Project Title:
(I1) Refined Analysis and Characterization Methods for Metals in Urban Residential Air, and their Application to Determining the Relationship Between Aerodynamic Particle Size and Metal Solubility

Principal Investigator and Co-investigators:
Chénier, M., J. Niu, N. Hassan, and P. Rasmussen (PI); University of Ottawa and Health Canada

Summary:
This study addresses the growing demand for information on exposures of urban populations to metal concentrations in airborne particulate matter (PM). Metals present in airborne PM have been implicated in a variety of cardio-respiratory illnesses associated with exposure to urban air pollution. Transition metals (e.g., Mn, Cr, Cu, Ni, and Zn) receive particular emphasis due to linkages between oxidative stress and impaired lung function.

The accuracy of exposure assessments is improved by the development of techniques for physical and chemical characterization of particle-bound metals. With respect to airborne PM, the degree of respiratory penetration is a direct function of the aerodynamic particle diameter, which may be characterized using cascade impactor systems or small portable PM_{10} and $PM_{2.5}$ monitors. Determination of total metal concentrations over the complete range of physical and aerodynamic size fractions of indoor dust (coarse, fine, ultrafine and nanoparticles) indicates a trend of increasing metal contents as particle size decreases. Thus, particle size is one of the most influential factors affecting metal bioaccessibility and is a critical sampling parameter in urban exposure studies.

Chemical characterization of particles, involves determination of the soluble fraction of particle-bound metal compounds under pH conditions of human body fluids, expressed as percentage of total metal content. For the determination of total metal content, the relative advantages and disadvantages of various high pressure microwave digestion versus hot water bath/ultrasonication techniques were evaluated using certified reference materials. For the determination of the soluble metal fraction, three different methods were evaluated: distilled water, ammonium acetate (0.01 M), and ammonium citrate (0.1 M). Preliminary application of the ammonium citrate method indicates that metal solubility is highly variable in size-fractionated samples of indoor dust, suggesting that the distribution of a metal amongst its component species (chemical forms) changes across the particle size spectrum.

Project Title:
(I1) Refined Analysis and Characterization Methods for Metals in Urban Residential Air, and their Application to Determining the Relationship Between Aerodynamic Particle Size and Metal Solubility

Poster Title:
Spatial Distribution of Metals in Windsor Part I: Indoor Dust

Principal Investigator and Co-investigators:
Nugent, M. (PhD candidate), University of Ottawa, P. Rasmussen (PI), University of Ottawa and Health Canada, A. Wheeler, M. Chénier, and M. Smith-Doiron, Health Canada

Summary:
This is a preliminary investigation of spatial relationships between settled indoor dust metal content and potential anthropogenic sources of metals in the Windsor, Ontario area. Specific goals were to (1) determine elemental associations in settled indoor dust (2) confirm the presence or absence of elemental spatial trends in indoor dust and, (3) investigate the potential influences of industrial sources of metals, major roads, highways and/or expressway.

Settled dust from 45 indoor environments was sampled in the city of Windsor, Ontario as part of the Border Air Quality Study (BAQS). The dust was collected using an ASTM vacuum method, sieved to 150 m and analyzed for total metal concentrations using ICP-MS. MS Excel was used to manage the dust metal data and calculate Pearson's correlation coefficient. ArcGIS (version 9.1) was used to analyze and visualize the data.

Some metals (Fe, Mn, Al and Tl) are strongly associated to one another which could indicate a common indoor or outdoor source. By using a simple interpolation method, spatial trends were found to exist for certain metals in indoor settled dust. In a SE to NW direction, concentrations of Fe, Mn, Al and Tl tend to increase. Several sampling locations in the city have high levels of Zn, Ni or Pb. The sources of these high levels are still unknown. No correlation was found between settled indoor dust metal concentrations and the distances to the nearest industrial source, or major roads and highways/expressway.

Windsor settled indoor dust metal content does not appear to be influenced by its proximity to known outdoor sources of metals, based on the present data set. It is acknowledged that spatial trends in the metal content of indoor dust may exist, however further detailed survey information, as well as a larger data set, is required. A soil survey will be undertaken to more fully characterize relationships between metal content of indoor particulate matter and the outdoor soil metal content in Windsor, Ontario.

Project Title:
(I2) Arsenic, Thallium and Mercury Speciation in Dust from Abandoned Gold Mine Tailings in Nova Scotia

Poster Title:
Solid-Phase Speciation of Arsenic in Historical Gold Mine Tailings from Nova Scotia

Principal Investigator and Co-investigators:
Walker, S.R. (Post Doc., GSC Atlantic & Queen's Univ.), H.E. Jamieson (P.I., Queen's Univ.), M.B. Parsons (Co-inv., GSC-Atlantic), and J.L. Campbell (Co-inv., Univ. Guelph)

Summary:
Determining the solid-phase speciation of As in near-surface tailings is critical for understanding the stability and potential toxicity of As. In this study, we are characterizing the mineral form and oxidation state of As in mine tailings and soils to better understand the bioaccessibility of As, and to evaluate the the feasibility of using mineralogical analysis as a surrogate for bioaccessibility assays.

In 2005 and 2006, a suite of 29 tailings and soil samples were collected from six abandoned gold mine sites in Nova Scotia where residents and recreational users may be exposed to historical mine wastes. Total As concentrations in the <150 µm fraction of these samples ranges from 195 to 313,000 ppm (mean 36,100 ppm). Ore processing techniques used at these sites include stamp milling, mercury amalgamation, and cyanidation, with or without prior gravity separation. At one mine, the gold ores were also processed using roasting followed by barrel chlorination. This set of 29 samples is undergoing concurrent *in vitro* bioaccessibility testing by other MITHE-RN researchers (Project #I5).

Most of the As in unweathered gold mine tailings from Nova Scotia is hosted in arsenopyrite (FeAsS), which is generally regarded as having relatively low toxicity. However, oxidation of arsenopyrite in the near-surface tailings has produced numerous secondary As phases that are less well understood in terms of stability and toxicity. Conventional powder X-ray diffraction (XRD) was used to identify some As-bearing phases (e.g. arsenopyrite and scorodite [$FeAsO_4 \cdot 2H_2O$]) in the most As-rich samples. Unfortunately, most As-bearing phases are below the detection limits of conventional XRD. Therefore, we are applying a combined petrographic and microanalytical approach (<10 µm spatial resolution) on target grains in polished thin sections. The techniques include synchrotron-based µ-XRD and µ-X-ray Absorption Near Edge Spectroscopy (XANES), and electron probe microanalysis (EPMA).

To date, approximately 40% of the samples have been analyzed using petrography, µ-XRD and µ-XANES. Petrographic analyses show that secondary As phases occur as both replacement textures (discrete grains and rims on relic sulfides) and as precipitates coating other grains (usually silicates). Arsenic in secondary phases occurs mainly as arsenate (As^{5+}) in a wide range of forms, including scorodite, amorphous Fe arsenate, kankite ($FeAsO_4 \cdot 3.5H_2O$), yukonite (poorly characterized Ca-Fe arsenate), and As bound to Fe oxyhydroxides. A few partially characterized samples from the roasting operation suggest a

very different As association in tailings and soils at this location, including the presence of mixed oxidation state As (As^{3+} and As^{5+}) in roaster-derived Fe oxides.

Data from this study are being used by the Historic Gold Mines Advisory Committee in Nova Scotia to help inform ongoing risk assessment and management decisions.

Project Title:
(I3) Pulmonary Absorption of Metals: Influence of Lung Surfactant Components on the Bioavailability and Toxicity of Inhaled Metals (*in vitro* studies)

Principal Investigator and Co-investigators:
Mantha, M., and C. Jumarie (PI); Département des. Sciences Biologiques, Université du Québec à Montréal, CP 8888, succ. Centre ville, Montréal. H3C 3P8

Summary:
Significant absorption of some metals may occur through inhalation, especially under occupational exposure but also from indoor air. The pulmonary absorption and toxicity of metal compounds are influenced by their solubility in biological fluids. In the terminal bronchioles, and more importantly in the alveoli, metals are subjected to interactions with surfactant synthesized by the alveolar type II (ATII) cells and to some extent by the bronchiolar Clara cells. Pulmonary surfactant consists of a mixture of 90% lipids (mostly phospholipids) and 10% proteins (SP-A, SP-B, SP-C and SP-D). Our research objectives are to estimate how surfactant components may modulate: i) metal toxicity; ii) metal transport in and through cell monolayers in various lung cell phenotypes.

Our study focuses on Cd, Ni, Pb and Hg and involve three different *in vitro* models: the human cell lines A549 (ATII cells) and H441 (Clara cells), and primary cultures of rat ATII cells. Because older primary cultures undergo trans-differentiation from ATII toward the ATI phenotype, experiments are also conducted at various days in culture. Serum-free culture medium and inorganic media are used to evaluate the impact of metal speciation on different endpoints.

Our results show that ^{109}Cd uptake in A549, H441 and rat ATII cells involves pH-sensitive specific transport leading to higher levels of accumulation under inorganic exposure conditions. In the two cell lines, a 2-fold increase was observed in ^{109}Cd uptake using a medium optimizing [Cd^{2+}] over chlorocomplexes formation. This increase was more pronounced in 3-day-old rat ATII cells but disapeared in older cultures. Uptake data obtained with metal mixtures in inorganic media suggest that Pb/Cd and Hg/Cd interactions involve chlorocomplexes in A549 cells whereas optimal Pb/Cd interactions in the H441 cells would rather involve the free cations. Toxicity data obtained with the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay demonstrate that Cd is more toxic compared to Hg in both human cell lines but A549 cells are more sensitive. The addition (1-5%) of either phosphatidyl choline or dipalmitoyl phosphatidyl choline, two lipids surfactant, did not protect from metal toxicity. Our results also show that: i) Hg increases the sensitivity of A549, but not H441 cells, to Cd whereas Pb lowers it; ii) Cd but not Pb increases sensitivity of both cell lines to Hg. Additivity between Cd and Hg was observed in A549 but not in H441 cells. Antagonist effect of Pb on Cd toxicity was also observed in A549 but not in H441 cells.

Our studies provide kinetic and toxicity data on different lung cell types: although comparable uptake levels are observed in alveolar and brochiolar cells, metal interaction for uptake and toxicity may strongly differ in both cell types.

Project Title:
(I4) Human Bioaccessibility of Metals Surrounding Northern Hotspots

Poster Title:
Bioaccessibility of arsenic in mine tailings from Eastern Canada

Principal Investigator & Co-investigators:
Laird, B.D., M. Corriveau, H. Jamieson, T. Van De Wiele, S.D. Siciliano (PI)

Summary:
The arsenic bioaccessibility of two size fractions (bulk and < 38 µm) of mine tailings from Goldenville, Lower Seal Harbour, and Montague, Nova Scotia were evaluated using an *in vitro* gastrointestinal model, the Simulator of the Human Intestinal Ecosystem (SHIME). Arsenic bioaccessibility, which ranged between 2 and 20% in the small intestine and 4 and 70% in the colon, was inversely related to arsenic concentration in the mine tailings. Additionally, arsenic bioaccessibility was significantly greater in the bulk fraction than in the < 38 µm fraction in both the small intestine and colon. Significant differences were observed in the arsenic bioaccessibility of mine tailings from the three Nova Scotia communities. Specifically, the arsenic bioaccessibility of tailings from Goldenville were higher than those from Lower Seal Harbour, which were higher than those from Montague. Colon microbes significantly increased the bioaccessibility of arsenic in mine tailings while being involved in the production of a black precipitate.

Project Title:
(I5) Bioaccessible and Bioavailable Cd, As and Ni in Foods and Soils

Poster Title:
A comparison of three *in vitro* methods to determine the gastrointestinal bioaccessibility of nickel from soil

Principle Investigators and Co-investigators:
Amendola, A.[1]; Hale, B.A.[1]; Drexler, J.W.[2]; and Dutton, M.[3]

[1] Department of Land Resource Science, University of Guelph, ON
[2] Department of Geology, University of Colorado at Boulder, CO
[3] Inco Ltd., Toronto, ON

Summary:
In the last 10 years, several *in vitro* methods have been developed to explain the relationship between soil metal concentrations and the bioaccessible fraction of these metals in the human gastrointestinal tract. There are several variables that influence the amount of metal that is bioavailable from the soil matrix, including metal speciation, soil type, and competing metal concentrations. First, this study focussed on using three *in vitro* bioaccessibility methods to determine the bioaccessible fraction of nickel from various soils: the SBRC (Solubility/Bioavailability Research Consortium) Method, the BARGE (Bioavailability Research Group Europe) Method, and a method developed by Golder Associates, which is based on the SBRC method. Each of these methods uses different ratios of soil weight to extraction solution volume, different methods of simulating GI tract movement, and different incubation times. It should be noted that the SBRC Method has been validated using an animal model for both arsenic and lead. Second, several certified reference materials were extracted using the three bioaccessibility methods, including NIST's SRM 2710 "Montana I" and SRM 2711 "Montana II", CANMET's "Sulphide Ore Mill Tailings" RTS-1, RTS-2, RTS-3, and the British Geological Survey's Bioaccessibility Reference Material. Third, the three bioaccessibility methods were carried out using both background and contaminated soils collected from Sudbury and Port Colborne. The results of these investigations will be presented. Future studies will include (i) addition of the intestinal or absorptive phase to the SBRC and Golder methods which only consider the gastric phase, wherein the solution pH is raised to that of the small intestine, (ii) simulation of intestinal absorption using Caco-2 cells, dialysis membranes, or pig intestines, and (iii) validation of the three bioaccessibility methods using an animal model.

Project Title:
(I5) Bioaccessible and Bioavailable Cd, As and Ni in Foods and Soils

Poster Title:
Bioaccessibility: Method Development for Improving Risk Assessments at Contaminated Sites

Principal Investigator and Co-investigator:
Lord-Hoyle, M., A. Campbell, L. Easton, I. Koch, J. Wragg, K. Reimer; Environmental Sciences Group, Royal Military College of Canada, Kingston, Ontario.

Summary:
Regulations and guidelines for contaminated site remediation in Canada are currently based on the total concentration of the target substance in a particular substrate (soil, sediments or water). Contaminants in soil, however, maybe be tightly bound and thus there is a growing trend to consider bioavailability – the fraction of a substance that is absorbed by the organism – in determining suitable risk based endpoints for site remediation in Canada. Bioavailability is usually measured by using *in vivo* methodologies, which tend to be expensive and time consuming; bioaccessibility measurements using simulated gastrointestinal conditions to estimate the soluble fraction of a substance are increasing in desirability for incorporation into risk assessment. Bioaccessibility measurements can be carried out with a simple extraction procedure and hence are more accessible, less expensive and quicker than *in vivo* studies to estimate bioavailability. For these measurements to be meaningful, however, it is important to compare bioaccessibility to *in vivo* bioavailability results and determine their accuracy. This poster presents a comparison of two bioaccessibility testing methods for arsenic (PBET vs. IVG). In addition, findings on how the composition of the substrate can affect the amount of a chemical that is available to the body will be discussed. These findings will be used to illustrate the importance of bioaccessibility testing in the risk assessment procedure.

Project Title:
(I5) Bioaccessible and Bioavailable Cd, As and Ni in Foods and Soils

Poster Title:
Contaminants and Soil Composition: Effects on Bioaccessibility

Principal Investigator and Co-investigator:
Meunier, L., I. Koch, J. Wragg, and K. Reimer; Environmental Sciences Group, Royal Military College of Canada, Kingston, ON

Summary:
Soil contaminants frequently pose a risk to human health. Various bioaccessibility methods have been developed to evaluate how much of a contaminant can be absorbed through the ingestion of contaminated soil. The results of these bioaccessibility tests can be applied to human health risk assessments (HHRAs). Research currently focuses on the complex physical, chemical and biological processes that govern bioaccessibility; though generalized models encompassing the relative impact of these processes with respect to contaminant source, level and species, and including soil composition and mineralogy are not yet available. Nevertheless, for a number of well characterized chemicals, where bioaccessibility has proven to be the rate-limiting step for the absolption of these contaminants, it is possible to achieve reasonable estimates of site-specific bioaccessibility for implementation in an HHRA.

The goal of this research project is therefore to establish links between soil composition and soil contaminant bioaccessibility. This poster presents an overview of the relationship between bioaccessibility of soil contaminants and soil composition, including contaminant-soil interactions, mineralogy, transport, pathways and exposure to soil contaminants, accumulation. and effects. At this point, previous studies on the bioaccessibility of metals and metalloids have yielded results useful to HHRAs, whereas studies on the bioaccessibility of organic contaminants are not as advanced. Arsenic and benzo(a) pyrene are presented as examples of substances of concern found in contaminated soil. Ultimately, conservative bioaccessibility data encompassing soil composition can be used to achieve meaningful risk assessments, from which appropriate remediation measures can be implemented.

Project Title:
(I5) Bioaccessible and bioavailable Cd, As and Ni in Foods and Soils

Poster Title:
Determining Bioaccessibility/Bioavailability of Metal Contaminants in Soils using Caco-2 Cells

Principle Investigators and Co-investigators:
Vasiluk, L., and B. Hale; Department of Land Resource Science, University of Guelph, Guelph, ON

Summary:
The uptake of soil-bound contaminants has been tested mainly in vivo, and the data suggest that contaminant bioavailability can be dramatically less than the total, due to a limited mobilization of the chemical from the soil matrix. Therefore, there is a possibility of overestimating the health risk posed by xenobiotics in soil and adjustments for oral bioavailability therefore need to be considered in human risk assessment. In the gastrointestinal tract, the uptake of contaminants depends on their solubilization in gastrointestinal fluids (bioaccessibility) followed by absorption into the intestinal epithelium (bioavailability). Due to increased attention to adjust ingestion exposure within risk assessments, there is an urgent need to validate against in vivo models an accurate and relatively inexpensive in vitro method for that could predict relative oral bioavailability of matrix-bound contaminants. This work chooses to evaluate a suite of soils and dusts using particle size separation combined with simulated gastrointestinal digestion in vitro and then characterization of the uptake of metals from the digestate into human cultured enterocytes, Caco-2 cells.
We chose to start our work by using naturally weathered soils receiving smelter emissions with elevated metal concentrations. We have included NIST SRM 2710 and 2584 in the study. The soils were subjected to gentle grinding and screened to exclude particles greater than 250 m, then bulk soil (<250 m) was separated into four fractions of particle size by sieving using fluorocarbon screens. We carried out two extractions of metals: EPA 3052 and PBET, with PBET followed by exposure of Caco-2 cell monolayers.
Particles of two fractions (<70 m and 150-250 m) comprised most of the weight of the bulk soil and both are moderately elevated in Cu and Ni. When Caco-2 cell monolayers were exposed to PBET digestate using these particle size fractions, Ni was absorbed at concentrations below detectable limit of graphite furnace atomic absorption spectroscopy. The bioavailability of Cu was higher, for particles 150-250 m. Our next step will be to work with another smelter-impacted soil with higher Ni concentrations and to explore the possibility that the concentration of Cu in the Caco-2 cells is not significantly different among different soil because cell absorptive capacity saturates at exposure concentrations lower than Cu concentrations in smelter soils.

Project Title:
(I6) Arsenic Speciation in the Environment

Poster Title:
Biomarken of Arsenic Exposure in Meadow voles *(Microtus pennsylavan;cus)* Living on an Abandoned Gold Mine Site

Principal Investigator and Co-investigators:
Saunders, J.[1], L. Knoppel[2], A. Yagrninas[3], I. Koch[1], and K. Reimer[1].

[1] Environmental Sciences Group
[2] Jacques Whitford
[3] Health Canada

Summary:
Arsenic is a relatively common natural element that is found in many environmental matrices. It is one .of the most frequently encountered inorganic contaminants, which when consumed by humans, even at low concentrations, can lead to carcinogenesis. The study of arsenic speciation and effects in a small teuestrial food chain is critical in understanding how arsenic behaves in mammals. Areas that contain highly elevated concentrations of arsenic, such as those impacted by abandoned gold mines, provide ideal study sites for ecological exposure assessments.

We conducted a biomonitoring study as part of a greater study to determine how the species (or chemical form) of arsenic changes at each level of a soil-plant-small mammal food chain. Meadow voles *(Microtus pennsylvanicus)* and deer mice *(Peromyscus maniculatus)* were captured at two sites at Seal Harbour, Nova Scotia, Canada, the site of a former gold mine, and at a reference site without anthropogenic arsenic influences, in August 2005. Mammals (n=63) were captured in Sherman live traps and after being anesthetized with isotluorane, blood was collected via cardiac puncture and blood smears prepared on glass slides. Cervical dislocations were then conducted and tissues (heart, liver, lungs, kidneys, stomach contents, stomach lining, intestines) were immediately dissected and frozen. Three biomarkers of arsenic exposure and effect were quantified: micronuclei in blood, and ATP and glutathione levels from liver. The relationship between total arsenic and arsenic species concentrations from voles and mice tissue and each biomarker will be discussed. The ultimate use of the results will be their incorporation into an ecological risk assessment. In addition, this data may be extrapolated for incorporation into a human health risk assessment.

Project Title:
(I7) Relation Between Methylmercury Exposure and Serum Paraoxonase Activity in Cree and Inuit Communities of Northern Québec

Principal Investigator and Co-investigators:
Ayotte, P. and É. Dewailly

Summary:
Methylmercury (MeHg) exposure has been linked to an increased risk of cardiovascular diseases, in particular myocardial infarction. Paraoxonase 1 (PON1), an enzyme located in the high density lipoprotein (HDL) fraction of blood lipids, may prevent cardiovascular diseases by metabolizing toxic oxidized lipids associated with both low density liproprotein (LDL) and HDL. MeHg and various metals/metalloids have been shown to inhibit PON1 activity *in vitro* but the relation between metal exposure and PON1 activity has not been studied in human populations. We hypothesized that mercury concentration in blood may be linked to decreased serum PON1 activity in Northern Quebec Aboriginal populations that are highly exposed to methylmercury through fish consumption. We measured serum PON1 activity in 900 Inuit adults who participated to the Nunavik Health Survey during the fall of 2004 and in 180 Cree adults from the community of Mistissini who were enrolled in the Multi-Community Environment-and-Health Longitudinal Study during the summer of 2005. Total mercury and selenium concentrations in blood, blood lipids, fatty acid profile in erythrocyte membranes and various socio-demographic and lifestyles habits had been determined in the course of these comprehensive health surveys. Mean serum PON1 activity was 10,772 U/L (range = 1,328-24,565 U/L) in the Inuit population and 11,165 U/L (range = 3,395-21,205 U/L) in Cree adults from Mistissini. Univariate analyses indicated that in the Inuit population, PON1 activities were positively correlated to blood selenium (Pearson's $r = 0.12$; $p < 0.001$) and blood mercury levels ($r = 0.10$, $p = 0.004$). However, in a multiple regression model adjusted for age of participants, alcohol consumption, blood HDL levels and eicosapentaenoic content of erythrocyte membranes, blood selenium concentrations remained positively associated with PON1 activities (standardized beta = 0.13, $p = 0.007$), whereas blood mercury concentrations were negatively associated with PON1 activities (standardized beta = -0.14, $p = 0.013$). Neither blood mercury nor blood selenium concentrations were associated with serum PON1 activities in the Cree population of Mistissini. Our results suggest that in the Inuit population, methylmercury exposure may have a slight inhibitory effect on PON1 activity, which seems to be offset by selenium intake.