: After adjustment for a number of factors (including sex, temperature & relative humidity), we found that urinary biomarker levels were significantly associated with daily concentrations of air pollutants. In contrast to the more immediate effects of air pollution on lung function and cardiovascular physiology, urinary biomarker responses were delayed by one to three days. Response from biomarkers of oxidative stress (MDA, 8-OHdG) predominated one day after exposure, while perturbations of inflammatory 8-isoprostane and VEGF levels were observed mostly on the second and third days. However, these responses were subtle and their associations with exposure to individual air pollutants presented complex patterns.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This study suggests that exposure to air pollution near an industrial site may influence markers of oxidative stress, even in a population of healthy, physically active volunteers.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: A better understanding of the relationship between human exposure to industrial air pollution and adverse health effects provides scientific evidence to inform decisions about where mitigation strategies may need to be employed to reduce human exposure.

3.16 Association between Hypertensive Disorders during Pregnancy and Subsequent Long-Term Risk of Hospitalization Due to End-Stage Renal Disease: A Population-Based Follow-Up

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OBJECTIVES/BACKGROUND/ISSUE(S): Hypertensive disorders in pregnancy (HDPs), including preeclampsia, affect approximately 5-8% of pregnant women in Canada. HDPs are associated with serious maternal and perinatal outcomes, and could also be related to adverse long-term consequences.

DESIGN/METHOD/DESCRIPTION: The objective is to examine the association between the long-term risk of hospitalization for end-stage renal disease (ESRD) and HDPs.

We selected a cohort of 1 641 016 women who delivered in Canadian hospitals between April 1993 and March 2003 from the Canadian Institute for Health Information's Discharge Abstract Database, after excluding chronic kidney disease, diabetes, lupus and other obstetric complications. We conducted a followup study on their subsequent hospitalizations from the thirteenth month after the delivery date until March 31, 2013. We calculated the corresponding person-years of observation for individual study and comparison groups, and examined the association of the occurrence of ESRD hospitalization with the various types of HDPs by estimating the crude, and adjusted odds ratios (aORs) and 95% confidence intervals (CI), as well as the median time of occurrence.

OUTPUTS/RESULTS: We found the subsequent ESRD hospitalization rates were 10.4 per 100 000 person-years in women with any HDP (102 836 women) and 1.6 per 100 000 person-years in women without HDPs (1 538 180 women), yielding an aOR of 5.7 [95% CI 4.7-6.9], with a median time 12.1 years (interquartile range [IR]: 8.4-15.5), and 10.3 years (IR: 5.8-13.2), respectively. Compared with women without any history of HDPs, women who had pre-existing hypertension with superimposed proteinuria had a 58-fold higher risk of subsequent ESRD hospitalization (95% CI: 38.6-89.5). Among women with preeclampsia, the rate of ESRD hospitalization was 11.3 per 100 000 person-years (54 204 women), with an aOR of 6.1 (95% CI: 4.8-7.7), and a median occurrence time of 9.4 years (IR: 5.7-12.8).

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Hypertensive disorders, especially preeclampsia during pregnancy, impose a significant impact on maternal and perinatal health, and further appear to increase the risk of subsequent ESRD hospitalization.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This study provides further evidence for assessing the impact of HDPs on subsequent ESRD and will be helpful for healthcare providers and policymakers to develop proper interventions.

3.17 Experiences and Lessons Learned in Data Management of a National Behavioural and Biological Surveillance System: The Tracks Surveillance System

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OBJECTIVES/BACKGROUND/ISSUE(S): The Tracks Surveillance System (Tracks) is a behavioural and biological surveillance system that monitors HIV and other sexually transmitted and blood-borne infections (STBBI) and associated risk behaviours among at-risk populations in participating sites across Canada. Lessons learned in data management for the Tracks have greatly improved the surveillance system's processes and data quality, thereby better informing policy development.

DESIGN/METHOD/DESCRIPTION: The Tracks data management procedures are described by a data processing cycle that provides for a progression from survey tool design to a fully cleaned and useable data set. Descriptions of tools/practices used at each stage of the cycle are described, as well as challenges and experiences encountered at different steps.

The Tracks team developed stringent standard operating procedures (SOPs) for data management and adopted electronic data collection tools to facilitate data collection, reduce time required for data entry and reduce errors from data collection/entry.

OUTPUTS/RESULTS: PHAC, in consultation with numerous stakeholders, oversees the development of each surveillance system protocol, questionnaire, procedures manual, survey tools, SOPs for data entry and cleaning, and data dictionaries for the Tracks Surveillance System. Documentation of these data management processes for all Tracks components has been developed. Data quality assessments are performed on a regular basis during and after data collection periods to ensure that all data entries are valid and complete. With these established data collection and cleaning procedures, data is now ready for analysis within 2 to 3 months.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The Tracks Surveillance System data processing cycle facilitates standardized collection and analysis of accurate, timely and relevant data on the prevalence of HIV and other STBBI and related behaviours in identified populations of interest.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Quality improvements in the Tracks Surveillance System data standards and protocols have resulted in more efficient processes and high quality surveillance data that can be used to monitor the HIV and STBBI epidemics and related risk behaviours in Canada, as well as inform policy and program development at local, provincial and national levels.

3.18 Evaluation of the Annual Canadian Biodosimetry Network Intercomparisons

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada is the lead of the Canadian Biodosimetry Network which provides biologically based dose assessments for potentially exposed individuals during a large scale event involving radiological or nuclear material. For such a network to function, it is essential to perform proficiency testing to ensure each laboratories ability to produce high quality assessments.

DESIGN/METHOD/DESCRIPTION: Annual intercomparisons are conducted to maintain the capacity and capabilities of this well-established biodosimetry network in conjunction with assessing efficient and effective analysis methods for emergency response.

These intercomparisons were conducted between laboratories in the Canadian National Biological Dosimetry Response Plan. Intercomparisons were performed over a six year period and comprised of the shipment of 10-12 irradiated, blinded blood samples for analysis by each of the participating laboratories. Dose estimates were determined by each laboratory using multiple biological endpoints. Dose estimates were returned to the lead laboratory for evaluation and comparison. This past year, several additional international laboratories took part as a method of participating in an organized intercomparison for quality control purposes.

OUTPUTS/RESULTS: Individual laboratories performed comparably from year to year with only slight fluctuations in the accuracy of dose estimates. Speed and accuracy of each method was compared. Some endpoints were proven to reduce the time of analysis without having a significant effect on the dose estimates while others, although faster, showed reduced accuracy of the dose estimates.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Annual intercomparisons are necessary to maintain a network of laboratories for emergency response biodosimetry as they verify the capacities and capabilities of the network. They are an excellent way to improve procedures as well as evoking confidence in the capabilities of the network.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The results of these intercomparisons demonstrate that Health Canada can confidently activate the Canadian Biodsimetry Network with the knowledge that partner laboratories are able to provide quality and timely dose assessments for emergency response.

3.19 Health Canada's Astronaut Biodosimetry Program

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OBJECTIVES/BACKGROUND/ISSUE(S): The Radiobiology Laboratory at Health Canada's (HC) Consumer and Clinical Radiation Protection Bureau (CCRPB) provides biologically based dose estimates for occupational exposures to ionizing radiation. As astronauts are exposed to higher than background levels of radiation, the Canadian Space Agency (CSA) has requested HC to provide expert advice to the astronaut program by monitoring dose estimates incurred during long-term space flights.

DESIGN/METHOD/DESCRIPTION: HC receives blood samples from astronauts prior to- and post-space flight in order to provide an important *in vivo* measurement of radiation-induced damage incurred during space flight. Measured cytogenetic endpoints include the Cytokinesis Block MicroNucleus (CBMN) assay, the Dicentric Chromosome Assay (DCA) and the Fluorescent *In Situ* Hybridization (FISH) assay. The FISH assay in particular is key to assessing long-lasting stable damage as it provides a measurement of stable translocations which are less likely to be expelled by the body in the long term. This long-lasting damage is believed to be the most likely to lead to an increased risk to the health of the astronauts during long-term flights (lasting 6 months or more). Using the pre-flight sample, HC prepares a personalized dose-calibration FISH curve for each astronaut. Post-flight samples are taken within 2 weeks and then again within 6 months. These post-flight samples are then analyzed for damage and a dose estimate is calculated in reference to pre-flight levels of damage.

Dose estimates are calculated for each astronaut based on a number of cytogenetic endpoints. As the program continues to evolve, the uncertainty of the dose estimates continues to be reduced, and the quality of the health risk assessments continues to improve.

OUTPUTS/RESULTS: The complexity of damage that results from high linear energy transfer (LET) radiation, such as that found in the space environment, requires a detailed scoring schema to capture as much information as possible from the FISH assay. To that end, in collaboration with NASA, HC has developed a clear, concise scoring schema that simplifies the scoring process and is easily transferrable to other laboratories.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: HC has implemented and manages a biodosimetry program for astronauts in partnership with the CSA. Guided by discussions with NASA, HC is harmonizing the manner in which data from the FISH assay is recorded to better facilitate the comparison of results with other international biodosimetry programs.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The results of this program help to better assess the potential health risks to astronauts during long-term space flights. As well, the harmonization of the FISH methods with international partners has improved the quality of the FISH assay which has implications for other Health Canada research programs.

3.20 Unintentional Poisonings in Children between 0-5 Years of Age: A Descriptive Epidemiological Analysis of Calls to the Ontario & Manitoba Poison Centres

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OBJECTIVES/BACKGROUND/ISSUE(S): Unintentional poisonings are one of the leading causes of preventable injuries amongst children. Such poisonings are the second most common reason for hospitalization in children aged 0-4 years, and the third leading cause of unintentional, injury-related death according to the Public Health Agency of Canada[1][2]. The objective of this study was to describe the epidemiology of unintentional poisonings in children between 0-5 years of age about whom medical advice was sought from the Ontario Poison Centre between January 2007 and December 2012 and the Manitoba Poison Centre between July 2012 and December 2012.

DESIGN/METHOD/DESCRIPTION: While the United States has a national database, National Poison Data System (NPDS), which compiles statistics on calls to accredited American poison centers, there is no comparable database for Canada. The Consumer Products Safety Directorate (CPSD) of Health Canada and the Ontario & Manitoba Poison Centres collaborated to accurately describe the spectrum and extent of injuries related to poisonings in their jurisdictions and to establish benchmarks by which to measure prevention activities under Canada's Food and Consumer Safety Action Plan (FCSAP).

130 867 telephone calls to the Ontario & Manitoba Poison Centres were analysed.

OUTPUTS/RESULTS: Two year olds were the most commonly represented group (31.7%), followed by 1 year olds (30.1%). The most common agents were found in consumer products (e.g. household cleaners), followed by over-the-counter drugs. The predominant site of exposure was the individual's own residence, though less frequently, 4 and 5 year olds were exposed in a school setting. For all substances, the route of exposure was mostly ingestion, which occurred in 85% to 95% of cases.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The results of this study provide evidence that consumer products are the most common poisoning agent category, thereby, identifying a need for further assessment by CPSD. The trends identified (i.e. children are predominately being poisoned at home via ingestion) provide further evidence to inform CPSD program activities.
CPSD has conducted analysis on the consumer product subset of this dataset to better understand poisoning trends by consumer products in young children.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The results of this study will be of interest to other Directorates within Health Canada as the data was categorized to best align with Acts and Regulations under Health Canada's purview. Though data are characterized at a high level, trends in poisoning categories may inform various health portfolio areas.

The increased understanding of unintentional poisonings of young children, for whom medical advice was sought from the Ontario & Manitoba Poison Centres, demonstrates the value of poison data as a key surveillance tool. The Health Portfolio, led by Health Canada, is working with poison centres across Canada to establish a Canadian surveillance system for poison information, and this research can help promote its development.

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[1] Public Health Agency of Canada. Child and Youth Injury in Review, 2009 Edition – Spotlight on Consumer Product Safety. Ottawa, 2009.
[2] Public Health Agency of Canada. Injury Surveillance On-Line: Leading Causes of Injury Deaths in Canada, Ottawa, 2005.

3.21 Maintaining Measles and Rubella Elimination in Canada: Challenges and Opportunities

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OBJECTIVES/BACKGROUND/ISSUE(S): Due to the success of immunization and timely surveillance, measles and rubella cases are rare in Canada. However, recent large outbreaks of measles have highlighted the risk of introducing disease through travel and immigration and gaps in immunization coverage for sub-populations that are under or unimmunized.

DESIGN/METHOD/DESCRIPTION: The goal is to develop a strategic framework to maintain elimination of measles and rubella in Canada. This will involve determining the policies and actions required to address the challenges of preventing measles and rubella outbreaks. We aim to identify evidence-based practices and establish policy guidance for immunization programs and coverage, disease surveillance, and outbreak management.

A review of the current evidence and policies will help PHAC determine the key areas of focus for maintaining measles and rubella elimination. Consultation with federal/provincial/ territorial partners and expert groups will assist in further refinement of key challenges and also to identify potential solutions and opportunities to improve immunization coverage and disease surveillance. As well, we have collaborated with the Canadian Agency for Drugs and Technologies in Health (CADTH) to conduct a systematic review on the cost-benefits of different public health interventions in response to measles importations to reduce secondary transmission. Results from the literature reviews and consultations will be synthesized to formulate policy options..

OUTPUTS/RESULTS: Key areas of focus include strengthening surveillance and outbreak response, enhancing immunization coverage monitoring and immunization programs, maintaining laboratory capacity and improving management of vaccine and immunoglobulin supply. For each focus area, current initiatives in Canada and strategies implemented by other countries will be reviewed. Potential strategies and guidance will be provided in the framework.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The framework will highlight key challenges and identify potential solutions and opportunities to maintain disease elimination that may be a model for other countries around the world as they achieve measles and rubella elimination goals.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The resulting framework will strengthen our current public health efforts in preventing vaccine-preventable diseases among Canadians. It will also contribute to our international obligation with the Pan-American Health Organization to fulfill their commitment to maintaining measles and rubella elimination for the Region of the Americas.

3.22 Development of Bead-Based Assays for Biomarkers of Disease and Chemical Exposure

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OBJECTIVES/BACKGROUND/ISSUE(S): Humans are exposed to a number of chemicals every day, some of which are linked to health effects ranging from inflammation to endocrine disruption. Health Canada monitors the exposure of Canadians to such chemicals through exposure routes such as air, water and consumer products. Biomonitoring studies are also conducted to measure levels of chemicals in human blood and urine. Higher throughput methods that permit the simultaneous measurement of many chemicals are needed since only a fraction of existing chemicals is currently being measured.

Health Canada researchers are developing assays for use on an existing high throughput laboratory instrument that uses antibodies bound to microbeads to allow the detection of multiple targets. Our goal is to adapt this technology so that it can detect potentially hazardous chemicals or their metabolites.

DESIGN/METHOD/DESCRIPTION: Initial studies tested the use of recognition molecules consisting of RNA or DNA instead of antibodies, which are typically used. The use of such alternatives was explored as one way to expand the number of targets measurable and was used to sensitively detect an indicator of inflammation. Recent work has focused on the detection of chemicals that naturally bind to proteins in the body to form protein-chemical adducts. These adducts can be used to monitor human exposure to those chemicals.

OUTPUTS/RESULTS: The initial studies demonstrated that RNA could be successfully used for detection of an indicator of inflammation over a higher concentration range than with a commercial kit using only antibodies. Studies on the detection of protein-chemical adducts indicate that the adducts can be measured at nanomolar concentrations under ideal conditions.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: A number of challenges were encountered during the development of the new detection systems, providing insight into the factors affecting their successful performance. Moreover, this research will provide information regarding the selectivity and specificity of this technology as well as whether it will be suitable in biomonitoring studies. Future experiments will evaluate protein-chemical adduct detection in serum samples.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The successful development of multiple assays that can test human samples for several chemical or protein-chemical adducts simultaneously would greatly aid Health Canada in its future biomonitoring studies, and could potentially accelerate the monitoring of a number of priority environmental contaminants under Health Canada's Chemicals Management Plan.

3.23 Mapping Connections: Highlights from the Synthesis Report of the National Population Health Study of Neurological Conditions

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OBJECTIVES/BACKGROUND/ISSUE(S): The National Population Health Study of Neurological Conditions (the Study), a suite of research initiatives funded by the Government of Canada, was implemented by Neurological Health Charities Canada and the Public Health Agency of Canada, in partnership with Health Canada and the Canadian Institutes of Health Research, to pursue the long-term goal of reducing the burden of neurological conditions in Canada.

DESIGN/METHOD/DESCRIPTION: With fourteen neurological conditions considered, the Study supported 13 research projects, each with their own design and methods, 3 national surveys and 7 microsimulation models. The impacts of neurological conditions (on individuals, families, caregivers), health services (use, gaps, recommended improvements), scope (prevalence, incidence, comorbidities), and risk factors (development, progression) were explored.

Key findings from the Study research projects, surveys, and microsimulation models were synthetized through a comprehensive and collaborative process under the oversight of a Scientific Advisory Committee and Synthesis Panel. The report on Study findings was released in September 2014.

OUTPUTS/RESULTS: Highlights include the following:

- Neurological conditions affect 3.6 million Canadians living in the community and more than half of Canadians requiring continuing care.
- With the aging population, these numbers will increase over the next 20 years, particularly for Alzheimer's disease and other dementias and Parkinson's disease/parkinsonism.
- The proportion of Canadians with a neurological condition reporting fair/poor health is almost 4 times higher than in the general population; the proportion is twice as high for mood or anxiety disorders, and 12 times as high for permanent unemployment.
- The need for informal assistance by Canadians with a neurological condition is common (39.6%).
- Health care costs are 3 to 7 times higher among individuals with certain neurological conditions compared to individuals without these conditions. For brain and spinal cord injuries, costs are even higher. Preventable risk factors are being identified so action can help reduce or mitigate the burden of neurological conditions.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This collaboration between experts, researchers, health professionals, and stakeholders increased awareness and generated new data and information. The report identified gaps in knowledge to guide future research, and areas for improvement to help tailor health care services.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This new evidence will support ongoing national surveillance and the development of programs and strategies to better address the needs of Canadians affected by neurological conditions, thus helping to reduce the burden for individuals, families, and the health care system.

3.24 Opportunities for Collaboration, Innovation and Efficiency: Involvement of Health Canada Laboratories in the Canadian Health Measure Survey

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OBJECTIVES/BACKGROUND/ISSUE(S): The biomonitoring component of the Canadian Health Measures Survey (CHMS) includes the analyses of several biomarkers in blood and urine samples. The Chemical Surveillance Bureau (CSB) works in close collaboration with laboratories to develop reliable and robust analytical methods that will then be included in the CHMS to analyze biomarkers in specified human matrices. Previously, certified commercial laboratories performed most of these analyses. However, as our analytical needs have evolved, several Health Canada laboratories have become involved in the CHMS. In addition to high quality biomonitoring data on over one hundred environmental chemicals, the CHMS database includes a wealth of information on physical health measures (e.g. height, weight, and blood pressure) as well as lifestyle and socio-economic factors.

DESIGN/METHOD/DESCRIPTION: Ideas and plans are being developed to engage laboratories and foster collaboration amongst research groups within Health Canada in method development, sample analysis and data analysis. In the proposed plan, research laboratories could participate in the CHMS by developing new analytical methods which could then be transferred to service laboratories within Health Canada or elsewhere for regular analyses. In addition CSB is planning to facilitate collaboration among scientists to perform in-depth analysis of the CHMS data on the factors influencing exposure to environmental chemicals in the general Canadian population.

OUTPUTS/RESULTS: In CHMS 2011-2013, 68 biomarker analyses were carried out by Health Canada laboratories. Involvement of laboratories and research groups within Health Canada has resulted in an expanded list of available analytical methods and significant efficiency and cost savings for the survey.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Collaboration with laboratories and research groups within Health Canada is expected to improve effective knowledge transfer while also creating opportunities for departmental laboratories to participate in the CHMS. At an operational level, internal collaborations will help to develop analytical chemistry expertise within our department, leading to efficiency and cost savings for Health Canada in managing the CHMS.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Health Canada's role in the biomonitoring component of the CHMS is in line with its mandate under the Chemical Management Plan. Human biomonitoring data collected in CHMS has been used in developing policies and regulations pertaining to Chemicals Management as well as in the human health risk assessment of environmental chemicals.

3.25 Use of Second-Generation Antipsychotics among Pediatric Patients, 2008-2012

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OBJECTIVES/BACKGROUND/ISSUE(S): The objectives of this study were to use data on drug prescription collected by IMS-Brogan to determine how second-generation antipsychotic drugs (SGAs) were used in Canadian children from 2008-2012 in terms of age differences, indications for use, and prescriber characteristics and thus provide context for ongoing pharmacovigilance of these drugs in this patient population.

DESIGN/METHOD/DESCRIPTION: Drug utilisation data were generated from two IMS-Brogan databases for the following drugs: quetiapine, risperidone, olanzapine, aripiprazole, clozapine, ziprasidone and paliperidone. None of these drugs had been newly authorised over this 5-year interval, although aripiprazole's authorised indications were broadened to include pediatric patients in 2011.

Drug recommendations by physician specialty and diagnosis were also analyzed for this five-year period.

OUTPUTS/RESULTS: SGA prescriptions for children increased by 27% between 2008 and 2012 (520,000 to 660,000). Children aged 13-18 years accounted for 63.6% of prescriptions, followed by children aged 6-12 years (34.0%) and children younger than 6 years (2.4%). Increased prescriptions for quetiapine and aripiprazole were the main reason for this increase. Attention Deficit Hyperactivity Disorder (ADHD) was the most common indication associated with the SGA prescriptions, followed closely by psychotic disorders and mood disorders. Among the10-19 year age group, 73% of prescriptions were prescribed by psychiatrists/ neurologists and10% of prescriptions were prescribed by pediatricians. In comparison, in the 3-9 year age group, 42% of prescriptions were prescribed by psychiatrists/neurologists and 27% by pediatricians.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Data showed that SGA use in children increased between 2008-2012, and that these drugs were recommended for a range of disorders. It should be noted that aripiprazole was authorised for use among 15-17 year olds for schizophrenia, and among 13-17 year olds to treat bipolar I disorder; aripiprazole's authorisation in 2011 to treat adolescents may explain the increased volume of prescriptions in both pediatric age groups. It is also important to note that although ADHD was the most common underlying diagnosis for prescribing SGAs, ADHD has yet to be an authorised indication for these products.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: As the use of SGAs increases, it is important to continue monitoring the trends of SGA use in the pediatric population, especially given the safety concerns associated with use of these products in children. Given the variation in prescriber speciality by age of children, the implementation of risk minimization strategies needs to consider multiple types of health care practitioner.

3.26 Biomonitoring of Newcomer Women from South and East Asia in Two Canadian Cities

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OBJECTIVES/BACKGROUND/ISSUE(S): Although Health Canada has established sound risk management activities for metals, preliminary studies indicate that levels of mercury, lead, and cadmium in Asian immigrants are higher than for the general Canadian population. These newcomers are exposed to metals in Canada through their diets, smoking, or culturally-specific behaviours. Given one in five people in Canada are foreign-born, and Asia has been Canada's largest source of immigrants during the past 5 years (at nearly 60%), exposures to these developmental toxicants may represent an important population health risk.

DESIGN/METHOD/DESCRIPTION: A new surveillance study will measure mercury, lead, and cadmium in the blood of women of reproductive age (WRA) in Vancouver (n = 300) and Toronto (n = 150) who have been Canadian residents for 1 to 5 years following their direct immigration from South or East Asia. Urinary cadmium will be measured in Vancouver. Determinants of metal concentrations will be evaluated using culturally-relevant questionnaires. Participants will receive and may discuss their biomarker results with a public health practitioner.

OUTPUTS/RESULTS: Descriptive statistics for the biomonitoring data will be calculated, and exploratory stepwise multiple regression analyses will evaluate factors associated with blood concentrations of metals. As appropriate, data for newcomer women will be compared with general Canadian biomonitoring surveys (e.g., MIREC for pregnant women, and CHMS for WRA).

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Comparing Asian immigrant data with other Canadian surveys will aid in determining whether risk management activities are effective or require adjustment. Regional health authorities will use study information to identify gaps in current health messaging and develop evidence-based public education tools. Finally, key findings will be incorporated into future strategies for biomonitoring of women and immigrants to Canada.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This targeted surveillance study provides a national perspective for Asian immigrants and informs risk management strategies for reducing lead exposures to the greatest extent practicable. Moreover, in Canada, a provisional blood guidance value for mercury has been established for women of reproductive age and children. Asian immigrants are frequently high consumers of fish and seafood that are predominant mercury exposure sources for Canadians, thus there may be opportunity for interventions for reducing these exposures.

3.27 Benzene Results from the Canadian Health Measure Survey (CHMS) 2009-2011

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OBJECTIVES/BACKGROUND/ISSUE(S): The Canadian Health Measure Survey (CHMS) latest report on biomonitoring of environmental chemicals, released in 2013, presents results for 91 biomarkers in 6400 Canadians from 3 to79 years. For the first time, it provides nationally representative Canadian data on benzene exposure: urinary metabolites and indoor air levels. Benzene has been identified by Health Canada as a priority indoor air contaminant. Chronic exposure to benzene has been shown to cause leukemia, a cancer of the blood or bone marrow, in industrial workers.

DESIGN/METHOD/DESCRIPTION: A benzene metabolite called S-PMA was measured in the urine of 2514 Canadians aged 3 to 79 years collected in Cycle 2 of the CHMS from 2009 to 2011. Within the same period, 5191 respondents of CHMS were asked to deploy an air sampler in their home for 7 consecutive days to measure benzene in their indoor air. These data provide baseline levels for benzene exposure in the Canadian population.

OUTPUTS/RESULTS: Benzene was detected in large proportions of participants (~78%) and in virtually all homes (~99%). Urinary concentrations of S-PMA were higher for smokers than for non-smokers. The indoor air measured in participant's homes had relatively low benzene concentrations. Mean concentrations for Canadian non-smokers fall below benchmark associated with negligible cancer risk level.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Benzene is being measured in blood, indoor air and tap water in Cycles 3 and 4 of the CHMS (2012-2015).

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Data for Cycle 3 (2012-2013) will become available in the summer of 2015. These additional data will be used to assess the trends in exposures to benzene.

3.28 Identification Process of the Pediatric Off-Label Use of Biological Products Licensed in Canada

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OBJECTIVES/BACKGROUND/ISSUE(S): The Policy Statement for the Off-label Use of Drugs in Children recently published in Pediatrics demonstrates that drugs not specifically indicated for children in their labelling are used based on the best available evidence and the importance of the benefit for the individual patient. The objective of this study is to identify the potential risks and possible benefits to pediatric population when treated with biologic products that are not indicated for this population. Note that the decision to treat a patient is practice of medicine and falls outside of Health Canada jurisdiction.

DESIGN/METHOD/DESCRIPTION: Canadian licensed biologic products either not indicated for or tested in children were identified by a search in the published literature, Canada's adverse reaction database and that of foreign regulators to identify adverse events reported and/or data supporting the effectiveness of these products when used in children. Results are being grouped by product class and individually for each drug examined.

This is a new issue that has only recently being discussed in Canada and other jurisdictions.

OUTPUTS/RESULTS: Following the identification of 42 Canadian licensed biological products used off-label in children a search of adverse events reported with such use was performed. Although, not expected to be reported to regulators, because these products are not licensed to be used in children, preliminary results showed adverse events reported in some jurisdictions where the products are licensed for pediatric population. The main source of adverse events, however, is studies published in the literature.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Interim results demonstrated that this is a valid approach to identify the risks and benefits of biological products used off-label in children.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The serious adverse events are to be included in the products Periodic Safety Update Reports and/or Periodic Safety Benefit-Risk Evaluation Report reviews; further, if necessary, to create a different approach when reviewing the Risk Management Plan for new drug submissions for the same product class.

Biomonitoring, Therapeutics and National and International Policy

4.01 Analysis of Available Online Training for Canadian Public Health Professionals

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OBJECTIVES/BACKGROUND/ISSUE(S): There has been limited application of online learning in public health. The aim of this study was to examine the trends and gaps in the online courses available for Canadian public health practitioners. Our second objective was to examine how the available courses compared to the Skills Online modules offered by the Public Health Agency of Canada.

DESIGN/METHOD/DESCRIPTION: Online courses were selected if they were related to public health and were offered by a Canadian institution. Elements collected for each course included cost, type of delivery, duration, language, accreditation, and source. The seven categories of Core Competencies for Public Health in Canada were matched accordingly for each course based on the available description. Collected information was stored into a database and analyzed for interpretation.

OUTPUTS/RESULTS: A total of 108 online courses were identified, of which universities were the leading providers (41%). Most (45 of 49) of free courses could be completed within 5 hours. Free courses did not provide any university credits and mainly provided certificate of completion (55%) or no credit (39%). Courses with learner fees took at least 25 hours to complete in most cases (56 of 59). Majority (68%) of the paid courses provided a university credit. Self-directed courses demonstrated a similar trend when compared to facilitated courses. Paid and facilitated courses had a more balanced coverage of the seven core competencies than free and self-directed courses. The Skills Online program was the main provider of facilitated courses outside of universities, and was also the only provider of facilitated courses in both English and French. Lastly, Skills Online was the only non-university provider to cover a comprehensive range of public health concepts at an affordable cost and extensive duration.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: To improve the awareness and access of online public health training to Canadian professionals, a web database of available course offerings is recommended.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Our study supports the federal government's role as the main contributor–apart from universities–of comprehensive online training to front line public health professionals in Canada. It also suggests key areas and issues where the Agency may need to partner with Canadian universities and NGOs to improve public health capacity.

4.02 Total Inorganic Arsenic Analysis in Pharmaceutical Drugs and Natural Health Products by High Pressure Liquid-Chromatography (HPLC) with Inductively Coupled Plasma Triple Quad Mass Spectrometry (ICPMSMS)

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OBJECTIVES/BACKGROUND/ISSUE(S): Part of the work of the Inspectorate Laboratory Programme is to provide reliable microbiological and chemical analyses and scientific expertise on these analyses in order to support inspections and investigations related to drugs and natural health products.

Arsenic trace analysis by ICP-MS and HPLC-ICP-MS are two tests that are commonly performed on drugs and Natural Health Products (NHPs). Reliable results are obtained for trace analysis of total inorganic arsenic using a method recently developed in the Longueuil laboratory for samples in various forms (tablets, capsules, herbal mixtures, algae containing NHPs, etc.).

DESIGN/METHOD/DESCRIPTION: The main objective was to develop/adapt a method to support relatively recent USP and Health Canada limits for total and inorganic arsenic (As^{+3}, As^{+5}) in drugs and NHP's. The regulatory level of total inorganic arsenic is about 5 times less than the total arsenic limits due to the high toxicity of inorganic arsenic relative to organic arsenic. This poses serious issues when analysing samples containing high concentrations of organoarsenic with conventional concentrated acid digestion techniques and ICP-MS. In effect, the digestion process tends to convert all organic arsenic into inorganic forms which may cause an overestimation of the inorganic arsenic content. To overcome that problem, a speciation method by HPLC-ICP-MS was developed. It includes an extraction process which maintains the total inorganic content present in the sample, followed by an HPLC-ICP-MS analysis which separates inorganic arsenic from organic forms.

A method consisting of two processes was developed. It comprises an extraction process to extract and maintain the inorganic content of arsenic present in the sample followed by a highly selective speciation technique (HPLC-ICP-MS, HPLC-ICP-MSMS) to separate those species.

OUTPUTS/RESULTS: Total Inorganic arsenic recovery results obtained from the developed methodology ranged from 90-110 % for algae and herb containing NHP samples and Drug samples spiked at the regulatory levels (0.03 ug/kg by weight / day) with %RSD (Relative Standard Deviation) <5%. The HPLC-ICP MSMS quantification limits were estimated at respectively 24 and 63 pg/ml for As^{+3} and As^{+5}.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The development/adaptation, validation, results and strategies to reliably measure/detect total inorganic arsenic content in pharmaceutical drugs and Natural Health Products by HPLC-ICP-MSMS will allow us to efficiently support inspections and investigations related to drugs and natural health products.

The new HPLC-ICPMSMS method developed by the Quebec Inspectorate Laboratory can now support inspection and compliance verification activities for drugs and NHPs related to the USP (United States Pharmacopeia) and NHP limits for total and inorganic arsenic.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The recently developed method will support the enforcement of drug and NHP regulations on total and inorganic arsenic.

4.03 Partner and Family Violence Among Older Canadians: Who is at Risk?

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OBJECTIVES/BACKGROUND/ISSUE(S): Interpersonal violence may decrease in later life, however older people constitute a susceptible group for partner and family violence. There is a dearth of original research on factors associated with violence in Canadian older adults.

DESIGN/METHOD/DESCRIPTION: The present study identifies individual (behavioural, health and socioeconomic) and interpersonal (partner/family relationships) factors associated with lifetime and current physical and non-physical violence experienced by older Canadians, while also accounting for the long-term impact of childhood adversities.

By using adapted statistical methods, we analyzed baseline data (2012) from International Mobility in Aging Study in a sample of community-dwelling individuals aged 65-74 years old, residing in Kingston (Ontario) and Saint-Hyacinthe (Quebec). Data included participant's information on current and previous experience of violence, individual and interpersonal characteristics, and adverse experience in the childhood.

OUTPUTS/RESULTS: Current violence of a non-physical nature was experienced by 18% of the sample. Women reported more lifetime non-physical (16.6% versus 10.3%) and physical violence by a partner (11.3% versus 0.5%) than men. Non-physical lifetime violence by family was more than twice frequent in women than men (12.1% versus 4.8%). Individuals reporting violence were more likely to live with family rather than alone/with a partner, have poor relationships and poorer health (obesity, decreased mobility), and consuming alcohol daily. Witnessing violence at home in childhood was particularly strongly associated with current and past violence by partner or family.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Our research suggests that a significant minority of older people is now experiencing or has experienced violence over their lifetimes by family and/or partner, and documents the impact of early adversity on subsequent partner and family violence in Canada. These findings identify some preventable factors associated with current and past violence. Future prevention and policies at the individual and interpersonal level may include approaches aiming at education, life skills training, and peer programs designed to reduce conflict and promote healthy relationships.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Through its Family Violence Initiative, PHAC aims to reduce occurrence of family violence in Canada by promoting public awareness of the risk and protective factors associated with family violence. This research aligns with our priorities with respect to elder abuse prevention, including interpersonal factors across the life span.

4.04 Outreach Strategy for Pesticide Buffer Zone Management Next to Wetland/Critical Habitat in South Okanagan/Simikameen Valley

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OBJECTIVES/BACKGROUND/ISSUE(S): The BC Okanagan and Similkameen valleys are home to a number of wetlands considered sensitive habitats as they host a number of species listed under the federal Species at Risk Act and the provincial Wildlife Act of BC. Recent studies show that agriculture pesticides are present in some wetlands and there is potential to impact amphibian health. For these reasons a project was launched by the BC Ministry of Environment and the BC Region for Pesticide Compliance and Enforcement-PMRA, to contact farmers who manage farms with or adjacent to wetlands. The objectives were to promote best management practices with regard to spray drift and run-off into wetlands, verify the quality and extent of amphibian habitat on private land, and to promote the important content on pesticide labels with respect to buffer zone directions for the protection of aquatic habitat.

DESIGN/METHOD/DESCRIPTION: To prepare for the outreach phase, maps of wetlands adjacent to orchards and vineyards were created identifying priority sensitive wetlands. A questionnaire was prepared as well as a spreadsheet of commonly used pesticides that had label statements for aquatic and terrestrial buffer zones. A number of publications were gathered to be provided as handout information such as importance of wetland habitat, how to mitigate pesticide spray drift, and how to use the PMRA –Health Canada label search tool. Many publications were in English, French and Punjabi.

OUTPUTS/RESULTS: Over a five day period teams of two people (one PMRA rep and one BCMOE rep), visited orchards and vineyards adjacent to sensitive wetlands. Each farmer contacted was surveyed on their pesticide use and their efforts, if any, to protect the sensitive habitat adjacent to their farm. Interviewers identified steps that growers could take to improve their stewardship such as; increase vegetation around habitat, reduce the number of operating nozzles when wind conditions change, complete an Environmental Farm Plan and using wind speed and direction to reduce drift into sensitive habitat.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The project successfully brought awareness of wetland protection to a 36 farmers that are in a position to make a difference. A number of growers had taken positive steps to protect wetlands such as removing land from production or planting natural vegetation to buffer the wetlands. With increased awareness and assistance from organizations such the BC Environmental Farm Plan Program and The Land Conservancy, farmers can be compensated for efforts made to protect, enhance, and restore sensitive habitats. Although this project focused on pesticide use and potential impacts, it is recognized that land development for purposes other than agriculture has a significant impact on the loss of wetlands.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Awareness of label buffer zones and the on-line tool Buffer Zone Calculator is growing but the department must continue to strive to promote its use and make farmers aware of these important label statements.

4.05 Modelling Home Care and Long Term Care Needs

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OBJECTIVES/BACKGROUND/ISSUE(S): The Strategic Policy Branch contributes to the evidence based evaluation of home care and facility-based long term care needs and costs for seniors in Canada, at both the individual and jurisdictional levels.

DESIGN/METHOD/DESCRIPTION: Statistics Canada Lifepaths micro simulation model is used to estimate home care and long term care needs based on parameter inputs from subject-matter experts. Simulated health outcomes (placement in long term care residence or receipt of home care) are currently based on age and gender. Other determinants of health/disability could also be added in future iterations of our simulations.

We have used Statistics Canada Lifepaths micro simulation model to estimate home care and long term care needs between 2015 and 2030. Eight policy scenarios were tested in comparison to the base case scenario which assumed that current home care and long term care policies would be maintained between 2015 and 2030.

OUTPUTS/RESULTS: The outcome of the simulation model is to evaluate the out-of-pocket costs borne by the individuals needing home care and/or long term care. Socio-economic factors are isolated to identify factors that affect an individual's capacity to pay for his/her health care needs in terms of home care and/or long term care. The costs borne by different levels of government and their fiscal capacity to deliver the same level of services in the future are also estimated. Subsidization to cover the cost of room and board (accommodation) of residential care services vary by province. Quebec and Alberta incurred the lowest costs per resident while provinces in the Atlantic region bore the highest subsidization cost per resident. Subsidization to cover the delivery of home care service had a lower variance across provinces. New Brunswick and Newfoundland and Labrador bore the lowest subsidization cost per home care client per year.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The first phase of the project consisted in evaluating out-of-pocket costs and unmet needs faced by individuals while the second phase is focusing on federal potential policy options.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The expected impact of this modelling exercise is to assess the extent of unmet needs of our seniors' population due to affordability and accessibility of care and services issues. Findings from these analyses are used to propose policy options to help alleviate these issues.

4.06 Physicians' Use of Electronic Medical Records: Evidence from the Commonwealth Fund 2012 International Health Policy Survey of Primary Care Physician

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OBJECTIVES/BACKGROUND/ISSUE(S): Electronic medical records (EMRs) are computer-based patient records which detail patient demographics, medical and drug history, and diagnostic information. They have the potential to increase productivity and quality of physicians' practices, and to improve communication among physicians and between physicians and their patients.

DESIGN/METHOD/DESCRIPTION: This study uses the data from the Commonwealth Fund 2012 International Health Policy Survey to examine the physicians' use of EMRs and its linkages with physicians' practice across 10 surveyed countries including Canada, focusing on the following research questions: (1) Who adopts EMR systems among physicians? (2) What functionalities of EMRs do physicians use? (3) How are the EMR systems reflected in physicians' practice?

OUTPUTS/RESULTS: The percentage of physicians using EMRs in Canada has increased from 37% in 2009 to 56% in 2012. However, Canada still lags behind many other countries. The young physicians were more likely to use EMRs in the majority of the surveyed countries including Canada. In Canada, the percentage of physicians using EMRs was higher in rural area than in urban area. The percentage of physicians reporting practices with 9 or more EMR functions has increased from 14% in 2009 to 26% in 2012 in Canada, but still much lower than Australia (90%), New Zealand (96%) and the United Kingdom (97%) with developed EMR adoption programs.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Canada has made significant progress in the use of EMRs in the primary care. However, Canada's performance is still far behind its international counterparts, especially the use of multiple EMR functions (e.g., electronic prescribing and ordering of tests; electronic accessing test results, medial alerts, clinical notes; computerized system for tracking lab tests, guidelines, alerts to provide patients with test results, preventive/follow-up care reminders; and computerized list of patients by diagnosis, medications, due for tests or preventive care).

The next step of research is to use the 2014 National Physician Survey to examine the physicians' use of EMRs and its impact on productivity and quality of physicians' services across Canadian jurisdictions.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This study will provide the most recent information and international comparison analysis to inform policy questions around EMR adoption and use among physicians in Canada. The demographic factors influencing physicians' use of EMRs can be considered in policy/program development.

4.07 Improving Effectiveness of the Chemicals Management Plan Public Outreach Through Chemical Awareness Learning Modules

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OBJECTIVES/BACKGROUND/ISSUE(S): A key goal of the Government of Canada's Chemicals Management Plan (CMP) is to inform Canadians about the potential risks and safe use of chemicals. The Regions and Programs Bureau (RPB) delivers various risk communication activities that increase public awareness of chemicals and associated health risks as part of the CMP program.

DESIGN/METHOD/DESCRIPTION: The CMP Evaluation from 2011 found that outreach to Canadians had been limited, with only a limited amount of proactive communications targeting Canadians undertaken. A review of environmental health educational tools suggested a new, better organized learning tool for chemicals awareness was needed to improve the consistency and effectiveness of public outreach to Canadians.

The **Chemical Awareness Learning Modules** (CALM) education and awareness program was developed to teach the risks and safe use of chemicals to service providers working with vulnerable populations, like nurses, community health officers and early childhood educators. These audiences would then be prepared to share the CMP's key messages with the public. Ten teaching modules were developed, taking advantage of existing Health Canada publications and other information relevant to the CMP.

OUTPUTS/RESULTS: The efficiency and effectiveness of regional CALM sessions were demonstrated during a pilot phase in 2013-14. RAPB was able to demonstrate a ninety percent (90%) reduction in program costs over prior efforts to achieve this objective by using regional employees to deliver the modules instead of contractors.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: These savings mean more sessions can be delivered to more Canadians each year. Ninety percent (90%) of CALM session participants report that their awareness and understanding of chemicals and their associated health issues has increased. Many respondents' qualitative comments show they intend to make many behaviour changes to reduce exposure to harmful chemicals.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The CALM modules raise public awareness of CMP and of strategies to reduce exposure to harmful chemicals. An outreach tool like CALM promotes public confidence in identifying and managing these risks and achieves RAPB's vision of excellence in program and service delivery through an integrated and horizontal approach.

4.08 WITHDRAWN

4.09 In-Depth Examination of Interferon Alpha-2 Products Using High Resolution Separation Techniques

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OBJECTIVES/BACKGROUND/ISSUE(S): Interferons (IFNs) are some of the most frequently prescribed therapeutic proteins for the treatment of diseases such as hepatitis, multiple sclerosis and leukemia. Like other therapeutic proteins, however, IFNs are susceptible to a number of process and product-related modifications during preparation, formulation or storage. Such modifications may result in the loss of therapeutic efficacy or unwanted immune reactions.

DESIGN/METHOD/DESCRIPTION: We have examined IFNα-2a and IFNα-2b products using methods based on high resolution separation techniques, in particular, capillary electrophoresis (CE) and high performance liquid chromatography (HPLC).

We have developed several methods that enable to separate and quantify process and product variants.

OUTPUTS/RESULTS: Size-exclusion HPLC was used to assess the formation of dimers, aggregates and denatured IFNα-2. Ion-exchange chromatography as well as a CE enabled monitoring charge variants while reversed-phase HPLC enabled detection of oxidised products and other variants. In addition, a CE method was developed to directly assess the integrity of the active ingredient in finished products containing excipients such as human serum albumin or polysorbate.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Developed methods will enable a more thorough assessment of the quality of biopharmaceuticals.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The development of reliable and validated analytical methods for the characterization of active ingredients or for measuring product quality enables the provision of advice to evaluators and policy makers as well as to manufacturers. In addition, the application of these methods should further our knowledge of the post-formulation quality of these complex preparations. Thus, the work is strongly aligned with sectoral and departmental strategic objectives with regard to the regulatory framework (product licensing, lot-release testing), post-marketing issues (surveillance and regulation of drug product utilization) and harmonization (development of an international strategy to guide development of partnerships with other international and national organizations).

4.10 Explaining Variations in Hospital Expenditures in Canada

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OBJECTIVES/BACKGROUND/ISSUE(S): Hospital expenditures constitute the largest share of total health care expenditures in Canada. Although existing studies have analyzed factors affecting hospital costs in specific provinces, no recent study investigates the potential causes of variation in hospital expenditures in Canada using nationwide hospital-level data.

DESIGN/METHOD/DESCRIPTION: This study investigates the factors explaining the variations in hospital expenditures and determines the level of cost efficiency in Canadian hospitals.

This study uses a sample of 424 hospitals over the fiscal years 2007-2008 to 2009-2010. A cost function is estimated and the parameters are then used to assess the cost efficiency of each hospital.

OUTPUTS/RESULTS: Empirical evidence shows that factors such as labor prices, inpatient days, outpatient visits and case-mix index, which reflect the complexity of medical conditions treated, are relevant in explaining expenditure variations. The estimated cost efficiency level for more than half of the hospitals range between 70 and 90 percent.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The average cost efficiency estimates of 75 percent suggest that there is a potential for cost savings of about $7 billion to $8 billion for the hospitals in the sample period. This study identifies the factors such as bed-occupancy rates, hospital concentration ratio, and average length of stay as the sources of inefficiency. A comparison of hospital groupings in terms of cost efficiency with respect to other factors such as nurse per bed ratio and administrative staff as a percentage of total staff provides additional information for policy purpose.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Health care expenditures constitute the “lion share” of most provincial budgets in Canada. Health Canada will use this study to help gain a better understanding of hospital expenditures in Canada, focussing on their cost efficiency for the sustainability of the Canadian health care system. Identifying the potential sources of inefficiency should allow developing policies that can help hospital management to reduce cost inefficiency and to increase the value for money spent on health care services.

4.11 Canadian Pesticide Sales Reporting and the Regulation of Pesticides in Canada

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OBJECTIVES/BACKGROUND/ISSUE(S): Historically, there was no national pesticide reporting system and limited information was available within Health Canada on pesticide sales in Canada. Since 2007, under the *Pest Control Products Act* and accompanying Pest Control Products Sales Information Reporting Regulations, the PMRA collects annual pesticide sales information from registrants of pest control products.

DESIGN/METHOD/DESCRIPTION: This program collects pesticide sales information to better inform the public and government organizations on the sales of pesticides in Canada. Sales data are considered a reasonable substitute for pesticide use data by the OECD, due to the expense of obtaining use data.

An electronic data collection and analysis system allows for registrants to report pesticide sales at the national and provincial level. The data allow the PMRA to answer questions related to pesticide sales in Canada from different provincial and federal government groups and to produce an annual public report.

OUTPUTS/RESULTS: The program experienced problems with data quality and reporting levels in the first year of reporting. Data quality was increased with targeted education. Compliance has increased since the inception of the program. Pesticide sales in Canada have been relatively consistent from 2007 to 2011, with small increases seen each year. The data show that the majority of pesticides sold in Canada are for agricultural uses, with just under one third of pesticides sold for non-agricultural uses, and a small portion sold for domestic uses.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The sales reporting program at the PMRA has proven to be a value added component in the regulation of pesticides in Canada, through collaboration with the incident reporting program, different pesticide evaluation streams, and the cost-recovery program at the PMRA, and with various departments at the federal and provincial levels of government. Continued collaboration with different groups and refinements to how the data are analyzed in the public report are expected in upcoming years.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This program has supplied sales data to help inform decision-making in various areas of pesticide regulation, such as, re-evaluation, incident reporting, and cost recovery, and in informing other government departments and the general public of the extent of pesticide sales in Canada.

4.12 The Changing Canadian Drug Scene in the Words of Drug Users

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OBJECTIVES/BACKGROUND/ISSUE(S): Monitoring of illicit drug use, abuse of new psychoactive substances (NPS), and prescription drug abuse supports the National Anti-Drug Strategy.

DESIGN/METHOD/DESCRIPTION: A two-year pilot project was conducted in major cities across Canada in 2012 and 2013. The objective was to monitor substance use among three high-risk drug using populations: street-entrenched adult drug users; street-involved youth drug users; and, recreational drug users. Estimates of the prevalence of use of known substances of abuse were obtained using face-to-face interviews of drug users. In addition, qualitative information on NPS and new patterns of drug use (including PDA) were derived from open-ended questions.

OUTPUTS/RESULTS: The results indicated that there were common patterns of drug use in this population. The most commonly abused substances were: cannabis, cocaine products and prescription drugs for street adults; cannabis and stimulants for street youth; cannabis, cocaine and ecstasy for recreational users. Street populations (adults and youths) reported abuse of prescription medications believed to have low abuse liability and street value such as antidepressants, antiepileptics or antipsychotics. Street-entrenched adult respondents more often reported using prescription opioids rather than street ones. Street youth reported abusing over-the-counter cough syrups containing dextromethorphan or pseudoephedrine and having cheap and easy access to prescription pills ($1-2 pills). Street youth reported the use of “Molly”. Though it seems to be related to ecstasy and MDMA, what Molly is remains unclear as each respondent defined it differently. The recreational drug using population was the one who most often reported the use of NPS, specifically substances from the 2-C family of drugs, DMT and Molly. Results about NPS of interest due to media coverage (e.g., bath salts or krokodil) are presented.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: These results document the drug use behaviours of three high-risk, drug-using populations. Some patterns of drug use were common across cities while some drug use behaviours were identified in only one or two cities. This knowledge complements existing surveillance activities.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The pilot project provided indications on how to develop an early warning system. Consideration will be given to the results and to the lessons learned in conducting this pilot project in our ongoing work towards drug control in Canada.

4.13 Estimating Medical Devices Expenditures in Canadian Hospitals

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OBJECTIVES/BACKGROUND/ISSUE(S): Medical technology is considered to be one of the key drivers of rising health costs in recent decades. However, the majority of technology-related spending is not well defined or quantified. The main difficulty in quantifying its importance is to find suitable proxies to medical technological innovation such as the wide range of medical devices used in hospitals and other settings.

DESIGN/METHOD/DESCRIPTION: This analysis sets out to measure medical devices expenditures using Canadian hospital data provided by the Canadian Institute for Health Information (CIHI). Expenses related to medical or surgical supplies used by all activity areas within the hospitals were estimated for the period between 2005 and 2012.

OUTPUTS/RESULTS: Canadian hospitals spent more than three and a half billion dollars on medical devices or six percent of total hospital expenditures in 2012. While medical devices are used all over the hospitals, the operating rooms and the diagnostic areas are the departments with the highest spending. In addition, spending in these activity areas increased the most between 2005 and 2012. Moreover, expenditure on medical devices varies with the teaching status, the size, and the geographic location of the hospitals.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: We expect that measuring medical devices expenditure in Canada will help identify areas where rapid technological change creates financial challenges to hospitals in the delivery of quality care.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This study provides further insight on the increasing cost of medical devices in Canadian hospitals, with additional evidence-based information to support policies and strategies on innovation and technology that could help manage costs, improve quality care delivery.

4.14 Health Impact Assessment (HIA) Framework of International Trade and Investment Rules

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OBJECTIVES/BACKGROUND/ISSUE(S): International trade makes up more than 60% of the Canadian economy and serves as a foundation for prosperity, good health and well-being in Canada. Health and trade are interconnected, as good health and economic prosperity are mutually reinforcing. At the same time, greater liberalization of the flows of goods, services and investment arising from new international trade agreements could place limitations on public policy, including the ability of the federal government to regulate for better health and safety, support sustainable health systems, and address health inequities.

DESIGN/METHOD/DESCRIPTION: Our aim is to develop a Health Impact Assessment (HIA) policy framework to maximize positive impacts of trade, while minimizing negative impacts on health and reducing health inequities in Canada. Design of this framework encompasses an extensive literature review, in-depth interviews and focus groups with key stakeholders, comparative analysis of mandatory impact assessment processes in Canada and other jurisdictions, and the development of analytical approaches, potential scenarios and policy alternatives.

OUTPUTS/RESULTS: Final products will include a research paper, policy tool kit comprised of a systematic guide and methodology to assess the impacts of trade and investment policies on health including qualitative and quantitative tools, and materials to strengthen knowledge transfer to target policy and research communities.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: To protect Canada's health interests in new trade and investment negotiations, federal and provincial health ministries need a health impact assessment framework to help answer the policy question, "What are the tangible benefits and costs to health from changes to rules in trade agreements"?

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Research on trade and on the health impacts of trade obligations is necessary to inform trade policy choices and its impact on health. This framework will help lay out a national approach to identifying the benefits and risks associated with governments' ability to monitor and to protect public health, to provide health services, to regulate environmental health, food products and consumer safety, or to ensure affordable access to medications and health technologies.

4.15 Determination of Supplier-to-Supplier and Lot-to-Lot Variability in Glycation of Recombinant Human Serum Albumin Expressed in *Oryza sativa*

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OBJECTIVES/BACKGROUND/ISSUE(S): The use of different engineered cells (e.g., plant, yeast) to produce the same recombinant human protein can result in expression-dependent chemical modifications (CMs) leading to variability of structure, stability and immunogenicity. Of particular interest are recombinant human proteins expressed in plant-based systems, which have shown particularly high CM variability. The use of plants to produce recombinant human therapeutic proteins is seen as highly desirable due to low-cost, high-yield systems and has been proposed for the production of human serum albumin (HSA), human transferrin, human growth hormone and the envelope protein of Japanese encephalitis virus. Recombinant HSA is of particular interest as it is the most abundant protein component of human plasma and, due to its abundance and specific properties, is utilized for numerous research studies and pharmaceutical applications. Obtaining HSA from human plasma has raised concerns about the possible transmission of infectious agents resulting in reduced use of plasma HSA (pHSA) as a drug excipient. These fears, as well as supply issues, have spawned the development of engineered versions of the protein from engineered cells of yeast and Asian rice, to produce artificial versions of the protein.

DESIGN/METHOD/DESCRIPTION: Recombinant human serum albumins (rHSA) produced in *Oryza sativa* (Asian rice) (OsrHSA) from a number of suppliers and different lots have been extensively characterized and compared to plasma-derived HSA (pHSA) and rHSA expressed in yeast (*Pichia pastoris* and *Saccharomyces cerevisiae*).

The heterogeneity of each sample was evaluated using size exclusion chromatography (SEC), reversed-phase high-performance liquid chromatography (RP-HPLC) and capillary electrophoresis (CE). Modifications of the samples were identified by liquid chromatography-mass spectrometry (LC-MS). The secondary and tertiary structure of the albumin samples were assessed with far U/V circular dichroism spectropolarimetry (far U/V CD) and fluorescence spectroscopy, respectively. Far U/V CD and fluorescence analyses were also used to assess thermal stability and drug binding.

OUTPUTS/RESULTS: High molecular weight aggregates in OsrHSA samples were detected with SEC and supplier-to-supplier variability and, more critically, lot-to-lot variability in one manufacturer-supplied product were identified. LC-MS analysis identified a greater number of hexose-glycated (addition of sugar) arginine and lysine residues on OsrHSA compared to pHSA or rHSA expressed in yeast. This analysis also showed supplier-to-supplier and lot-to-lot variability in the degree of glycation at specific lysine and arginine residues for OsrHSA. Both the number of glycated residues and the degree of glycation correlated positively with the quantity of non-monomeric species and the chromatographic profiles of the samples. Tertiary structural changes were observed for most OsrHSA samples which correlated well with the degree of arginine/lysine glycation.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: All OsrHSA samples showed elevated levels of arginine and lysine hexose glycation compared to rHSA expressed in yeast, suggesting that the extensive glycation of the recombinant proteins is a by-product of either the expression system or purification process and not a random occurrence. The extensive glycation of OsrHSA observed could have implications for therapeutic use of the protein since plant-specific sugars such as α-1,3- fucose and β-1,2-xylose have been associated with adverse immune reactions. Further studies will be conducted to determine if this variability in glycation is seen with other recombinant human proteins expressed in Asian rice.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This study shows that the human serum albumin proteins when produced in plant systems are modified with extensive addition of sugars. The knowledge gained from this study should allow for the better regulation of products generated in plant systems.

4.16 Analysis of Meningococcal Serogroup A Polysaccharide by ICP-MS

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OBJECTIVES/BACKGROUND/ISSUE(S): The Glycobiology Laboratory in the Centre for Biologics Evaluation Regulatory Research Division (BGTD) routinely develops methods to analyze the carbohydrate (sugar) components of biological therapeutics. One group of biological therapeutics are polysaccharide (sugar)-based meningococcal vaccines which are used to induce immune resistance against the bacterium *Neisseria meningitidis*. The active components of most meningococcal vaccines are four antigenic polysaccharides (Men A, C, Y, W-135) derived from the bacterial capsule. Development of analytical methods to measure the polysaccharide content of meningococcal and other polysaccharide-based vaccines is essential for their evaluation during new drug submissions and lot release testing.

DESIGN/METHOD/DESCRIPTION: The goal of this study was to develop a method using inductively coupled-mass spectrometry (ICP-MS) to quantify the phosphorus-containing Men A polysaccharide (N-acetylmannosamine-6-phosphate) in meningococcal vaccines by direct measurement of phosphorus. Several production lots from three commercially available meningococcal vaccines were analyzed by both the new ICP-MS method and by chromatographic methods previously developed by the Glycobiology Laboratory.

OUTPUTS/RESULTS: The analysis of several vaccines for Men A polysaccharide content by ICP-MS produced results comparable to those obtained by our established chromatographic methods. When compared with chromatographic methods, the new ICP-MS method reduced sample preparation time and did not require a Men A polysaccharide standard.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: ICP-MS is currently used as a complementary technique to the chromatographic methods previously developed for polysaccharide vaccine analysis in the Glycobiology Laboratory. The utility of this ICP-MS method for meningococcal Men A vaccine analysis has been expanded to include other vaccines such as *Haemophilus influenzae* (Hib, PRP polyribosyl phosphate) vaccines and meningococcal Men X (N-acetylglucosamine-4-phosphate polymer) vaccines.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This project illustrates the capability of the Glycobiology Laboratory (RRD-CBE-BGTD) to develop methods to analyze new biological therapeutics for human use in Canada.

4.17 Collaborative Approach to Promote Prudent Use of Medically-Important Antimicrobial Drugs in Food Animal Production

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OBJECTIVES/BACKGROUND/ISSUE(S): Antimicrobial drugs play an important role in maintaining human health and animal welfare. The global emergence of antimicrobial resistance and the lack of antimicrobial pipelines have nevertheless, drawn much attention toward the protection of precious antimicrobial resources. One obvious approach is to ensure the prudent antimicrobial use in all sectors wherever these drugs are used, including agri-food. The current regulatory guidance for safety, effectiveness and quality requirements for pre-market approval of veterinary antimicrobials is comprehensive and meets the needs in protecting human health and the food supply. However, the conditions of use of certain existing antimicrobial products in food animals (e.g., the historically-authorized growth promotion indications) may not meet current regulatory requirements and appropriate measures can be taken to bring their labelling at par with current standards.

DESIGN/METHOD/DESCRIPTION: An integrated approach involving multiple lines of scientific convergence and policy recommendations is being applied. This approach involved reviewing all existing food animal antimicrobial products and putting them in the context of their importance in human medicine for prioritization purpose, This was accompanied by considerations of regulatory efforts made by foreign regulatory agencies, recommendations from the World Health Organization, and inputs from the federal/provincial/territorial partners as well as stakeholders including veterinarians, pharmaceutical industry and food animal producers.

OUTPUTS/RESULTS: The Veterinary Drugs Directorate recognizes the critical importance of prudent antimicrobial use to limit resistance development. Our approach identified the need to modify some use conditions of the approved food animal antimicrobial products. Thus, the Directorate issued, on April 10, 2014, a Notice to Stakeholders highlighting two concrete measures: Removal of growth promotion and/or production claims of medically-important antimicrobials; and Development of options to strengthen the veterinary oversight of antimicrobial use in food animals. These measures were aimed at promoting prudent use of antimicrobials in food animals. Our approach also revealed the necessity to sustain surveillance efforts on antimicrobial use and resistance.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: There is ongoing consultation with the impacted parties to specify the procedures for implementation of these measures with anticipated target date of December 2016, which aligns with similar measures being implemented by the United States Food and Drug Administration.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Antimicrobial resistance is a priority public health issue. Our initiative provides an example to demonstrate a collaborative approach between the regulator and stakeholders to address the complex issue associated with appropriate risk management of antimicrobial use and resistance.

4.18 Rapid and Accurate Determination of the Potency of MMRV Vaccine by Quantitative Polymerase Chain Reaction

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OBJECTIVES/BACKGROUND/ISSUE(S): All human vaccine lots 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. MMRV vaccines are designed to protect children against measles, mumps, rubella, and varicella-chicken pox. There is increasing demand for this vaccine, and an urgent need to develop faster, more accurate quality control assays. This work describes the development of a new method allowing rapid and accurate measurement of the MMRV vaccine. Future application of the method to routine vaccine quality control could improve the availability of the MMRV vaccine.

DESIGN/METHOD/DESCRIPTION: The potency of the MMRV vaccine is currently determined by the $TCID_{50}$ (measles, mumps, and rubella) and plaque assay (varicella) techniques. These techniques require the use of inhibitory antibodies, as well as 3 cell lines, and that the infection with the cells proceeds for 7-10 days. Limitations of these techniques are: 1) assay duration; 2) labour intensity; and, 3) ambiguous results, leading to variation within and between labs. In 2010, Public Health Agency of Canada (PHAC) implemented a two dose schedule in routine immunization of children against varicella. MMR is already routinely administered in two doses. 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.

We developed a new assay to evaluate the potency of MMRV vaccines using quantitative PCR in combination with a step resulting in a more efficient infection of the cells allowing potency results to be obtained 24 hours after infection.

OUTPUTS/RESULTS: The length of assay time has not only been substantially reduced, but the need for inhibitory antibodies is also eliminated and we have modified it into a high-throughput 96-well plate format. Using the qPCR technique we have also automated the data acquisition reducing variation between and within labs.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Our assay provides a high-throughput method with reduced variation and results obtained in 24 hours with acceptable accuracy and reproducibility with no need for inhibitory antibodies. Further evaluation of this assay will need to be done to demonstrate statistically acceptable reproducibility and accuracy as compared to the traditional methods.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: With increasing demand for MMRV vaccines this allows manufacturing and Health Canada to rapidly and confidently release vaccine lots while assuring vaccine quality.

4.19 Results of a Survey on Use of Hand Sanitizers by Adults in Ottawa, Canada

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OBJECTIVES/BACKGROUND/ISSUE(S): Estimating human exposures to chemicals found in personal care products requires information on the frequency of use and amount of product used. This type of information is not always available for all products of interest, including the use of hand sanitizers. The Existing Substances Risk Assessment Bureau (ESRAB) in collaboration with the New Substances Assessment and Control Bureau (NSACB) conducted a survey on the use of hand sanitizers during the 2013 Health Canada Science Forum in order to fill this data gap.

DESIGN/METHOD/DESCRIPTION: The goal of the survey was to quantify how much hand sanitizer is used per application as well as how often participants use the product. This information will be added to ESRAB and NSACB's compilation of default exposure factors for cosmetic and personal care products for use in human exposure assessments conducted under Canada's Chemicals Management Plan.

At the 2013 Health Canada Science Forum, a booth was set up with 3 types of hand sanitizers (liquid from a squeeze bottle, liquid in with a pump dispenser, and foam with a pump dispenser) and a scale. Participants of the forum were asked to answer a short questionnaire on frequency of use, and to use one of the appointed hand sanitizer products. The product was weighed before and after each participant's use in order to determine the amount of product used per application. Information was then compiled and analyzed.

OUTPUTS/RESULTS: Approximately 180 participants between the ages of 20 and 59 years from Health Canada responded to the questionnaire and about 160 participants used one of the hand sanitizers. The frequency of use of the product ranged from multiple times per day to never or infrequently. The amount of products used across the 3 types of hand sanitizers ranged from 0.04-4.54g/application, with the average amount used being highest for the liquid pump dispenser.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: This is the first/largest survey in North America that targeted the use of hand sanitizer in adults. It is also the first source of data related to the use of hand sanitizers in children and will contribute to exposure factor development related to consumer use of hand sanitizers. This work will improve consistency in exposure assessments and provide a source of exposure factors for researchers and regulators across the department.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Development of exposure factors for estimating potential human exposure to substances present in cosmetics and personal care products such as those developed from this project provides a consistent approach as well as data that is relevant to the use of products by Canadians. This in turn supports and strengthens ongoing risk assessment work under the Chemicals Management Plan as well as other programs assessing or managing risks associated with this product sector.

4.20 Developing a Framework to Protect Sensitive Habitats from Agricultural Pesticide Use

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OBJECTIVES/BACKGROUND/ISSUE(S): Prior to allowing the sale, import or use of a pesticide in Canada, Health Canada's Pest Management Regulatory Agency (PMRA) assesses potential environmental risks to non-target organisms and their habitat from the use of pesticides. When potential risks are identified, mitigation measures (mandatory and/or voluntary) are used to reduce the risks to acceptable levels.

DESIGN/METHOD/DESCRIPTION: Concerns raised by stakeholders have indicated that PMRA's current approach to protecting sensitive non-target habitats from the use of pesticides may unintentionally be acting as a disincentive to the protection of existing habitat and creation of new habitat in agricultural landscapes. A key factor contributing to this problem is that the types of habitats considered to be sensitive, and the extent to which each type requires protection, are not clearly defined.

This project aims to develop a comprehensive national framework for classifying farmed and unfarmed habitats found in agro-ecosystems based on a combination of physical and functional (i.e. ecological goods and services) attributes. This framework will then be used to inform the development of policy options for protecting sensitive agro-habitats from pesticide exposure.

OUTPUTS/RESULTS: The policy approach will be focused on identifying new tools and resources which will make it easier for growers and applicators to identify habitat types requiring protection and comply with required mitigation measures, as well as, foster increased engagement in environmentally sustainable farming practices by landowners.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Establishing a common language to classify agro-habitats, as well as criteria to determine their relative value will strengthen PMRA's ability to make informed management decisions regarding the extent of habitat protection required.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This approach will enable the Agency to continue to meet its obligations under the *Pest Control Product Act* to protect important habitats and support sustainable pest management strategies, while also addressing stakeholder needs and supporting the competitiveness of the agricultural sector.

4.21 Molecular Interaction and Amyloid-like Aggregates of the Conserved Hydrophobic Region of the Prion Protein in presence of Dodecylphosphocholine Micelles

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OBJECTIVES/BACKGROUND/ISSUE(S): Aggregation of a misfolded form of normal cellular proteins is associated with neurodegenerative diseases such as Alzheimer, Huntingdon, and prion diseases. Past failed trials that tested potential therapeutics aimed at reducing plaque depositions are raising the issue that amyloid may not be the appropriate target but a side product of these diseases. Regardless of the wealth of information on these proteins, the mechanism that governs the misfolding of these proteins remains unknown at the molecular level. In an attempt to fill this knowledge gap, we have extensively studied the structural behaviour of the Prion protein (PrP) in a membrane mimicking environment known to be involved in its inter-conversion to the disease associated form.

DESIGN/METHOD/DESCRIPTION: A domain of the prion protein referred to as the conserved hydrophobic region has been produced in Escherichia Coli and studied by NMR spectroscopy in Dodecylphosphocholine (DPC) micelles as a membrane mimicking system. Various techniques were used to characterize the structure and the conformational behaviour in this model membrane environment.

OUTPUTS/RESULTS: In this study, we showed that the peptide PrP(110-136) containing the conserved hydrophobic region of the PrP protein has a high affinity for DPC micelles. The results showed that the peptide inserts into the micelle, thus adopting an alpha-helical conformation. Also, we found that the peptide can interact with the micelle surface. The surface bound species readily forms fibril-like aggregates, while the pure peptide remains in solution in the absence of DPC micelles. Proofs of the formation of amyloid fibrils were obtained with the observation of birefringence with optical microscopy, and with Thioflavin-T binding that showed the characteristic absorption with fluorescence spectroscopy.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Our findings shed light on the early stage of the interconversion and allowed us to propose a mechanism for the process that can explain the onset of the amyloid plaques in cases of sporadic forms of the disease, as well as the genetic (inherited) form such as the Gerstmann–Sträussler–Scheinker syndrome.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The knowledge developed with this study will complement the current understanding of this class of diseases that will assist decision-making in policy development for drug production and future drug submission review.

4.22 Structural Impact of Gly52 Mutations in the Catalytic Domain of Diphtheria Toxin: How does CRM197 stimulate the immune system?

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OBJECTIVES/BACKGROUND/ISSUE(S): The Centre for Biologics Evaluation at Health Canada is responsible for evaluating and conducting regulatory research on vaccines, subsequent entry biologics and emerging issues to maximize the safety and efficacy of products available to the Canadian population. Vaccines against bacterial infections such as Meningococcal-C or Haemophilus- influenzae-type-b (Hib) are produced by linking cell-surface polysaccharides from the disease-associated bacteria to a carrier protein. The latter is selected for its ability to stimulate an immune response in order to enhance the production of neutralizing antibodies against the target bacteria. Cross-reacting-material 197 (CRM197) is used as a carrier protein for this property.

DESIGN/METHOD/DESCRIPTION: CRM197 is a naturally occurring, non-toxic mutant of diphtheria toxin. It results from a single nucleotide mutation changing glycine 52 to a glutamate residue (G52E). This residue is located besides the catalytic site of the catalytic domain of diphtheria toxin (DTA) thus inducing a perturbation of the local conformation. This suggests that the lack of toxicity results from a loss of catalytic activity. Here, we present a study of the structure of DTA, CRM197 and mutants (G52A, G52S, G52N, G52Q) using nuclear magnetic resonance spectroscopy. We investigated the effects on the structure and stability of the catalytic domain by mutating position 52 with amino acids of increasing size.

OUTPUTS/RESULTS: In this study, we present the three-dimensional structure of DTA in solution derived from chemical shifts measured by NMR spectroscopy. Analysis of the mutants at position 52 indicates that the catalytic domain of diphtheria toxin does not tolerate the smallest conformational perturbation. The latter destabilizes the three- dimensional fold leading to protein aggregation.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Our finding provide strong indication that the lack of toxicity observed for CRM197 is the result of a highly perturbed conformation near the active site producing an improperly folded catalytic domain.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The intent of this study is to develop a better understanding at the molecular level of the impact of the naturally occurring G52E mutation on the diphtheria toxin. This will allow for better explanation as to how carrier protein used in the Toxoids allow for immunization with acceptable safety.

4.23 Analysis of Commercial Pesticide Importation Data to aid Compliance and Enforcement

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OBJECTIVES/BACKGROUND/ISSUE(S): More than 80% of pesticides used in Canada are imported (McEwan and Deen, 1997). Results from annual inspections showed that unregistered pesticides offered for sale constituted the most common violation in the marketplace. Preventing entry of these products into Canada is a better long-term solution. Since 2012, the Pest Management Regulatory Agency (PMRA) and other participating government agencies have been receiving commercial import data from the Canada Border Services Agency (CBSA) under the Single Window Initiative. We analysed these data to identify trends in pesticide imports and to gather evidence to respond to non-compliant importations.

DESIGN/METHOD/DESCRIPTION: Information from 200 transactions involving pesticides is daily received from the CBSA and manually organised based on the Harmonized System codes. Key metrics including volume, categories (insecticide, herbicide, fungicide, and sanitizer), importers, countries of origin and ports of entry into Canada, are considered.

Monthly summaries are sent to the regional inspectors, who identify non-compliant imported pesticides in their respective regions. Subsequent actions are taken based on the risks, the non-compliance history of the importer or any incoherent information identified from the data.

OUTPUTS/RESULTS: Over 30 million kilograms of pesticides are yearly imported into Canada. 44% of all pesticides imported are herbicides followed by sanitizers (25%). Pesticides originate from the USA (85%), Europe (6%) and China (4%).The majority of pesticides enter into Canada through Ontario (over 60%).We found that the importation of pesticides was seasonal. This finding allowed the PMRA's Pesticide Compliance Program to better target and plan inspections when pesticides are still in warehouses. Since 2013, 142 inspections originating from the data were conducted with 84 violations confirmed as of April 01, 2014.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Import data analysis enabled the Pesticide Program to better understand the trends and patterns of pesticide importation and better target compliance verifications. Once the requisite software is available, predictive analysis using the data will facilitate the identification of non-compliance situations.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: PMRA's work using the CBSA data from the border raised interest among other Health Canada regulatory programs and assisted with the delivery of compliance and enforcement activities.

4.24 Identification of Three Novel Biomarkers of Islet Regenerative Function in Human Multipotent Mesenchymal Stromal Cells

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OBJECTIVES/BACKGROUND/ISSUE(S): Multipotent mesenchymal stromal cells (MSCs) are proposed as a novel therapy for treating diabetes by promoting the regeneration of damaged islets of Langerhans, which are known as the insulin-producing clusters. The clinical promise of such treatments may be hampered by a high degree of donor related variability in MSC function and a lack of standards for comparing potency.

DESIGN/METHOD/DESCRIPTION: This study identifies markers of cultured human MSCs that are directly associated with islet regenerating function. MSC cultures from six healthy bone marrow donors were demonstrated to have differing capacities to reduce blood glucose levels in a murine diabetic model, which led to their classification into islet regenerative (R) or non-regenerative (NR) groups. MSC cultures from both groups were then directly compared for their protein and small non-coding RNA molecule (microRNA) profiles.

OUTPUTS/RESULTS: A total of 1410 proteins and 1362 microRNAs were quantified from both groups resulting in the identification of 612 proteins with increased abundance and 10 microRNAs with decreased expression in R MSC cultures. Eleven proteins whose altered abundance levels were associated with reduced expression of microRNAs were selected for further study. Further validation showed that EMILIN-1 and ILK are significantly associated with islet regeneration, and that HDGF may also be a valuable marker.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Our study is the first to identify markers of cultured human MSCs whose abundance levels are associated with the biological potency of islet regenerating function.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This research may provide a means to standardize MSC-based health products using biomarkers that are directly linked to product potency.

4.25 Rapid Classification of Authentic and Counterfeit Viagra Using Focal-Plan-Array Fourier Transform Infrared Spectroscopy

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OBJECTIVES/BACKGROUND/ISSUE(S): The distribution of counterfeit drugs is a serious worldwide issue. In order to prevent the health risks associated with counterfeit drug consumption, government regulatory and law enforcement agencies require fast and reliable scientific measures to distinguish the genuine and counterfeit products. Infrared imaging by focal-plane-array Fourier transform infrared (FPA-FTIR) spectroscopy may be a useful tool for this purpose because it allows for the rapid chemical characterization of tablets and other solid dosage forms.

DESIGN/METHOD/DESCRIPTION: The aim of this project is to develop methodology to rapidly distinguish between authentic and counterfeit erectile dysfunction drugs using FPA-FTIR spectroscopy.

Infrared imaging of 28 authentic Viagra™ pills (8 pills of 25 mg, 10 pills of 50 mg and 10 pills of 100 mg) and 13 counterfeit erectile dysfunction drugs was undertaken. Multiple images were collected from the coating and the core of pills to analyze the chemical composition, presence/absence of the active and non-active ingredients and their distribution.

OUTPUTS/RESULTS: Multivariate analysis of the spectral data contained within the infrared images allowed for discrimination between the authentic and counterfeit drugs.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The results of this study demonstrate that infrared imaging can provide a rapid (within minutes) and effective means of characterizing counterfeit drugs and distinguishing them from authentic products.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The implementation of infrared imaging may assist regulatory and law enforcement agencies in protecting consumers against drug counterfeiting.

4.26 Rapid Identification and Classification of Pathogenic *Escherichia coli* by Focal-Plan-Array Fourier Transform Infrared (FPA-FTIR) Spectroscopy

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OBJECTIVES/BACKGROUND/ISSUE(S): Pathogenic strains of *Escherichia coli (E. coli)* cause serious symptoms in humans. These toxin-producing *E. coli* (VTEC) are transmitted to humans through contaminated food and water.

DESIGN/METHOD/DESCRIPTION: The VTEC can be categorized into subspecies level based on serotypes and different symptoms that it causes in human (pathotypes). To address the need for rapid and efficient methods for the identification of such a diverse group of bacteria, infrared spectroscopy with a sophisticated detector (FPA-FTIR) was used to investigate the fingerprint of the whole organism in a non-destructive manner.

A total of 98 clinical isolates of VTEC, equally distributed among 4 pathotypes [EHEC (serotype O157:H7), EPEC (16 serotypes), STEC (20 serotypes), and UPEC (19 serotypes)], were selected as reference strains for this study. For each isolate, infrared images of intact cells taken from pure cultures were acquired by FPA-FTIR spectroscopy and processed to generate high-quality infrared spectral fingerprints. A set of four replicate spectra of each isolate was included in a spectral library, yielding a library size of 392 replicates.

OUTPUTS/RESULTS: Using specific mathematical analyzing techniques (multivariate analysis), we successfully differentiated the different serotypes solely based on their infrared spectral fingerprints.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: FPA-FTIR fingerprinting has the potential for rapid identification of pathogenic *E. coli* at the serotype level.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: These findings demonstrate the potential of infrared spectral fingerprinting as a rapid and reliable tool for screening and discriminating microorganisms of clinical or foodborne origin, at a pathotype and serotype level. This may significantly reduce the time of identification of the unknown microorganism; therefore, enhancing the efficiency of the treatment in epidemic.

4.27 Medications and Adverse Effects during Extreme Heat Events: Vulnerability Assessment Using PHAC Pharmacological Surveillance Databases

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OBJECTIVES/BACKGROUND/ISSUE(S): Common and mild side effects (e.g. mild dehydration) are often associated with prescribed medications (e.g. anti-hypertensives, anticholinergics). Under extreme heat conditions, these side effects can be exacerbated thereby increasing risks for more serious health outcomes (e.g. heat stroke).To define and address this risk, we need to describe the prevalence of medication usage.

DESIGN/METHOD/DESCRIPTION: The Centre of Foodborne, Environmental and Zoonotic Infectous Diseases (CFEZID), developed a pharmacy syndromic surveillance (PSS) pilot study to assess the utility of over-the-counter medications and prescription drugs for public health. We analyzed the PSS database to identify the geographic and demographic medication usage patterns and prevalence of certain diseases and mapped out vulnerable populations. This pilot project aims to outline the feasibility, uncertainties and challenges of using this new dataset.

An initial vulnerability assessment of a subset of the Canadian population was investigated using PSS, medications with potential adverse interactions during an extreme heat event were categorized using a series of systematic literature reviews produced by the Institut National de Santé Publique (INSPQ). The drug identification numbers (DINs) in HC's Drug Product Database were identified and medication usage was analyzed by active ingredients, age, gender, dispensing date and postal Forward Sortation Areas (FSAs) of the store.

OUTPUTS/RESULTS: Preliminary analysis has demonstrated that:

a. Prescription rates of medications can be tracked
b. Prevalence of chronic diseases in Canada can be estimated by drug dispensation data and can be further stratified by age and gender.

Analysis of prescription usage by FSA can provide a useful tool in mapping out vulnerable populations.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The rate of prescription can be tracked using the PSS which could be considered a potential tool to inform public health program design and emergency response planning. This pilot project has revealed both the utility of this new dataset as well as methodological challenges that can be addressed in the future.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Understanding medication usage can further increase the capacity of Canadian communities to assess their vulnerability to extreme heat events. This will provide regional and local health authorities with evidence-based information that supports the implementation of public health policies and health promotion and prevention of heat-related illness and deaths.

4.28 Pesticide Label Database

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OBJECTIVES/BACKGROUND/ISSUE(S): The PMRA requires quick access to pesticide label information in order to respond to various enquiries from the public, stakeholders, media, as well to as to respond to the information needs of evaluators in the Agency. The main problem is that pesticide labels are stored in a format that only allows for a basic text based search. This is inefficient due to label complexity and variable terminology. Label search results require a significant amount of time and resources. As a result, search results would often vary with the knowledge and experience of the evaluator.

DESIGN/METHOD/DESCRIPTION: The original purpose of the project was to provide evaluators with quick and easy access to accurate and complete label search results, and to standardize search results across the Agency. In order to accomplish this, a relational database was designed: the Pesticide Labels Database (PLD). Label content was extracted into defined database fields, and each field was assigned a relationship within the database in order to allow for relational queries. Additional programming and data entry has allowed the project to expand beyond its original scope, and meet the evolving information needs of the Agency.

OUTPUTS/RESULTS: Web reports were programmed to allow evaluators to access data from complex searches in a fraction of the time and with a much higher degree of certainty than was previously possible. Time is saved gathering label information for alternative because of the significantly reduced number of false positives returned in a given search. For example, PLD search results for products registered for use on the crop “oats” results in 60% fewer false positives. Further use of the database revealed greater potential for use in Agency risk assessments than originally anticipated.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The PLD has been embraced by users, and is now a standard tool for the Agency. Agency-wide release of the tool is scheduled for fiscal 2014-2015.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The PLD enables rapid output of accurate and complete data from pesticide labels. It will result in significantly decreased response time and resources required to respond to the information needs of the PMRA (e.g. media line enquiries, ministerials, enquiries from other governmental departments and branches, etc.).

4.29 Challenges in the Application of Pharmacogenetics in Pharmacovigilance: Donepezil as a Case Example

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OBJECTIVES/BACKGROUND/ISSUE(S): During early clinical drug development, genetic intervariability In subjects is often explored to help establish drug tolerability or response; however the application of this pharmacogenetic data in post-market risk management practices has been limited by challenges in data collection/interpretation/validation and implementation in medical practice.

DESIGN/METHOD/DESCRIPTION: While pharmacogenetic considerations arising out of pre-market studies are currently included in the Canadian Product Monograph (CPM) for many drugs, the use of genetic intervariability data to predict adverse drug reaction (ADR) susceptibility is often difficult to establish. A recent assessment of the risk of muscle tissue breakdown, termed rhabdomyolysis, in patients taking a drug for the treatment of Alzheimer (i.e., donepezil) highlighted the need for considering a role for pharmacogenetic data in pharmacovigilance.

OUTPUTS/RESULTS: Genetic variability in drug metabolizing enzymes such as CYP2D6, are thought to play a significant role in explaining the differences in response and tolerability to donepezil. For example, patients who have a mutated CYP2D6 have been shown to have a 32% slower clearance of donepezil. Since a large proportion of subjects reporting rhabdomyolysis with donepezil were from Japan, and occurred after dosage increase, CYP2D6 genetic variations were reviewed in this specific population.

This association could not be further explored as no genetic data was collected along with ADR reports. An overview of drug labels that include pharmacogenetic data was also performed to better understand the scope as well as potential applications of this information.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: While the role of pharmacogenetics in drug safety is expanding, there are limitations hampering the broad integration of pharmacogenetic data into the risk management life cycle of a drug.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Health Canada should continue to be involved in the development of pharmacogenetic information networks and guidance. As mandatory adverse event reporting in healthcare facilities becomes a reality, there could be an opportunity for Health Canada to solicit pharmacogenetic data, when available.

4.30 Development of the Human Pathogen and Toxin Regulations in Collaboration with Academia

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OBJECTIVES/BACKGROUND/ISSUE(S): The Public Health Agency of Canada (the Agency), with input from stakeholders, has developed a new program and regulatory framework to support the full implementation of the Human Pathogen and Toxins Act (HPTA). The Agency's goal was to develop a framework for individuals working with human pathogens and toxins that would allow the best and most innovative science at Canadian universities and other science and technology facilities to proceed in a way that is as safe and secure as possible.

DESIGN/METHOD/DESCRIPTION: The HPTA established a safety and security regime to protect the health and safety of the public against risks posed by human pathogens and toxins. While the HPTA builds upon Canada's existing biosafety program for imported human pathogens and toxins, there is currently limited oversight over domestically acquired agents. The proposed Human Pathogens and Toxins Regulations (HPTR) address this gap and will bring the HPTA into full force.

OUTPUTS/RESULTS: Throughout the consultation process the academic sector expressed unique concerns and needs relating to HPTA implementation. In general the unique risks faced by the academic sector include:

- Biosafety 'rules' often not compatible with research and innovation objectives
- High volume of student turnover and mobility
- Hard to control/manage researchers and laboratories
- Variable internal accountability systems for biological risk

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The Agency learned that academia would benefit from enhanced engagement to inform the regulatory development and to ensure the framework did not impact Canada's science and innovation capacity. The Agency has held sector specific consultation with academia, participated in conferences and has held discussions with academic managers, researchers and biosafety personnel, to more completely understand their context.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The Agency has used this input to develop a regulatory framework that is proportionate to risk and performance based. The Agency will continue to work with provinces and territories in an effort to eliminate overlap where appropriate and to minimize administrative and cost burden where possible.

4.31 Preliminary Risk Assessment of Blood Product Adverse Reaction Reports

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OBJECTIVES/BACKGROUND/ISSUE(S): Health Canada has had a blood product surveillance system in place for several years; however, recent enhancements have been undertaken to put in place a screening and assessment process in order to ensure the earliest possible identification of safety issues related to these products. Manufacturers of blood products are required to report to Health Canada all serious adverse reactions (AR) within 15 days of becoming aware of the reaction.

DESIGN/METHOD/DESCRIPTION: The Canada Vigilance Program receives AR reports from health professionals, consumers, or the manufacturers for all marketed health products, including blood products (e.g., immunoglobulins, coagulation factors, fibrin sealants, etc...). The reports are sent to the bureau responsible for their evaluation. The MBBNHPB has implemented further enhancements to the surveillance and assessment of these products.

A preliminary risk assessment of all serious AR reports is completed to detect possible safety issues associated with blood products. If a safety issue is identified, the appropriate risk minimization steps are implemented and immediate regulatory actions are taken. Where appropriate, additional scientific evaluations may be undertaken to validate potential safety signals in order to establish a relationship between the blood product and the AR.

OUTPUTS/RESULTS: The enhancements made to the blood product surveillance system were implemented in early 2014 and are now part of on-going surveillance activities for blood products. To date, the program has received 404 AR reports of which 332 were serious. Thus far, no new safety issues were identified.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: These enhancements will enable Health Canada to identify and evaluate emerging blood product safety issues and trends in a timely manner. Preliminary data suggests that this enhanced approach has the potential to add significant value to the existing blood surveillance system.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The enhancements made to the surveillance system are intended to further improve the safety of blood products in Canada by raising awareness of the need to report ARs to fractionated blood products and strengthening Health Canada's ability to take timely action to manage identified risks.

4.32 The Native Conformation of Influenza Haemagglutinin as a Surrogate for Vaccine Potency

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OBJECTIVES/BACKGROUND/ISSUE(S): Following the outbreak of the novel H1N1 influenza virus in 2009 manufacturers quickly began developing monovalent vaccines to target this specific influenza strain. Simultaneously regulators streamlined processes to evaluate the safety and effectiveness of these vaccines so that they could be approved for release. Despite these efforts, the majority of the North American public were unable to access suitable vaccines until the peak of the virus outbreak had passed. Following the conclusion of the 2009 H1N1 influenza pandemic, analysis of the public health response highlighted several areas for improvement including the development of alternative approaches for potency evaluation of influenza vaccines.

DESIGN/METHOD/DESCRIPTION: In this work, we developed a rapid assay using group specific monoclonal antibodies to detect the native conformation of influenza haemagglutinin (HA) antigen as a surrogate for vaccine potency based on using a sandwich ELISA methodology. The results were compared to potency values as determined by SRID, the current standard used for influenza potency testing, hemagluttination inhibition, SDS-page and an animal model.

OUTPUTS/RESULTS: This novel ELISA method accurately measured the potency of one manufacturer's product as determined by the conventional release test (SRID), however it significantly underreported the potency of a second manufacturer's vaccine. This disparity was also observed using other approaches such as haemagglutination inhibition and a synthetic receptor-based potency assay, with both also underreporting the potency of the same product. By SDS-page analysis we demonstrate that these two vaccines have significant differences in their respective HA monomeric, trimeric and oligomeric content and these differences in the quaternary structures are the likely root cause of the disparity in potency results between the various assays. However in an animal model of vaccine efficacy we find no significant difference in the immune response generated by each vaccine. This work highlights the difficulty in using an assay that depends solely on receptor binding or a single epitope as a surrogate for vaccine potency.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Simple replacement of current potency testing regime (SRID) with an ELISA based method does not seem feasible given the variability between the vaccine products of different manufacturers.

4.33 Continuous Improvement Toward a Real-Time, Risk-Based Surveillance and Risk Management Program for Human Cells, Tissues, and Organs Adverse Reactions in Canada

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OBJECTIVES/BACKGROUND/ISSUE(S): Human Cells, Tissues and Organs (CTO) are a special category of health products regulated by Health Canada. CTO are minimally manipulated products (e.g., stem cells, musculoskeletal tissue, kidney, etc.) transplanted from donors to patients, and in most cases, the product is unique. These products pose distinct risks to transplant patients, and thus are governed by their own set of regulations.

As with all marketed health products, Health Canada continuously monitors the safety of CTO; however, Health Canada's current CTO adverse reaction monitoring, evaluation, and risk management program (CTO Program) faces several challenges, including under-reporting, poor quality reports, timely receipt of important safety information and the risk of disease transmission from donors to patients

DESIGN/METHOD/DESCRIPTION: To address these challenges, Health Canada is actively working on multiple strategies to enhance the current national CTO Program.

OUTPUTS/RESULTS: Several projects, within the enhanced CTO Program, have been initiated. Health Canada is piloting a risk-based review process for CTO adverse reaction reports that includes evaluation within one business day of receipt, tracking and assessment of compliance with Canadian regulations, and collection of missing information. Strategies to improve adverse reaction reporting are also underway, including a rapid feedback form to quickly obtain critical information, and updates to the adverse reaction reporting guidance document. A rapid risk communication strategy is under development and outreach efforts to improve adverse reaction reporting and increase stakeholder participation are ongoing, including presentations at national scientific fora and direct communication with stakeholders. New risks have been identified, such as allergy and malignancy, and efforts to strengthen the national standards and regulations are in progress.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The pilot phase of the enhanced CTO Program has been completed. Going forward, the department will continue its improvement efforts, with a focus on the quantity, quality, and types of reports received and the creation of an Organ Safety Network in Canada. Future work will also focus on increasing communication between Health Canada and the transplant community.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Ultimately, the enhanced CTO adverse reaction monitoring, evaluation, and risk management program is expected to strengthen Health Canada's ability to maximize the safety of CTOs transplanted in Canada.

4.34 The Saga of Using Mass Spectrometry in Protein Quantitation of Influenza Vaccines: To Label or Not to Label

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OBJECTIVES/BACKGROUND/ISSUE(S): The quality control of complex biological products often includes quantifying key proteins. For example, influenza vaccines are controlled by indirectly measuring the viral surface protein, hemagglutinin. We have been exploring the use of mass spectrometry to quantify these proteins as well as numerous other key proteins in these products.

DESIGN/METHOD/DESCRIPTION: This study is based on the mass spectral observation that the three most intense peptides from all proteins digested with the enzyme trypsin, have the same relative response within 15%. The validation of this method has been explored by producing, in-house, polypeptides that only include the peptides being measured and those from reference standard proteins. By necessity the key sequences are the same concentration and we may use their relative responses and the known concentration of the standard protein to more rigorously quantify these critical proteins. Further, we have purchased stable isotope labelled peptides that have the identical sequence to those from the vaccines and compared their relative intensities by tandem mass spectrometry.

The new methods were developed and run in parallel with standard methods for numerous annual and pandemic influenza vaccines.

OUTPUTS/RESULTS: The results indicate that the concentration determination of key proteins using mass spectrometry is very complex and requires careful validation. The assumptions regarding the purity of standard proteins and peptides must be carefully scrutinized.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The current official methods require months to prepare reagents and only quantify hemagglutinin. The methods being developed here may be applied within one week and will quantify not only hemagglutinin, but also neuraminidase and residual proteins from the production process (for example, the egg protein ovalbumin). The key advantages of this method are its rapidity and the wide range of proteins that are identified and quantified. These are exactly the required attributes for quality control of pandemic vaccines and the investigation of products associated with unexpected adverse events.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: The lessons learned in these studies may be applied to many other biological products for the identification and quantitation of critical proteins. This method will provide a new tool to allow Health Canada to confidently review products in emergency situations such as pandemic influenza outbreaks.

4.35 Biologics: Expenditure Trends and Challenges Ahead

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OBJECTIVES/BACKGROUND/ISSUE(S): Overall drug spending growth has slowed in the last few years, however spending on biologics has increased rapidly. This study presents an analysis of expenditure trends of biologics in provincial public drug plans. It also discusses potential impact of biologics on overall drug spending and challenges in managing costs in the future.

DESIGN/METHOD/DESCRIPTION: The study examines expenditure trends of biologics compared to non-biologics by using the CIHI's National Prescription Drug Utilization Information System (NPDUIS) database from 2004/05 to 2011/12. Drugs are identified and aggregated by the Anatomical-Therapeutic-Chemical level 3. To examine factors contributing to cost growth, the increases in drug costs are decomposed into increases driven by changes in utilization, average unit cost, therapeutic class, etc. Trends are also compared across provincial public plans to determine whether differences exist.

OUTPUTS/RESULTS: Cost of biologics in public plans rose 230% (in current dollars) from 2004/05 to 2011/12. Its share of total drug cost was more than doubled over the same period of time. Biologics have become a major driver of drug cost growth in public plans. It accounted for 91.2% of total drug cost growth in 2011/12. The rapid cost growth was mainly driven by increasing cost per claim. Immunosuppressant and ocular vascular disorder agents accounted for 85% of cost growth of all biologics in that period. More new biologics will likely be introduced in coming years with potential huge costs. Biologics alone will contribute over 5 percentage points to total drug cost growth in 2021/22, if recent trends continue in the next decade.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Biologics have become a major driver of cost growth in recent years, and their impact on public drug plan spending will likely increase further as more new drugs become available.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: Given the rapid cost growth of biologics, it will be critical to better manage the approval, listing and cost of biologics, in addition to the current policy focus on generic pricing, to avoid future cost escalation.

4.36 Canada's Computed Tomography (CT) Survey: Development of Diagnostic Reference Levels (DRLs)

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OBJECTIVES/BACKGROUND/ISSUE(S): CT is a valuable tool in medicine, providing essential information that supports the diagnosis, treatment and monitoring of patients. This value is reflected in the increasing number of CT scanners and examinations performed in Canada; however, it is important to ensure that as more CT imaging is performed, examinations are carried out with the lowest levels of exposure possible (optimisation). A commonly accepted method to assist with imaging optimisation is the implementation of Diagnostic Reference Levels (DRLs).

DESIGN/METHOD/DESCRIPTION: DRLs are "benchmarks" calculated from CT exam dose information, for common exams (Head, Chest, Abdomen etc.) and attempt to provide a reasonable measure or "target" of what would be considered good and reasonable practice. They have proven to be an effective measure in reducing patient exposure for frequently used protocols, while allowing imaging staff sufficient room for adapting clinical needs.

A close collaboration with provinces and territories was established from the onset, and encouraged a high degree of participation. Survey booklets were mailed to and completed for each confirmed diagnostic CT scanner across Canadian centres. Facilities were given ~ 16 weeks to complete the booklets which provided all necessary tables to collect information on equipment specifications and imaging practice.

OUTPUTS/RESULTS: Overall, the national survey resulted in the capture of information on 387 CT scanners (76% of 510 units in Canada) corresponding to 27 774 imaging samples. Data gathered covers an extensive range of CT vendors, models and capabilities – providing a large snapshot of real CT practice in Canada. Analysis is underway, and will produce appropriate, standardised exposure benchmarks per common CT protocol.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Survey results will support the development of national DRLs and support federal/provincial/territorial efforts towards radiation protection of patients.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: CT equipment are governed under the *Radiation Emitting Devices (RED) Act*; however, there is no explicit standard for CT equipment in the *RED Regulations*. Thus, survey results will provide context and intelligence to future reviews of current federal legislation applicable to CT equipment, as well as support the development of up-to date guidance on radiation protection of patients.

4.37 A Framework for Supply Chain Integrity

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OBJECTIVES/BACKGROUND/ISSUE(S): Health products that are determined to be safe, effective, and capable of being manufactured with reliable quality are authorized for manufacturing by health product regulators. A broad network of manufacturers conducts the following activities in relation to health products: distribution, fabrication, importation, processing, package/label, or wholesale. This network is termed the supply chain. The integrity of the supply chain is maintained by ensuring that supply chain safeguards are present, functioning, and being used as intended. As a supply chain loses integrity, Canadians begin to access unapproved or substandard health products from unapproved or substandard facilities.

The objective of this project was to develop a tool that highlights, distinguishes and organizes these concepts in a way that can easily be applied in an operational context.

DESIGN/METHOD/DESCRIPTION: A conceptual framework approach was selected for this project. A conceptual framework organizes ideas and concepts in order to make them accessible for application in operational contexts. A conceptual framework was developed using a taxonomical approach that aims to organize actors within the health product supply chain into groups using characteristics. A set of characteristics was developed to describe actors within the health product supply chain.

Supply chain integrity issues are diverse, complex and multi-faceted. Regulators will be aided by the development of a conceptual framework for understanding supply chain integrity that describes the types of actors participating in the supply chain and shows how a health product regulator must tailor its activities to the characteristics of actors in order to maintain the integrity of the supply chain.

OUTPUTS/RESULTS: Three characteristics were identified as describing a supply chain: compliance, sincerity and licence status. These characteristics are used to develop four distinct groups of actors and each group is defined as operating in a supply chain class: legitimate supply chain, legitimate, with problems supply chain, quasi-illegitimate supply chain and illegitimate supply chain. These characteristics and categories form the basis of the supply chain integrity conceptual framework, which is the key output of this project.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Health product regulators work in partnership with manufacturers, health professionals, and consumers to maintain and strengthen a supply chain that produces safe and effective health products. The framework helps to understand how regulatory activities to support the supply chain can be divided into two main categories: bolstering the two legitimate supply chains and undermining the two illegitimate supply chains. Contemporary supply chain issues are better understood because this framework helps to deconstruct the multiple issues intertwined within the overarching subject.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This discussion paper has highlighted two broad themes where the Inspectorate can focus on improving our activities with respect to the two illegitimate supply chains. The first theme is related to improving internal capacity, while the second theme is related to improving processes within regulated parties.

4.38 Public Health Disease Surveillance Informatics Systems: Evaluating Environmental Public Health Information Suite (ELPHIS)

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OBJECTIVES/BACKGROUND/ISSUE(S): Preventing and optimally managing waterborne illness risks and illness is ultimately the goal of the environmental health's Safe Drinking Water Program at First Nations and Inuit Health Branch (FNIHB)-Alberta. To aid in achieving this goal, a public health disease surveillance informatics system has been in evolution since its design in 2006. This analysis describes the results of a formal program evaluation of the most recent upgrade of Environmental Public Health Information Suite (ELPHIS) brought online in June 2013. ELPHIS is a suite of applications used to manage data regarding drinking water quality for First Nations communities in Alberta Region. The evaluation was undertaken to systematically estimate the quality and value of the system and to identify further enhancements and changes to strengthen the system. A Centres for Disease Control (CDC) evaluation framework was adapted and applied to ELPHIS.

DESIGN/METHOD/DESCRIPTION: The CDC and Prevention's Guidelines for Evaluating Public Health Surveillance Systems served as the organizational tool to guide the development of comprehensive evaluation materials that included the Checklist for Evaluating Public Health Surveillance Systems (Disease Surveillance Informatics Systems). Based on this checklist, and informed by other evaluation resources and input from stakeholders, nine system attributes were used as the system quality criteria and included: simplicity, flexibility, data quality, timeliness, and acceptability. Data collection approaches included key informant interviews, logic, protocols reviews, and on-site observations.

OUTPUTS/RESULTS: ELPHIS was found to possess high degrees of all of the attributes applied in the evaluation, and therefore was found to be a quality and high value system. Recommendations for ‘in-field’ access to the system were made by users. Arguments for expansion of the system for use in other FNIHB regions were collected.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: Public health informatics systems are rarely evaluated for quality. This evaluation is the first undertaken of an environmental health water informatics system in Canada and sheds light on the importance of systematic program evaluation tools applied. ELPHIS is a quality informatics system that is simple to use, adaptable to change, acceptable to users and communities, and allows for timely water information to be available to decision-makers that will enhance our ability to achieve the goal of preventing and optimally managing waterborne risk and illness in our populations served.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: This Informatics System is being considered for adoption nationally across all Regions of FNIHB.

4.39 The Assessment of Anti-Biofilm Claims for Hard Surface Disinfectants: Challenges for Industry and Regulatory Evaluators

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OBJECTIVES/BACKGROUND/ISSUE(S): Historically, the pre-market regulatory approval of hard surface disinfectants had depended on the demonstration of efficacy against pure cultures of the single cell (planktonic) forms of the test microorganisms. However, it is now widely recognized that microorganisms commonly exist as complex communities called biofilms with their resident microbes demonstrating increased resistance to disinfection. Significant work, done by test laboratories and research centers, has recently led to the generation of biofilms *in vitro* , that has enabled the development of standardized test methods for anti-biofilm claims. The challenge that regulators and industry currently face is which test methods to choose as relevant for various proposed anti-biofilm applications. Also, the fact that many disinfectants approved previously based on efficacy on the planktonic forms are unable to meet the requirements of these new methodologies is yet another obstacle fabricators face.

DESIGN/METHOD/DESCRIPTION: A guidance document for hard surface disinfectants was prepared that enables selection of the best test method for a proposed biofilm application. The methods are biofilm reactor-based and vary on the type of continuous flow that produce controlled shear forces modelling different fluid dynamics. So far, three different types (High, Intermediate, and Low shear) reactor-based methods) are approved by standard setting agencies. Biofilms generated under high shear are more robust and show a higher degree of resistance to disinfection compared to those generated under intermediate or low shear.

OUTPUTS/RESULTS: The availability of standardized biofilm testing methods has enabled regulatory agencies develop pre-market guidance documents for stakeholders. The new guidance document on hard surface disinfectants, published by Health Canada, recognizes the approved test methods for biofilm disinfection. Submissions from industry with anti-biofilm claims are now carefully scrutinized.

IMPACTS/OUTCOMES/CONCLUSIONS/IMPLICATIONS/NEXT STEPS: The guidance document will assist in the regulation of hard surface disinfectants. Although an ideal hard surface disinfectant is one that kills ≥99.999% (≥5 log10 reduction) of the target microorganisms in a biofilm within a 10 minute exposure, It appears that accommodations may be required for disinfectants with anti-biofilm claims, depending on their application. A strong focus on harmonization with the US EPA's requirements is expected to strengthen and provide some level of uniformity in the enforcement of the overall approach in regulating hard surface disinfectants with anti-biofilm claims.

IMPACT ON THE DEPARTMENT/POLICIES/REGULATIONS: As the Non-prescription Drugs Evaluation Division builds their knowledge of these new and emerging anti-biofilm test methodologies and determines what anti-biofilm claims would be appropriate for hard surface disinfectants, it is anticipated that it will also contribute towards the improvement of efficacy and labelling guidance. The development of a harmonized international approach for efficacy and labelling requirements would also be expected to facilitate the introduction of such products into the Canadian market.

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