INTRUMENTAL APPROACHES FOR IMPROVING THE DETECTION LIMIT FOR SELECTED PCDD CONGENERS IN SAMPLES FROM THE GENERAL U.S. POPULATION AS BACKGROUND LEVELS CONTINUE TO DECLINE

Turner WE, Welch SM, DiPietro ES, Whitfield WE, Cash TP, McClure PC, Needham LL and Patterson DG Jr.

Organic Analytical Toxicology Branch, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Highway, NE, Mail stop F-17, Atlanta, GA 30341.

Introduction

The U.S. Environmental Protection Agency (EPA)[1] reported in 2000 that the release of "dioxin-like" compounds into the environment decreased by almost 80% from 1987-1995. Aylward and Hays[2] in a review of studies from the United States, Canada, Germany, and France observed that mean 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) human serum levels have decreased by almost a factor of 10 over the past 30 years. TCDD levels were about two parts per trillion (ppt or pg/g fat) in the general U.S. population in 2000 and based on known pharmacokinetics in humans predicted that mean background TCDD levels will decrease to 0.5-1 ppt by 2015.[2] The decline in background levels of dioxins, furans, and PCBs in both the environment and in humans is consistent with the assumption that regulatory efforts over the past several decades were in fact successful.

Our laboratory is currently analyzing a random one-third sub-sample of people aged 12 years and older from the 2003-2004 National Health and Nutrition Examination Survey (NHANES) conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC) for PCDDs/PCDFs/cPCBs and PCBs. The sampling plan for NHANES is a complex, stratified, multistage, probability-cluster design that selects a representative sample of the civilian, noninstitutionalized U.S. population. This current PCDDs/PCDFs/cPCBs and PCBs data will be part of the 4th *National Report on Human Exposure to Environmental Chemical*, another report in a series of two-year cycle biomonitoring exposure assessments of the U.S. population to environmental chemicals designed to establish reference ranges and track trends in levels of exposure over time that began in 1999-2000. While one of the objectives is to obtain geometric means and distribution percentiles by age, sex and race/ethnicity, it is often impossible to determine the 95th percentiles for some congeners due to the "ultra-trace" levels encountered. As serum levels in the U.S. continue to diminish, our method for measuring these environmental toxicants will continue to be "pushed to the limit" of its analytical capability. In previous articles[3,4] we investigated some of the variables influencing the quantification of fg/g concentrations of PCDDs/PCDFs and cPCBs in human serum and explored parameters that can affect the method detection limit (MDL). As previously observed, increasing the amount of serum used for analysis would be the most obvious and practical way to improve the MDL. However, in NHANES the amount of serum is restricted to ~7±3 g for PCDDs/PCDFs/cPCBs and PCBs. Here we report the outcome of our efforts to improve the detection limits for TCDD and 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PnCDD). This was accomplished by analyzing the first 832 NHANES sample extracts twice. In the first injection all seventeen 2,3,7,8-substituted PCDDs/PCDFs and four cPCBs were quantified using six multiple-ion detection (MID) groups and in the second injection TCDD and PnCDD were quantified in two MID descriptors in which the dwell times for the native masses were increased. Also reported are our preliminary results using the new Thermo Electron DFS double focusing magnetic sector high-resolution mass spectrometer and cryogenic zone compression gas chromatography coupled with magnetic sector mass spectrometry.

Materials and Methods

Sample Preparation

Serum samples were prepared according to the procedure reported by Turner et al.[5] Samples were spiked with $^{13}C_{12}$-labled internal standards followed by C_{18} solid-phase extraction (SPE) and a multicolumn automated cleanup and enrichment procedure using a Fluid Management Systems Power-Prep/6. An analytical run is comprised of two

method blanks, eight unknown samples and two quality control samples. PCBs were eluted from the AX-21 carbon in the forward direction with hexane and dichloromethane (1:1) and PCDDs/PCDFs/cPCBs were eluted in the reverse direction with toluene. One µL of dodecane "keeper" was added to each of the eluants and solvent evaporated to about 350 µL using a Caliper TurboVap II. Residual solvent was transferred to silanized autosampler vials and evaporated to one µL. Before analysis by high-resolution gas chromatography (HRGC) and high-resolution mass spectrometry (HRMS) the vials were reconstituted with 5-µL of ^{13}C-labeled external standard in nonane.

Mass Spectrometry

A Leap Technology GC Pal autosampler was used to make 2-µL injections into an Agilent 6890 gas chromatograph (GC). The GC was operated in the splitless injection mode with a flow of 1 mL/min He through a DB-5ms column (30 m x 0.25 mm x 0.25 µm film). Selected congeners were quantified by isotope-dilution mass spectrometry (IDMS) using selected ion monitoring (SIM) at 10,000 resolving power (10% valley) on two Thermo Electron MAT 95 XP (5kV) magnetic sector field mass spectrometers (with "sensitivity" upgrade part# 1150760 installed) operated in the electron impact (EI) mode at 40 eV.[6] Two separate HRMS quantification schemes were employed with the MAT 95s: one for all seventeen 2,3,7,8-substituted PCDDs/PCDFs and four cPCBs using six MID groups and another for TCDD and PnCDD only in two MID descriptors. Autosamplers were used to inject all 832 sample extracts back to back. In addition, 100 background level sample extracts previously run on the MAT 95 XP for 21 congeners were reanalyzed on a Thermo Electron DFS (5kV) magnetic sector double focusing mass spectrometer using SIM with six MID groups at 13,000 resolving power operated in the EI mode at 40 eV.

The total lipid content of each specimen was estimated from its total cholesterol and triglyceride values using a "summation" method.[7]

Results and Discussion

The TCDD and PnCDD congeners were selected for replicate analyses because these congeners had a very low frequency of detectable results in previous cycles of NHANES and because they have highest assigned toxicity equivalency factors (TEFs) of 1 and their scientific importance. Table 1 shows a comparison of method detection limits (ppt, pg/g lipid) observed for sample weights between 4 and 10 g using the MAT 95 XP. Overall, we were able

Table 1. Serum detection limits (ppt or pg/g lipid) for six MID groups TCDD/PnCDD and two MID groups TCDD/PnCDD on a MAT 95 XP HRMS. Assumptions: 70 % recovery and total lipid 0.6 %.

Sample Weight (g)	Six MID Groups TCDD/PnCDD MDL (ppt)	Two MID Groups TCDD/PnCDD MDL (ppt)
4.0	4.46	1.78
4.5	3.97	1.59
5.0	3.57	1.43
5.5	3.25	1.30
6.0	2.98	1.19
6.5	2.75	1.10
7.0	2.55	1.02
7.5	2.38	0.95
8.0	2.23	0.89
8.5	2.10	0.84
9.0	1.98	0.79
9.5	1.88	0.75
10.0	1.79	0.72

to lower the MDL for TCDD and PnCDD 2.5 fold by using the two MID group descriptor. Table 2 shows the percent nondetects for TCDD and PnCDD for injection 1 and injection 2. Using the two MID group descriptors we observed a 29% increase in the number of detects for TCDD and 16% for PnCDD. In time, we will analyze all of the more than 2000 NHANES 2003-2004 samples extracts in duplicate. However, it is difficult to foresee what impact this obvious improvement in MDL for TCDD and PnCDD will have in the final NHANES 2003-2004 report for these congeners because there are many complicating factors involved. For example, TCDD and PnCDD concentrations on average tend to increase with age and vary, to some extent, between sexes and we do not know the final statistical weighting that each sample represents in the U.S. population or the demographic characteristics (age, sex and race/ethnicity) of the samples. The MDL is also proportional to sample weight and influenced by analyte recovery. One can only speculate whether the extra work of making two injections was worth the effort and result in obtaining estimates of least the upper percentiles for TCDD and PnCDD.

Table 2. The percent nondetects for TCDD and PnCDD for sample injection 1 and injection 2.

	Nondetects for injection 1	**Nondetects for injection 2**
TCDD (n=832)	73 %	44 %
PnCDD (n=832)	53%	37%

Recently we performed experiments to evaluate the detection limits of the new DFS with six MID groups. Based on running standards, the instrumental detection limits (IDL) for all 21 PCDD/PCDF/cPCB congeners were approximately 5 fold lower than for the MAT 95 XP. When we ran about 100 sample extracts on the DFS previously analyzed on the MAT 95 XP, we observed about a 3 fold improvement in the MDL for all congeners. The difference in the IDL and the MDL observed on the DFS is mostly likely due to chemical noise and matrix effects in actual sample extracts compared to pure standards. These preliminary results from the Thermo Electron DFS appear promising and may provide us another means to improve the MDL for all 21 congeners in a single injection in the future.

It has been recognized that thermal modulation can enhance the sensitivity of gas chromatographic measurements by increasing the signal-to-noise ratio. Recently we reported our initial results using cryogenic zone compression coupled with magnetic sector mass spectrometry for analysis of PCDDs/PCDFs in human serum.[8] The gas chromatograph was an Agilent 6890 operated in splitless mode and the mass spectrometer was a MAT 95 XP. The modulation device was the Zoex Corporation loop modulator which utilizes liquid nitrogen as the cryogen and consists of one cold jet and one hot jet with 2 loops of DB-5 column passing through the jets, creating a quadruple jet or dual-stage system. Figure 1 shows a 3D plot of the total ion current (TIC) resulting from an injection of a human serum extract in which 7 dioxin congeners and 1 furan congener were monitored. We have continued to make some progress in experiments using cryogenic zone compression coupled to the MAT 95 XP to develop a method for TCDD and PnCDD with MDLs about 10 fold lower than we are currently able to achieve by the conventional 6 MID group technique. At this time, however, the method is not rugged enough to apply to studies with large numbers of samples. Ultimately we would also like to add 1,2,3,6,7,8-HxD, 2,3,4,7,8-PnCDF and 3,3',4,4',5-PCB to this method so we could quantify the 5 congeners that contribute the most to the total TEQ among the 21 PCDDs/PCDFs/cPCBs in background level samples. Details of our ongoing development of this new cryogenic zone compression technique are reported elsewhere in these proceedings.[9]

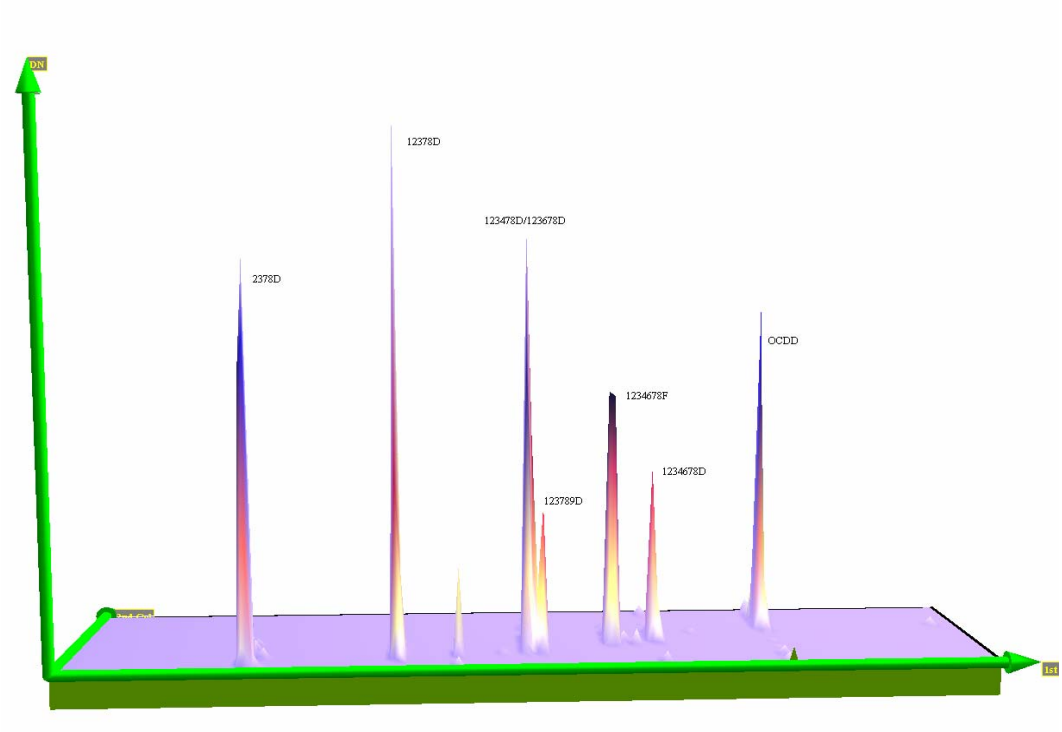

Figure 1. 3D plot showing the total ion current (TIC) from an injection of a human serum extract in which 7 dioxin congeners (2 unresolved) and 1 furan congener were monitored.

References

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