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Association of DDT and heptachlor epoxide in human blood with diabetic nephropathy

Abstract: Six organochlorine pesticides and pesticide metabolites in human blood were tested to determine their relationships with diabetic nephropathy. The data were derived from the National Health and Nutrition Examination Survey (NHANES) 1999–2004 (unweighted, n=2992, population estimate=133,088,752). The six chemicals were *p,p′*-DDT (dichlorodiphenyltrichloroethane), *p,p′*-DDE (dichlorodiphenyltrichloroethylene), beta-hexachlorocyclohexane, oxychlordane, *trans*-nonachlor and heptachlor epoxide. In this research, total diabetes included diagnosed and undiagnosed diabetes (glycohemoglobin, A1c ≥6.5%), and nephropathy was defined as a urinary albumin to creatinine ratio >30 mg/g, representing microalbuminuria and macroalbuminuria. The pesticide *p,p′*-DDT and pesticide metabolite heptachlor epoxide were significantly associated with total diabetes with nephropathy, with odds ratios of 2.08 (95% CI 1.06–4.11) and 1.75 (95% CI 1.05–2.93), respectively. Organochlorine pesticides are thought to act through the constitutive androstane receptor/pregnane X receptor disease pathway, but this is not well established. When *p,p′*-DDT and heptachlor epoxide were both elevated, the odds ratio for diabetic nephropathy was 2.76 (95% CI 1.31–5.81), and when six of six organochlorine pesticides and pesticide metabolites, were elevated, the odds ratio for diabetic nephropathy was 3.00 (95% CI 1.08–8.36). The differences in the odds ratios for these groups appear to be due to differences in the mean heptachlor epoxide concentration of each category. Organochlorine pesticides and pesticide metabolites are known to have estrogenic, antiestrogenic or antiandrogenic activity. The constitutive androstane receptor/pregnane X receptor pathway is thought to interact with the aryl hydrocarbon receptor pathway, and the associations noted may be due to that interaction.

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Keywords: diabetes; kidney disease; National Health and Nutrition Examination Survey (NHANES); organochlorine pesticides.

DOI 10.1515/reveh-2015-0003
Received January 27, 2015; accepted February 25, 2015; previously published online March 30, 2015

Introduction

Diabetic nephropathy is associated both with risk factors caused by environmental pollution and those found naturally in the environment. Natural and man-made environmental risk factors for diabetic nephropathy include cadmium, iron, lead, arsenic, polychlorinated organic compounds (otherwise known as persistent organic pollutants), environmental tobacco smoke, nitrogen compounds and contrast agents (1–4). Persistent organic pollutants are thought to act through two disease pathways, the aryl hydrocarbon receptor pathway and the constitutive androstane receptor/pregnane X receptor pathway (5).

It is well established that dioxins, furans and dioxin-like polychlorinated biphenyls (dl-PCBs) act through the aryl hydrocarbon receptor pathway (6). We studied 23 of the 29 chlorinated dioxin-like compounds that were reported to have toxic equivalency factors by Van den Berg et al. (7). Concentrations in human blood and toxic equivalency factors of each chemical were used to calculate total toxic equivalency (TEQ) for each person. The natural log of total toxic equivalency [ln(TEQ+1)] was then used as a continuous variable to test its association with diabetic nephropathy and with diabetes without nephropathy. A total of 11.9% of the sample had no compounds above the maximum limit of detection and were assigned a TEQ of 0 fg/g. The median TEQ was 34.54 fg/g, and the 75th percentile had a TEQ of 81.66 fg/g. The odds ratio for log-transformed toxic equivalency and diabetic nephropathy was 2.35 (95% CI 1.57–3.52), whereas the odds ratio for log-transformed toxic equivalency and diabetes without nephropathy was 1.44 (95% CI 1.11–1.87). Comparing the 75th percentile of TEQ to 0 fg/g TEQ we found that the odds ratio was 6.96 for diabetic nephropathy, and 2.94

for diabetes without nephropathy. The magnitude of the odds ratio for diabetic nephropathy suggests this association with TEQ is due to reverse causation. Reverse causality is when the expected effect precedes the expected cause, that is, diabetic nephropathy would precede a rise in dioxin-like chemicals in the blood. However, it is also possible that high levels of dioxin and dioxin-like chemicals may be both a case of reverse causality and a risk factor for diabetic nephropathy. We hypothesize that there is a negative feedback loop whereby a rise in dioxin-like chemicals in blood would cause the beginnings of diabetic kidney disease, which in turn would promote a build up of more dioxin-like chemicals as the kidneys become less efficient at removing toxins from the blood (8). Alternatively, diabetic nephropathy may be associated with another condition that affects metabolism of dioxin-like chemicals. Lee et al. (9) found elevated dioxins and furans to be significantly associated with fatty liver and gamma-glutamyltransferase (GGT), which suggests liver function may be involved.

Non-dioxin-like polychlorinated biphenyls and organochlorine pesticides are presumed to act through the constitutive androstane receptor/pregnane X receptor pathway (5), but less is known about this disease pathway than the aryl hydrocarbon receptor pathway. Organochlorine pesticides have estrogenic, antiestrogenic or antiandrogenic activity (10). The properties of specific organochlorine pesticides and pesticide metabolites are not easily determined. Notably, DDT has estrogen agonist activity, whereas its metabolite, DDE, has antiestrogenic properties (11).

We investigated the association of six organochlorine pesticides and pesticide metabolites, and diabetic nephropathy. The six chemicals were: *p,p′*-DDT (dichlorodiphenyltrichloroethane), *p,p′*-DDE (dichlorodiphenyltrichloroethylene), beta-hexachlorocyclohexane, oxychlordane, *trans*-nonachlor, and heptachlor epoxide. DDT, an insecticide, was banned in the US in 1973, but was used in Mexico until 2000. Hispanics in the US are known to have higher concentrations of DDT in their blood than other races/ethnicities. DDE is a metabolite of DDT. Beta-hexachlorocyclohexane is a component of technical-grade lindane, a fungicide and insecticide, banned for many agricultural uses in 1985. Technical-grade chlordane includes *cis*-chlordane, *trans*-chlordane, heptachlor and *trans*-nonachlor. Use of technical-grade chlordane was restricted in 1983 and banned in 1988. *Trans*-nonachlor is a component of technical-grade chlordane. Oxychlordane is a metabolite of chlordane. Heptachlor, an insecticide, was banned in the US in 1988, and heptachlor epoxide is a metabolite of heptachlor (12). In a previous study, all six of these pesticides and pesticide metabolites were associated with diabetes, without regard to whether or not the subjects had nephropathy (13).

The purpose of this paper is to elaborate on the analyses of six organochlorine pesticides and pesticide metabolites and diabetic nephropathy that we have conducted, and to compare those findings to the results obtained for dioxins, furans and dl-PCBs and diabetic nephropathy (8).

Methods

The methods used in this study followed those used in our study of dioxins, furans and dl-PCBs (8). Both studies utilized data available from the National Health and Nutrition Examination Survey (NHANES) 1999–2004. The persons included in each study deferred for two reasons. First, organochlorine pesticides and dioxin-like compounds were determined in the same one-third subsample in 1999–2000 and 2001–2002, but in separate subsamples in 2003–2004. Second, the organochlorine pesticide study only included six compounds, and the study of dioxin-like chemicals included 23 compounds. The study of organochlorine pesticides had less missing data excluded and therefore more people in the study (unweighted n=2992 vs. unweighted n=2588). Detailed information on the methodology of the NHANES 1999–2004, including laboratory assessment, can be found at the National Center for Health Statistics website (14). Nephropathy was defined as a urinary albumin to creatinine ratio >30 mg/g, representing both microalbuminuria and macroalbuminuria (15). Total diabetes was defined as either diagnosed or undiagnosed diabetes. Undiagnosed diabetes was defined as persons who had glycohemoglobin (A1c) ≥6.5% (16). We did not use fasting plasma glucose for determination of undiagnosed diabetes because it was only measured on a fasting subsample of participants and would have reduced by half the number of persons in our analyses.

Six organochlorine pesticides and pesticide metabolities 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 ≥20 years old. The unweighted number of participants in our study was 2992, which represented 133,088,752 non-incarcerated US adults (Table 1). The pesticides and pesticide metabolities were measured in serum by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry, and the concentrations were expressed per g of blood, rather than adjusting for lipids, as recommended by Schisterman et al. (17). Each person had a sample-specific limit of detection.

When possible, the organochlorine pesticides were classified as having three levels: the first quartile (used as the reference group), fourth quartile and an intermediate category including the second and third quartiles (*p,p′*-DDE and *trans*-nonachlor, Table 2). When the proportion of the sample, below the maximum limit of detection, was >25%, the reference group included everyone with concentrations below the maximum limit of detection (*p,p′*-DDT, beta-hexachlorocyclohexane, oxychlordane, and heptachlor epoxide). In cases where the proportion below the maximum limit of detection was >75% of the sample, the organochlorine pesticides were classified as having two levels, above and below the maximum limit of detection (*p,p′*-DDT and beta-hexachlorocyclohexane). Hence, the

Table 1: Diabetes categories and number of organochlorine pesticides and pesticide metabolites elevated.

	Unweighted n	Population estimate	Proportion, %
Diabetes category			
Normal A1c (<5.7%)			
– Without nephropathy	2028	99,786,292	75.0
– With nephropathy	176	6,560,075	4.9
Pre-diabetes (A1c 5.7%–6.4%)			
– Without nephropathy	385	14,453,160	10.8
– With nephropathy	62	2,033,872	1.5
Total diabetes (diagnosed or A1c ≥6.5%)			
– Without nephropathy	216	7,060,864	5.3
– With nephropathy	125	3,194,489	2.4
Number of compounds elevated			
0	1307	70,462,934	52.9
1	408	19,534,623	14.7
2	293	11,179,093	8.4
3	284	10,870,009	8.2
4	286	9,610,653	7.2
5	257	7,506,336	5.6
6	157	3,925,104	2.9
DDT/heptachlor epoxide elevated			
Neither elevated	1905	93,944,553	70.6
One elevated	718	29,613,283	22.2
Both elevated	369	9,530,916	7.2
Total sample	2992	133,088,752	100

Table 2: Categories for six organochlorine pesticides and pesticide metabolites.

	Unweighted n	Population estimate	Proportion, %
Beta-hexachlorocyclohexane, ng/g			
<0.1018	2219	107,733,733	80.9
≥0.1018	773	25,355,019	19.0
p,p′-DDE, ng/g			
<0.8340	608	33,268,792	25.0
0.8340–3.8410	1276	66,541,411	50.0
≥3.8411	1108	33,278,548	25.0
p,p′-DDT, ng/g			
<0.0860	2398	117,901,470	88.6
≥0.0860	594	15,187,281	11.4
Oxychlordane, ng/g			
<0.0470	848	39,069,218	29.4
0.0470–0.1443	1210	60,624,731	45.6
≥0.1444	934	33,394,803	25.1
Trans-nonachlor, ng/g			
<0.0611	717	33,269,889	25.0
0.0611–0.2227	1329	66,494,595	50.0
≥0.2228	946	33,324,267	25.0
Heptachlor epoxide, ng/g			
<0.0550	2020	94,604,261	71.1
0.0550–0.0607	110	4,996,657	3.8
≥0.0608	862	33,487,833	25.2

proportion considered elevated was 11% for *p,p′*-DDT, 19% for beta-hexachlorocyclohexane and 25% for the other four organochlorine pesticides and pesticide metabolites (Table 2). We also counted the number of compounds elevated and used those persons with no compounds elevated as the reference group for the analyses (Table 1).

We tested the associations of six organochlorine pesticides and pesticide metabolities, and the number of compounds elevated, with total diabetes with nephropathy and total diabetes without nephropathy, in logistic regression models adjusted for participant age, sex, race/ethnicity, education, poverty-to-income ratio, energy adjusted fruit and vegetable consumption, physical activity, family history of diabetes and body mass index. Definitions of the covariates, and categories used were previously described in Everett and Thompson (8). We choose to include body mass index in our models because we found previously that including it made our results more conservative (8).

We used SAS version 9.3 for all analyses (18). 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 1) 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 oversampled and a complex sampling design was employed, sampling weights provided by the NHANES for the organochlorine pesticide subsample were used to compute population estimates based on weighted parameter estimates and standard errors (14).

Results and discussion

Of the persons with total diabetes (diagnosed and undiagnosed diabetes), 31.1% had nephropathy (unweighted n=125, Table 1). We excluded persons with pre-diabetes (16) from our analyses, as we have noted effects of dioxins and dioxin-like chemicals in the pre-diabetes range (19). We also excluded those with nephropathy who had normal A1c (<5.7%), from our reference group. Analyses of organochlorine pesticides and pre-diabetes could be the topic of a future study.

The proportion of the US population with one or more of the six pesticides and pesticide metabolites elevated was 47.1% (Table 1). Four of the compounds were significantly associated with diabetes without nephropathy. These were beta-hexachlorocyclohexane, oxychlordane, *trans*-nonachlor and heptachlor epoxide (Table 3), with elevated heptachlor epoxide having an odds ratio of 4.12

Table 3: Association of six organochlorine pesticides and pesticide metabolites with total diabetes, with and without nephropathy.[a]

	Total diabetes without nephropathy		Total diabetes with nephropathy	
	Odds ratio	95% CI	Odds ratio	95% CI
Beta-hexachlorocyclohexane, ng/g				
<0.1018	1.00	–	1.00	–
≥0.1018	1.89	1.08–3.31	1.49	0.90–2.46
p,p′-DDE, ng/g				
<0.8340	1.00	–	1.00	–
0.8340–3.8410	2.32	0.93–5.82	0.60	0.27–1.31
≥3.8411	2.66	0.95–7.44	0.78	0.34–1.79
p,p′-DDT, ng/g				
<0.0860	1.00	–	1.00	–
≥0.0860	1.82	0.90–3.66	2.08	1.06–4.11
Oxychlordane, ng/g				
<0.0470	1.00	–	1.00	–
0.0470–0.1443	1.23	0.58–2.59	1.17	0.46–2.98
≥0.1444	2.87	1.18–6.98	2.39	0.75–7.65
Trans-nonachlor, ng/g				
<0.0611	1.00	–	1.00	–
0.0611–0.2227	0.99	0.50–1.94	0.94	0.30–2.97
≥0.2228	2.50	1.10–5.67	1.54	0.45–5.22
Heptachlor epoxide, ng/g				
<0.0550	1.00	–	1.00	–
0.0550–0.0607	0.70	0.21–2.31	1.43	0.23–8.87
≥0.0608	4.12	2.78–6.10	1.75	1.05–2.93

[a]Adjusted for age, gender, race/ethnicity, education, poverty income ratio, body mass index, energy adjusted fruit and vegetable consumption, physical activity and family history of diabetes.

Table 4: Association of the number of organochlorine pesticides and pesticide metabolites elevated with total diabetes, with and without nephropathy.[a]

	Total diabetes without nephropathy		Total diabetes with nephropathy	
	Odds ratio	95% CI	Odds ratio	95% CI
Number of compounds elevated				
0	1.00	–	1.00	–
1	2.63	1.17–5.95	0.59	0.14–2.46
2	3.61	1.45–9.00	0.65	0.23–1.84
3	4.48	1.78–11.33	1.32	0.49–3.56
4	4.63	1.84–11.63	2.02	0.94–4.30
5	5.94	2.13–16.56	1.69	0.70–4.07
6	8.72	2.79–27.27	3.00	1.08–8.36
0	1.00	–	1.00	–
≥1	3.81	1.88–7.72	1.23	0.61–2.48
DDT/heptachlor epoxide elevated				
Neither elevated	1.00	–	1.00	–
One elevated	3.51	2.07–5.93	1.27	0.72–2.24
Both elevated	4.82	2.42–9.62	2.76	1.31–5.81

[a]Adjusted for age, gender, race/ethnicity, education, poverty income ratio, body mass index, energy adjusted fruit and vegetable consumption, physical activity and family history of diabetes.

(95% CI 2.78–6.10) for total diabetes without nephropathy. When the number of compounds elevated was evaluated, each individual number from 1 to 6 was associated with total diabetes without nephropathy compared to the group with none of the compounds elevated (Table 4). One or more compounds elevated had an odds ratio of 3.81 (95% CI 1.88–7.72) for total diabetes without nephropathy. Hence, results obtained previously for the six organochlorine pesticides and diabetes, without regard to nephropathy (13), were strongly influenced by persons who had diabetes, but not nephropathy.

The pesticide *p,p′*-DDT and pesticide metabolite heptachlor epoxide were significantly associated with total diabetes with nephropathy, with odds ratios of 2.08 (95% CI 1.06–4.11) and 1.75 (95% CI 1.05–2.93), respectively (Table 3). When *p,p′*-DDT and heptachlor epoxide were both elevated, the odds ratio for diabetic nephropathy was 2.76 (95% CI 1.31–5.81), and when six of six organochlorine pesticides and pesticide metabolites were elevated, the odds ratio for diabetic nephropathy was 3.00 (95% CI 1.08–8.36). The differences in the odds ratios for these groups appeared to be due to differences in the mean heptachlor epoxide concentration of each category. The heptachlor epoxide elevated group (unweighted n=862) had a mean of 0.128 ng/g (95% CI 0.115–0.140 ng/g) heptachlor epoxide, the DDT/heptachlor epoxide elevated group (unweighted n=369) had a mean of 0.166 ng/g (95% CI 0.139–0.193 ng/g) heptachlor epoxide, and the six of six compounds elevated group (unweighted n=157) had a mean of 0.207 ng/g (95% CI 0.162–0.251 ng/g) heptachlor epoxide. In contrast, the *p,p′*-DDT elevated group (unweighted n=594) had a mean of 0.35 ng/g (95% CI 0.28–0.42 ng/g) *p,p′*-DDT, the DDT/heptachlor epoxide elevated group (unweighted n=369) had a mean of 0.28 ng/g (95% CI 0.20–0.36 ng/g) *p,p′*-DDT, and the six of six compounds elevated group (unweighted n=157) had a mean of 0.27 ng/g (95% CI 0.21–0.33 ng/g) *p,p′*-DDT, indicating little difference in DDT concentration among these categories.

Whereas the results indicate significant associations of DDT and heptachlor epoxide with diabetic nephropathy, they do not suggest reverse causality. This difference between dioxin-like chemicals and organochlorine pesticides and pesticide metabolites may be due to different disease pathways involved. However, we can only speculate on this point. The findings are important as, at

the time of the study, 7.2% of the US population had both elevated DDT and elevated heptachlor epoxide in their blood. Given that the data for this study was collected in 1999–2004, and the pesticides of interest were banned in the 1970s and 1980s, an update to the current decade would be useful.

Conclusion

Natural and man-made environmental risk factors for diabetic nephropathy include cadmium, iron, lead, arsenic, polychlorinated organic compounds, environmental tobacco smoke, nitrogen compounds and contrast agents. We have conducted research on diabetic nephropathy and two classes of polychlorinated organic compounds, namely dioxins, furans and dl-PCBs, and organochlorine pesticides. Dioxin and dioxin-like compounds are highly associated with diabetic nephropathy, so much so that it appears to be a case of reverse causality. However, it is also possible that high levels of dioxin and dioxin-like chemicals may be both a case of reverse causality and a risk factor for diabetic nephropathy. We also tested the association of six organochlorine pesticides and found *p,p′*-DDT and heptachlor epoxide were associated with diabetic nephropathy. We further found higher odds ratios for diabetic nephropathy among those with both elevated DDT and elevated heptachlor epoxide and when six of six compounds were elevated. The odds ratios for these categories appears to be due to higher mean heptachlor epoxide concentrations in these groups and not differences in DDT concentrations. Notably, when either DDT or heptachlor epoxide were elevated, but not both, the odds ratio for diabetic nephropathy was not significantly different from the reference group of neither elevated (odds ratio 1.27, 95% CI 0.72–2.24, Table 4). Combinations of environmental pollutants and diabetic nephropathy should be studied more frequently.

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