A Strong Dose-Response Relation Between Serum Concentrations of Persistent Organic Pollutants and Diabetes

Results from the National Health and Examination Survey 1999–2002

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OBJECTIVE — Low-level exposure to some persistent organic pollutants (POPs) has recently become a focus because of their possible link with the risk of diabetes.

RESEARCH DESIGN AND METHODS — Cross-sectional associations of the serum concentrations of POPs with diabetes prevalence were investigated in 2,016 adult participants in the National Health and Nutrition Examination Survey 1999–2002. Six POPs (2,2′,4,4′,5,5′-hexachlorobiphenyl, 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin, 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin, oxychlordane, *p,p*′-dichlorodiphenyltrichloroethane, and *trans*-nonachlor) were selected, because they were detectable in ≥80% of participants.

RESULTS — Compared with subjects with serum concentrations below the limit of detection, after adjustment for age, sex, race and ethnicity, poverty income ratio, BMI, and waist circumference, diabetes prevalence was strongly positively associated with lipid-adjusted serum concentrations of all six POPs. When the participants were classified according to the sum of category numbers of the six POPs, adjusted odds ratios were 1.0, 14.0, 14.7, 38.3, and 37.7 (P for trend < 0.001). The association was consistent in stratified analyses and stronger in younger participants, Mexican Americans, and obese individuals.

CONCLUSIONS — There were striking dose-response relations between serum concentrations of six selected POPs and the prevalence of diabetes. The strong graded association could offer a compelling challenge to future epidemiologic and toxicological research.

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Abbreviations: AhR, aryl hydrocarbon receptor; DDE, *p,p*′-dichlorodiphenyltrichloroethane; HCB, hexachlorobenzene; HpCDD, 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin; LOD, limit of detection; NHANES, National Health and Nutrition Examination Survey; OCDD, 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin; PCB, polychlorinated biphenyl; PCB153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran; POP, persistent organic pollutant; SUMPOP, sum of POP levels; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEF, toxic equivalency factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Persistent organic pollutants (POPs) have become widespread environmental contaminants and now represent a global problem (1). The toxicity of these pollutants in humans and wildlife is enhanced by their persistence in the environment and their bioaccumulation potential in the tissues of animals and humans through the food chain (1). POPs include a variety of man-made chemicals. Some POPs, including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), and several organochlorines used as pesticides have been highlighted by international organizations as being chemicals of concern (2).

Low-level exposure to some POPs has recently been associated with an increased risk of diabetes (3). Prospective cohort studies of subjects exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most potent dioxin congener of POPs, or other POPs in occupational or accidental settings have reported increased risk of diabetes, modified glucose metabolism, or insulin resistance (4–10). The U.S. Department of Veterans Affairs added type 2 diabetes to the list of presumptive diseases associated with the exposure to dioxin-containing Agent Orange in Vietnam (11).

However, whether similar associations exist in the general population with lifetime exposure to very low doses of a mixture of various POPs is not known. Given that almost everyone has measurable amounts of POPs, the public health significance of a relation of mixed dioxins with diabetes may be substantial despite a relatively modest association with any individual dioxin.

TCDD-mediated diabetes might reflect decreased expression of the insulin-responsive glucose transporter GLUT4. Several animal studies have demonstrated a TCDD-mediated decrease in glucose transport in vitro and in vivo (12–16). The stimulation of tumor necrosis factor-α expression by TCDD in adipose tis-

sue and other cell types (17,18) is relevant to insulin resistance and diabetes (19–21). Recently, a genome-wide gene expression study reported that aryl hydrocarbon nuclear receptor translocator was most substantially reduced in type 2 diabetic islets; dioxin is a ligand of the aryl hydrocarbon receptor (AhR), which is a partner of aryl hydrocarbon nuclear receptor translocator (22).

In this study, we investigated associations of prevalent diabetes with the serum concentrations of POPs. We could not study all 49 POPs measured in the National Health and Examination Survey (NHANES) 1999–2000 and 2001–2002 (23) because a majority of participants had serum concentrations under the limit of detection (LOD) of many kinds of POPs. We therefore selected six POPs (2,2′,4,4′,5,5′-hexachlorobiphenyl [PCB153], 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin [HpCDD], 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin [OCDD], oxychlordane, *p,p*′-dichlorodiphenyltrichloroethane [DDE], and transnonachlor) for which at least 80% of study subjects had concentrations more than the LOD.

RESEARCH DESIGN AND METHODS

— The 1999–2000 and 2001–2002 NHANES (public use dataset, http://www.cdc.gov/nchs/about/major/nhanes/datalink.htm) conducted by the Centers for Disease Control and Prevention were designed to be nationally representative of the noninstitutionalized U.S. civilian population on the basis of a complex multistage probability sample. Details of the NHANES protocol and procedures are available elsewhere (24,25).

PCDDs, PCDFs, PCBs, and organochlorine pesticides were measured in serum from a random one-third subsample of subjects aged ≥12 years in 1999 and 2000. In 2001 and 2002, dioxins, furans, and coplanar PCBs were measured in a random one-third subsample of people aged ≥20 years, and organochlorine pesticides and other PCBs were measured in a random one-third subsample of people aged ≥12 years. For this analysis, data from the two surveys were aggregated. A total of 2,016 study participants aged ≥20 years with information on serum concentrations of six POPs were analyzed.

The NHANES standardized home interview was followed by a detailed physical examination in a mobile evaluation clinic or the participant's home. Information on demographic characteristics, ethnicity, and medical history of diabetes was obtained in a household interview. Information on history of diabetes included questions about prior diagnoses of diabetes by a physician and current use of insulin and oral hypoglycemic agents. We substituted median values of nonsubjects for missing BMI (measured as kilograms divided by the square of height in meters), waist circumference, or poverty income ratio in 291 subjects; exclusion of these 291 individuals did not change any conclusions.

Venous blood and urine samples were collected and shipped weekly at −20°C. PCDDs, PCDFs, PCBs, and organochlorine pesticides were all measured as individual chemicals by high-resolution gas chromatography/high-resolution mass spectrometry using isotope dilution for quantification. All analytes were measured in ~5 ml of serum using a modification of the method of Turner et al. (26). The POPs were provided by NHANES and adjusted for serum total cholesterol and triglycerides. Plasma fasting glucose was measured using a modified hexokinase enzymatic method.

For each POP, the reference group was subjects with serum concentrations under the LOD, and subjects with detectable values were further categorized using the 25th, 50th, 75th, and 90th percentiles. It should be noted that these analyses have an individual LOD that varied with the extractable sample volume. Only the maximum observed LOD was provided by NHANES. A higher sample volume results in a lower LOD and a better ability to detect low levels of a substance. To evaluate the summary effect of six POPs, the category number of each POP (0 assigned to the nondetectable category, and 1 through 5 assigned to successively increasing categories) was added to make the sum of POP levels (SUMPOPs), producing a value of 0–30, which was itself categorized at its 25th, 50th, 75th, and 90th percentiles, making five groups.

Participants were considered to have diabetes if *1*) their fasting plasma glucose was ≥126 mg/dl or their nonfasting plasma glucose was ≥200 mg/dl or *2*) they reported a history of physician-diagnosed diabetes. Exclusion of nonfasting subjects did not greatly change the estimates. However, 34.5% of the 2,016 study subjects did not fast for at least 8 h, and their exclusion substantially limited stratified analysis. Thus, we present results based on 2,016 study subjects. Logistic regression models were used to calculate multivariate-adjusted odd ratios (ORs). Adjusting variables were sex, race/ethnicity, age (years), poverty income ratio (continuous), BMI (continuous), and waist circumference (continuous). Subgroup analyses stratified by age, sex, race/ethnicity, poverty income ratio, or BMI were performed. All statistical analyses were performed with SAS 9.1 and SUDAAN 9.0. Estimates of the main results were calculated to account for stratification and clustering (27), adjusting for age, race and ethnicity, and poverty income ratio instead of using sample weights; this adjustment is regarded as a good compromise between efficiency and bias (27,28). The rarity of diabetes in the least exposed category of SUMPOPs and in the stratified analyses presented a statistical challenge because of possible unreliability of the absolute risk estimate at very low risk, resulting in very wide confidence limits for ORs relative to this natural reference category. To address this statistical problem, we present ORs for prevalent diabetes according to SUMPOPs in two alternative formats, one using the lowest category and one the second-lowest category as the referent. We did not do this in the stratified analyses because the number at risk in the different POP categories varied considerably across strata, making the strategy of shifting referent category confusing. There were zero cases in the least-exposed categories in some strata, yielding an infinite OR; we conservatively estimated ORs by artificially adding one case in those lowest exposure categories.

RESULTS

— Age was the closest correlate of serum concentrations of all POPs with correlation coefficients ranging from 0.44 (HpCDD) to 0.74 (oxychlordane) (Table 1). For example, the mean age of subjects with PCB153 nondetectable or <25th percentile was 35 years compared with a mean age of 68 years for subjects with concentrations >90th percentile among those with detectable concentrations. Men tended to have lower concentrations of most POPs, especially OCDD. Both non-Hispanic white race/ethnicity and poverty income ratio were inversely associated with most POPs, especially DDE. After adjustment for age, the relations of BMI with POPs were variable depending on POPs. There were significant positive associations of BMI with

Table 1—*Spearman correlation coefficients* among six POPs with age, sex, race and ethnicity, poverty income ratio, BMI, and waist circumference*

	PCB153	HpCDD	OCDD	Oxychlordane	DDE	*trans*-Nonachlor
Age	+0.69†	+0.44†	+0.48†	+0.74†	+0.50†	+0.73†
Men‡	+0.01	−0.16†	−0.30†	−0.08†	−0.06	+0.01
Non-Hispanic white race‡	+0.04	−0.11†	−0.11†	−0.04	−0.47†	−0.13†
Poverty income ratio‡	+0.11†	−0.03	−0.09†	−0.02	−0.21†	−0.07†
BMI‡	−0.09†	+0.19†	+0.13†	+0.04	+0.06†	+0.03
Waist circumference‡	−0.08†	+0.11†	+0.04	+0.01	−0.01	+0.01

*Detectable values of each POP were individually ranked to calculate correlation coefficients. All nondetectable values were ranked as 0. †$P < 0.01$. ‡Age adjusted.

HpCDD, OCDD, or DDE, whereas BMI was inversely associated with PCB153 (Table 1). There were strong correlations in serum concentrations among all POPs, e.g., $r = 0.92$ was seen for oxychlordane and *trans*-nonachlor, and correlation of each of these with PCB153 was about 0.7. HpCDD and OCDD had a correlation of 0.78. All other pairwise correlations of POPs were in the range of 0.37 to 0.53.

Prevalence of diabetes ($n = 217$) was unrelated to TCDD, which was detectable in only 7% of the sample. However, diabetes was strongly positively associated with all six POPs detectable in at least 80% of the sample, especially PCB153, oxychlordane, and *trans*-nonachlor (Table 2), after adjustment for age, sex, race/ethnicity, poverty income ratio, BMI, and waist circumference. Additional adjustment for triglyceride, cholesterol, saturated fat intake, and cigarette smoking did not materially change the results (data not shown). When the study subjects were classified according to SUMPOPs, adjusted ORs for diabetes were 1.0, 14.0, 14.7, 38.3, and 37.3 (P for trend < 0.001) (Table 2). After exclusion of nonfasting subjects, adjusted ORs were 1.0, 16.9, 15.8, 36.7, and 38.0 (P for trend < 0.001). Examination of the alternate analysis using the second-lowest exposure category as referent highlights the large increase in risk between the lowest and second-lowest exposure categories, with a further significant 2.5-fold increase in ORs at >75th percentile of SUMPOPs. The association between SUMPOPs and fasting glucose levels among nondiabetic subjects showed a weak positive trend (adjusted means 88.3, 89.5, 89.9, 89.5, and 90.7 mg/dl, P for trend = 0.08).

After stratification by age, sex, race/ethnicity, poverty income ratio, or BMI, the prevalence of diabetes increased with increasing concentration category of the six POPs in most subgroups (Table 3). Although most P values for interaction were nonsignificant, the associations tended to be stronger among younger subjects, Mexican Americans, and obese subjects. It was interesting that there was no association between obesity and diabetes among subjects with nondetectable levels of POPs, despite the substantial numbers at risk in each BMI category.

CONCLUSIONS — An inference that observed associations are causal should be made carefully in a cross-sectional study such as this one. It may be that metabolic changes caused by diabetes slow metabolism and/or excretion of POPs, leading to a greater accumulation. The fact that diabetes was associated with all six POPs investigated, despite different toxicological profiles, could lend credence to such an alternative possibility. However, we think that the relation between POPs and diabetes observed in this study may be causal for several reasons. First, our finding is basically consistent with prospective cohort studies whose study subjects were exposed to high doses of POPs in occupational or accidental settings, despite a difference in strength of association (4–10). As we discuss later, the strength of association in the current study subjects with large chronic lifetime exposure to low doses of POPs could be stronger than in those with short-term exposure to high doses of POPs. Second, the idea that dioxin exposure may cause diabetes is in line with the known biology of these pollutants. Third, reverse causality is unlikely because the metabolism of POPs in mammalian systems is intractable; the half-life of the compounds ranges from 7 to 10 years in humans (29,30). Supporting our assertion, one human study reported that the rate of elimination of POPs from blood was not associated with the duration of diabetes (31). Fourth, the associations of diabetes with all the POPs investigated may be reasonably explained by the high correlations among serum concentrations of various POPs in the human body. Yet it is entirely possible that the six POPs studied here are not themselves causally related to diabetes. Rather, they could be surrogates of exposure to a mixture of POPs. Finally, >90% of POPs comes from animal foods in the general population without occupational or accidental exposures (1), but diabetic patients tend to alter their diet toward consuming more plant foods than animal foods. Thus, dietary changes after diagnosis of diabetes would seem to be a negative confounder, not a positive one. Another scientifically interesting finding was that obesity did not increase the prevalence of diabetes among subjects with nondetectable levels of POPs even though there were sufficient numbers of study subjects at risk in each BMI category.

In the U.S., the serum concentrations of POPs in the general population have been decreasing over several decades (32). Thus, the current dramatic increase in type 2 diabetes incidence may be puzzling if the striking association between serum concentration of POPs and diabetes shown in this study is causal. This puzzle may be explained by the epidemic of obesity in the U.S.; our study showed that the association between POPs and diabetes was much stronger among obese subjects compared with that of lean subjects. As people get fatter, the retention and toxicity of POPs related to the risk of diabetes may increase.

The concept of toxic equivalency factors (TEFs), a measure of ability to bind to the AhR, has been developed to facilitate risk assessment and regulatory control of exposure to complex PCDD, PCDF, and PCB mixtures (33,34). However, we did not use TEFs to calculate the cumulative effect of POPs because the strength of association of each POP observed in this study did not appear to be correlated with the TEF of each POP, leading us to hypothesize that binding to the AhR may not

Table 2—*Adjusted ORs and 95% CI of prevalent diabetes by category of six POPs*

Analyte	Not detectable	Detectable*					P_{trend}
		<25th*	25th to <50th	50th to <75th	75th to <90th	≥90th	
PCB153							
Conc. (ng/g of lipid)	—	14.3	36.7	60.2	93.6	164	
Cases/*n*	10/413	20/397	48/404	67/401	42/237	30/164	
Prevalence (%)	2.4	5.0	11.9	16.7	17.7	18.3	
Adjusted OR (95% CI)†	Referent	2.5 (1.1–6.0)	4.3 (2.2–8.6)	5.9 (3.0–11.9)	5.9 (3.1–11.3)	6.8 (3.0–15.5)	<0.001
HpCDD							
Conc. (pg/g of lipid)	—	20.7	37.8	60.8	97.5	170	
Cases/*n*	12/263	19/436	44/439	56/439	40/262	46/177	
Prevalence (%)	4.6	4.4	10.0	12.8	15.3	26.0	
Adjusted OR (95% CI)†	Referent	1.0 (0.5–2.1)	1.7 (0.8–3.8)	1.8 (0.9–3.3)	1.6 (0.6–3.9)	2.7 (1.3–5.5)	0.007
OCDD							
Conc. (pg/g of lipid)	—	194	323	514	805	1,485	
Cases/*n*	13/390	30/401	47/410	46/408	50/241	31/166	
Prevalence (%)	3.3	7.5	11.5	11.3	20.8	18.7	
Adjusted OR (95% CI)†	Referent	1.7 (0.8–3.6)	2.2 (1.0–5.0)	1.6 (0.7–3.7)	2.7 (1.2–6.2)	2.1 (0.9–5.2)	0.094
Oxychlordane							
Conc. (ng/g of lipid)	—	8.5	15.4	25.1	39.1	65.5	
Cases/*n*	11/359	11/404	32/422	63/416	51/249	49/166	
Prevalence (%)	3.1	2.7	7.6	15.1	20.5	29.5	
Adjusted OR (95% CI)†	Referent	0.8 (0.3–2.2)	1.9 (0.7–5.5)	3.1 (1.0–10.1)	4.5 (1.4–14.3)	6.5 (2.0–21.4)	<0.001
DDE							
Conc. (ng/g of lipid)	—	112	292	717	1,560	3,700	
Cases/*n*	—	16/502	35/506	56/502	57/304	53/202	
Prevalence (%)	—	3.2	6.9	11.2	18.8	26.2	
Adjusted OR (95% CI)†	—	Referent	1.5 (0.7–3.1)	1.6 (0.8–3.4)	2.3 (1.0–5.5)	4.3 (1.8–10.2)	<0.001
trans-Nonachlor							
Conc. (ng/g of lipid)	—	11.0	21.7	35.7	60.6	114	
Cases/*n*	4/203	11/451	28/455	60/451	60/274	54/182	
Prevalence (%)	2.0	2.7	6.6	14.0	23.0	32.4	
Adjusted OR (95% CI)†	Referent	1.2 (0.4–3.2)	2.5 (1.0–6.1)	4.9 (2.0–11.8)	7.6 (2.9–19.8)	11.8 (4.4–31.3)	<0.001
Sum of six POPs							
Cases/*n*	—	2/463	34/505	53/527	65/275	63/246	
Prevalence (5)	—	0.4	6.7	10.1	23.6	25.6	
Adjusted OR (95% CI)†	—	Referent	14.0 (3.0–65.0)	14.7 (3.4–63.9)	38.3 (8.0–183.1)	37.7 (7.8–182.0)	<0.001
Adjusted OR‡ (95% CI)†	—	0.07 (0.02–0.33)	Referent	1.1 (0.6–1.7)	2.7 (1.5–4.9)	2.7 (1.5–4.8)	<0.001

*Percentiles were computed among detectables, retaining all nondetectables as the lowest category. †Adjusted for age, sex, race and ethnicity, poverty income ratio, BMI, and waist circumference. ‡ORs with an alternative reference category are presented to more precisely estimate the confidence limits in the higher categories, given theoretical statistical instability in the lowest category, which has only two cases.

Table 3—*Adjusted ORs and 95% CIs of prevalent diabetes by category of the sum of six POPs after stratified by age, sex, race and ethnicity, poverty income ratio, or BMI*

	Category of the sum of six persistent organic pollutants						
	<25th	25th to <50th	50th to <75th	75th to <90th	≥90th	P_{trend}	$P_{interaction}$
Age							
20–39 years							
Cases/*n*	1/388	8/258	1/78	1/10	0/0		
Adjusted OR (95% CI)	Referent	7.6 (0.9–65.2)	3.4 (0.2–61.8)	19.6 (0.9–434.6)	—	0.092	
40–59 years							
Cases/*n*	0/62	16/188	22/228	12/69	12/39		
Adjusted OR (95% CI)	Referent	5.4 (0.7–42.4)	4.6 (0.6–36.2)	10.4 (1.3–86.3)	18.0 (2.1–154.1)	<0.001	
≥60 years							
Cases/*n*	1/13	10/59	30/221	52/196	51/207		
Adjusted OR (95% CI)	Referent	2.3 (0.2–21.0)	1.5 (0.2–12.4)	3.9 (0.5–32.8)	3.8 (0.4–32.3)	<0.001	<0.001
Sex							
Men							
Cases/*n*	0/212	15/237	34/267	33/107	19/80		
Adjusted OR (95% CI)	Referent	11.7 (1.5–90.5)	16.0 (2.0–125.0)	45.6 (5.6–369.0)	27.3 (3.2–230.0)	<0.001	
Women							
Cases/*n*	2/251	19/268	19/260	32/168	44/166		
Adjusted OR (95% CI)	Referent	8.0 (1.8–36.0)	7.2 (1.5–33.8)	18.0 (3.7–87.4)	26.2 (5.2–131.3)	<0.001	0.985
Race/ethnicity							
Non-Hispanic whites							
Cases/*n*	2/224	14/236	18/266	27/137	18/106		
Adjusted OR (95% CI)	Referent	5.6 (1.2–26.4)	3.4 (0.7–16.9)	9.4 (1.8–48.6)	6.8 (1.2–37.7)	0.026	
Non-Hispanic blacks							
Cases/*n*	0/78	5/82	10/81	8/38	18/74		
Adjusted OR (95% CI)	Referent	3.8 (0.4–34.6)	5.6 (0.6–51.2)	8.1 (0.8–84.6)	6.0 (0.6–64.4)	0.246	
Mexican Americans							
Cases/*n*	0/111	8/134	20/149	24/83	24/54		
Adjusted OR (95% CI)	Referent	4.9 (0.6–40.9)	7.4 (0.9–60.9)	16.4 (1.9–140.9)	26.5 (3.0–238.2)	<0.001	0.073
Poverty income ratio							
< Median (2.18)							
Cases/*n*	0/215	21/212	31/224	39/124	40/128		
Adjusted OR (95% CI)	Referent	19.1 (2.5–145.0)	22.7 (2.9–177.3)	65.1 (8.1–525.1)	69.8 (8.5–571.7)	<0.001	0.805
≥ Median (2.18)							
Cases/*n*	2/248	13/293	22/303	26/151	23/118		
Adjusted OR (95% CI)	Referent	5.1 (1.1–23.7)	4.5 (1.0–21.1)	10.7 (2.2–51.7)	8.8 (1.7–44.9)	<0.001	
BMI							
<25 kg/m^2							
Cases/*n*	0/176	6/171	4/145	8/71	9/60		
Adjusted OR (95% CI)	Referent	6.2 (0.7–55.5)	3.0 (0.3–33.1)	11.6 (1.0–132.4)	16.2 (1.4–192.2)	0.013	
25 to <30 kg/m^2							
Cases/*n*	1/158	14/181	20/206	25/117	23/106		
Adjusted OR (95% CI)	Referent	11.0 (1.4–85.3)	9.2 (1.1–73.9)	20.6 (2.5–171.5)	19.8 (2.3–170.5)	0.002	
≥30 kg/m^2							
Cases/*n*	1/129	14/153	29/176	32/87	31/80		
Adjusted OR (95% CI)	Referent	11.5 (1.5–90.8)	19.4 (2.4–155.6)	53.4 (6.4–445.5)	50.1 (5.8–433.1)	<0.001	0.504

Adjusted for age, sex, race and ethnicity, poverty income ratio, BMI, and waist circumference; continuous values of age, BMI, and waist circumference were adjusted in all models. In the subgroups with zero cases in the lowest category, we artificially added one case to that category; covariates were evaluated at the median values of noncases in the category.

be the critical pathway. In fact, the AhR hypothesis does not explain all aspects of toxicity, notably the extreme variation of toxicity between different animal species (35).

In most previous epidemiological studies (4–10), only TCDD was evaluated as a risk factor for diabetes because TCDD is the most potent congener of these POPs. We did not examine TCDD here because so few individuals had detectable levels. In women and non-Hispanic blacks in the NHANES 1999–2002, only the 95th TCDD percentiles could be characterized, which were 6.4 and 7.4 pg/g lipid, respectively (23). The remainder of the U.S. population is likely to have even lower levels of this hallmark dioxin.

Dose-response relations shown in this study were surprisingly strong compared with the weak to modest associations shown in the previous epidemiological studies (4–10). Our study had two important design features lacking in other studies: first, we selected those POPs for which we were sure those with nondetectable levels would have very low levels and could serve as the reference group; and second, we evaluated a composite of POP levels. In our study, the risk of prevalent diabetes increased consistently across the range of SUMPOPs. In this situation, the selection of the reference group is statistically critical to the estimated strength of ORs. For example, if we pooled the lower four categories of POPs as the referent group and compared it with the highest category, the OR would be substantially underestimated. In fact, most previous epidemiological studies on POPs were performed with subjects who had exposure to higher concentrations of POPs in occupational or accidental settings, taking the general population as the reference group. However, our current result suggests that this kind of approach may not be valid because there may be a much clearer dose-response relation in the lower concentrations of background concentrations of POPs in the general population. Interestingly, this observation appeared to be in good agreement with the dose-response relation of TCDD observed in experimental studies. According to experimental studies, the administered dose of TCDD linearly increased the hepatic TCDD concentrations; however, the induction of cytochrome P-450 enzymes (CYP1A1 and CYP1A2), one of the most sensitive responses to TCDD and its structural analogs, increased nonlinearly as a function of the hepatic concentration of TCDD, reaching the maximum effect (36). Similar findings were observed with some PCBs (37). Humans are currently regarded as a less-susceptible species with respect to TCDD or other congeners based on findings of previous epidemiological studies with subjects having high exposure to POPs (38). However, the chronic exposure to low concentrations of POPs in the general population may be more detrimental in developing adverse health effects than previously thought. Along these lines, it is worthwhile to note that the most consistent dose-response associations between POPs and diabetes appeared to occur in epidemiological studies with subjects having lower serum concentrations of TCDD than in occupational settings (4,8), conceivably because of the statistical artifact of not identifying a true low-risk subgroup. Unlike prior studies, in this study, we analyzed several POPs simultaneously so that we could estimate the cumulative effect of exposure mixtures. In most previous studies, only serum concentrations of TCDD were measured. Although TCDD is well known to be the most potent POP because of a strong affinity to AhR, other mechanisms might also be involved in the toxicity of POPs for diabetes (39). Thus, other POPs, as well as TCDD, might be relevant in the pathogenesis of diabetes.

This study has several limitations. The current findings should be interpreted with caution because of the cross-sectional nature of this study, despite both strength and consistency of associations. The NHANES dataset did not allow us to differentiate type 1 from type 2 diabetes, and the association of POP levels with diabetes prevalence might differ by diabetes type. Only 11 subjects were aged <40, so most subjects probably had type 2 diabetes. Experimental studies have shown that TCDD could cause hypoinsulinemia through an alteration of pancreatic membrane tyrosine phosphorylation, suggesting that POPs may be involved in the pathogenesis of type 1 diabetes as well as type 2 diabetes (40). Also, misclassification bias is possible because some subjects with a higher POP value but a lower sample volume could be classified in the reference group or vice versa. Such misclassification would be nondifferential if (as is likely) sample volume is unrelated to prevalence of diabetes. Finally, because diabetes was extremely rare in those with the least exposure to POPs, the reference category may not be stable and ORs could be overestimated.

In summary, there were striking monotonic and additive dose-response relations between serum concentrations of six selected POPs and the prevalence of diabetes. These cross-sectional findings, although not definitive, are sufficiently provocative that further study should be done. A prospective study of the relation between dioxin exposure and diabetes is needed because both the exposure and the disease have substantial prevalence and the public health significance could be marked.

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