**Relating Metals Exposure to Birth and Early Childhood Outcomes via the Metabotype of Cord Blood**

**Christiani: 0.9% Saline Buffer Addition**

A total of 200 serum samples and 2 CHEAR Serum Reference Material aliquots were thawed on ice for sample preparation, 150 L of the thawed serum sample were transferred to labeled tubes on ice where they were mixed with 100 L of NMR 0.9% Saline Solution containing 2.5 mM formate. Samples were randomly assigned to one of two pools, either Pool 1 or Pool 2. An aliquot of 15 L of each study sample was added to create their corresponding pools. A 150 L aliquot was taken from each pool to make 10 aliqouts. Another nine pools from the CHEAR reference material samples were aliquoted and processed along with the study samples and study pools. Pool aliquots were processed identically to the study samples, as described above. Sample tubes were vortexed for 4 min on a multi-tube vortexer and centrifuged at 16,000 rcf for 4 min. A volume of 250 L was taken from each sample supernatant and transferred into a pre-labeled 3mm NMR tubes for data acquisition on a 600 MHz spectrometer.

1H NMR spectra of urine samples were acquired on a Bruker 600 MHz NMR spectrometer (located at David H Murdock Research Institute, Kannapolis, NC, USA) using a 5 mm cryogenically cooled ATMA inverse probe and ambient temperature of 25 ℃. A 1D CPMG presaturation pulse sequence (cpmgpr1d) was used for data acquisition. For each sample 128 transients were collected into 64k data points using a spectral width of 12.0 ppm, 2s relaxation delay, and an acquisition time of 4.5 s per FID. The water resonance was suppressed using resonance irradiation during the relaxation delay. NMR spectra were processed using TopSpin 3.5 software (Bruker-Biospin, Germany). Spectra were zero filled, and Fourier transformed after exponential multiplication with line broadening factor of 0.5. Phase and baseline of the spectra were manually corrected for each spectrum. Spectra were referenced internally to the Formate signal. The quality of each NMR spectrum was assessed for the level of noise and alignment of identified markers. Spectra were assessed for missing data and underwent quality checks. NMR signals were library matched to metabolites and the concentrations were determined relative to 1 mM Formateusing Chenomx NMR Suite 8.1 Professional (Chenomx, Edmonton, Alberta, Canada) software.

Due to sample volume limitations: study samples, study pools, and CHEAR reference samples were prepared using 150 uL of sample and diluted with 100 ul of 0.9% Saline buffer. This is a deviation from the CHEAR Proficiency testing in which 400 ul of CHEAR reference sample was used and diluted with 300 uL of 0.9% Saline buffer. Samples were then transfered into a 3 mm NMR tube, in this study, as opposed to a 5 mm NMR tube for the Proficiency testing.

Data for this study were acquired on a 600 MHz Bruker NMR spectrometer. Data for the procficiency testing were acquired on a 700 MHz Bruker NMR spectrometer.