MONO-ORTHO AND DI-ORTHO SUBSTITUTED PCB CONGENERS AND TYPE 2 DIABETES IN THE ANNISTON COMMUNITY HEALTH SURVEY

Silverstone AE[1], Weinstock RS[1], Rosenbaum PF[1], Foushee RS[2], Pavuk M[3] for the Anniston Environmental Health Research Consortium.

1. SUNY Upstate Medical University, Syracuse, NY 13210, 2. University of Alabama, Birmingham, AL, 35294
3. Agency for Toxic Substances and Disease Registry, CDC, Atlanta, GA 30343.

Abstract
Associations between the mono-ortho and di-ortho substituted polychlorinated biphenyls (PCBs) and the prevalence of type 2 diabetes were investigated in adult participants of the cross-sectional community study in Anniston, Alabama, where PCBs were produced between 1929 and 1972. Among study participants with PCB measurements, 205 were classified as having diabetes, 169 as having pre-diabetes and 392 were classified as not having diabetes. We excluded participants with pre-diabetes from the statistical analyses in this study. The median for the sum of mono-ortho PCBs was 1.07 ng/g whole weight for individuals with diabetes and 0.58 ng/g whole weight for those without diabetes. Similarly, in those with diabetes the median for di-ortho PCBs was higher than for those without diabetes (5.20 vs 3.43 ng/g whole weight). We found strong statistically significant associations between diabetes and the sum of mono-ortho PCBs for the second, third and fourth quartiles, relative to the first quartile, after adjustment for the diabetes risk factors in participants less than 55 years of age (OR=3.55 for the second, OR=2.66 for the third, and OR=4.16 for the fourth quartile). Similar, but somewhat weaker associations, were also observed for the di-ortho PCBs for the third and fourth quartiles (OR=1.95 and OR=3.19, respectively).

Introduction
The increasing prevalence of obesity and sedentary lifestyle are likely to be the major contributors to the increase in diabetes prevalence worldwide.[1] The role of environmental factors, including the exposure to toxic chemicals, have been a topic of intense interest.[2] Polychlorinated biphenyls (PCBs), dioxins, and chlorinated pesticides have been implicated in the increase in diabetes prevalence or insulin resistance and several positive associations have been reported.[3-5] These data come primarily from cross-sectional studies and, overall, results remain inconclusive.[6] The city of Anniston, Alabama contained a Monsanto/Solutia PCB production facility that produced PCBs between 1929 and 1972. It is estimated that about 50% of the total US production of PCBs occurred at this facility. High PCB levels in both the environment and people have been reported in earlier investigations.[7] The cross-sectional health survey of the community was conducted by the Anniston Environmental Health Research Consortium which was funded by the Agency for Toxic Substances and Disease Registry. The present study examined associations between biomarkers of environmental exposure to PCBs and type 2 diabetes in the adults from Anniston. Two different groups of PCB congeners, mono-ortho PCBs and di-ortho substituted PCBs, with different structure-mechanism activities, were evaluated in the present study. Mono-ortho PCBs are one of the PCB groups that exert their effects through the AhR receptor, similar to dioxins, while di-ortho PCBs are non-dioxin like in that they have toxicities independent of the AhR receptor and may affect various cytochrome CYP-450 enzymes or thyroid homeostasis or have other adverse effects.[8]

Materials and Methods

Study Design and Population

3,320 households were randomly selected from a commercial list of all residential sites within the city limits. Of the households that were successfully contacted, 1,110 individuals were randomly selected to participate in the survey. Of these 1,110 residents, 774 visited the study office and 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 (smoking, diet), health conditions, and individual medications were recorded. The study was reviewed and approved by the appropriate Institutional Review Boards.

Laboratory and Statistical Analyses

Fasting glucose and lipid panels were analyzed in the Clinical Chemistry Laboratory of the Jacksonville (Alabama) Medical Center. The 35 major ortho-substituted PCB congeners were measured by the Division of Laboratory Sciences at the Centers for Disease Control and Prevention's National Center for Environmental Health using high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry.[9] Study specimens were analyzed in batches of 24 intermixed with quality control (QC, n=3) and method blank (n=3) samples. Serum total lipids were calculated using the enzymatic "summation" method using triglyceride and total cholesterol measurements. For the mono-ortho PCB congeners, we summed the six congeners with assigned WHO toxic equivalency factors (PCBs 105, 118, 156, 157, 167, and 189). We did not include the mono-ortho congeners 28, 66, and 74. We also summed the di-ortho PCB congeners (PCBs 44, 49, 52, 87, 99, 101, 110, 128, 138+158, 146, 153, 170, 172, 180, and 194). Values below the detection limit were substituted with the congener-specific limit of detection divided by the square root of 2. The sums for PCB groups were divided into quartiles, with the first quartile serving as the referent category. Quartile cut points were based on values from the entire group of participants with valid whole weight PCB measurements (n=766). Diabetes was defined as a self-report of doctor-diagnosed diabetes or a fasting glucose above 125 mg/dL among those with negative self-reports. Self reported diabetes was confirmed by questionnaire responses related to treatment and management of disease and by the review of the medication lists. Pre-diabetes was defined as having a fasting glucose level between 100 and 125 mg/dL and no previously diagnosed diabetes or use of glucose control medications. Diabetes free participants were defined as individuals with a negative self-report of diabetes and a fasting glucose level below 100 mg/dL, as well as the absence of use of diabetes medications. A positive family history of diabetes was defined as diabetes in siblings, parents, grandparents, or aunts and uncles. Multivariable logistic regression models were used to calculate odds ratios of diabetes and the 95% confidence intervals. The pre-diabetes group was excluded from the analysis contrasting the odds of disease between individuals with and without diabetes using quartiles of the sum of the mono-ortho and di-ortho substituted PCB congeners.

Results and Discussion

Forty-seven percent of study participants were non-white (all African-American except 4 Native Americans) and 70% were female. The mean age was 54.8 years, with a range of 18 to 93 years. Approximately 30% of the participants had less than a high school education, 40% were high school graduates without college and most had low income (data not shown). Most of the participants (52%) had an elevated body mass index (BMI, $\geq$30 kg/m^2) while 21% of the participants had a BMI in the normal range ($<$25 kg/m^2). Diabetes was present in 27% of the participants, including those who self-reported (n=177) and those with diabetes (n=30) detected based on a fasting glucose level >125 mg/dl (Table 1).

PCB levels varied by diabetes status; the median sum of mono-ortho PCB congeners was 1.07 ng/g whole weight for those with diabetes versus 0.59 ng/g whole weight for those without diabetes. For the sum of di-ortho congeners, the median for those with diabetes was 4.08 ng/g whole weight while for participants without diabetes

it was 3.43 ng/g whole weight. Age specific geometric means for the sum of 35 PCB congeners for all participants of the Anniston Community Health Study were about three times higher than the corresponding geometric means for each age group (20-39, 40-59, 60+ years) in the 2003-2004 National Health and Nutrition Examination Survey.[10]

A statistically significant increase in the likelihood of diabetes with PCB exposure was observed in results from the unadjusted logistic regression on all participants combined. Odds ratios for the fourth quartile were 5.06 for the mono-ortho PCB and 3.64 for the di-ortho PCBs relative to the first quartile (referent) and each 95% confidence interval excluded the null value. These associations were attenuated (odds ratios 1.93 and 1.48, respectively) and the corresponding confidence intervals included the null value when adjusting for diabetes risk factors.

Models stratified by age for mono-ortho and di-ortho PCBs are presented in Table 2 for participants less than 55 years old (n=288) and those 55 years and older (n=292; 55 was the median age of the cohort). In the younger study participants, we found the likelihood of diabetes to be 3.55 (95% CI 1.62-7.75), 2.66 (95% CI 1.12-6.32), and 4.16 (95% CI 1.32-13.1) times higher among those in the second, third, and fourth quartile of mono-ortho PCBs in comparison to the referent category. The adjustment for established diabetes risk factors had relatively minor effect in the younger age group. The unadjusted odds ratios for the corresponding quartiles were 3.74, 3.17 and 5.15, respectively (data not shown). The odds ratios for the older age groups were elevated, but each confidence interval included the null value. We also calculated the odds ratios for the mono-ortho PCBs TEQ quartiles which were similar to the odds ratios calculated for the sum of mono-ortho PCBs in the younger age group (OR=2.33, 95% CI 1.13-4.79 for the second quartile, OR=2.43, 95% CI 1.04-5.64 for the third quartile, OR=5.42, 95% CI 1.90-15.4 for the fourth quartile; data not shown). The odds ratios for the di-ortho PCBs were similarly elevated for the younger age group but the increases were less pronounced than for the mono-ortho PCB congeners and were statistically significantly increased only for the fourth quartile (OR=3.19, 95% CI 1.06-9.59). In the older participants, di-ortho PCBs were not statistically significantly associated with diabetes in any of the models.

We investigated the association of PCB levels with the prevalence of diabetes in a community with high environmental exposures to PCBs for two major PCB congener groups, mono-ortho and di-ortho substituted PCBs. Overall, we found that individuals with diabetes had elevated levels of both mono-ortho and di-ortho PCB congeners relative to those without diabetes. This association was highly significant (especially for mono-ortho PCBs) in participants younger than 55 years and was not substantially attenuated by the adjustment for the established risk factors of diabetes.

Acknowledgements
Members of the Anniston Environmental Health Research Consortium include (in addition to paper's authors): David Baker, Shirley Baker, Bessie Jones, community representatives; Scott Bartell, UC Irvine; Lain Bian, James Olson, Peter Rogerson, University at Buffalo; Fred Biasini, Russell Foushee, Max Michael, Alan Percy, University of Alabama Birmingham; David O. Carpenter, Anthony DeCaprio, University at Albany; Jane Cash, Martha Lavender, Christie Shelton, Jacksonville State University; Germaine Buck-Louis, NICHD; Howard Frumkin ATSDR/CDC; Mark Hermanson, University of Pennsylvania; Roberto Izquierdo Upstate Medical University, SUNY; Glenn Johnson, University of Utah; Rhoda Johnson, University of Alabama, Tuscaloosa; Kirsten Moysich, Roswell Park Cancer Institute and John Stone, Tuskegee University. We would also like to acknowledge Andreas Sjödin, Wayman Turner and Larry Needham at the National Center for Environmental Health, Division of Laboratory Sciences, for their expert chemical analyses for this study.

References
1. Zimmet P, Alberti KG, Shaw J.. *Nature* 2001;414(6865):782-7.
2. Longnecker MP, Daniels JL.. *Environ Health Perspect* 2001;109 Suppl 6:871-6.
3. Wang SL, Tsai PC, Yang CY, Leon Guo Y. *Diabetes Care* 2008;31(8):1574-9.
4. Lee DH, Lee IK, Jin SH, Steffes M, Jacobs DR, Jr. *Diabetes Care* 2007;30(3):622-8.
5. Lee D-H, Lee I-K, Song K, et al. *Diabetes Care* 2006;29(7):1638-1644.
6. Porta M. Persistent organic pollutants and the burden of diabetes. *Lancet* 2006;368(9535):558-9.
7. ATSDR. 2000: http://www.atsdr.cdc.gov/HAC/PHA/annpc/ann_toc.html.
8. Hansen LG.. *Environ Health Perspect* 1998;106 Suppl 1:171-89.
9. Sjödin A, Jones RS, Lapeza CR, Focant J-F, MCGahee EE, Patterson D Jr. *Anal Chem* 2004; 76: 1921.
10. Pavuk M, Olson JR, Sjödin A, Bonner M, Dearwent SM, Turner WJ, Needham LL. *Organohalogen Compd* 2009 – current Dioxin meeting.

Table 1. Selected demographic characteristics of the Anniston Community Health Survey participants by diabetes status.

Variable		No Diabetes (n=396)	Pre-diabetes (n=171)	Diabetes (n=207)
Mean Age (SD), years		51.4 (16.8)	57.6 (13.9)	58.7 (14)
Gender	Male	109 (28%)	100 (59%)	154 (74%)
	Female	287 (72%)	71 (41%)	53 (26%)
Race	White	226 (57%)	88 (52%)	97 (47%)
	Non-White	170 (43%)	83 (48%)	110 (53)
Family History of Diabetes				
	Yes	219 (57%)	103 (62%)	151 (74%)
	No	165 (43%)	62 (38%)	53 (26%)
BMI (kg/m^2)				
	<25	127 (32%)	18 (10%)	25 (12%)
	25-29.9	106 (27%)	56 (33%)	43 (21%)
	30-39.9	116 (32%)	78 (46%)	95 (46%)
	≥40	36 (9%)	18 (11%	44 (21%)

Table 2. Odds ratios and 95% confidence intervals of reported diabetes and mono-ortho and di-ortho PCBs quartiles in the Anniston Community Health Survey stratified by age group (<55, ≥ 55 years of age).

	Adjusted Odds Ratios[a] (95% Confidence Intervals)			
∑ Mono-ortho PCBs[b] Quartiles (ng/g whole weight)	I. (0.02-12)	II. (0.13-0.30)	III. (0.31-0.74)	IV. (0.75-30.2)
<55 years	1.00 (referent)	3.55 (1.62-7.75)	2.66 (1.12-6.32)	4.16 (1.32-13.1)
≥55 years	1.00 (referent)	1.66 (0.41-6.66)	2.56 (0.64-10.1)	1.87 (0.45-7.87)
∑ Di-ortho PCBs[c] Quartiles (ng/g whole weight)	I. (0.06-0.93)	II. (0.94-2.01)	III. (2.02-4.51)	IV. (4.51-108)
<55 years	1.00 (referent)	2.00 (0.93-4.27)	1.95(0.82-4.65)	3.19 (1.06-9.6)
≥55 years	1.00 (referent)	2.87 (0.66-12.5)	2.49 (0.57-11)	1.65 (0.37-7.4)

a. Odds ratios adjusted for BMI, race, gender, family history of diabetes, lipid lowering medications, b. Mono-ortho PCBs included PCB 105, 118, 156, 157, 167, 189 (only mono-ortho PCBs with the assigned WHO TEFs included; additional mono-ortho PCBs 28, 66, and 74 measured in the study participants not included); Di-ortho PCBs included PCB 44, 49, 52, 87, 99, 101, 110, 128, 138+158, 146, 153, 170, 172, 180, 194.