SAME SAMPLE CLEANUP FOR PCDDs/PCDFs/cPCBs, PCNs AND PBDDs/PBDFs IN HUMAN SERUM AND QUANTIFICATION BY GC/ID-HRMS

Turner WE, Whitfield WE, Cash TP, DiPietro ES, Anderson CJ, McClure PC, and Sjodin A

Centers for Disease Control and Prevention (CDC), National Center for Environmental Health and Agency for Toxic Substance and Disease Registry and the (NCEH/ATSDR), 4770 Buford Hwy NE, Atlanta, GA 30341, USA

Introduction

Beginning in 1999-2000 the scope of the National Health and Nutrition Examination Survey (NHANES), conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC), was expanded to include an ongoing biomonitoring exposure assessment of the U.S. population to selected environmental chemicals. Biomonitoring or body burden data from these surveys can be used to determine which chemicals get into Americans and at what concentrations; establish reference ranges; assess the effectiveness of public health efforts to reduce exposure; and track, over time, trends in levels of exposure. The sampling plan for NHANES is a complex, stratified, multistage, probability-cluster design that selects a representative sample of the civilian, non-institutionalized U.S. population. One of our biggest analytical challenges is to expand the list of environment toxicants to be measured in each NHANES 2-year cycle using the limited amount serum allocated to the laboratory (typically 6-8 mL) in our one third-subset of NHANES specimens. Presented here is a recently developed method for **7** polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 dibenzofurans (PCDFs), 4 coplanar PCBs (cPCBs), 6 polychlorinated naphthalenes (PCNs) and 7 polybrominated dibenzo-*p*-dioxins, (PBDDs) and 10 polybrominated dibenzofurans (PBDFs) in human serum using the Fluid Management Systems Power-Prep/6 for sample cleanup and enrichment and quantification by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry (GC/ID-HRMS) using Thermo Fisher Scientific DFS Magnetic Sector Mass Spectrometers.

Materials and Methods

This new method is a modification of our previously described method for serum PCDDs/PCDFs/cPCBs.[1] The serum samples spiked with $^{13}C_{12}$-labeled internal standards (Cambridge Isotopes Laboratory) and the analytes of interest are isolated in hexane using a C_{18} solid phase extraction (SPE) procedure which is followed by an automated Fluid Management Systems Power-Prep/6 cleanup and enrichment procedure using multi-layered silica gel (acidic, basic, and neutral silica) and alumina columns coupled to AX-21 carbon columns. PCDDs/PCDFs/cPCBs, PCNs and PBDDs/PBDFs are all collected in the reverse direction with toluene from the AX-21 columns.

Following sample cleanup, excess solvent for each eluant is evaporated to 350 µL using a Biotage TurboVap II Evaporation System and the remaining solvent is transferred to silanized auto sampler vials containing 1µL of dodecane "keeper" and evaporated to "dryness" using a Biotage TurboVap LV Evaporation System. Before quantification, the extracts are reconstituted with 5 µL $^{13}C_{12}$-labeled external standards.

PCDD/PCDF/cPCB and PCN congeners are analyzed at the same time by injecting 2 µL of the sample extract into a Thermo Trace GC Ultra gas chromatograph equipped with a DB-5ms capillary column (30m x 0.25 mm x 0.25 µm film thickness) coupled to a Thermo Fisher Scientific DFS mass spectrometer operated in EI mode using selected ion monitoring (SIM) at 10,000 resolving power and a 7 group SIM descriptor. PBDD/PBDF congeners are analyzed by injecting another 2 µL of the sample extract into a Agilent 6890 gas chromatograph equipped with a Rtx-1614 capillary column (15m x 0.25 mm x 0.10 µm film thickness) coupled to a Thermo Electron DFS mass spectrometer operated at 40 eV in EI mode using selected ion monitoring (SIM) at 10,000 resolving power and a 6 group SIM descriptor. The concentration of each analyte is calculated from a daily linear calibration curve. An analytical run consists of eight unknown serum samples, two method blanks, and

two quality control samples. 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.[2] Whole-weight and lipid-adjusted detection limits are reported for each analyte in every sample, corrected for sample weight and analyte recovery.

Results and Discussion

Table 1 is a list of the 17 PCDDs/PCDFs, 4 cPCBs, 6 PCNs and 17 PBDDs/PBDFs that can be measured in human serum using the same sample cleanup method.

Table 1. Analyte list for PCDD/PCDF/cPCB, PCN and PBDDs/PBDF.

Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans		**Polybrominated Dibenzo-*p*-dioxins and Dibenzofurans**
1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin (OCDD)		2,3,7,8-Tetrabromorodienzo-*p*-dioxin (TBDD)
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin (HpCDD)		1,2,3,7,8-Pentabromodibenzo-*p*-dioxin (PeBDD)
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD)		1,2,3,4,7,8-Hexabromorodibenzo-*p*-dioxin (HxBDD)
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD)		1,2,3,6,7,8-Hexabromodibenzo-*p*-dioxin (HxBDD)
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin (HxCDD)		1,2,3,7,8,9-Hexabromodibenzo-*p*-dioxin (HxBDD)
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin (PeCDD)		1,2,3,4,6,7,8-Heptabromoodibenzo-*p*-dioxin (HpBDD)
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)		1,2,3,4,6,7,8,9-Octabromodibenzo-*p*-dioxin (OBDD)
1,2,3,4,6,7,8,9-Octachlorodibenzofuran (OCDF)		2,3,7,8,-Tetrabromodibenzofuran (TBDF)
1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF)		1,2,3,7,8-Pentabromodibenzofuran (PeBDF)
1,2,3,4,7,8,9-Heptachlorodibenzofuran (HpCDF)		2,3,4,7,8-Pentabromodibenzofuran (PeBDF)
1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF)		1,2,3,4,7,8-Hexabromodibenzofuran (HxBDF)
1,2,3,6,7,8-Hexachlorodibenzofuran (HxCDF)		1,2,3,6,7,8-Hexabromodibenzofuran (HxBDF)
1,2,3,7,8,9-Hexachlorodibenzofuran (HxCDF)		1,2,3,7,8,9-Hexabromodibenzofuran (HxBDF)
1,2,3,7,8-Pentachlorodibenzofuran (PeCDF)		2,3,4,6,7,8,-Hexabromodibenzofuran (HxBDF)
2,3,4,6,7,8-Hexachlorodibenzofuran (HxCDF)		1,2,3,4,6,7,8-Heptabromodibenzofuran (HpBDF)
2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)		1,2,3,4,7,8,9-Heptabromodibenzofuran (HpBDF)
2,3,7,8-Tetrachlorodibenzofuran (TCDF)		1,2,3,4,6,7,8,9-Octabromodibenzofuran (OBDF)
Coplanar Polychlorinated Biphenyls - cPCBs		**Polychlorinated Naphthalenes**
3,3',4,4'-Tetrachlorobiphenyl (PCB 77)		1,2,3,4-Tetrachlorinated naphthalene (PCN 27)
3,4,4',5-Tetrachlorobiphenyl (PCB 81)		1,2,3,5,7- and 1,2,4,6,7-Pentachlorinated naphthalene (PCN 52 & 60)
3,3',4,4',5-Pentachlorobiphenyl (PCB 126)		1,2,3,4,5,7- and 1,2,3,5,6,8-Hexachlorinated naphthalene (PCN 64 & 68)
3,3',4,4',5,5'-Hexachlorobiphenyl (PCB 169)		1,2,3,4,6,7- and 1,2,3,5,6,7-Hexachlorinated naphthalene (PCN 66 & 67)
		1,2,3,5,7,8-Hexachlorinated naphthalene (PCN 69)
		1,2,3,4,5,6,7-Heptachlorinated naphthalene (PCN 73)

To increase our analytical throughput for measuring polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides, we previously developed and validated a combined PCB/OC GC/ID-HRMS method[3] for the simultaneous quantification of 38 PCBs and 13 OC pesticides in a single analysis without sacrificing data quality. Consequently, we were able to double our analytical throughput by modifying mass spectrometric parameters alone and have routinely used this method for over 7 years. Using an analogous approach, we have now also successfully combined the quantification of PCDDs/PCDFs/cPCBs and PCNs into a single analysis without increasing the overall run time because the PCNs tended to elute earlier than the PCDDs/PCDFs/cPCBs. Figure 1 and Figure 2 show the reconstructed ion chromatograms for PCDDs/PCDFs/cPCBs and PCNs respectively on a 30 m DB-5ms column.

Figure 3 shows a reconstructed ion chromatogram for PBDDs/PBDFs on a 15 m Rtx-1614 column. In the analysis of PBDDs/PBDFs we determined that it was imperative to use a short 15 m high-temperature column and a deactivated single goose-neck GC liner to minimize the degradation of OBDD and OBDF. During the development of this method, we observed that the instrumental detection limits for PBDDs/PBDDs were approximately 30 fold higher than for PCDDs/PCDFs on the DFS mass spectrometer.

Figure 1. A reconstructed ion chromatogram for PCDDs/PCDFs/cPCBs on a 30 m DB-5ms column.

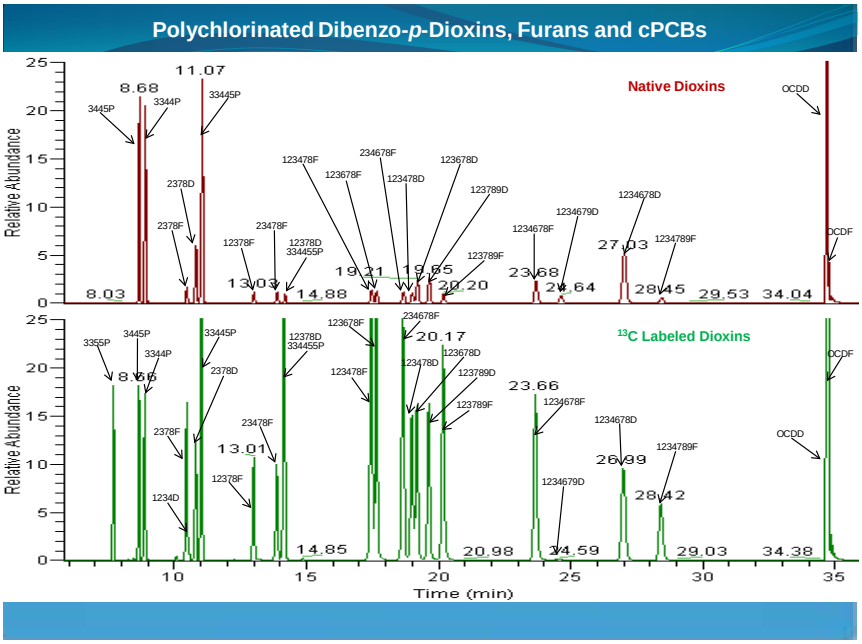


Figure 2. A reconstructed ion chromatogram for PCNs on a 30 m DB-5ms column.

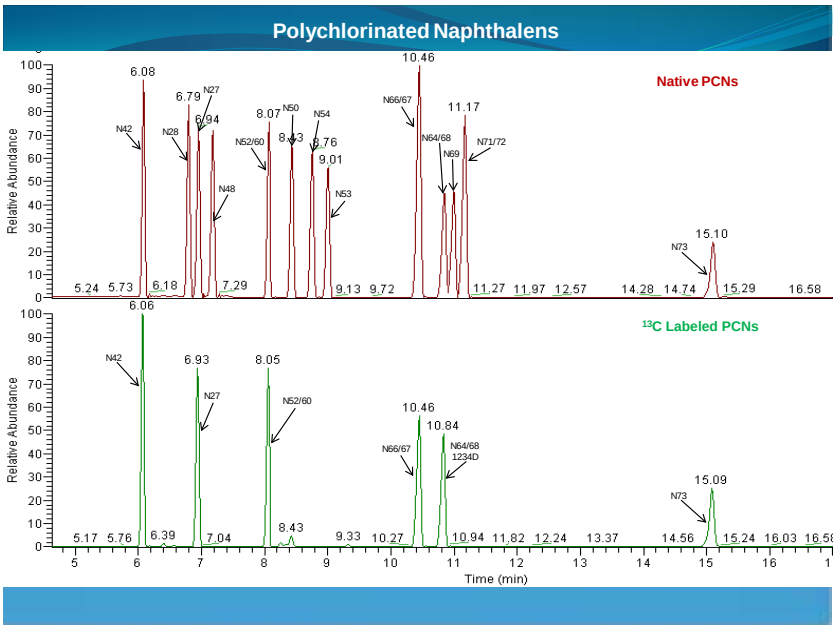
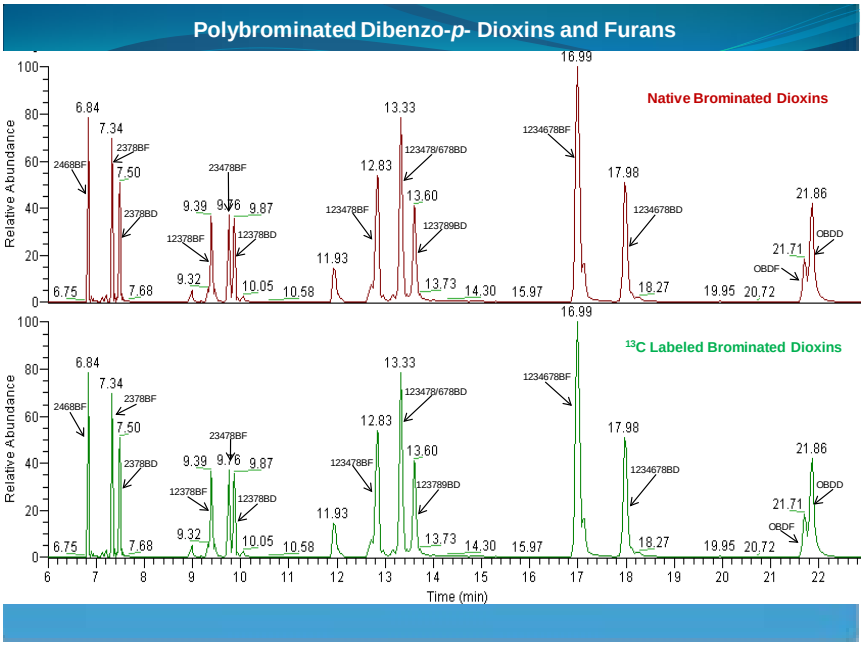


Figure 3. A reconstructed ion chromatogram for PBDDs/PBDFs on a 15 m Rtx-1614 column.



In preliminary data from U.S. adult serum specimens (non-NHANES specimens) we observed that 1,2,3,5,7-/1,2,4,6,7-pentaPCN (PCN52/PCN60) and 1,2,3,4,6,7-/1,2,3,5,6,7-hexaPCN (PCN66/PCN67) to be the most frequently detected congeners, as previously reported by Asplund[4] in specimens from Sweden. In the same specimens, we did not detect PBDDs and only occasionally a few PBDFs with 1,2,3,4,6,8,9-HeptaPBDF being the most commonly detected PBDF congener. During the cleanup method development, obtaining consistent recoveries of OBDF and OBDD from serum has been problematic and the presence of background contamination of 1,2,3,4,6,8,9-HeptaPBDF in blanks has resulted in an increase in the detection limit for this congener compared to the other PBDDs/PBDFs.

References

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