DIOXINS, DIBENZOFURANS, AND NON-ORTHO POLYCHLORINATED BIPHENYLS IN A SUBSET OF THE ANNISTON COMMUNITY HEALTH SURVEY

Pavuk M[1]*, Dutton N[2], Turner WE[3], Sjödin A[3], Bartell S[4] for the Anniston Environmental Health Research Consortium

[1]Agency for Toxic Substances and Disease Registry (ATSDR), Centers for Disease Control and Prevention (CDC), Atlanta, GA 30341; [2] Oak Ridge Institute for Science and Education (ORISE) and ATSDR, CDC, Atlanta, GA 30341 [3]National Center for Environmental Health, CDC, Atlanta, GA 30341; [4]University of California Irvine, Irvine, CA 92697.

Introduction

Anniston, Alabama, was the site of a production facility that manufactured polychlorinated biphenyls (PCBs) from the late 1920s until 1971. About half of the total U.S. production of PCBs occurred at this facility. Numerous investigations have examined, in varied detail and scope, exposure to PCBs in Anniston residents. Levels of several dioxin-like compounds, including non-ortho-substituted PCBs (no-PCBs), in only a small number of individuals were reported previously by ATSDR (n=10)[1]. Historically, high levels of PCBs were reported in both environmental media[1] and people in the area[2]. We have previously reported on associations between PCBs and diabetes[3], hypertension[4], and lipids[5].

In general, the commercially produced PCBs (different types of Aroclor ® products) have been shown not to be contaminated with PCDD, PCDF, or no-PCB congeners[1]. The batch of late production Aroclor 1254 that contained traces of PCB 126 was not produced in the Anniston plant. However, heating or burning PCBs is known to produce PCDFs and PCDDs. In addition, PCB-containing waste or refuse from the production contained in landfills and other environmental deposits in Anniston has not been systematically tested for the presence of dioxin-like compounds. It should be noted that uncontrolled burning of residential waste (outdoor/backyard trash burning) is considered to be the single largest source for releasing dioxins[6] and dioxin-like PCBs, such as PCB 169[7], in the United States.

We present here the results of analyses of PCDDs, PCDFs, and no-PCBs in human sera from a subset of Anniston Community Health Survey (ACHS) volunteers. The purpose of this pilot study was to determine if serum concentrations of dioxin-like compounds in this sub-sample were similar or unusually different from background concentrations in the general U.S population.

Materials and Methods

Study Design and Population

Anniston residents aged 18 years or older were eligible and were selected through a stratified random sample. A pool of 3,320 eligible addresses was randomly selected from a commercial list of all residential properties in Anniston, with oversampling (two-thirds of all eligible) in west Anniston. One adult resident was randomly selected from each of the 1,823 successfully contacted households. Out of 1,110 respondents, 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, self-reported health behaviors and health conditions, and individual medications were recorded. The data for these analyses were collected in spring 2007 when a subset of ACHS participants were randomly selected and asked to donate 20 ml of blood for dioxin analyses; 67 volunteers provided blood samples, and 65 samples were analyzed. The study was reviewed and approved by the appropriate Institutional Review Boards.

Laboratory and Statistical Analyses

After blood samples were centrifuged, the serum was aliquoted and stored at -20°C until shipment on dry ice to the laboratory. Seven PCDD, 10 PCDF, and 3 no-PCB congeners (PCBs 81, 126, and 169) were measured in the serum by the laboratory at the Centers for Disease Control and Prevention's National Center for Environmental Health. Serum samples were spiked with a mixture of $^{13}C_{12}$-labeled PCDDs/PCDFs and no-PCBs as internal standards. The analytes were isolated from serum by a C_{18} solid-phase extraction followed by a multicolumn

automated cleanup and enrichment procedure[8]. Samples were processed in batches of 10, which included a method blank and two (2) quality control samples that were aliquots of pooled bovine sera spiked with PCDDs, PCDFs, and no-PCBs. The analytes were separated on a DB-5 MS capillary column (Agilent JW Scientific DB-5 MS p/n 122-5532; Agilent Technologies, Santa Clara, CA) and quantified using selected ion monitoring, high-resolution (10,000 resolving power) mass spectrometry[8]. Quantification was done by isotope dilution mass spectrometry using calibration standards containing $^{13}C_{12}$-labeled and unlabeled analytes. 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[9].

Serum total lipids were calculated using the enzymatic "summation" method using triglyceride and total cholesterol measurements[10]. 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 are multiplied by the respective congener concentration to give the congener's toxic equivalency (TEQ) under the World Health Organization scheme; congener-specific TEQs are summed for a total TEQ[11]. Thus, the dioxin-like toxicity contribution of each chemical class can be compared. Presented percentiles were compared by race or age groups[12].

Results and Discussion

Of the 65 participants, 47 self-identified as African-American and 18 as White. A majority of the sub-sample was female (83%, n=54); this proportion was the same for both race groups. The median age was 61 years for all participants, 59 years for African-Americans (range: 28 to 79), and 68.5 years for Whites (range: 48 to 78).

Table 1. TEQs in pg/g lipid for Anniston participants and in the U.S. general population by race group.

TEQ	Anniston (2007) 50th percentile (95% CI)		NHANES (2001-2) [a] 95th percentile (95% CI)	
	White (n=18)	African American (n=47)	Non-Hispanic White (n=640)	Non-Hispanic Black (n=212)
PCDD TEQ	16.0 (13.1-19.5)	16.3 (12.5-17.9)	37.4 (29.4-44.3)	44.7 (29.7-55.7)
PCDF TEQ	3.2 (2.8-4.2)	3.4 (2.5-4.3)	9.2 (8.0-10.6)	10.4 (8.0-12.1)
no-PCB TEQ	3.3 (2.2-7.5)	11.5 (4.5-19.6)	12.4 (10.4-14.2)	13.6 (10.9-17.7)
mo-PCB TEQ	1.8 (0.7-2.5)	3.9 (1.9-6.1)	2.7 (2.3-3.3)	3.5 (2.1-4.6)
Total TEQ	27.5 (18.7-33.8)	38.6 (32.1-48.5)	56.7 (50.6-66.4)	71.1 (49.3-82.8)

[a] Patterson et al. 2008[13]. The TEQs for Anniston participants were calculated using TEF values as re-evaluated in 2005[14].

Table 1 shows 50th percentiles for the four groups of dioxin-like chemicals and total dioxin TEQ for Anniston participants and 95th percentiles for NHANES ($\geq$20 years of age) by race. Because the concentrations for so many congeners were below LOD in the NHANES results, lower percentiles (e.g., 50th or 75th) for many of these TEQs were not presented in the reports on NHANES 2001-2 or 2003-4[13,15]. As such, we use the NHANES 95th percentiles as a marker of unusual exposure. The median mo-PCB TEQ for African-American participants in Anniston was higher than the 95th percentile for non-Hispanic blacks in NHANES, although the difference was not statistically significant. Otherwise, the 50th-percentile TEQs for the Anniston sample were no higher than the NHANES 95th percentiles; however, the differences between median TEQs in Anniston and NHANES 95th percentiles were small when comparing no-PCBs for African-Americans and mo-PCBs for Whites. The NHANES 95th percentiles for PCDD TEQ were 56.7 and 71.1 pg/g lipid for non-Hispanic whites and blacks, respectively. The 95th percentile for total TEQ for African-American participants from Anniston (107.9 pg/g lipid; not shown in Table 1) was about 1.5 times higher than the corresponding total TEQ from NHANES 2001-2. Median PCDD and PCDF TEQs in Anniston were substantially lower than the NHANES 95th percentiles in both race groups and do not seem to indicate unusual exposure to these compounds. In contrast, mo-PCBs for Anniston African-Americans are clearly elevated, with the median concentration reaching the 95th percentile among non-Hispanic blacks in the US population. Congener-specific results for mo-PCB congeners by race and sex were reported recently[2].

Geometric mean TEQs were only available for age-, race- and sex-specific serum pools from NHANES 2001-2. Geometric mean TEQs (pg/g lipid, using 2005 TEFs) for PCDDs, PCDFs, no-PCBs, mo-PCBs, and total TEQs for women ages 60 and older were as follows: 20.8, 4.8, 4.5, 1.8, and 37.5, respectively, for non-Hispanic blacks and 18.5, 4.6, 4.1, 1.4, and 34.1, respectively, for non-Hispanic whites[13]. These groups are fairly comparable to this Anniston sub-sample demographically; no geometric mean is available for a summary group by race/ethnicity (i.e., all sexes and ages). The median no-PCB and mo-PCB TEQs for African-Americans in the Anniston subset (11.5 and 3.9 pg/g lipid, respectively) appear elevated in relation to the corresponding geometric mean estimates from the NHANES 2001-2 serum pool for non-Hispanic black women ages 60 and older, but the difference is not statistically significant.

Table 2. Concentrations of non-ortho PCBs in pg/g lipid in Anniston and in the U.S. general population.[a]

Congener	Anniston (2007) 50th percentile (95% CI)		NHANES (2003-4) [a] 95th percentile (95% CI)	
	40-59 years (n=27)	60+ years (n=34)	40-59 years (n=379)	60 + years (n=446)
PCB 81	1.7 (1.2-2.8)	2.5 (2.1-3.4)	13.1 (<LOD-16.30)	14.1(<LOD-17.4)
PCB 126	78.9 (21.5-144)	78.9 (28.5-170)	69.4 (47.6-101)	126 (102-188)
PCB 169	26.3 (17.3-38.6)	42.3 (29.4-57.3)	31.9 (27.9-48.5)	63.4 (52-86.1)

[a] Patterson et al., 2009[15]. LOD=limit of detection.

In Table 2, we show the 50th percentiles from the Anniston sample and 95th percentiles from NHANES 2003-4 for no-PCB congeners 81, 126 and 169 in the 40-59 years and the 60 years and older age groups. The 50th percentile for PCB 126 for 40- to 59-year-old Anniston group exceeded the 95th percentile for the same age group in NHANES, although the difference was not statistically significant. The NHANES 95th percentile for PCB 126 was 126 pg/g lipid for ≥60-year-old group, while in the corresponding Anniston age group, the 95th percentile was 523 pg/g and the 90th percentile was 264 pg/g lipid (not shown in Table 2). These results suggest that while still elevated (particularly for PCBs 126 and 129 in the younger age group, where the medians found in the Anniston sample were close to the NHANES 95th percentiles), the concentrations of no-PCB congeners measured in Anniston participants were mostly below the 95th percentile for NHANES 2003-4. It may also suggest that concentrations of dioxin-like compounds may have decreased since 1997-8 from those found in sampling for litigation efforts (median PCB 126 was 756 ng/g lipid)[1]. However, both the current (2007) and the 1997-8 Anniston samples were comprised of volunteers and do not represent any particular neighborhood or the city overall. These comparisons have to be taken with much caution as they represent results of a pilot study based on a small sample size.

Median concentrations for PCB 81 were below the limit of detection for NHANES 2003-4, the median concentration of PCB 126 was two to four times higher in Anniston than in NHANES by age group, and the median concentration of PCB 169 were about 40% lower in the ≥60-year-old group in NHANES than in Anniston (data not shown). The 50th percentile for NHANES was only reported for PCB 126 for the non-Hispanic whites (14.4 pg/g lipid); the medians for PCBs 81 and 126 were below LOD for both race groups, and the median for PCB 169 was below LOD for non-Hispanic blacks.

The median PCB 126 concentration for Anniston African-Americans was 105 pg/g lipid, five times higher than the median measurements from NHANES 2003-4 for the 40- to 59-year-old group (17.8 pg/g lipid) and three times higher than the median for the ≥60-year-old group (31.5 pg/g lipid); the median PCB 126 concentration for Anniston African-Americans fell between the 90th percentile (92.7 pg/g lipid) (not shown in Table 2) and the 95th percentile for the ≥60-year-old group. Age-by-race categories were not presented for the NHANES 2003-4 data.

This is the largest sample of Anniston residents to date in which PCDDs, PCDFs, and coplanar PCBs have been measured. Earlier measurements of a small group of individuals (n=10) found much higher PCDD, PCDF, no-PCB, and total TEQs[1]. The present study indicates that 65 volunteers from the ACHS had total TEQs that generally fell within the 95th percentiles for U.S. background concentrations. However, while median PCDDs

and PCDFs TEQs in Anniston were similar to or lower than the NHANES 95th percentiles, median TEQs for no-PCBs and mo-PCBs among African-Americans in Anniston approached or surpassed the NHANES 95th percentiles, and no-PCB and mo-PCBs appeared elevated even in the White participants. Within the Anniston sample, median TEQs for mo-PCBs and especially no-PCBs were higher in African-Americans than in Whites, with the majority of the comparative increase in total dioxin TEQ among African-Americans due to the higher levels of no-PCBs – specifically PCB126, and to a lesser extent, PCB 169.

These data suggest that while no-PCBs generally were not part of commercially produced Aroclor products, Anniston residents who are at least 40 years old are likely to have concentrations of no-PCBs (especially PCB 126) that are several times higher than in corresponding age groups from NHANES. Anniston residents may have been exposed to no-PCBs through PCB waste from manufacture of Aroclors, burning of trash, or other sources. We cannot exclude the possibility of recent exposure to these compounds currently present in environmental deposits around Anniston, but the higher levels observed in the oldest age group suggests that past exposures are more relevant. The sample size is small and presented data must be considered exploratory in nature. These limited inferences can only be confirmed with a much larger sample of Anniston residents. A follow-up study funded by the National Institutes of Health has been planned and sample collection is underway.

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