Environmental Research 132 (2014) 126–131

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

# Dioxins, furans and dioxin-like PCBs in human blood: Causes or consequences of diabetic nephropathy?

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ARTICLE INFO

*Article history:*
Received 10 August 2013
Received in revised form
29 March 2014
Accepted 31 March 2014

*Keywords:*
Dioxins
Furans
Polychlorinated biphenyls
Diabetic nephropathy
Kidney disease

ABSTRACT

Nephropathy, or kidney disease, is a major, potential complication of diabetes. We assessed the association of 6 chlorinated dibenzo-*p*-dioxins, 9 chlorinated dibenzofurans and 8 polychlorinated biphenyls (PCBs) in blood with diabetic nephropathy in the 1999–2004 National Health and Nutrition Examination Survey (unweighted $N=2588$, population estimate = 117,658,357). Diabetes was defined as diagnosed or undiagnosed (glycohemoglobin $\geq 6.5\%$) and nephropathy defined as urinary albumin to creatinine ratio $>30$ mg/g, representing microalbuminuria or macroalbuminuria. For the 8 chemicals analyzed separately, values above the 75th percentile were considered elevated, whereas for the other 15 compounds values above the maximum limit of detection were considered elevated. Seven of 8 dioxins and dioxin-like compounds, analyzed separately, were found to be associated with diabetic nephropathy. The chemicals associated with diabetic nephropathy were: 1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin; 1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin; 2,3,4,7,8-Pentachlorodibenzofuran; PCB 126; PCB 169; PCB 118; and PCB 156. Three of the 8 dioxins and dioxin-like compounds; 1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin; 2,3,4,7,8-Pentachlorodibenzofuran and PCB 118; expressed as log-transformed continuous variables; were associated with diabetes without nephropathy. When 4 or more of the 23 chemicals were elevated the odds ratios 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. Log-transformed toxic equivalency (TEQ) was associated with both diabetic nephropathy, and diabetes without nephropathy, the odds ratios were 2.35 (95% CI = 1.57–3.52) for diabetic nephropathy, and 1.44 (95% CI = 1.11–1.87) for diabetes without nephropathy. As the kidneys function to remove waste products from the blood, diabetic nephropathy could be either the cause or the consequence (or both) of exposure to dioxins, furans and dioxin-like PCBs.



## 1. Introduction

Nephropathy, or kidney disease, is a major, potential complication of diabetes. Among persons with Type 1 or Type 2 diabetes, approximately 25–40% develop nephropathy within 20–25 years of diagnosis, making diabetic nephropathy the single leading cause of incident chronic kidney disease (Fukami and Yamagishi, 2012). Diabetic nephropathy is also responsible for a substantial number of patients (the majority of whom are of African American race) initiating renal replacement therapy due to end-stage renal disease (Komorowsky et al., 2012). Moreover, in the Atherosclerosis Risk in Communities Study, 80% of the increased risk for declining renal function in African Americans with diabetes was due to individual- and/or environmental-level risk factors including education, household income, health insurance, fasting glucose level, mean systolic blood pressure, smoking history, and physical activity level (Adler, 2006). Additional environmental-level risk factors have been identified for diabetic nephropathy specifically, as well as more generally for chronic kidney disease and diabetes and include cadmium, iron, lead, arsenic, polychlorinated organic compounds, nitrogen compounds, and contrast agents (Edwards and Prozialeck, 2009; Haswell-Elkins et al., 2008; Marchewka and Grzebinoga, 2009; Obert et al., 2011). Similarly, lead, cadmium, arsenic, mercury, uranium, industrial chemicals, elevated ambient temperatures, infections and smoking have been implicated as environmental risk factors for chronic kidney disease specifically (Alebiosu, 2003; Alebiosu and Ayodele, 2005; Mercado and Jaimes, 2007; Nordberg et al., 2009; Satarug et al., 2000; Soderland et al., 2010).

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http://dx.doi.org/10.1016/j.envres.2014.03.043
0013-9351/

Furthermore, dioxins, furans, polychlorinated biphenyls (PCBs), pesticides (particularly insecticides), bisphenol-A, phthalates, polybrominated diphenyl ether, air pollution, lead, cadmium and arsenic have been implicated as environmental risk factors for diabetes specifically (Alonso-Magdalena et al., 2010, 2011; Carpenter, 2011; Casals-Casas and Desvergne, 2011; De Coster and van Larebeke, 2012; Frithsen and Everett, 2012; Meeker, 2010; Otles and Cagindi, 2010; Thayer et al., 2012; Codru et al., 2007; Lee et al., 2006; Rignell-Hydbom et al., 2007, 2009; Rylander et al., 2005; Ukropec et al., 2010; Uemura et al., 2008; Vasiliu et al., 2006; Wang et al., 2008).

Recent reviews of persistent organic pollutants and diabetes have been published by Carpenter (2008), Crinnion (2011), Everett et al. (2011), Frithsen and Everett (2012) and Wang et al. (2010). These reviews concluded that the role of environmental contaminants or pollutants in diabetes is not yet well understood. The strongest body of epidemiological evidence is that linking certain persistent organic pollutants to diabetes. Some of these chemicals such as DDT, heptachlor and PCBs are no longer produced as they have been recognized as hazardous to humans and/or the environment. There is now sufficient epidemiologic evidence showing an association between several environmental contaminants and diabetes to warrant large-scale prospective epidemiologic studies. Future research should focus on the effect of low-level exposures that are typical for the majority of the population.

Subsequent studies include a Japanese cross-sectional study of 11 PCBs and diabetes using the Saku Control Obesity Program (Tanaka et al., 2011). In this study, PCB 146 and 180 were associated with diabetes (diagnosed and undiagnosed) in this middle-aged, overweight and obese sample. A cross-sectional study using the Helsinki Birth Cohort tested the association of PCB 153 with diabetes and while the *p*-value for trend was significant, none of the individual categories were significantly elevated (Airaksinen et al., 2011). Everett and Thompson (2012) investigated cross-sectional associations of 23 dioxins, furans and dioxin-like PCBs with diabetes (diagnosed and undiagnosed) and pre-diabetes in the 1999–2004 United States National Health and Nutrition Examination Survey (NHANES). In this study, having multiple compounds elevated was highly associated with diabetes, whereas, having one compound elevated was highly associated with pre-diabetes. Of the 8 dioxin-like compounds tested separately; 1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD); 1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin (OCDD); 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF); PCB 169; PCB 118; and PCB 156 were associated with diabetes (diagnosed and undiagnosed).

Notable, recent longitudinal studies include that of Lee et al. (2010) who evaluated 22 PCBs and incident diabetes using a case–control design based on the Coronary Artery Risk Development in Young Adults cohort. Five dioxin-like PCBs were tested (PCB 105, 118, 156, 157 and 167), but none were statistically significant. A low-dose effect was found for 4 PCBs (PCB 74, 178, 180 and 187) in which the second quartile had a significantly elevated odds ratio. Another longitudinal study by Lee et al. (2011) using the Swedish Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) cohort investigated 14 PCBs and one dioxin. The fifth quintile was significantly elevated for PCB 74, 180, 194, 206 and 209. A low-dose effect was also observed with the second quintile of dioxin-like PCB 105 being significantly elevated. The purpose of the current study was to determine relationships of dioxins, furans and dioxin-like PCBs in human blood with diabetic nephropathy among United States (U.S.) adults.

## 2. Materials and methods

We used data derived from the National Health and Nutrition Examination Survey (NHANES) 1999–2004 to investigate the associations of dioxins and dioxin-like compounds with diabetic nephropathy, and with diabetes without nephropathy as such data include a representative sample of non-institutionalized U.S. adults. Detailed information on the methodology of the NHANES 1999–2004, including laboratory assessment, can be found at the National Center for Health Statistics website (CDC, 2013). Nephropathy was defined as urinary albumin to creatinine ratio $>30$ mg/g, representing both microalbuminuria and macroalbuminuria (Molitch et al., 2004). 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 glycohemoglobin (A1c) $\geq 6.5\%$ who had not been diagnosed as having diabetes (ADA, 2010). We did not use fasting plasma glucose for determination of undiagnosed diabetes because glucose was only measured on a fasting subsample of participants and would have reduced by half the number of persons in our analyses.

Six chlorinated dibenzo-*p*-dioxins, 9 chlorinated dibenzofurans, and 8 dioxin-like polychlorinated biphenyls (Table 1) were measured in nonfasting blood samples of a one-third, stratified random, subsample of participants 12 years old and older. We evaluated persons in this subsample who were $\geq 20$ years old. The unweighted number of participants in our study was 2588 which represented a total of 117,658,357 non-incarcerated U.S. adults (Table 2). The 23 dioxins and dioxin-like compounds 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. (2005). Each person had a sample-specific limit of detection.

Values below or equal to, the maximum limit of detection observed (Table 1) were set to zero for the purposes of our analyses. Eight of the 23 dioxins and dioxin-like compounds had $>25\%$ of their values above the maximum limit of detection, and were analyzed separately. The 8 dioxins and dioxin-like chemicals were categorized as less than or equal the maximum limit of detection, greater than or equal the 75th percentile, and an intermediate category, greater than the maximum limit of detection and less than the 75th percentile. We also evaluated the number of compounds elevated. A compound was considered elevated if its concentration was above the 75th percentile for the 8 chemicals listed in Table 4, or above the maximum limit of detection for the other 15 compounds. Finally, total toxic equivalency (TEQ) was calculated by multiplying concentrations and toxic equivalency factors established by the World Health Organization (Van den Berg et al., 2006). The 8 dioxins, furans, and dioxin-like PCBs analyzed separately in Table 4, were also analyzed as log-transformed, continuous variables [ln(concentration+1)], as was TEQ [ln(TEQ+1)], *p*-values for the log-transformed variables (Table 3), and odds ratios for log-transformed TEQ (Table 5) were reported.

**Table 1**
Dioxins and dioxin-like compounds investigated.

| | Maximum limit of detection |
|---|---|
| Dioxins | |
| 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) | 47.73 fg/g |
| 1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin (PeCDD) | 53.81 fg/g |
| 1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD) | 88.60 fg/g |
| 1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin (HxCDD) | 81.46 fg/g |
| 1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin (HpCDD) | 157.33 fg/g |
| 1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin (OCDD) | 1945.82 fg/g |
| Furans | |
| 2,3,7,8-Tetrachlorodibenzofuran (TCDF) | 51.90 fg/g |
| 1,2,3,7,8-Pentachlorodibenzofuran (PeCDF) | 47.87 fg/g |
| 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) | 51.12 fg/g |
| 1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF) | 47.31 fg/g |
| 1,2,3,6,7,8-Hexachlorodibenzofuran (HxCDF) | 49.29 fg/g |
| 1,2,3,7,8,9-Hexachlorodibenzofuran (HxCDF) | 47.16 fg/g |
| 2,3,4,6,7,8-Hexachlorodibenzofuran (HxCDF) | 55.23 fg/g |
| 1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF) | 71.63 fg/g |
| 1,2,3,4,6,7,8,9-Octachlorodibenzofuran (OCDF) | 165.11 fg/g |
| PCBs | |
| 3,4,4′,5-Tetrachlorobiphenyl (PCB 81) | 274.85 fg/g |
| 3,3′,4,4′,5-Pentachlorobiphenyl (PCB 126) | 86.48 fg/g |
| 3,3′,4,4′,5,5′-Hexachlorobiphenyl (PCB 169) | 114.98 fg/g |
| 2,3,3′,4,4′-Pentachlorobiphenyl (PCB 105) | 0.048 ng/g |
| 2,3′,4,4′,5-Pentachlorobiphenyl (PCB 118) | 0.041 ng/g |
| 2,3,3′,4,4′,5-Hexachlorobiphenyl (PCB 156) | 0.048 ng/g |
| 2,3,3′,4,4′,5′-Hexachlorobiphenyl (PCB 157) | 0.048 ng/g |
| 2,3′,4,4′,5,5′-Hexachlorobiphenyl (PCB 167) | 0.048 ng/g |

**Table 2 Diabetes categories, number of compounds elevated, and toxic equivalency (TEQ) quartiles.**

| | Unweighted *N* | Population estimate | Proportion (%) |
|---|---|---|---|
| Diabetes category | | | |
| Normal A1c (< 5.7%) | | | |
| Without nephropathy | 1788 | 89,282,573 | 75.9 |
| With nephropathy | 156 | 6,497,735 | 5.5 |
| Pre-diabetes (A1c 5.7–6.4%) | | | |
| Without nephropathy | 312 | 11,463,344 | 9.7 |
| With nephropathy | 50 | 1,585,421 | 1.3 |
| Total diabetes (diagnosed or A1c≥6.5%) | | | |
| Without nephropathy | 184 | 6,143,212 | 5.2 |
| With nephropathy | 98 | 2,686,073 | 2.3 |
| Number of compounds elevated[a] | | | |
| 0 | 946 | 47,558,488 | 40.4 |
| 1 | 335 | 16,809,962 | 14.3 |
| 2 | 235 | 11,035,270 | 9.4 |
| 3 | 163 | 7,372,429 | 6.3 |
| 4–8 | 519 | 20,513,596 | 17.4 |
| 9–13 | 317 | 11,828,906 | 10.0 |
| ≥14 | 73 | 2,539,706 | 2.2 |
| Toxic equivalency (TEQ fg/g) | | | |
| < 13.87 | 593 | 29,382,702 | 25.0 |
| 13.87–34.53 | 585 | 29,417,231 | 25.0 |
| 34.54–81.65 | 615 | 29,400,551 | 25.0 |
| ≥81.66 | 795 | 29,457,873 | 25.0 |
| Total sample | 2588 | 117,658,357 | 100 |

[a] Note that only 11.9% of the sample (unweighted $N=284$) had no compounds above the maximum limit of detection. The number of compounds elevated does not include values above the maximum limit of detection, and below the 75th percentile, for the 8 chemicals shown in Tables 3 and 4. For the other 15 chemicals, values above the maximum limits of detection are included.

**Table 3**
Association of 8 log-transformed dioxin and dioxin-like compounds[a], and log-transformed toxic equivalency[b], with total diabetes, with and without nephropathy[c].

| | Total diabetes without nephropathy *p*-value | Total diabetes with nephropathy *p*-value |
|---|---|---|
| 1,2,3,6,7,8-HxCDD (fg/g) | 0.3032 | 0.0270 |
| 1,2,3,4,6,7,8-HpCDD (fg/g) | 0.0584 | 0.2183 |
| 1,2,3,4,6,7,8,9-OCDD (fg/g) | 0.0319 | 0.0073 |
| 2,3,4,7,8-PeCDF (fg/g) | 0.0103 | 0.0107 |
| PCB 126 (fg/g) | 0.0503 | 0.0167 |
| PCB 169 (fg/g) | 0.4635 | 0.0016 |
| PCB 118 (ng/g) | 0.0099 | 0.0282 |
| PCB 156 (ng/g) | 0.1664 | 0.0142 |
| Toxic equivalency (TEQ fg/g) | 0.0054 | < 0.0001 |

[a] As a continuous variable, ln(concentration+1).
[b] As a continuous variable, ln(TEQ+1).
[c] Adjusted for age, gender, race/ethnicity, education, poverty income ratio, energy adjusted fruit and vegetable consumption, physical activity and family history of diabetes.

We tested the associations of 8 individual chemicals, the number of compounds elevated, and TEQ with total diabetes with nephropathy and with total diabetes without nephropathy in ordinal regression models adjusted for participant age, sex, race/ethnicity, education, poverty-to-income ratio, energy adjusted fruit and vegetable consumption, physical activity and family history of diabetes. Race/ethnicity was classified as non-Hispanic white, non-Hispanic black, Mexican American, and other Hispanic. Education was classified as less than 9th grade, 9–12th grade without a diploma, high school graduate/GED or equivalent, some college or associate degree, and college graduate or above. 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 (US Census Bureau, 2013a). Poverty-to-income ratio was top coded at 5, and values below 1.00 were below the official poverty threshold (US Census Bureau, 2013b). 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

**Table 4**
Association of 8 dioxins and dioxin-like compounds with total diabetes, with and without nephropathy.[a]

| | Total diabetes without nephropathy | | Total diabetes with nephropathy | |
|---|---|---|---|---|
| | Odds ratio | 95% CI | Odds ratio | 95% CI |
| 1,2,3,6,7,8-HxCDD (fg/g) | | | | |
| < 88.60 | 1.00 | – | 1.00 | – |
| 88.60–299.45 | 1.19 | 0.60–2.38 | 1.44 | 0.54–3.86 |
| ≥ 299.46 | 1.56 | 0.70–3.46 | 6.20 | 2.09–18.38 |
| 1,2,3,4,6,7,8-HpCDD (fg/g) | | | | |
| < 157.33 | 1.00 | – | 1.00 | – |
| 157.33–367.07 | 1.75 | 0.75–4.10 | 1.41 | 0.61–3.29 |
| ≥ 367.08 | 2.27 | 1.08–4.77 | 1.76 | 0.62–4.96 |
| 1,2,3,4,6,7,8,9-OCDD (fg/g) | | | | |
| < 1945.82 | 1.00 | – | 1.00 | – |
| 1945.82–2908.62 | 1.50 | 0.89–2.52 | 1.78 | 0.77–4.08 |
| ≥ 2908.63 | 1.61 | 1.06–2.43 | 4.02 | 1.68–9.62 |
| 2,3,4,7,8-PeCDF (fg/g) | | | | |
| < 51.12 | 1.00 | – | 1.00 | – |
| 51.12–51.73 | < 0.001[b] | – | < 0.001[b] | – |
| ≥ 51.74 | 2.05 | 1.18–3.58 | 3.14 | 1.30–7.55 |
| PCB 126 (fg/g) | | | | |
| < 86.48 | 1.00 | – | 1.00 | – |
| 86.48–214.53 | 1.27 | 0.61–2.65 | 1.25 | 0.56–2.80 |
| ≥ 214.54 | 2.32 | 1.15–4.69 | 2.42 | 1.20–4.87 |
| PCB 169 (fg/g) | | | | |
| < 114.98 | 1.00 | – | 1.00 | – |
| 114.98–167.32 | 1.13 | 0.50–2.57 | 4.63 | 1.74–12.28 |
| ≥ 167.33 | 1.40 | 0.59–3.32 | 3.84 | 1.28–11.48 |
| PCB 118 (ng/g) | | | | |
| < 0.041 | 1.00 | – | 1.00 | – |
| 0.041–0.089 | 2.82 | 1.45–5.47 | 2.89 | 0.90–9.26 |
| ≥ 0.090 | 3.64 | 1.49–8.91 | 6.64 | 1.87–23.57 |
| PCB 156 (ng/g) | | | | |
| < 0.048 | 1.00 | – | 1.00 | – |
| 0.048–0.055 | 1.54 | 0.65–3.63 | 7.47 | 2.35–23.74 |
| ≥ 0.056 | 1.57 | 0.78–3.14 | 3.35 | 1.36–8.27 |

[a] Adjusted for age, gender, race/ethnicity, education, poverty income ratio, energy adjusted fruit and vegetable consumption, physical activity and family history of diabetes.
[b] No cases in this concentration range.

**Table 5**
Association of the number of compounds elevated and log-transformed toxic equivalency, with total diabetes, with and without nephropathy.[a]

| Number of compounds elevated | Total diabetes without nephropathy | | Total diabetes with nephropathy | |
|---|---|---|---|---|
| | Odds ratio | 95% CI | Odds ratio | 95% CI |
| 0 | 1.00 | – | 1.00 | – |
| 1 | 0.75 | 0.30–1.89 | 0.30 | 0.04–2.14 |
| 2 | 0.84 | 0.35–2.00 | 2.42 | 0.64–9.10 |
| 3 | 2.02 | 0.81–5.03 | 1.54 | 0.25–9.55 |
| 4–8 | 1.92 | 0.79–4.69 | 6.00 | 1.60–22.54 |
| 9–13 | 2.73 | 1.20–6.23 | 6.82 | 1.93–24.09 |
| ≥14 | 2.41 | 0.87–6.72 | 20.30 | 3.14–131.47 |
| Toxic equivalency[b] (TEQ fg/g) | 1.44 | 1.11–1.87 | 2.35 | 1.57–3.52 |

[a] Adjusted for age, gender, race/ethnicity, education, poverty income ratio, energy adjusted fruit and vegetable consumption, physical activity and family history of diabetes.
[b] As a continuous variable, ln(TEQ+1).

(CDC, 2013). An individual was considered to have a family history of diabetes if one of their parents, grandparents, brothers or sisters had diabetes.

We used SAS version 9.3 for all analyses (SAS Institute Inc., 2013). The surveylogistic procedure was used for all regression models as this procedure allows for appropriate population-level estimates from the complex sample design used in the NHANES. The "normal" group (A1c $<5.7\%$ and urinary albumin to creatinine ratio $<30$ mg/g, Table 2) was used as the referent for all regression models. Our analyses incorporated both the stratification and clustering aspects of the sampling design. The proper weighting procedures include adjustments for nonresponse and poststratification. Moreover, as minorities were oversampled and a complex sampling design was employed, sampling weights provided by the NHANES for the dioxins subsample were used to compute population estimates based on weighted parameter estimates and standard errors (CDC, 2013).

## 3. Results and discussion

Of the participants with total diabetes, 30.4% had nephropathy (Table 2). Seven of 8 dioxins and dioxin-like compounds, analyzed separately, were found to be associated with diabetic nephropathy specifically (Tables 3 and 4). The chemicals associated with diabetic nephropathy were 1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin; 1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin; 2,3,4,7,8-Pentachlorodibenzofuran; PCB 126; PCB 169; PCB 118; and PCB 156. In contrast, only 3 of the 8 log-transformed, dioxins and dioxin-like compounds (1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin, 2,3,4,7,8-Pentachlorodibenzofuran, and PCB 118) were associated with diabetes without nephropathy (Table 3).

We did not use body mass index and waist circumference as covariates in our models because adjusting for measures of obesity is controversial in studying the association between persistent organic pollutants and diabetes. There is growing evidence that obesity is on the causal pathway between persistent organic pollutants and diabetes and this relationship is potentially confounded by the consumption of fatty food, which is associated with obesity and increased persistent organic pollutants levels. However, adipose tissue serves as a reservoir of persistent organic pollutants, thereby reducing the circulating persistent organic pollutants levels. This effect might have a positive role in limiting the exposure to target tissues for diabetes, such as pancreatic beta cells. Comparing the results presented in Tables 3 and 4, there were no differences for total diabetes with nephropathy. However, for total diabetes without nephropathy, 3 of the 8 dioxins and dioxin-like compounds were associated using log-transformed data (Table 3), whereas 5 of the 8 compounds were associated using categories (Table 4). Further, when body mass index and waist circumference were added as covariates there were no differences for total diabetes with nephropathy, and only 2 of the compounds, 2,3,4,7,8-Pentachlorodibenzofuran and PCB 118, were associated with total diabetes without nephropathy, when using categories (data not shown). Hence, adding body mass index and waist circumference as covariates made the results more conservative.

In Everett and Thompson (2012), the odds ratio for 4 or more compounds elevated and total diabetes, without regard to whether or not a person had nephropathy, was 2.62 (95% CI 1.29–5.31). In the present study, when 4 or more of the 23 chemicals were elevated, the odds ratios 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. The only number of compounds elevated category, which was significant for total diabetes without nephropathy, was 9–13 compounds (Table 5). Log-transformed toxic equivalency (TEQ) was significantly associated with both diabetic nephropathy, and diabetes without nephropathy. The odds ratio for diabetic nephropathy was 2.35 (95% CI=1.57–3.52), and the odds ratio for diabetes without nephropathy was 1.44 (95% CI=1.11–1.87).

A key longitudinal, re-analysis of the Ranch Hand Study was published by Kerger et al. (2012). Evaluating 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, the most toxic of the dioxin-like compounds, they concluded that the associations previously reported for these U.S. Vietnam war veterans were due to reverse causation. Reverse causality is when the expected effect precedes the expected cause. In our case, diabetic nephropathy would precede a rise in dioxin-like chemicals in blood. As used by Kerger et al. (2012), the onset of diabetes would precede a rise in 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in blood. One of the lines of reasoning used was to compare incident diabetes by serum dioxin decile calculated separately for the Ranch Hand group and for the Comparison group. Median serum dioxin ranged from 2.9 ppt for the first decile to 101 ppt in the tenth decile for Ranch Hand group, and from 1.3 ppt for the first decile to 8.5 ppt in the tenth decile for the Comparison group. Incident diabetes for these deciles were 9.9% and 12.0% for the first decile of the Ranch Hand and Comparison groups, respectively, and 28.4% and 27.6% for the tenth decile of the Ranch Hand and Comparison groups, respectively. If the data were plotted by serum dioxin concentration, the Comparison group would only overlap the first four deciles of the Ranch Hand group, but would show a steeper slope of incident diabetes and serum dioxin. Kerger et al. (2012) hypothesized that reverse causation effects relate to increased lipolysis and lipolytic metabolism during diabetes progression and/or periods of poor diabetes control. This study is especially relevant to investigations of diabetic nephropathy and dioxin-like compounds because the kidneys function to remove toxins from the blood. Impaired kidney function could result in dioxin-like chemicals being retained in the blood stream for longer times than would be the case in persons who do not have diabetic kidney disease or nephropathy.

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 (Haws et al., 2006), which has been hypothesized to antagonize the peroxisome proliferator-activated receptor and thus contribute to the pathophysiology of diabetes (Remillard and Bunce, 2002). However, Ruzzin (2012) argued that the effects associated with persistent organic pollutants' exposure may occur independently of Ah receptor activation. He further stated that potential modes of action of persistent organic pollutants may include activation of constitutive androstane receptor or steroid xenobiotic receptor, and competitive binding to nuclear receptors.

Gennings et al. (2012) studied the body burden of 42 environmental chemicals and "wellness" using the 2001–2002 NHANES. Wellness was defined using 28 clinical, serum biomarkers, and the environmental chemicals were assigned to one of six disease pathways. Nine dioxins, furans and dioxin-like PCBs were presumed to affect the Ah receptor pathway. The dioxins and dioxin-like chemicals were calculated to represent 17% of the total body burden of environmental chemicals in NHANES participants. Persistent organic pollutants, other than dioxin-like chemicals, were assigned to a different pathway that interacted with the Ah receptor pathway. Three non-dioxin-like PCBs, 3 metabolites of organochlorine pesticides, and one metabolite of an organophosphate pesticide (3,5,6-trichloropyridinol), were presumed to affect the constitutive androstane receptor/pregnane X receptor pathway. These compounds were calculated to represent an additional 18% of the total body burden of environmental chemicals in the U.S. population.

The constitutive androstane receptor/pregnane X receptor pathway appears to not behave the same as the Ah receptor pathway. Six organochlorine pesticides, and pesticide metabolites,

presumed to affect the constitutive androstane receptor/pregnane X receptor pathway, have been evaluated using the 1999–2004 NHANES (unweighted $N=2992$) to test their association with diabetic nephropathy (Everett and Thompson, 2013). The proportion of the sample with diabetic nephropathy was 2.4%, and proportion having diabetes without nephropathy was 5.3%. Only *p,p′*-DDT (dichlorodiphenyltrichloroethane) was associated with diabetic nephropathy, with *p,p′*-DDT $\geq 0.0860$ ng/g having an odds ratio of 2.20 (95% CI 1.12–4.34) compared to *p,p′*-DDT $< 0.0860$ ng/g. Beta-hexachlorocyclohexane, oxychlordane, *trans*-nonachlor, and heptachlor epoxide were associated with diabetes without nephropathy, but not with diabetic nephropathy, as might be expected given the relative sizes of each group. A metabolite of DDT, *p,p′*-DDE (dichlorodiphenyltrichloroethylene) was not associated with either diabetic nephropathy or diabetes without nephropathy. The proportion of the U.S. population estimated to have *p,p′*-DDT $\geq 0.0860$ ng/g was 11.4%. Additional research is needed to determine what pathway is affected by elevated levels of DDT.

As the kidneys function to remove waste products from the blood, diabetic nephropathy could be either the cause or the consequence (or both) of exposure to dioxins, furans, and dioxin-like PCBs. Evaluating diabetes with and without nephropathy in cross-sectional and, importantly, longitudinal investigations, may provide additional insights into the nature of both the cause and the effect of environmental pollution on diabetes. Our finding that 1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin; 2,3,4,7,8-Pentachlorodibenzofuran, and PCB 118 were associated with total diabetes without nephropathy suggests these environmental toxicants may be especially important factors in the etiology of diabetes, however future investigations are needed to confirm this assertion. Additionally, the cross-sectional differences between those with diabetic nephropathy and those with total diabetes without nephropathy suggest that these two groups should be analyzed separately in future investigations.

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