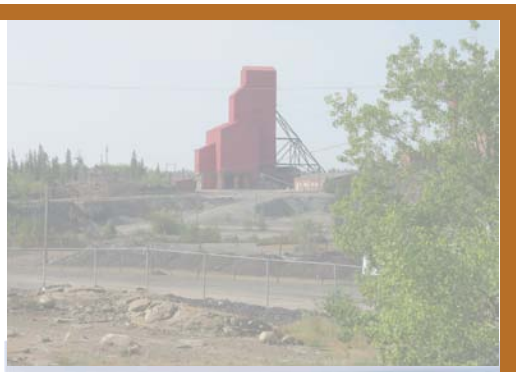

APPENDIX G

LABORATORY ANALYSIS PLAN

Appendix G – Laboratory Analysis Plan

Description of Laboratory Analysis

All assays of biological specimen will be determined by the Centre de toxicologie du Québec (CTQ) of L'Institut national de santé publique du Québec (INSPQ). The laboratory is accredited under ISO 17025 and uses numerous external quality control programs, including the German External Quality Assurance Scheme (EQAS) and Centre for Disease Control's (CDC) Lead and Multi-element Proficiency Testing (LAMP). While serving as the reference laboratory for human toxicology in the province of Quebec, it also collaborates on a number of national and international biomonitoring studies such as the Canadian Health Measures Study (CHMS) and the Maternal Infant Research on Environmental Contaminants (MIREC).

Analytical Methods

The analytic method of choice for chemicals in biological fluids is dependent on the specific chemical of interest. The method chosen for a particular study balances the availability of established reference levels with sensitivity of detection and experience. Under the direction of CTQ, the current *Evaluation of Environmental Contaminant Exposure in Children (under 15) in Flin Flon, Manitoba and Creighton, Saskatchewan*, uses urine and blood samples to estimate internal exposure of study participants. The biological samples will be analyzed using inductively coupled plasma with detection by mass spectrometry (ICP-MS) or cold vapour atomic absorption spectrometry (CVAAS). The specific analytic methods to be undertaken and respective limits of quantification for each of these assays are reported in Table 1 below. Reproducibility for each analyte is shown in the right column. The concentrations provided in parenthesis refer to the concentration at which the reproducibility was assessed using the same method and on identical material. The between-run-precision represents the coefficient of variation of a particular analyte analysed at least once on 10 different runs usually on different days. In general, the coefficients of variation of results are of the order of 3 to 5%.

Table 1: Overview of biomarker analyses

Biomarker	Analytic method	Detection limit	Reproducibility
Urinary Total and Inorganic Arsenic	ICP-MS	0.05 µmol/L (Inorganic) 0.003 µmol/L (Total)	~ 5% (0.5 µmol/L)
Urinary Inorganic Mercury	Cold Vapour Atomic Absorption	0.5 nmol/L	5% (12 nmol/L)
Blood lead	ICP-MS	0.001 µmol/L	~ 3% (0.3 µmol/L)

While details specific laboratory procedures are proprietary property of CTQ, the following laboratory protocols will be used in the analysis.

- Analysis of urinary arsenic will be conducted according to CTQ protocol "*Analytical method for the determination of metals in urine by inductively coupled plasma mass spectrometry*" protocol number M-558 based on approach developed by Fitchett and colleagues[1]. 'Inorganic arsenic' refers to the species found in urine as a result of inorganic arsenic exposure: Sum of As+3, As+5, MAA and DMAA in urine.;

[1] Fitchett AW, Daughtrey EH et Mushak P. 1975. Anal. Chim. Ac., 79, 93-99.

- Analysis of urinary inorganic mercury will be conducted according to CTQ protocol "Analytical method for the determination of inorganic mercury in urine with the FIMS 100 module from Perkin Elmer" protocol reference number M-568; and,

- Analysis of lead in blood will be conducted according to protocol *"Analytical method for the determination of metals in blood, serum or plasma by inductively coupled plasma mass spectrometry*" protocol reference number M-557.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a sensitive technique for the multi-element analysis of trace elements in solution. ICP-MS combines the strengths of two established technologies: 1) the ion source (or ICP), a well proven analytical tool that operates in excess of 6000 degrees Kelvin, and 2) a quadrapole mass spectrometer that acts as a detector and separates the metal elements and their isotopes so they can be individually measured.

All determinations using ICP-MS are to be conducted using the Perkin-Elmer SCIEX - ELAN 6000. Diluted urine samples are converted into an aerosol by using an argon nebulizer flow within a spray chamber. A portion of the aerosol is transported through the spray chamber and then through the central channel of the plasma, where it is raised to temperatures of 6000K by the ICP torch. The increase in temperature dries the sample to a solid and then to a gas. The thermal energy atomizes and ionizes the sample. Once inside the mass spectrometer, the ions pass through the ion optics, then through the mass-analyzing quadrupole before being detected as they strike the surface of the detector. Signals resulting from the detection of the ions are processed into digital information that is used to indicate the intensity of the ions and subsequently the concentration of the element such as arsenic and lead.

Cold Vapour Atomic Absorption

Urine samples will be used to determine internal exposure to inorganic mercury using cold vapour atomic absorption spectrometry based largely on protocol developed by Gou and Bassner[2]. Since mercury in urine is found almost entirely in the inorganic form, microwave digestion will not necessary. Decomposition of mercury compounds is achieved by adding mixed bromate-bromide reagent and concentrated hydrochloric acid (HCl). Further decomposition of mercury compounds is achieved by adding of potassium permanganate online. The mercury vapor (reduced from inorganic mercury compounds by sodium tetrahydroborate) is measured by the spectrophotometer Perkin-Elmer Flow Injection Mercury System with the FIMS 100.

Quality Control

For each series, the control samples will be analyzed as follows:

- Calibration curve at several levels
- Reference materials
- Duplicate intra-series (a random sample analyzed 2 times in one series of analysis)

[2] Guo T, Baasner J. Determination of mercury by flow-injection cold vapor atomic absorption spectrometry. Analytica Chimica Acta 1993;278:189–196.

- Duplicate inter-series (a random sample analyzed 2 times on 2 different sets of analysis)
- White reactive
- Sample added (a random sample and enriched elements of interest.

Reference materials used in this study come from the programs of comparisons interlaboratory headed the Laboratory of Human Toxicology / INSPQ. These materials were validated according to pre-established protocols and complying with ISO 17025. Reference materials and certified reference materials from outside agencies recognized. When a series of tests is completed, the technologist will check the raw data generated by the device, the stability of readings and the results of reference materials. For a series to be accepted, the results of reference materials must be within 2 standard deviations of the target value. In cases where these criteria are not met, the analysis is to be repeated. In general, the coefficients of variation of results are of the order of 5% on all tests.