

EXPOSURE TO DIOXINS AND DIOXIN-LIKE COMPOUNDS IN A FOLLOW-UP STUDY OF THE ANNISTON COMMUNITY HEALTH SURVEY (ACHS II)

Birnbaum LS¹, Yang E², Sjödin A³, Jones RS³, Lewin MD⁴, Pavuk M⁴

¹National Cancer Institute, NIH, Research Triangle Park, NC; ²ORISE and ATSDR, CDC, Atlanta, GA;

³National Center for Environmental Health, CDC, Atlanta, GA; ³Agency for Toxic Substances and Disease Registry (ATSDR), Centers for Disease Control and Prevention (CDC), Atlanta, GA.

Introduction

Anniston, Alabama was the site of a production facility where approximately half of the total U.S. production of polychlorinated biphenyls (PCBs) occurred, from the 1930s to 1970s. Earlier ATSDR investigations detailing the extent of exposure to PCBs in Anniston communities found high concentrations of PCBs present in the environment and local residents. We conducted the Anniston Community Health Survey (ACHS) from 2005-2007 to investigate further PCB exposure and potential health effects in 765 participants [1-2]. Several studies conducted in the ACHS cohort found positive associations between PCBs and diabetes, hypertension, and lipids [3-5]. We have also previously reported on concentrations of dioxin-like compounds in a pilot study (subset) of the Anniston Community Health Survey participants (ACHS, n=65), and found elevated concentrations of non-ortho-substituted PCBs [6].

The commercially produced PCBs (different types of Aroclor products) have been shown to be contaminated with small amounts (10-1,000 pg/mg) of polychlorinated dibenzofurans (PCDFs). The heating or burning of PCBs is known to produce PCDFs, smaller amounts polychlorinated terphenyls (PCTs) and polychlorinated quaterphenyls (PCQs), and traces of polychlorinated dibenzo-*p*-dioxins (PCDDs) [7]. Today, uncontrolled burning of residential waste (outdoor/backyard trash burning) is considered to be the single largest source for releasing dioxins and dioxin-like PCBs, such as PCB 169, in the United States, in contrast to a larger contribution of historical releases by various industrial operations in the past [8-9]. We present here the toxic equivalence factors of PCDDs, PCDFs, mono-ortho and non-ortho PCBs TEQs, and concentrations of non-ortho PCBs in human sera from a follow-up study of the Anniston Community Health Survey (ACHS II) participants.

Materials and Methods

Study Design and Population

All surviving ACHS participants that had a PCB measurement were eligible for ACHS II. A total of 359 follow-up study participants were enrolled in 2014, which accounted for 82% of those located and successfully contacted. Details of the subjects' recruitment and enrollment were published elsewhere [10]. Data collection occurred in 2014, followed by analytical testing in 2015. Demographic information, medical and family history, self-reported health behaviors and health conditions, and individual medications were recorded. In addition to ortho-PCBs measured in ACHS, participants of ACHS II were tested for dioxins, dioxin-like PCBs, flame retardants and other chemicals, and heavy metals that were not measured in the first phase of the study from 2005-2007. The study was reviewed and approved by the appropriate Institutional Review Boards.

Laboratory and Statistical Analyses

Generally, 18-20 mL of serum was collected from each participant for dioxin analyses. After blood samples were centrifuged, the sera were aliquoted and stored at -20°C until shipped on dry ice to the laboratory at the Centers for Disease Control and Prevention's National Center for Environmental Health, where they were stored at -70°C before analyses. Seven PCDD, ten PCDF, and three non-ortho PCB congeners (PCBs 81, 126, and 169) were measured in the sera by the laboratory. Serum samples were spiked with a mixture of ¹³C₁₂-labeled PCDDs/PCDFs and non-ortho PCBs as internal standards. The analytes were isolated from serum by a C₁₈ solid-phase extraction followed by a multicolumn automated cleanup and enrichment procedure [11]. Samples were processed in batches of 10, which included a method blank and two quality control samples that were aliquots of pooled bovine sera spiked with PCDDs, PCDFs, and non-ortho PCBs. The analytes were quantified using selected ion monitoring, high-resolution (10,000 resolving power) mass spectrometry. The mono-ortho-substituted PCB congeners (mo-PCBs) were measured by the same laboratory using high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry as described previously [12].

Serum total lipids were calculated by the enzymatic “summation” method using triglyceride and total cholesterol measurements [13]. Values below detection limits were substituted with the congener-specific limit of detection divided by the square root of 2. PCDD, PCDF, and PCB congeners have each been assigned a potency value relative to 2,3,7,8-TCDD (toxic equivalency factor, TEF). The TEF values were multiplied by the respective congener concentration to give the congener’s toxic equivalency (TEQ) under the World Health Organization scheme; congener-specific TEQs were summed for a total TEQ [14]. Thus, the dioxin-like toxicity contribution of each chemical class could be compared. The differences among the TEQ percentiles were statistically significant at $\alpha < 0.05$ when confidence intervals were mutually exclusive [15]. This was a conservative but robust method for rejecting a null hypothesis. A total of 338 participants were included in the statistical analyses, having met inclusion criteria and quality control, as well as having available covariate information.

Results and Discussion

Table 1 shows demographic characteristics of ACHS II participants by race. All study participants were either White or African American; one participant who reported being American Indian and White was included in the statistical analyses as White. The median age for Whites was about 3 years higher than that for African Americans, but the overall range between the two groups was roughly identical (26-87 years). Their total years of residence in Anniston were also similar. African Americans have a slightly higher BMI than Whites, which was statistically significant, with the majority of the cohort being obese (BMI > 30). The cohort was also largely female (72.4%).

Table 1. Demographics of participants of ACHS II.

Characteristic	African-Americans (n=172)	Whites (n=166)	Total (n=338)
		Median (Range)	
Age (years)	61 (26-86)	64 (27-87)	63 (26-87)
		Median (Interquartile Range)	
BMI (kg/m ²)	31.5 (26-37.2)	29 (25.3-35) ^a	30 (26-36.2)
Total years of residence	54 (40-64)	55 (40-64)	54 (41-64)
		Percentage (non-missing)	
Female	132 (76.7%)	113 (68.1%) ^a	245 (72.4%)
Reside in west Anniston	162 (94.2%)	132 (79.5%) ^a	294 (87%)

^ap < 0.05 for African-Americans compared with whites using Fisher’s exact test.

Table 2. Dioxin TEQs in pg/g lipid (90th percentile) for Anniston participants and in the U.S. general population by race group.

TEQ	Anniston (2014) 90 th percentile (95% CI)		NHANES (2001-2) ^a 90 th percentile (95% CI)	
	African American (n=172)	White (n=166)	Non-Hispanic Black (n=212)	Non-Hispanic White (n=640)
PCDD TEQ	21.6 (18.8-31.1)	16.8 (15.4-22.3)	29.4 (22.9-43.9)	27.5 (21.1-32.2)
PCDF TEQ	5.6 (4.9-7.3)	4.1 (3.6-4.8)	7.9 (6.2-9.5)	7.3 (6.5-8.7)
Non-ortho PCB TEQ	32.3 (29.4-48.4) ^b	10.3 (7.3-13.1)	9.8 (6.6-12.1)	8.4 (7.5-9.4)
Mono-ortho-PCB TEQ	6.8 (5.4-7.7) ^b	3.2 (2.6-3.9) ^b	2.5 (1.8-3.2)	2.1 (1.7-2.4)
Total TEQ	69.2 (64.8-96.9) ^b	31.7 (29.7-40.0)	47.6 (38.1-64.5)	43.6 (36.9-51.2)

^aPatterson et al. 2008¹⁶; 2005 WHO TEFs were used to calculate TEQs¹⁴.

^bDifference statistically significant at $\alpha < 0.05$ compared to NHANES when confidence intervals mutually exclusive.

Table 2 shows 90th percentiles for the four groups of dioxin-like chemicals and total dioxin TEQ for Anniston participants and for NHANES 2001-2 (≥ 20 years of age) by race. We compared our data with the NHANES 90th

percentiles as a marker of unusual exposure because the concentrations for many congeners were below LOD in the NHANES results (e.g., 50th or 75th percentiles¹⁶⁻¹⁷). As shown in Table 2, non-ortho PCB TEQ, mono-ortho PCB TEQ, and total dioxin TEQ were significantly higher in Anniston African Americans than in NHANES non-Hispanic Blacks (p<0.05). Anniston Whites had a significantly higher non-ortho PCB TEQ (p<0.05).

Because the samples for ACHS II were collected 12 years later than in NHANES 2001-2, we expected to see lower concentrations of both PCDD TEQ and PCDF TEQ in ACHS II (assuming the absence of a major source(s) of PCDDs and PCDFs) as shown in Table 2. Total dioxin TEQ was also lower in Anniston Whites than in NHANES Whites. Generally, declining concentrations of dioxins have been observed in the populations of the U.S. and other industrialized countries since the 1980s.

The 90th percentiles of non-ortho PCB TEQ were about three times higher in Anniston African-Americans than in NHANES 2001-2 non-Hispanic blacks (32.3 vs. 9.8 pg/g lipid). Compared to NHANES 2001-2 non-Hispanic Whites, Anniston Whites also have elevated levels, albeit to a lesser degree (10.3 vs. 8.4 pg/g lipid). Mono-ortho PCB TEQ was about two times higher in Anniston African-Americans at the 90th percentile and still marginally elevated in Whites compared to NHANES 2001-2.

Within the ACHS II cohort, total dioxin TEQ was over two times higher in Anniston African-Americans than in Whites because of increases in non-ortho PCB TEQ and to a lesser degree, mono-ortho PCB TEQ. PCDD TEQ was also marginally elevated in Anniston African-Americans vs. Whites, but that was not statistically significant (confidence intervals overlapped).

Consequently, the proportion of non-ortho PCB TEQ contribution to the total dioxin TEQ in Anniston African-Americans, was about two and a half times higher (47%) than in Anniston Whites or NHANES non-Hispanic Blacks and non-Hispanic Whites (~20%). Proportions of mono-ortho PCB TEQ were about two times higher in both African-Americans and Whites (10% vs 5%).

Table 3. Non-ortho PCBs concentrations in pg/g lipid (50th percentile) for Anniston studies participants in 2007 and 2014.

Congener	Anniston (2007) 50 th percentile (95% CI)		Anniston (2014) 50 th percentile (95% CI)	
	African American (n=26)	White (n=9)	African American (n=26)	White (n=9)
PCB 126	107 (39.6-239)	17.5 (7.3-70.1)	94.1 (29.1-191.1)	16.0 (0-64.0)
PCB 169	31.6 (20.9-48.4)	16.8 (9.1-70.1)	37.6 (24.9-72.4)	20.8 (14.0-44.8)

In Table 3, we compared 50th percentiles of non-ortho PCBs, PCBs 126 and 169, from a pilot study conducted in Anniston in 2007 (a subset of ACHS, n=65) and from the same participants of ACHS II. Of these, 35 participants had data for the two time points. We found that PCB 126 concentrations decreased slightly over time from 2007 to 2014. For PCB 169, a slight increase was observed for both African Americans and Whites. None were statistically significant. The small sample size precludes us from making any firm inferences with these observations. Earlier we have shown that the concentrations of PCBs 126 and 169 in Anniston residents have concentrations of no-PCBs several times higher than those in the general US population (NHANES 2003-4).¹⁸

Re-analyses of archived Aroclor samples over the last decade or so by several different groups clearly showed the presence and ranges of PCBs 126 and 169 in most of commercial products analyzed (mostly in Aroclor 1232, 1242, 1248, 1254) [19-21]. The present data suggest that while non-ortho PCBs generally constituted a small mass of the produced Aroclor products, it is plausible that Anniston residents were exposed to these congeners from the production and disposal of PCB-containing waste. Some contribution from burning of trash, cultural practices, or other sources cannot be excluded.

In conclusion, this is the largest sample of Anniston residents to date in which PCDDs, PCDFs, and coplanar PCBs have been measured. The results support the findings from the pilot study of 65 volunteers from the ACHS

whose blood were collected in 2007. We still observed elevated levels of total dioxin TEQs, mainly due to elevation in non-ortho PCB TEQs and mono-ortho TEQs in African Americans. The concentrations of PCBs 126 and 169 were elevated in both 2007 and 2014 samples relative to NHANES 2001-2, but the median concentrations measured in 2014 were lower than in 2007.

Acknowledgements

We thank all study participants and community members who participated in the study. The study was funded by the National Cancer Institute through interagency agreements with the Centers for Disease Control and Prevention (CDC) (IAA#: 11-AT1-001-00; IAA#: 12-AT-12-ANNISTON) and by ATSDR. Data collection for this study was funded via contract from ATSDR to the University of Alabama at Birmingham (UAB) (CDC Contract No. 200-2011-40834). This research was also supported in part by an appointment to the Research Participation Program at the CDC administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and CDC/ATSDR and by the Intramural Program of the NCI. The contents of this publication are solely the responsibility of the authors and do not necessarily represent ATSDR's or CDC's official views.

References

1. Agency for Toxic Substances and Disease Registry. (2000). Health Consultation: Evaluation of soil, blood & air data from Anniston, Alabama. Monsanto Company, Anniston, Calhoun County, Alabama. CERCLIS No. ALD004019048. U.S. Department of Health and Human Services, Atlanta.
2. Pavuk M, Olson JR, Sjödin A, Wolff P, Turner WE, Shelton C, Dutton ND, Bartell S; Anniston Environmental Health Research Consortium. (2014); *Sci Total Environ.* 473-474: 286-97
3. Silverstone AE, Rosenbaum PF, Weinstock RS, Bartell SM, Foushee HR, Shelton C, Pavuk M. (2012); *Environ. Health Perspect.* 120(5): 727-32.
4. Goncharov A, Bloom M, Pavuk M, Birman I, Carpenter DO. (2010); *J. Hypertension* 28(10): 2053-60.
5. Aminov Z, Haase RF, Pavuk M, Carpenter DO; Anniston Environmental Health Research Consortium. (2013); *Environ Health* 12(108).
6. Pavuk M, Dutton N, Turner WE, Sjödin A, Bartell S (2014). Dioxins, dibenzofurans, and non-ortho polychlorinated biphenyls in a subset of the Anniston Community Health Survey. *Organohalogen Compd* 76:1195-1198; 2014.
7. Kannan N, Tanabe S, Wakimoto T, Tatsukawa R. (1987) Coplanar polychlorinated biphenyls in aroclor and kanechlor mixtures. *J Assoc Off Anal Chem.* 70(3):451-4.
8. Environmental Protection Agency. (2013). "Human Health." Wastes - Non-Hazardous Waste - Municipal Solid Waste. <http://www.epa.gov/waste/nonhaz/municipal/backyard/health.htm>.
9. Brown JE Jr., Frame GM II, Olson DR, Webb JL. (1995); *Organohalogen Compounds* 26: 427-30.
10. Birnbaum LS, Dutton ND, Cusack C, Mennemeyer ST, Pavuk M. (2016) *Environ Sci Pollut Res Int.* 23(3):2014-21.
11. Turner W, DiPietro E, Lapeza C, Green V, Gill J, Patterson DG. (1997); *Organohalogen Compounds* 31: 26-31.
12. Sjödin A, Jones RS, Lapeza CR, Focant J-F, McGahee, EE, Patterson D Jr. (2004); *Anal. Chem.* 76: 1921-7.
13. Bernert, JT, Turner WE, Patterson DG Jr., Needham, LL. (2007); *Chemosphere* 68: 824-31.
14. Van den Berg M, Birnbaum LS, Denison M, et al. (2006); *Toxicol. Sci.* 93: 223-41.
15. Schenker N, Gentleman JF. (2001); *The American Statistician*; 55: 182-6.
16. Patterson DG Jr., Turner WE, Caudill SP, Needham LL. (2008); *Chemosphere* 73(1 Suppl): S261-77.
17. Patterson DG Jr, Wong LY, Turner WE, Caudill SP, Dipietro ES, McClure PC, Cash TP, Osterloh JD, Pirkle JL, Sampson EJ, Needham LL. (2009); *Environ Sci Technol.* 43(4): 1211-8.
18. Pavuk M, Dutton N, Sjödin A, Jones RS, Lewin MD, Birnbaum LS. Abstract #3419. Society of Toxicology (SOT) 2016, 55th Annual Meeting and Tox Expo, New Orleans, LA, March 13-17, 2016.
19. Johnson GW, Hansen LG, Hamilton MC, Fowler B, Hermanson MH (2008). *Environ Toxicol Pharmacol.* 25(2):156-63.
20. Kodavanti PR, Kannan N, Yamashita N, Derr-Yellin EC, Ward TR, Burgin DE, Tilson HA, Birnbaum LS. (2001) *Environ Health Perspect.* 109(11):1153-61.
21. Rushneck DR, Beliveau A, Fowler B et al. (2004). *Chemosphere.* 54(1):79-87.