Supplementary Appendix 1. Methodology of chemical measurements

All descriptions are from the NHANES websites:

https://wwwn.cdc.gov/nchs/nhanes/search/default.aspx

1. Serum creatinine

The DxC800 modular chemistry side uses the Jaffe rate method (kinetic alkaline picrate) to determine the concentration of creatinine in serum, plasma, or urine. The creatinine calibration is traceable to an isotope dilution mass spectrometry (IDMS) reference method. The method on the DxC800 is IDMS Standardized. A precise volume of sample is introduced into a reaction cup containing an alkaline picrate solution. Absorbance readings are taken at 520 nm between 19 and 25 seconds after sample injection. Creatinine from the sample combines with the reagent to produce a red color complex. The absorbance rate has been shown to be a direct measure of the concentration of the creatinine in the sample.

2. Urine albumin

A solid-phase fluorescent immunoassay for the measurement of human urinary albumin is described by Chavers et al. (Chavers, BM, Kidney Int. 1984; 25:576–578). The fluorescent immunoassay is a non-competitive, double-antibody method for the determination of human albumin in urine. Antibody to human albumin is covalently attached to derivatized polyacrylamide beads. The solid-phase antibody is reacted with a urine specimen, and the urine albumin-antigen complexes with the solid-phase antibody. This complex then reacts with fluorescein-labeled antibody. The unattached fluorescent antibody is then removed by washing during centrifugation. The fluorescence of the stable solid-phase antibody complex is determined with a fluorometer; the fluorescence is directly proportional to the amount of urine albumin present. The standard curve is 0.5–20 μg/mL of albumin.

Results of the fluorescent immunoassay (FIA) are reproducible, and the test is accurate and sensitive for the detection of human urinary albumin excretion. It is especially useful for the measurement of low levels of urinary albumin not detectable by dipstick methods. The FIA

assay resembles the radio-immunoassay (RIA) in technique and sensitivity without the potential health hazards associated with the handling of isotopes in the laboratory (Chavers, BM, Kidney Int. 1984; 25:576–578).

3. Urine creatinine

Creatinine is produced by creatine and creatinine phosphate as a result of muscle metabolic processes. It is then excreted by glomerular filtration during normal renal function. Creatinine may be measured in both serum and urine. Creatinine measurement is useful in the diagnosis and treatment of renal diseases, in monitoring renal dialysis, and as a calculation basis for other urinary analytes (e.g. total protein, microalbumin).

In this enzymatic method creatinine is converted to creatine under the activity of creatininase. Creatine is then acted upon by creatinase to form sarcosine and urea. Sarcosine oxidase converts sarcosine to glycine and hydrogen peroxide, and the hydrogen peroxide reacts with chromophore in the presence of peroxidase to produce a color product that is measured at 546 nm (secondary wavelength = 700 nm). This is an endpoint reaction that agrees well with recognized HPLC methods, and it has the advantage over Jaffe picric acid-based methods that are susceptible to interferences from non-creatinine chromogens.

4. Acrylamide & Glycidamide

This procedure describes a method to measure hemoglobin adducts of acrylamide and its primary metabolite glycidamide in human whole blood or erythrocytes. Specifically, the reaction products with the N-terminal valine of the hemoglobin protein chains (N-[2-carbamoylethyl]valine and N-[2-hydroxycarbamoyl-ethyl]valine for acrylamide and glycidamide adducts, respectively) are measured.

This method is based on modified Edman reaction, which uses the effect of N-alkylated amino acids being able to form Edman products in neutral or alkaline conditions without changing the pH to acidic conditions required in conventional Edman reaction procedures. It was first described for N-terminal hemoglobin adducts of ethylene oxide, propylene oxide and styrene oxide and later optimized to increase yield of Edman products of these adducts. This optimized method was then successfully applied to adducts produced by other chemicals

such as acrylamide, glycidamide and acrylonitrile. This optimized method was further refined and modified in-house to increase sensitivity and enable automation.

The procedure described here consists of 4 parts: Preparation of the specimen for measurement of hemoglobin adducts of acrylamide and glycidamide; Total hemoglobin measurement in the sample solution used for hemoglobin adduct measurements; Modified Edman reaction in the sample solution and isolation of Edman products and Analysis of Edman products by HPLC/MS/MS and results processing

Because results are reported in pmol adduct per gram of hemoglobin, the amount of hemoglobin used for the modified Edman reaction needs to be known. Therefore, this procedure includes a measurement procedure for total hemoglobin. It is a commercial assay kit based on a well-established procedure commonly used in clinical chemistry.

Quantitation of the acrylamide and glycidamide hemoglobin adduct is performed using octapeptides with the same amino acid sequence as the N-terminal of the beta-chain of hemoglobin and with acrylamide and glycidamide attached at the valine (AA-VHLTPEEK, GA-VHLTPEEK) and the corresponding stable isotope labeled AA-Val(13C5 15N)-HLTPEEK and GA-Val(13C5 15N)-HLTPEEK as internal standards. Total hemoglobin measurement is performed using calibrators provided with the manufacture’s assay kit.

5. Cotinines

Serum cotinine and hydroxycotinine are measured by an isotope-dilution high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometric (ID HPLC-APCI MS/MS) method. Briefly, the serum sample is spiked with methyl-D3-cotinine and methyl-D3-hydroxycotinine as internal standards. The sample is basified and then applied to a supported liquid extraction (SLE) plate. The analytes are extracted with an isopropanol/methylene chloride mixture, the organic extract is concentrated, and the residue is injected onto a C18 HPLC column. The eluent from these injections is monitored by APCI-MS/MS. The m/z 80 product ion from the m/z 177 quasi-molecular ion is measured for cotinine and the m/z 80 product ion from the m/z 193 quasi-molecular ion is measured for hydroxycotinine. Additional ions for the internal standards and for confirmation are also monitored for the respective compounds. Analyte concentrations are derived from the area ratios of native-to-labeled compounds in the sample by comparisons to a standard curve.

6. Dioxins, Furans, Coplanar PCB (Serum)

Serum specimens (1–1.5 mL) to be analyzed for PCBs and persistent pesticides are spiked with 13C12-labeled internal standards and the analytes of interest are isolated in hexane using a C18 solid phase extraction (SPE) procedure followed by extraction through neutral silica and Florosil SPE columns. PCBs and pesticides are eluted from the Florosil column with hexane and 1:1 dichloromethane /hexane. For PCBs and pesticides, each analytical run consists of nine unknown specimens, one method blank, and two quality control samples. Before quantification, the vials are reconstituted with 10μL 13C-labeled external standard. Sample extracts are then analyzed simultaneously for PCBs and pesticides by HRGC/ID-HRMS where 1 μL is injected, using a GC Pal (Leap Technology) auto sampler, into a Hewlett-Packard 6890 gas chromatograph operated in the splitless injection mode with a flow of 1 mL/min helium through a DB-5ms capillary column (30 m x0.25 mm x0.25 μm film thickness) where analytes are separated prior to entering a Thermo Finnigan MAT95 XP (5 kV) magnetic sector mass spectrometer operated in EI mode at 40 eV, using selected ion monitoring (SIM) at 10,000 resolving power (10% valley). Two ion current responses corresponding to two masses are monitored for each native (12C) compound and it corresponding 13C-internal standard. The instrumental response factor for each analyte is calculated as the sum of the two 12C-isomers divided by the sum of two 13C-isomers.

Calibration of mass spectrometer response factor vs. concentration is performed using calibration standards containing known concentrations of each 12C compound and its corresponding 13C internal standard. The concentration of each analyte is derived by interpolation from individual linear calibration curves and is adjusted for sample weight. The validity of all mass spectrometry data are evaluated using a variety of established criteria, such as signal-to-noise ratio ≥ 3 for the smallest native ion mass, instrument resolving power $\geq$ 10,000, chromatographic isomer specificity index with 95% limits, relative retention time ratio of native to isotopically labeled analyte within 3 parts-per-thousand compared to a standard, response ratios of the two 12C and 13C ions must be within ± 20 % of their theoretical values and analyte recovery $\geq$10 % and $\leq$ 120%. In addition, the calculated mean and range of each analyte in the quality control sample must be within their respective confidence intervals. The method detection limit (MDL) for each analyte is calculated correcting for sample weight and recovery. The total lipid content of each specimen is

estimated from its total cholesterol and triglycerides values using a “summation” method. Analytical results for PCBs and pesticides are reported on a whole-weight [ng/g or parts-per-billion (ppb)] and lipid-adjusted basis [ng/g or ppb]. International toxicity equivalents (I-TEQs) are also reported for PCDDs, PCDFs, cPCBs and other “dioxin-like” PCBs, based on the WHO-TEF system. Prior to reporting results, all quality control (QC) data undergo a final review by a Division of Laboratory Science quality control officer.

7. Blood metals

This method directly measures lead (Pb), cadmium (Cd), total mercury (Hg), manganese (Mn) and selenium (Se) content of whole blood specimens using mass spectrometry after a simple dilution sample preparation step.

During the sample dilution step, a small volume of whole blood is extracted from a larger whole blood patient specimen after the entire specimen is mixed (vortexed) to create a uniform distribution of cellular components. This mixing step is important because some metals (e.g., Pb) are known to be associated mostly with the red blood cells in the specimen and a uniform distribution of this cellular material must be produced before a small volume extracted from the larger specimen will accurately reflect the average metal concentration of all fractions of the larger specimen. Coagulation is the process in which blood forms solid clots from its cellular components. If steps are not taken to prevent this process from occurring, i.e., addition of anti-coagulant reagents such as EDTA in the blood collection tube prior to blood collection, blood will immediately begin to form clots once leaving the body and entering the tube. These clots prevent the uniform distribution of cellular material in the blood specimen even after rigorous mixing, making a representative sub-sample of the larger specimen unattainable. It is important that prior to or during sample preparation the analyst identify any sample having clots or micro-clots (small clots). Clotted samples are not analyzed by this method due to the inhomogeneity concerns (i.e., all results for the sample are processed as “not reportable”).

Dilution of the blood in the sample preparation step prior to analysis is a simple dilution of 1 part sample + 1 part water + 48 parts diluent. The effects of the chemicals in the diluent are to release metals bound to red blood cells making them available for ionization, reduce ionization suppression by the biological matrix, prevent clogging of the sample introduction

system pathways by undissolved biological solids, and allow introduction of internal standards to be utilized in the analysis step. Tetramethylammonium hydroxide (TMAH, 0.4% v/v) and Triton X-100TM (0.05%) in the sample diluent solubilizes blood components. Triton X-100TM also helps prevent biological deposits on internal surfaces of the instrument's sample introduction system and reduce collection of air bubbles in sample transport tubing. Ammonium pyrrolidine dithio carbamate (APDC) in the sample diluent (0.01%) aids in solubilizing metals released from the biological matrix. Ethyl alcohol in the sample diluent (1%) aids solubility of blood components and aids in aerosol generation by reduction of the surface tension of the solution. The internal standards, rhodium, iridium, and tellurium, are at a constant concentration in all blanks, calibrators, QC, and samples. Monitoring the instrument signal ratio of a metal to its internal standard allows correction for instrument noise and drift, and sample-to-sample matrix differences.

Liquid samples are introduced into the mass spectrometer through the inductively coupled plasma (ICP) ionization source. The liquid diluted blood sample is forced through a nebulizer, which converts the bulk liquid into small droplets in an argon aerosol. The smaller droplets from the aerosol are selectively passed through the spray chamber by a flowing argon stream into the ICP. By coupling radio-frequency power into flowing argon, plasma is created in which the predominant species are positive argon ions and electrons and has a temperature of 6000-8000 K. The small aerosol droplets pass through a region of the plasma and the thermal energy vaporizes the liquid droplets, atomizes the molecules of the sample and then ionizes the atoms. The ions, along with the argon, enter the mass spectrometer through an interface that separates the ICP (at atmospheric pressure, ~760 torr) from the mass spectrometer (operating at a pressure of 10-5 torr). The ions first pass through a focusing region, then the dynamic reaction cell (DRC), the quadrupole mass filter, and finally are selectively counted in rapid sequence at the detector allowing individual isotopes of an element to be determined.

Generally, the DRC operates in one of two modes. In 'vented' (or 'standard') mode the cell is not pressurized and ions pass through the cell to the quadrupole mass filter unaffected. In 'DRC' mode, the cell is pressurized with a gas for the purpose of causing collisions and/or reactions between the fill gas and the incoming ions. In general, collisions or reactions with the incoming ions selectively occur to either eliminate an interfering ion, change the ion of interest to a new mass, which is free from interference, or collisions between ions in the beam and the DRC gas can focus the ion beam to the middle of the cell and increase the ion signal.

In this method, the instrument is operated in DRC mode when analyzing for manganese, mercury, and selenium. For selenium, the DRC is pressurized with methane gas (CH4, 99.999%) which reduces the signal from 40Ar2+ while allowing the 80Se+ ions to pass relatively unaffected through the DRC on toward the analytical quadrupole and detector. Manganese and mercury are both measured when the DRC is pressurized with oxygen gas (O2, 99.999%). They are analyzed at the same flow rate of oxygen to the DRC cell to avoid lengthening analysis time due to pause delays that would be necessary if different gas flows were used for the two analytes. The oxygen reduces the ion signal from several interfering ions (37Cl18O+, 40Ar15N+, 38Ar16O1H+, 54Fe1H+) while allowing the Mn+ ion stream to pass relatively unaffected through the DRC on toward the analytical quadrupole and detector. In the case of mercury, collisional focusing of the mercury ions occurs, increasing the observed mercury signal at the detector by approximately a factor of two (2x).

Once ions pass through the DRC cell and electrically selected for passage through the analytical quadrupole, electrical signals resulting from the ions striking the discrete dynode detector are processed into digital information that is used to indicate the intensity of the ions. The intensity of ions detected while aspirating an unknown sample is correlated to an elemental concentration through comparison of the analyte: internal standard signal ratio with that obtained when aspirating calibration standards. This method was originally based on the method by Lutz (Lutz et al., 1991). The DRC portions of the method are based on work published by Tanner (Tanner, et al. 1999; 2002).

8. Urine metals

This method directly measures multiple metals in urine specimens using mass spectrometry after a simple dilution sample preparation step. Liquid samples are introduced into the mass spectrometer through the inductively coupled plasma (ICP) ionization source, reduced to small droplets in an argon aerosol via a nebulizer, and then the droplets enter the ICP. The ions first pass through a focusing region, followed by the dynamic reaction cell (DRC), the quadrupole mass filter, and finally are selectively counted in rapid sequence at the detector allowing individual isotopes of an element to be determined.

9. Urine arsenics

Arsenobetaine, arsenocholine, monomethylarsonic acid, dimethylarsinic acid, arsenous (III) acid, arsenic (V) acid

The concentration of speciated arsenics is determined by using high performance liquid chromatography (HPLC) to separate the species coupled to an ICP-DRC-MS to detect the arsenic species. This analytical technique is based on separation by anion-exchange chromatography (IC), followed by detection using quadrupole ICP-MS technology, and includes DRC™ technology (Baranov VI et al., 1999), which minimizes or eliminates many argon-based polyatomic interferences (Tanner S et al., 2000) will require 0.5 mL of urine. Arsenic species column separation is largely achieved due to differences in charge-charge interactions of each negatively charged arsenic component in the mobile phase, with the positively-charged quaternary ammonium groups bound at the column's solid-liquid interface. Upon exit from the column, the chromatographic eluent goes through a nebulizer, where it is converted into an aerosol upon entering the spray chamber.

Carried by a stream of argon gas, a portion of the aerosol is transported through the spray chamber and then through the central channel of the plasma, where it is heated to temperatures of 6000-8000° K. This thermal energy atomizes and ionizes the sample. The ions and the argon enter the mass spectrometer through an interface that separates the ICP, which is operating at atmospheric pressure (approximately 760 torr), from the mass spectrometer, which is operating at approximately 10-5 torr.

The mass spectrometer permits detection of ions at each mass-to-charge ratio in rapid sequence, which allows the determination of individual isotopes of an element. Once inside the mass spectrometer, the ions pass through the ion optics, then through the DRC™, and finally through the mass-analyzing quadrupole before being detected as they strike the surface of the detector. The ion optics uses an electrical field to focus the ion beam into the DRC™.

The DRC™ component is pressurized with an appropriate reaction gas and contains a quadrupole. In the DRC™, elimination or reduction of argon-based polyatomic interferences takes place through the interaction of the reaction gas with the interfering polyatomic species in the incoming ion beam. The quadrupole in the DRC™ allows elimination of unwanted reaction by-products that would otherwise react to form new interferences.

10. Polycyclic Aromatic Hydrocarbons (Urine)

The specific analytes measured in this method are monohydroxylated metabolites of PAHs (OH-PAHs), namely 1-hydroxynaphthalene, 2-hydroxynaphthalene, 2-hydroxyfluorene, 3-hydroxyfluorene, 1-hydroxyphenanthrene, 2- & 3-hydroxyphenanthrene, and 1-hydroxypyrene. The analytical procedure involves enzymatic hydrolysis of glucuronidated/sulfated OH-PAH metabolites in urine, extraction by on-line solid phase extraction, and separation and quantification using isotope dilution high performance liquid chromatography-tandem mass spectrometry (on-line SPE-HPLC-MS/MS) (Wang et al., 2016).

11. Per(Poly)fluoroalkyl Substances (Serum)

Solid phase extraction coupled to High Performance Liquid Chromatography-Turbo Ion Spray ionization-tandem Mass Spectrometry (online SPE-HPLC-TIS-MS/MS) is used for the quantitative detection of perfluorooctane sulfonamide (PFOSA), 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSA-AcOH), 2-(N-ethyl-perfluorooctane sulfonamido) acetic acid (Et-PFOSA-AcOH), perfluorobutane sulfonate (PFBuS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDeA), perfluoroundecanoate (PFUA), and perfluorododecanoate (PFDoA). Briefly, after dilution with formic acid, one aliquot of 100 µL of serum is injected into a commercial column switching system allowing for concentration and chromatographic separation of the analytes. Detection and quantification are done using tandem mass spectrometry (Kuklenyik Z, et al. 2005).

12. Perchlorates

This method is a quantitative procedure for the measurement of nitrate, perchlorate, and thiocyanate in human urine using ion chromatography coupled with electrospray tandem mass spectrometry. Chromatographic separation is achieved using an IonPac AS16 column with sodium hydroxide as the eluent. The eluent from the column is ionized using an electrospray interface to generate and transmit negative ions into the mass spectrometer. Comparison of relative response factors (ratio of native analyte to stable isotope labeled

internal standard) with known standard concentrations yields individual analyte concentrations.

13. Pesticides (Serum)

Thirty-eight ortho-substituted polychlorinated biphenyls (PCBs), 13 persistent chlorinated pesticides, and selected pesticide metabolites are measured in serum by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry (HRGC/ID-HRMS). All serum specimens are handled using Universal Precautions.

Serum specimens (1–1.5 mL) to be analyzed for PCBs and persistent pesticides are spiked with 13C12-labeled internal standards and the analytes of interest are isolated in hexane using a C18 solid phase extraction (SPE) procedure followed by extraction through neutral silica and Florosil SPE columns. PCBs and pesticides are eluted from the Florosil column with hexane and 1:1 dichloromethane /hexane. For PCBs and pesticides, each analytical run consists of nine unknown specimens, one method blank, and two quality control samples. Before quantification, the vials are reconstituted with 10μL 13C-labeled external standard. Sample extracts are then analyzed simultaneously for PCBs and pesticides by HRGC/ID-HRMS where 1 μL is injected, using a GC Pal (Leap Technology) auto sampler, into a Hewlett-Packard 6890 gas chromatograph operated in the splitless injection mode with a flow of 1 mL/min helium through a DB-5ms capillary column (30 m x 0.25 mm x 0.25 μm film thickness) where analytes are separated prior to entering a Thermo Finnigan MAT95 XP (5 kV) magnetic sector mass spectrometer operated in EI mode at 40 eV, using selected ion monitoring (SIM) at 10,000 resolving power (10% valley). Two ion current responses corresponding to two masses are monitored for each native (12C) compound and it corresponding 13C-internal standard. The instrumental response factor for each analyte is calculated as the sum of the two 12C isomers divided by the sum of two 13C- isomers.

Calibration of mass spectrometer response factor vs. concentration is performed using calibration standards containing known concentrations of each 12C compound and its corresponding 13C internal standard. The concentration of each analyte is derived by interpolation from individual linear calibration curves and is adjusted for sample weight. The validity of all mass spectrometry data are evaluated using a variety of established criteria, such as signal-to-noise ratio ≥ 3 for the smallest native ion mass, instrument resolving power

≥ 10,000, chromatographic isomer specificity index with 95% limits, relative retention time ratio of native to isotopically labeled analyte within 3 parts-per-thousand compared to a standard, response ratios of the two 12C and 13C ions must be within ± 20 % of their theoretical values and analyte recovery ≥10 % and ≤ 120%. In addition, the calculated mean and range of each analyte in the quality control sample must be within their respective confidence intervals. The method detection limit (MDL) for each analyte is calculated correcting for sample weight and recovery. The total lipid content of each specimen is estimated from its total cholesterol and triglycerides values using a "summation" method. Analytical results for PCBs and pesticides are reported on a whole-weight [ng/g or parts-per-billion (ppb)] and lipid-adjusted basis [ng/g or ppb]. International toxicity equivalents (I-TEQs) are also reported for PCDDs, PCDFs, cPCBs and other "dioxin-like" PCBs, based on the WHO-TEF system. Prior to reporting results, all quality control (QC) data undergo a final review by a Division of Laboratory Science quality control officer.

14. Pesticides (Urine), Phenols (urine)

Bisphenol A (BPA) and Alkylphenols (APs) have been previously measured in biological matrixes by using gas chromatography (GC) or high performance liquid chromatography (HPLC) coupled with different detection techniques. To achieve enhanced sensitivity and selectivity, the phenols have been derivatized to alkyl or acyl derivatives before GC-mass spectrometry (GC/MS) analysis (Brock, et al., 2001; Jeannot, et al., 2002; Kojima, et al., 2003; Lerch and Zinn, 2003; Louter, et al., 1997; Rinken, 2002; Schonfelder, et al., 2002; Zafra, et al., 2002; Rosenfeld and Moharir, 1991). We have developed a sensitive method for measuring BPA, 4-tert-octylphenol (tOP), benzophenone-3 (BP-3), one chlorophenols triclosan, and four parabens. The method uses solid phase extraction (SPE) coupled on-line to HPLC and tandem mass spectrometry (MS/MS). With the use of isotopically labeled internal standards, the detection limits in 100 μL of urine are 0.1-2 nanograms per milliliter (ng/mL), sufficient for measuring urinary levels of phenols in non-occupationally exposed subjects.

15. Phytoestrogens (Urine)

The test principle utilizes high performance liquid chromatography-atmospheric pressure photoionization-tandem mass spectrometry (HPLC-APPI-MS/MS) for the quantitative

detection of genistein, daidzein, equol, O-desmethylangolensin, enterodiol, and enterolactone. Human urine samples are processed using enzymatic deconjugation of the glucuronidated phytoestrogens followed by size-exclusion filtration. Phytoestrogens are then separated from other urine components by reversed phase HPLC, detected by APPI-MS/MS, and quantified by isotope dilution. Assay precision is improved by incorporating carbon-13 labeled internal standards for each of the analytes, as well as a 4-methylumbelliferyl glucuronide and 4-methylumbelliferyl sulfate standards to monitor deconjugation efficiency. This selective method allows for rapid detection of phytoestrogens in human urine with limits of detection in the low parts per billion (ng/mL) range.

16. Phthalates, urine

The test principle utilizes high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS) for the quantitative detection in urine of the following metabolites: monoethyl phthalate (MEP), monobutyl phthalate (MBP), mono-isobutyl phthalate (MiBP), mono(3-carboxypropyl) phthalate (MCPP), mono(2-ethylhexyl) phthalate (MEHP), monobenzyl phthalate (MBzP), monoisononyl phthalate (MNP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), monocarboxyoctyl phthalate (MCOP), monocarboxynonyl phthalate (MCNP), and cyclohexane-1,2-dicarboxylic acid-mono (hydroxy-isononyl) ester (MHNCH) (Silva, et al., 2007). Urine samples are processed using enzymatic deconjugation of the glucuronidated metabolites followed by on-line solid phase extraction (SPE) coupled with reversed phase HPLC-ESI-MS/MS. Assay precision is improved by incorporating isotopically labeled internal standards of the phthalate metabolites and MHNCH. In addition, 4-methyl umbelliferyl glucuronide is used to monitor deconjugation efficiency. This selective method allows for rapid detection of metabolites of commonly used phthalates and DINCH in human urine with limits of detection in the low ng/mL range.

17. Volatile Organic Compounds (Blood)

An automated analytical method was developed using capillary gas chromatography (GC) and mass spectrometry (MS) with selected-ion monitoring (SIM) detection and isotope-

dilution. This method quantifies levels of individual VOCs and Trihalomethanes (THMs) and methyl tert-butyl ether (MTBE) in whole blood to low-parts-per-trillion range. Because non-occupationally exposed individuals have blood VOC concentrations within this range, this method is applicable for determining these quantities and investigating cases of sustained or recent low-level exposure.

18. Volatile Organic Compounds (Urine)

This method is a quantitative procedure for the measurement of VOC metabolites in human urine using ultra performance liquid chromatography coupled with electrospray tandem mass spectrometry (UPLC-ESI/MSMS) as described by Alwis et al (2012). Chromatographic separation is achieved using an Acquity UPLC® HSS T3 (Part no. 186003540, 1.8 µm x 2.1 mm x 150 mm, Waters Inc.) column with 15 mM ammonium acetate and acetonitrile as the mobile phases. The eluent from the column is ionized using an electrospray interface to generate and transmit negative ions into the mass spectrometer. Comparison of relative response factors (ratio of native analyte to stable isotope labeled internal standard) with known standard concentrations yields individual analyte concentrations.