

# POLYCHLORINATED BIPHENYLS, DIOXINS, AND DIABETES IN THE ANNISTON COHORT

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## Introduction

Anniston, Alabama is the site of a former Monsanto Chemical Company production facility that manufactured polychlorinated biphenyls (PCBs). Between 1932 and 1971, the facility produced all commercial and experimental Aroclor mixtures, each containing different individual PCB congeners, accounting for more than half of the total PCB production in the country. High concentrations of PCBs have been reported in people<sup>1</sup> and environmental media.<sup>2</sup> We have reported previously on PCB exposure and diabetes in Anniston residents from the Anniston Community Health Survey (ACHS).<sup>3</sup> Increased risk of diabetes was observed for the sum of 35 ortho-substituted PCBs in the ACHS data that were collected in 2005-7. We conducted a follow up study to ACHS – ACHS II to study the changes in exposure, diabetes and diabetes at the second time point. The data and samples from the follow up study were collected in 2014. Dioxins and dioxin-like non-ortho PCBs were added to the ACHS II analytical plan to expand on the exposure profile of the Anniston cohort.<sup>4</sup>

Associations between exposure to PCBs, along with other persistent organic pollutants, and diabetes have been studied extensively. Substantial evidence associates persistent organic pollutants (POP) with metabolic disturbances related to diabetes, but longitudinal studies with repeated measures are few.<sup>5-6</sup> Recent analyses of several established cohorts reported additional data on biomarkers of diabetes risk, but the mechanism of action is still not well delineated.<sup>7-8</sup> Toxicological studies have explored inflammatory response and insulin signaling disruption with PCB 77,<sup>9</sup> glucose homeostasis and pancreas cell function after exposure to Aroclor 1254,<sup>10</sup> and disruption of adipogenesis with PCB 126<sup>11</sup> to list just a few by which exposure to PCBs may lead to the development of diabetes. Here we present results of analyses on associations between the prevalent diabetes and PCBs and dioxin TEQs from the follow up study (ACHS II) of the Anniston project.

## Materials and methods

### *Study Design and Population*

Methods for the ACHS and ACHS II were described in detail elsewhere.<sup>3-4</sup> For the follow up study, all surviving participants with PCB measurements were eligible to participate (n=766). Prior to enrollment, we were able to ascertain mortality status of 114 participants; 69 participants moved to distant addresses outside of study area. We successfully contacted 438 participants of the remaining participants (with the current address in the study area) and of these, a total of 359 participants enrolled in the follow up study (82%). Sufficient amount of sera for dioxins analyses were collected from 338 participants who were included in the statistical analyses here. They also provided a fasting blood sample for measurements of glucose, PCBs and lipid levels, and had their height, weight, waist circumference, and blood pressure measured using a standardized protocol. Demographic information, medical and family history, as well as self-reported health behaviors, health conditions, and individual medications were recorded. The studies were reviewed and approved by the appropriate Institutional Review Boards.

### *Laboratory and Statistical Analyses*

Generally, 18-20 mL of sera were collected from each participant for dioxin analyses. After blood samples were centrifuged, the sera were aliquoted and stored at -20°C until shipment on dry ice to the laboratory, where they

were stored at  $-70^{\circ}\text{C}$  until analyzed. Seven PCDD, ten PCDF, and three no-PCB congeners (PCBs 81, 126, and 169) were measured in the sera by the laboratory at the Centers for Disease Control and Prevention's National Center for Environmental Health. The analytes were separated on a DB-5 MS capillary column (Rxi 5Sil MS; Restek, Bellefonte, PA) and quantified using selected ion monitoring, high-resolution (10,000 resolving power) mass spectrometry.<sup>12</sup> The 35 major ortho- and mono-ortho-substituted PCB congeners were measured by the same laboratory using high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry in both studies as described previously.<sup>13</sup> Serum total lipids were calculated by the enzymatic "summation" method using triglyceride and total cholesterol measurements.<sup>14</sup> The 2005 WHO Toxic Equivalency Factors (TEF) were used to calculate the congeners' toxic equivalency (TEQ) and total dioxin TEQ.<sup>15</sup> For the particular sum of TEQs, only congener concentrations above the limit of detection were used in the summation.

Diabetes was defined as self-report of physician-diagnosed diabetes or fasting glucose  $\geq 125$  mg/dL or being on glycemic control medication; non-diabetes was defined as a fasting glucose  $< 125$  mg/dL and the absence of glycemic control medications.<sup>3</sup> For the present analyses, we did not exclude participants with prediabetes. Glycemic control medication was verified by a nurse during the study office visit. Logistic regression models were used to contrast diabetes status (diabetic, non-diabetic) with the exposure variables: the sum of PCBs, total dioxin TEQ and its subcomponents (PCDD TEQ, PCDF TEQ, mono-ortho PCBs TEQ and non-ortho PCBs TEQ). The PCDD TEQ is sum of seven dioxin congener TEQs, PCDF TEQ is the sum of ten dibenzofuran congener TEQs.<sup>15</sup> For these analyses, the non-ortho PCBs TEQ was the sum of PCB 126 TEQ and PCB 169 TEQ; only 3.4% of participants had PCB 81 above the method's limit of detection. These congeners were also modeled separately as elevated concentrations were reported in some Anniston residents. Sum of PCBs and all TEQ variables were modeled as the logarithm to base 10 ( $\log_{10}$ ). Whole weight PCB variables of the sum of 35 PCB congeners (ng/g whole weight) were adjusted for log-transformed total lipids. Other covariates in adjusted logistic regression models included age, race (African-American or White), sex (female or male), BMI (continuous), current smoking status (yes or no), and family history of diabetes. Odds ratios (OR) and 95% Confidence intervals (CI) are presented.

## Results and Discussion

Participants with diabetes were older by almost 4 years and had statistically significantly higher BMI than non-diabetics ( $p=0.015$ ). There was also a significantly higher proportion of African Americans among those with diabetes (60% vs 46%). Females represented majority of the participants (73%); no major difference in proportion of females among diabetics and non-diabetics was noted. Glucose levels were elevated in diabetics as would be expected. Similarly, there was a significantly higher proportion of those with family history of diabetes among diabetics. Smoking and total lipids were not significantly different.

**Table 1.** Demographics and exposure variables of ACHS II participants (2014).

Demographics	Diabetics (n=118)	Non-diabetics (n=220)
Age (years)	65.15 $\pm$ 11.04* ( $p= 0.0062$ )	61.34 $\pm$ 13.88
BMI (kg/m <sup>2</sup> )	33.19 $\pm$ 8.96* (0.015)	30.82 $\pm$ 7.60
African Americans	71 (60.17%)* ( $p= 0.012$ )	101 (45.91%)
Females	86 (72.88%)	159 (72.27%)
Total lipid (mg/dL)	615.71 $\pm$ 172.95	626.75 $\pm$ 144.84
Smoking Status	19 (16.10%)	52 (43.70%)
Family History of Diabetes	94 (79.66%)* ( $p= 0.0003$ )	133 (60.45%)
Girth (inches)**	43.76 $\pm$ 6.17* ( $p<0.0001$ )	40.86 $\pm$ 5.97
Glucose Level (mg/dL)	137.49 $\pm$ 77.01* ( $p<0.0001$ )	85.10 $\pm$ 12.97
<b>Exposures</b>		
Sum PCBs (ng/g lipid)	541.84	494.37
PCDD TEQ (pg/g lipid)	10.05* ( $p= 0.014$ )	8.72
PCDF TEQ (pg/g lipid)	2.50	2.26
Mono-ortho PCB TEQ (pg/g lipid)	2.20	1.99

Non-ortho PCB TEQ (pg/g lipid)	4.42	3.98
Total dioxin TEQ (pg/g lipid)	20.45* (p=0.044)	17.77
<b>Demographics</b>	<b>Diabetics (n=118)</b>	<b>Non-diabetics (n=220)</b>

Demographic results are presented in Mean  $\pm$  Standard Error for continuous variables and Number Count (%) for categorical variables. Exposure variables are presented as Least Square Geometric Means (GM).

\*Statistical difference between Diabetics and Non-diabetics (p-value<0.05) for phase 2. Analyses of covariance used for comparison of least square geometric means adjusted for age, sex, race, BMI, family history of diabetes, and smoking status.

The sum of 35 PCBs was higher in diabetics than non-diabetics in a sample of ACHS II participants but the difference was not statistically significant. However, PCDD TEQ and total dioxin TEQ were statistically significantly higher in diabetics than in non-diabetics in ACHS II sample. Other groups of TEQs were similar between diabetic and non-diabetics.

**Table 2.** Prevalence odds ratios (OR) and 95% confidence intervals (CI) of diabetes in ACHS II participants (2014).

Sum of PCBs* (ng/g lipid)	Unadjusted			Adjusted**		
	n	OR (95% CI)	p-value	n	OR (95% CI)	P-Value
Whole Weight	118/338	2.05 (1.28, 3.29)	0.0030	118/336	1.45 (0.70, 3.03)	0.32
Lipid Weight	118/338	2.08 (1.30, 3.33)	0.0023	118/336	1.45 (0.70, 3.03)	0.32
<b>TEQ (pg/g lipid)</b>						
PCB 126	104/267	1.71 (1.04, 2.79)	0.034	104/265	1.36 (0.69, 2.67)	0.37
PCB 169	108/298	2.08 (0.95, 4.57)	0.067	108/296	1.40 (0.49, 4.03)	0.53
PCDD	118/337	4.94 (2.06, 11.86)	0.0003	118/335	4.69 (1.34, 16.36)	0.015
PCDF	118/336	3.61 (1.46, 8.98)	0.0057	118/334	2.49 (0.78, 7.90)	0.12
Mono-ortho PCBs	118/338	2.15 (1.34, 3.43)	0.0014	118/336	1.49 (0.71, 3.14)	0.29
Non-ortho PCBs	117/313	1.70 (1.10, 2.62)	0.017	117/311	1.28 (0.69, 2.35)	0.43
Total Dioxin	118/338	3.59 (1.84, 7.01)	0.0002	118/336	2.68 (0.98, 7.33)	0.056

\*PCB sum contains 35 congeners. The PCB sums and TEQs were all log<sub>10</sub> transformed. All TEQ variables are in pg/g lipid. n=diabetics/total.

\*\*Adjusted models account for age, sex, race, BMI, family history of diabetes, and smoking status (and total lipid for whole weight sum of PCBs).

We applied unconditional logistic regression models to contrast the odds ratios for diabetes and exposure to PCBs and dioxins in a sample of Anniston residents that participated in ACHS II in 2014 (Table 2). For the sum of PCBs, we observed increased odds ratios in unadjusted analyses (OR~2.1) for both lipid and wet weight PCBs. After adjustment for risk factors of diabetes, those associations remained marginally elevated at OR~1.45 but were no longer statistically significant. Similar results were reported for ACHS (data not shown) with stronger associations being reported for those below median age (1.98 (0.85, 4.64), in agreement with the results reported for the whole cohort at phase 1.<sup>3</sup> Age, BMI, and family history of diabetes were significantly associated with diabetes in most models (data not shown).

In ACHS II, we measured dioxins, dibenzofurans, and non-ortho PCB congeners and calculated corresponding TEQs as well as total dioxin TEQs. For non-ortho PCBs 126 and 169, the associations observed were similar to results reported for the sum of PCBs. The statistically significant association with diabetes from unadjusted logistic regression models was attenuated after adjustment for risk factors of diabetes. However, stronger associations with diabetes were observed for the PCDD TEQ, PCDF TEQ, and the total dioxin TEQ. The association with diabetes remained statistically significant even after adjustment for the covariates for PCDD TEQ with odds ratios above 4.00 for both unadjusted and adjusted analyses. Total dioxin TEQ and PCDF TEQ were also elevated in the adjusted model [(OR=2.68 (0.98, 7.33) and 2.49 (0.78, 7.90), respectively] but the confidence interval included one. It should be noted that the PCDD TEQ represented about 50% of the total TEQ and the non-ortho PCBs represented about 20%.<sup>16</sup>

In conclusion, we found elevated odd ratios for associations between diabetes and the sum of ortho-substituted PCBs, and non-ortho PCBs 126 and 169 that got attenuated after adjustment for major risk factors for diabetes.

The strongest association was observed for the PCDD TEQ, which remained statistically significant after the adjustments. PCDF TEQ and the total dioxin TEQ were also elevated. This suggests the importance of measuring all dioxin-like compounds including dioxin and furans, and non-ortho PCBs in addition to 35 ortho-substituted PCBs, even though they are found in generally lower concentrations (pg/g vs ng/g). Further analyses are warranted to examine incident cases of diabetes in contrast to prevalent cases studied here.

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