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Organophosphate pesticides and progression of chronic kidney disease among children: A prospective cohort study

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ABSTRACT

Background: Growing evidence suggests that exposure to environmental chemicals, such as pesticides, impacts renal function and chronic kidney disease (CKD). However, it is not clear if pesticides may affect CKD progression and no studies exist in children.

Objectives: The objective of this study was to examine associations between serially measured urinary OP pesticide metabolites and clinical and laboratory measures of kidney function over time among children with CKD.

Methods: This study used data on 618 participants enrolled in the CKD in Children study (CKiD), a cohort study of pediatric CKD patients from the US and Canada. Children were followed over an average of 3.0 years (standard deviation (SD) = 1.6) between 2005 and 2015. In serially collected urine samples over time, six nonspecific dialkyl phosphate (DAP) metabolites of OP pesticides were measured. Biomarkers of tubular injury (kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL)) and oxidant stress (8-hydroxy-2′-deoxyguanosine (8-OHdG) and F_2-isoprostane) were determined in the same specimens. Estimated glomerular filtration rate (eGFR), proteinuria, and blood pressure were assessed annually.

Results: DAPs were associated with increased KIM-1 and 8-OHdG throughout follow-up. A standard deviation increase in $\sum$diethyl metabolites was associated with increases of 11.9% (95% Confidence Interval (CI): 4.8%, 19.4%) and 13.2% (95% CI: 9.3%, 17.2%) in KIM-1 and 8-OHdG over time, respectively. DAPs were associated with lower eGFR at baseline and higher eGFR over subsequent years.

Conclusions: These findings provide preliminary evidence suggesting that urinary DAP metabolites are associated with subclinical kidney injury among children with CKD, which may signal the potential for clinical events to manifest in the future. The results from this study are significant from both a clinical and public health perspective, given that OP pesticide exposure is a modifiable risk factor.

1. Introduction

Chronic kidney disease (CKD) is a serious condition associated with significant comorbidities, mortality, and healthcare costs. Due to medical and technological advances, survival has improved, leading to greater prevalence, which is approximately 55–60 per million children in western nations (Becherucci et al., 2016) Although still relatively uncommon in children, CKD is associated with substantial morbidity, long-term health consequences, and healthcare costs, especially as it progresses to end stage kidney disease (ESKD) (US Renal Data System 2018 Annual Data Report, 2019a, b, c, d). More research is needed on the determinants of CKD progression to ESKD and prevention strategies. Identification of potentially modifiable risk factors is a key goal.

Progression of CKD to ESKD occurs over time and factors such as blood pressure, hyperuricemia, and anemia have been shown to accelerate the rate of kidney function decline in children (Rodenbach et al., 2015; Staples et al., 2010; US Renal Data System 2018 Annual Data Report, 2019a; Warady et al., 2015; Wong et al., 2012). Notably, few potentially modifiable risk factors have been identified in relation to CKD progression. However, there is growing evidence suggesting that non-traditional factors including exposure to environmental chemicals, such as pesticides, may affect renal function (Sullivan and Krieger, 2001), which motivates the hypothesis that they could play a role in CKD progression as well.

Organophosphate (OP) and organochlorine pesticides have emerged as potential contributors to CKD of unknown etiology (CKDu), which is defined as unexplained CKD that occurs in the absence of typical risk factors or presentation such as diabetes and glomerular nephritis (Pearce and Caplin, 2019). Reports of farmworkers with occupational histories of pesticide application in Central America, India, and Sri Lanka motivated this hypothesis (Herrera et al., 2014; Jayatilake et al., 2013; Valcke et al., 2017), but substantive evidence is still lacking (Pearce and Caplin, 2019). Epidemiologic studies conducted among farmworkers and their wives have also documented links between self-reported pesticide application and ESKD (Lebov et al., 2016; Lebov et al., 2015). Several studies have identified associations between serum biomarkers of pesticide exposure and CKDu and correlates of reduced kidney function in clinical populations in India (Ghosh et al., 2017; Siddarth et al., 2014; Siddharth et al., 2012). Results from toxicological studies have been consistent and indicate that this may occur through increased oxidant stress (Poovala et al., 1999; Poovala et al., 1998; Shah and Iqbal, 2010). For example, one OP, diazinon, enhances renal lipid peroxidation and diminishes antioxidant capacity (Shah and Iqbal, 2010). However, *meta*-analyses and reports from working groups have concluded that there is insufficient evidence to support a contributing role of pesticides in CKDu, citing the need for more high-quality studies using biomonitoring for exposure assessment (Crowe et al., 2019; González-Quiroz et al., 2018; Valcke et al., 2017; Wesseling et al., 2020).

The general (i.e., non-occupational) population is exposed to OP pesticide residues primarily through diet (Cequier et al., 2017; Lu et al., 2008; Quirós-Alcalá et al., 2012; Trasande, 2017). Exposure is prevalent, with at least 74% of children in the US having detectable levels of at least one dialkyl phosphate (DAP) metabolite, and children having significantly higher levels than adults (Barr Dana et al., 2004; Jain, 2016). However, no studies have examined OP pesticide exposure in relation to renal function among children with CKD. Unlike other established risk factors for CKD progression, exposure to OP pesticides is potentially modifiable, given that studies have shown that exposure levels can be reduced by consuming organic foods (Bradman et al., 2015; Lu et al., 2008; Lu et al., 2006).

Therefore, we conducted this study with the following objectives: (1) to examine the associations between serially measured urinary OP pesticide metabolites and clinical measures of kidney function (i.e., eGFR, proteinuria, systolic blood pressure (SBP), diastolic blood pressure (DBP), and time to ESKD or renal replacement therapy (RRT)) and laboratory measures of subclinical kidney damage (i.e., urinary

biomarkers of tubular injury (kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL)) over time in a well-characterized cohort of children and adolescents with CKD; and (2) to determine a potential mechanism of action by evaluating longitudinally assessed oxidative stress biomarkers (i.e., 8-hydroxy-2′-deoxyguanosine (8-OHdG) and F_2-isoprostane.

2. Material and methods

2.1. Study population

The CKD in Children (CKiD) study is a multi-centered prospective cohort study of children aged 6 months to 16 years with mild-to-moderate CKD with the overall goal of identifying predictors and sequelae of CKD progression (Furth et al., 2006). The CKiD study procedures and protocol have been previously described (Furth et al., 2006). Briefly, pediatric patients with prevalent CKD were recruited at a network of sites in the US and Canada. Children were enrolled, assessed 3–6 months after study entry (i.e., baseline), and annually thereafter. These annual assessments included a physical examination, biospecimen collection (e.g., urine, serum, etc.), and questionnaire administration and were conducted until the initiation of RRT for treatment of ESKD. Biospecimens were stored in a central biorepository for future use in ancillary studies. The New York University (NYU) School of Medicine Institutional Review Board deemed this project exempt from review due to data collection being complete and the dataset de-identified.

2.2. Exposure measures

Urine samples serially collected at annual visits were used for exposure assessment. Six DAP metabolites of OP pesticides, dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), diethylthiophosphate (DETP), dimethylphosphorodithidate (DMDTP), and diethyldithiophosphate (DEDTP), and creatinine were analyzed at the Wadsworth Center, New York State Department of Health, Albany, NY. The target DAP metabolites were analyzed by solid phase extraction. Briefly, 0.5 mL of urine was transferred into a 15-mL polypropylene tube, and 2.5 ng each of isotope-labeled internal standard (IS) mixture (six ISs for each of the six target analytes) was added. The urine samples were mixed with 1.5 mL of 2% formic acid (v/v). The samples were then passed through Oasis WAX cartridges (50 mg/3 mL) that were conditioned with methanol (3 mL) and HPLC-grade water (3 mL). After loading samples, cartridges were washed with HPLC-grade water (3 mL) and acetonitrile/HPLC-grade water (3 mL; 50:50, v/v). Analytes were recovered by elution with 3 mL of 5% ammonia in methanol. The extracts were evaporated to near-dryness under nitrogen (Organomation Associates Inc., West Berlin, MA), fortified in 250 μL of acetonitrile/20 mM ammonium acetate (90:10, v/v), and filtered through a 0.22 μm nylon filter (Spin-X, Costar, Corning, NY) prior to instrumental analysis.

Chromatographic separation of the analytes was achieved with a Waters ACQUITY Class I HPLC system (Waters, Milford, MA) fitted with a Luna HILIC column (3 μm, 100 × 3 mm, Phenomenex). Injection volume was 1.5 μL, and mobile phase flow was 400 μL/min. The mobile phase consisted of acetonitrile (A) and 20 mM ammonium acetate in acetonitrile at pH 8.5 (B). The gradient started with 99% B and held for 6 min, linearly decreased to 50% within 0.1 min and held for 1.4 min, and reverted to initial condition within 0.1 min and held for 2.4 min. The total run time was 10 min. Mass spectrometric analysis was performed on an Applied Biosystems API 5500 electrospray triple quadrupole mass spectrometer (ESI-MS/MS; Applied Biosystems, Foster City, CA, USA). The turbo ion spray source setting in the negative ionization mode were: curtain gas (CUR) 30 psi; collision gas (CAD) 10 psi; source temperature 550 °C; ion source gas 1 70 psi; ion source gas 2 70 psi; and ion spray voltage –3500 V. Nitrogen was used as CUR and CAD.

For all exposures, measures below the limit of detection (LOD) were

substituted by the LOD/√2 (Hornung and Reed, 1990). DAP metabolites were analyzed singularly and as molar sums: ∑dimethyl (DM) metabolites (DMP, DMTP, and DMDTP), ∑diethyl (DE) metabolites (DEP and DETP), and ∑DAP (DMP, DMTP, DMDTP, DEP, and DETP). All calculations excluded DEDTP because it was detected in < 10% of samples.

2.3. Outcomes

2.3.1. Clinical renal function measures

Clinical measures of renal function included eGFR, urinary protein-to-creatinine ratio (UPCR), SBP, and DBP, all measured at each annual visit. EGFR was calculated using the modified Schwartz (i.e., bedside) equation (Schwartz et al., 2009). Methods for determining UPCR, SBP, and DBP have been described previously (Fuhrman et al., 2017; Wong et al., 2009). Laboratory analysis of total urinary protein was determined using an immunoturbidimetric assay at the central CKiD laboratory (University of Rochester). UPCR was then calculated as the ratio of the total urinary protein concentration to urinary creatinine concentration. SBP and DBP were measured in the right arm by auscultation using an aneroid sphygmomanometer three times at 30-second intervals (Flynn et al., 2008). The mean BP was computed and standardized to z-scores according to the National High Blood Pressure Education Program Fourth Report (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004).

Time to ESKD/RRT was determined by the start date of dialysis or transplantation (Warady et al., 2015).

2.3.2. Laboratory measures of renal damage

Laboratory measures of subclinical renal damage included urinary tubular injury biomarkers: kidney injury molecule-1 (KIM-1), representing damage to the proximal tubule and neutrophil gelatinase-associated lipocalin (NGAL), representing damage to the distal tubule. KIM-1 and NGAL were assayed in stored urine samples at the NYU High Throughput Biology Laboratory. They were quantified by solid phase sandwich ELISAs using the Quantikine Human KIM-1 Immunoassay and Quantikine Human Lipocalin-2 Immunoassay, respectively (R&D Systems, Minneapolis, MN), according to manufacturer instructions. All analyses were conducted in duplicate. Intra-assay coefficients of variation (CV) ranged from 3.6% to 3.7% for KIM-1 and 2.3%-3.9% for NGAL. Inter-assay CVs ranged from 0.7% to 4.3% for KIM-1 and 0.6%-4.8% for NGAL.

2.3.3. Oxidant stress biomarkers

8-OHdG and F_2-isoprostane were measured in urine samples with sufficient volume at the NYU High Throughput Biology Laboratory (N = 2465 samples; N = 618 individuals and N = 1287 samples; N = 522 individuals, respectively) using previously described methods (Jacobson et al., 2020). Briefly, 8-OHdG was quantified by competitive ELISA using the OxiSelect™ Oxidative DNA Damage ELISA (Cell Biolabs, Inc., San Diego, CA) and F_2-isoprostane by a competitive enzyme-linked immunoassay, the OxiSelect™ 8-*iso*-Prostaglandin $F_{2\alpha}$ ELISA kit.

2.4. Statistical analysis

Distributions of DAP metabolites were examined by calculating percent detection, geometric means, and selected percentiles. For comparison, we examined urinary levels of DAP metabolites among National Health and Nutrition Examination Survey (NHANES) samples from 2011 to 2012 for individuals aged 6–19 years (Centers for Disease Control and Prevention, 2012).

In preparation for modeling, urinary concentrations of DAP metabolites were natural log-transformed due to their right skewed distributions to reduce the influence of measures at the upper tail. Outcomes of interest that were right skewed: UPCR, KIM-1, NGAL, 8-OHdG and F_2-isoprostane, were natural-log transformed before multivariable analysis

in order to approximate normal distributions.

To estimate longitudinal associations between repeated measures of DAPs and renal function measures and oxidative stress biomarkers, linear mixed effects models (LMMs) were fit with random intercepts and a first-order autoregressive correlation structure to account for both the intra-individual correlation of measures over time and inter-individual variation (formula shown in Supplemental Material). Separate models were fit for each exposure-outcome combination. Models were run for each individual DAP metabolite as well as each molar sum (i.e., ∑DM, ∑DE, and ∑DAP) and all outcomes of interest (i.e., eGFR, UPCR, SBP, DBP, KIM-1, NGAL, 8-OHdG, and F_2-isoprostane). Models were fit with DAP metabolites expressed on the volume basis (ng/ml or nmol/L) and urinary creatinine was controlled for as a covariate (Barr et al., 2004). All models additionally adjusted for continuous time since the baseline visit, a quadratic term for time, and baseline covariates hypothesized to act as confounders and/or key predictors of the outcome(s) based on previous literature: sex, race/ethnicity, age, glomerular versus non-glomerular or CAKUT categorization, birthweight, premature birth, angiotensin converting enzyme inhibitor (ACEI) use, angiotensin receptor blocker (ARB) use, BMI z-score, and SBP z-score (in models for all outcomes except blood pressure) (Supplemental Figure) (Furth et al., 2006; Kataria et al., 2017; Kataria et al., 2015; Malits et al., 2018). In addition, to examine whether associations varied over time as CKD progressed, we fit all models with cross-product interaction terms between all DAP metabolites and follow-up time. If the interaction with time was significant (p < 0.05), we reported time-specific associations. Lastly, in all models, the log-transformed exposure variables were standardized so that the estimates represented the average change in the outcome in response to a 1-standard deviation (SD) change in the log-transformed exposure, which facilitates the interpretation of associations across exposures. In the presentation of results in the text for outcomes that were natural log-transformed (i.e., UPCR, KIM-1, NGAL, 8-OHdG and F_2-isoprostane), the β-coefficients were exponentiated so that they could be interpreted as the percent change in the outcome response to a 1-SD increase in the log-transformed exposure.

We also fit extended Cox proportional hazards models, which accommodate time-varying exposures, to estimate associations between longitudinal DAP metabolites and time to RRT (Kleinbaum and Klein, 2011). Time at risk started at the baseline visit and continued until the initiation of RRT or censorship at the end of follow-up. Follow-up time for this analysis was available outside of the clinical study period due to linkages with medical records, with a median of 5.7 years (IQR: 3.4, 8.0). The proportional hazards assumption was checked for all covariates using visual inspection of log–log survival curves and testing of Schoenfeld residuals and covariate-by-time interaction terms.

Finally, we conducted several sensitivity analyses. First, we considered several parameterizations of exposure including cumulative averages, peak concentrations, and quartiles. We also examined the potential for non-linear relationships by using generalized additive mixed-effect models (GAMMs). Second, we evaluated associations between DAP metabolites and rates of change in renal outcomes using a two-step approach: (1) estimating the individual slope over time using the individual repeated outcome measures and (2) using this slope as the outcome in a model with urinary DAPs. Third, we examined whether the associations between DAP metabolites and the outcomes of interest varied by kidney function level by stratifying by eGFR categories at baseline. Separate models were run among those with eGFR levels ≥ 45 mL/min/1.73 m² (eGFR category G3a and higher) and < 45 mL/min/1.73 m² (eGFR category G3b and lower) (KDIGO, 2013 clinical practice guideline for the evaluation and management of chronic kidney disease 2013). Third, residential zip code was available on 80% of children. We examined the impact of controlling for zip-code level poverty measures (i.e., percent below the poverty line) using American Community Survey data in our analyses. Fourth, we examined the impact of using an alternate method for accounting for variation in urinary creatinine excretion: the covariate-adjusted standardization plus covariate

adjustment method (O'Brien et al., 2015). Lastly, because dietary restrictions may be warranted for certain CKD patients according to disease type (glomerular vs. non-glomerular) which could theoretically affect OP exposure, we stratified our analysis by disease type.

Analyses were performed using complete case analysis. All analyses were conducted in R version 3.5.0 for Windows (Team, 2013), using the package 'nlme' (Pinheiro et al., 2018) version 3.1–137 for LMM models.

3. Results

Between 2005 and 2015, 618 CKiD participants were included in this study. There were no markedly different demographic or clinical characteristics between the subgroup included in this study (59% of the total enrollment) and the complete CKiD cohort (data not shown) (Atkinson et al., 2020;). They were followed for an average of 3.0 years (SD = 1.6), constituting 2469 total visits (mean number of visits ± SD: 4.0 ± 1.6). Most children were male (63.8%), white (58.3%), and had non-glomerular disease (89.2%) (Table 1). Median age at baseline was 11.0 (interquartile range (IQR): 7.6, 14.6). CKD severity at baseline was moderate, with an average eGFR = 51.9 mL/min/1.73 m^2 (SD = 19.8) and 39.1% with stage 3B or more advanced disease (eGFR ≤ 44). Median urinary protein: creatinine ratio was 0.30 at baseline (IQR: 0.1, 0.8), and SBP and DBP were slightly above average (mean z-scores = 0.32 ± 1.1 and 0.52 ± 1.0, respectively).

At least one DAP metabolite was detected in 100% of samples. DMTP was the most prevalent metabolite detected (99.9%) and had the greatest concentrations compared with the other five DAPs, whereas DEDTP was rarely detected (9.9%) (Table 2). DM and DE metabolites were moderately correlated (Spearman rho = 0.52, p < 0.0001). All DAP metabolites exhibited substantial within-person variation over time, with intraclass correlation coefficients ranging from 0.18 to 0.28. ΣDAP concentrations tended to decrease over the course of follow-up (Table 3). Although total urinary DAP concentrations did not vary across most demographic strata, in bivariate analyses, levels tended to be greater among Black and Hispanic participants compared with White and those of other race (Supplemental Table 1). In addition, DAP

Table 1
Characteristics of study population, Chronic Kidney Disease in Children Study (CKiD), 2005–2015.

	Total (N = 618)
Sex, n (%)	
Female	224 (36.3)
Male	394 (63.8)
Race/Ethnicity, n (%)	
White	360 (58.3)
Black	102 (16.5)
Hispanic	85 (13.8)
Multiracial/Other	71 (11.5)
Age (years) at visit 1, median (IQR)	11.0 (7.6, 14.6)
BMI z-score at visit 1, mean (SD)	0.41 (1.2)
Type of kidney disease, n (%)	
Non-Glomerular	551 (89.2)
Glomerular	67 (10.8)
ARB use, n (%)	47 (8.7)
ACEI use, n (%)	241 (44.8)
Birthweight, kg, mean (SD)	3.1 (0.8)
Premature, n (%)	62 (11.5)
eGFR (ml/min/1.73 m^2) at visit 1, mean (SD)	51.9 (19.8)
UPCR at visit 1, median (IQR)	0.30 (0.1, 0.8)
SBP Z-score at visit 1, mean (SD)	0.32 (1.1)
DBP Z-score at visit 1, mean (SD)	0.52 (1.0)

Abbreviations: SD: standard deviation; ACEI: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; BMI: body mass index; eGFR: estimated glomerular filtration rate; UPCR: urinary protein to creatinine ratio; IQR: interquartile range; SBP: systolic blood pressure; DBP: diastolic blood pressure.
Percent missing for covariates: age, sex, race/ethnicity, glomerular disease type, ARB/ACEI use (0); birthweight (5.5); prematurity (4.2); BMI (3.2); SBP (7.3).

concentrations were highest among those aged ≤ 8 years and decreased with age. Overall, urinary concentrations of DAP metabolites among CKiD participants were lower than those of contemporaneous NHANES samples (Table 2).

Associations between DAP metabolites and eGFR varied over time (Fig. 1). At baseline, DAPs were associated with reductions in eGFR (for a SD increase in ΣDAP, β = −1.01 mL/min/1.73 m^2, 95% Confidence Interval (CI): −1.66, −0.36) (Table 4). Estimates for DE metabolites (DE and DETP) showed the strongest associations that extended out to 1 year after baseline. However, at years 2 through 4 after baseline, DAP metabolites were associated with increases in eGFR (Supplemental Table 2). For example, for a SD increase in ΣDAP, eGFR increased by 0.54 mL/min/1.73 m^2 (95% CI: 0.02, 1.06) at year 2, 0.89 mL/min/1.73 m^2 (95% CI: 0.32, 1.47) at year 3, and 0.69 mL/min/1.73 m^2 (95% CI: 0.09, 1.29) at year 4. All DAP metabolites exhibited this same trend. Overall, DAP metabolites were not associated with UPCR, SBP, or DBP (Table 4 and Supplemental Tables 3 and 4). In addition, DAP metabolites were not associated with time to RRT (Table 5).

DEP and ΣDE metabolites were associated with increased concentrations of KIM-1 that persisted over time (Table 6). For an SD increase in ΣDE, there was an expected increase in KIM-1 of 11.9% (95% CI: 4.8%, 19.4%) (computed from Table 6). DM metabolites also had positive point estimates in association with KIM-1 but were smaller in magnitude and not statistically significant. DAP metabolites were not associated with NGAL concentrations.

DAP metabolites were consistently associated with increases in urinary 8-OHdG excretion (Table 7). DE metabolites showed the strongest associations with 8-OHdG. For example, a SD increase in DEP was associated with a 12.2% increase (95% CI: 8.3%, 16.2%) in 8-OHdG (computed from Table 7). For DETP, associations were apparent within the first 2 years after baseline and tended to attenuate over time (Supplemental Table 5). DAP metabolites were not statistically significantly associated with increased F_2-isoprostane, although some metabolites, such as DMTP and DMDTP, had positive point estimates.

In sensitivity analyses, modeling the association between DAP metabolites and renal outcomes with different parameterizations of exposures (i.e., cumulative averages, peak exposure, quartiles, and allowing for non-linear relationships using GAMMs) yielded results consistent with the primary analysis (data not shown). When we used a two-step approach to examine rates of change over time, results were null for eGFR and biomarkers for tubular injury. However, we observed an inverse association between urinary DAPs and rate of UPCR change over time, suggesting that those with higher urinary DAP concentrations had decreased rates of UPCR over time compared with those with lower concentrations (Supplemental Table 6). When stratified by baseline eGFR levels, associations between exposure biomarkers and eGFR, KIM-1, and 8-OHdG did not materially change (Supplemental Tables 7–9), although associations tended to be stronger in magnitude among those with eGFR ≥ 45. Results were consistent when analyses were repeated controlling for zip-code level poverty measures () and when we implemented the covariate-adjusted standardization plus covariate adjustment method for accounting for variation in creatinine excretion (Supplemental Tables 12–13). Finally, results were consistent in direction when the population was stratified by CKD disease type (i.e., glomerular vs. non-glomerular), although estimates were imprecise among those with glomerular disease because this comprised only 11% of the cohort (n = 67) (Supplemental Tables 14–16).

4. Discussion

In a longitudinal cohort of children with CKD, we found that higher levels of DAP metabolites were associated with increased urinary excretion of KIM-1 over time, suggesting subclinical kidney injury. However, although DAP metabolites were associated with lower eGFR at baseline, there was a trend of higher eGFR over follow-up and no association with UPCR, SBP, DBP, or time to RRT. On the biomarker level,

Table 2

Distributions of urinary metabolites of OP pesticides over time, Chronic Kidney Disease in Children Study (CKiD), 2005–2015 and comparison with National Health and Nutrition Examination Survey (NHANES) samples, 2011–2012.

| Biomarker | LOD | %<LOD | GM[b] | Percentile | | | | ICC | NHANES 2011-2012[a] | |
				25th	50th	75th	95th		%<LOD[b]	GM[c]
Individual OP pesticide metabolites										
DMP (ng/ml)	0.0532	22.4	1.03	0.54	1.78	3.91	14.33	0.18	1.9	2.54
DEP (ng/ml)	0.0544	1.7	1.11	0.55	1.06	2.15	6.95	0.24	1.1	2.49
DMTP (ng/ml)	0.00492	0.08	1.36	0.49	1.19	3.44	17.99	0.26	5.1	1.53
DETP (ng/ml)	0.01796	3.6	0.17	0.08	0.16	0.37	1.40	0.28	27.1	0.30
DMDTP (ng/ml)	0.0024	2.6	0.12	0.05	0.11	0.30	1.95	0.22	43.8	0.21
DEDTP (ng/ml)	0.0334	90.1	–	<LOD	<LOD	<LOD	<LOD		95.9	–
OP pesticide groups										
∑DM (nmol/L)[d]	–	–	23.8	9.64	24.80	60.71	261.87	0.26	–	36.2
∑DE (nmol/L)[e]	–	–	8.76	4.40	8.38	16.56	53.50	0.26	–	19.3
∑DAP (nmol/L)[f]	–	–	37.8	16.44	36.85	79.72	299.23	0.26	–	64.8

Abbreviations: DAP, dialkyl phosphate; DE, diethyl; DM, dimethyl; DMP, dimethylphosphate; DEP, diethylphosphate; DMTP, dimethylthiophosphate; DETP, diethylthiophosphate, DMDTP, dimethylphosphorodithidate; DEDTP, diethyldithiophosphate; LOD, limit of detection; ICC, intraclass correlation coefficient; NHANES, National Health and Nutrition Examination Survey.

[a] Among participants aged 6–19 years.

[b] Limit of detection for all DAP metabolites measured by NHANES was 0.1 ng/ml.

[c] Geometric means were not calculated for chemicals with ≥ 50% of measures < LOD.

[d] ∑DM is the molar sum of DMP, DMTP, and DMDTP.

[e] ∑DE is the molar sum of DEP and DETP.

[f] ∑DAP is the molar sum of DMP, DEP, DMTP, DETP, and DMDTP.

Table 3

Distributions of urinary ∑DAP (nmol/L) by CKiD study visit, 2005–2015.

| | Geometric mean | Percentiles | | |
		25th	50th	75th
Baseline	48.0	20.2	43.4	109.1
Year 1	39.7	16.7	38.8	87.2
Year 2	41.9	19.2	38.5	80.2
Year 3	31.4	14.7	32.1	67.7
Year 4	30.5	13.3	33.8	61.3
Year 5	27.8	13.5	25.8	51.3

Abbreviations: DAP, dialkyl phosphate.

DAPs were not associated with NGAL, but ΣDE and especially DEP was associated with increased KIM-1 concentrations. Lastly, all DAP metabolites, again especially DE metabolites, were associated with greater urinary concentrations of 8-OHdG over time, indicative of increased oxidant stress.

Despite an inconsistent association that varied over time with eGFR, our findings suggest no other associations with clinical renal outcomes, but evidence of subclinical renal damage as assessed by tubular injury and oxidant stress biomarkers. Although there was no association with time to RRT, proteinuria, or blood pressure, we did observe an inverted J-shaped association with eGFR that persisted across several parameterizations of exposure, stratifications by baseline eGFR and glomerular disease type, and control for area-level socioeconomic status. Based on studies showing associations between pesticide exposures and ESKD (Lebov et al., 2016; Lebov et al., 2015), we hypothesized that such an association with eGFR could be due to OP exposure-induced ESKD and thus discontinued follow-up in those who reached this endpoint in the CKiD study. In other words, if those vulnerable to OP exposure dropped out of the study over time due to ESKD/RRT (i.e., CKiD exclusion criteria), it may appear that DAPs were positively associated with renal function among the remaining participants in the study in the later years of follow-up. However, the lack of an association between DAPs and time to ESKD/RRT does not support this possibility. At present, we do not have a satisfactory explanation for the inverted J-shaped trajectory of eGFR changes over time and OP exposure.

Our findings of increased 8-OHdG, indicating oxidant-mediated DNA damage, are consistent with the hypothesis that OPs affect renal function through induction of oxidant stress. *In vitro* studies have identified oxidative stress as the central mechanism by which OPs induce acute kidney injury (Poovala et al., 1999; Poovala et al., 1998). In addition, toxicologic studies among diazinon and chlorpyrifos-treated rats and mice have shown dose-dependent increases in reactive oxygen species and oxidative stress in tissues and declines in renal antioxidant enzyme activity (Jafari et al., 2012; Ma et al., 2013; Shah and Iqbal, 2010). Our findings of diethyl metabolites associated with increased 8-OHdG are particularly consistent with this given that diazinon and chlorpyrifos are two species that metabolize to diethyl metabolites (Centers for Disease Control and Prevention, 2009). Finally, although we did not observe associations with lipid peroxidation (i.e., F_2-isoprostane), this was likely due to the smaller sample size.

In addition, the documentation of elevated urinary KIM-1 but not NGAL in relation to OP exposure is consistent with what is known about the localization of these biomarkers along the nephron. While NGAL is primarily expressed in the distal tubule, KIM-1 is detected in the proximal convolute tubule (Bank et al., 2017; Bieniaś et al., 2015). Because of greater bulk uptake of environmental chemicals by the proximal segments, this may account for the stronger association between OP exposure and urinary excretion of KIM-1 in the CKID cohort.

Our study benefited from prospective collection of biospecimens over time and throughout the course of CKD among a well characterized cohort of children. We measured urinary biomarkers of OP pesticides multiple times within each individual, which allowed us to examine the potential impact of cumulative exposure over time. By leveraging the biobank of serially collected samples, this statistical approach compensates for the substantial intra-individual variability in these measures due to their non-persistence and short biologic half-lives (Casas et al., 2018). This was a significant improvement on previous studies that have primarily utilized single spot urine samples. This longitudinal study design and serial collection of biologic specimen also allowed for the repeated measurement of tubular injury and oxidative stress biomarkers over time. In conjunction with the clinical renal and cardiovascular measures collected within the CKiD study protocol, this provided for a comprehensive picture of the trajectory of renal function over time.

Limitations of this study included the use of urine as the matrix for measuring biomarkers of exposure (i.e. OP pesticides) and effect (i.e., tubular injury and oxidant stress). Kidney dysfunction is known to affect urinary solute excretion, and those with severe CKD have been shown to have reduced excretory capacity owing to impaired glomerular filtration
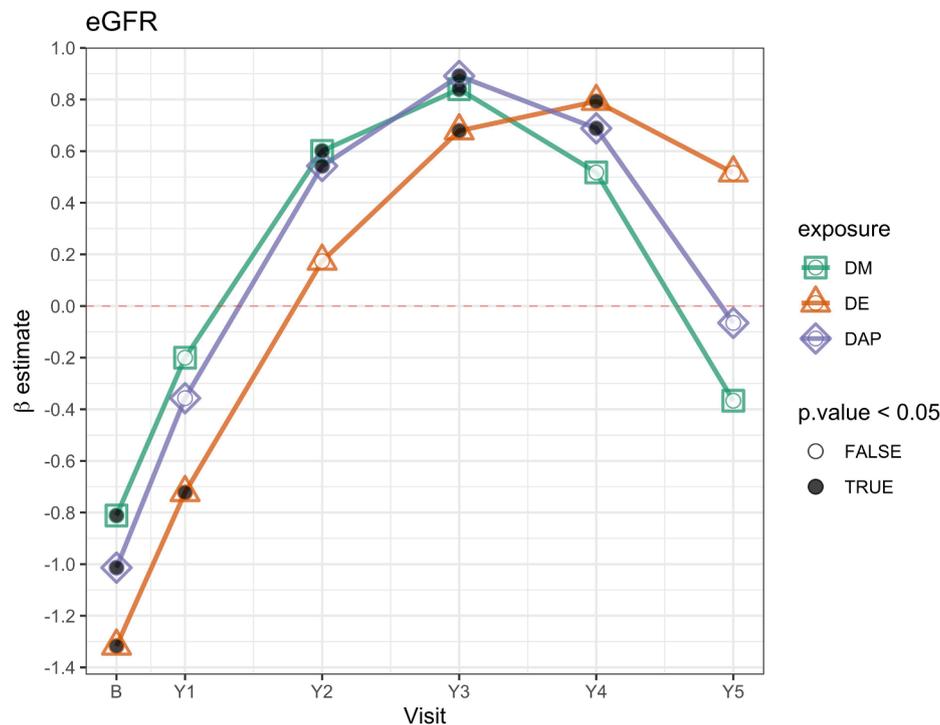
eGFR

Fig. 1. Associations between ln-transformed DAP metabolite groups and eGFR by study visit shown as β-coefficients on the y-axis. Estimates were derived from adjusted linear mixed-effects models and correspond to a 1- standard deviation change in each ln-transformed DAP exposure.

Table 4
Associations between ln-transformed chemical exposures and eGFR, ln-transformed urinary protein-to-creatinine ratio, SBP z-score, and DBP z-score from linear mixed-effects models.

	eGFR (ml/min/1.73 m²)[a] (N = 2024)				Ln-UPCR[a] (N = 1969)		
	β	95% CI	p		B	95% CI	p
DMP*	−0.629	−1.296, 0.038	0.064	DMP	−0.001	−0.036, 0.034	0.956
DEP*	−1.306	−1.983, −0.629	<0.0001	DEP	−0.029	−0.066, 0.008	0.133
DMTP*	−0.87	−1.515, −0.225	0.008	DMTP	−0.006	−0.041, 0.029	0.744
DETP*	−0.973	−1.611, −0.335	0.003	DETP	−0.047	−0.084, −0.010	0.012
DMDTP*	−0.652	−1.313, 0.009	0.053	DMDTP*	0.014	−0.047, 0.075	0.66
∑DM*	−0.812	−1.465, −0.159	0.015	∑DM	−0.005	−0.042, 0.032	0.792
∑DE*	−1.317	−1.982, −0.652	<0.0001	∑DE*	−0.038	−0.075, −0.001	0.052
∑DAP*	−1.014	−1.665, −0.363	0.002	∑DAP	−0.017	−0.054, 0.020	0.36
	SBP Z-score[b] (N = 2035)				DBP Z-score[b] (N = 2034)		
	β	95% CI	p		B	95% CI	p
DMP	−0.009	−0.050, 0.032	0.671	DMP	0.016	−0.023, 0.055	0.432
DEP	−0.008	−0.049, 0.033	0.694	DEP	0.001	−0.038, 0.040	0.954
DMTP*	0.007	−0.062, 0.076	0.839	DMTP	0.028	−0.013, 0.069	0.169
DETP	−0.008	−0.051, 0.035	0.721	DETP	0.006	−0.035, 0.047	0.76
DMDTP	−0.007	−0.048, 0.034	0.739	DMDTP	0.003	−0.036, 0.042	0.878
∑DM*	−0.014	−0.046, 0.040	0.68	∑DM	0.026	−0.015, 0.067	0.211
∑DE	−0.008	−0.051, 0.035	0.723	∑DE	0.004	−0.037, 0.045	0.831
∑DAP	−0.003	−0.046, 0.040	0.9	∑DAP	0.023	−0.018, 0.064	0.267

Abbreviations: eGFR: estimated glomerular filtration rate; UPCR: urinary protein to creatinine ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure; DAP, dialkyl phosphate; DE, diethyl; DM, dimethyl; DMP, dimethylphosphate; DEP, diethylphosphate; DMTP, dimethylthiophosphate; DETP, diethylthiophosphate, DMDTP, dimethylphosphorodithidate; DEDTP, diethyldithiophosphate.
β: estimate per SD change.
  * Exposure has significant interaction with time (p < 0.05) and estimate for exposure at baseline is presented here; time-specific estimates were plotted in Fig. 1 and Supplemental Tables 2–4.
  [a] The model controlled for time since baseline, age, gender, race/ethnicity, glomerular status, birthweight, low birthweight prematurity, ARB, ACEI, BMI-Z score, SBP Z-score (all measured at each patient's first visit) and creatinine.
  [b] The model controlled for time since baseline, age, gender, race/ethnicity, glomerular status, birthweight, low birthweight, prematurity, ARB, ACEI, BMI-Z score (all measured at each patient's first visit) and creatinine.

and/or diminished tubular secretion (Weaver et al., 2016). Therefore, urinary biomarker concentrations may be affected, especially in those with the most advanced CKD. Consistent with this theory is our observations of decreasing urinary DAP concentrations over the course of

follow-up among CKiD children and lower urinary levels of DAPs among CKiD children compared with healthy children from NHANES. However, we note that these differences may also be due to variation in residential proximity to agriculture, diet, home and garden OP pesticide use, and

Table 5

Adjusted Hazard Ratios (HR) and 95% Confidence Intervals (CI) from Cox Proportional Hazards Models for the associations of ln-transformed chemical exposures with time to renal replacement therapy and/or end-stage renal disease among 538 CKiD participants (n = 179 events) with baseline exposure data.

	HR	95% CI
DMP	0.99	0.85, 1.16
DEP	1.15	0.84, 1.58
DMTP	0.95	0.76, 1.20
DETP	0.89	0.70, 1.13
DMDTP	0.83	0.68, 1.02
∑DM	0.95	0.76, 1.20
∑DE	1.02	0.74, 1.41
∑DAP	0.99	0.75, 1.30

Abbreviations: DAP, dialkyl phosphate; DE, diethyl; DM, dimethyl; DMP, dimethylphosphate; DEP, diethylphosphate; DMTP, dimethylthiophosphate; DETP, diethylthiophosphate, DMDTP, dimethylphosphorodithidate; DEDTP, diethyldithiophosphate.

All models controlled for age, gender, race/ethnicity, glomerular status, birthweight, low birthweight, prematurity, ARB, ACEI, BMI-Z score, SBP Z-score (all measured at each patient's first visit) and creatinine.

Table 6

Associations between ln-transformed chemical exposures and ln-transformed tubular injury biomarkers from linear mixed-effects models.

	ln-NGAL (N = 1925)			ln-KIM-1 (N = 1925)		
	β	95% CI	P	β	95% CI	p
DMP	−0.018	−0.096, 0.060	0.649	0.049	−0.012, 0.110	0.114
DEP	−0.073	−0.155, 0.009	0.077	0.123	0.060, 0.186	<0.0001
DMTP	−0.012	−0.092, 0.068	0.77	0.03	−0.033, 0.093	0.349
DETP	−0.029	−0.109, 0.051	0.486	0.046	−0.017, 0.109	0.152
DMDTP	−0.07	−0.147, 0.007	0.077	−0.008	−0.069, 0.053	0.805
∑DM	−0.002	−0.082, 0.078	0.961	0.051	−0.012, 0.114	0.11
∑DE	−0.065	−0.147, 0.017	0.124	0.112	0.047, 0.177	0.001
∑DAP	−0.024	−0.106, 0.058	0.56	0.059	−0.006, 0.124	0.069

Abbreviations: NGAL: Neutrophil gelatinase-associated lipocalin; KIM-1: Kidney Injury Molecule-1; DAP, dialkyl phosphate; DE, diethyl; DM, dimethyl; DMP, dimethylphosphate; DEP, diethylphosphate; DMTP, dimethylthiophosphate; DETP, diethylthiophosphate, DMDTP, dimethylphosphorodithidate; DEDTP, diethyldithiophosphate.

All models controlled for time since baseline, age, gender, race/ethnicity, glomerular status, birthweight, low birthweight, prematurity, ARB, ACEI, BMI-Z score, SBP Z-score (all measured at each patient's first visit) and creatinine.

β: estimate per SD change.

Table 7

Associations between ln-transformed chemical exposures and ln-transformed oxidative stress biomarkers from linear mixed-effects models.

	ln-8-OHdG (N = 2029)			ln-F$_2$-Isoprostane (N = 1045)		
	β	95% CI	P	β	95% CI	p
DMP	0.039	0.006, 0.072	0.024	−0.045	−0.151, 0.061	0.403
DEP	0.115	0.080, 0.150	<0.0001	−0.063	−0.171, 0.045	0.252
DMTP	0.066	0.031, 0.101	<0.0001	0.094	−0.018, 0.206	0.1
DETP*	0.149	0.094, 0.204	<0.0001	0.052	−0.050, 0.154	0.315
DMDTP	0.081	0.048, 0.114	<0.0001	0.099	−0.015, 0.213	0.092
∑DM	0.065	0.030, 0.100	<0.0001	0.02	−0.090, 0.130	0.721
∑DE	0.124	0.089, 0.159	<0.0001	−0.045	−0.151, 0.061	0.41
∑DAP	0.088	0.053, 0.123	<0.0001	0.008	−0.104, 0.120	0.887

Abbreviations: 8-OHdG: 8-hydroxy-2′-deoxyguanosine; DAP, dialkyl phosphate; DE, diethyl; DM, dimethyl; DMP, dimethylphosphate; DEP, diethylphosphate; DMTP, dimethylthiophosphate; DETP, diethylthiophosphate, DMDTP, dimethylphosphorodithidate; DEDTP, diethyldithiophosphate.

All models controlled for time since baseline, age, gender, race/ethnicity, glomerular status, birthweight, low birthweight, prematurity, ARB, ACEI, BMI-Z score, SBP Z-score (all measured at each patient's first visit) and creatinine.

β: estimate per SD change.

* Exposure has significant interaction with time ($p < 0.05$) and estimate for exposure at baseline is presented here; time-specific estimates are shown in Supplemental Table 5.

confidence intervals, it is a more accurate reflection of the true exposure environment over time. Another limitation was that we had missing data on covariates and outcomes that led to different sample sizes for each regression, most notably F$_2$-isoprostane. Finally, we did not have data on dietary information, which could act as a confounder between OP pesticide exposure and renal function. Because OP pesticide exposure is related to consumption of conventional produce (Curl Cynthia et al., 2003), those with greater fruit and vegetable consumption have greater OP pesticide exposure levels (Berman et al., 2020; Cequier et al., 2017). Overall, those who eat diets rich in fruits and vegetables may have healthier lifestyles and better renal function (Banerjee et al., 2019), which could confound associations with OP exposure. Therefore, without controlling for dietary factors, this could bias results in the direction of improved renal function. Another possible way this could affect results is through potential dietary restrictions. For example, potassium restriction to prevent hyperkalemia and the associated reduced protein intake could potentially explain the positive effect of OP exposure and maintenance of eGFR. However, the majority of patients in the CKiD cohort had non-glomerular disease (i.e., CAKUT). These children, in contrast to those with glomerular disorders, generally have sustained urine output and normal serum potassium levels and do not require potassium restriction. Moreover, there was no difference in effect of OP exposure in the non-glomerular versus glomerular subgroups, arguing against an effect of potassium restriction. Finally, we also note that there were other correlates of exposure that were not measured in this study that may have acted as confounding variables, such as climate, urbanicity, drinking water supply, and other environmental conditions.

From a public health perspective, these findings are significant because in contrast to many CKD risks which are not amenable to modification, OP body burdens can be ameliorated through consumption of organic foods. Studies have shown that interventions that introduce organic in the place of conventional produce effectively and rapidly reduce pesticide and herbicide exposure, evidenced by lower and even non-detectable levels of urinary pesticides (Bradman et al., 2015; Lu et al., 2006). Additional studies specifically among children

the use of OP pesticides in school settings. We observed similar trends in our earlier study of bisphenol A and phthalate exposure in this cohort (Malits et al., 2018). Further study is needed to explain these differences in chemical exposure levels. Despite these differences in exposure, in sensitivity analyses, we observed that associations between DAPs and renal outcomes did not vary by baseline renal function. This suggests that our observed associations were not artifacts of any potentially impaired excretion among those with the most reduced renal function. In addition, urinary DAPs reflect recent exposure and showed low reliability over time owing to their non-persistence, which posed a unique challenge in statistical analyses. However, unlike previous studies that have used a single spot urine sample to quantify exposure (Kataria et al., 2015; Trasande et al., 2020), our study incorporated this variability into all estimates. Although this likely decreased precision and led to larger

with CKD are needed to confirm these observations in this vulnerable subgroup given potential differences in excretion.

This study has potential implications for CKDu research. Despite the lack of consistent associations with clinical endpoints, we documented renal injury on the subclinical level that indicates tubular damage and thus the potential for clinically apparent changes with extended follow-up. Specifically, elevated KIM-1 and 8-OHdG have been linked with faster CKD decline to ESKD, incident albuminuria, cardiovascular disease, and premature mortality (Alderson et al., 2016; Bhavsar et al., 2012; Park et al., 2017; Peralta et al., 2012; Schei et al., 2018; Walther et al., 2019). This supports the hypothesis that subclinical injury over time may eventually manifest as CKDu in the absence of traditional risk factors for CKD like hypertension or obesity. While we note that this study was not comprised of patients with CKDu, and that CKD diagnosed in children differs from CKDu in that the causes are well understood (including congenital anomalies of the kidney and urinary tract (CAKUT), hereditary nephropathies, and chronic glomerulonephritis (Becherucci et al., 2016; Harambat et al., 2012)); in documenting an association between OP pesticide exposure and subclinical evidence of kidney injury, it still may be of practical relevance to that line of research (Valcke et al., 2017). Furthermore, this is the first study on pesticides and CKD conducted among children, which lays the groundwork for further studies of whether the origins of CKDu may emerge in early life.

5. Conclusions

In conclusion, in a cohort of children with CKD followed over time, urinary DAP metabolites were associated with increased KIM-1 and 8-OHdG concentrations over the course of follow-up, showing a trend of subclinical tubular injury and promotion of oxidant stress in the absence of consistent evidence for clinically apparent renal outcomes. The increased excretion of tubular injury biomarkers indicate the need for ongoing surveillance for an adverse effect of OP pesticides on CKD progression in pediatric patients. These findings provide preliminary evidence for urinary DAP metabolites, serving as non-specific biomarkers of OP pesticides, ubiquitous environmental chemicals, to impact subclinical kidney damage among children with CKD.

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CRediT authorship contribution statement

Melanie H. Jacobson: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Yinxiang Wu:** Conceptualization, Formal analysis, Investigation, Methodology, Writing - review & editing. **Mengling Liu:** Conceptualization, Methodology, Project administration, Visualization, Writing - review & editing. **Kurunthachalam Kannan:** Data curation, Formal analysis, Methodology, Resources, Software, Supervision, Validation. **Adela Jing Li:** Data curation, Formal analysis. **Morgan Robinson:** Data curation, Formal analysis. **Bradley A. Warady:** Data curation, Project administration. **Susan Furth:** Data curation, Project administration. **Howard Trachtman:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - review & editing. **Leonardo Trasande:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106597.

References

Alderson, H.V., Ritchie, J.P., Pagano, S., Middleton, R.J., Pruijm, M., Vuilleumier, N., Kalra, P.A., 2016. The Associations of Blood Kidney Injury Molecule-1 and Neutrophil Gelatinase-Associated Lipocalin with Progression from CKD to ESRD. Clin. J. Am. Soc. Nephrol. 11, 2141–2149.

Atkinson, M.A., Ng, D.K., Warady, B.A., Furth, S.L., Flynn, J.T., 2020. The CKiD study: overview and summary of findings related to kidney disease progression. Pediatric Nephrol.

Banerjee, T., Crews, D.C., Tuot, D.S., Pavkov, M.E., Burrows, N.R., Stack, A.G., Saran, R., Bragg-Gresham, J., Powe, N.R., Powe, N., Banerjee, T., Tuot, D., Hsu, C.-Y., McCulloch, C., Crews, D., Hsu, R., Grubbs, V., Bibbins-Domingo, K., Shlipak, M., Peralta, C., Rubinsky, A., Coresh, J., Saran, R., Shahinian, V., Gillespie, B., Morgenstern, H., Heung, M., Herman, W., McClellan, W., Bragg-Gresham, J., Steffick, D., Tilea, A., Yin, M., Robinson, I., Zivin, K., Kurtz, V., Wyncott, A., Burrows, N.R., Eberhardt, M., Geiss, L., Mondesire, J., Moore, B., Patel, P., Pavkov, M., Rolka, D., Saydah, S., Shrestha, S., Waller, L., 2019. Poor accordance to a DASH dietary pattern is associated with higher risk of ESRD among adults with moderate chronic kidney disease and hypertension. Kidney Int. 95, 1433–1442.

Bank, J.R., van der Pol, P., Vreeken, D., Monge-Chaubo, C., Bajema, I.M., Schlagwein, N., van Gijlswijk, D.J., van der Kooij, S.W., Reinders, M.E.J., de Fijter, J.W., van Kooten, C., 2017. Kidney injury molecule-1 staining in renal allograft biopsies 10 days after transplantation is inversely correlated with functioning proximal tubular epithelial cells. Nephrol., Dialysis, Transplant. : Off. Publ. Eur. Dialysis Transplant Association - European Renal Association 32, 2132–2141.

Barr Dana, B., Bravo, R., Weerasekera, G., Caltabiano Lisa, M., Whitehead Ralph, D., Olsson Anders, O., Caudill Samuel, P., Schober Susan, E., Pirkle James, L., Sampson Eric, J., Jackson Richard, J., Needham Larry, L., 2004. Concentrations of dialkyl phosphate metabolites of organophosphorus pesticides in the U.S. population. Environ. Health Perspect. 112, 186–200.

Barr, D.B., Wilder, L.C., Caudill, S.P., Gonzalez, A.J., Needham, L.L., Pirkle, J.L., 2004. Urinary creatinine concentrations in the US population: implications for urinary biologic monitoring measurements. Environ. Health Perspect. 113, 192–200.

Becherucci, F., Roperto, R.M., Materassi, M., Romagnani, P., 2016. Chronic kidney disease in children. Clin. Kidney J. 9, 583–591.

Berman, T., Barnett-Itzhaki, Z., Göen, T., Hamama, Z., Axelrod, R., Keinan-Boker, L., Shimony, T., Goldsmith, R., 2020. Organophosphate pesticide exposure in children in Israel: Dietary associations and implications for risk assessment. Environ. Res. 182, 108739.

Bhavsar, N.A., Köttgen, A., Coresh, J., Astor, B.C., 2012. Neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule 1 (KIM-1) as predictors of incident CKD stage 3: the Atherosclerosis Risk in Communities (ARIC) Study. Am. J. Kidney Dis. 60, 233–240.

Bieniaś, B., Zajączkowska, M., Borzęcka, H., Sikora, P., Wieczorkiewicz-Płaza, A., Wilczyńska, D., 2015. Early markers of tubulointerstitial fibrosis in children with idiopathic nephrotic syndrome: preliminary report. Medicine (Baltimore) 94, e1746.

Bradman, A., Quirós-Alcalá, L., Castorina, R., Schall Raul, A., Camacho, J., Holland Nina, T., Barr Dana, B., Eskenazi, B., 2015. Effect of organic diet intervention on pesticide exposures in young children living in low-income urban and agricultural communities. Environ. Health Perspect. 123, 1086–1093.

Casas, M., Basagana, X., Sakhi, A.K., Haug, L.S., Philippat, C., Granum, B., Manzano-Salgado, C.B., Brochot, C., Zeman, F., de Bont, J., Andrusaityte, S., Chatzi, L.; Donaire-Gonzalez, D., Giorgis-Allemand, L., Gonzalez, J.R., Gracia-Lavedan, E., Grazuleviciene, R., Kampouri, M., Lyon-Caen, S., Panella, P., Petraviciene, I., Robinson, O., Urquiza, J., Vafeiadi, M., Vernet, C., Waiblinger, D., Wright, J., Thomsen, C., Slama, R., Vrijheid, M., 2018. Variability of urinary concentrations of non-persistent chemicals in pregnant women and school-aged children. Environ. Int. 121, 561-573.

Centers for Disease Control and Prevention. Fourth national report on human exposure to environmental chemicals. Atlanta, GA; 2009.

Centers for Disease Control and Prevention. https://wwwn.cdc.gov/Nchs/Nhanes/2011-2012/OPD_G.htm. 2012.

Cequier, E., Sakhi, A.K., Haug, L.S., Thomsen, C., 2017. Exposure to organophosphorus pesticides in Norwegian mothers and their children: Diurnal variability in concentrations of their biomarkers and associations with food consumption. Sci. Total Environ. 590–591, 655–662.

Crowe, J., Brooks, D., R, C.-R., M, G.-Q., Jakobsson, K., Kimmel, P., Mendley, S., Trottier, B., Joubert, Bonnie R., 2019. Third International Workshop on Chronic Kidney Diseases of Uncertain/Non-traditional Etiology in Mesoamerica and Other Regions. San Jose, Costa Rica.

Curl Cynthia, L., Fenske Richard, A., Elgethun, K., 2003. Organophosphorus pesticide exposure of urban and suburban preschool children with organic and conventional diets. Environ. Health Perspect. 111, 377–382.

Flynn, J.T., Mitsnefes, M., Pierce, C., Cole, S.R., Parekh, R.S., Furth, S.L., Warady, B.A., 2008. Chronic Kidney Disease in Children Study, G. Blood pressure in children with chronic kidney disease: a report from the Chronic Kidney Disease in Children study. Hypertension (Dallas, Tex : 1979), 52, 631–637.

Fuhrman, D.Y., Schneider, M.F., Dell, K.M., Blydt-Hansen, T.D., Mak, R., Saland, J.M., Furth, S.L., Warady, B.A., Moxey-Mims, M.M., Schwartz, G.J., 2017. Albuminuria, proteinuria, and renal disease progression in children with CKD. Clinical J. Am. Soc. Nephrol. 12, 912–920.

Furth, S.L., Cole, S.R., Moxey-Mims, M., Kaskel, F., Mak, R., Schwartz, G., Wong, C., Munoz, A., Warady, B.A., 2006. Design and methods of the Chronic Kidney Disease in Children (CKiD) prospective cohort study. Clin. J. Am. Soc. Nephrol. 1, 1006–1015.

Ghosh, R., Siddarth, M., Singh, N., Tyagi, V., Kare, P.K., Banerjee, B.D., Kalra, O.P., Tripathi, A.K., 2017. Organochlorine pesticide level in patients with chronic kidney disease of unknown etiology and its association with renal function. Environ. Health Prev. Med. 22, 49.

González-Quiroz, M., Pearce, N., Caplin, B., Nitsch, D., 2018. What do epidemiological studies tell us about chronic kidney disease of undetermined cause in Meso-America? A systematic review and meta-analysis. Clinical Kidney J. 11, 496–506.

Harambat, J., van Stralen, K.J., Kim, J.J., Tizard, E.J., 2012. Epidemiology of chronic kidney disease in children. Pediatric Nephrol. 27, 363–373.

Herrera, R., Orantes, C.M., Almaguer, M., Alfonso, P., Bayarre, H.D., Leiva, I.M., Smith, M.J., Cubias, R.A., Torres, C.G., Almendárez, W.O., Cubias, F.R., Morales, F. E., Magaña, S., Amaya, J.C., Perdomo, E., Ventura, M.C., Villatoro, J.F., Vela, X.F., Zelaya, S.M., Granados, D.V., Vela, E., Orellana, P., Hevia, R., Fuentes, E.J., Mañalich, R., Bacallao, R., Ugarte, M., Arias, M.I., Chávez, J., Flores, N.E., Aparicio, C.E., 2014. Clinical characteristics of chronic kidney disease of nontraditional causes in Salvadoran farming communities. MEDICC Rev. 16, 39–48.

Hornung, R.W., Reed, L.D., 1990. Estimation of average concentration in the presence of nondetectable values. Appl. Occup. Environ. Hyg. 5, 46–51.

Jacobson, M.H., Liu, M., Wu, Y., Furth, S., Warady, B., Trachtman, H., Trasande, L., 2020. Oxidant stress and renal function among children with chronic kidney disease: a repeated measures study. Sci. Rep. 10, 3129.

Jafari, M., Salehi, M., Ahmadi, S., Asgari, A., Abasnezhad, M., Hajigholamali, M., 2012. The role of oxidative stress in diazinon-induced tissues toxicity in Wistar and Norway rats. Toxicol. Mech. Methods 22, 638–647.

Jain, R.B., 2016. Levels of dialkylphosphate metabolites in urine among general U.S. population. Environ. Toxicol. Pharmacol. 43, 74–82.

Jayatilake, N., Mendis, S., Maheepala, P., Mehta, F.R., 2013. Chronic kidney disease of uncertain aetiology: prevalence and causative factors in a developing country. BMC Nephrol. 14, 180.

Kataria, A., Levine, D., Wertenteil, S., Vento, S., Xue, J., Rajendiran, K., Kannan, K., Thurman, J.M., Morrison, D., Brody, R., 2017. Exposure to bisphenols and phthalates and association with oxidant stress, insulin resistance, and endothelial dysfunction in children. Pediatr. Res. 81, 857.

Kataria, A., Trasande, L., Trachtman, H., 2015. The effects of environmental chemicals on renal function. Nat. Rev. Nephrol. 11, 610.

KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease, 2013. Chapter 1: Definition and classification of CKD. Kidney international supplements. 3, 19–62.

Kleinbaum, D., Klein, M.J.N.Y., 2011. Springer-Verlag. Survival Analysis: A self-learning text, 2005.

Lebov, J.F., Engel, L.S., Richardson, D., Hogan, S.L., Hoppin, J.A., Sandler, D.P., 2016. Pesticide use and risk of end-stage renal disease among licensed pesticide applicators in the Agricultural Health Study. Occup. Environ. Med. 73, 3–12.

Lebov, J.F., Engel, L.S., Richardson, D., Hogan, S.L., Sandler, D.P., Hoppin, J.A., 2015. Pesticide exposure and end-stage renal disease risk among wives of pesticide applicators in the Agricultural Health Study. Environ. Res. 143, 198–210.

Lu, C., Barr Dana, B., Pearson Melanie, A., Waller Lance, A., 2008. Dietary intake and its contribution to longitudinal organophosphorus pesticide exposure in urban/suburban children. Environ. Health Perspect. 116, 537–542.

Lu, C., Toepel, K., Irish, R., Fenske Richard, A., Barr Dana, B., Bravo, R., 2006. Organic diets significantly lower children's dietary exposure to organophosphorus pesticides. Environ. Health Perspect. 114, 260–263.

Ma, P., Wu, Y., Zeng, Q., Gan, Y., Chen, J., Ye, X., Yang, X., 2013. Oxidative damage induced by chlorpyrifos in the hepatic and renal tissue of Kunming mice and the antioxidant role of vitamin E. Food Chem. Toxicol. 58, 177–183.

Malits, J., Attina, T.M., Karthikraj, R., Kannan, K., Naidu, M., Furth, S., Warady, B.A., Vento, S., Trachtman, H., Trasande, L., 2018. Renal Function and exposure to Bisphenol A and phthalates in children with Chronic Kidney Disease. Environ. Res. 167, 575–582.

National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 114, 555–576.

O'Brien, K.M., Upson, K., Cook, N.R., Weinberg, C.R., 2015. Environmental chemicals in urine and blood: improving methods for creatinine and lipid adjustment. Environ. Health Perspect. 124, 220–227.

Park, M., Hsu, C.-Y., Go, A.S., Feldman, H.I., Xie, D., Zhang, X., Mifflin, T., Waikar, S.S., Sabbisetti, V.S., Bonventre, J.V., Coresh, J., Nelson, R.G., Kimmel, P.L., Kusek, J.W.,

Rahman, M., Schelling, J.R., Vasan, R.S., Liu, K.D., 2017. Urine Kidney Injury Biomarkers and Risks of Cardiovascular Disease Events and All-Cause Death: The CRIC Study. Clin. J. Am. Soc. Nephrol. 12, 761.

Pearce, N., Caplin, B., 2019. Let's take the heat out of the CKDu debate: more evidence is needed. Occup. Environ. Med. 76, 357–359.

Peralta, C.A., Katz, R., Bonventre, J.V., Sabbisetti, V., Siscovick, D., Sarnak, M., Shlipak, M.G., 2012. Associations of urinary levels of kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) with kidney function decline in the Multi-Ethnic Study of Atherosclerosis (MESA). Am. J. Kidney Dis. 60, 904–911.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Team, R.C.J.R.F.S.C.h.C.R.-p.o.p.n, 2018. nlme: linear and nonlinear mixed effects models. R package version 3.1–137.

Poovala, V.S., Huang, H., Salahudeen, A.K., 1999. Role of reactive oxygen metabolites in organophosphate-bidrin-induced renal tubular cytotoxicity. J. Am. Soc. Nephrol. : JASN 10, 1746–1752.

Poovala, V.S., Kanji, V.K., Tachikawa, H., Salahudeen, A.K., 1998. Role of oxidant stress and antioxidant protection in acephate-induced renal tubular cytotoxicity. Toxicol. Sci. : Off. J. Soc. Toxicol. 46, 403–409.

Quirós-Alcalá, L., Bradman, A., Smith, K., Weerasekera, G., Odetokun, M., Barr, D.B., Nishioka, M., Castorina, R., Hubbard, A.E., Nicas, M., Hammond, S.K., McKone, T.E., Eskenazi, B., 2012. Organophosphorous pesticide breakdown products in house dust and children's urine. J. Eposure Sci. Environ. Epidemiol. 22, 559–568.

Rodenbach, K.E., Schneider, M.F., Furth, S.L., Moxey-Mims, M.M., Mitsnefes, M.M., Weaver, D.J., Warady, B.A., Schwartz, G.J., 2015. Hyperuricemia and Progression of CKD in Children and Adolescents: The Chronic Kidney Disease in Children (CKiD) Cohort Study. Am. J. Kidney Dis. : Off. J. National Kidney Found. 66, 984–992.

Schei, J., Fuskevåg, O.-M., Stefansson, V.T.N., Solbu, M.D., Jenssen, T.G., Eriksen, B.O., Melsom, T., 2018. Urinary Markers of Oxidative Stress Are Associated With Albuminuria But Not GFR Decline. Kidney Int. Rep. 3, 573–582.

Schwartz, G.J., Munoz, A., Schneider, M.F., Mak, R.H., Kaskel, F., Warady, B.A., Furth, S. L., 2009. New equations to estimate GFR in children with CKD. J. Am. Soc. Nephrol. : JASN 20, 629–637.

Shah, M.D., Iqbal, M., 2010. Diazinon-induced oxidative stress and renal dysfunction in rats. Food Chem. Toxicol. : Int. J. Published British Ind. Biol. Res. Assoc. 48, 3345–3353.

Siddarth, M., Datta, S.K., Mustafa, M.D., Ahmed, R.S., Banerjee, B.D., Kalra, O.P., Tripathi, A.K., 2014. Increased level of organochlorine pesticides in chronic kidney disease patients of unknown etiology: Role of GSTM1/GSTT1 polymorphism. Chemosphere 96, 174–179.

Siddharth, M., Datta, S.K., Bansal, S., Mustafa, M., Banerjee, B.D., Kalra, O.P., Tripathi, A.K., 2012. Study on organochlorine pesticide levels in chronic kidney disease patients: Association with estimated glomerular filtration rate and oxidative stress. J. Biochem. Mol. Toxicol. 26, 241–247.

Staples, A.O., Greenbaum, L.A., Smith, J.M., Gipson, D.S., Filler, G., Warady, B.A., Martz, K., Wong, C.S., 2010. Association between clinical risk factors and progression of chronic kidney disease in children. Clin. J. Am. Soc. Nephrol. 5, 2172–2179.

Sullivan, J.B., Krieger, G.R., 2001. Clinical Environmental Health and Toxic Exposures. Lippincott Williams & Wilkins.

Team, R.C., 2013. R: A language and environment for statistical computing.

Trasande, L., 2017. When enough data are not enough to enact policy: The failure to ban chlorpyrifos. PLoS Biol. 15 e2003671–e2003671.

Trasande, L., Aldana, S.I., Trachtman, H., Kannan, K., Morrison, D., Christakis, D.A., Whitlock, K., Messito, M.J., Gross, R.S., Karthikraj, R., Sathyanarayana, S., 2020. Glyphosate exposures and kidney injury biomarkers in infants and young children. Environ. Pollut. 256, 113334.

US Renal Data System 2018 Annual Data Report, 2019a. Chapter 6: CKD among Children and Adolescents. Am. J. Kidney Dis. 73, S125–S132.

US Renal Data System 2018 Annual Data Report, 2019b. Chapter 7: ESRD among Children, Adolescents, and Young Adults. American Journal of Kidney Diseases. 73, S463–S500.

US Renal Data System 2018 Annual Data Report, 2019c. Chapter 7: Healthcare Expenditures for Persons with CKD. Am. J. Kidney Dis. 73, S133–S158.

US Renal Data System 2018 Annual Data Report, 2019d. Chapter 9: Healthcare Expenditures for Persons with ESRD. Am. J. Kidney Dis. 73, S519–S528.

Valcke, M., Levasseur, M.E., Soares da Silva, A., Wesseling, C., 2017. Pesticide exposures and chronic kidney disease of unknown etiology: an epidemiologic review. Environ. Health 16, 49.

Walther, C.P., Shah, M., Navaneethan, S.D., 2019. Estimated GFR Decline and Tubular Injury Biomarkers With Intensive Blood Pressure Control. Am. J. Kidney Dis. : Off. J. National Kidney Found. 73, 4–7.

Warady, B.A., Abraham, A.G., Schwartz, G.J., Wong, C.S., Munoz, A., Betoko, A., Mitsnefes, M., Kaskel, F., Greenbaum, L.A., Mak, R.H., Flynn, J., Moxey-Mims, M.M., Furth, S., 2015. Predictors of Rapid Progression of Glomerular and Nonglomerular Kidney Disease in Children and Adolescents: The Chronic Kidney Disease in Children (CKiD) Cohort. Am. J. Kidney Dis. : Off. J. National Kidney Found. 65, 878–888.

Weaver, V.M., Kotchmar, D.J., Fadrowski, J.J., Silbergeld, E.K., 2016. Challenges for environmental epidemiology research: are biomarker concentrations altered by kidney function or urine concentration adjustment? J. Eposure Sci. Environ. Epidemiol. 26, 1.

Wesseling, C., Glaser, J., Rodríguez-Guzmán, J., Weiss, I., Lucas, R., Peraza, S., da Silva, A.S., Hansson, E., Johnson, R.J., Hogstedt, C., Wegman, D.H., Jakobsson, K., 2020. Chronic kidney disease of non-traditional origin in Mesoamerica: a disease

primarily driven by occupational heat stress. Revista panamericana de salud publica = Pan Am. J. Public Health 44 e15–e15.

Wong, C.J., Moxey-Mims, M., Jerry-Fluker, J., Warady, B.A., Furth, S.L., 2012. CKiD (CKD in children) prospective cohort study: a review of current findings. Am. J. Kidney Dis. : Off. J. National Kidney Found. 60, 1002–1011.

Wong, C.S., Pierce, C.B., Cole, S.R., Warady, B.A., Mak, R.H., Benador, N.M., Kaskel, F., Furth, S.L., Schwartz, G.J., 2009. Association of proteinuria with race, cause of chronic kidney disease, and glomerular filtration rate in the chronic kidney disease in children study. Clin. J. Am. Soc. Nephrol. 4, 812–819.