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Persistent Organic Pollutants and Risk of Type 2 Diabetes: A Prospective Investigation Among Middle-aged Women in Nurses' Health Study II

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Abstract

Background—Exposure to persistent organic pollutants (POPs) may predispose to the development of type 2 diabetes (T2D), but prospective evidence is needed.

Objectives—We investigated the association between plasma-POP concentrations in the late 1990s and incident T2D during more than 11 years of follow-up in the Nurses' Health Study II.

Discussion—Three organochlorine pesticides and 20 polychlorinated biphenyls (PCBs) were measured in banked plasma from 793 case–control pairs of T2D. In a multiviarate-adjusted model, T2D ORs(95%CIs) comparing extreme POP tertiles (high vs. low) were 1.66(1.24, 2.21; P_{trend}=0.001) for hexachlorobenzene (HCB), 3.64(2.59, 5.13; P_{trend}<0.001) for β-hexachlorocyclohexane (β-HCH), 1.56(1.14, 2.13; P_{trend}<0.001) for p,p′-dichlorodiphenyldichloroethylene (*p,p*'-DDE), and 2.00(1.45, 2.74; P_{trend}<0.001) for PCB toxicity equivalents (PCB-TEQ). Adjustment for previous weight change and concurrent body mass index attenuated these associations, but that for PCB-TEQ remained (OR[95% CI]=1.74[1.12, 2.70]; P_{trend}=0.008). When analysis was stratified by weight change before blood draw, ORs(95% CIs) of T2D comparing extreme (high vs. low) POP groups were 1.97(1.01, 3.85; P_{trend}=0.01) for HCB, 2.67(1.34, 5.34; P_{trend}<0.001) for β-HCH, and 2.41(1.22, 4.75; P_{trend}<0.001) for PCB-TEQ in the lowest weight gain group. In the highest weigh-gain group, corresponding estimates were 1.29(0.74, 2.25; P_{trend}=0.46) for HCB, 1.44(0.79, 2.65; P_{trend}=0.20) for β-HCH, and 0.91(0.51, 1.65; P_{trend}=0.65) for PCB-TEQ (all $P_{interaction}$<0.05).

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Conclusions—Our findings suggest that elevated PCB exposures may have diabetogenic potentials. These data also highlight the impact of lifestyle factors, especially history of weight gain, on circulating POP concentrations and their associations with subsequent T2D risk.

Keywords

persistent organic pollutant; type 2 diabetes; weight change

INTRODUCTION

Persistent organic pollutants (POPs), such as organochlorine compounds (OCs), are resistant to degradation in the environment and highly persistent in the human body (Porta et al. 2008). Although the production and use of the OCs have been banned for decades, the background exposure remains to be a public health concern due to their persistence and adverse effects on, e.g., reproduction, and endocrine functions (EPA 2009). Emerging evidence has also linked POPs to the pathogenesis of type 2 diabetes (T2D), but most studies are cross-sectional and limited in size, with equivocal findings for different POPs (Lee et al. 2014; Taylor et al. 2013).

The exposure, retention, and elimination of POPs in adults are affected by demographic, lifestyle, and biological factors (Milbrath et al. 2009; Thomsen et al. 2010; Zong et al. 2016), some of which, such as age, smoking, breastfeeding, and overweight, are also known risk factors for T2D. For example, adipose tissue is the major storage site for the highly-lipophilic OCs (La Merrill et al. 2013), and changes in body weight can impact on POP retention and blood concentrations (Lim et al. 2011; Wolff et al. 2007). However, the impact of these factors on associations between POP exposures and T2D risk has yet to be considered in prospective studies.

In the present study, we conducted a nested case-control study on plasma-POPs (including 3 organochlorine pesticides [OCPs] and 20 polychlorinated biphenyls [PCBs]) and T2D risk in the Nurses' Health Study II (NHSII). We hypothesized that long-term background POP exposure is an independent risk factor of T2D among middle-aged American women. In addition, we analyzed the associations of plasma-POP concentrations with age, smoking, duration of breastfeeding, and body weight, and their potential modification of POP associations with T2D risk.

METHODS

Study population and sample collection

The NHSII is a prospective cohort study established in 1989, when 116,430 female registered nurses aged 25–42 years were enrolled. Blood samples were collected between 1995 and 2000 among 29,611 women at age of 32–52 years. Briefly, participants had their blood drawn and shipped overnight with an icepack to a central biorepository. Samples were processed immediately upon arrival, and then separated into plasma, red blood cells, and buffy coat. All samples were stored in liquid nitrogen freezers (≤−130°C) until shipment for analysis.

Through June 2011, a total of 793 T2D cases were identified among participants who were free of T2D, cardiovascular disease, or cancer at blood sample collection. For each case, one control was randomly selected among women who remained free of T2D at the case's date of diagnosed using the risk-set sampling scheme (Prentice and Breslow 1978). We matched cases and controls for age at blood sample collection, date/time of sample collection, race (white or other), fasting status when blood was drawn (≥8h or not), and menopausal status and hormone replacement therapy use. T2D cases diagnosed within the first year since blood sample collection were excluded to minimize reverse causation bias. This study was approved at the Committee on the use of Human Subjects in Research at Harvard School of T.H. Chan Public Health and Brigham and Women's Hospital. Informed consent was provided by all participants involved in this research.

Laboratory measurements

Plasma (250 μl) was deproteinized by adding formic acid (250 μl), and then 50 μl of the solution was mixed with internal standards (isotope labeled POPs) and extracted with 400 μl hexane. The solution is cleaned in an activated silica gel (150 mg) in glass cartridges. The analytes and internal standard substances were eluted with petroleum benzene, and the eluent was concentrated to 15 μl under a gentle stream of nitrogen using iso-octane as keeper. The analytes and internal standard substances were separated and quantified by gas chromatography with mass spectrometry detection (GC-MS) on an Agilent GC 7890A and an Agilent MSD 5975 C. Using a CTC-CombiPAL injector, 1.2 μl of the sample was injected splitless. Separation was performed on a Phenomenex Zebron ZB-XLB 60 m column (ID 0.25 mm, film 0.25 μm) and an oven temperature program between 75°C and 310°C. Calibration was performed by using plasma calibration standards of the analytes, processed in the same manner as the samples to be analyzed. Calculation of results was done by using calibration curves on the basis of the ratios of the analyte area-under-the-peak (AUP) and the AUP of the corresponding isotope-labeled standard. Blank samples and control samples are analyzed in each series, and their results were assessed by using control charts. External quality assurance was performed by the continuous and successful participation in the German External Quality Assessment Scheme (G-EQUAS) for the parameters PCB28, PCB52, PCB101, PCB138, PCB153, PCB180, DDE, DDT, and HCB in plasma.

Among the 44 different POPs identified, we excluded 20 POPs with ≥50% participants having values below the limit of detection (LOD, 0.01 μg/L) to enable meaningful variations in POP concentrations for statistical analyses, and further excluded γ-hexachlorocyclohexane due to a high coefficient of variation (CV; of 48%). As a result, 23 POPs remained in the analysis, including hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), *p,p*'-dichlorodiphenyldichloroethylene (*p,p*'-DDE), and PCB-28, 74, 99, 105, 118, 138, 146, 153, 156, 157, 163, 167, 170, 178, 180, 183, 187, 190, 196, and 203. Values below LODs were replaced with LODs divided by the square root of two (Caudill et al. 2007). In a pilot study on stability over time, intraclass correlation coefficients of POP concentrations in samples from 40 study participants collected 1–2 years apart were between 0.60 for HCB to 0.96 for PCB-187.

Each pair of matched case-control blood samples was analyzed in the same run, and assayed by the same technician in random sequence under identical conditions. Duplicates of blinded quality control samples (n=64) were run along with the case-control samples to allow the calculation of intra-assay CVs. The mean CV for POP measurements was 3.7%, ranging from 0.4% for PCB-169 to 7.8% for PCB-170.

Hemoglobin A1c (HbA1c), total cholesterol, and triacylglycerol were measured on the Roche P Modular system (Roche Diagnostics, Indianapolis, IN). Total adiponectin concentrations were measured using by enzyme-linked immunosorbent assay (ALPCO, Salem, NH), and fasting insulin was measured by radioimmunoassay (Linco, St Louis, MO, USA). The intra-assay CVs for these measurements ranged from 0.7% for insulin to 4.0% for total adiponectin.

Ascertainment of T2D

Participants were asked whether they received a physician-diagnosis of diabetes at baseline and on all biennial follow-up questionnaires. Those reporting a diabetes diagnosis were sent a validated supplementary questionnaire regarding any symptoms, diagnostic tests, and treatment (Manson et al. 1991). We used one of the following American Diabetes Association 1998 criteria to confirm self-reported T2D diagnosis: (1) an elevated glucose concentration (fasting plasma glucose ≥7.0 mmol/l, random plasma glucose ≥11.1mmol/l, or plasma glucose ≥11.1mmol/l after an oral glucose load), and at least one symptom; (2) no symptoms, but elevated glucose concentrations on two separate occasions; or (3) treatment with insulin or oral hypoglycemic medication. In validation studies, 61 of 62 self-reported cases of T2D randomly selected in the Nurses' Health Study were confirmed after an endocrinologist reviewed the medical records without the information from the supplementary questionnaire.(Manson et al. 1991) Only confirmed T2D cases were included in the current study.

Assessment of covariates

At enrollment, participants responded to a questionnaire inquiring about body weight, height, demographic and lifestyle information, and medical history. Similar follow-up questionnaires have been administered biennially thereafter to update these variables. Information on cigarette smoking, physical activity, family history of diabetes, and oral contraceptive use was also assessed in the questionnaires at baseline and during follow-up. Previous weight change was calculated as the difference between body weight at cohort enrollment in 1989 and at blood draw. For cases and their matched controls, subsequent weight change was the weight difference from blood draw to diagnosis year of the index cases. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Lifetime breastfeeding duration in months was recorded in 1993. We further adjusted for updated parity between the 1993 questionnaire and the blood draw. We defined and adjusted for states of residence as coastal, lake, and inland. Physical activity as metabolic equivalent tasks (METs, hours per week) was estimated based on average time per week in the past year spent on leisure-time physical activities. Dietary data were obtained using a validated food frequency questionnaire (FFQ) in 1991 and then updated every 4 years using the same FFQ (Salvini et al. 1989).

Statistical methods

Lipid-adjusted plasma-POP concentrations (in ng/g lipid) constituted the primary exposure parameters. Total serum lipid content was calculated using the Phillips formula (Phillips et al. 2008). Given the highly skewed distributions, POP values were log-transformed before the analysis, and transformed back to the original scale for presentation in the tables to facilitate the interpretation of effect estimates.

We used Spearman correlation coefficients (r_s) to evaluate correlations of individual POPs with 1) plasma concentrations of total adiponectin, insulin, and HbA1c, and 2) foods and beverages that have well-documented relationship with T2D (Ley et al. 2016), including whole grains, nuts, yogurt, green leafy vegetables, fruits high in anthocyanins (berries, grapes, and apples), coffee, alcohol, white rice, potato/French fries, red/processed meat, sugar sweetened beverages, and fruit juices, in addition to animal fat as a potential dietary source of POPs. These analyses were restricted to the control group, who were more representative of the overall NHSII cohort.

In the main analysis for T2D risk, we calculated PCB toxicity equivalency (PCB-TEQ) concentration per the U.S. Environmental Protection Agency recommendations (EPA 2010), by multiplying the concentrations of 5 dioxin-like PCBs, including PCB-105, 118, 156, 157, and 167, with their corresponding toxicity equivalence factor and then summing up to obtain an overall weighted concentration. Individual PCBs were also grouped using alternative approaches. Thus, we summed PCBs according to their estrogenic/antiestrogenic effects as previously suggested (Wolff et al. 1997). We also calculated the sum of individual PCBs that showed significant associations with T2D risk in the study of Lee *et al* (Lee et al. 2010).

The associations between plasma-POP concentrations and T2D risk were evaluated using conditional logistic regression. In addition to matching factors, we further adjusted for demographic and lifestyle confounding factors, including family history of diabetes (yes, or no), oral contraceptive use (never used, past user, or current user), smoking status (never, former, or current), alcohol intake (abstainer, <5.0 g/day, 5.0–14.9 g/day, or ≥15.0 g/day), physical activity (<3 METs-hr/week, 3–8.9 METs-hr/week, 9–17.9 METs-hr/week, 18–26.9 METs-hr/week, or ≥27 METs-hr/week), lifetime breastfeeding duration (never breastfeeding/no child, ≤11 months, or ≥12 months, or missing), number of births after 1993 (0, 1,or ≥2), and states of residence (coastal, lake, or inland). In the fully-adjusted model, we further controlled for previous weight change (in tertiles) and concurrent BMI (< 23.0 kg/m^2, 23.0–24.9 kg/m^2, 25.0–29.9 kg/m^2, 30.0–34.9 kg/m^2, or ≥ 35.0 kg/m^2). Covariates were derived from the questionnaire administered in 1995, or available records closest to 1995 (such as breastfeeding). Participants were categorized into tertiles according to the distribution of POP concentrations among controls. P values for linear trend were calculated by modeling the median values of each tertile as a continuous variable.

Based on previous analysis on PCBs and body fat distribution (Zong et al. 2015), we also summed PCBs according to the number of chlorine substitutions as PCB with ≤4 chlorines (PCB4Cls), PCB with 5 chlorines (PCB5Cls), PCB with 6 chlorines (PCB6Cls), PCB with 7 chlorines (PCB7Cls), and PCB with ≥8 chlorines (PCB8Cls, see Table S1), because the number of chlorine substitutions is a major determinant of lipophilicity and thus the

elimination half-lives of POPs in humans. Among controls, we also calculated least-squared means of the plasma-PCB concentrations according to age, lifetime breastfeeding duration, previous weight change, concurrent BMI, and smoking status.

We analyzed the association between plasma-POP concentrations and subsequent weight gain, and further adjusted for subsequent weight gain when modeling the association between POPs and T2D risk. In sensitivity analyses, we adjusted for plasma fasting insulin, adiponectin, or HbA1c to evaluate whether these intermediate outcomes could potentially explain the associations between POPs and T2D risk. To account for potential over-adjustment by standardizing POP values to blood lipid levels (Lee et al. 2010), we analyzed the association between crude POP concentration (μg/L) and T2D risk, before and after further adjustment for total blood lipids in the model. To assess any effect modification by predictors of POP burdens, we performed stratified analyses in multivariate-adjusted models. Statistical significance of effect modification (P for interaction) was evaluated by likelihood ratio test (LRT) comparing models with and without multiplicative interaction terms between tertiles of POP concentrations and each potential effect modifier. We also performed a factor analysis for the 23 POPs, after imputing values below LOD and rescaling the imputed values to be within the range of 0–0.01 μg/L. Three factors with eigenvalues >1 were rotated by an orthogonal transformation (Varimax rotation function in SAS). We subsequently determined the associations between factor scores and T2D risk.

All P values were two-sided. Data were analyzed with the Statistical Analysis Systems software package, version 9.4 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Characteristics of cases and controls at sample collection are presented in Table 1. Cases were more likely to have a family history of diabetes, to use oral contraceptives, to live in states around the Great Lakes area, or to be smokers, but less likely to breastfeed and had lower levels of physical activity compared to controls. Cases gained more weight since study enrollment in 1989 to blood draw, and had higher concurrent BMI than controls. Concentrations of HCB, β-HCH, *p,p*'-DDE, and PCB-28, 74, 99, 105, 118, and 138 were higher in cases than controls, whereas PCB-156, 157, 170, 178, 180, 187, 190, 196, and 203 were lower.

Among controls, we observed inverse correlations of HCB, *p,p*'-DDE, and most PCBs (PCB-74, 138, 146, 153, 156, 157, 163, 167, 170, 178, 180, 183, 187, 190, 196, and 203) with plasma fasting insulin (all $r_s < -0.10$, P<0.05), but not HbA1c ($r_s < 0.08$, see Table S2) after multivariate adjustment for covariates. In addition, *p,p*'-DDE, and PCB-28, 99, 105, and 183 were weakly and inversely correlated with total adiponectin concentrations. POPs were not significantly correlated with intakes of foods and beverages (all $r_s \leq 0.11$) known as T2D risk factors (see Table S3).

In models adjusting for matching factors only (Table 2), HCB, β-HCH, *p,p*'-DDE, and PCB-TEQ were all associated with a higher T2D risk, and ORs (95%CIs) for the highest POP tertiles ranged from 1.55 (1.18, 2.04; P_{trend}=0.02) for *p,p*'-DDE to 3.40 (2.52, 4.57;

P_{trend}<0.001) for β-HCH in comparison with the reference group (the lowest tertile). These findings remained significant after further adjustment for other confounding factors (model 2), but attenuated when previous weight change and concurrent BMI were controlled for (model 3). In the fully-adjusted model, positive trends remained for β-HCH and PCB-TEQ (P_{trend}≤0.01), but significantly increased T2D risk in the highest POP tertiles remained significant only for PCB-TEQ (OR [95%CI] = 1.74 [1.12, 2.70]).

T2D risks according to tertiles of individual PCBs were presented in sTable 1. PCB-74, 105, 138, 157, and 167 were significantly, positively associated T2D risk. When PCBs were grouped according to their estrogenic/antiestrogenic effects or those identified by Lee *et al* (Table 3), the group of antiestrogenic, immunotoxic, dioxin-like, moderately persistent PCBs (i.e., the sum of PCB-74, 105, 118, 156, and 167 that are collectively named Group 2A) were associated with higher T2D risk in the fully-adjusted model. Other groups showed largely inverse associations in multivariate-adjusted models, which were attenuated after previous weight change and concurrent BMI were further controlled for. When PCBs were grouped according to the number of chlorine substitutions (Table 4), both PCB4Cls and PCB5Cls were associated with a higher T2D risk.

When exploring impacts of factors associated with POP storage among controls, we found that older age, shorter breastfeeding durations and less weight gain were each independently associated with higher circulating POP concentrations in the control group (Table 5). Comparing extreme groups, POP concentrations were 19% (for HCB) to 64% (for β-HCH) higher in older participants (≥50 years vs. <40 years). They were 17% (for HCB) to 23% (for *p,p*'-DDE) lower among women with longer breastfeeding duration (≥12 months vs. no children or breastfeeding), and 15% (for HCB and PCB8Cls) to 39% (for β-HCH and PCB4Cls) lower for those with more previous weigh gain (>11.3 kg vs. <2.3 kg). Concurrent BMI was positively correlated with plasma OCPs and PCBs with fewer chlorine substitutions (≤4 and 5 chlorines), but the opposite was observed for highly-chlorinated PCBs (≥7 chlorines).

Plasma-POP concentrations were not associated with higher subsequent weight gain among controls (see Table S4), and further adjusting for subsequent weight change did not significantly change the association between POPs and T2D (see Table S5). Adjustment for insulin and HbA1c concentrations at baseline somewhat strengthened the positive associations of β-HCH and PCB-TEQ with T2D risk (see Table S4), whereas adjusting for adiponectin slightly attenuated the results. When crude pollutant concentrations (μg/L) were modeled, HCB, β-HCH, and PCB-TEQ were again positively associated with T2D (see Table S5) in models that considered previous weight change and concurrent BMI, and the trends remained significant for β-HCH and PCB-TEQ after controlling for total lipids.

We also performed analyses stratified by age, breastfeeding duration, previous weight change, and concurrent BMI (see Table 6 and Table S7). Consideration of effect modification by previous weight change resulted in positive associations of HCB, β-HCH and PCB-TEQ with T2D risk becoming stronger among women with relatively stable body weight from enrollment to blood draw ($P_{interaction}$<0.05). The T2D ORs (95% CIs) comparing extreme (high vs. low) POP groups ranged from 1.97 (1.01, 3.85; P_{trend}=0.01 for

HCB) to 2.67 (1.34, 5.34; P_{trend}<0.001 for β-HCH) in the lowest weight gain group, and were 0.91 (0.51, 1.65; P_{trend}=0.65 for PCB-TEQ) to 1.44 (0.79, 2.65; P_{trend}=0.20 for β-HCH) in the highest weigh gain group.

In the factor analysis, three factors were generated with 77% variance explained overall (see Table S8). Factor 1 was primarily correlated with highly chlorinated PCBs (PCB-170 to 203), factor 2 was loaded on PCBs with fewer chlorines (PCB-74 to 138, 157, and 167), and factor 3 was loaded on OCPs. PCB-146, 153, 156, and 163 were loaded on both factor 1 and factor 2, thus not considered in interpretation. In the fully-adjusted model (see Table S9), factor 2 was associated with a higher T2D risk, and the OR (95% CI) comparing extreme tertiles was 1.74 (1.13, 2.68, P_{trend}=0.01; high vs. low).

DISCUSSION

In this study of middle-aged U.S. women, plasma concentrations of PCBs, when summarized using the toxicity equivalence score, were significantly associated with a higher T2D risk. Age, breastfeeding history, previous weight change, and concurrent BMI were strong predictors of plasma-POP concentrations. Moreover, the associations of HCB, β-HCH, and PCB-TEQ with T2D risk were more pronounced among women who maintained a stable weight than those who gained weight before the blood draw.

Our findings are largely consistent with previous prospective studies that demonstrated overall positive associations between POPs and diabetes risks, although findings for individual POPs were less consistent. For example, positive associations were observed between total serum-PCBs and incident diabetes among women, but not among men, living in around the Great Lake (Vasiliu et al. 2006), whereas among 471 fish consumers living in that area, serum concentrations of *p,p*'-DDE, but not total PCBs, were associated with a higher diabetes risk (Turyk et al. 2009). In a cohort of Swedish women, serum PCB-153 and *p,p*'-DDE concentrations were used as surrogates of overall POP exposure, and *p,p*'-DDE concentrations were associated with T2D risk after excluding cases diagnosed within the first 6 years (Rignell-Hydbom et al. 2009). More recently, Lee *et al* reported that 6 to 11 out of the 19 measured POPs showed positive trends toward increased T2D risk among an elderly population in Sweden (Lee et al. 2011), and a potentially non-linear association was observed for summed ranks of 31 POPs in young U.S. adults (Lee et al. 2010). However, both HCB and total PCBs were associated with T2D risk in a monotonic fashion according to a meta-analysis of prospective studies (Wu et al. 2013). Inconsistencies in previous studies regarding congener-specific findings could likely be explained by small sample sizes, insufficient adjustment for confounding factors, differential background exposure status, lack of lipid adjustment, different individual POPs included in early investigations, and differences in other population characteristics that may affect POP retention in the body (Lee et al. 2014). Given that humans are typically exposed to a mixture of various POPs with shared sources, similar metabolism, and modes of action (Lee et al. 2014), these studies collectively support a pathogenic role of POP exposure in T2D development.

Because adipose tissue is both the primary storage site and a target organ for lipophilic POPs' obesogenic effects, the relationships between POP concentrations and body weight

are complicated (Wolff et al. 2007). Our previous analysis of NHANES data showed that highly-chlorinated POPs were more likely to be inversely associated with body fat, mainly among younger participants, whereas POPs with shorter half-lives appeared to be associated with higher BMI, especially among older participants (Hooper et al. 2007; Mondal et al. 2014; Wang et al. 2009; Zong et al. 2015). Interestingly, we found that weight gain was consistently associated with lower concentrations of all POP groups, possibly due to dilution by the expanded adipose tissue compartment. This notion is supported by findings in short-term intervention studies that showed elevated serum-POP concentrations following weight loss (Lim et al. 2011). Such complicated relationship highlighted the importance of incorporating both concurrent body weight and history of weight change when investigating the association between POP exposures and obesity-related disorders. In our study, an inverse association with T2D was observed for the more lipophilic, highly-chlorinated PCB before body weight adjustment, reflecting the existence of confounding by concurrent compartmental distribution. While participants with recent excess weight gain would be likely to have lower circulating POP concentrations, a higher T2D risk was found, thus obscuring the true associations between blood concentrations of POPs and T2D risk when weight change history is not taken into account (Wolff et al. 2007). These considerations may also help explain the observed stronger associations between POPs and T2D risk among women with stable body weight, for whom the associations were less subject to the impact from weight change trajectories. In our study, POP concentrations did not show positive associations with subsequent weight gain, and further adjusting for later weight gain did not change the POP-T2D associations. In this regard, more studies are warranted to examine the confounding and mediating effects of body weight on POPs and obesity-related disorders.

The observed associations of age and lifestyle factors with plasma POPs are in line with existing studies that investigated their roles as determinants of body POP burden. Age strongly predicts POP concentrations because chronological age reflects higher environmental POP concentrations in early years, longer lifetime exposure, slower hepatic elimination during aging, and combined effects of other factors such as smoking and body fat accumulation (Milbrath et al. 2009). Lactation is a major route for POP elimination among women, and we and others have consistently demonstrated an inverse association between longer lifetime breastfeeding duration and POP burden (Zong et al. 2016), which are further elucidated by studies showing reduced circulating POP concentrations among breastfeeding mothers (Thomsen et al. 2010). In addition, the inverse association between smoking and certain POPs could be explained by that smoking could accelerate dioxin and dioxin-like compound elimination by inducing dioxin-degrading enzymes (Milbrath et al. 2009). Diet is considered as one major source of current POP exposure, but we did not observe strong correlations of POPs with food and beverage intakes, possibly because our FFQs are less specific in regard to long-term intakes of individual foods contaminated by POPs.(Kvalem et al. 2009)

In addition to a large sample size, our study is also strengthened by the use of a prospective study design and a long follow-up duration. Repeated assessments and careful adjustment for body weight, lifestyle factors, and health status helped control for confounding by these factors. However, our research is also subject to some limitations. For example, our

participants were all middle-aged female nurses of predominant Caucasian origin, thus limiting the generalizability of findings to men and other ethnic groups. Second, we evaluated POP concentrations in single blood samples, which may not represent life-time exposures, even for these persistent substances. However, in NHSII participants, POP concentrations were highly reproducible in blood samples collected 1–2 years apart, suggesting that one measurement of POP concentrations may properly represent the long-term exposure status before blood draw. Such reproducibility also makes reverse causation bias by undiagnosed diabetes unlikely. Furthermore, we excluded diabetes cases occurred within the first year of blood collection to further minimize this bias. Last, we cannot entirely exclude the role of residual or unmeasured confounding given the observational study design.

CONCLUSIONS

We found that plasma concentrations of OCPs and PCBs in the late 1990s during subsequent years were associated with a higher T2D risk among middle-aged U.S. women. Age, breastfeeding history, previous weight change, and concurrent body weight were among the primary determinants of circulating POP concentrations. The associations between plasma-POPs and T2D were more pronounced among women who maintained a stable body weight. These findings highlight the impact of lifestyle factors, especially recent weight gain, on circulating POP concentrations and their associations with subsequent T2D risk. The complicated interplay between POP concentrations and body weight on T2D risk warrants careful investigation in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

1. We observe strong associations of plasma OCPs and PCBs with their determinants.
2. Mono-ortho dioxin-like PCBs are associated with higher type 2 diabetes risk.
3. The POP-T2D associations are stronger among people without previous weight gain.

Table 1

Characteristics of T2D cases and controls in the NHSII at blood sample collection.

Variable [a]	Case	Control	P value [b]
Age at blood sample collection (years)	45.3±4.4	45.3±4.4	0.97
Caucasians	756(95.3%)	766(96.6%)	0.20
Fasting ≥as hr at blood sample collection	547(69.0%)	570(71.9%)	0.21
Family history of diabetes	257(32.4%)	133(16.7%)	<0.001
Menopausal status and hormone uses			
Pre-menopause	538(78.2%)	538(78.2%)	>0.99
Postmenopausal, never use	29(4.1%)	29(4.1%)	
Postmenopausal, current or past use	122(17.7%)	122(17.7%)	
Use of oral contraceptive			0.03
Current user	106(13.4%)	94(11.9%)	
Former user	668(84.2%)	661(83.4%)	
Never used	19(2.4%)	38(4.8%)	
Lifetime breastfeeding duration			0.03
No breastfed, no children	287(38.1%)	254(33.5%)	
≤54 months	256(34.0%)	245(32.3%)	
≥45 months	211(28.0%)	259(34.2%)	
Number of birth after 1993	0.08±0.35	0.06±0.29	0.38
States of residence			0.04
Coastal states	124(15.6%)	163(20.6%)	
Lake states	442(55.7%)	412(52.0%)	
Inland states	227(28.6%)	218(27.5%)	
Physical activity (MET-hr/week)	16.1±25.8	20.7±28.2	0.001
Smoking status (%)			
Never smoked	500(63.1%)	515(65.0%)	0.02
Former smoker	194(24.5%)	213(26.9%)	
Current smoker	99(12.5%)	65(8.2%)	
Alcohol intake (g/day)			<0.001
Abstainers	350(44.1%)	264(33.3%)	
0.1–4.9	348(43.9%)	376(47.4%)	
5.0–9.9	51(6.4%)	87(11.0%)	
≥10	44(5.6%)	66(8.3%)	
Previous weight change (kg)	9.7±11.6	4.6±10.3	<0.001
BMI (kg/m^2)	32.6±6.8	25.5±5.4	<0.001
Persistent organic pollutants (ng/g lipid)			
HCB	18.0(14.5, 22.4)	16.6(13.3, 21.1)	<0.001
β-HCH	14.3(8.84, 22.7)	9.84(6.08, 16.2)	<0.001
p,p'-DDE	312(180, 501)	272(150, 481)	0.002
PCB-28	2.05(1.25, 3.47)	1.58(1.18, 2.64)	<0.001
PCB-74	12.6(8.51, 17.0)	10.2(6.83, 14.7)	<0.001

Variable [a]	Case	Control	P value [b]
PCB-99	7.94(4.92, 12.5)	6.14(3.85, 9.51)	<0.001
PCB-105	2.94(1.69, 5.07)	1.96(1.31, 3.36)	<0.001
PCB-118	15.1(9.40, 25.5)	11.2(7.28, 18.5)	<0.001
PCB-138	25.2(16.3, 37.5)	22.9(15.2, 33.1)	<0.001
PCB-146	4.04(2.76, 5.77)	4.13(2.80, 6.02)	0.15
PCB-153	39.4(27.6, 58.2)	41.1(28.2, 58.4)	0.22
PCB-156	6.27(4.12, 9.05)	6.69(4.53, 9.93)	0.005
PCB-157	1.52(1.05, 2.39)	1.63(1.18, 2.53)	0.001
PCB-163	7.35(5.03, 10.5)	7.60(5.36, 11.3)	0.10
PCB-167	1.71(1.16, 2.67)	1.60(1.19, 2.46)	0.32
PCB-170	7.55(5.45, 10.2)	8.64(5.97, 12.6)	<0.001
PCB-178	1.49(1.11, 2.22)	1.90(1.34, 2.75)	<0.001
PCB-180	23.2(16.8, 31.8)	28.3(19.5, 40.4)	<0.001
PCB-183	2.87(1.92, 4.11)	2.87(1.86, 4.15)	0.84
PCB-187	6.31(4.44, 9.19)	7.34(5.04, 10.5)	<0.001
PCB-190	1.38(1.05, 1.94)	1.53(1.18, 2.21)	<0.001
PCB-196	1.46(1.08, 2.16)	1.74(1.23, 2.55)	<0.001
PCB-203	3.26(2.30, 4.60)	4.09(2.82, 5.83)	<0.001

Abbreviations: T2D, type 2 diabetes; NHSII, Nurses' Health Study II; BMI, body mass index; HCB, hexachlorobenzene; β-HCH, β-hexachlorocyclohexane; *p,p*'-DDE, *p,p*'-dichlorodiphenyldichloroethylene; PCBs, polychlorinated biphenyls.

[a] Continuous variable are shown as mean ± standard deviation or median(interquartile ranges), and categorical variables are shown as number (percentages. For menopausal status and hormone uses, values were based on non-missing data).

[b] *p*-values are based on Student's *t*-test for variables expressed as mean ± standard deviation, Wilcoxon rank–sum test for variables expressed as median (interquartile ranges), or Pearson χ^2 test for variables expressed as percentages.

Table 2

Associations between plasma-POP concentrations and T2D.[a]

	Tertile 1	Tertile 2	Tertile 3	P_{trend}
HCB				
Median (Range, ng/g)	12.35(1.50, 14.47)	16.56(14.48, 19.41)	23.51(19.42, 162.4)	
$N_{case}/N_{control}$	198/264	269/265	326/264	
Model 1 [b]	Ref	1.42 (1.09, 1.84)	1.73 (1.33, 2.24)	<0.001
Model 2 [c]	Ref	1.32 (0.99, 1.77)	1.66 (1.24, 2.21)	0.001
Model 3 [d]	Ref	0.82 (0.55, 1.23)	0.94 (0.62, 1.40)	0.98
β-HCH				
Median (Range, ng/g)	4.58(0.89, 7.59)	9.84(7.60, 13.40)	20.29(13.44, 324.2)	
$N_{case}/N_{control}$	157/264	213/265	423/264	
Model 1	Ref	1.54 (1.15, 2.06)	3.40 (2.52, 4.57)	<0.001
Model 2	Ref	1.66 (1.20, 2.30)	3.64 (2.59, 5.13)	<0.001
Model 3	Ref	0.85 (0.55, 1.31)	1.49 (0.94, 2.36)	0.01
p,p'-DDE				
Median (Range, ng/g)	126.0(6.93, 179.8)	271.6(181.3, 392.7)	618.1(394.9, 3432)	
$N_{case}/N_{control}$	200/264	305/265	288/264	
Model 1	Ref	1.58 (1.22, 2.04)	1.55 (1.18, 2.04)	0.02
Model 2	Ref	1.64 (1.23, 2.19)	1.56 (1.14, 2.13)	0.05
Model 3	Ref	1.34 (0.90, 1.99)	0.91 (0.58, 1.41)	0.22
PCB-TEQ				
Median (Range)[e]	4.32(1.51, 5.71)	7.28(5.73, 9.49)	13.1(9.40, 145.4)	
$N_{case}/N_{control}$	205/264	240/265	348/264	
Model 1	Ref	1.24 (0.95, 1.61)	1.86 (1.43, 2.44)	<0.001
Model 2	Ref	1.26 (0.94, 1.68)	2.00 (1.45, 2.74)	<0.001
Model 3	Ref	1.14 (0.77, 1.68)	1.74 (1.12, 2.70)	0.008

Abbreviations: POP, persistent organic pollutants; T2D, type 2 diabetes; HCB, hexachlorobenzene; β-HCH, β-hexachlorocyclohexane; *p,p*'-DDE, *p,p*'-dichlorodiphenyldichloroethylene; PCBs, polychlorinated biphenyls; TEQ, toxicity equivalents.

[a] Relative risk was estimated using conditional logistic regression;

[b] Model 1, automatically adjusted for matching factors, including age, ethnicity, time of sample collection, fasting status, and menopausal status and post-menopausal hormone use;

[c] Model 2, further adjusted for family history of diabetes, oral contraceptive use, lifetime breastfeeding duration, number of birth after 1993, states of residence, smoking status, alcohol use, and physical activity;

[d] Model 3, further adjusted for previous weight change and concurrent BMI.

[e] The TEF values were multiplied by 10,000.

Table 3

Associations between PCB groups and T2D. [a]

Group 1B (estrogenic, weak phenobarbital-type inducers, persistent, PCB187)[b]				
Median (Range, ng/g)	4.18(0.97, 5.73)	7.34(5.74, 9.06)	12.59(9.09, 59.22)	
N case/N control	332/264	261/265	200/264	
Model 1	Ref	0.73 (0.57, 0.94)	0.54 (0.41, 0.71)	<0.001
Model 2	Ref	0.74 (0.56, 0.97)	0.54 (0.40, 0.74)	<0.001
Model 3	Ref	0.92 (0.63, 1.35)	1.04 (0.68, 1.59)	0.80
Group 2A (antiestrogenic, immunotoxic, dioxin-like, moderately persistent: PCB-74, 105, 118, 156, 167)				
Median (Range, ng/g)	19.14(5.23, 25.89)	32.33(25.92, 42.46)	56.38(42.50, 577.11)	
N case/N control	191/264	244/265	358/264	
Model 1	Ref	1.40 (1.07, 1.84)	2.20 (1.66, 2.91)	<0.001
Model 2	Ref	1.36 (1.01, 1.84)	2.27 (1.63, 3.16)	<0.001
Model 3	Ref	1.14 (0.76, 1.70)	1.73 (1.10, 2.71)	0.01
Group 2B (antiestrogenic and immunotoxic, dioxin-like, persistent: PCB170)				
Median (Range, ng/g)	5.10(0.99, 7.01)	8.64(7.02, 10.95)	14.38(10.99, 45.28)	
N case/N control	344/264	284/265	165/264	
Model 1	Ref	0.75 (0.59, 0.97)	0.44 (0.33, 0.58)	<0.001
Model 2	Ref	0.71 (0.53, 0.94)	0.39 (0.28, 0.54)	<0.001
Model 3	Ref	1.12 (0.77, 1.61)	1.29 (0.83, 2.00)	0.27
Group 3 (phenobarbital-type inducers, persistent: PCB-99, 153, 180, 183, 196, 203)				
Median (Range, ng/g)	51.65(8.68, 68.99)	86.17(69.08, 106.5)	140.7(107.0, 633.2)	
N case/N control	312/264	251/265	230/264	
Model 1	Ref	0.76 (0.59, 0.98)	0.69 (0.53, 0.90)	0.01
Model 2	Ref	0.75 (0.57, 1.00)	0.64 (0.47, 0.87)	0.006
Model 3	Ref	0.90 (0.61, 1.31)	1.25 (0.81, 1.92)	0.22
POPs associated with T2D by Lee et al[c] PCB-74, 146, 153, 170, 178, 180, 183, 187, 196, 203				
Median (Range, ng/g)	67.69(12.87, 91.55)	111.5(91.63, 138.5)	182.8(138.6, 813.4)	
N case/N control	318/264	247/265	228/264	
Model 1	Ref	0.73 (0.57, 0.94)	0.67 (0.52, 0.87)	0.005
Model 2	Ref	0.70 (0.53, 0.94)	0.62 (0.46, 0.85)	0.005
Model 3	Ref	0.84 (0.57, 1.23)	1.31 (0.85, 2.02)	0.12

Abbreviations: T2D, type 2 diabetes; HCB, hexachlorobenzene; β-HCH, β-hexachlorocyclohexane; *p,p*'-DDE, *p,p*'-dichlorodiphenyldichloroethylene; PCBs, polychlorinated biphenyls; TEQ, toxicity equivalents.

[a] Relative risk was estimated using conditional logistic regression, Models were adjusted the same as table 2.

[b] Wolff et al. 1997.

[c] Lee et al. 2010.

Table 4

Associations between PCB groups defined by the number of chlorine substitutions of PCB congeners and T2D risk. [a]

Component	Tertile 1	Tertile 2	Tertile 3	P_{trend}
PCB with ≤ 4 chlorines				
Median (Range, ng/g)	7.14(1.85, 9.64)	12.17(9.66, 15.37)	20.86(15.38, 127.2)	
$N_{case}/N_{control}$	168/264	235/265	390/264	
Model 1	Ref	1.55 (1.17, 2.05)	2.73 (2.05, 3.62)	<0.001
Model 2	Ref	1.46 (1.07, 2.00)	2.78 (2.00, 3.85)	<0.001
Model 3	Ref	1.25 (0.82, 1.92)	1.79 (1.14, 2.81)	0.007
PCB with 5 chlorines				
Median (Range, ng/g)	10.65(3.14, 15.19)	19.79(15.21, 27.07)	37.38(27.18, 505.1)	
$N_{case}/N_{control}$	160/264	243/265	390/264	
Model 1	Ref	1.65 (1.26, 2.18)	2.97 (2.22, 3.97)	<0.001
Model 2	Ref	1.76 (1.30, 2.38)	3.13 (2.25, 4.34)	<0.001
Model 3	Ref	1.39 (0.92, 2.09)	1.70 (1.08, 2.67)	0.03
PCB with 6 chlorines				
Median (Range, ng/g)	50.38(7.78, 68.34)	86.50(68.43, 107.97)	143.3(108.1, 785.0)	
$N_{case}/N_{control}$	271/264	244/265	278/264	
Model 1	Ref	0.90 (0.70, 1.16)	1.02 (0.79, 1.32)	0.70
Model 2	Ref	0.89 (0.67, 1.19)	0.95 (0.70, 1.29)	0.86
Model 3	Ref	0.95 (0.65, 1.40)	1.43 (0.93, 2.19)	0.05
PCB with 7 chlorines				
Median (Range, ng/g)	29.62(7.19, 39.62)	49.30(39.76, 61.39)	82.20(61.41, 274.1)	
$N_{case}/N_{control}$	364/264	263/265	166/264	
Model 1	Ref	0.62 (0.47, 0.81)	0.39 (0.30, 0.52)	<0.001
Model 2	Ref	0.57 (0.43, 0.77)	0.36 (0.26, 0.49)	<0.001
Model 3	Ref	0.98 (0.66, 1.46)	1.00 (0.65, 1.54)	0.99
PCB with ≥8 chlorines				
Median (Range, ng/g)	3.58(1.68, 4.66)	5.85(4.66, 7.20)	9.69(7.20, 71.31)	
$N_{case}/N_{control}$	384/264	239/265	170/264	
Model 1	Ref	0.50 (0.38, 0.66)	0.34 (0.25, 0.46)	<0.001
Model 2	Ref	0.49 (0.37, 0.67)	0.35 (0.25, 0.49)	<0.001
Model 3	Ref	0.89 (0.60, 1.32)	1.05 (0.67, 1.67)	0.76

Abbreviations: T2D, type 2 diabetes; PCBs, polychlorinated biphenyls.

[a] Relative risk was estimated using conditional logistic regression, Models were adjusted the same as table 2.

Table 5

Multivariate-adjusted plasma POP concentrations (ng/g lipid) as stratified by major determinants of POP concentrations among controls (N=793).[a]

	Age at baseline			**P_{trend}**	**Direction**
	<40 years	**40–50 years**	**>50 years**		
HCB	15.0(13.9, 16.2)	17.3(16.8, 17.8)	17.8(16.6, 19.1)	0.003	Increase
β-HCH	6.62(5.66, 7.75)	10.0(9.36, 10.6)	10.8(9.35, 12.6)	<0.001	Increase
p,p'-DDE	182(156, 212)	299(281, 319)	267(231, 309)	0.002	Increase
PCB with ≤ 4 chlorines	9.52(8.58, 10.6)	12.2(11.7, 12.7)	13.4(12.1, 14.8)	<0.001	Increase
PCB with 5 chlorines	15.8(13.9, 17.9)	20.8(19.8, 21.9)	21.7(19.3, 24.5)	0.001	Increase
PCB with 6 chlorines	63.2(56.8, 70.3)	89.6(85.8, 93.6)	87.3(79.0, 96.6)	0.001	Increase
PCB with 7 chlorines	33.7(30.7, 37.0)	51.3(49.4, 53.3)	52.4(48.0, 57.2)	<0.001	Increase
PCB with ≥8 chlorines	4.23(3.85, 4.65)	6.14(5.91, 6.4)	6.60(6.04, 7.21)	<0.001	Increase
	Lifetime breastfeeding duration [b]				
	No breastfed/children	≤11	≥12		
HCB	18.1(17.3, 19.0)	17.7(16.9, 18.5)	15.4(14.7, 16.1)	<0.001	Decrease
β-HCH	11.7(10.7, 12.8)	10.9(9.9, 11.9)	7.11(6.49, 7.79)	<0.001	Decrease
p,p'-DDE	318(290, 349)	311(284, 341)	211(192, 231)	<0.001	Decrease
PCB with ≤ 4 chlorines	14.6(13.7, 15.6)	13.5(12.6, 14.3)	8.89(8.35, 9.47)	<0.001	Decrease
PCB with 5 chlorines	23.0(21.3, 24.9)	23.3(21.5, 25.2)	15.5(14.3, 16.7)	<0.001	Decrease
PCB with 6 chlorines	99.8(93.4, 107)	100(93.7, 107)	63.3(59.4, 67.6)	<0.001	Decrease
PCB with 7 chlorines	54.9(51.9, 58.1)	54.7(51.7, 57.9)	38.9(36.8, 41.2)	<0.001	Decrease
PCB with ≥8 chlorines	6.20(5.86, 6.56)	6.35(6.00, 6.72)	5.27(4.99, 5.58)	<0.001	Decrease
	Previous weight change, median(interquartile range), kg				
	Low	Middle	High		
	0.45(−2.27, 2.27)	6.35(4.54, 7.71)	13.6(11.3, 18.1)		
HCB	18.4(17.7, 19.1)	16.4(15.7, 17.1)	15.4(14.5, 16.3)	<0.001	Decrease
β-HCH	10.5(9.74, 11.4)	9.05(8.30, 9.88)	8.56(7.58, 9.68)	0.003	Decrease
p,p'-DDE	308(285, 334)	258(237, 282)	238(211, 269)	0.002	Decrease
PCB with ≤ 4 chlorines	12.9(12.3, 13.6)	11.5(10.8, 12.2)	10.8(10.0, 11.8)	0.002	Decrease

	Age at baseline			P_{trend}	Direction
	<40 years	**40–50 years**	**>50 years**		
PCB with 5 chlorines	21.9(20.6, 23.4)	19.4(18.0, 20.8)	18.2(16.4, 20.1)	0.001	Decrease
PCB with 6 chlorines	94.7(89.6, 100)	79.3(74.6, 84.2)	76.7(70.5, 83.5)	<0.001	Decrease
PCB with 7 chlorines	55.1(52.6, 57.8)	44.9(42.6, 47.3)	42.9(39.8, 46.1)	<0.001	Decrease
PCB with ≥8 chlorines	6.59(6.29, 6.92)	5.50(5.22, 5.80)	5.29(4.92, 5.69)	<0.001	Decrease
	BMI at baseline				
	<25 kg/m^2	25–30 kg/m^2	>30 kg/m^2		
HCB	16.2(15.7, 16.8)	17.7(16.8, 18.6)	19.1(17.9, 20.4)	<0.001	Increase
β-HCH	8.01(7.47, 8.60)	10.9(9.88, 12.1)	14.5(12.7, 16.6)	<0.001	Increase
p,p'-DDE	238(221, 255)	308(279, 341)	381(332, 437)	<0.001	Increase
PCB with ≤ 4 chlorines	10.8(10.3, 11.3)	12.8(11.9, 13.7)	15.4(14.1, 16.9)	<0.001	Increase
PCB with 5 chlorines	17.5(16.5, 18.6)	22.4(20.6, 24.4)	28.4(25.3, 31.8)	<0.001	Increase
PCB with 6 chlorines	83.4(79.4, 87.6)	89.0(83.0, 95.5)	85.5(77.7, 94.0)	0.41	
PCB with 7 chlorines	51.0(48.9, 53.2)	47.8(45.0, 50.8)	42.7(39.3, 46.3)	0.003	Decrease
PCB with ≥8 chlorines	6.34(6.08, 6.62)	5.63(5.30, 5.98)	5.01(4.62, 5.44)	<0.001	Decrease
	Smoking status				
	Never	Former	Current		
HCB	17.3(16.8, 17.9)	16.6(15.8, 17.4)	16.2(14.8, 17.7)	0.08	
β-HCH	9.71(9.09, 10.4)	8.79(7.94, 9.74)	11.4(9.5, 13.7)	0.71	
p,p'-DDE	283(265, 302)	252(227, 279)	291(242, 350)	0.43	
PCB with ≤ 4 chlorines	12.6(12.0, 13.1)	11.0(10.2, 11.8)	10.8(9.51, 12.2)	0.001	Decrease
PCB with 5 chlorines	21.6(20.5, 22.8)	17.8(16.4, 19.4)	18.1(15.6, 21.1)	0.005	Decrease
PCB with 6 chlorines	87.1(83.3, 91.1)	79.4(74.0, 85.3)	90.1(79.3, 102)	0.46	
PCB with 7 chlorines	49.5(47.6, 51.5)	47.1(44.3, 50.1)	47.8(42.8, 53.4)	0.26	
PCB with ≥8 chlorines	6.03(5.80, 6.27)	5.71(5.37, 6.07)	5.73(5.13, 6.40)	0.17	

Abbreviations: POP, persistent organic pollutants; T2D, type 2 diabetes; HCB, hexachlorobenzene; β-HCH, β-hexachlorocyclohexane; *p,p*'-DDE, *p,p*'-dichlorodiphenyldichloroethylene; PCBs, polychlorinated biphenyls; TEQ, toxicity equivalents.

[a]Least squared means are estimated in general linear model with adjustment for age at blood draw, ethnicity, menopausal status and post-menopausal hormone use, time of blood draw, fasting status, family history of diabetes, oral contraceptive use, lifetime breastfeeding duration, number of birth after 1993, states of residence, smoking status, alcohol use, physical activity, previous weight change, and concurrent BMI where feasible.

[b] missing data for breastfeeding n=39.

Table 6

Associations between POP and T2D by previous weight change history. [a]

Component	Previous weight change	Tertile 1	Tertile 2	Tertile 3	P_{trend}	$P_{interaction}$
HCB	Low	Ref	1.13 (0.56, 2.28)	1.97 (1.01, 3.85)	0.01	0.04
	Middle	Ref	0.83 (0.48, 1.45)	0.82 (0.46, 1.45)	0.55	
	High	Ref	1.53 (0.91, 2.59)	1.29 (0.74, 2.25)	0.46	
β-HCH	Low	Ref	0.82 (0.39, 1.72)	2.67 (1.34, 5.34)	<0.001	0.004
	Middle	Ref	0.86 (0.48, 1.56)	0.89 (0.47, 1.68)	0.81	
	High	Ref	1.11 (0.62, 1.97)	1.44 (0.79, 2.65)	0.20	
p,p'-DDE	Low	Ref	1.81 (0.97, 3.37)	1.29 (0.66, 2.51)	0.97	0.07
	Middle	Ref	1.12 (0.64, 1.96)	0.82 (0.44, 1.53)	0.39	
	High	Ref	0.88 (0.51, 1.52)	0.60 (0.33, 1.09)	0.07	
PCB-TEQ	Low	Ref	1.20 (0.59, 2.44)	2.41 (1.22, 4.75)	0.002	0.01
	Middle	Ref	0.67 (0.37, 1.18)	0.95 (0.52, 1.74)	0.82	
	High	Ref	1.17 (0.69, 1.99)	0.91 (0.51, 1.65)	0.65	

Abbreviations: POP, persistent organic pollutants; T2D, type 2 diabetes; HCB, hexachlorobenzene; β-HCH, β-hexachlorocyclohexane; *p,p*'-DDE, *p,p*'-dichlorodiphenyldichloroethylene; PCBs, polychlorinated biphenyls; TEQ, toxicity equivalents.

[a]Relative risk was estimated using unconditional logistic regression after adjusting for matching factors, including age, time of sample collection, menopausal status and post-menopausal hormone use, family history of diabetes, oral contraceptive use, lifetime breastfeeding duration, number of birth after 1993, states of residence, smoking status, alcohol use, physical activity, and concurrent BMI. Statistical significance of effect modification (P for interaction) was evaluated by LRT comparing models with and without multiplicative interaction term between tertiles of POP levels and the effect-modifier at issue.