Polychlorinated Biphenyls, Polychlorinated Dibenzo-*p*-dioxins, Polychlorinated Dibenzofurans, Pesticides, and Diabetes in the Anniston Community Health Survey Follow-up (ACHS II)

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Abbreviations: PCBs, polychlorinated biphenyls; PCDD, polychlorinated dibenzo-p-dioxins; PCDF, polychlorinated dibenzofurans; TEQ, Dioxin toxic equivalent; p,p'- DDE, dichloro-diphenyl dichloroethylene; TCDD, 2, 3, 7, 8-tetrachloro dibenzo-p-dioxin, β-HCCH, beta-hexachlorocyclohexane; HCB, hexachlorobenzene.

Abstract

Dioxins and dioxin-like compounds measurements were added to polychlorinated biphenyls (PCBs) and organochlorine pesticides to expand the exposure profile in a follow-up to the Anniston Community Health Survey (ACHS II, 2014) and to study diabetes associations. Participants of ACHS I (2005-2007) still living within the study area were eligible to participate in ACHS II. Diabetes status (type-2) was determined by a doctor's diagnosis, fasting glucose ≥125 mg/dL, or being on any glycemic control medication. Incident diabetes cases were identified in ACHS II among those who did not have diabetes in ACHS I, using the same criteria. Thirty-five ortho-substituted PCBs, 6 pesticides, 7 polychlorinated dibenzo-*p*-dioxins (PCDD), 10 furans (PCDF), and 3 non-ortho PCBs were measured in 338 ACHS II participants. Dioxin toxic equivalents (TEQs) were calculated for all dioxin-like compounds. Main analyses used logistic regression models to calculate odds ratios (OR) and 95% confidence intervals (CI). In models adjusted for age, race, sex, BMI, total lipids, family history of diabetes, and taking lipid lowering medication, the highest ORs for diabetes were observed for PCDD TEQ: 3.61 (95% CI: 1.04, 12.46), dichloro-diphenyl dichloroethylene (p,p'-DDE): 2.07 (95% CI 1.08, 3.97), and *trans*-Nonachlor: 2.55 (95% CI 0.93, 7.02). The OR for sum 35 PCBs was 1.22 (95% CI: 0.58-2.57). To complement the main analyses, we used BKMR and g-computation models to evaluate 12 mixture components including 4 TEQs, 2 PCB subsets and 6 pesticides; suggestive positive associations for the joint effect of the mixture were found but were not significant. These results add support to earlier findings for diabetes associations with PCBs, PCDDs, *trans*-Nonachlor and p,p'-DDE.

Keywords: Persistent organic pollutants, PCBs, Pesticides, Diabetes, Longitudinal study, Mixture analysis, BKRM, g-computation

1. Introduction

The Swann Chemical Company (1929-1935) and Monsanto Company (1935-1971) operated a production plant in Anniston, AL that manufactured polychlorinated biphenyls (PCBs) between 1929 and 1971. The facility produced all commercial and experimental Aroclor® mixtures, containing a number of individual PCB congeners, accounting for about half of the total PCB production in US (Erickson and Kaley, 2011). Elevated concentrations of PCBs have been previously reported in Anniston residents (ATSDR 2000, Pavuk et al., 2014a) and environmental media (Hermanson et al., 2003). Our previous report on PCB exposure and diabetes in Anniston residents from the Anniston Community Health Survey (ACHS I) noted increased risk of diabetes for the sum of 35 ortho-substituted PCBs in data collected from 2005-2007 (Silverstone et al., 2012). While we were not able to review and verify the medical records, most of the diabetes was assumed to be type 2 diabetes based on late onset. This risk was more pronounced in those younger than 55 years old (median age of the cohort) and in females (Silverstone et al., 2012). Analyses with the toxicological/structure-activity subsets of PCB congeners did not reveal additional information; dioxin-like PCB congeners were limited to mono-ortho congeners that are highly correlated with other non-dioxin like PCBs and have weak affinity to the aryl-hydrocarbon receptor (Ah-R) pathway (Gourronc et al., 2018; Larsson et al., 2015). We conducted a follow-up study to ACHS I, ACHS II, in 2014, about 8 years after the baseline. The measurements of serum polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like non-ortho PCBs (non-ortho-PCBs) were added to ACHS II to expand the exposure profile of the Anniston cohort (Birnbaum et al., 2016).

Associations between exposure to PCBs and type 2 diabetes, along with other persistent organic pollutants (POPs), have been studied extensively, and have been the subject of several in-depth reviews (Lee et al., 2014; Lind et al., 2018; Taylor et al., 2013; Thayer et al, 2012). Strong associations between various PCBs, dioxin congeners, and pesticides first reported in data from the National Health and

Nutrition Examination Survey (NHANES) databases by Lee at al. (2006, 2007, and corroborated by Everett et al. (2007), gave impetus to a score of cross-sectional investigations around the world evaluating metabolic disturbances related to diabetes and exposure to mostly non-dioxin like PCBs, organochlorine pesticides, and other POPs such as polybrominated diphenyl ethers (PBDEs) (Airaksinen et al., 2011; Arrebola et al., 2013; Everett and Thompson, 2012; Gasull et al., 2012; Han et al., 2019; Henriquez-Hernandez et al., 2017; Huang et al., 2015; Kim et al., 2018; Marushka et al., 2018; Nakamoto et al., 2013; Persky et al., 2012; Raffetti et al., 2018; Silverstone et al., 2012; Tanaka et al., 2011). A smaller number of longitudinal studies have investigated the relationship between POPs and diabetes incidence prospectively, with less consistent results (Berg et al., 2021; Charles et al., 2022; Lee et al. 2010, 2011; Magliano et al., 2021; Rignell-Hydbom et al., 2009; Turyk et al., 2009, 2015; Suarez-Lopez et al, 2015; Tornevi et al., 2019; Vasiliu et al., 2006; Wu et al., 2013; Zong et al., 2018). This body of research was built on earlier investigations focused on the examination of the association between 2, 3, 7, 8-tetrachloro dibenzo-p-dioxin (TCDD, prototypical "dioxin") and diabetes in occupational studies and veterans' cohorts with higher than background exposures (Calvert et., 1999 Longnecker and Michalek 2000, Michalek and Pavuk 2008, Steenland et al., 1999, 2001; Vena et al., 1998).

The potential mechanism of action has been elucidated in more detail for dioxin-like PCBs. Exposure to PCBs 77 and 126 which are strong Ah-R agonists, resulted in impaired glucose and insulin tolerance in mice on low and high fat diets (Baker et al., 2015). Human pre-adipocytes treated with PCB 126 had significantly reduced ability to fully differentiate (to adipocytes), downregulating transcription factor PPAR-γ and late adipocyte differentiation genes (Gadupudi et al., 2015). Furthermore, exposure to PCB 126 activated the pro-inflammatory response pathway, which is recognized as a causative factor in the development of type 2 diabetes (Gourronc et al., 2018). A number of potential mechanisms leading to insulin resistance for non-dioxin like PCBs have been investigated by Kim et al. (2019).

Traditional approaches to study multi-pollutant exposures are often limited due to potential issues including, multicollinearity, model misspecification, and the inability to evaluate multiple correlated exposures and pollutants as a single mixture in contrast to modeling associations with individual chemical compounds/analytes (Gibson et al., 2019; Taylor et al., 2013). To address these limitations, advanced statistical methods, such as the Bayesian Kernel Machine Regression (BKMR) (Bobb et al., 2015, 2018) and quantile-based g-computation (g-comp) (Keil et al., 2020), have been introduced to the field. BKMR is a semiparametric statistical method that can be employed to estimate the overall mixture effect and individual chemical impact within a mixture on health outcomes, exploring potential nonlinearity and non-additivity (Bobb et al., 2015, 2018). Quantile g-computation is a causal inference method that uses a weighted quantile regression approach and can generate a marginal structural estimate for the overall joint exposure effect on the change in the outcome (Snowden, 2011; Keil et al, 2020). A growing number of epidemiologic studies have applied BKMR to evaluate the effect of exposure to POPs, mostly per- and polyfluoroalkyl substances (PFAS) on gestational diabetes and glucose homeostasis, thyroid function, or hypertensive disorders (Preston et al., 2020, 2022; Xu et al., 2022; Zhang et al., 2022a). A few studies evaluated dioxin-like compounds and PCBs using mixture methods studying various health outcomes such hyperuricemia, breast cancer, neurodevelopment measures or cognitive function (Yim et al., 2022; Parada et al., 2021; Sasaki et al., 2023; Zhang et al., 2022b).

In the present ACHS II study, we examined associations between diabetes and PCDDs, PCDFs, and non-ortho PCBs, in addition to ortho-substituted PCBs and chlorinated pesticides. Cross sectional associations with prevalent diabetes in the ACHS II sample (for dioxins, PCBs, and pesticides) were examined as well as association with incident diabetes (for PCBs and pesticides) in members of the Anniston cohort to further elucidate possible relationships between environmental exposures to POPs and diabetes. Additionally, the broad exposure assessment results available in the Anniston cohort gave

us an impetus to also perform complementary Bayesian kernel machine regression (BKMR) and quantile g-computation analyses to assess the joint POPs mixture effects and the relative importance of mixture components on diabetes.

2. Materials and methods

2.1 Study Design and Population

Methods for the ACHS I and ACHS II have been described in detail in previous publications (Pavuk et al., 2014b; Birnbaum et al., 2016). For the follow up study, all surviving participants of ACHS I with PCB measurements were eligible to participate (n=765). Prior to enrollment, we were able to ascertain that 114 participants had died; in addition, 69 participants were found to have moved outside the study area. Of the remaining participants, 438 with a current address in the study area were successfully contacted. Of these, a total of 359 enrolled as participants in the follow-up study (82%) (Birnbaum et al., 2016). Sufficient volumes of sera for dioxin analyses were collected from 338 participants who have been included in the statistical analyses presented here. The participants also provided a fasting blood sample for measurements of glucose, POPs, and lipid levels, and had their height, weight, waist circumference, and blood pressure measured using a standardized protocol. During the study office visit, demographic information, medical and family history, as well as self-reported health behaviors and health conditions were recorded. Individual medications including glycemic control medication (oral and injectable; name, dose, frequency) were recorded and verified by a nurse (participants had to bring the medication to the study office).

Diabetes was defined as self-report of physician-diagnosed diabetes, or fasting glucose ≥125 mg/dL, or being on any glycemic control medication. Non-diabetes was defined as a fasting glucose <125 mg/dL and the absence of glycemic control medications. Reported diabetes was type II diabetes;

158 we could not verify medical records if any were type I. For the present analyses, we excluded
159 participants with prediabetes (glucose between 100 and 124mg/dL) to be consistent with reporting from
160 ACHS I (Silverstone et al. 2012). The studies were reviewed and approved by the appropriate
161 Institutional Review Boards.
162
163 *2.2 Laboratory Analyses*
164 The sera were isolated by centrifugation using red top vacutainer tubes and shipped on dry ice to
165 the Division of Laboratory Sciences at the CDC, National Center for Environmental Health (NCEH).
166 Participant samples were stored at −70°C. Serum samples were first measured for PCDD/F and non-
167 ortho PCBs based on published methodology (Turner et al. 1997) using 20 g of serum (median: 20g;
168 range: 2.5–20.7 g; 10^{th} percentile: 14:0 g). The samples were then measured for ortho-PCBs and
169 pesticides according to published methodology (Sjödin et al., 2004; Jones et al., 2012) using 2g of
170 serum. Each analytical batch for ortho-PCBs/pesticides was defined as 24 unknowns, 3 quality controls,
171 and 3 method blanks, while for PCDD/F and non-ortho-PCBs, each analytical batch included 8
172 unknowns, 2 quality controls, and 2 method blanks. Measurements of target organohalogen compounds
173 were made by gas chromatography–isotope dilution high-resolution mass spectrometry. Serum total
174 lipids were calculated by the enzymatic "summation" method using triglyceride and total cholesterol
175 measurements (Bernert et al., 2007). The 2005 WHO Toxic Equivalency Factors (TEF) were used to
176 calculate the congeners' toxic equivalency (TEQ) and total dioxin TEQ (Van den Berg et al., 2006).
177
178 *2.3 Statistical Analysis*
179 Statistical analyses were conducted using SAS System 9.4 (SAS Institute, Inc., Cary, NC), and
180 SPSS (IBM SPSS Statistics for Windows, Version 28.0, Armonk, NY: IBM Corp). Descriptive
181 statistics for demographic characteristics and exposure variables were calculated for those with diabetes,

prediabetes, or no diabetes; differences between groups were compared using a two-tailed t-test or one-way ANOVA for continuous variables and chi-square tests for categorical variables. General linear models were used to calculate geometric mean concentrations of the chemical exposures by diabetes status with control for age, sex, race, BMI, smoking status and family history of diabetes. Spearman's correlation coefficients were run for all exposure variables. As these POPs correlations were expected to be high, we also conducted hierarchical cluster analysis (HCA), performed via ClustOfVar package in R (Chavent et al., 2012). It provides hierarchical and k-means clustering of a set of variables. The center of a cluster of variables is a synthetic variable which is the first principal component calculated by PCAmix. The homogeneity of a cluster is defined as the squared correlation between the variables and the center of the cluster.

Unconditional logistic regression models were used to contrast diabetes status (diabetes, no diabetes) with the exposure variables. Chemical exposures included: six pesticides (hexachlorobenzene [HCB], β-HCCH, *trans*-Nonachlor, Oxychlordane, pp'-DDE, Mirex), the sum of 35 PCB congeners, total dioxin TEQ and its subcomponents (PCDD TEQ, PCDF TEQ, mono-ortho PCBs TEQ and non-ortho PCBs TEQ). These summary exposure groups were created as follows, PCDD TEQ (sum of 7 dibenzo-dioxin congeners: 2,3,7,8-TCDD, 1,2,7,8-PCDD, 1,2,3,4,7,8-HCDD, 1,2,3,6,7,8-HCDD, 1,2,3,7,8,9-HCDD, 1,2,3,4,6,7,8-HCDD, OCDD), PCDF TEQ (sum of 10 dibenzo-furan congeners: 2,3,7,8-TCDF, 1,2,3,7,8-PCDF, 2,3,4,7,8-PCDF, 1,2,3,4,7,8-HCDF, 1,2,3,6,7,8-HCDF, 1,2,3,7,8,9-HCDF, 2,3,4,6,7,8-HCDF, 1,2,3,4,6,7,8-HCDF, 1,2,3,4,7,8,9-HCDF, OCDF), mono-ortho PCBs TEQ (sum of PCBs 105, 118, 156, 157, 167, and 189), non-ortho PCBs TEQ (sum of PCBs 81, 126, 169) (van den Berg et al., 2006). In addition to sum of PCBs, we used structure-activity groups based on the chlorine substitution. The subsets were the di-ortho, and the tri- and tetra- ortho PCB congeners, while the mono-ortho and non-ortho PCBs substituted groups were already included with the dioxin TEQs above. For the individual congeners and pesticides, we used LOD/square root2 to substitute levels

below LOD (Hornung and Reed, 1990). For the main analysis, three logistic regression models were applied with co-variables selected based on the literature review of POPs and diabetes associations, and variables available in the Anniston study (Turyk et al., 2009, Lee et al., 2014; Zong et al., 2018). Model 1 analyses were adjusted for basic demographic variables: age, race (African American or White), sex (female or male), and log-transformed total lipids. Model 2 was adjusted for additional covariables including, family history of diabetes (yes or no), lipid lowering medication (yes or no), current smoking status (yes or no), BMI (kg/m^2), access to health insurance during last year (yes or no), and education (high school or less, more than high school). Model 3 was a more parsimonious model, with adjustment for age, race, BMI, lipid lowering drugs and family history of diabetes. Appropriate covariables for model 3 were ascertained using a backwards stepwise procedure and a likelihood *p*-value for removal of 0.10. Sum of PCBs, PCB groups, pesticides and all TEQ variables were modeled as whole weight variables and logarithmically transformed to base 10 ($\log_{10}$). Odds ratios (OR) and 95% confidence intervals (CI) are presented for diabetes associations with exposure variables modeled as continuous variables (all exposure compounds). We also ran exploratory models stratified by sex (male, female) and race (African American, White), but reduced sample size has limited those inferences. Included covariables were identical to those in model 3, the parsimonious model described above. Interaction terms were assessed for the sum PCB and PCB/TEQ subgroups using the likelihood ratio *p* value for removal of > 0.10 in a backward stepwise procedure.

Odds ratios for incident cases of diabetes versus non-diabetes group were calculated using the same regression models 1 and 2 as described above but using the exposure variables and time-sensitive covariates (e.g., current smoking) from the baseline ACHS rather than the follow-up study. Of the 37 incident diabetes cases reported between the baseline and the follow up studies, 24 had nurse-verified use of glycemic medication (63.2%). To complement the main statistical analyses, we used the Bayesian kernel machine regression (BKMR) to evaluate the joint and individual effects of exposure to

PCBs, dioxins and pesticides on the odds of diabetes and to estimate the relative contributions of different mixture components (Bobb et al., 2015, 2018). BKMR uses a kernel function to flexibly model both the overall joint effect of an exposure mixture and to estimate individual exposure-outcome associations. To determine the joint association, the algorithm subtracts the mean value of the outcome when the mixture concentrations are at the 25th percentile from the mean value of the outcome when the mixture concentrations are at the 75th percentile while holding the covariates constant (the percentiles are modifiable).

Given the sample size for the main analyses (n=310), and large number of assessed exposures [dioxin-like compounds (20), PCBs (35), pesticides (6); for a total of 62 analytes] we elected to use the same structure-activity based dioxin TEQs and PCB groups as described above to reduce the number of exposure variables to 12. We have also used those groups in our hypertension outcomes analyses (Pavuk et al., 2019) and this strategy is similar to what was done in other studies assessing mixtures, e.g., Xu et al., 2022, Preston et al., 2022, as a way to maintain the robustness of the analytical method. Thus, we included the same two groups of non-dioxin-like PCBs: the di-ortho and tri- and tetra- ortho substituted PCBs, four TEQ groups: PCDD, PCDF, non-ortho, and mono-ortho PCB TEQs, as well as six individual pesticides (which do not have a common mode of toxicity) in BKMR analyses.

Additionally, the variable selection option in BKMR was used to estimate posterior inclusion probabilities (PIPs) for each exposure to identify the relative importance of these mixture components to the overall mixture (Bobb et al., 2018). We used the hierarchical variable selection function, which is recommended in the presence of higher group correlations. For the dichotomous diabetes outcome (diabetes versus no diabetes), we used the probit extension of BKMR (Bobb et al., 2018). Models were run for 50,000 iterations using the Markov chain Monte Carlo sampler. The model convergence was checked by visually inspecting trace plots. Possible nonlinearity in dose-response functions and interactions were also examined among the mixture component. Consistent with the main analyses, all

exposure variable concentrations were log10 transformed for the BKMR models due to sensitivity to extreme values. To facilitate comparability across the different statistical approaches we included the same set of covariates in all models.

To investigate consistency of findings across different multipollutant approaches, we also employed quantile g-computation as a second complementary method (Snowden, 2011; Keil et al., 2020). Quantile g-computation provides a single estimate of the overall marginal structural effect of the exposure mixture on the outcome and weights for the individual mixture components. The weights represent the exposures' relative contributions to the overall mixture effect. The positive and negative relative weights each sum to 1.0. The overall effect estimate (psi (ψ)) was computed for exposure to dioxins, PCBs, and pesticides mixture in relation to diabetes using a one-quantile change of all mixture components, assuming a Gaussian distribution. The mixture slope and overall model confidence bounds were iterated by 500 bootstraps; no boot option was used to obtain relative weights. Prior knowledge from the BKMR, including possible nonlinearity or non-additivity, was fed to the quantile g-computation if necessary.

Mixture analyses were conducted using R (version 4.2.1; R Development Core Team) with the packages "bkmr," for BKMR and "qgcomp," for quantile g-computation; https://cran.r-project.org/web/packages/qgcomp/).

3. Results

3.1 Study Population Demographics

The demographic comparisons between diabetes, pre-diabetes, and participants with no diabetes are shown in Table 1. Participants with diabetes and pre-diabetes were older by 5 and 6 years compared to those with no diabetes. While 51% of the 2014 cohort was African American, 60.7% of those with

278 diabetes diagnoses were African American. Females represented most of the participants (72%),
279 however, no major difference in the proportions of females with and without diabetes or pre-diabetes
280 were noted. Glucose levels, as expected, were elevated in participants with diabetes and pre-diabetes as
281 well as mean insulin. Significant differences by diabetes status were not observed for educational level
282 or access to health insurance. There was a significantly higher proportion of positive family history
283 reports of diabetes among participants with diabetes (78% vs 59%). Smoking status, total lipids,
284 triglycerides, and total cholesterol were not significantly different across the three groups. There were
285 significantly higher proportion of participants on lipid lowering medication among those with pre-
286 diabetes (61%) or diabetes (48%) compared to those without diabetes (31%).
287
288 *3.2 Geometric Means Comparison*
289 In Table 2, we compared geometric means of pesticides, major PCBs and dioxin-like chemical
290 groups (sum of PCBs and summary TEQs) that were adjusted for age, sex, race, BMI, smoking status,
291 and a family history of diabetes. Geometric means of studied chemicals and subgroups were, in general,
292 higher in those with diabetes for all chemicals. PCDD TEQ was significantly higher for those with
293 diabetes compared to those without diabetes as were *trans*-Nonachlor and p,p'-DDE. There were no
294 significant differences for those with prediabetes relative to those without diabetes. All other studied
295 chemical groups did not have significant differences by diabetic status (*p* values from 0.06 to 0.87).
296 Table S1 provides similar results for the ACHS I cohort overall. The summed PCB levels were generally
297 lower at time 2 (ACHS II) than at time 1, whereas the remaining PCB subgroups and pesticides changes
298 did not fit a particular pattern.
299
300 *3.3 Logistic Regression Analyses*

Table 3 summarizes the associations for prevalent diabetes in 2014 for the entire cohort using continuous exposure variables (PCBs, dioxin TEQ groups, and pesticides). In model 1, the odds ratio for sum of PCBs was 1.13 (95% CI: 0.56, 2.29) while the fully adjusted OR in model 2 was 1.22 (95% CI: 0.58-2.57). Odds ratios for the PCB subsets (mono-ortho, di-ortho, and tri- and tetra-ortho) were similar, ranging from 1.09 to 1.39 with confidence intervals that all included the null. The model 3 results for the summary PCB and subgroups were similar to those observed in models 1 and 2. While the results for PCBs were not significantly associated with diabetes, the model 1 ORs for PCDD TEQ, total dioxin TEQ, p,p'-DDE, and *trans*-Nonachlor were elevated with the null value excluded from the CI. In the fully adjusted model 2, the highest ORs for diabetes showing statistical significance were for PCDD TEQ 3.61 (1.04, 12.46) and p,p'-DDE 2.07 (1.08, 3.97). In model 3, *trans*-Nonachlor and p,p'-DDE ORs remained significantly associated with diabetes As shown in Table S2, increasing age, African American ethnicity/race, having a positive family history of diabetes, taking lipid lowering medication, and having an elevated BMI were significantly associated with prevalent diabetes in a fully adjusted model without chemical exposures.

3.4 Exploratory Analyses with Stratified Groups

Exploratory logistic regression models stratified by sex and race using continuous POP exposure variables were run with results presented in Table S3. Odds ratios for the sum of 35 PCBs were 4.23 (95% CI: 1.10, 16.35) for Whites compared to 0.80 (95% CI: 0.35, 1.81) for African Americans. The highly chlorinated tri- and tetra-ortho PCB group OR also was significantly elevated in Whites at 7.76 but with a very wide 95% CI: 1.95, 30.86. Interaction terms for both the sum PCB and highly chlorinated subgroup and race were not significant ($p > 0.05$) in their respective adjusted models. African Americans had elevated levels of p,p'-DDE relative to Whites, but the CI included the null. For the sex specific analyses, ORs for p,p'-DDE were 2.16 (95% CI: 1.06, 4.41) for females compared to 0.94 (95%

CI: 0.22, 3.96) for males. The odds ratios for oxychlordane and *trans*-Nonachlor were higher for males than females, with significantly elevated ORs noted for *trans*-Nonachlor in males.

3.5 Incident diabetes

There were 37 incident diabetes cases identified 'post baseline' out of 212 'at risk persons' enrolled in the follow up study. Persons with diabetes at baseline and with pre-diabetes were excluded from these longitudinal analyses. Demographic characteristics and laboratory measurements for incident analyses are shown in Table S4; statistical significance was noted only for a family history of diabetes. In logistic regression modeling of incident diabetes (Table 4), the highest OR reported was for *trans*-Nonachlor in Model 1 [1.28 (95% CI: 0.29, 5.61)]. The odds ratio for p,p'-DDE was above the null but non-significant [1.12, (95% CI: 0.47, 2.72)]. Odds ratios for the sum of PCBs and the PCB subgroups were all below 1.0. None of the reported associations were statistically significant in the adjusted models 1 and 2.

3.6 *Mixture Analysis*

Spearman's correlation coefficients (Figure 1a) indicated that the exposures investigated in this study were highly correlated, especially among PCBs groups. The highest correlation coefficient was seen among the di-ortho and tri-tetra-ortho PCBs at 0.98. The mono-ortho TEQ also was highly correlated with the tri-tetra-ortho PCBs (0.90), the di-ortho PCBs (0.95) as well as the non-ortho PCB TEQ at 0.88. Among the pesticides, only *trans*-Nonachlor and oxychlordane showed a high correlation (0.80). The dioxins and furans were also highly correlated 0.84. Mirex was less correlated with other pesticides than it was with the tri-tetra and di-ortho PCBs (0.72 and 0.73, respectively). The dioxin and furan TEQs generally showed mid-range correlations with both the pesticides and the PCB subgroups.

348 Because of the high correlations among the POPs, the 12 mixture components were grouped via
349 hierarchical cluster analysis for use in the BKMR analyses (see Fig 1b). The group and individual
350 conditional PIPs from the BKMR diabetes model are summarized in Table S5. Group 3 (PIP=0.74)
351 included p,p'-DDE, PCDF TEQ, PCDD TEQ, HCB, and β-HCCH. Group 1 (PIP =0.46) was composed
352 of all the PCB subgroups (di-ortho and tri-tetra-ortho PCBS, mono-ortho TEQ, and non-ortho PCB
353 TEQ) plus Mirex while group 2 (PIP = 0.56) included *trans*-Nonachlor and Oxychlordane. For the joint
354 effects on diabetes, the highest conditional PIPs were noted for *trans*-Nonachlor and Oxychlordane
355 (0.50), p,p'- DDE (0.49), non-ortho PCB TEQ (0.39), and PCDD TEQ (0.28), indicating their relatively
356 large influence within the mixture. The group PIPs were higher than the individual conditional PIPs
357 suggesting additive effects of combining structure activity groups modulated by high correlation.
358 As shown in Figure 2a, the overall diabetes BKMR analysis indicated that the 12 component
359 POP mixture was positively associated with the prevalence of diabetes in ACHS II. The joint effect OR
360 for diabetes was 1.40 with 95% CI (-1.13, 3.93), as exposure to the mixture of POPs increased from the
361 25th to the 75th percentile. The BKMR model also explored potential interactive effect among the 12
362 mixture components (Figure 2b). In those analyses, the associations of each dioxin TEQ and PCB group,
363 and the individual pesticides with diabetes were mainly unchanged while holding the other components
364 within the mixture at fixed percentiles, indicating no synergistic or multiplicative interactions.
365 Univariate exposure-response curves from BKMR are depicted in Figure S1. For these single
366 variable exposure plots, the strongest positive associations with diabetes were observed for p,p'-DDE,
367 PCDD TEQ, the non-ortho PCB TEQ, and *trans*-Nonachlor. The exposures showing inverse
368 associations with diabetes included Oxychlordane, β-HCCH, the di-ortho PCBs, and mono-ortho PCB
369 TEQ. Little evidence of a nonlinear relationship was observed.
370 Results from the quantile g-computation were similar to our overall diabetes BKMR results,
371 suggesting a positive but non-significant association. The overall marginal structural effect for each

quantile change in all mixture components was ψ = 0.28 (95% CI -0.15, 0.70; see Figure 3a). This value can also be interpreted as an OR of 1.32 (95% CI: -1.12, 3.76). The scaled effect size in the positive direction had value of 1.78 while the scaled effect size in the negative direction was -1.47, somewhat smaller, given the overall positive association.

The relative weights for 12 mixture components are shown in Figure 3b. Individual weights represent the relative contribution of each mixture component to the partial positive or negative scaled mixture effect. The relative weights are constrained to sum to 1 in each direction. The largest positive weight was assigned for tri- tetra-PCBs (0.37), followed by p,p'-DDE, *trans*-Nonachlor and PCDF TEQ (0.22, 0.18, and 0.09, respectively), whereas the di-ortho PCBs demonstrated the largest negative weight (0.65), followed by oxychlordane and β-HCCH. Given no evidence of nonlinearity or non-additivity shown from BKMR, we did not include any polynomial or interaction terms of exposures in the model.

4. Discussion

4.1 Short summary of findings

In our study of an aging U.S. cohort equally representing African Americans and Whites, serum concentrations of p,p'-DDE, *trans*-Nonachlor, tri- tetra-PCBs, and PCDDs TEQs were significantly associated with a higher diabetes risk in single exposure logistic regression models. Age, race, family history of diabetes, and BMI were significant predictors of POP concentrations and diabetes status. Mixture effect analyses using BKMR and g-computation also provided suggestive evidence for a positive joint mixture effect of PCBs, dioxins, and pesticides. Several pesticides, including p,p'-DDE and *trans*-Nonachlor, along with PCDD TEQ and non-ortho PCB TEQ were assigned higher relative contributions to the overall mixture effects in both mixture analyses; \a similar observation was made for the BKMR individual models in which the other exposures were fixed at a specific percentile. The mixture analyses identified several inverse associations with diabetes (e.g., di-ortho PCBs,

Oxychlordane, β-HCCH, mono-ortho PCB TEQ) not observed in the single exposure models, that likely decreased overall positive association of the mixture.

4.2 Diabetes in ACHS

In ACHS I, we found positive associations with prevalent diabetes between PCB groups and diabetes overall, among women, and those younger than 55 years old (Silverstone et al. 2012). In ACHS II, we found ORs for the sum of 35 PCBs to be similar (ACHS II OR=1.22) to what was observed in ACHS I (OR=1.23), but with no differences observed between men and women. Women had elevated odds of p,p'-DDE in both ACHS I and II while inverse associations for men in the follow-up study were observed for some TEQs, dioxin-like PCBs, and pesticides (β-HCCH, p,p'-DDE) but the confidence intervals were wide. More limited inferences can be made for men in ACHS II as the total male sample size was n=93 compared to n=245 for women. The follow-up cohort demographic composition remained similar to that at baseline, however; 72% vs 70% were female, and 49% vs 54% were White, respectively (Silverstone et al., 2012). Median age increased from 55 to 61 years over the two studies (n=114 confirmed dead), and the prevalence of diabetes increased from 27% in ACHS I to almost 40% in ACHS II.

As noted above, the sum 35 PCB ORs were similar in both ACHS I and II, with the null value included within the confidence interval. In ACHS II, the associations with PCBs (sum 35 and higher chlorinated tri- and tetra-ortho PCBs) were significantly elevated in Whites relative to African Americans (Table S3), although neither interaction term was statistically significant. In the ACHS II analyses stratified by race (also excluding prediabetes) inferences were limited by the smaller sample size and wide confidence intervals.

4.3 Studies Examining Association of POP Exposure and Diabetes Risk

Although PCB levels were several times higher in 2014 in ACHS II participants than in NHANES 2013-2014, PCDD/F levels were more similar to the US general population as characterized in NHANES (Yang et al., 2018). This is consistent with PCDD/PCDF concentrations found in Anniston residents primarily originating from background exposure, such as food (Health Canada 2006). Despite PCDD/PCDF levels being closer to the general U.S. population, one of the strongest associations noted between chemical exposures and diabetes in Anniston was found for this group of POPs, as opposed to sum of 35 PCBs, where associations were more modest. Lee at al. (2007) also observed elevated diabetes with PCDD and PCDF groups but to a lesser degree than pesticides, dioxin-like PCBs, and non-dioxin-like PCBs in re-analyses of earlier NHANES data (Lee et al., 2006). The original 2006 Lee report presented data only for two PCDD congeners, hepta- and octa-dibenzo-p-dioxins (HpCDD, OCDD), which showed significant associations with diabetes. Odds ratios for organochlorine pesticides were elevated in both Lee studies, either as a group or, for individual pesticides (Lee at al., 2006, 2007). The strongest association was for DDE (p=0.02), but elevated ORs also were observed for *trans*-Nonachlor and oxychlordane (Lee et al., 2006). The ACHS II data show reasonable agreement with the NHANES findings given that the Anniston population has different demographic characteristics (median age 61 years, half African American, about 70% female).

Previous literature has shown that background dioxin concentrations can have a significant association with diabetes after adjusting for diabetes risk factors (Longnecker and Michalek, 2000). This is reflected in our ACHS II analysis of those with and without diabetes, where dioxins are significantly associated with diabetes; PCDD and total dioxin TEQ had ORs of 3.45 (95% CI: 1.07, 11.16) and 2.65 (95% CI (1.06, 6.62), respectively.

Our findings also are generally consistent with previous prospective studies that demonstrated overall positive associations between POPs and diabetes risks (Lee et al. 2010, 2011; Rignell-Hydbom et al., 2009; Turyk et al., 2009; Vasiliu et al., 2006, Tornevi et al., 2019, Charles et al., 2022). While

444 individual PCB findings were less consistent, further agreement on p,p'- DDE and several other
445 pesticides emerged. In a study of middle-aged U.S. women (Zong et al., 2018), plasma concentrations of
446 dioxin-like mono-ortho PCBs, p,p'-DDE, HCB and β-HCCH were significantly associated with higher
447 type 2 diabetes risk. Age, breastfeeding history, previous weight change, and concurrent BMI were
448 strong predictors of plasma-POP concentrations. HCB was also significantly associated with type 2
449 diabetes in both cross-sectional and longitudinal assessments of matched case-control pairs in the
450 Swedish Västerbotten Intervention Program diabetes sub-study. Additionally, the cross-sectional
451 analyses in that study found significantly elevated risks of diabetes with p,p'-DDE, the sum of dioxin
452 like PCBs (congeners 118 and 156) as well as the sum of non-dioxin-like PCBs (Tornevi et al., 2019).
453 In the longitudinal Tromsø Study from northern Norway, *cis*-nonachlor, *cis*-heptachlor epoxide and
454 p,p'-DDT were each observed to have significant associations with diabetes at various time points
455 across the study period (Charles et al., 2022). Results from the French D.E.S.I.R. cohort were similar to
456 the Anniston incidence analyses; hazard ratios for their 200 incident diabetes cases did not differ
457 significantly from one for organochlorine pesticides or PCBs (Magliano et al., 2021).
458 A sex-specific association with diabetes was also noted between total serum-PCBs and incident
459 diabetes among women, but not among men, from the Great Lakes area (Vasiliu et al., 2006), as well as
460 in the baseline Anniston cohort (women OR=1.52; men OR=0.68) for PCBs. In the Anniston follow-up
461 cross sectional analyses, ORs for p,p'-DDE but not PCBs were elevated in women. A similar finding
462 was reported in 471 fish consumers from the Great Lakes area where serum concentrations of p,p′-DDE,
463 but not total PCBs, were associated with a higher diabetes risk (Turyk et al., 2009). In a cohort of 50–
464 59-year-old Swedish women, p,p′-DDE concentrations, but not PCB 153, were associated with diabetes
465 after excluding cases diagnosed within the first 6 years after study start (Rignell-Hydbom et al., 2009)
466 [4^{th} vs. 1^{st} quartile, OR 5.5 (95% CI: 1.2, 25)]. In a pilot study of 44 women with type 2 diabetes and 44
467 matched controls from the Norwegian Women and Cancer Study, p,p'-DDE was found to be a

significant predictor of prevalent cases of type 2 diabetes (Berg et al., 2021). Both non-dioxin and dioxin-like PCBs (congeners not specified), along with *cis*-nonachlor were also associated with prevalent type 2 diabetes, but not incident cases in this pilot project. Our prevalent diabetes results for p,p'-DDE were consistent with this study with a significant association with diabetes among women.

Finally, in an elderly population in Sweden, Lee et al. reported that 6 to 11 out of the 19 measured POPs showed positive trends towards increased diabetes risk (Lee et al., 2011). Additionally, a potentially non-linear association was observed for summed ranks of 31 POPs in young U.S. adults in the CARDIA study, including pp'-DDE (Lee et al., 2010). In the earlier meta-analysis of prospective studies (Wu et al., 2013), the sum of PCBs (OR=1.70) and HCB (OR=2.00) showed the strongest evidence with diabetes risk, with p,p'-DDE summary risk being more modest 1.25 (95% CI: 0.94, 1.66). PCBs were not divided into lower or higher chlorinated groups in that review. We also reported positive associations with *trans*-Nonachlor and oxychlordane in Anniston I cohort similar to results reported by Lee et al. (2010); only *trans*-Nonachlor was statistically significant in the ACHS II cohort.

Some inconsistencies in previous studies regarding congener-specific PCB findings and specific pesticides could likely be explained by small sample sizes, insufficient adjustment for confounders, differential background exposure status, lack of lipid adjustment, varying individual POPs included in early investigations, or differences in other population characteristics that may affect POP retention in the body (Lee et al., 2014). Because many POPs are used in the same industrial processes and products, and ingestion of foods contaminated by POPs released and accumulated in the environment is the primary source of exposures, humans are typically exposed to similar POP mixtures (Lee et al., 2014; Pavuk et al., 2014a). Therefore, these studies collectively support an overall, pathogenic role of POP exposure in diabetes development, and different findings on individual POPs may be affected by persistence, retention in the body, and distribution among tissues (Birnbaum, 1985).

491 Our results suggest that a family history of diabetes remains an important risk factor and/or
492 potential confounder of POPs on diabetes risk. Genetic susceptibility has been shown to play a key role
493 in modifying the risk of environmental chemicals on diabetes (Franks, 2011). While several previous
494 studies on diabetes have not accounted for family history of diabetes (Zong et al., 2018, Turyk et al.,
495 2009; Tornevi et al. 2019), one prospective cohort study in US women included family history of
496 diabetes as an effect modifier, but specifically for gestational diabetes (Rahman et al., 2019).
497 Studies have also suggested heterogeneous associations for PCBs by degree of chlorination,
498 where heavily chlorinated PCBs were more likely to be associated with obesity, insulin resistance, lipid
499 abnormalities, and diabetes (Lee et al., 2011, 2010). It is believed that the degree of chlorination is an
500 important determinant for the toxicity of chlorinated POPs; those with a greater number of chlorine
501 atoms persist longer in the environment and in the body and may be more toxic (Lee et al., 2010). While
502 this pattern was not consistent across studies (Kim et al., 2014), it was present in Whites in the Anniston
503 II cohort who showed higher chlorinated PCBs strongly related to diabetes (Table S3).

504 *4.4 Mixture Analyses*

505 We used two different statistical approaches to mixtures; our findings from the BKMR models
506 were in good general agreement with the results from the quantile g-computation models. For the overall
507 joint effect, both methods were suggestive of a modest positive association between diabetes and the
508 mixture of dioxins, PCBs, and pesticides. The OR for joint effect on diabetes in BKMR was 1.40 (95%
509 CI: -1.13, 3.93) and similar to the structural marginal effect estimate from the g-computation when
510 interpreted as OR of 1.32 (95% CI: -1.12, 3.76). The magnitude of effect from each mixture model was
511 generally lower than that observed in the single exposure logistic regression models likely due to the
512 mixture analyses accounting for the negative associations not observed in single exposure models. The
513 identification of the relative importance of individual mixture components on the outcome was similar
514 but differences were noted. As the summary statistics used were not the same, a direct comparison was

515 difficult. PCDD TEQ, p,p'-DDE, non-ortho PCBs, and *trans*-Nonachlor were the strongest contributors
516 to the mixture effects in the BKRM model while the tri-tetra PCBs, p,p'-DDE, *trans*-Nonachlor, and
517 PCDF TEQ were the top four in g-computation models. The hierarchical group PIPs showed stronger
518 effects on diabetes in BKMR then individual conditional PIPs. We did not observe any major departures
519 from linearity or strong suggestion of interactive effects in BKMR. No noticeable changes were seen in
520 single exposure effects on diabetes when all other exposures were fixed at three different percentiles.
521 The discrepancy in the rank of the most influential dioxin or PCB components between BKMR and
522 quantile g-computation is likely attributable to variations in techniques for handling the presence of
523 highly correlated exposures and smaller individual effects within these statistical methods. In the
524 presence of highly correlated chemicals within a mixture, BKMR is likely to exclude some covariates
525 from the correlated clusters, while quantile g-computation is still subject to multicollinearity and might
526 provide relevant weights in different directions for the correlated exposures. We aimed to attenuate
527 some of the higher correlations by using *a-priori* groupings based on structural and biological, as well as
528 toxicological effects (Safe, 1997-1998; van den Berg et al., 2006).

529 It has been argued that even if individual chemicals have small, clinically negligible effects, the
530 joint effect could be significant and clinically relevant (Silva et al., 2002). The two mixture approaches
531 showed that hierarchical groupings modulate simple additivity among highly correlated groups with
532 similar and/or different toxicological properties as seen in this study and that of Yim et al., 2022. The
533 overall strengths of multiple methodological approaches were in the clear visualization of dose-response
534 curves for the joint and individual effects, the agreement of the overall mixture effects using two
535 approaches, and the evaluation of non-additivity and potential interactive effects.

536 In contrast to BKMR, quantile g-computation can generate a single interpretable slope estimate
537 for the overall effect a per quintile increase in all mixture components per change in the outcome. G-
538 computation also is insensitive to outliers because of quantization (Keil et al., 2020). As in other

539 traditional statistical methods, prior knowledge about nonlinearity and interactions must be known for
540 accurate model specification. This can be assessed by using BKMR, as was done in the present study,
541 making the use of the two methods complementary.

542 Several recent studies have used BKMR with a focus on gestational diabetes and glycemic
543 function with exposure to PFAS (Preston et al., 2020, Xu et al., Yu et al., 2021, Zhang et al., 2022a).
544 The authors noted limited consistency in identifying which PFAS analytes contributed most to the joint
545 mixture effects based on group and conditional PIPs across different study designs and populations.
546 While methodologically relevant, direct comparisons with the present study are not feasible. Multiple
547 statistical approaches, including G-computation and BKRM have been used in recent years to study
548 various groups of chemicals from PCBs and dioxins to heavy metals, with a variety of health outcomes
549 (e.g. Parada et al., 2021, Yim et al., 2022, Wu et al., 2023). To our knowledge this is the first study to
550 examine diabetes in an adult cohort with exposures to a mixture of PCBs, dioxins, and organochlorine
551 pesticides.
552
553 *4.5 Potential Mechanism of Action*
554 While the precise molecular mechanism has yet to be elucidated, experimental studies and
555 animal models support a diabetogenic effect of POPs through adipogenesis (Tang-Peronard et al., 2011;
556 Gadupudi et al., 2015; Janesick and Blumberg, 2016), gluconeogenesis (Gadupudi et al. 2016a-b),
557 insulin resistance and β-cell dysfunction (Kim et al., 2014; Lee et al., 2008; Zhang et al., 2015), as well
558 as lipid abnormalities (Lee et al., 2011 Robledo et al., 2015). Exposure to POPs of various classes,
559 including PCBs, have been linked with activation of peroxisome proliferator-activated receptor-α
560 (PPAR-α) (Shipley et al., 2004; Pyper et al., 2010) and receptor-γ (Janesick and Blumberg, 2016;
561 Kamstra et al., 2014) among other nuclear receptors including LXR, FXR, CAR, PXR (Shi et al., 2019;
562 Kublbeck et al., 2020; Wahlang et al., 2019). These are ligand-activated transcription factors involved in

563 gene expression, lipid metabolism, glucose homeostasis, and inflammation. Also, studies have
564 demonstrated that sub-chronic exposure to POP-mixtures at low-doses similar to the background
565 concentrations observed in human populations can induce mitochondrial dysfunction (Ruzzin et al.,
566 2010; López-Armada et al., 2013), which can lead to insulin resistance and secretory dysfunction of
567 pancreatic β-cells (Shi et al., 2019; Szendroedi et al., 2012). Mitochondrial dysfunction also can trigger
568 metabolic dysfunctions, such as insulin resistance leading to diabetes (Hotamisligil, 2006; Lim et al.
569 2009, 2010; Shen et al., 2011).

570 The common cellular mechanism of dioxin-like compounds is the action of the aryl hydrocarbon
571 receptor (AhR) (Budinsky et al., 2014). Based on the potencies of dioxin-like compounds to activate
572 various AhR-dependent endpoints, a toxic equivalence factor (TEF) approach for the risk assessment of
573 mixtures was established, with the most toxic component (2,3,7, 8-TCDD, TEF = 1) as a reference. The
574 TEQ is then computed as the sum of the concentrations of individual dioxin or PCB isomers multiplied
575 by their TEFs (Van den Berg et al., 2006). We used this methodology to characterize exposure in
576 ACHS-II for hypertension outcomes (Pavuk at al., 2019, Yang et al., 2018) and in the present study.

577

578 *4.6 Strengths and weaknesses*

579 Notable strengths of this present study include follow up data in a well characterized cohort
580 comprised of approximately 50% African Americans. The cohort was also of middle to lower socio-
581 economic status and education. We were able to expand the exposure profile in ACHS II to include
582 PCDDs, PCDFs and non-ortho PCBs. While the sample size was generally adequate, inferences in some
583 stratified analyses were limited by loss to follow up (e.g., death, moved out of the area). Selection bias,
584 if any, had only minor effect on racial or sex composition of the follow-up sample which remained
585 similar to the baseline. We collected comprehensive questionnaire and extensive biomarker data that
586 allowed for control of a variety of confounding variables, including family history of diabetes.

587 Despite a relatively modest sample size in the follow-up, large number of participants in our
588 study population had diabetes (almost 40%). However, for the incidence diabetes, we may have been
589 underpowered to detect modest associations between POPs and diabetes even with eight years of follow
590 up and with cohort median age over 60 years old (37 incidence cases; n=212 at risk in incidence analysis
591 vs n=338 in prevalence analyses). We were unable to conclusively verify type II diabetes via medical
592 record review and assumed late onset diabetes based on reported age of diagnosis.
593 Nonetheless, most of the POPs in our analysis have relatively long biological half-lives in
594 humans and therefore these measures likely represent an individual's exposure over years (Megson et
595 al., 2013, Patterson et al., 2009). The Anniston cohort is based at one of the two former PCB production
596 sites in the United States. PCB concentrations are substantially higher in this cohort than they are in
597 NHANES participants, and closer to occupational exposures (Pavuk et al., 2014). Dioxins were only
598 modestly elevated (Yang et al., 2018) compared to NHANES, while the pesticide levels were
599 comparable to concentrations measured during the corresponding time period in NHANES (Rosenbaum
600 et al., 2017).
601 Additionally, capturing higher than average levels of these legacy POPs may have increased our
602 ability to detect subtle associations between these mixture components and our outcome. Finally, the
603 Anniston cohort population consists of approximately equal frequencies of non-Hispanic White
604 individuals and African Americans, living in a small town in south-eastern Alabama, an area with
605 generally middle to lower educational attainment and socioeconomic status. From this perspective, the
606 ACHS cohort and may be more generalizable with respect to diabetes risk factors than some other high-
607 socioeconomic status cohorts. However, the underlying biological mechanisms linking exposure to the
608 dioxin/PCB/pesticide mixture with diabetes are unlikely to differ in other populations as these
609 compounds are detected in all developed economies.

We evaluated associations with diabetes, which we assessed via reported physician diagnosis, clinical laboratory measurements of glucose and insulin, and detailed nurse-verified glycemic medication review. The use of BKMR allowed us to model both individual and joint effects of exposure to pesticides, PCBs and dioxins on (type-2) diabetes, visually assessing exposure–response functions and examining potential interactions among different mixture components. In addition, we used quantile g-computation to assess the robustness of our BKMR results and found that results were quite similar across methodologies, especially for the overall joint mixture effects.

5. Conclusions

Our follow-up study results add to the body of literature that has researched associations between exposure to PCBs, other POPs, and diabetes. We found elevate odds ratios for, p,p'-DDE, trans-Nonachlor, some PCBs, and PCDDs TEQ for prevalent diabetes, but those was attenuated for the incident diabetes in single exposure logistic regression models. We observed positive overall joint effects of the PCBs, dioxins, and pesticide mixture on diabetes with BKMR (OR of 1.40) and quantile g computation (OR of 1.32), although neither reached statistical significance. Both mixture methods were in general agreement in identifying the strongest components, however the magnitude of effect was generally lower than that seen in the single exposure regression models. Future studies should further examine the joint effects of exposure to POPs mixtures and build on this work by incorporating repeated exposure and outcome measures.

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648
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657

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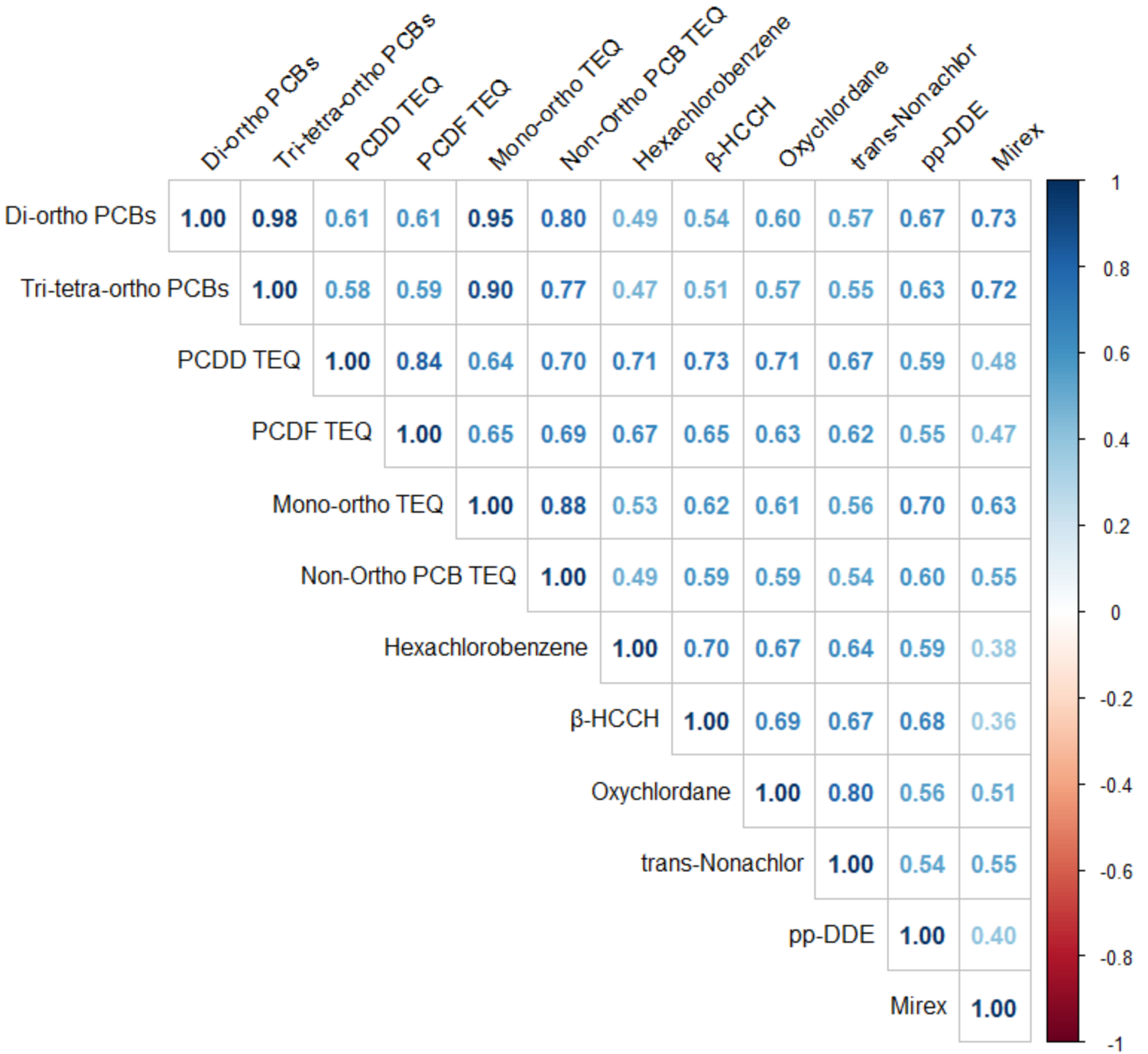
Figure 1. a. Spearman Correlation Coefficients. b. Hierarchical clustering showing 3 mixture component-groups for BKMR modeling.

Figure 2. BKMR results for diabetes, ACHS II: a. The overall joint effects. b. Single variable effects consistent with no interaction and no additivity when holding all other components to a fixed quantile.

Figure 3. Quantile G computation, ACHS II a. Slope and 95% confidence bands for joint effects of mixture components on diabetes; MSM is marginal structural model. The overall effect was Ψ=0.28 (95% CI: -0.15, 0.70). b. Relative weights - positive weights are more influential in the overall mixture.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Oxychlordane
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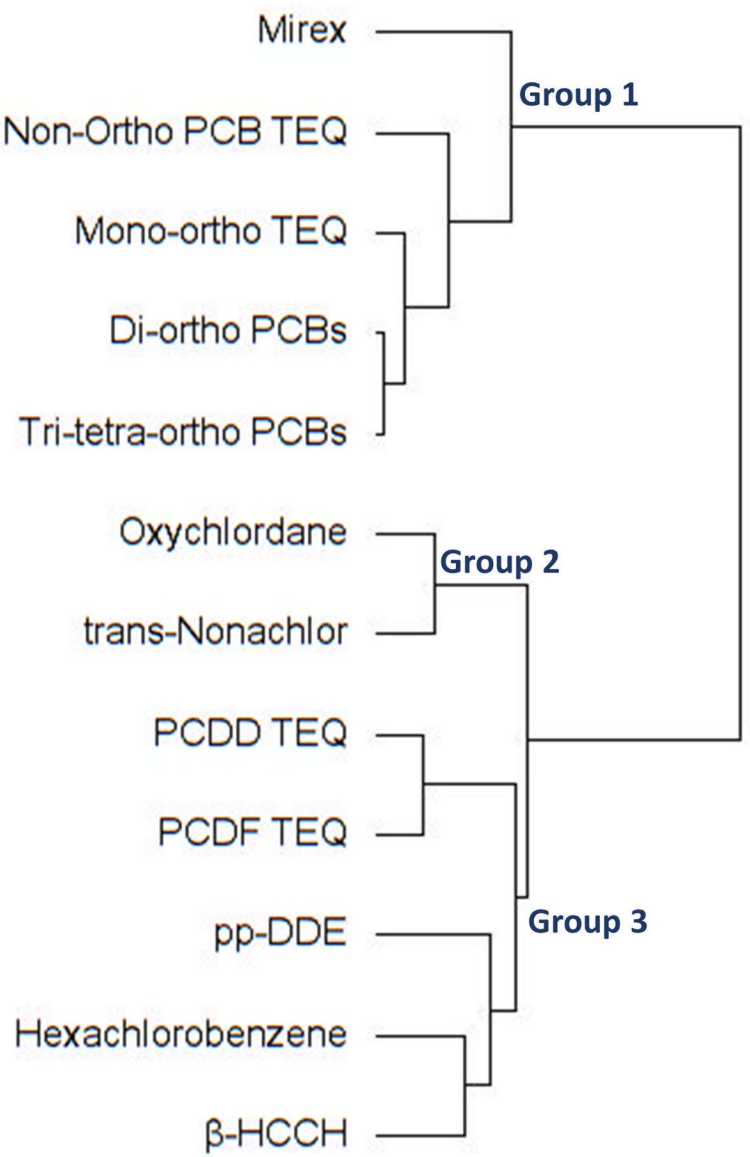
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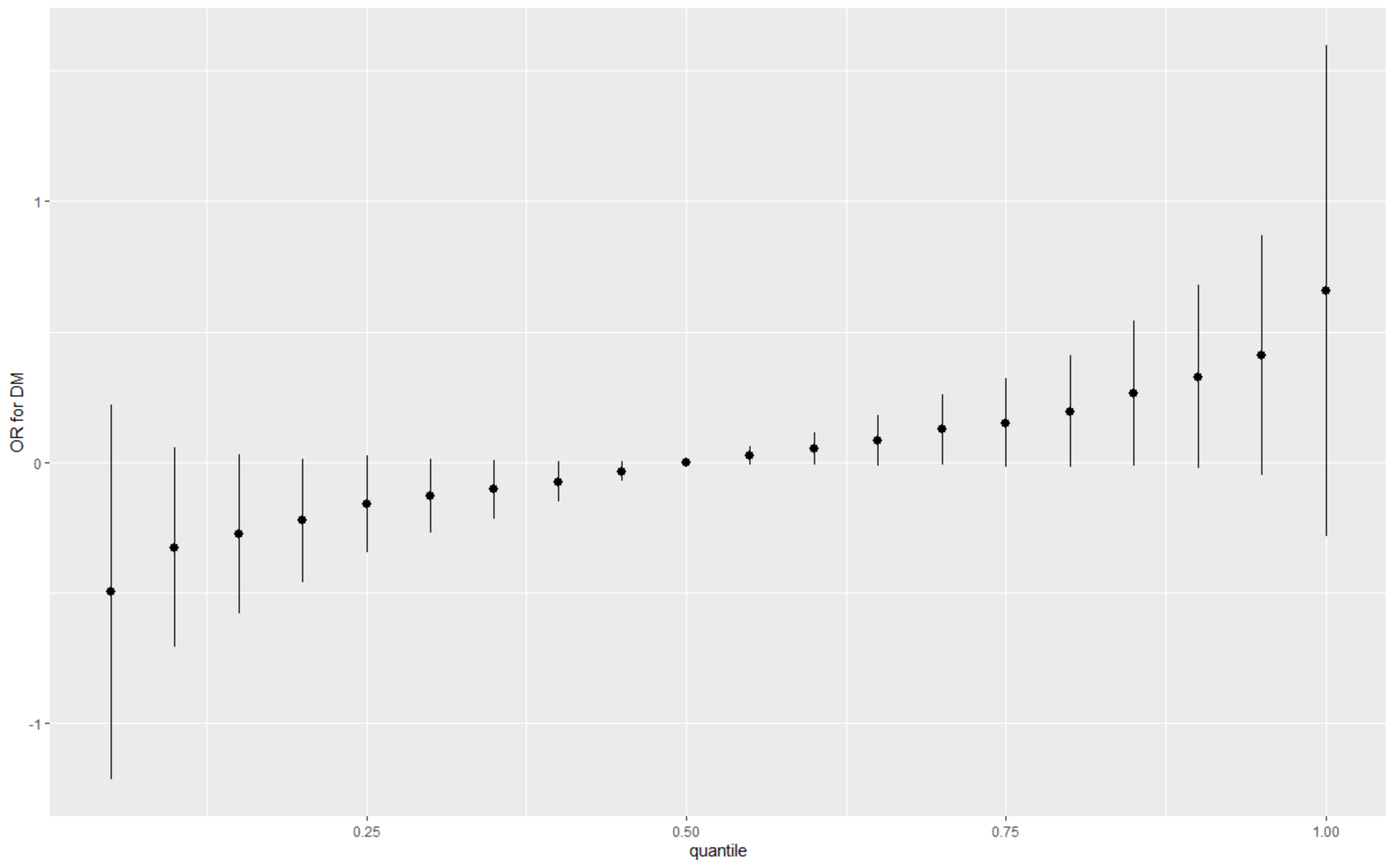
Group 1
Group 2
Group 3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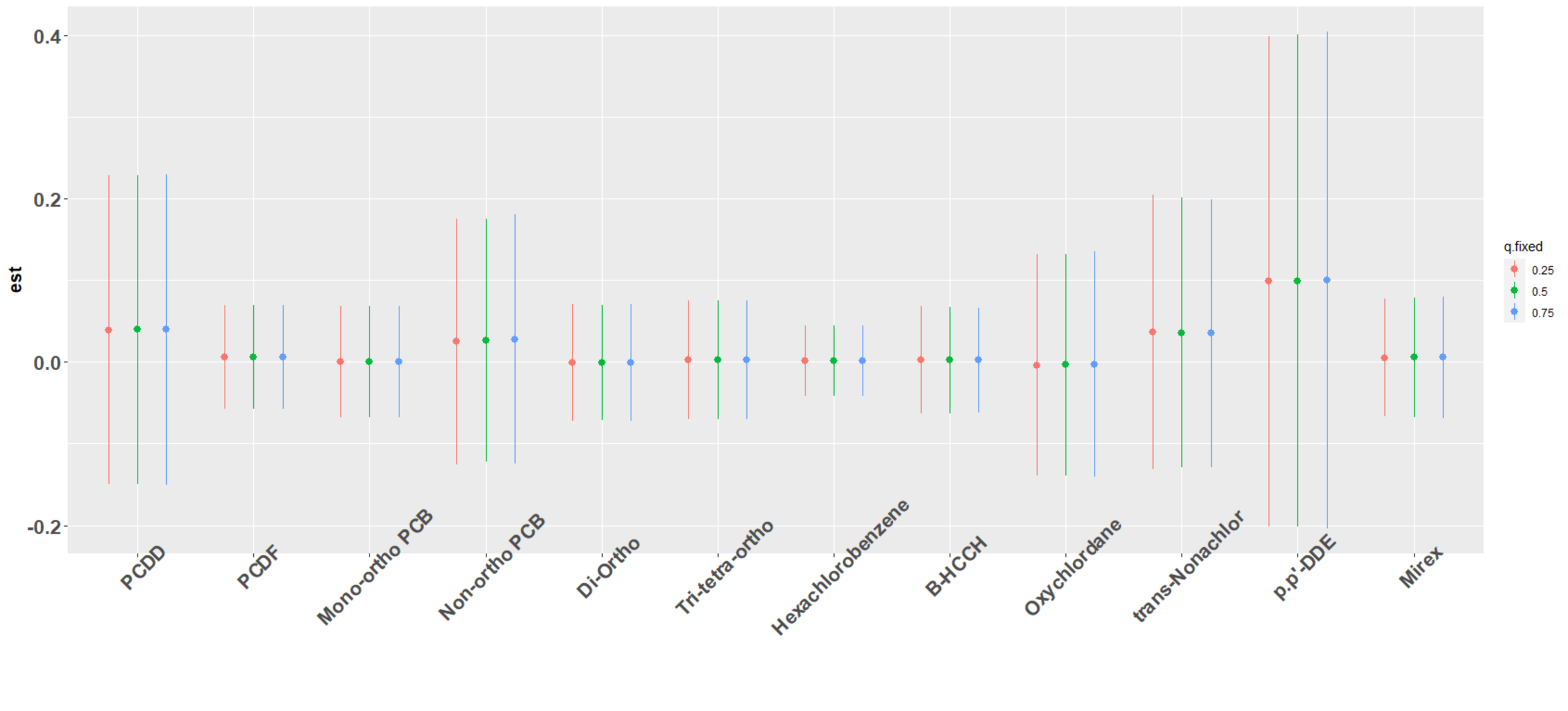
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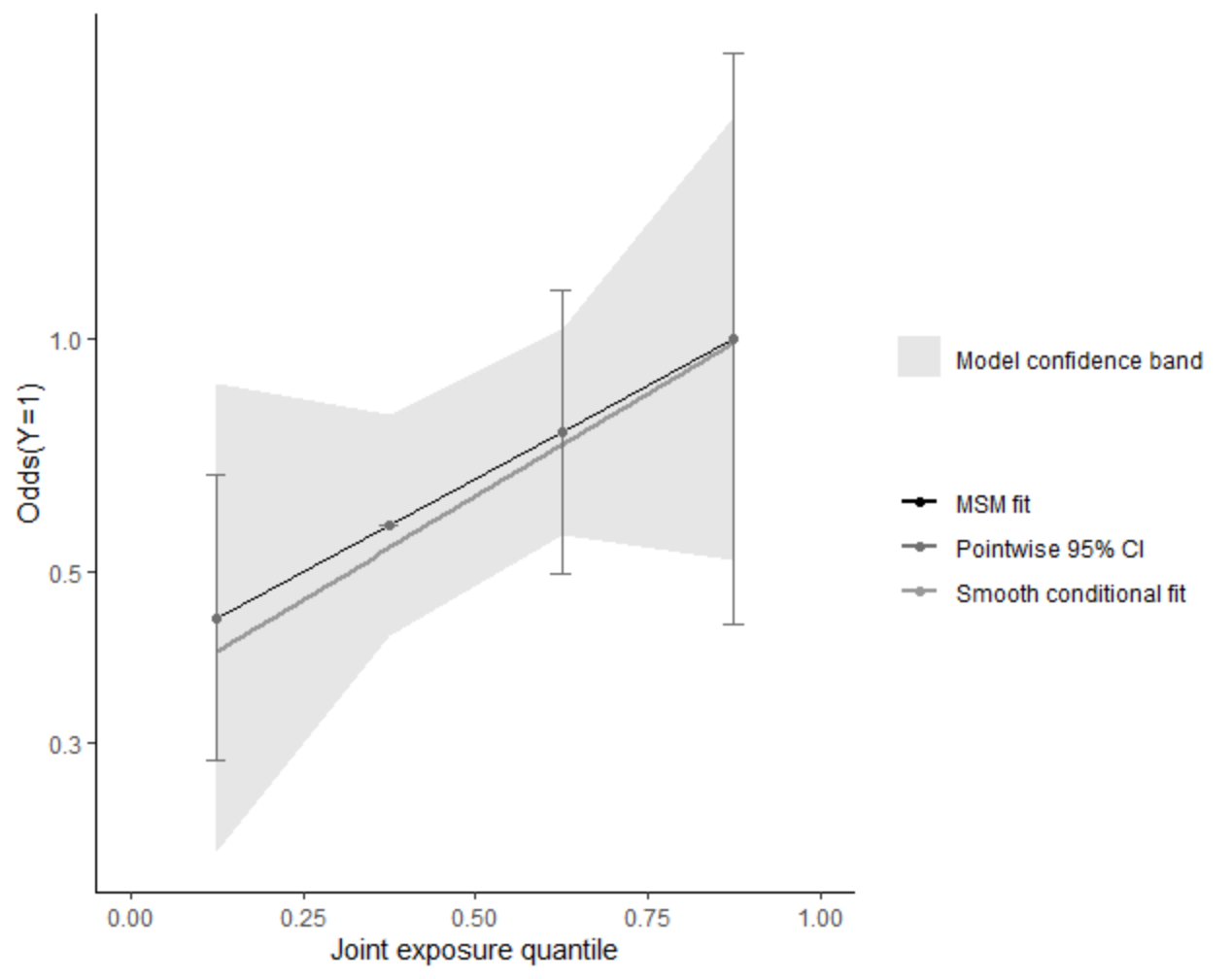
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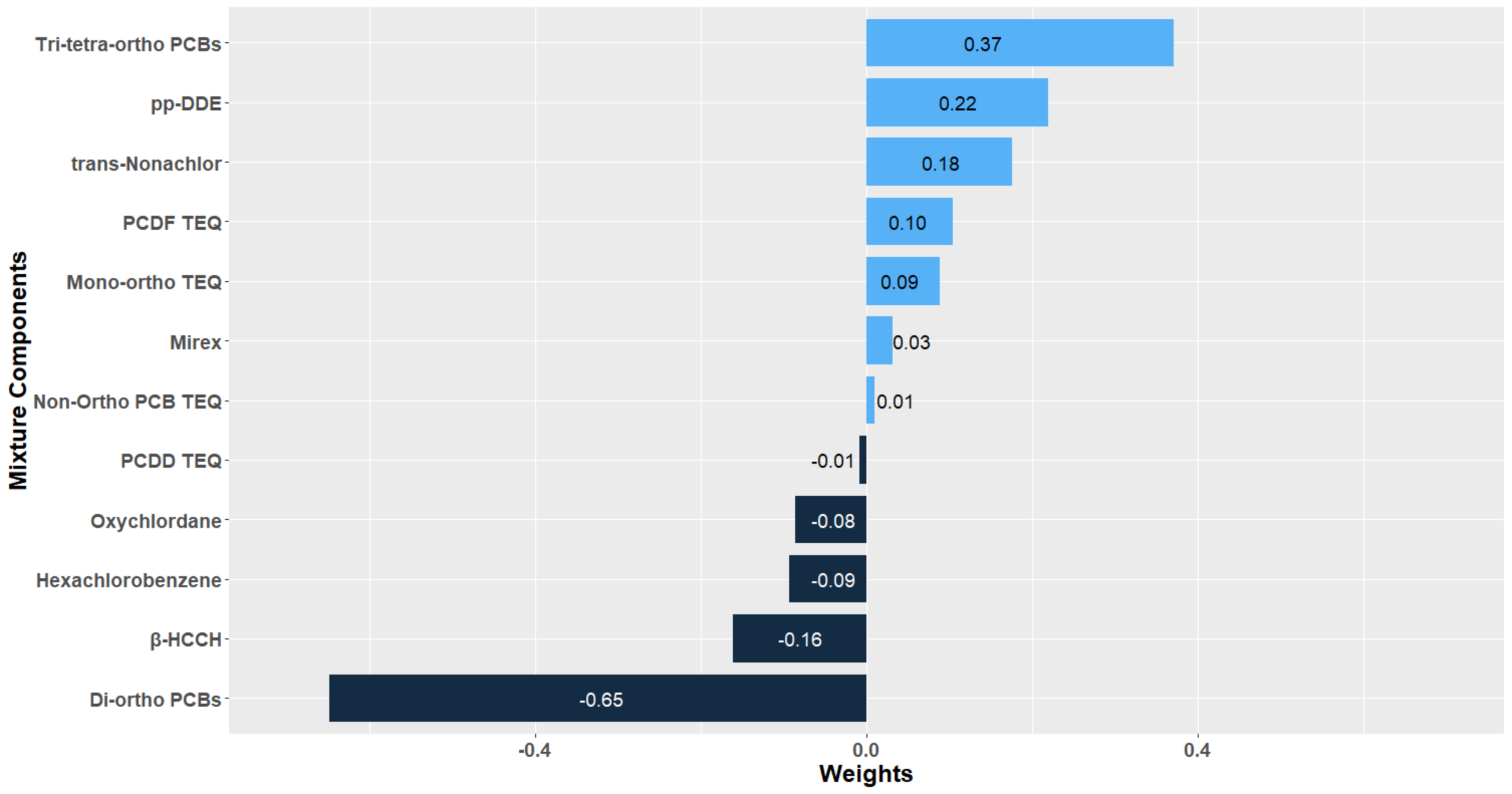
Pointwise 95% CI
Smooth conditional fit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Mirex
Non-Ortho PCB TEQ
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Oxychlordane
Hexachlorobenzene
β-HCCH
Di-ortho PCBs
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Mixture Components
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Weights

Tables

Table 1. Demographic and clinical characteristics (mean (SD) or n (percent)) of participants in ACHS II (2014)

Characteristic	No Diabetes (n=175)	Pre-diabetes (n=28)	Diabetes (n=135)	p-value
Age in years	60.21 (13.2) [a]	66.79 (14.2)	65.06 (11.8)	**0.0010**
Female	125 (71.4%)	19 (67.9%)	101 (74.8%)	0.6818
African Americans	83 (47.4%) [b]	7 (25.0%)	82 (60.7%)	**0.0011**
Years residing in Anniston	49.48 (16.1) [a]	54.43 (17.8)	54.27 (17.0)	**0.0298**
Lifetime alcohol use (12 or more alcoholic drinks in lifetime)	123 (70.3%)	19 (67.9%)	88 (65.2%)	0.6447
Smoking status (currently smoking)	41 (23.4%)	6 (21.4%)	24 (17.8%)	0.4795
Family history of diabetes	104 (59.4%) [b]	18 (64.3%)	105 (77.8%)	**0.0028**
Physical activity (physically active in last month)	76 (43.4%)	8 (28.6%)	45 (33.3%)	0.1064
Education level (more than high school)	63 (36.0%)	8 (28.6%)	48 (35.5%)	0.7423
Healthcare access (had health insurance last year)	153 (87.4%) [b]	28 (100%)	126 (93.3%)	**0.0434**
Annual income (>$25,000)	52 (29.7%) [b]	12 (42.9%)	25 (18.5%)	**0.0099**
BMI – kg/m^2	30.92 (7.69)	30.69 (5.83)	32.78 (9.02)	0.1098
Girth (inches)	40.64 (5.89) [a]	42.41 (6.21)	43.35 (6.26)	**0.0006**
Glucose level (mg/dL)	81.29 (9.80) [a]	107.45 (6.81)	131.15 (73.98)	**<0.0001**
Insulin (UI/ml)	355.9 (445.64) [a]	554.2 (531.9)	465.5 (516.48)	**0.0411**
Total lipid (mg/dL)	623.39 (140.87)	639.51 (163.9)	618.8 (170.9)	0.8127
Total triglyceride (mg/dL)	121.34 (76.48)	153.11 (100.32)	141.59 (96.81)	0.0538
Glycemic meds	0 (0%) [b]	0 (0%)	78 (56.78%)	**<0.0001**
Lipid lowering meds	54 (30.86%) [b]	17 (60.71%)	65 (48.15%)	**0.0006**

Variables with missing values: Girth (3: 2 African American, 1 White).

[a] $p < 0.05$ using the one-way ANOVA test

[b] $p < 0.05$ comparing participants with no diabetes, pre-diabetes, and diabetes using Chi-square test of independence

Table 2. Geometric means (95% confidence intervals (CI)) by diabetes status, adjusted for age, sex, race, BMI, smoking status, and family history of diabetes in general linear models[a].

Chemical Groups	No Diabetes (n=175)	Pre-Diabetes (n=28)	Diabetes (n=135)	Total (n=338)
Sum of PCBs	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**
Whole Weight[c] (pg/g)	2754 (2393, 3169)	2443 (1803, 3311)	2897 (2460, 3419)	2691 (2338, 3090)
PCB Subsets				
Di-Ortho	2051 (1771, 2365)	1737 (1270, 2371)	2103 (1778, 2494)	1958 (1694, 2259)
Tri-tetra-ortho	741.3 (635.3, 862.9)	668.3 (479.7, 928.9)	803.5 (672.9, 961.6)	736.2 (632.4, 855.0)
Summary TEQs (pg/g)				
PCDD	50.93 (46.34, 55.84)	52.23 (42.65, 63.82)	57.54 (51.64, 64.26)[b]	53.45 (48.75, 58.61)
PCDF	13.55 (12.30, 14.96)	14.22 (11.53, 17.53)	14.45 (12.91, 16.18)	14.09 (12.79, 15.48)
Mono-ortho PCB	8.37 (7.19, 9.77)	7.14 (5.11, 9.95)	8.83 (7.37, 10.56)	8.09 (6.95, 9.41)
Non-ortho PCB	19.18 (15.92, 23.17)	18.54 (12.27, 28.05)	20.84 (16.90, 25.76)	19.49 (16.18, 23.55)
Total Dioxin	97.94 (87.49, 109.6)	103.0 (80.53, 131.8)	111.2 (97.49, 127.1)	103.9 (92.89, 116.4)
Pesticides				
Hexachlorobenzene	50.58 (47.42, 53.95)	49.77 (43.25, 57.27)	52.60 (48.74, 56.75)	50.93 (47.86, 54.32)
B-HCCH	39.81 (34.75, 45.70)	43.95 (32.73, 59.15)	42.85 (36.55, 50.23)	42.16 (36.89, 48.30)
Oxychlordane	109.1 (98.62, 121.1)	123.3 (99.31, 153.1)	119.9 (106.6, 134.8)	117.2 (106.2, 129.7)
trans-Nonachlor	198.1 (176.1, 222.8)	253.5 (197.6, 325.0)	234.4 (204.6, 269.1)[b]	227.5 (203.2, 255.2)
p,p'-DDE	1541 (1309, 1815)	1258 (881.0, 1794)	2004 (1655, 2432)[b]	1573 (1336, 1849)
Mirex	64.41 (56.10, 73.96)	67.92 (50.35, 91.52)	72.11 (61.37, 84.72)	68.07 (59.42,78.16)

[a] All variables were log transformed. Summed totals, PCBS and TEQS, do not include substitutions for <LOD while the individual pesticides include substitutions.

[b] p - value ≤ 0.05 in comparison of participants with diabetes to those without diabetes. There were no significant differences in the comparisons of prediabetes to no diabetes.

[c] Contains 35 congeners.

Table 3. Odds Ratios (OR) and 95% Confidence Intervals (CI) of diabetes prevalence (excluding prediabetes) of ACHS II participants (2014).

Chemical Groups	n[b]	OR (95% CI)[c]	OR (95% CI) [d]	OR (95% CI)[e]
		Model 1	Model 2	Model 3
Summary TEQs				
PCDD	135/309	**3.45 (1.07, 11.16)**	**3.61 (1.04, 12.46)**	2.86 (0.98, 8.36)
PCDF	135/308	1.66 (0.56, 4.96)	1.70 (0.55, 5.30)	1.65 (0.58, 4.65)
Mono-ortho PCB	135/310	1.36 (0.72, 2.57)	1.23 (0.63, 2.40)	1.21 (0.65, 2.28)
Non-ortho PCB	133/288	1.51 (0.86, 2.64)	1.23 (0.67, 2.25)	1.19 (0.69, 2.06)
Total Dioxin	135/310	**2.65 (1.06, 6.62)**	2.24 (0.85, 5.89)	2.01 (0.85, 4.77)
PCB Groupings				
Sum 35 PCBs[a]	135/310	1.13 (0.56, 2.29)	1.22 (0.58, 2.57)	1.28 (0.64, 2.57)
Mono-ortho PCB	135/310	1.38 (0.72, 2.67)	1.26 (0.63, 2.51)	1.24 (0.65, 2.36)
Di-ortho PCB	135/310	1.09 (0.56, 2.14)	1.14 (0.56, 2.34)	1.15 (0.58, 2.26)
Tri, tetra-ortho PCB	134/309	1.22 (0.63, 2.34)	1.39 (0.69, 2.80)	1.39 (0.72, 2.68)
Pesticides				
Hexachlorobenzene	134/308	2.05 (0.38, 11.10)	1.84 (0.31, 11.12)	1.65 (0.37, 7.30)
β-HCCH	135/310	1.74 (0.88, 3.43)	1.25 (0.60, 2.62)	1.17 (0.61, 2.22)
Oxychlordane	133/302	2.08 (0.75, 5.83)	1.85 (0.62, 5.54)	1.75 (0.67, 4.60)
trans-Nonachlor	125/287	**3.04 (1.17, 7.92)**	2.55 (0.93, 7.02)	**2.64 (1.04, 6.71)**
p,p'-DDE	134/309	**2.13 (1.16, 3.91)**	**2.07 (1.08, 3.97)**	**2.15 (1.23, 3.70)**
Mirex	135/310	1.33 (0.65, 2.71)	1.60 (0.73, 3.52)	1.57 (0.77, 3.21)

[a] PCB sum contains 35 congeners. The Pesticides, PCB sums/groupings and TEQs were all $\log_{10}$ transformed.

[b] n=participants with diabetes/total (excluding pre-diabetes)

[c] Model 1 adjusted for age, sex, race, and total lipid

[d] Model 2 adjusted for age, sex, race, BMI, family history of diabetes; smoking status, education, health care access, lipid lowering drugs, and total lipid.

[e] Model 3 adjusted for age, race, BMI, lipid lowering drugs, family history of diabetes for all models except p,p'-DDE (all listed variables except race included in that model).

Table 4. OR (95% CI) of diabetes **incidence** (excluding prediabetes and diabetes diagnosis in ACHS I) in participants from ACHS II (2014).

Chemicals[a] Whole Weight	**[b]Diabetes/ Total**	**Model 1 OR (95% CI) [c]**	**Model 2 OR (95% CI) [d]**
Sum 35 PCBs	37/212	0.44 (0.14, 1.42)	0.46 (0.13, 1.58)
Mono-ortho PCBs	37/212	0.43 (0.14, 1.32)	0.35 (0.10, 1.16)
Di-ortho PCBs	37/212	0.43 (0.14, 1.36)	0.41 (0.12, 1.42)
Tri- tetra-ortho PCBs	37/212	0.47 (0.16, 1.36)	0.53 (0.17, 1.60)
Pesticides			
p,p'-DDE	37/212	1.12 (0.47, 2.72)	0.98 (0.37, 2.61)
trans-Nonachlor	37/209	1.28 (0.29, 5.61)	1.13 (0.24, 5.44)

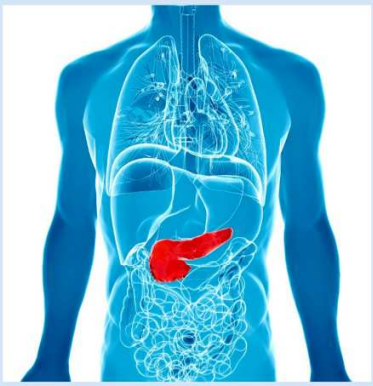
[a] The PCB sums and Pesticides were all $\log_{10}$ transformed. [Smoking variable was from the baseline in ACHS I, all other covariables from time 2].

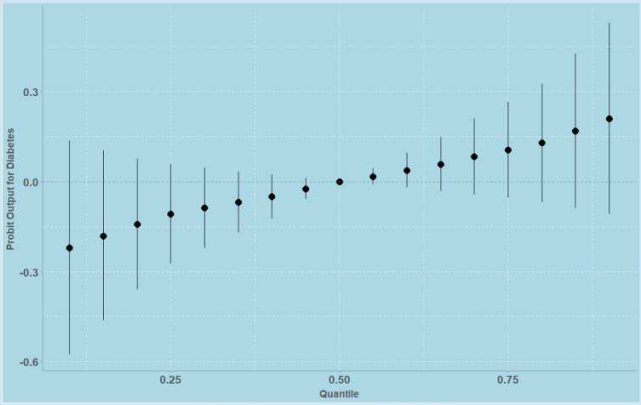
[b] Number participants with incident diabetes/total (excluding diabetes at baseline and pre-diabetes).

[c] Model 1 adjusted for age, sex, race, and total lipid.

[d] Model 2 adjusted for age, sex, race, total lipid, BMI, family history of diabetes; smoking status, education, health care access, and lipid lowering drugs.

Diabetes and Exposure to PCBs, Dioxins, and Pesticides in Anniston Cohort





Joint mixture effects (95% CI)

- **Logistic regression -> Single exposure models**
- **BKMR and G comp -> Evaluation of mixtures and increase robustness**