Environment-Wide Association Study of CKD

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Abstract

Background and objectives Exposure to environmental chemicals has been recognized as one of the possible contributors to CKD. We aimed to identify environmental chemicals that are associated with CKD.

Design, setting, participants, & measurements We analyzed the data obtained from a total of 46,748 adults who participated in the National Health and Nutrition Examination Survey (1999–2016). Associations of chemicals measured in urine or blood (n=262) with albuminuria (urine albumin-to-creatinine ratio $\geq$30 mg/g), reduced eGFR ($< 60$ ml/min per 1.73 m²), and a composite of albuminuria or reduced eGFR were tested and validated using the environment-wide association study approach.

Results Among 262 environmental chemicals, seven (3%) chemicals showed significant associations with increased risk of albuminuria, reduced eGFR, or the composite outcome. These chemicals included metals and other chemicals that have not previously been associated with CKD. Serum and urine cotinine, blood 2,5-dimethylfuran (a volatile organic compound), and blood cadmium were associated with albuminuria. Blood lead and cadmium were associated with reduced eGFR. Blood cadmium and lead and three volatile compounds (blood 2,5-dimethylfuran, blood furan, and urinary phenylglyoxylic acid) were associated with the composite outcome. A total of 23 chemicals, including serum perfluorooctanoic acid, seven urinary metals, three urinary arsenics, urinary nitrate and thiocyanate, three urinary polycyclic aromatic hydrocarbons, and seven volatile organic compounds, were associated with lower risks of one or more manifestations of CKD.

Conclusions A number of chemicals were identified as potential risk factors for CKD among the general population.

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Introduction

CKD is a global public health problem (1–3). The high prevalence of CKD worldwide cannot solely be explained by well-known causes such as diabetes mellitus, hypertension, and GN. Recently, environmental factors have been recognized as important risk factors for the development and progression process of CKD (4,5). Production and use of consumer chemicals have significantly increased in recent decades (6), and chemicals can cause adverse outcomes for human health (7,8). Epidemiologic studies have revealed that at the levels of current exposure, many chemicals are closely associated with human diseases, including neurologic, endocrinologic, and neoplastic diseases (9,10).

Various genetic and environmental factors are suggested as potential risk factors for developing CKD (11). Indeed, several environmental chemicals, including melamine and heavy metals like lead or cadmium, have long been known to be risk factors for kidney injury and CKD (12). Recently, consumer chemicals such as phthalates and bisphenol A have also been reported to be associated with CKD not only among adults, but also among children or adolescents (13). In some populations, other environmental chemicals, including perfluoroalkyl acids, dioxins, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls, have been suggested as a new risk factors for CKD (14,15). However, the associations of these environmental chemicals with kidney disease parameters and CKD are not consistent according to the time points, populations, and clinical circumstances. In addition, our knowledge on the role of chemicals in the cause of CKD is quite limited, considering the growing number of chemicals being introduced in the market and used in daily lives.

In this study, the associations between various environmental chemicals measured in the general United States population and the prevalence of CKD were evaluated. For this purpose, a genome-wide association study methodology was applied for environmental chemicals instead of various genetic phenotypes, i.e., an environment-wide association study (EWAS) (16). By utilizing EWAS, hundreds of bio-monitored chemicals were tested simultaneously for their association with CKD. The results of this study will help to identify a list of potential chemicals with significant association with CKD, which can be

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Materials and Methods

Study Participants and Data Analyzed

We analyzed 46,748 adults (age ≥18 years) who participated in the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2016. Among 92,062 eligible participants, 38,714 young individuals (age <18 years) were initially excluded. In addition, 6600 participants who did not have data for either urinary albumin-to-creatinine ratio (ACR) or eGFR were finally excluded. This cross-sectional, observational cohort study was approved by the Institutional Review Board of Seoul National University Boramae Medical Center (approval number 07–2019–16). Information on the environmental chemicals as well as demographic and laboratory data were obtained from the NHANES database (https://www.cdc.gov/nchs/nhanes/index.htm; demographic, examination, questionnaire, and laboratory data set) in March 2019.

We defined hypertension as an average systolic BP of >140 mm Hg or diastolic BP of >90 mm Hg measured at least twice, history of hypertension, or currently taking antihypertensive medications. Diabetes mellitus was defined as fasting glucose level of >126 mg/dl, random glucose level of >200 mg/dl, or history of diabetes mellitus. Corrected serum creatinine levels were used in the survey of 1999–2000 and 2005–2006 (17,18). Serum and urine creatinine levels were measured using the Jaffe rate methods (kinetic alkaline picrate) with calibration to an isotope dilution mass spectrometry reference method. Urinary albumin levels were measured using solid-phase fluorescent immunoassay. We calculated eGFR using the CKD Epidemiology Collaboration equations (19). Three CKD outcomes were assessed: albuminuria (urinary ACR ratio ≥30 mg/g), reduced eGFR (<60 ml/min per 1.73 m²), and a composite outcome of albuminuria or reduced eGFR.

Measured Chemicals

In NHANES, environmental chemicals were measured in randomly selected subsamples within specific age groups. Measurements of chemicals in serum were made in samples from participants aged ≥12 years. Urine chemicals were measured in a representative one-third subsample. In the discovery set, a total of 262 environmental (minimal number of observations above 500) chemicals were included in the analysis. These chemicals could be grouped as follows: blood acrylamide and glycicamide (n=2), serum and urinary cotinines (n=2), serum dioxins (dioxins, furans, coplanar polychlorinated biphenyls; n=59), blood metals (n=4), urinary metals (n=13), urinary arsenics (n=8), urinary polycyclic aromatic hydrocarbons (n=11), serum perfluoralkyl and polyfluoroalkyl substances (n=12), urinary perchlorate/nitrate/thiocyanate (n=3), serum (n=9) and urinary pesticides (n=54), urinary pheno- nols (n=8), urinary phytoestrogens (n=6), urinary phthalates (n=15), and blood (n=29) and urinary volatile organic compounds (n=27). Information on environmental chemicals and measurement methods is given in Supplemental Appendices 1–3.

Statistical Analyses

The associations between various environmental chemicals measured in the urine or blood, and CKD were assessed by the EWAS approach proposed by Patel et al. for type 2 diabetes mellitus (16,20). EWAS refers to the association study of various exposomes and disease outcomes similarly to a genome-wide association study of SNPs and disease. In general, an EWAS of environmental chemicals for specific disease requires a multiple-cycle population study such as NHANES. An EWAS integrates the multiple survey results between chemical and disease using meta-analysis methods, and validates the results using other populations. Figure 1 presents the outline of the analytic approach used in this study.

We utilized the nine NHANES surveys (1999–2016) to analyze the association between environmental chemicals and CKD. The data sets from nine NHANES cycles (1999–2016) during the 18-year period were divided into the discovery and the validation sets. To reduce errors derived from chronological order, we assigned cycles in an alternate manner into the discovery set (1999–2000, 2003–2004, 2007–2008, 2011–2012, and 2015–2016) and validation set (2001–2002, 2005–2006, 2009–2010, and 2013–2014). In the discovery set, which is composed of data from the five NHANES cycles, the association between each environmental chemical and CKD was tested through survey weighted logistic regression with covariates of age, age-squared, sex, diabetes mellitus, hypertension, body mass index, race/ethnicity, smoking, and socioeconomic status. Family poverty-to-income ratio as a continuous variable was used for socioeconomic status adjustment. Considering the wide range of ages among NHANES participants, we added the age-squared term as a covariate to model the nonlinear effect of differing ages on disease outcomes. Imputation of missing values was not considered. The appropriate sample weights of the smallest subpopulation among variables were selected and adjusted among weights of mobile examination center or subsample weights of each chemicals (21). Then, the estimates from the five NHANES cycles in the discovery set were combined to obtain the meta-analytical results of the combined association and P values. Random-effects models were applied in the meta-analysis. To correct for multiple comparisons, we applied the false discovery rate in the meta-analysis. False discovery rate is one of the correction methods in multiple comparisons and is known to be less conservative compared with other correction approaches (22,23).

Heavy metal–induced nephropathy is known to share the common pathologic pathway of oxidative stress, and interaction and synergistic effects between heavy metals were recently reported (24,25). Interactions between significant heavy metal chemicals were tested with integration of interaction variable into each logistic regression from nine NHANES cycles. Pearson correlations between validated chemicals were summarized using correlation matrix (R library, corplot). Environmental chemicals with false discovery rate <1% were considered as potential risk factors for CKD. Potential risk factors of environmental chemical for CKD identified in the discovery set were tested in the validation set, which is composed of the four NHANES cycles. P<0.05 was considered a significant cut-off value. We also performed a meta-analysis using further confirmed in follow-up epidemiologic studies and experimental mechanistic investigations.
random-effects models to combine the results of each replication of NHANES cycle in the validation set.

Continuous variables were expressed as the mean and SD (median and interquartile range, if a variable did not show normal distribution), and categorical variables were presented as frequencies with percentages. For chemicals measured in urine, creatinine-corrected concentrations were used to adjust the urinary dilution. For this purpose, all urinary chemical levels were divided by the urinary creatinine concentration. All variables were tested for normal distribution using the Kolmogorov–Smirnov test. Most environmental chemical concentrations showed right-skewed distributions; therefore, they were log-transformed. All chemical concentrations were standardized to a mean of 0 and SD of 1 to compare their effect size.

R (version 3.5.2 for Windows) and SPSS software (version 21.0; IBM, Armonk, NY) were used for all analyses. A two-tailed P value <0.05 was considered significant.

Results

Participant Characteristics

A total of 46,748 adult participants were included in this analysis. Participant demographic and clinical characteristics are summarized in Table 1. The number of male participants was 25,709 (48%). Mean age of participants was 47±19 (range 18–85) years. Race/ethnicity of the included participants was 8,979 (19%) Mexican American, 20,674 (44%) white, 9,555 (20%) black, and 3,769 (8%) other. Mean body mass index was 29±7 (range 12.0–130.2) kg/m², mean serum creatinine level was 0.89±0.37 mg/dl, median value of urinary ACR was 6.9 (interquartile range, 4.4–13.6) mg/g, and mean eGFR was 94±24 ml/min per 1.73 m². Hypertension and diabetes were prevalent in 39% and 13% of participants, respectively.

EWAS Analysis for CKD

The Manhattan plots of EWAS for CKD defined by the different criteria developed in the discovery set are presented in Figure 2. Seven (3%) chemicals were associated with increased prevalence of any of the three CKD outcomes, and 23 (9%) chemicals were associated with decreased prevalence of CKD. For albuminuria, four and one chemicals were associated with increased and decreased risk of CKD, respectively. For reduced eGFR, two and 22 chemicals were associated with increased and decreased risk of CKD, respectively. For composite CKD outcome, five and four chemicals were associated with increased and decreased risk of CKD, respectively.

Figure 2A shows the Manhattan plot of EWAS of albuminuria. Serum cotinine, urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanonol, blood cadmium, urinary cadmium, serum perfluorooctanoic acid, and eight blood volatile organic compounds were discovered. Among them, blood cadmium, serum cotinine, urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanonol, serum perfluorooctanoic acid, and blood 2,5-dimethylfuran were validated in the validation set. Those chemicals that showed significant associations with albuminuria are summarized in Table 2. Contrary to other discovered and validated chemicals, serum perfluorooctanoic acid was associated with a decreased risk of albuminuria.

Figure 2B shows the Manhattan plot of EWAS of reduced eGFR. Five urinary arsenics, two blood metals, seven
urinary metals, two perchlorates, two urinary phthalates (mono-benzyl and mono-carboxynonyl phthalates), three urinary polycyclic aromatic hydrocarbons, and six blood and six urinary volatile organic compounds were identified as significant factors in the discovery set. Among these, many chemicals were also identified as significant in the validation set, which included two blood metals (lead and cadmium), seven urinary metals (barium, cadmium, cobalt, cesium, molybdenum, lead, and thallium), three urinary arsenics (arsenocholine, arsenous acid, and arsenic acid), two urinary perchlorates (nitrate and thiocyanate), three urinary polycyclic aromatic hydrocarbons (1-phenanthrene, 2-phenanthrene, and 1-pyrene), and one blood and six urinary volatile organic compounds showed associations of decreased prevalence of reduced eGFR.

Figure 2C shows the Manhattan plot of EWAS of composite CKD outcome. Serum cotinine, two blood metals (lead and cadmium), four urinary metals (barium, cesium, molybdenum, and thallium), serum perfluorooctane sulfonamide, urinary nitrate, one phthalate (mono-carboxynonyl phthalate), one urinary polycyclic aromatic hydrocarbon (2-fluorene), and seven blood and one urinary volatile organic compounds were discovered in the discovery set. Of these, validated environmental chemicals were two blood metals (lead and cadmium), three urinary metals (barium, cesium, and thallium), urinary nitrate, and two blood (2,5-dimethylfuran and furan) and one urinary volatile organic compound (phenylglyoxylic acid). Environmental chemicals that were significantly associated with composite CKD outcomes are shown in Table 4. Blood lead and cadmium and two blood (2,5-dimethylfuran and furan) and one urinary volatile organic compound (phenylglyoxylic acid) were associated with an increased risk of composite CKD outcome. Three urinary metals (barium, cesium, and thallium) and urinary nitrate

### Table 1. Characteristics of National Health and Nutrition Examination Survey (NHANES) participants included in an environment-wide association study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All Participants (n=46,748)</th>
<th>Discovery Set (n=25,281)</th>
<th>Validation Set (n=21,467)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>47 (19)</td>
<td>47 (19)</td>
<td>47 (19)</td>
</tr>
<tr>
<td>Sex, male, %</td>
<td>22,656 (48)</td>
<td>12,295 (49)</td>
<td>10,361 (48)</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>80 (21)</td>
<td>80 (21)</td>
<td>80 (21)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29 (7)</td>
<td>28 (7)</td>
<td>29 (7)</td>
</tr>
<tr>
<td><strong>Race/ethnicity, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexican American</td>
<td>8979 (19)</td>
<td>4867 (19)</td>
<td>4112 (19)</td>
</tr>
<tr>
<td>Other Hispanic</td>
<td>3771 (8)</td>
<td>2307 (9)</td>
<td>1464 (7)</td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>20,674 (44)</td>
<td>10,544 (42)</td>
<td>10,130 (47)</td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>9555 (20)</td>
<td>5307 (21)</td>
<td>4248 (20)</td>
</tr>
<tr>
<td>Other</td>
<td>3769 (8)</td>
<td>2256 (9)</td>
<td>1513 (7)</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>6094 (13)</td>
<td>3433 (14)</td>
<td>2661 (12)</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>18,122 (39)</td>
<td>9985 (40)</td>
<td>8137 (38)</td>
</tr>
<tr>
<td>Systolic BP, mm Hg (n=42,319)</td>
<td>124 (20)</td>
<td>125 (20)</td>
<td>124 (19)</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg (n=42,319)</td>
<td>70 (13)</td>
<td>70 (13)</td>
<td>70 (13)</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl (n=22,723)</td>
<td>107 (36)</td>
<td>108 (38)</td>
<td>105 (33)</td>
</tr>
<tr>
<td>Serum albumin, g/dl</td>
<td>4.2 (0.4)</td>
<td>4.3 (0.4)</td>
<td>4.2 (0.4)</td>
</tr>
<tr>
<td>Uric acid, mg/dl</td>
<td>5.4 (1.5)</td>
<td>5.4 (1.5)</td>
<td>5.4 (1.5)</td>
</tr>
<tr>
<td>BUN, mg/dl</td>
<td>13 (6)</td>
<td>13 (6)</td>
<td>13 (6)</td>
</tr>
<tr>
<td>Serum creatinine, mg/dl</td>
<td>0.89 (0.37)</td>
<td>0.89 (0.37)</td>
<td>0.90 (0.37)</td>
</tr>
<tr>
<td>eGFR, ml/min per 1.73 m²</td>
<td>94 (24)</td>
<td>94 (24)</td>
<td>94 (24)</td>
</tr>
<tr>
<td>Urinary albumin-to-creatinine, mg/g</td>
<td>6.9 (4.4–13.6)</td>
<td>7.0 (4.5–13.9)</td>
<td>6.9 (4.4–13.2)</td>
</tr>
<tr>
<td><strong>Cigarette smoking</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>9225 (20)</td>
<td>4894 (19)</td>
<td>4331 (20)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>10,801 (23)</td>
<td>5870 (23)</td>
<td>4931 (23)</td>
</tr>
<tr>
<td>Never smoker</td>
<td>23,875 (51)</td>
<td>12,969 (51)</td>
<td>10,906 (51)</td>
</tr>
<tr>
<td>Others (refused, do not know, missing)</td>
<td>2847 (6)</td>
<td>1548 (6)</td>
<td>1299 (6)</td>
</tr>
<tr>
<td><strong>Socioeconomic status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family income-to-poverty ratio, median (IQR)</td>
<td>2.5 (1.6)</td>
<td>2.5 (1.6)</td>
<td>2.5 (1.6)</td>
</tr>
<tr>
<td>eGFR&lt;60 ml/min per 1.73 m², %</td>
<td>4315 (9)</td>
<td>2338 (9)</td>
<td>1977 (9)</td>
</tr>
<tr>
<td>eGFR&lt;45 ml/min per 1.73 m², %</td>
<td>1590 (3)</td>
<td>816 (3)</td>
<td>693 (3)</td>
</tr>
<tr>
<td>eGFR&lt;30 ml/min per 1.73 m², %</td>
<td>421 (1)</td>
<td>230 (1)</td>
<td>191 (1)</td>
</tr>
<tr>
<td>ACR&gt;30 mg/g, %</td>
<td>5642 (12)</td>
<td>3177 (13)</td>
<td>2465 (11)</td>
</tr>
<tr>
<td>ACR&gt;300 mg/g, %</td>
<td>958 (2)</td>
<td>554 (2)</td>
<td>404 (2)</td>
</tr>
</tbody>
</table>

Data are shown as mean and SD or numbers and proportion (%). PIR, poverty-income ratio; ACR, albumin-to-creatinine ratio.

*Median (interquartile range).*
were associated with decreased risk of composite CKD outcomes.

Blood cadmium consistently shows significant association with increased prevalence of CKD in all CKD definition criteria. Blood lead showed a significant association with increased prevalence of CKD in high degrees of albuminuria (Table 5). Moreover, the odds ratios increased gradually with the increase of albuminuria and decrease of eGFR. A combined effect of blood lead and cadmium was also shown in various CKD categories defined by albuminuria (Supplemental Table 1). Blood (2,5-dimethylfuran and furan) and urinary (phenylglyoxylic acid) volatile organic compounds were associated with an increased risk of CKD defined by albuminuria or eGFR (Table 4).
Increased blood 2,5-dimethylfuran levels were significantly associated with increased risk of CKD defined by low levels of albuminuria (urinary albumin-to-creatinine ratio ≥30 mg/g) and CKD composite outcome (Supplemental Table 2).

Correlation between environmental chemicals is summarized with a correlation matrix (Supplemental Figures 1 and 2). Serum cotinine, urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), urine Cotinines

<table>
<thead>
<tr>
<th>Metal (blood)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>1.28 (1.20 to 1.36)</td>
<td>6.28×10⁻¹¹</td>
</tr>
<tr>
<td>Cotinine, serum</td>
<td>1.17 (1.09 to 1.25)</td>
<td>2.55×10⁻⁴</td>
</tr>
<tr>
<td>Nitrosamine metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), urine</td>
<td>1.21 (1.10 to 1.34)</td>
<td>5.00×10⁻¹³</td>
</tr>
<tr>
<td>Perfluoroalkyl substances</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfluorooctanoic acid, serum</td>
<td>0.69 (0.57 to 0.83)</td>
<td>3.05×10⁻³</td>
</tr>
<tr>
<td>Volatile organic compounds (blood)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood 2,5-dimethylfuran</td>
<td>1.26 (1.11 to 1.42)</td>
<td>5.00×10⁻³</td>
</tr>
</tbody>
</table>

Table 2. Chemicals significantly associated with albuminuria in an environment-wide association study

A total of 262 chemicals were evaluated, and only those that met an FDR<1% in the discovery cohort and P<0.05 in the validation cohort are included in the table. Albuminuria is defined as urine albumin-to-creatinine ratio ≥30 mg/g. Each chemical is evaluated per SD increment in log-transformed blood concentration or log-transformed chemical-to-creatinine ratio. 95% CI, 95% confidence interval; FDR, false discovery rate.

Discussion

Our results, using the largest data set accumulated on the general United States population, clearly show that several chemicals exposed during daily lives are significantly associated with CKD. Among the 262 environmental chemicals, 30 (11%) chemicals were associated with any of the three CKD outcomes. Five (2%) chemicals were associated with albuminuria, 24 (9%) chemicals were associated with reduced eGFR, and nine (3%) chemicals were associated with composite CKD outcomes, respectively. Blood lead and cadmium, serum cotinine, urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, blood volatile organic compounds including 2,5-dimethylfuran and furan, and urinary phenylglyoxylic acid are associated with increased prevalence of CKD. In particular, blood lead and cadmium showed significant associations with both CKD criteria defined by albuminuria and eGFR, and the discovered volatile organic compounds showed significant associations with CKD defined by albuminuria. These chemical risk factors were identified among hundreds of chemicals measured in the general adult population using the EWAS approach. This EWAS approach provides a powerful and effective tool to identify potential risk factors for adverse outcomes of concern, from multiple candidate chemicals. In addition, visualization of the results using Manhattan plots is helpful to present the association between various environmental chemicals and a specific disease. To date, the EWAS approach has been applied to several diseases, such as type 2 diabetes mellitus, hypertension, metabolic syndrome, and semen quality (16,20,26–28).

In this study, we found that blood cadmium was consistently associated with CKD in all definition categories, and in almost all ranges of albuminuria and eGFR. Our observation also supports that coexposure to blood lead and cadmium additionally increases the risk of CKD, especially CKD defined by albuminuria. In fact, lead and cadmium exposed through environmental or occupational sources have been linked to adverse kidney effects (29), through glomerular and tubular damage and interstitial fibrosis (30–36).

Volatile organic compounds are a group of chemicals that have been used as solvents, degreasers, and cleaning agents in the industry and consumer products. Although several methods, such as detection in exhaled breath, have been suggested for assessing exposure to volatile organic compounds, their parents or metabolites in blood and urine levels have been widely used as proxy values of external exposure amount (37,38). It has been reported that some volatile organic compounds, such as furans, are typically elevated in patients with CKD with or without dialysis (39,40). Chang et al. (41) reported that occupational exposure to volatile organic compounds was associated with increased risk of CKD up to 3.84-fold. Neghab et al. (42) investigated whether occupational volatile organic compound exposure at a petrol station was significantly associated with elevated serum creatinine levels. In our investigation, two blood volatile organic compounds (furan and 2,5-dimethylfuran) and one urinary volatile organic compound (phenylglyoxylic acid) were associated with CKD as defined by albuminuria or eGFR.

Several chemicals, including perfluorooctanoic acid, were found to be associated with decreased CKD prevalence in our study. Perfluorooctanoic acid is consistently
Seafood consumption may increase serum fluorooctanoic acid levels (49,50) and should also be carefully controlled as a confounder of the causal relationship, because fish provides polyunsaturated fatty acids that are beneficial for preventing chronic diseases, including cardiovascular disease (51–53). Therefore, the association between fluorooctanoic acid and decreased CKD prevalence observed in the present population warrants further investigation with a more refined analytical design, e.g., inclusion of fluorooctanoic acid–specific confounders in the model.

Previous studies reported associations between urinary excretion of heavy metals and increased eGFR (54–60). Jin et al. (61) observed similar findings among the 11 metals (cadmium, lead, mercury, total arsenic, dimethylarsinic acid, barium, cobalt, cesium, molybdenum, thallium, and tungsten), and urinary excretion rates of lead and cadmium decreased according to the decrease of eGFR at a similar blood lead or cadmium concentration status. These associations between urinary concentrations of heavy metals and eGFR might result from the decreased urinary excretion of chemicals in CKD patients with decreased eGFR (62,63).

Although this study used the largest number of participants, with hundreds of chemicals in consideration, as a
Cross-sectional association study, it has several limitations, and the observation should be interpreted with caution. First, single measurements of environmental chemicals may not represent true exposure profiles that can be compared with health outcomes. Second, we included one chemical in each logistic regression model, thus, potential interactions among the chemicals, in terms of toxicological modes of action and commonality of the exposure sources, could not be reflected and may lead to false negative or positive associations.

Our findings suggest that increased exposure to heavy metal lead, cadmium, or volatile organic compounds can be associated with increased prevalence of CKD. For each chemical that showed significant associations with CKD,

### Table 4. Chemicals significantly associated with a composite CKD outcome of albuminuria or reduced eGFR in an environment-wide association study

<table>
<thead>
<tr>
<th>Environmental Chemicals</th>
<th>Meta-Analysis (Discovery Data Set)</th>
<th>Meta-Analysis (Validation Data Set)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds Ratio (95% CI) FDR</td>
<td>Odds Ratio (95% CI) P Value</td>
</tr>
<tr>
<td><strong>Metals (blood)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td>1.27 (1.19 to 1.35) 6.51×10^{-12}</td>
<td>1.18 (1.09 to 1.27) 2.77×10^{-5}</td>
</tr>
<tr>
<td>Lead</td>
<td>1.27 (1.12 to 1.45) 3.53×10^{-3}</td>
<td>1.12 (1.00 to 1.24) 4.24×10^{-2}</td>
</tr>
<tr>
<td><strong>Metals (urine)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barium, urine</td>
<td>0.77 (0.71 to 0.84) 3.80×10^{-7}</td>
<td>0.76 (0.69 to 0.84) 1.87×10^{-7}</td>
</tr>
<tr>
<td>Cesium, urine</td>
<td>0.76 (0.66 to 0.87) 1.76×10^{-3}</td>
<td>0.73 (0.65 to 0.81) 5.98×10^{-9}</td>
</tr>
<tr>
<td>Thallium, urine</td>
<td>0.73 (0.66 to 0.81) 1.72×10^{-7}</td>
<td>0.75 (0.67 to 0.83) 2.66×10^{-8}</td>
</tr>
<tr>
<td>Nitrates, thiocyanates, perchlorates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary nitrate</td>
<td>0.74 (0.62 to 0.87) 4.16×10^{-3}</td>
<td>0.79 (0.68 to 0.93) 4.70×10^{-3}</td>
</tr>
<tr>
<td><strong>Volatile organic compounds (blood)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood 2,5-dimethylfuran</td>
<td>1.24 (1.12 to 1.36) 6.73×10^{-4}</td>
<td>1.12 (1.02 to 1.22) 1.67×10^{-2}</td>
</tr>
<tr>
<td>Blood furan</td>
<td>1.21 (1.11 to 1.32) 3.23×10^{-4}</td>
<td>1.12 (1.02 to 1.23) 2.03×10^{-2}</td>
</tr>
<tr>
<td><strong>Volatile organic compounds (urine)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylglyoxylic acid</td>
<td>1.32 (1.15 to 1.51) 1.38×10^{-3}</td>
<td>1.18 (1.01 to 1.38) 3.26×10^{-2}</td>
</tr>
</tbody>
</table>

A total of 262 chemicals were evaluated, and only those that met an FDR<1% in the discovery cohort and P<0.05 in the validation cohort are included in the table. Albuminuria is defined as urine albumin-to-creatinine ratio ≥30 mg/g and reduced eGFR is defined as <60 ml/min per 1.73 m². Each chemical is evaluated per SD increment in log-transformed blood concentration or log-transformed chemical-to-creatinine ratio. 95% CI, 95% confidence interval; FDR, false discovery rate.

### Table 5. Associations of blood lead and cadmium with albuminuria and reduced eGFR

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Exposure</th>
<th>Meta-Analysis (Discovery Data Set)</th>
<th>Meta-Analysis (Validation Data Set)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Odds Ratio (95% CI) FDR</td>
<td>Odds Ratio (95% CI) P Value</td>
</tr>
<tr>
<td><strong>Urine albumin-to-creatinine ratio, mg/g</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥30</td>
<td>Lead</td>
<td>1.23 (1.07 to 1.42) 3.99×10^{-2}</td>
<td>1.08 (0.97 to 1.20) 1.51×10^{-1}</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>1.28 (1.20 to 1.36) 6.28×10^{-11}</td>
<td>1.16 (1.06 to 1.27) 9.03×10^{-4}</td>
</tr>
<tr>
<td>≥300</td>
<td>Lead</td>
<td>1.39 (1.22 to 1.59) 3.41×10^{-5}</td>
<td>1.38 (1.16 to 1.63) 2.10×10^{-4}</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>1.42 (1.27 to 1.58) 6.02×10^{-8}</td>
<td>1.27 (1.00 to 1.61) 5.41×10^{-2}</td>
</tr>
<tr>
<td><strong>eGFR, ml/min per 1.73 m²</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>Lead</td>
<td>1.35 (1.24 to 1.48) 9.45×10^{-10}</td>
<td>1.27 (1.11 to 1.45) 4.08×10^{-4}</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>1.30 (1.19 to 1.42) 2.65×10^{-7}</td>
<td>1.20 (1.10 to 1.30) 1.17×10^{-5}</td>
</tr>
<tr>
<td>&lt;45</td>
<td>Lead</td>
<td>1.60 (1.59 to 1.85) 5.45×10^{-9}</td>
<td>1.63 (1.42 to 1.88) 4.12×10^{-12}</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>1.81 (1.58 to 2.09) 1.80×10^{-14}</td>
<td>1.64 (1.42 to 1.89) 1.65×10^{-11}</td>
</tr>
<tr>
<td>&lt;30</td>
<td>Lead</td>
<td>1.98 (1.50 to 2.62) 7.40×10^{-5}</td>
<td>2.25 (1.75 to 2.90) 3.67×10^{-10}</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>2.43 (1.99 to 2.96) 2.59×10^{-16}</td>
<td>1.91 (1.54 to 2.37) 3.54×10^{-9}</td>
</tr>
<tr>
<td><strong>Composite CKD outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite 1</td>
<td>Lead</td>
<td>1.27 (1.12 to 1.45) 3.53×10^{-3}</td>
<td>1.12 (1.00 to 1.24) 4.24×10^{-2}</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>1.27 (1.19 to 1.25) 6.51×10^{-12}</td>
<td>1.18 (1.09 to 1.27) 2.77×10^{-5}</td>
</tr>
<tr>
<td>Composite 2</td>
<td>Lead</td>
<td>1.43 (1.29 to 1.58) 7.15×10^{-10}</td>
<td>1.45 (1.29 to 1.63) 4.91×10^{-10}</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>1.48 (1.35 to 1.63) 2.38×10^{-14}</td>
<td>1.42 (1.28 to 1.57) 2.48×10^{-11}</td>
</tr>
<tr>
<td>Composite 3</td>
<td>Lead</td>
<td>1.73 (1.54 to 1.95) 1.52×10^{-18}</td>
<td>1.90 (1.59 to 2.28) 3.60×10^{-12}</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>2.01 (1.76 to 2.30) 2.49×10^{-22}</td>
<td>1.61 (1.35 to 1.90) 4.51×10^{-8}</td>
</tr>
</tbody>
</table>

Albuminuria is defined as urine albumin-to-creatinine ratio ≥30 mg/g and reduced eGFR is defined as <60 ml/min per 1.73 m². Each chemical is evaluated per SD increment in log-transformed blood concentration or log-transformed chemical-to-creatinine ratio. 95% CI, 95% confidence interval; FDR, false discovery rate.
further studies that investigate the pathophysiological mechanisms of nephrotoxicity of these environmental chemicals, using in vitro or in vivo experimental models, and that validate the association of exposure to the environmental chemicals and CKD in other populations are warranted. Prospective, longitudinal, cohort studies with multiple repetitive sampling could help to support the causality of the observed relationship.

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Disclosures
All authors have nothing to disclose.

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Supplemental Material
This article contains the following supplemental material online at http://cjasn.asnjournals.org/lookup/suppl/doi:10.2215/CJN.06780619/-/DCSupplemental. Supplemental Table 1. Interaction of blood lead and cadmium with the risk of CKD in whole data set according to the degree of albuminuria, eGFR, and composite categories. Supplemental Table 2. Blood 2,5-dimethylfuran and CKD: meta-analysis results from discovery data set and validation data set according to the degree of albuminuria, eGFR, and composite categories. Supplemental Table 3. Serum perfluorooctanoic acid and CKD: meta-analysis results from discovery data set and validation data set according to the degree of albuminuria, eGFR, and composite categories. Supplemental Figure 1. Correlation matrix between environmental chemicals associated with increased risk of CKD. Supplemental Figure 2. Correlation matrix between environmental chemicals associated with decreased risk of CKD. Supplemental Appendix 1. Measurement of chemicals. Supplemental Appendix 2. Sample weights. Supplemental Appendix 3. List of chemicals.

References


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J.L. and S.O. contributed equally to this work.

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Supplementary Appendix 1. Methodology of chemical measurements

All descriptions are from the NHANES websites:


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 its 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.

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, arsenecholine, 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), perfluoroctane sulfonate (PFOS), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perflurononanoate (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 its 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 \( \geq 3 \) 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.