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Benchmark Dose for Dioxin Based on Gestational Diabetes Mellitus Using Coexposure Statistical Methods and an Optimized Physiologically Based Toxicokinetic Model

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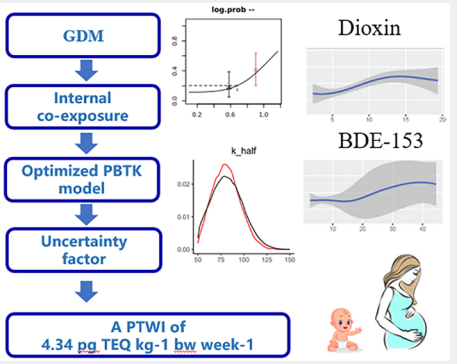
ABSTRACT: Dioxins are ubiquitous endocrine-disrupting substances, but determining the effects and benchmark doses in situations of coexposure is highly challenging. The objective of this study was to assess the relationship between dioxin andgestational diabetes mellitus (GDM), calculate the benchmark dose (BMD) of dioxin in coexposure scenarios, and derive a daily exposure threshold using an optimized physiologically based toxicokinetic (PBTK) model. Based on a nested case-control study including 77 cases with GDM and 154 controls, serum levels of 29 dioxin-like compounds (DLCs) along with 10 perfluoroalkyl acids (PFAAs), seven polybrominated diphenyl ethers (PBDEs), and five non-dioxin-like polychlorinated biphenyls (ndl-PCBs) were measured at 9−16 weeks of gestation. Bayesian machine kernel regression (BKMR) was employed to identify significant chemicals, and probit and logistic models were used to calculate BMD adjusted for significant chemicals. A physiologically based toxicokinetic (PBTK) model was optimized using polyfluorinated dibenzo-*p*-dioxins and dibenzofurans (PFDD/Fs) data by the Bayesian−Monte Carlo Markov chain method and was used to determine the daily dietary exposure threshold. The median serum level of total dioxin toxic equivalent (TEQ) was 7.72 pg TEQ/g fat. Logistic regression analysis revealed that individuals in the fifth quantile of total TEQ level had significantly higher odds of developing GDM compared to those in the first quantile (OR, 8.87; 95% CI 3.19, 27.58). The BKMR analysis identified dioxin TEQ and BDE-153 as the compounds with the greatest influence. The binary logistic and probit models showed that the BMD_{10} (benchmark dose corresponding to a 10% extra risk) and $BMDL_{10}$ (lower bound on the BMD_{10}) were 3.71 and 3.46 pg TEQ/g fat, respectively, when accounting for coexposure to BDE-153 up to the 80% level. Using the optimized PBTK model and modifying factor, it was estimated that daily exposure should be below 4.34 pg TEQ kg^{-1} bw $week^{-1}$ in order to not reach a harmful serum concentration for GDM. Further studies should utilize coexposure statistical methods and physiologically based pharmacokinetic (PBTK) models in reference dose calculation.



KEYWORDS: *dioxin, gestational diabetes mellitus, coexposure, benchmark dose, physiologically based toxicokinetic model*

1. INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (dl-PCBs) are persistent organic pollutants that belong to a family of organic compounds called dioxins or dioxin-like chemicals (DLCs). These toxic chemicals are ubiquitous in the global environment, and more than 90% of human exposure to dioxins is through the diet.[1] There is increasing evidence that certain persistent organic pollutants (POPs) such as dioxin, polychlorinated biphenyls (PCB), polybrominated diphenyl ethers (PBDE), and perfluoroalkyl substances (PFAS) may contribute to the etiology of gestational diabetes mellitus (GDM).[2−8] Dioxins can bind to aryl hydrocarbon receptors (AHR) with high affinity, activate transcription of target genes coding for xenobiotic-metabolizing enzymes in liver, and elicit genomic and nongenomic pathways in pancreatic β cells.[9,10] Emerging evidence has shown some analogues of PBDE and PCB activated AHR and had measurable dioxin-like potency.[11,12] Dioxin, PBDE, and PFAS may also interfere with peroxisome proliferator-activated receptors (PPARs), steroid receptors, and thyroid homeostasis, leading to glucose metabolism disorders and GDM.[13−15] Although mechanisms of POPs leading to GDM are currently not well documented, it is crucial to identify significant chemicals and establish safe exposure levels.

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The benchmark dose (BMD) method, commonly used in toxicology and risk assessment, is a powerful tool for estimating the dose−response relationship between a chemical and its associated health effect.[16,17] However, its application in coexposure scenarios, where individuals are simultaneously exposed to multiple pollutants, has been limited. For chemicals with similar modes of action (MoA), methods such as the relative potency factor (RPF) and toxicity equivalency factor (TEF) can be utilized to calculate the weighted sum of pollutant concentrations as independent variables in BMD calculations.[18,19] Otherwise, practical statistical methods such as Bayesian kernel machine regression (BKMR), weighted quantile sum regressions, and shrinkage methods are used to estimate health effects of exposure to multipollutant mixtures.[20] Recent studies have emphasized the importance of utilizing the BMD method in coexposure scenarios, but there are very few discussions on the exposure threshold of a single compound in the presence of coexposure of several kinds of POPs.[21,22] Besides, the PBTK model is a more comprehensive approach that can be taken to assess the risks associated with dioxin exposure by improving the accuracy of exposure level estimation and accounting for uncertainties. Implementing it with the Bayesian Monte Carlo methodology, the PBTK model can be further enhanced in the Chinese population and the assessment of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs).[23,24]

In our nested case-control study, we collected serum levels of DLCs, PFASs, PBDEs, and PCBs, as well as gestational glycemic measurements from 231 pregnant women. Our study is the first to use the occurrence of GDM to compute the daily exposure threshold for dioxins with BMD approaches in coexposure scenarios and an optimized PBPK model for DLCs, which should provide a reliable estimate for quantifying the health risks of dioxin exposure.

2. METHODS

2.1. Study Population and Data Collection

The present study utilized exposure data sets from a prospective nested case-control study, as previously described.[2] Briefly, recruitment of participants was carried out at the Maternal and Child Health Hospital in the Xicheng District of Beijing, China, between August 2013 and June 2015. Eligible participants were healthy pregnant women without a family history of diabetes or prediabetes. Participants were enrolled if they agreed to provide a blood sample between 9 and 13 weeks of gestation and were routinely screened for gestational diabetes mellitus (GDM) at 24−28 weeks of gestation. Ultimately, a total of 77 pregnant women were diagnosed with GDM within the study population, and two healthy women were selected from the cohort to serve as paired controls for each case.

2.2. Blood Collection and Chemical Analysis

The study analyzed 17 polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), 12 dioxin-like polychlorinated biphenyls (dl-PCBs), including four non-ortho PCBs and eight mono-ortho PCBs, five non-dioxin-like polychlorinated biphenyls (ndl-PCBs), 10 perfluoroalkyl acids (PFAAs), and seven polybrominated diphenyl ethers (PBDEs). The detection of chemicals in maternal whole blood samples and detailed lists of chemicals was carried out using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS), as previously described.[25−27] Toxic equivalent (TEQ) values for PCDD/Fs and dl-PCBs were calculated using the WHO2005-TEF values (pg TEQ/g fat).[19] In addition, serum total cholesterol and triglycerides were measured using an automatic biochemistry analyzer, and the total serum lipids were estimated using the following formula: total lipids (mg/dL) = 2.2 × cholesterol + triglycerides + 62.3.

2.3. Assessment of Glycemic Measures

A 75 g oral glucose tolerance test (OGTT) without prior plasma glucose screening was administered for diagnosing GDM. Fasting venous blood samples were collected before 9 am after an overnight fast of at least 8 h. Blood samples were then taken at intervals of 1 and 2 h after the administration of a 75 g glucose load to measure postprandial glucose concentrations. GDM was diagnosed if one or more of the following threshold values were met or exceeded: 5.1 mmol/L (fasting blood glucose, FBG), 10.0 mmol/L (1 h postprandial blood glucose, 1h-PBG), and/or 8.5 mmol/L (2 h postprandial blood glucose, 2h-PBG), in accordance with the criteria established by the International Association of Diabetes and Pregnancy Study Groups.[28]

2.4. Statistical Analyses

2.4.1. Stage I: Estimating Association between Participants' Outcomes and Serum Dioxin. The participants' baseline characteristics were reported as the mean ± standard deviation (SD) for each group, and the chemical levels were presented as the mean (IQR). The comparison of the baseline characteristics between the cases and controls was carried out utilizing the Mann−Whitney U tests for continuous variables. Individual serum dioxins TEQ for PCDDs, PCDFs, dl-PCBs, and PCDD/Fs were modeled as continuous variables for logistic regression and then categorized into quintiles according to their distributions among the whole population. Simple and multivariable conditional logistic regression analyses were conducted to assess the association between dioxins and GDM risk. Multivariate linear regression models were used to examine the association between dioxin TEQ and continuous outcomes, such as fasting blood glucose (FBG), 1 h postprandial blood glucose (1h-PBG), and 2 h postprandial blood glucose (2h-PBG). The models were adjusted for body mass index, serum triglyceride, and total cholesterol in adjusted model 1 and further adjusted for the sum of PFAS, PBDE, and ndl-PCBs levels in adjusted model 2. Considering the distributions of dioxin TEQ were right-skewed, they were log transformed to approximate normal distributions.

2.4.2. Stage II: Bayesian Kernel Machine Regression (BKMR) Model. BKMR is a nonparametric method used in mixture analysis to identify and estimate the effects of multiple exposures or predictors on health outcomes. It extends the kernel machine regression model to accommodate nonlinear and interactive effects using the Gaussian kernel.

The BKMR model for continuous variable is given by

$$Y_i = h(z_{i1}, ..., z_{iM}) + \beta x_i + \epsilon_i$$

where Y_i is a continuous, normally distributed health end point, h is a flexible function (usually a Gaussian kernel) of the predictor variables $z_{i1},\cdots,z_{iM}$, x_i is a vector of covariates assumed to have a linear relationship with the outcomes, and β is the transpose of the vector of the corresponding coefficients.

The BKMR model for a dichotomous variable is given by[29]

$$\Phi^{-1}(\mu_i) = \alpha + \beta_z z_i + \beta_x x_i$$

where Φ is the cumulative distribution function (CDF) for the standard normal distribution and $\mu_i = P(Y_i = 1)$ is the probability of an event. Markov chain Monte Carlo (MCMC) sampling with 50,000 iterations was run, and the first 10,000 were dropped. We set the threshold of the posterior inclusion probability (PIP) to 0.5 and calculated the PIP for each chemical. The marginal nonlinear dose−response curve between the individual pollutant and the health outcome, by fixing the health effect of other mixture components at the 25th, 50th, and 75th quantiles, was used to find potential nonlinear associations and interactions. Furthermore, the overall impact of the mixture was assessed by plotting the expected change in glycemic measures as the quantiles of all exposure biomarkers increased simultaneously. The BKMR model fitting was performed using the bkmr software implemented as an R package.

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2.4.3. Stage III: Benchmark Dose Estimation. For univariate BMD calculation, dioxin exposures were divided into 10 groups, and the median concentration of dioxin and the mean and standard deviation of the outcomes for each group were calculated. US EPA BMD software 3 (BMDS 3) and a website-based Bayesian BMD system were used for BMD calculations.[30] The benchmark response (BMR) is a predefined threshold for measuring the magnitude of an adverse effect compared to the background. In our study, the BMR level was set at a 10% relative deviation from the background mean and the BMR type was relative deviation for continuous model and extra risk for dichotomous model.[31]

Based on the results of our study, GDM exhibited a lower BMDL, a suitable BMD/BMDL ratio, and a significantly higher PIP compared to 1h-PBG and 2h-PBG. As a result, it was chosen as the preferred end point for subsequent BMD calculations in coexposure scenarios. A generalized linear model was fitted for the relationship between GDM and coexposure to POPs, with a probit and a logistic link function applied for GDM:

$$\Phi^{-1}(\mu_i) = \alpha + \beta_z z_i + \beta_x x_i$$

where Φ is the cumulative distribution function (CDF) for the standard normal distribution, $\mu_i = P(Y_i = 1)$ is the probability of an event, z_i is a vector of chemicals, x_i is a vector of covariates assumed to have a linear relationship with the outcomes, and β_z and β_x are the transpose of the vectors of the corresponding coefficients. The BMD and BMDL values for dioxin are calculated while fixing the exposure levels of other chemicals at specified percentiles. We employed a Markov chain Monte Carlo (MCMC) simulation procedure using WinBUGS (MRC Biostatistics Unit, Cambridge, UK). We performed a convergence test using the Gelman–Rubin method to obtain the potential scale reduction factors and their 95% confidence interval. The corresponding BMDL level was determined as the lower 95% simulated sample value (n = 10,000) upon convergence.

2.5. Estimation of Daily Intake Threshold of Dioxin

2.5.1. Bayesian Optimization of PBTK Model. Our study utilizes the concentration- and age-dependent model (CADM), which has been modified and utilized in the European Food Safety Authority (EFSA) dioxin reference dose derivation.[23,24] Partitioning between fat and liver is concentration-dependent, and the liver burden follows a Michalis–Menten relationship with body burden, as shown in Figure S1. This study aimed to optimize the PBTK model for PCDD/Fs and Chinese residents using the Bayesian–Markov chain Monte Carlo (MCMC) method. To implement the Bayesian–MCMC method, we recoded the CADM model using the "mrgsolve" package in R. The consistency between the original and recorded models was evaluated by calculating the R^2 value, which was greater than 99%. Prior distributions for parameters were obtained from literature sources.[24,32] We performed an extensive search for dioxin exposure data sets and collected five population-based PCDD/Fs dietary intake and human milk concentration data.[33−36] In addition, we obtained unpublished data including 16 individual samples with dietary exposure and feces concentration measured.

The delayed rejection adaptive metropolis (DRAM) sampling was used to update parameters. Three Markov chains of 20,000 iterations were run, with the first 5000 iterations as "burn-in" iterations. The Gelman–Rubin method was used, and the Gelman–Rubin–Brooks diagram visually displayed the convergence test results. Three Markov chains used different parameter starting points, respectively: (1) the parameter nonlinearly fitted by the Nelder–Mead method; (2) the parameters used by EFSA; and (3) the lower boundary of the parameters. Model fitting was assessed using root-mean-squared error (RMSE). To validate the model, we collected unpublished data from three districts in Hubei Province, China, which included 46 men (n > 5 per district) with dietary exposure and measured serum PCDD/Fs concentrations. The optimized model with the best fitting parameters from three MCMC simulations was used to derive the human exposure associated with BMD and BMDL at the mean age of pregnant women (29 years), assuming breastfeeding for 12 months.

2.5.2. Uncertainty Factors and Modifying Factors. To provide evidence for provisional tolerable weekly intake (PTWI) calculations, uncertainty factors and modifying factors were applied. The uncertainty factor used in institutional standards and our studies was shown in Table S1. For example, a factor of 10 was applied when extrapolating from LOAEL to NOAEL and a factor of 3 for sensitive populations (sperm quality in children <10 years old and TSH in infants) in U.S. Environmental Protection Agency (US EPA) data.[37] Due to the large sample size, BMD method, and prospective design in human studies, an uncertainty factor of 1 was applied in our study. However, bioaccessibility percentages of PCDD/Fs vary across food groups such as rice, vegetables, and meat, with boiling resulting in lower bioaccessibility percentages of 4.9%, 1.9%, and 7.8%, respectively, and frying resulting in higher percentages of 17.7%, 15.2%, and 26.6%, respectively, which differs from the bioaccessibility (>95%) in carriers such as corn oil, eggs, and breast milk reported by EFSA.[23,38] Therefore, a modifying factor of 0.5 was applied to account for these differences.

3. RESULTS

3.1. Demographic Characteristics

The general characteristics of the study participants are shown in Table 1. Cases and controls were comparable in terms of age, BMI, and total cholesterol, while triglycerides in GDM subjects were significantly higher than those in healthy controls. PCDFs were the major contributors to the TEQ of dioxins with medians of 4.43 pg TEQ/g fat (IQR, 3.30–5.97), followed by PCDDs with medians of 1.87 pg TEQ/g fat (0.88–3.61 pg) and dl-PCBs with medians of 1.06 pg TEQ/g fat (0.20–1.83) in the study population. The median serum concentrations of TEQ of PCDDs, PCDFs, dl-PCBs, and PCDD/Fs and total dioxins in GDM cases were higher than those in controls and they were statistically significant. For

Table 1. Basic Characteristics and Dioxin Levels among Women with and without GDM[a]

variable	GDM	non-GDM	*P* value
Basic Characteristics			
n	77	154	
age (years)	29.23 ± 3.12	28.95 ± 2.76	0.73
BMI (kg/m^2)	22.38 ± 2.97	21.70 ± 2.77	0.11
triglycerides (mmol/L)	1.67 ± 0.74	1.42 ± 0.52	0.01
total cholesterol (mmol/L)	4.45 ± 0.97	4.32 ± 0.85	0.28
FBG (mmol/L)	4.84 ± 0.66	4.42 ± 0.37	<0.001
1h-PBG (mmol/L)	10.07 ± 1.59	7.83 ± 1.35	<0.001
2h-PBG (mmol/L)	8.38 ± 1.41	6.59 ± 1.07	<0.001
TEQ Values (pg TEQ/g fat)			
PCDDs TEQ	1.70 (0.81, 3.12)	2.67 (1.09, 4.88)	0.009
PCDFs TEQ	4.13 (2.96, 5.53)	5.04 (4.00, 7.01)	0.001
dl-PCB TEQ	0.96 (0.14, 1.70)	1.29 (0.58, 2.22)	0.003
PCDD/Fs TEQ	5.77 (4.73, 8.02)	8.32 (6.08, 11.53)	<0.001
DLCs TEQ	6.89 (5.82, 9.22)	9.86 (7.38, 13.17)	<0.001
∑ndl-PCBs (ng/g lipids)	35.92 (25.83, 47.34)	39.54 (27.2, 57.35)	0.10
∑PFAAs (ng/mL)	15.01 (11.10, 18.64)	15.41 (11.92, 19.28)	0.27
∑PBDEs (pg/g wet weight)	71.69 (53.52, 96.71)	87.37 (62.01, 122.29)	<0.001

[a]Basic characteristics are presented as mean ± standard deviation (SD) for each group while chemical levels are presented as mean (IQR). *P* value indicates the significance of the difference between the two groups, as determined by Mann–Whitney U tests.

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Table 2. Association between Dioxin TEQ and GDM: Odds Ratios (ORs) with Corresponding 95% Confidence Intervals (CIs)[a]

	quintile 1	quintile 2	quintile 3	quintile 4	quintile 5	continuous model
			PCDDs			
incidence	11/35	14/32	10/36	20/26	22/25	
unadjusted	reference	1.39 (0.55, 3.57)	0.88 (0.33, 2.35)	2.45 (1.02, 6.14)	2.8 (1.17, 6.99)	1.28 (1.02, 1.63)
adjusted model 1	reference	1.64 (0.64, 4.37)	0.85 (0.31, 2.33)	2.6 (1.05, 6.7)	3.14 (1.27, 8.17)	1.27 (1.01, 1.49)
adjusted model 2	reference	2.12 (0.79, 5.92)	0.98 (0.35, 2.77)	3.04 (1.17, 8.32)	3.77 (1.46, 10.31)	1.30 (1.02, 1.68)
			PCDFs			
incidence	10/36	10/36	16/30	17/29	24/23	
unadjusted	reference	1.00 (0.37, 2.72)	1.92 (0.77, 4.98)	2.11 (0.85, 5.45)	3.76 (1.56, 9.61)	2.18 (1.23, 4.01)
adjusted model 1	reference	0.94 (0.34, 2.64)	1.92 (0.75, 5.14)	2.29 (0.89, 6.12)	4.06 (1.63, 10.73)	2.30 (1.27, 1.43)
adjusted model 2	reference	1.00 (0.35, 2.85)	1.95 (0.74, 5.3)	2.44 (0.93, 6.69)	3.48 (1.36, 9.43)	1.99 (1.09, 3.81)
			dl-PCBs			
incidence	10/36	12/34	18/29	14/31	23/24	
unadjusted	reference	1.27 (0.49, 3.38)	2.23 (0.91, 5.74)	1.63 (0.64, 4.27)	3.45 (1.43, 8.82)	1.39 (1.12, 1.77)
adjusted model 1	reference	1.14 (0.42, 3.13)	2.09 (0.83, 5.5)	1.72 (0.66, 4.64)	3.61 (1.46, 9.45)	1.42 (1.13, 1.54)
adjusted model 2	reference	1.13 (0.41, 3.14)	2.23 (0.86, 6.02)	1.84 (0.68, 5.15)	3.58 (1.40, 9.72)	1.44 (1.13, 1.86)
			PCDD/Fs			
incidence	7/39	10/36	15/32	19/26	26/21	
unadjusted	reference	1.55 (0.54, 4.67)	2.61 (0.98, 7.57)	4.07 (1.55, 11.71)	6.9 (2.68, 19.75)	4.93 (2.42, 10.46)
adjusted model 1	reference	1.68 (0.57, 5.18)	2.61 (0.95, 7.75)	4.32 (1.61, 12.71)	7.89 (2.94, 23.55)	5.50 (2.62, 1.61)
adjusted model 2	reference	1.70 (0.57, 5.33)	3.12 (1.10, 9.57)	4.34 (1.59, 13)	7.06 (2.57, 21.45)	4.84 (2.27, 10.76)
			Total TEQ			
incidence	7/39	11/35	14/33	17/28	28/19	
unadjusted	reference	1.75 (0.62, 5.23)	2.36 (0.88, 6.89)	3.38 (1.28, 9.77)	8.21 (3.18, 23.63)	7.18 (3.26, 16.68)
adjusted model 1	reference	1.92 (0.66, 5.93)	2.56 (0.92, 7.69)	3.71 (1.37, 11.01)	10.29 (3.78, 31.4)	8.54 (3.72, 1.68)
adjusted model 2	reference	1.83 (0.61, 5.75)	2.80 (0.99, 8.61)	3.78 (1.36, 11.48)	8.87 (3.19, 27.58)	7.44 (3.19, 18.44)

[a]All dioxin TEQ were natural logarithm transformed. The covariates in the first adjusted model consisted of BMI, serum triglyceride, and total cholesterol. In the second adjusted model, additional covariates such as ∑PFAAs, ∑ndl-PCBs, and ∑PBDEs were included.

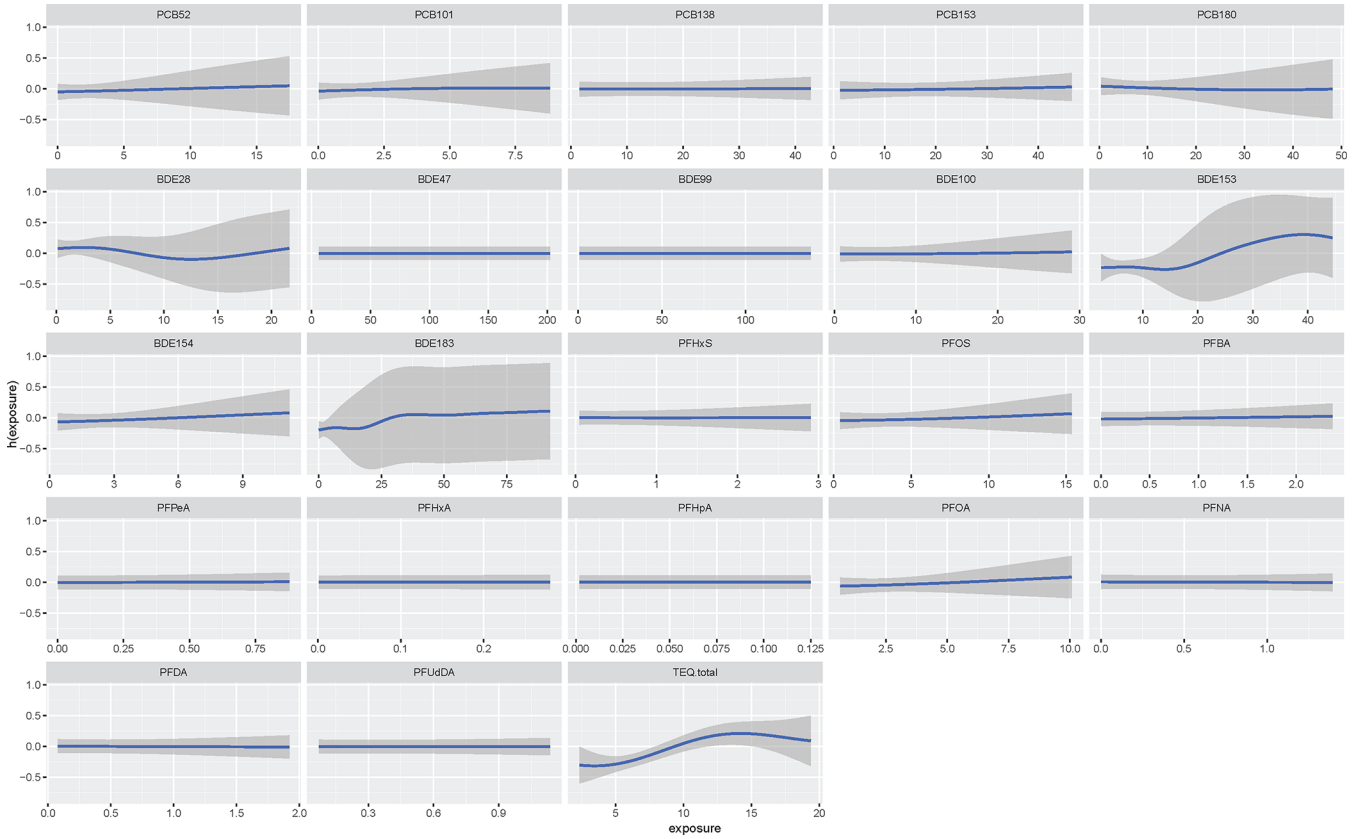

Figure 1. Univariate dose−response associations between chemicals and GDM with 95% confidence bands (shaded areas) using BKMR model.

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Table 3. BMD_{10}, $BMDL_{10}$, and Corresponding Daily Exposure Threshold of Dioxin TEQ Adjusted for BDE-153

model	BDE-153 exposure quantile	BMD_{10} (pg TEQ/g fat)	$BMDL_{10}$ (pg TEQ/g fat)	unadjusted exposure threshold (pg TEQ kg^{-1} bw $week^{-1}$)	adjusted exposure threshold (pg TEQ kg^{-1} bw $week^{-1}$)
logistic model	20%	6.47	5.34	3.57	7.14
	40%	5.64	4.75	3.08	6.16
	60%	5.08	4.26	2.73	5.46
	80%	4.52	3.71	2.38	4.76
probit model	20%	6.06	4.98	3.29	6.58
	40%	5.25	4.41	2.87	5.74
	60%	4.71	3.96	2.52	5.04
	80%	4.15	3.46	2.17	4.34

other POPs, cases and controls were similar in ∑PFAAs and ∑ndl-PCBs, while ∑PBDEs in GDM patients were significantly higher than those in heathy controls. Weak correlations were observed between specific pairs of POPs, such as PCDFs and ndl-PCBs as well as PCDFs and PBDEs, as shown in Figure S2.

3.2. Association between Serum Dioxin and Outcomes

Table 2 presents the results of the association between TEQ and GDM risk in different models. The association remained positive and statistically significant in both crude and adjusted models, and the effect estimates were only slightly attenuated by adjustment for other POPs. In the fully adjusted model, each unit increase in the log-transformed TEQ of PCDDs, PCDFs, dl-PCBs, PCDD/Fs, and DLCs was associated with estimated ORs of 1.30 (95% CI 1.02, 1.68), 1.99 (95% CI 1.09, 3.81), 1.44 (95% CI 1.13, 1.86), 4.84 (95% CI 2.27, 10.76), and 7.44 (95% CI 3.19, 18.44), respectively. Further analysis of the ORs of GDM by quintiles showed that individuals in the highest quintiles of dioxin TEQ of PCDDs (OR = 3.77, 95% CI 1.46, 10.31), PCDFs (OR = 3.48, 95% CI 1.36, 9.43), and dl-PCBs (OR = 3.58, 95% CI 1.40, 9.72) had significantly higher odds of developing GDM compared to those in the lowest quintiles in the fully adjusted models. Notably, TEQ of PCDD/Fs and DLCs showed the strongest association with GDM in the highest quintiles (OR = 7.06, 95% CI 2.58, 21.45; OR = 8.87, 95% CI 3.19, 27.58) compared to the lowest quartile. Table S2 shows the unadjusted and adjusted linear regression coefficients (β) for continuous glycemic outcomes associated with TEQ of dioxins. The fully adjusted model demonstrated a significant positive association between the increase in PCDFs, PCDD/Fs, and total TEQ and the rise in 1 h postprandial plasma glucose. Similarly, a significant positive association between the increase in PCDDs, PCDD/Fs, and total TEQ and the elevation in 2 h postprandial plasma glucose was observed. However, no significant correlation was found between dioxin TEQ and fasting blood glucose levels.

3.3. Identification of the Most Significant POPs

In Figure 1, it is evident that the total TEQ (PIP = 1.00) and BDE-153 (PIP = 0.88) demonstrate comparable univariate dose−response relationships with GDM. However, the PIPs of the other POPs were less than 0.5. Confident intervals with high precision were observed for dioxin TEQ, while more uncertainty was found for BDE-153. Figure S3 depicts binary interactions between POPs, where we observed no significant difference in the predictor−response function of total TEQ for other POPs fixed at different quantiles. Therefore, we excluded the interaction term in the dioxin TEQ BMD analysis. In Figure S4, we assessed the combined effects of POPs and estimated the posterior mean and associated 95% confidence intervals of the altered GDM risk (expressed as a z-score) when POPs were fixed at a particular percentile compared to when they were all at their 50th percentile. Our analysis revealed a simultaneous increase in GDM risk with an increase in POPs. For 1h-PBG, dioxin TEQ (PIP = 0.86) were the most significant POPs, while for 2h-PBG, no chemicals had a PIP > 0.5. Additionally, we found that the estimated 1h-PBG and 2h-PBG increased with a simultaneous increase in POPs in Figure S5.

The figure shows the univariate relationship between each covariate and the outcome, where all of the other exposures are fixed to a particular percentile.

3.4. Benchmark Dose Calculation

Table S3 summarizes the results of using BMDS software and the Bayesian BMD website to estimate the univariate association between total TEQ and glycemic measures in this study. The probit model had the lowest AIC and the highest posterior weight (29%) and the logistic model had the second largest posterior weight (28%), as shown in Figure S6 and Figure S7. Bayesian modeling suggested modest effects of dioxin TEQ on GDM in various models, while for continuous outcomes, many models were unable to fit parameters or had large BMD/BMDL ratios. GDM was the most sensitive outcome with a reasonable BMD/BMDL ratio, thus further analysis was conducted on GDM in coexposure scenarios.

Table 3 shows the BMD_{10} and $BMDL_{10}$ values for total TEQ adjusted for BDE-153. In the probit model, the BMD_{10}/$BMDL_{10}$ for total TEQ were 6.06/4.98 pg TEQ/g fat at 20% BDE-153 exposure and 4.15/3.46 pg TEQ/g fat at 80% exposure. The logistic model had BMD_{10}/$BMDL_{10}$ values of 6.47/5.34 pg TEQ/g fat at 20% BDE-153 exposure and 4.52/3.71 pg TEQ/g fat at 80% exposure. Figure S8 shows that coexposure to BDE-153 can influence the BMD and BMDL for DLCs, with a decrease in BMD values when assumed BDE-153 exposure increases.

3.5. Bayesian Optimization of PBTK Model and Daily Exposure Threshold Calculation

Figure S9 shows the Gelman−Rubin−Brooks plots of three chains, suggesting good convergences among chains for each parameter. Corrected scale reduction factors were calculated for the three chains based on the method of Brooks and Gelman, and the values were between 1.0 and 1.02, which corresponded to an equilibrium posterior parameter distribution. The prior and posterior distributions of the means and standard deviations for the estimated parameters were shown in Table S4 and Figure S10. The medians of posterior distributions for K_half, fmin, fmax, and fab were close to prior estimates, while the standard deviation was substantially lower than prior distributions. However, the posterior distribution of

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Table 4. Point of Departure of Dioxin in Our Study and Comparisons with US EPA and EFSA[a]

institution	human study outcomes	POD type	BMD software	sample size	point of departure (pg TEQ/g fat)	unadjusted dietary exposure threshold (pg TEQ kg^{-1} bw $week^{-1}$)	adjusted dietary exposure threshold (pg TEQ kg^{-1} bw $week^{-1}$)
			Our Study				
	GDM	$BMDL_{10}$	BMDS	231	3.08	1.96	3.92
	GDM	$BMDL_{10}$	Bayesian BMD	231	1.98	1.19	2.38
	GDM	$BMDL_{10}$ adjusted for other POPs	WinBUGS	231	4.30	2.17	4.34
	GDM	NOAEL	-	231	6.39	2.73	5.46
			Institutional Standards				
US EPA (2010)	sperm concentration, total sperm motility, TSH concentration	LOAEL	-	51/71	39	4.7	4.7
EFSA (2018)	sperm concentration	NOAEL	-	133	7	2	2

[a]The derivations from the point of departure to the dietary exposure threshold in our study and institutional standards is conducted using the PBTK model. TSH, thyroid-stimulating hormone; NOAEL, no-observed-adverse-effect level; LOAEL, least-observed-adverse-effect level.

k_e and k_a, rate constants for dioxin elimination, had flatter distributions and were substantially greater than prior information, indicating that the PCDD/Fs elimination was faster and more variational than TCDD. As shown in Figure S11, the root-mean-square error (RMSE) of the optimized parameters was lower than the original parameters for population-based data sets (39.9% improvement), individual data sets (10.6% improvement), and independent population-based data sets (66.6% improvement). Therefore, the optimized model predicted the data reasonably well and can be used to derive estimated dietary dioxin exposure from serum dioxin level.

The optimized model was used to derive the human dietary exposure associated with the BMDs and BMDLs at the mean age of pregnant women, as presented in Table 3. After considering the uncertainty and modifier factor, the daily exposure threshold for dioxin TEQ was 4.76 and 4.34 pg TEQ kg^{-1} bw $week^{-1}$ for the probit and logistic models, respectively, corresponding to the BMDL when assuming 80% exposure to BDE-153. Table 4 shows the daily exposure thresholds derived by different methods in our study, as well as comparisons with institutional standards. Our PTWI estimate of 4.34 pg TEQ kg^{-1} bw $week^{-1}$ is slightly lower than the PTWI in US EPA (2010) for sperm concentration, total sperm motility, and TSH concentration but is higher than the PTWI in EFSA (2018) for sperm concentration. The flowchart of the process of the dioxin risk assessment is shown in Figure S12.

4. DISCUSSION

In this study, we revealed a robust positive association between serum dioxins and GDM and provided an estimation of the daily exposure threshold for dioxins-induced GDM based on a nested case control study. We identified new dioxin BMD_{10} and $BMDL_{10}$ values of 4.15 and 3.46 pg TEQ/g fat for GDM adjusted for covariates and coexposure POPs. Furthermore, we utilized a Bayesian−MCMC optimized PBTK model based on dioxin TEQ and additional factors to derive a reference dose of 4.34 pg TEQ kg^{-1} bw $week^{-1}$. These findings provide crucial information for future risk assessments and highlight the need for more comprehensive monitoring and regulation of dioxin exposure, particularly in vulnerable populations such as pregnant women.

GDM is defined as glucose intolerance of varying severity in pregnant women, and its global prevalence has increased remarkably to 14.0%.[39] It has both short-term and long-term consequences for the mother and child.[40] Apart from lifestyle, age, and genetic factors, a systemic review and meta-analysis have found exposure to certain POPs, including PCBs, PBDEs, PFASs, and phthalates (PAEs), increased the risk of GDM.[5] Although few studies have explored the association between dioxins and GDM, the effects of dioxins on glucose metabolism have been widely studied. It is worth noting that associations between PCDD/Fs and diabetes are consistent.[7,41,42] Two cohort studies established after dioxin contamination incidents in Seveso, Italy and Yucheng, Taiwan, China found that high dioxin levels were associated with diabetes in women but not in men.[43,44] However, determining the direction of the association between dioxins and diabetes is challenging due to lipolysis in diabetes patients, which may release tissue dioxin into the blood and elevate serum dioxin levels.[45] Our study utilized a prospective design to obtain precise estimates of prediagnosis dioxin levels and provided potential causal evidence for the associations between exposure to dioxins and GDM compared to cross-sectional studies. Our results suggest that exposure to dioxin significantly increases the risk of GDM, with the strongest associations observed in the highest quintiles of the TEQ of PCDD/Fs and DLCs. Moreover, the study provides evidence that dioxin exposure is associated with postprandial plasma glucose levels but not fasting blood glucose.

Both *in vitro* and *in vivo* studies have demonstrated that AHR plays a physiological function in glucose metabolism.[46−48] Dioxins exert toxicological effects through the AHR-mediated pathway and then suppress the function of peroxisome proliferator-activated receptor (PPAR) γ, leading to insulin resistance.[49,50] Dioxin also affects pancreatic β-cells and reduces their function.[10] In addition, POPs are reported to increase AHR transactivating (AHRT) bioactivity, and a cohort study has linked serum AHRT activity with an increased risk of GDM.[51−53] However, several POPs, including PCBs, furans, PAHs, and PBDEs, can activate AHR, albeit with different potencies.[54] For example, *in vitro* and *in silico* studies have promoted the dioxin-like potency of prototype HO and MeO analogues of PBDEs.[55,56] Therefore, it is crucial to explore the potential synergistic effects of POPs as mixtures

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and their mechanisms of action on glucose metabolism and GDM risk.

Multiple statistical methods have been applied to estimate the overall effect and identify significant pollutants. Commonly used methods include nonparametric approaches (e.g., classification and regression tree) and regularized regression methods (e.g., least absolute shrinkage and selection operator).[20] Another approach, Bayesian kernel machine regression (BKMR), is a flexible tool that can handle nonlinear and interactive effects between pollutants, control for confounding variables, estimate overall effects, and allow for probabilistic inference and uncertainty quantification within a Bayesian framework.[57] Our study found that the chemicals with the highest PIP for GDM were dioxin and BDE-153 with similar dose−response curves, suggesting that they may influence glycemic measures through similar pathways. BDE-153 has been shown to disrupt glucose homeostasis and alter lipid metabolism in both animal and human studies.[58−60] However, the large uncertainties observed for BDE-153 suggest that further research is needed to better understand its role in the development of GDM. In our study, identifying significant chemicals from high-dimensional data and exploring potential interactions provides the basis for BMD calculations.

Traditionally, the no-observed-adverse-effect level (NOAEL), or the lowest-observed-adverse-effect level (LOAEL), is used as the internal exposure limits derivation for dioxin and other POPs.[23,37] However, BMD modeling is now considered the preferred approach to identify and manage the risk of many chemicals, which can make use of complete data and has a higher quantitative sensitivity and accuracy.[61] Choosing an appropriate model for BMD calculation depends on several factors, such as the biological plausibility, the goodness of fit, and comparison of alternative models. Our study found that the dose−response relationships for GDM were robust across models, and probit and logistic models had the lowest posterior weight. The S-shaped curve is supported by previous research showing a positive dose−response relationship between TCDD and health outcomes at low levels of exposure (<10 pg/g fat) but not at high levels.[62] However, in the investigation of continuous outcomes, most models have failed to fit parameters or have had unreasonably large BMD/BMDL ratios. It may be due to factors such as measurement error, nonlinear relationships, inadequate sample size, and observed chemical concentration range.

Through screening significant chemicals and analyzing dose−response relationships, we used generalized linear models to calculate the BMD of dioxins for GDM in coexposure scenarios. Conventional BMD methods and software are typically used to estimate the association between a single exposure and an outcome, which may not be suitable for addressing the complexities of mixed exposure scenarios in human populations.[17] Limited statistical methods have estimated BMD in coexposure scenarios, such as the delta method in BKMR,[21] reverse processing in principal component analysis (PCA),[63] covariate-specific BMD,[61] and comparisons of BMD intervals.[64] A widely accepted method is to calculate the weighted sums by relative potency and then use a univariate BMD model.[18,19] In our study, when BDE-153 was assumed at 80% exposure level, BMD_{10} and $BMDL_{10}$ were 4.15/3.46 pg TEQ/g fat, respectively. This is the first BMD value for dioxins in a mixture exposure scenario, and the BMD estimation was made more accurate by adjusting for levels of other POPs. It is essential to consider the potential influence of other pollutants on the target pollutant's potency when assessing health risks associated with exposure to environmental pollutants.

PBTK models are essential for connecting dietary exposure to chemicals with their internal levels in the body. The original PBTK model for dioxin was developed and optimized on TCDD while the half-lives for different DLCs differed in a range of 6.8−11.6 years.[24,65,66] Corresponding to BMDs of dioxin TEQ, a suitable model for PCDD/Fs and dl-PCBs was of great significance. We applied Bayesian−MCMC methods, in which the joint posterior distribution of the parameter is proportional to the prior distribution of the parameter and the likelihood of PCDD/Fs exposure data of Chinese residents. The result showed the model performed well in the training data set and validating data set in estimation of dietary exposure based on serum concentration. Therefore, the Bayesian approach holds great promise for advancing PBTK modeling in risk assessments as it allows for better integration of animal experiments and population data sets.

In our study, the daily exposure threshold for the total TEQ of dioxin was 4.34 pg TEQ kg^{-1} bw $week^{-1}$. In 2010, the US EPA set the oral PTWI for TCDD at 4.7 pg kg^{-1} bw $week^{-1}$, based on two studies linking TCDD exposure to decreased sperm concentration and motility in men and elevated thyroid-stimulating hormone levels in newborns.[37] In 2018, the EFSA developed an oral PTWI for PCDD/Fs at 2 pg TEQ kg^{-1} bw $week^{-1}$ based on a cohort study that associated serum dioxin with decreased sperm concentration.[23,67] Strict regulatory controls on major industrial sources and national monitoring programs have contributed to a significant reduction in human exposure in recent years, leading to a decrease in plasma and human milk levels.[23,68] In China, the 95th percentile of the population dietary exposure level to PCDD/Fs and dl-PCBs was estimated at 0.06 and 1.14 pg TEQ kg^{-1} bw $week^{-1}$, so the probability of the adverse health risk in the Chinese population is relatively low.[68] Our study provides evidence for a newly proposed PTWI based on endocrine outcomes. However, it is important to also consider the potential impact of dioxin exposure on potent and sensitive outcomes, including immune system impairment, neurodevelopmental effects, carcinogenesis, and others, where relatively low POD values might be identified.[61,69,70]

There are several strengths in our study. First, the outcomes of this study were based on a longitudinal study to establish cause−effect relationships. Second, Bayesian kernel machine regression and BMD calculation in coexposure scenarios allows for the identification of significant POPs and the assessment of their effects while adjusting for the effects of other POPs, which is a powerful tool for calculating the point of departure through data sets on mixture exposure. Third, PBTK is a robust and versatile tool that can be updated continuously to include new and relevant data, thus improving the accuracy and relevance of risk assessments. However, limitations do exist in our study. One is the small sample size, which may limit the accuracy of coefficient estimation and benchmark dose analysis. Second, although we were able to adjust for potential confounding variables, residual confounding by dietary intake and physical activity may be present and cause bias in our estimate. Moreover, additional epidemiological studies are required to confirm the association between dioxin and GDM.

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CONCLUSIONS

In summary, this study provides evidence of a significant association between dioxin exposure and gestational diabetes mellitus in pregnant women. The study also demonstrates the usefulness of coexposure statistical methods and an optimized physiologically based toxicokinetic (PBTK) model in assessing the relationship between dioxin and GDM and estimating a daily exposure threshold. The study found that the daily exposure threshold for dioxin should be below 4.34 pg TEQ kg^{-1} bw $week^{-1}$ to avoid harmful serum concentrations for GDM, which is close to EFSA and US EPA's dioxin reference dose. Therefore, this study suggests that dioxin reference doses based on endocrine disruptors should be considered for pregnant women.

ASSOCIATED CONTENT

Data Availability Statement

Data will be made available on request.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/envhealth.4c00017.

Supplementary results obtained from the data analysis process, comprehensive details of the risk assessment methodology, and illustrative method flowcharts (PDF)

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Notes

All procedures performed in the study involving animals followed the ethical standards and were approved by the Ethics Committee of China National Center for Food Safety Risk Assessment.

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