Age Specific Dioxin TEQ Reference Range

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Introduction

Worldwide public concern of the toxicity of polychlorinated dibenzo-p-dioxins (PCDDs) and Dibenzofurans (PCDFs) has catalyzed extensive research in many toxicologically related areas. However, to date there have been no large-scale studies that have statistically been able to describe the overall dioxin reference levels of the U.S. population. Nor has there been significant information on background levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), which has been called the most toxic of the dioxin compounds and contributes the most to the dioxin toxicity equivalent (TEQ).

Attempts at determining a reference level have been made, however, the levels of PCDDs, PCDFs and PCBs in the environment have been declining in past 2 decades[1, 2, 3]. Therefore estimates from years past may not be a valid measure of today's background dioxin levels. In addition, PCDDs and PCDFs are lipophilic compounds that tend to accumulate in the lipid stores of the body and are resistant to metabolism in the body. Thus it is plausible to hypothesize that older persons will have higher dioxin levels than younger persons.

The purpose of this research is to 1) to determine the relationship between overall blood dioxin TEQs and TCDD with respect to age and 2) present a current estimate of age-specific reference ranges for dioxin TEQ and TCDD for a sample of U.S. studies.

Methods and Materials

Age-specific reference range levels were calculated using preexisting data on serum dioxin levels in 4 studies (Table 1). Demographic data on age, race, and gender were also collected. Each study was conducted independently and was not intended to represent a population based sample, with the exception of Louisiana. A total of 588 serum dioxin samples from participants with no known exposure to dioxin-like compounds other than exposure to background levels of dioxin were included in this compilation. Known exposure to dioxin is described as documented epidemiological evidence of previous occupational, diet, or residential proximity to potential industrial exposure sources. All blood samples were collected in 1996 through 2001.

All samples for each study were analyzed at the dioxin laboratory of the Centers for Disease Control and Prevention. Eight Polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 dibenzofurans (PCDFs), and 4 non-ortho substituted or coplanar polychlorinated biphenyls (cPCBs) were measured in serum by high-resolution gas chromatography/ isotope-dilution high-resolution mass spectrometry (HRGS/ID-HRMS). These measurements did not, however, include the mono-ortho-substituted PCBs which may add substantially to the sum TEQ. Serum samples were spiked with $^{13}C_{12}$-labeled internal standards and the analytes of interest were isolated using a C_{18} solid phase extraction (SPE) procedure followed by a multi-column automated cleanup and enrichment procedure. The analytes were separated by HRGC using a DB-5 ms capillary column and quantified by ID-HRMS using selected ion monitoring (SIM) at 10,000 resolving power.

The concentration of each analyte is calculated from an individual standard linear calibration. Each analytical run is conducted blinded and consists of three unknown serum samples, a method blank, and a quality control sample. After all data are reviewed using comprehensive quality assurance and quality control (QA/QC) procedures, the analytical results are reported on both a whole-weight and lipid-adjusted basis. Serum total lipids are calculated using an enzymatic 'summation' method. International toxicity equivalents (I-TEQs) are also reported for PCDDs, PCDFs, and cPCBs , based on the WHO-TEQ system. Limits of detection (LODs), on a whole-weight and lipid-adjusted basis, are reported for each sample, corrected for sample weight and analyte recovery. Measurements below the LOD were assigned a value of LOD/2 and measurements for analytes that could not be reported because of QA/QC procedures were assigned a value of zero.

Results

The mean dioxin TEQ increases as age increases (Table 2). The sharpest increase is observed among the age group 60+. The other estimates such as standard deviation, median and 95th percentile, follow the same trend. When stratifying among the 4 studies the same trend in age occurs, although the frequency of participants in each age category is not the same for each study (data not shown). In a simple linear regression of TEQ onto age, a significant correlation is indicated ($p< 0.0001$, $R^2=0.39$), however, the relationship between age and TEQ is clearly not linear and the range of TEQ values increases with age. A plot of TEQ transformed by a base 10 logarithmic function results in a more linear relationship with age and in a more uniform variance across the age range ($P< 0.0001$, $R^2=0.41$). The curve resulting from a back transformation of the regression of log10 (TEQ) onto age is overlaid on the same plot as the simple linear regression fit (Figure 1), illustrating that a more appropriate description of the relationship between total dioxin TEQ and age is non-linear.

Since TCDD is the major contributor to dioxin TEQ many researchers have chosen to measure only the TCDD. Therefore we present both the total dioxin TEQ and TCDD levels. Although smaller in magnitude the mean TCDD levels exhibit similar non-linear increases as age increases (Table 3). The TCDD and age relationship is again portrayed as a non-linear curve (Figure 2).

Discussion

This compilation of 4 studies found overall dioxin TEQ and TCDD levels increased with age. The relationship was defined as non-linear for both total TEQ and TCDD. Given the dioxin TEQ distribution is skewed and fits lognormal distribution well, log transformation was applied. Further residual plot diagnoses showed that back-transformed regression fits both the dioxin TEQ and TCDD data well. Although the back-transformed regression may over correct the skewness, the non-linear relationship between dioxin TEQ and age is an improvement over the ill-fitted linear regression.

Many studies commonly use a single population estimate such as the mean or 95th percentile to describe a population's dioxin body burden. In Table 2, the 95th percentile for the oldest age-group is about 6 times larger than the 95th percentile in the youngest age-group. If the overall 95th percentile was solely used to describe this sample's dioxin exposure most people's exposure would be subject to misclassification. Therefore separate estimates by age category must be taken into consideration when attempting to describe a population's dioxin body burden.

Consideration of the following study limitations needs to be taken into account. Three of the 4 studies included in this compilation were conducted independently and were not intended to represent a population based sample. In addition the age distribution among the different studies was not consistent. Although this research attempts to provide a current estimate of dioxin reference levels the studies included in this compilation represent data collected between 1996 and 2001. Background levels today may be somewhat different than what was observed during this 5 year time period.

Given the limitations of this research it is important to take into account that to date there are no other current age-specific reference levels for populations with no known exposure to dioxin-like compounds in the U.S, while this compilation includes data from 588 unexposed persons in 4 states. In addition, serum analysis for each study was analyzed at the same laboratory consequently reducing potential bias.

Overall this research shows that consideration of age-specific reference levels is more appropriate than a single estimate for the entire population. Misclassification is reduced when age-specific reference ranges are used in dioxin exposure assessment. In addition this compilation of 588 participants with no known dioxin exposure provides a reasonable estimate of the most recent age-specific reference levels available to date in the US.

Table 1: Description of studies included in the calculation of age-specific dioxin reference levels

State(s)	Year Sample Collected	# Selected for Reference Level Calculation[1]	Study Description
North Carolina	1996	29	Assess variability in gene expression biomarkers in relation to serum dioxin concentrations in persons with no known source of dioxin exposure
Missouri	1997	128	Assess dioxin levels in persons who resided near incineration site and a comparison group. No difference in dioxin levels were evaluated among the sampled groups.
New York	1997	16	Assess dioxin exposure among anglers that consume fish from Lake Ontario fish and wildlife compared to persons who do not consume fish and wildlife from this Lake. A higher dioxin TEQ was observed among frequent consumers compared to non-consumers.
Louisiana	2001	415	Assess dioxin levels among persons who resided near potential industrial sources of dioxin exposure compared to persons who resided in locations distant to the potential exposure. No difference in dioxin levels were evaluated among the sampled groups.

1 Study participants from each study were eligible for inclusion with the exception of those believed to be exposed to dioxin or dioxin-like compounds and found to have a higher dioxin TEQ than a comparison sample. With the exception of New York, the number selected for calculation of the reference level is the same as the original study sample size.

Table 2: Dioxin TEQ reference range by age group based on studies from LA, MO, NC, & NY

Age Group	N	Mean	Std	Median	P75	P90	P95	Min	Max
15-29	116	6.4	6.0	5.4	7.8	11.7	14.0	0.0	53.9
30-44	199	11.8	6.9	9.8	16.6	21.1	23.2	0.2	50.4
45-59	160	16.9	9.6	14.9	22.3	29.5	32.8	0.8	55.4
60+	113	36.1	24.9	32.3	45.6	69.2	85.4	3.4	146.4
All	588	16.8	16.4	12.1	20.8	33.7	48.0	0.0	146.4

Table 3: Dioxin TCDD reference range based on 4 studies from LA, MO, NC and NY

Age Group	N	Mean	Std	Median	P75	P90	P95	Min	Max
15-29	116	1.0	1.1	0.8	1.0	1.9	2.4	0.3	9.7
30-44	199	1.4	1.3	1.0	1.7	2.9	4.0	0.2	10.9
45-59	160	1.9	1.6	1.4	2.6	4.1	5.0	0.3	9.3
60+	113	3.9	3.7	3.2	5.8	7.5	10.9	0.3	22.6
All	588	1.9	2.3	1.1	2.5	4.6	6.0	0.2	22.6

Figure 1. Dioxin TEQ versus **Age for Studies** from **LA, MO, NC, and NY**

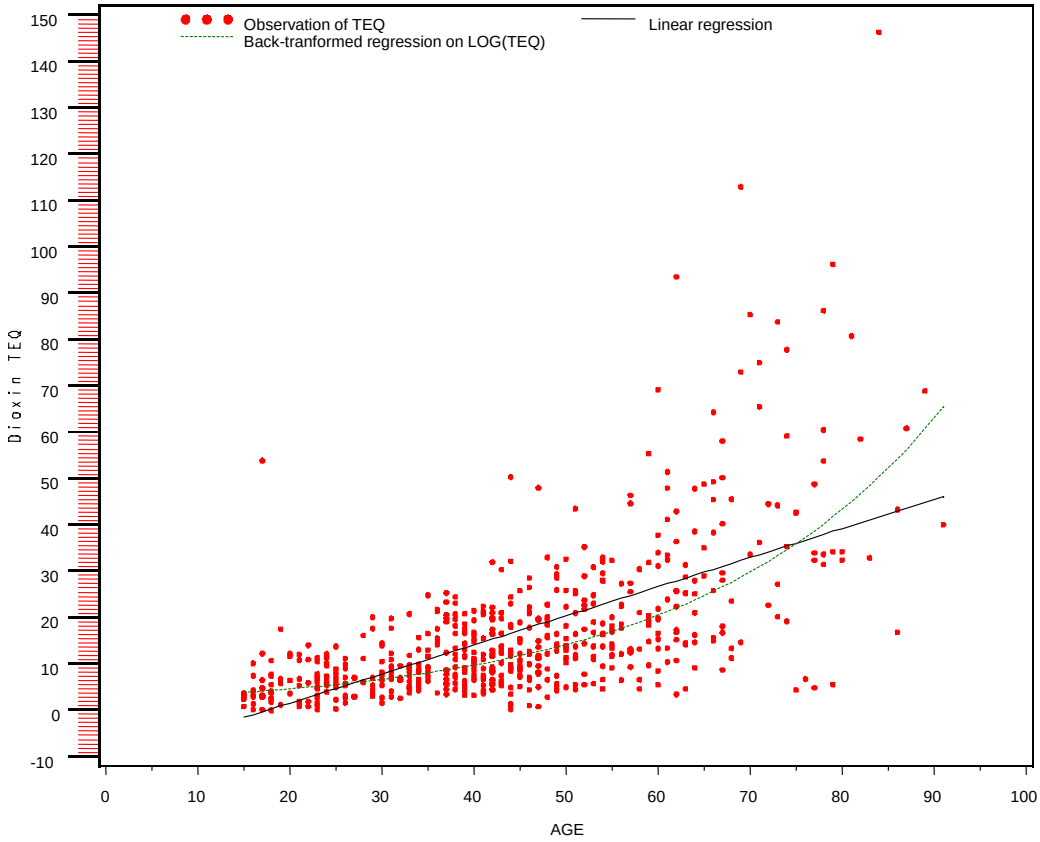
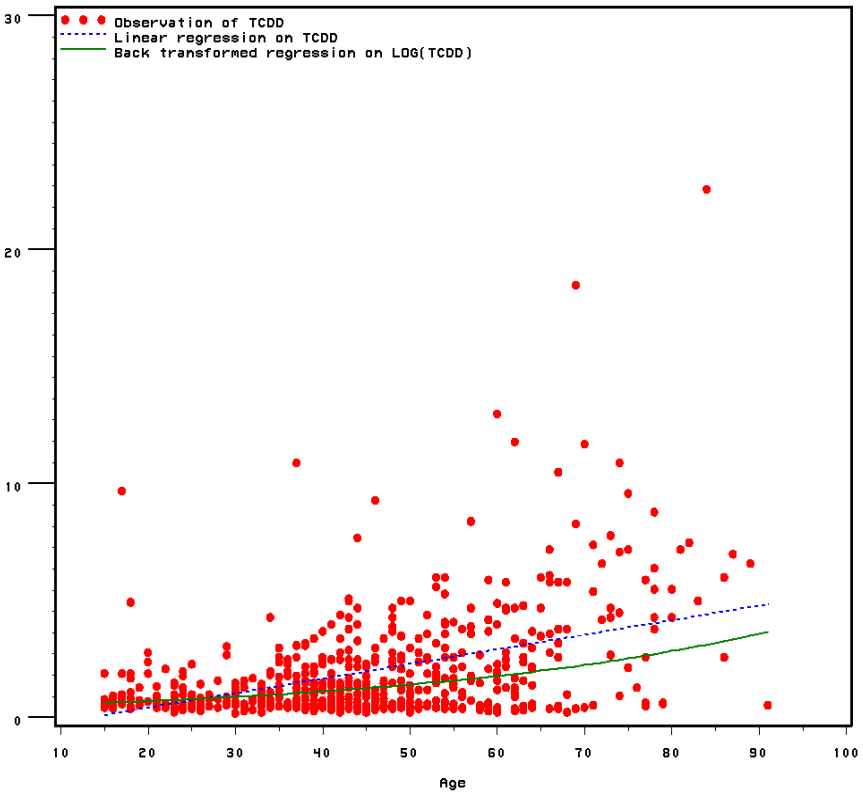


Figure 2. 2378 –TCDD (ppt, lipid adjusted) Versus Age for Studies From LA, MO, NC and NY



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