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# Association of dioxins, furans and dioxin-like PCBs in human blood with nephropathy among US teens and young adults

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**Abstract:** We assessed the association of three chlorinated dibenzo-*p*-dioxins, a chlorinated dibenzofuran, and four dioxin-like polychlorinated biphenyls (PCBs) in human blood with nephropathy (microalbuminuria or macroalbuminuria) among teens and young adults (12–30 years old) having normal glycohemoglobin (A1c <5.7%). The data were derived from the 1999–2004 National Health and Nutrition Examination Survey (unweighted n=1504, population estimate=38,806,338). In this paper, nephropathy refers to normal A1c with nephropathy. In an all-adult sample (Everett CJ, Thompson OM. Dioxins, furans and dioxin-like PCBs in human blood: causes or consequences of diabetic nephropathy? Environ Res 2014;132:126–31), the cut-offs for these chemicals being considered elevated, were defined as the 75th percentile. Using these same cut-offs again, the proportion of those with one or more of the eight dioxin-like compounds elevated was 9.9%. The four chemicals associated with nephropathy were 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin, PCB 126, PCB 169, and PCB 156. The proportion with one or more of these four dioxin-like chemicals elevated was 3.9% (unweighted n=46) and the odds ratio (OR) for nephropathy was 7.1 [95% confidence interval (CI) 1.8–28.1]. The association was strong among females (OR 17.4, 95% CI 3.4–88.6), but among males there were no cases of nephropathy when one or more of the four dioxin-like chemicals were elevated, and therefore no association. In a separate analysis, elevated toxic equivalency, defined using the eight dioxin-like chemicals ($TEQ_8$), was associated with nephropathy. $TEQ_8$ ≥50.12 fg/g included 2.6% of the sample (unweighted n=28) and had an OR of 5.8 (95% 1.3–25.9) for nephropathy. As found in the analysis of one or more of four dioxin-like chemicals elevated, $TEQ_8$ ≥50.12 fg/g was associated with nephropathy among females (OR 11.9, 95% CI 1.6–87.2), but not males. Trends for least-squares means also differed by gender, but there were no significant differences in mean $TEQ_8$ between normal subjects and those having nephropathy in either males or females. We also evaluated pre-diabetes (A1c 5.7–6.4%) without nephropathy and found no associations when one or more of four dioxin-like compounds were elevated, or when $TEQ_8$ was ≥50.12 fg/g. In this study, associations of dioxin-like chemicals with nephropathy were found among females at an early age. Prospective studies are needed to determine if dioxin-like compounds cause nephropathy, or if these relationships are cases of reverse causation.

**Keywords:** dioxins; dioxin-like polychlorinated biphenyls; furans; kidney disease; pre-diabetes.

## Introduction

Kidney disease, or nephropathy, is a major complication of diabetes, which may take years to develop after a person is diagnosed with diabetes (1). Associations of dioxins, furans and dioxin-like polychlorinated biphenyls (PCBs) with diabetic nephropathy have been reported (2). The associations found were strong enough that the effect may be a case of reverse causation. Dioxins, furans, and dioxin-like PCBs, induce responses in animal models that are characterized by severe weight loss, thymic atrophy, hepatotoxicity, edema, fetotoxicity, teratogenicity, reproductive toxicity, immunotoxicity, and enzyme induction. These responses are subsequent to aryl hydrocarbon (Ah) receptor binding (3), which has been hypothesized to antagonize the peroxisome proliferator-activated receptor and thus contribute to the pathophysiology of diabetes (4).

While diabetic nephropathy is usually presumed to follow diagnosis of incident diabetes, it is also possible that a person can have nephropathy (urinary albumin to creatinine ratio >30 mg/g) before they are diagnosed with incident diabetes (5). Specifically, 12.1% of those in

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an all-adult sample, that have pre-diabetes, also have nephropathy, and could progress to incident diabetes with nephropathy very easily. Both pre-diabetes without nephropathy, and pre-diabetes with nephropathy are associated with dioxin-like chemicals. When one or more of 23 dioxin-like chemicals are elevated, pre-diabetes without nephropathy has an odds ratio (OR) of 1.54 [95% confidence interval (CI) 1.04–2.26], and pre-diabetes with nephropathy has an OR of 4.70 (95% CI 1.17–18.92) compared to those with normal glycohemoglobin (A1c) without nephropathy (5). This is important because total diabetes (diagnosed and undiagnosed diabetes) with nephropathy, is highly associated with dioxins, furans, and dioxin-like polychlorinated biphenyls (PCBs), so much so that it appears to be a case of reverse causality. When four or more of 23 dioxin-like chemicals were elevated, the ORs were 7.00 (95% CI 1.80–27.20) for diabetic nephropathy, and 2.13 (95% CI 0.95–4.78) for diabetes without nephropathy (2).

The question that remains is how would a patient progress to pre-diabetes with nephropathy over the course of their life? Do dioxin-like chemicals promote pre-diabetes without nephropathy first, and from there on to pre-diabetes with nephropathy, or is the path to normal A1c with nephropathy first, and then to pre-diabetes with nephropathy? The third possibility, going directly from normal A1c without nephropathy to pre-diabetes with nephropathy seems very unlikely. Considering the difference in mean age of the diabetes categories in the all-adult sample (Supplemental Table 1), analyzed in Everett and Thompson (2), one finds the difference between normal A1c without nephropathy and pre-diabetes without nephropathy is 14.4 years, and the difference between normal A1c without nephropathy and normal A1c with nephropathy is 6.5 years. This suggests development of nephropathy is a faster process than development of pre-diabetes. However, normal A1c with nephropathy is not associated with one or more of 23 dioxin-like chemicals elevated in the all-adult sample. In a fully-adjusted model (not previously reported) the OR was 1.20 (95% CI 0.65–2.23).

To shed more light on this subject, we have undertaken an analysis of a teen and young adult sample (12–30 years old) using the National Health and Nutrition Examination Survey (NHANES), 1999–2004. In this study, "normal" refers to normal glycohemoglobin (A1c <5.7%) without nephropathy, "nephropathy" refers to normal A1c with nephropathy, and "pre-diabetes" refers to A1c 5.7–6.4% without nephropathy. The objective of this study was to determine associations of eight dioxin-like chemicals with nephropathy and pre-diabetes in this younger sample.

## Materials and methods

Data from the National Health and Nutrition Examination Survey (NHANES) 1999–2004 were used to investigate associations of dioxins and dioxin-like chemicals with nephropathy and pre-diabetes, among a representative sample of non-institutionalized US teens and young adults (12–30 years old). Detailed information on the methodology of the NHANES 1999–2004, including laboratory assessment, can be found at the National Center for Health Statistics website (6). Nephropathy was defined as urinary albumin to creatinine ratio >30 mg/g, representing both microalbuminuria and macroalbuminuria (7). Total diabetes was defined as either diagnosed or undiagnosed diabetes. Diagnosed diabetes was determined by self-report answer to the NHANES question: "Other than during pregnancy, have you ever been told by a doctor or health professional that you have diabetes or sugar diabetes?" Those who answered "borderline" were considered to not have diabetes. Undiagnosed diabetes was defined as persons who had A1c ≥6.5% who had not been diagnosed as having diabetes (8). We used this definition of total diabetes because it is possible for someone with diagnosed diabetes to have an A1c <6.5%. This occurs when the disease is well controlled. Once a person is diagnosed with diabetes they will have the disease for the rest of their life. The distribution of our teen and young adult sample by diabetes, nephropathy, and age categories is shown in Table 1.

**Table 1:** Proportions in the teen and young adult sample, by disease category and age.

| Disease category | Unweighted n | Population estimate | Proportion, % |
|---|---|---|---|
| Normal A1c (<5.7%) without nephropathy | | | |
| Age 12–19 | 798 | 10,689,230 | 27.5 |
| Age 20–30 | 534 | 24,029,918 | 61.9 |
| Total | 1332 | 34,719,148 | 89.5 |
| Normal A1c (<5.7%) with nephropathy[a] | | | |
| Age 12–19 | 90 | 1,475,371 | 3.8 |
| Age 20–30 | 32 | 1,557,166 | 4.0 |
| Total | 122 | 3,032,538 | 7.8 |
| Pre-diabetes (A1c 5.7–6.4%) without nephropathy | | | |
| Age 12–19 | 23 | 200,297 | 0.5 |
| Age 20–30 | 14 | 479,980 | 1.2 |
| Total | 37 | 680,278 | 1.8 |
| Pre-diabetes (A1c 5.7–6.4%) with nephropathy[a] | | | |
| Age 12–19 | 2 | 12,066 | 0.03 |
| Age 20–30 | 1 | 5048 | 0.01 |
| Total | 3 | 17,114 | 0.04 |
| Total diabetes (diagnosed or A1c ≥6.5%) without nephropathy[b] | | | |
| Age 12–19 | 4 | 54,356 | 0.1 |
| Age 20–30 | 6 | 302,904 | 0.8 |
| Total | 10 | 357,260 | 0.9 |
| Grand totals | | | |
| Age 12–19 | 917 | 12,431,321 | 32.0 |
| Age 20–30 | 587 | 26,375,016 | 68.0 |
| Total | 1504 | 38,806,338 | 100 |

[a]Albumin to creatinine ratio >30 mg/g. [b]None of those with total diabetes had nephropathy. Of the 10 persons with total diabetes, four were diagnosed and on insulin (presumably Type 1 diabetics), one diagnosed and not on insulin, and five undiagnosed (A1c ≥6.5%).

Three chlorinated dibenzo-*p*-dioxins, one chlorinated dibenzofuran, and four dioxin-like polychlorinated biphenyls (PCBs) were measured in nonfasting blood samples of a one-third random, subsample of participants 12 years old and older. We evaluated persons in this subsample who were 12–30 years old. The unweighted number of participants in our study was 1504, which represented a total of 38,806,338 non-incarcerated US teens and young adults. Due to the weighting of the NHANES data, persons 12–19 years old represented 32.0% of the sample, and subjects 20–30 years old represented 68.0% of the sample (Table 1). The eight dioxins and dioxin-like chemicals were measured in serum by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry and the concentrations were expressed per gram of blood, rather than adjusting for lipids, as recommended by Schisterman et al. (9). Each person had a sample-specific limit of detection.

In the all-adult sample (2), eight of 23 dioxins and dioxin-like chemicals had >25% of their values above the maximum limit of detection and were analyzed separately. In the current study we used these same eight compounds. Whereas in the all adult sample the cut-offs for being considered elevated were defined as the 75th percentile, in the teen and young adult sample we used these previously identified cut-offs (Table 2), except that used for 2,3,4,7,8-pentachlorodibenzofuran which was raised because it had a higher maximum limit of detection than before. When possible we categorized the eight dioxins and dioxin-like chemicals as: 1) less than or equal the maximum limit of detection, 2) greater than the cut-off for the elevated concentration as previously defined, and 3) an intermediate category, greater than the maximum limit of detection and less than the cut-off for the elevated concentration. Logistic regressions were used to calculate ORs for the categories studied.

We also analyzed total toxic equivalency ($TEQ_8$) for the eight dioxin-like chemicals in our study. Toxic equivalency is calculated by multiplying concentrations and toxic equivalency factors established by the World Health Organization (10) and summing the products for all the chemicals being studied. $TEQ_8$ was correlated with total toxic equivalency using 23 chlorinated dioxin-like chemicals ($TEQ_{23}$) in the all-adult sample (2) with an $r^2$=0.92. We evaluated least squares means of $TEQ_8$ by group, and ORs for elevated $TEQ_8$ categories defined by the 75th, 90th, 95th, and 97.5th percentiles.

We tested the associations of the eight individual chemicals in regression models adjusted for participant age, gender, race/ethnicity, body mass index (BMI) Z-score, poverty-to-income ratio, energy adjusted fruit and vegetable consumption, and physical activity. Race/ethnicity was classified as non-Hispanic white, non-Hispanic black, Mexican American, and other Hispanic. BMI Z-score was defined as nine categories ranging from –2 to +2, which varied by sex and age (11), and analyzed as a continuous variable in the regression models. Poverty-to-income ratio was analyzed as a continuous variable, and was the ratio of a family's income to their appropriate poverty threshold based on family size (12). Poverty-to-income ratio was top coded at 5, and values below 1.00 were below the official poverty threshold (13). Number of fruit and vegetable servings consumed per day was determined from questions asked during the NHANES dietary interview and adjusted for energy intake (kcal/day). Physical activity was defined as moderate or vigorous activity over the past 30 days, versus sedentary, from two NHANES questions (6). Of the covariates used in Everett and Thompson (2), education level of teens was not indicative of socio-economic status, and family history of diabetes was not recorded for teens. Therefore, these two variables were not used in the current study.

**Table 2:** Proportions in teen and young adult sample by levels of dioxin-like chemicals.

| | Unweighted n | Population estimate | Proportion, % |
|---|---|---|---|
| 1,2,3,6,7,8-HxCDD, fg/g[a] | | | |
| <121.41 | 1350 | 32,914,096 | 84.8 |
| 121.41–299.45 | 147 | 5,530,256 | 14.2 |
| ≥299.46 | 7 | 361,985 | 0.9 |
| 1,2,3,4,6,7,8-HpCDD, fg/g[b] | | | |
| <161.08 | 1144 | 27,773,131 | 71.6 |
| 161.08–367.07 | 311 | 9,509,643 | 24.5 |
| ≥367.08 | 49 | 1,523,564 | 3.9 |
| 1,2,3,4,6,7,8,9-OCDD, fg/g[c] | | | |
| <1945.82 | 1381 | 34,851,655 | 89.8 |
| 1945.82–2908.62 | 73 | 2,110,308 | 5.4 |
| ≥2908.63 | 50 | 1,844,375 | 4.8 |
| 2,3,4,7,8-PeCDF, fg/g[d] | | | |
| <54.02 | 1478 | 38,041,349 | 98.0 |
| ≥54.02 | 26 | 764,989 | 2.0 |
| PCB 126, fg/g | | | |
| <86.48 | 1178 | 28,476,721 | 73.4 |
| 86.48–214.53 | 294 | 9,074,062 | 23.4 |
| ≥214.54 | 32 | 1,255,554 | 3.2 |
| PCB 169, fg/g | | | |
| <133.01 | 1477 | 37,685,604 | 97.1 |
| 133.01–167.32 | 9 | 482,163 | 1.2 |
| ≥167.33 | 18 | 638,571 | 1.6 |
| PCB 118, ng/g | | | |
| <0.042 | 1394 | 34,929,011 | 90.0 |
| 0.042–0.089 | 84 | 3,095,051 | 8.0 |
| ≥0.090 | 26 | 782,275 | 2.0 |
| PCB 156, ng/g | | | |
| <0.048 | 1490 | 38,252,492 | 98.6 |
| 0.048–0.055 | 2 | 28,319 | 0.1 |
| ≥0.056 | 12 | 525,527 | 1.4 |
| One of four chemicals elevated[e] | | | |
| None elevated | 1458 | 37,283,987 | 96.1 |
| One or more elevated | 46 | 1,522,350 | 3.9 |

[a]1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD). [b]1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin (HpCDD). [c]1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin (OCDD). [d]2,3,4,7,8-Pentachlorodibenzofuran (PeCDF). [e]1,2,3,6,7,8-HxCDD, PCB 126, PCB 169, or PCB 156 elevated.

We used SAS version 9.3 for all analyses (14). The survey logistic procedure was used for all logistic regression models, and the surveyreg procedure used for linear regressions, as these procedures allow for appropriate population-level estimates from the complex sample design used in the NHANES. The "normal" group was used as the referent for all regression models. Our analyses incorporated both the stratification and clustering aspects of the sample design. The proper weighting procedures include adjustments for nonresponse and poststratification. Moreover, as minorities were over sampled and a complex sampling design was employed, sampling weights provided by the NHANES for the dioxins, furans, and dioxin-like PCBs subsample were used to compute population estimates based on weighted parameter estimates and standard errors (6).

## Results

There were several differences between the teen and young adult sample (12–30 years old) and the all-adult sample (≥20 years old) analyzed previously (2). First, the proportion with one or more of the eight dioxin-like chemicals elevated was 9.9% in the teen and young adult sample, and 53.5% in the all-adult sample. Further, the proportion with nephropathy was 7.8%, and the proportion with pre-diabetes was 1.8% in the teen and young adult sample (Table 1), vs. 5.5%, and 9.7%, respectively, in the all-adult sample. The unweighted N for nephropathy among females was 87, and the unweighted N for males was 35 in the teen and young adult sample. Only 0.04% (unweighted n=3) had "pre-diabetes with nephropathy" in the teen and young adult sample, which was too few to analyze. Those with "total diabetes" (0.9%) were equally divided between diagnosed (unweighted n=5) and undiagnosed (A1c ≥6.5%, unweighted n=5), and most of those with diagnosed diabetes appeared to be Type 1 diabetics as they were on insulin (Table 1).

The proportion of values below the maximum limit of detection (MLOD) for individual dioxin-like compounds ranged from 71.6% to 98.6%. To calculate $TEQ_{8,}$ values below the MLOD were set to zero, and 55.4% (unweighted n=929, population estimate=21,484,130) of the sample had all eight of the dioxin-like chemicals below the MLOD, and therefore a $TEQ_8$ of zero. The proportion elevated by individual dioxin-like chemical ranged from 0.9% to 4.8% (Table 2). These proportions are low because we used the same cut-offs as we did in the all-adult sample (except that used for 2,3,4,7,8-pentachlorodibenzofuran).

Four of the eight dioxin-like compounds tested were associated with nephropathy, these were 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD), PCB 126, PCB 169, and PCB 156 (Table 3). PCB 126 had 3.2% of the sample elevated (unweighted n=32) and an OR of 8.9 (95% CI 2.0–39.7) for nephropathy. When one or more of the four were elevated the OR for nephropathy was 7.1 (95% CI 1.8–28.1), this group included 3.9% of the sample (unweighted n=46). The confidence intervals for the ORs were wide due to the small number subjects with these chemicals elevated. None of the eight dioxin-like chemicals were associated with pre-diabetes. In most instances there were no cases of pre-diabetes in the elevated concentration range (Table 3). We also tested one or more of four dioxin-like chemicals elevated by gender and found females had an association with nephropathy (OR=17.4, 95% CI 3.4–88.6), but males did not.

$TEQ_8$ and $TEQ_{23}$ are correlated. The equation relating the two in the all-adult sample is:

**Table 3:** Association of eight dioxins and dioxin-like chemicals with nephropathy, and pre-diabetes, in logistic regression models.[a]

| | Nephropathy[b] | | Pre-diabetes[c] | |
|---|---|---|---|---|
| | Odds ratio | 95% CI | Odds ratio | 95% CI |
| 1,2,3,6,7,8-HxCDD, fg/g[d] | | | | |
| <121.41 | 1.0 | – | 1.0 | – |
| 121.41–299.45 | 0.2 | 0.1–0.5 | 0.7 | 0.0–15.8 |
| ≥299.46 | 51.1 | 4.1–641.6 | <0.001[e] | – |
| 1,2,3,4,6,7,8-HpCDD, fg/g[f] | | | | |
| <161.08 | 1.0 | – | 1.0 | – |
| 161.08–367.07 | 0.5 | 0.2–1.0 | 0.3 | 0.1–0.9 |
| ≥367.08 | 3.7 | 0.7–20.3 | 0.9 | 0.3–3.1 |
| 1,2,3,4,6,7,8,9-OCDD, fg/g[g] | | | | |
| <1,945.82 | 1.0 | – | 1.0 | – |
| 1,945.82–2,908.62 | 0.7 | 0.2–2.9 | 0.4 | 0.1–2.7 |
| ≥2,908.63 | 2.9 | 0.5–17.0 | 0.6 | 0.2–2.4 |
| 2,3,4,7,8-PeCDF, fg/g[h] | | | | |
| <54.02 | 1.0 | – | 1.0 | – |
| ≥54.02 | 0.9 | 0.2–5.3 | <0.001[e] | – |
| PCB 126, fg/g | | | | |
| <86.48 | 1.0 | – | 1.0 | – |
| 86.48–214.53 | 0.8 | 0.4–1.5 | 0.3 | 0.1–1.0 |
| ≥214.54 | 8.9 | 2.0–39.7 | <0.001[e] | – |
| PCB 169, fg/g | | | | |
| <133.01 | 1.0 | – | 1.0 | – |
| 133.01–167.32 | <0.001[e] | – | <0.001[e] | – |
| ≥167.33 | 9.4 | 1.02–87.6 | <0.001[e] | – |
| PCB 118, ng/g | | | | |
| <0.042 | 1.0 | – | 1.0 | – |
| 0.042–0.089 | 0.7 | 0.2–2.7 | 1.8 | 0.6–5.2 |
| ≥0.090 | 7.0 | 0.7–72.4 | <0.001[e] | – |
| PCB 156, ng/g | | | | |
| <0.048 | 1.0 | – | 1.0 | – |
| 0.048–0.055 | <0.001[e] | – | –[i] | – |
| ≥0.056 | 17.9 | 2.1–152.6 | <0.001[e] | – |
| One of four chemicals elevated[j] | | | | |
| None elevated | 1.0 | – | 1.0 | – |
| One or more elevated | 7.1 | 1.8–28.1 | <0.001[e] | – |

[a]Adjusted for age, gender, race/ethnicity, BMI Z-score, poverty income ratio, energy adjusted fruit and vegetable consumption, and physical activity. [b]Normal A1c (<5.7%) with nephropathy. [c]Pre-diabetes (A1c 5.7–6.4%) without nephropathy. [d]1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD). [e]No cases in this concentration range. [f]1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin (HpCDD), [g]1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin (OCDD). [h]2,3,4,7,8-Pentachlorodibenzofuran (PeCDF). [i]No data in this concentration range. [j]1,2,3,6,7,8-HxCDD, PCB 126, PCB 169, or PCB 156 elevated.

$$TEQ_{23}=1.56*TEQ_8-11.86 \quad r^2=0.92$$

The 75th percentile of $TEQ_{23}$ reported in Everett and Thompson (2) was 81.66 fg/g. Using the above equation, the comparable level of $TEQ_8$ is 59.95 fg/g, which is 73.4% of the $TEQ_{23}$ value.

Least squares means for $TEQ_8$ were calculated by disease category (Table 4). The trend was for $TEQ_8$ for

**Table 4:** Mean toxic equivalency ($TEQ_8$) by disease category and gender, as determined in three linear regression models using SAS proc surveyreg.[a]

| | Toxic equivalency (fg/g) least squares means | |
|---|---|---|
| | $TEQ_8$ estimate | 95% CI |
| Whole sample | | |
| Normal[b] | 5.66 | 3.76–7.56 |
| Nephropathy[c] | 10.61 | 0.96–20.26 |
| Pre-diabetes[d] | 1.03 | −3.72 to 5.79 |
| Males | | |
| Normal | 5.11 | 3.16–7.06 |
| Nephropathy | 3.92 | −0.83 to 8.68 |
| Pre-diabetes | 1.99 | −4.60 to 8.58 |
| Females | | |
| Normal | 5.86 | 3.09–8.62 |
| Nephropathy | 13.73 | 1.41–26.06 |
| Pre-diabetes | −0.86 | −7.64 to 5.93 |

[a]Adjusted for age, gender, race/ethnicity, BMI Z-score, poverty income ratio, energy adjusted fruit and vegetable consumption, and physical activity. [b]Normal A1c (<5.7%) without nephropathy. [c]Normal A1c (<5.7%) with nephropathy. [d]Pre-diabetes (A1c 5.7–6.4%) without nephropathy.

**Table 5:** Odds ratios for elevated toxic equivalency ($TEQ_8$) by disease categories and gender, in a series of logistic regression models.[a]

| | Elevated $TEQ_8$, fg/g | Odds ratio | 95% CI |
|---|---|---|---|
| ≥75th Percentile | | | |
| Normal[b] | ≥12.70 | 1.0 | – |
| Nephropathy[c] | | 1.1 | 0.5–2.6 |
| Pre-diabetes[d] | | 0.5 | 0.2–1.6 |
| ≥90th percentile | | | |
| Normal | ≥25.41 | 1.0 | – |
| Nephropathy | | 1.6 | 0.6–4.7 |
| Pre-diabetes | | 0.8 | 0.2–2.4 |
| ≥95th percentile | | | |
| Normal | ≥35.26 | 1.0 | – |
| Nephropathy | | 2.6 | 0.6–11.6 |
| Pre-diabetes | | 1.3 | 0.4–4.9 |
| ≥97.5th percentile | | | |
| Normal | ≥50.12 | 1.0 | – |
| Nephropathy | | 5.8 | 1.3–25.9 |
| Pre-diabetes | | 1.4 | 0.2–11.3 |
| ≥97.5th percentile, male | | | |
| Normal | ≥50.12 | 1.0 | – |
| Nephropathy | | <0.001[e] | – |
| Pre-diabetes | | 4.0 | 0.4–38.9 |
| ≥97.5th percentile, female | | | |
| Normal | ≥50.12 | 1.0 | – |
| Nephropathy | | 11.9 | 1.6–87.2 |
| Pre-diabetes | | <0.001[e] | |

[a]Adjusted for age, gender, race/ethnicity, BMI Z-score, poverty income ratio, energy adjusted fruit and vegetable consumption, and physical activity. [b]Normal A1c (<5.7%) without nephropathy. [c]Normal A1c (<5.7%) with nephropathy. [d]Pre-diabetes (A1c 5.7–6.4%) without nephropathy. [e]No cases in this concentration range.

nephropathy to have a higher mean than the normal group in the whole sample and among females. However, there were no significant differences in mean $TEQ_8$ between disease categories. Because 55.4% of the sample had $TEQ_8$=0, the data were not normally distributed and the comparison of means assuming a normal distribution invalid.

When elevated $TEQ_8$ was defined as ≥12.70 fg/g, ≥25.41 fg/g or ≥35.26 fg/g the OR for nephropathy was not significant (Table 5). However, when elevated $TEQ_8$ was defined as ≥50.12 fg/g the OR for nephropathy was 5.8 (95% CI 1.3–25.9). The $TEQ_8$ ≥50.12 fg/g group included 2.6% of the sample (unweighted n=28). The association in the whole sample appeared to be entirely due to an association among females (OR=11.9, 95% CI 1.6–87.2), and there were no associations with pre-diabetes at any level of elevated $TEQ_8$ in the whole sample or by gender.

We also calculated sensitivity and specificity for nephropathy when $TEQ_8$ was defined as ≥50.12 fg/g in the whole sample and for females. However, the design variables and sample weights could not be used in these calculations. Sensitivity was 4.00% (exact 95% CI 1.31–9.09%) and specificity 98.33% (exact 95% CI 97.51–98.94%) in the whole sample, and sensitivity 5.68% (exact 95% CI 1.87–12.76%) and specificity 98.03% (exact 95% CI 96.72–98.92%) among females. In addition, we calculated comparable values for nephropathy when one or more of four dioxin-like chemicals were elevated. For the whole sample, sensitivity was 6.40% (exact 95% CI 2.80–12.22%), and specificity 97.24% (exact 95% CI 96.24–98.04%). For females, sensitivity was 9.09% (exact 95% CI 4.01–17.13%) and specificity 96.77% (exact 95% CI 95.19–97.94%). All four relationships were significantly different from chance at p<0.0001.

## Discussion

We are not aware of any studies of dioxin-like chemicals that have been conducted using NHANES 1999–2004 data on subjects 12–19 years old, other than those done to assess prevalence in the "Third National Report on Human Exposure to Environmental Chemicals" (15). Teens were more intensively sampled in the NHANES than were young adults, and all of our analyses, except calculation of sensitivity and specificity, were conducted using the

design variables and subject sampling weights calculated for the NHANES 1999–2004.

Given the proportion with pre-diabetes was so much less in the teen and young adult sample (1.8%) than in the all-adult sample (9.7%), and the proportion with nephropathy was actually higher (7.8% vs. 5.5%), it would appear progression to nephropathy would be more likely than progression to pre-diabetes in teens and young adults.

The results presented support the hypothesis that the relationship between dioxin-like compounds and nephropathy, among teens and young adults, is a case of a threshold effect rather a continuous graded effect. We considered three estimates of an at-risk population, these were 9.9% of the population having one or more of eight dioxin-like chemicals elevated, 3.9% of the population having one or more of four chemicals elevated (the four dioxin-like chemicals with significant ORs), and 2.6% of the population having $TEQ_8$ ≥50.12 fg/g. Because it relates to specific dioxin-like chemicals (1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin, PCB 126, PCB 169, and PCB 156), we consider the best estimate of the at-risk population to be 3.9% (unweight n=46, population estimate=1,522,350 teens and young adults).

The ORs (7.1, 95% CI 1.8–28.1) for one or more of four dioxin-like chemicals elevated and nephropathy has a wide confidence interval and is therefore somewhat unstable. This makes the logistic regression more likely to generate a false positive result. However, we maintain that a logistic regression that shows the elevated dioxin-like compounds category has an OR significantly >1.00 is sufficient to put forth the hypothesis that nephropathy in teens and young adults is associated with dioxin-like chemicals.

Calculation of least-squares means by disease category is problematic because the data for our study are not normally distributed. The eight dioxin-like chemicals used in the calculation of mean $TEQ_8$ were all below the maximum limit of detection for 55.4% of the persons included (unweighted n=929, population estimate=21,484,130). Thus the data are zero-inflated, as values below the maximum limit of detection for each of the eight dioxin-like chemicals were assigned values of zero for the purposes of calculating $TEQ_8$. The term "zero-inflated" means that there are zeros in excess of those included in the theoretical distribution for the statistical test being conducted. In this case the normal distribution presumed in SAS proc surveyreg. There are "zeromodel" procedures in some statistical tests, for example for zero-inflated count data (zero-inflated negative binomial regression) analyzed with SAS proc countreg. We used SAS proc surveyreg, because it could handle the design variables and sample weights used in the NHANES, but there is no provision for zero-inflation in the regression analysis using this statistical procedure.

Associations of dioxin-like compounds with nephropathy were found among females, but not males. Estrogen is known to have a protective effect with respect to chronic kidney disease (16, 17), and dioxin-like chemicals are known to act through the Aryl hydrocarbon receptor (3). Lee et al. (18) noted: "There are interactions between the Aryl hydrocarbon receptor and estrogen receptor signaling pathways (19), suggesting that dioxins could have indirect effects on some estrogen-mediated endpoints as well". This difference between females and males is striking and raises questions about women's health and environmental pollutants.

In this study, associations of dioxin-like chemicals with nephropathy were found among females at an early age. Prospective studies are needed to determine if dioxin-like compounds cause nephropathy, or if these relationships are cases of reverse causation. In contrast to the results for nephropathy, there was no evidence of an association of dioxin-like chemicals with pre-diabetes. While progression to pre-diabetes occurs, there is no evidence that it is associated with dioxin-like chemicals before the age of 30 years old in either males or females. While we assume everyone begins life as "normal", we cannot rule out the possibility of pre-natal exposures to dioxin-like chemicals. As the youngest persons in our sample were 12 years old, we do not have any way to assess what happens at an age younger than that. This NHANES study of dioxin-like chemicals is unique as subjects 12–19 years old were included. Progression to "pre-diabetes with nephropathy" appears to occur later in life. In the all-adult sample (2), the mean age of those with "pre-diabetes with nephropathy" was 63.0 years old (Supplemental Table 1), which was not significantly different from the mean age for "total diabetes (diagnosed and undiagnosed diabetes) with nephropathy." The recommendation that all persons with nephropathy be excluded from longitudinal studies of incident diabetes and dioxin-like chemicals (5) remains appropriate, and is not altered by the results of this study.

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