

## **SURVEY OF SERUM CONCENTRATIONS OF DIOXINS, FURANS, AND COPLANAR POLYCHLORINATED BIPHENYLS IN A SMALL NON-RANDOM SAMPLE OF U.S. RESIDENTS**

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### **Introduction**

This cross sectional assessment of serum dioxin concentrations was conducted as part of a larger study to examine the relationship between dioxin exposure and gene expression in peripheral blood mononuclear cells. Recent reports indicate that environmental levels of dioxins have declined since the mid-1980's<sup>1</sup>. Except for the recent National Health and Nutrition Examination Survey (NHANES), there has been little systematic surveillance of serum dioxins levels in the US general population. Here, we report the serum concentrations of 22 congeners of dioxins and dioxin-like compounds and their relationship with age, sex, smoking, and meat consumption.

### **Methods and Materials**

**Study Subjects:** From April to June 1996, 29 volunteers were solicited through advertisements placed in area newspapers. Human subjects approval was obtained from the NIEHS Institutional Review Board (Approval #OH86-E-0037). All volunteer contacts were handled by a third party laboratory, CODA, Inc, Research Triangle Park, NC, under contractual agreement NO1ES-45376. Informed consent was obtained from each volunteer prior to interview or the collection of venous blood. All subjects were required to meet the weight, age and medical requirements for blood donation. A standardized questionnaire, administered by the staff, was used to obtain information about the sex, age, race, monthly consumption of fried or grilled meat, and cigarette smoking history of each subject. All subjects were compensated \$20.00 for their participation.

**Serum preparation:** Each subject provided approximately 50 ml of blood which was collected in 3-4 15-ml red top Vacutainer tubes without additives. Within two hours of acquisition, the blood samples were transferred to a different site for serum separation. The procedure for acquisition of the serum has been previously described<sup>2</sup>. Briefly, the blood was allowed to clot at room temperature for 20-30 minutes, centrifuged for 10 minutes at 1000 xg and the serum transferred into a Wheaton sample bottle (Fisher Scientific, GA) pre-rinsed with HPLC-grade acetone, toluene,

hexane and acetone. The volume of serum obtained ranged from 5 to 15 ml. The sealed Wheaton bottles were placed upright in a -20 C freezer until shipment on dry ice to National Center for Environmental Health (NCEH) for congener analysis.

**Congener analysis:** The analytes consisted of 8 polychlorinated dibenzo-*p*-dioxins (PCDDs), including TCDD, 10 polychlorinated dibenzofurans (PCDFs) and 4 coplanar polychlorinated biphenyl congeners (cPCBs). The serum samples were analyzed by the NCEH at the U.S. Centers for Disease Control and Prevention by high resolution gas chromatography/isotope dilution high resolution mass spectrometry<sup>3</sup>. After spiking with labelled internal standards, analytes were separated by C18 solid phase extraction followed by a cleanup and enrichment procedure. This was followed by further separation by gas chromatography (Hewlett-Packard 6890) equipped with a DB-5ms capillary column (30 m x 0.25 µm film). Quantification was accomplished by mass spectrometer (Finnigan MAT95) using selected ion monitoring. Each analysis included three unknown serum samples, a method blank, and a quality control sample.

**Data reporting and analysis:** All serum congener concentrations were lipid adjusted based on measured triglyceride and total cholesterol levels<sup>4</sup>. The TEQs were calculated based on the WHO TEFs<sup>5</sup>. For congeners having serum concentrations below the detection limit (DL), a value of ½ the DL was used in all analyses. Statistical analyses were performed using SPSS 11.5.0. Exposure variables required log transformation to achieve normality.

The small number of subjects and the lack of cotinine measurements precluded the use of smoking as a continuous variable. Therefore, smokers were grouped as Current, Never, and Past smokers based on interview data. Respondents were classified as Past smokers if they had smoked any cigarettes within the past five years OR they had smoked more than 500 cigarettes over the course of their lifetime.

## Results and Discussion

The demographic characteristics for the 29 North Carolina volunteers are shown in Table 1. There was a slight preponderance of males (n=16) and the cohort was predominantly white (n=22) with only three current smokers. Age was not statistically different in any of the groupings assessed: males *vs* females; white *vs* other races (the “Combined other” category); Never *vs* Past *vs* Current smokers; and Never/Past *vs* Current smokers. Males reported meat consumption levels that were statistically higher than the levels reported by females (8.4 days each month compared to 4.7 days).

Table 2 shows lipid adjusted serum dioxin levels. Interview results indicated that the volunteers had no known risk factors for dioxin exposure *eg.* employment in an occupation likely to result in exposure to combustion products. The arithmetic mean TEQ for this cohort was 15 pg/g with a maximum of 33.2 pg/g. It should be noted that the NC volunteers, along with four other US cohorts sampled between 1995 and 1997, has been used to derive dioxin reference values. The combined cohorts had an arithmetic mean total TEQ of 21 pg/g, suggesting that North Carolina volunteers have lower dioxin exposures than people living in other parts of the country<sup>4</sup>.

**Table 1 Demographic characteristics of 29 North Carolina volunteers**

		Male	Female	All
Sex		16	13	29
Race	White	11	11	22
	Black	2	1	3
	Other <sup>1</sup>	3	1	4
Mean Years of Age (SD)		35.9 (9.1)	34.6 (7.9)	35.3 (8.5)
	White			33.0 (5.3; n=22)
	Combined other <sup>2</sup>			36.0 (9.2; n=7)
	Never smoked			34.9 (8.3; n=19)
	Past smokers			33.9 (9.5; n=7)
	Current smoker			41.0 (7.0; n=3)
Meat consumption		<b>8.4<sup>3,4</sup> (8.0)</b> <b>(2 to 30)</b>	<b>4.7<sup>3,4</sup> (6.2)</b> <b>(0 to 24)</b>	6.8 <sup>3</sup> (7.4) (0 to 30)
Smoking	Current	2	1	3
	Past	3	4	7
	Never	11	8	19

*Bolded italic results are statistically significant*

<sup>1</sup>Hispanic, Asian, and American Indian

<sup>2</sup>Black, Hispanic, Asian, and American Indian

<sup>3</sup>Estimated number of days each month that meat was consumed (SD);  
(minimum to maximum reported days of meat consumption)

<sup>4</sup>p=0.04 Mann Whitney U test; males vs female meat consumption

**Table 2. Lipid adjusted serum dioxin levels (pg/g)**

	TCDD	PCDDs	PCDFs	cPCBs	Total TEQ
Arithmetic Mean (SD) (95% CI)	2.4 (2.1) (1.6-3.2)	8.4 (3.8) (6.9-9.8)	4.7 (2.3) (3.9-5.6)	1.9 (1.3) (1.4-2.4)	15.0 (6.0) (12.7-17.3)
Geometric Mean (SD) (95% CI)	1.8 (2.0) (1.4-2.4)	7.6 (1.6) (6.4-9.0)	4.3 (1.5) (3.7-5.1)	1.6 (1.8) (1.3-2.0)	14.0 (1.5) (12.0-16.2)
Minimum value	0.3	2.7	1.7	0.3	4.7
Maximum value	6.2	18.3	14.2	6.2	33.2
Mean % Total TEQ	14.7	55.1	31.9	13.0	
Median % Total TEQ	12.0	56.8	30.9	11.1	
Minimum % Total TEQ	5.9	34.4	18.1	5.6	
Maximum % Total TEQ	43.4	72.9	49.7	39.2	

PCDDs contributed the highest proportion (an average of 55%) of the total TEQ with cPCBs contributing the least (13%). The most prevalent congener was 1,2,3,7,8 pentachlorodibenzo-*p*-dioxin which accounted for an average of 30% of the total TEQ with a maximum contribution of

47%. Figure 1 graphically depicts the relative contribution of the major category of dioxins for each individual.

Table 3 examines the relationship between the measured serum dioxin levels, expressed as lipid adjusted TEQ, and several covariates. The relationship between covariates and serum dioxins were investigated for TCDD, PCDDs, PCDFs, cPCBs and Total TEQ.

The variables, "Race" and "Meat consumption", were not significantly associated with the concentration of any of the serum dioxin categories.

A small sex-related difference was found with males having significantly higher levels of serum PCDDs than females (5.0 pg/g compared to 3.7 pg/g,  $p=0.040$ ). A male with a PCDF TEQ of 14.6 pg/g, a value or more than double any of the other furan measurements, was identified as an outlier. Exclusion of the outlier resulted in a male/female difference in PCDF levels that was not quite significant ( $p=0.065$ ). The levels of TCDD, PCDDs, cPCBs and Total TEQ were comparable in males and females.

Consistent with earlier studies, the concentrations of TCDD, PCDDs, and PCDFs significantly increase with age (Spearman's correlation coefficients of 0.361 to 0.440). The exception was cPCBs which showed no relationship with age.

Significant differences in the serum concentrations of all dioxin categories, except cPCBs, were found when smoking habits were categorized as "Never", "Past" and "Current" smokers. *Post hoc* analysis using the Dunnett T3 multiple comparison test did not indicate significant differences between pairs of groups except that "Current" smokers had significantly higher concentrations of PCDD than those who had never smoked. Grouping "Past" smokers with "Never" smokers showed a similar pattern of higher non-cPCB dioxin exposure among the smokers. However, these results should be interpreted with caution since there are only three individuals who were currently smoking. Age is an unlikely explanation for the observed differences since the ages of the various smoking groups were similar.

In summary, these serum measurements from the mid 1990's suggest that residents of the US receive environmental dioxin exposure during the course of their lives. Although the consumption of dietary lipids is generally thought to be the primary source of exposure to dioxins, the current results suggest that cigarette smoking may also contribute. We did not find a relationship between diet, sex, race and dioxin concentrations. While these observations are intriguing, possible biases due to the small number of volunteers surveyed should be acknowledged.

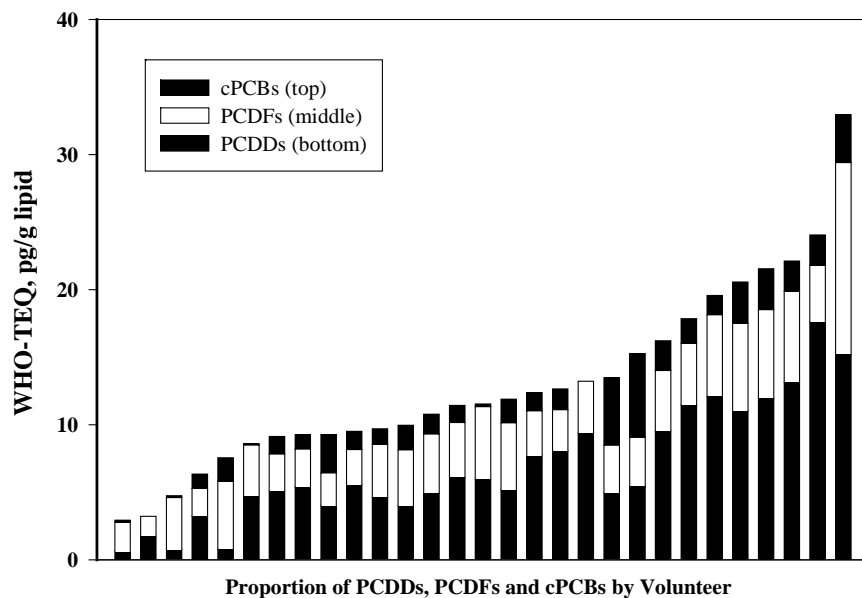
### Acknowledgements

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Figure 1. Serum concentrations of PCDD, PCDFs and PCBs in 29 North Carolina volunteers



**Table 3 Serum dioxins and covariates among 29 North Carolina volunteers**

Covariate	Category	n	TCDD <sup>1</sup>	PCDDs <sup>1</sup>	PCDFs <sup>1</sup>	cPCBs <sup>1</sup>	Total TEQ <sup>1</sup>
Categorical variables							
Sex	Male	16	1.9 (1.8)	8.1 (1.5)	<b>5.0<sup>3</sup> (1.5)</b>	1.6 (1.5)	15.0 (1.4)
	Female	13	1.8 (2.4)	7.0 (1.7)	<b>3.7 (1.4)</b>	1.7 (2.1)	12.8 (1.6)
Race	White	22	2.2 (1.5)	7.4 (1.6)	7.4 (1.6)	1.6 (1.7)	13.6 (1.5)
	Other	7 <sup>2</sup>	1.7 (2.2)	8.4 (1.5)	4.5 (1.3)	1.6 (2.0)	15.1 (1.4)
Smoking	Never	19	<b>1.5<sup>4</sup> (1.7)</b>	6.9 <sup>4</sup> (1.4)	<b>4.0<sup>5</sup> (1.4)</b>	1.7 (1.7)	<b>13.1<sup>4</sup> (1.3)</b>
	Past	7	<b>2.1 (2.8)</b>	7.9 (1.8)	<b>4.2 (1.5)</b>	1.3 (2.1)	<b>13.5 (1.7)</b>
	Current	3	<b>4.1 (1.0)</b>	12.8 (1.2)	<b>7.6 (1.8)</b>	2.1 (1.8)	<b>22.9 (1.4)</b>
Smoking	Never/Past	22	<b>1.7<sup>6</sup> (2.0)</b>	<b>7.1<sup>6</sup> (1.5)</b>	<b>4.1<sup>6</sup> (1.4)</b>	1.6 (1.8)	<b>13.2<sup>6</sup> (1.4)</b>
	Current	3	<b>4.1 (1.0)</b>	<b>12.9 (1.2)</b>	<b>7.6 (1.8)</b>	2.1 (1.8)	<b>22.9 (1.4)</b>
Continuous variables: Spearman's correlation coefficients							
Age		29	<b>0.381</b> <i>p=0.042</i>	<b>0.440</b> <i>p=0.017</i>	<b>0.361</b> <i>p=0.054</i>	0.225 p=0.241	<b>0.425</b> <i>p=0.021</i>
Meat Consumption		29	-0.067 p=0.729	-0.098 p=0.613	0.113 p=0.560	-0.141 p=0.467	-0.117 p=0.545

***Bolded italic results are statistically significant***

<sup>1</sup>pg/g lipid adjusted; log transformed data;

results are given as geometric mean (geometric SD)

<sup>2</sup>"Other" includes three Black, two Asian, one Hispanic and one American Indian participant

<sup>3</sup>p<0.05 Student's t test

<sup>4</sup>p<0.10 and p>0.05 One-way ANOVA of Never, Past and Current smokers

<sup>5</sup>p<0.05 One-way ANOVA of Never, Past and Current smokers

<sup>6</sup>p<0.05 Student's t test comparing Never/past vs Current smokers