TEMPORAL CHANGES OF SERUM CONCENTRATIONS OF POLYCHLORINATED BIPHENYLS AND ORGANOCHLORINE PESTICIDES IN A RESIDENTIAL COHORT

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Introduction

Anniston, Alabama, is a city where polychlorinated biphenyls (PCBs) were manufactured from 1929 until 1971. The city's current population is about 23,000 people[1]. The Anniston Community Health Survey (ACHS) was a cross-sectional study conducted in response to concerns among Anniston community members over whether exposure to polychlorinated biphenyls (PCBs) had increased their body burden of these contaminants and negatively affected their health. Data were collected for ACHS from 2005 to 2007.

Compared with the general U.S. population as described by the National Health and Nutrition Examination Survey (NHANES) 2003-4, the summed serum concentrations of 35 ortho-substituted PCBs were about three times higher for African-American ACHS participants and two times higher for White ACHS participants[2].

The Anniston Community Health Survey: Follow-Up and Dioxin Analyses (ACHS-II) is follow-up study to ACHS, the second of two cross-sectional surveys and sample collection components combined to construct a longitudinal design. A major goal of ACHS-II is to obtain repeated measures of the 35 ortho-PCB congeners analyzed in the ACHS in order to evaluate whether concentrations of these compounds decreased over time. The original ACHS PCB panel was selected because it represents the majority of steady-state and episodic PCB congeners by mass that are found in humans[3] and did not include non-ortho PCBs. Other main goals of ACHS-II are the measurement of additional analytes (including non-ortho PCBs, polychlorinated dioxins, furans, polybrominated diphenyl ethers, and heavy and trace metals) and evaluation of changes in health indicators since ACHS[4].

Materials and methods

Surviving ACHS participants with valid PCB results were eligible to enroll in ACHS-II. All participants gave written, informed consent prior to data collection. The ACHS-II protocol and data collection instruments were reviewed and approved by the Institutional Review Boards at the Centers for Disease Control and Prevention (CDC) and University of Alabama at Birmingham (UAB).

The research team collected data from participants in 2014, an average of eight years after ACHS. Details of data collection have been published elsewhere[4]. Briefly, study staff recorded participants' medications and then measured participants' height, weight, waist circumference, and blood pressure at the study office in Anniston or at participants' homes. Registered nurses then determined which participants were eligible to provide fasting blood samples and drew 125mL blood from those participants (or a smaller amount from participants for whom drawing the full quantity was deemed inadvisable). Fasting venous blood samples were drawn by one of the nurses, centrifuged within 2 hours, and then frozen within 1 hour. Whole blood and serum specimens were stored at the study office at -20°C until they were shipped weekly to CDC, where specimens were kept at -70°C until needed for analysis. Trained study staff also interviewed participants using a questionnaire to gather information on demographics, health conditions, health-related behaviors, and other topics. Of 359 eligible participants, 348 had blood specimens drawn. Serum concentrations of PCBs and chlorinated pesticides at follow-up have been measured in 229 participants at this time.

The National Center for Environmental Health (NCEH) Division of Laboratory Sciences measured the same 35 ortho-substituted PCBs (congeners 28, 44, 49, 52, 66, 74, 87, 99, 101, 105, 110, 118, 128, 138-158, 146, 149, 151, 153, 156, 157, 167, 170, 172, 177, 178, 180, 183, 187, 189, 194, 195, 196-203, 199, 206, and 209) and nine organochlorine pesticides (hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCCH), γ-HCCH, o,p'-dichlorodiphenyltrichloroethane (DDT), p,p'-DDT, p,p'-dichlorodiphenyldichloroethene (DDE), oxychlordane, trans-nonachlor, and mirex) as were measured at baseline. We present changes in organochlorine concentrations between ACHS and ACHS-II for PCBs 28, 74, 99, 118, 153, 156, 167, 180, 189, 194, and 206, as well as for p,p'-DDT, p,p'-DDE and hexachlorobenzene. PCB congeners and pesticides were measured using high-resolution gas chromatography/isotope dilution high-resolution mass spectrometry (HRGC/ID-HRMS) as reported previously[5]. Briefly, serum specimens (2 mL) were fortified with $_{13}C^{12}$-labeled internal standards and diluted with concentrated formic acid and water using a 215 liquid handler (Gilson Inc.; Middleton, WI) for automation. Automated solid phase extraction (SPE) using silica and silica/sulfuric acid lipid degradation were performed on the Rapid Trace SPE work station (Caliper Life Sciences Inc.; Hopkinton, MA). Samples were then injected into a Hewlett-Packard 6890 gas chromatograph equipped with a DB-5 ms capillary column (30 m × 0.25 mm× 0.25 μm film thickness) coupled to a Thermo Finnigan MAT95 XP mass spectrometer operated in EI mode using selected ion monitoring at 10,000 resolving power. The concentration of each analyte was calculated from its calibration curve.

Congener-specific LODs were reported from the laboratory for each participant based on serum specimen volume and weight. For calculation of geometric means (GM), any concentration that was less than the congener- and participant-specific LOD and had no measured value was left with a value of zero. When measured concentrations below the LODs were reported from the laboratory, those measurements (rather than substituted values) were included in the dataset and analyses because a reported result is the best available estimate of the true value[6].

Results and discussion

Laboratory analyses of PCBs and pesticides are still in progress, so we present results from up to 229 participants who have results available from both baseline and follow-up at this time.

Of the 229 participants for whom PCB 153 measurements at both baseline and follow-up are currently available, the majority were female (76%) (Table 1). Most participants identified as White (60%), with the rest identifying as African-American. The mean age at baseline in 2007-2009 was 55.5 years, resulting in a mean age at follow-up of 62.9. The mean BMI, about 32, appeared to be the same at both points in time. Of the participants who reported being current smokers at baseline, 10 reported that they did not currently smoke at follow-up and 4 said they had not smoked ≥100 cigarettes in their lifetimes; 6 who were not current smokers at baseline had become current smokers by follow-up. As of follow-up, the mean length of time that these participants reported living in Anniston was more than 50 years (not shown), showing that this study captured many long-term residents.

Table 1: Selected demographic and behavioral characteristics of participants for whom both baseline and follow-up measurements of PCB 153 are currently available (n=229).

Characteristic	**ACHS: baseline (2005-2007)**	**ACHS-II: follow-up (2014)**
	N (%, non-missing)	
Self-identified race		
African-American	92 (40.2%)	
White	137 (59.8%)	
Female	173 (75.6%)	
Current smoker	56 (24.8%) (3 missing)	48 (21.1%) (1 missing)
	Mean ± standard error	
Age in years	55.5 ± 0.8	62.9 ± 0.8
BMI – kg/m^2	32.2 ± 0.5	32.1 ± 0.5

We present changes over time in the serum concentrations of selected PCBs, pesticides, and pesticide metabolites as geometric means of individuals' ratios of follow-up to baseline concentration (Table 2). The

geometric means for selected PCBs, reported here in pg/g whole weight, ranged from 11.2 for PCB 189 to 711.6 for PCB 153 at baseline, and from 8.5 for PCB 28 to 608.2 for PCB 153 at follow-up. The geometric means ratios of follow-up to baseline concentrations were <1 for all selected analytes presented here, indicating estimated average decreases in these concentrations. The estimated average decreases over time are statistically significant at the α=0.05 level for all presented analytes except PCBs 189 and 194 (for which the 95% confidence intervals for the geometric mean ratios include 1).

Table 2: Baseline and follow-up geometric mean concentrations and geometric mean ratio of follow-up to baseline concentrations of selected ortho-PCB congeners, pesticides, and pesticide metabolites (pg/g whole weight).

Analyte	N	Geometric mean concentration at baseline (95% CI)	Geometric mean concentration at follow-up (95% CI)	Geometric mean ratio of follow-up to baseline concentration (95% CI)
PCB28	165	14.5 (13.3-15.8)	8.5 (7.5-9.6)	0.55 (0.51-0.60)
PCB74	226	78.6 (70.9-87.1)	62.2 (54.1-71.5)	0.77 (0.73-0.81)
PCB99	229	96.4 (84.7-109.6)	72.5 (60.9-86.2)	0.72 (0.68-0.76)
PCB105	200	33.5 (29.2-38.5)	27.4 (23.0-32.5)	0.71 (0.67-0.76)
PCB118	229	182.7 (159.3-209.5)	136.4 (114.2-163.0)	0.73 (0.69-0.76)
PCB153	229	711.6 (632.3-800.8)	608.2 (522.6-707.9)	0.83 (0.79-0.97)
PCB156	226	82.9 (74.6-92.1)	78.9 (68.9-90.3)	0.95 (0.91-0.99)
PCB167	215	33.8 (30.1-37.9)	30.5 (26.3-35.4)	0.92 (0.87-0.97)
PCB180	228	491.7 (438.9-550.8)	449.5 (390.5-517.3)	0.91 (0.87-0.95)
PCB189	188	11.2 (10.3-12.2)	10.2 (9.2-11.4)	0.96 (0.91-1.01)
PCB194	220	118.9 (106.0-133.3)	112.0 (97.0-129.4)	0.96 (0.92-1.01)
PCB206	225	95.9 (84.1-109.4)	89.7 (76.5-105.2)	0.91 (0.87-0.96)
HCB	226	60.4 (57.6-63.3)	52.2 (49.6-54.9)	0.86 (0.82-0.89)
p,p'-DDT	139	36.6 (33.4-40.2)	32.8 (29.2-36.9)	0.62 (0.58-0.67)
p,p'-DDE	228	2231 (1992-2499)	1982 (1698-2314)	0.82 (0.78-0.87)

As anticipated, among the PCBs, the most pronounced decreases in serum concentration over time were observed for congeners with lower chlorination: PCBs 28, 105, 99, 118, and 74, which had geometric mean ratios ranging from 0.55 to 0.77 and each have 3 to 5 chlorine substituents. Congeners with lower chlorination generally are more quickly eliminated than are the more highly chlorinated congeners, such as PCBs 194 and 206[3]. Interestingly, three mono-ortho PCBs have not declined much in this sample of Anniston residents (PCB 189, with geometric mean ratio at 0.96, PCB 156 at 0.95, and PCB 167 at 0.92).

In contrast to PCBs, chlorinated pesticides were not manufactured in Anniston. We present organochlorine pesticide data for context and comparison. The estimated average decreases in serum concentrations of DDT, DDE, and HCB appeared similar to those observed for PCB 153 and congeners with fewer chorine substituents. Of the chlorinated pesticides, a greater proportional decrease was seen for DDT than for HCB, but while DDT itself seems to be metabolized more quickly than HCB, the metabolite DDE remains and appears to be eliminated at a rate similar to HCB.

Notably, the follow-up-to-baseline ratios for all measured analytes had ranges with maxima >1 in this set of participants. That is, for each analyte, there was at least one individual in this set of participants whose serum concentration increased between baseline and follow-up. We will examine those individuals and potential sources of ongoing exposure in more detail once all samples are analyzed. However, these preliminary results exhibit an overall pattern of estimated decreases in most of these analytes, suggesting that levels of these PCBs and pesticides are decreasing for the study population as a whole.

Decreases in serum concentrations over time suggest that these participants generally have not been measurably exposed to these PCBs or pesticides since baseline or, at least, that elimination since baseline has been higher on

average than any new exposure to these compounds during the follow-up period. These preliminary results lend support to the hypothesis that the high levels of PCBs documented in this cohort[2] are mainly due to historical exposures, rather than to recent exposures to soil still contaminated with PCBs[7] or other sources persisting in the environment.

Unadjusted geometric means are presented here, though demographic and some health-related behavior (e.g., smoking) data are available and may be used to adjust future analyses.

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