A Screening Method for PCDD/Fs and Non-ortho PCBs in Biological Matrixes

Chia-Yang Chen*, J. Ronald Hass, and Phillip W. Albro****

*Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7400, USA
** Triangle Laboratories, Inc. 801 Capitola Drive, Durham, NC 27713, USA

Introduction

A quick, sensitive, and relatively inexpensive analytical method is essential for a large-scale epidemiological study or long-term monitoring. It is also crucial for environmental, toxicological, or other large studies, since more samples can be analyzed under a limited budget (that is, get more information at less expense). Currently, most analyses of PCDD/Fs and PCBs are very costly due to labor-intensive and time-consuming sample preparation procedures plus the use of high-resolution mass spectrometry (HRMS) for identifying analytes. This prevents extended surveillance of the general population for exposure to PCDD/Fs and PCBs.

Traditional Soxhlet extractions[1] use large volumes of organic solvents (200–500 mL per sample) and generally take 8–16 hours. In addition, thimbles and glassware require extraction before use. Blending methods[2,3] are much faster (1–3 minutes per blend and commonly just need 2 blends), but can only extract one sample each time. Moreover, they need 150–300 mL of solvents per sample, which could take a lot of time to concentrate before further cleanup. Conventional multiple liquid-liquid extractions of serum samples[4] are tedious and use comparatively large volumes of solvents. Solid-phase extractions of plasma/serum[3,5] are more rapid, use less solvent, and get cleaner extracts. However, blockage of cartridges might happen when sample sizes increase.

According to the literature reviewed by the authors, seven dioxin-like compounds—2,3,7,8-TCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, 3,3',4,4'-TCB (PCB #77), 3,3',4,4',5-PeCB (PCB #126), and 3,3',4,4',5,5'-HxCB (PCB #169) —can account for 93 $\pm$ 5.4% (n = 132) of the total TEQs in reported human blood or plasma samples.[6–12] Accordingly, it would be sufficient to estimate the total exposure to dioxin-like compounds by just measuring these seven chemicals. For the needs of our future research, 1,2,3,7,8-PeCDF and OCDF were also investigated.

A rapid and relatively inexpensive screening method has been developed for detecting the nine compounds in five rat tissues: (1) serum, liver, and adipose tissue from maternal rats; (2) placentas and whole fetuses.

Materials and Methods

Native and $^{13}C_{12}$-labeled standards of PCDD/Fs and non-*ortho* PCBs were from Cambridge Isotope Laboratories, Andover, MA. A vacuum manifold (Baker spe-24G column processor with Teflon solvent guides, J.T. Baker), a KNF Neuberger vacuum pump (model #53), and one-gram Supelclean LC-18 cartridges (Supelco, Inc.) were used for solid-phase extraction (SPE). An FS-30 ultrasonic cleaner (Fisher Scientific) was used to sonicate samples. A Centrific centrifuge was from Fisher Scientific.

Control serum, liver, adipose, fetus, and placenta tissues of the 21st gestational day (GD 21) Long Evans (LE) rats were from the National Exposure Research Laboratory, the U.S. Environmental Protection Agency (U.S. EPA), Research Triangle Park, NC. Rat livers containing exempt amounts of ^{3}H (tritium) were from the same source. All tissues were stored in sealed plastic tubes at –21 ^{o}C and were thawed at room temperature before extraction.

Extraction of Rat Serum. 12 pg of each native compound (in methanol) and 500 pg/congener of $^{13}C_{12}$-labeled internal standards (in methanol) were spiked into 3 mL of control rat serum. After gently rotating in a mixer at 30 rpm for an hour, the serum sample was mixed with an equal volume of 88% formic acid and was rotated at 30 rpm for 10 more minutes. The serum/formic acid mixture was hand-shaken vigorously with 3-mL *n*-heptane for 2 minutes. After centrifugation, the upper *n*-heptane layer was collected. The serum/formic acid mixture was extracted one more time as above.

Extraction of Solid Tissues. 0.6 g of liver, 1.2 g of fat, 5.0 g of placenta, and 5.2 g of whole fetus were used. 12 pg of each native compound (in methanol) and 500 pg/congener of $^{13}C_{12}$-labeled internal standards (in methanol) were spiked onto 0.15-gram Celite (diatomaceous earth). The mixture of the Celite, solid tissue, and eight times the sample-weight of anhydrous sodium sulfate granules was ground to a powder by a pestle in a mortar. The powder was transferred to a 50-mL centrifuge tube. The mortar and pestle were rinsed with 30 mL of acetonitrile and the rinse was added into the centrifuge tube. The tube was sealed with a Teflon-lined screw cap and the sample was sonicated for 15 minutes in an FS-30 ultrasonic cleaner. The supernate was collected after centrifugation. The sediment was extracted as above, but 20 mL of acetonitrile was used instead of 30 mL. The combined supernate was blown down to 10 mL with a gentle stream of nitrogen in a 65 ^{o}C water bath. The concentrated extract was mixed with 10 mL of 88% formic acid and 10 mL of HPLC water. The mixture passed through a 1-g LC-18 cartridge at a flow rate of about one mL/min. After the sample passed through the cartridge, 6 mL of HPLC water was passed to wash out the residue of formic acid in the cartridge. The cartridge was dried using vacuum pumping for two hours before eluting with two parts of 3-mL *n*-heptane. The *n*-heptane eluent was collected for further cleanup.

Cleanup of Extracts. The extract (in *n*-heptane) either from serum or from solid tissues, without further concentration, was loaded to a dual-column unit—3.0 g of 40% H_2SO_4-coated silicic acid and 1.0 g of activated acidic alumina. The silicic acid column was removed after eluting with 5-mL *n*-heptane and the flow was 0.53–0.55 mL/min. The alumina column was eluted with 2 additional portions of 5-mL *n*-heptane and 2 portions of 5-mL 20% methylene chloride ($MeCl_2$)/*n*-heptane and the flow was 1.2–1.4 mL/min. The eluent of 20% $MeCl_2$/*n*-heptane was collected and was concentrated to dryness by a speedvac.

Analysis. A Hewlett-Packard (HP) 5890 series II gas chromatography (GC) combined with a VG Autospec HRMS (using electron impact ionization mode) were employed for the analysis. A 30-m DB-5MS column (0.25-mm i.d., 0.5 μm film thickness) was installed and helium was the carrier gas. The temperature program was as follows: initial temperature 175 ^{o}C for 1 min, ramp at 40 ^{o}C/min to 270 ^{o}C and hold for 1 min, ramp at 5 ^{o}C/min to 295 ^{o}C, and ramp at 10 ^{o}C/min to 350 ^{o}C. Dried samples in ½ dram vials were reconstituted with 10 uL of the recovery standard (50 ng/mL of $^{13}C_{12}$-1,2,3,7,8,9-HxCDD in toluene) and 2 uL of the re-dissolved residues was injected. Data were acquired at 5,000 resolution (defined by full width at 5% peak maximum) using selected ion recording (SIR) mode. The two most abundant molecular ions [either (M and M+2) or (M+2 and M+4)] were monitored for both native analytes and ^{13}C-labeled internal standards. One

exception was that the (M+4 and M+6) ions for 3,3',4,4',5-PeCB was measured to avoid the interference from 2,3,7,8-TCDD.

Determination of lipid contents and MDLs. A modified Folch's method[13] was used to determine lipid contents of the various tissues. To determine the statistical method detection limit (MDL), eight spiked samples of rat control tissues were analyzed. The signal height to noise height (S/N) ratio and the noise level of each analyte were measured to calculate the MDL at 99% confidence level (CL).

Results and Discussion

The lipid contents of LE rat tissues were serum 0.71 $\pm$ 0.15%, liver 6.5 $\pm$ 0.22%, adipose 86 $\pm$ 4.6%, placenta 4.0 $\pm$ 0.15%, and fetus 1.4 $\pm$ 0.05%. We found that acetonitrile only extracted about 12% of total lipids. Consequently, 3-g acidic-coated silicic acid can handle the extracted lipids easily. We also analyzed liver samples from the rats that were administered ^{3}H-labeled 2,3,7,8-TCDD. We demonstrated that two extractions with acetonitrile could extract approximately 97% of the endogenous TCDD.

The recoveries of internal standards of MDL samples are serum 74 $\pm$ 9.4%, liver 52 $\pm$ 7.4%, adipose 48 $\pm$ 4.6%, placenta 66 $\pm$ 9.4%, and fetus 51 $\pm$ 9.2% for 2,3,7,8-TCDD. Except for the MDL samples of serum and placenta, the recoveries of internal standards of other MDL samples were 10–20% lower than those we observed in the samples for method development. The MDL samples of serum were injected on GC/HRMS within four days after finishing sample preparation. However, all other MDL samples of solid tissues were not injected until 1.5–2 months later, because of the tight schedule of the HRMS. Therefore, the causes of lower recoveries could be (1) the volatile loss of the analytes or (2) analytes sticking on the vials for a long time and hard to re-dissolve. Because the samples were stored in a dark cabinet, photo-degradation of the analytes was unlikely.

The MDLs (whole-weight basis) at 99% CL are serum 1.3 ppt, liver 5.4 ppt, adipose 4.3 ppt, placenta 0.73 ppt, and fetus 1.2 ppt for 2,3,7,8-TCDD. The detection limits (S/N >2.5, whole-weight basis) of the MDL samples are serum 0.87 $\pm$ 0.19 ppt, liver 3.9 $\pm$ 1.0 ppt, adipose 2.8 $\pm$ 0.56 ppt, placenta 0.40 $\pm$ 0.09 ppt, and fetus 0.66 $\pm$ 0.17 ppt for 2,3,7,8-TCDD.

Extracting serum samples with *n*-heptane, a relatively non-toxic solvent, is simple and rapid (2.5–3 hr/10 sample/analyst). Sonication of tissues with acetonitrile gets fewer co-extractives than other less-polar solvents do and reduces the burden on the following cleanup steps. Using only two adsorbents, the acid-coated silicic acid and acidic alumina, simplifies the cleanup steps ($\approx$ 2 hr/10 sample/analyst). Not needing to separate hexa- and hepta-congeners enables us to run a sample within 15 minutes on a conventional GC. The method also uses less organic solvent in the sample preparation ($\approx$ 62.5 mL per serum sample and $\approx$ 131 mL per solid tissue sample). It saves the cost for purchasing solvents, reduces the amount of hazardous waste, and decreases solvent residues that may interfere with trace analysis.

Acknowledgements

The authors express their gratitude to Dr. Jonathan Hamm and Dr. Linda Birnbaum, the U.S. EPA, for providing rat tissues. The authors also wish to thank Robert Schoonhoven, the Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, for the assistance in operating the scintillation counter.

References

1. Tondeur, Y., Beckert, W.F., Billets, S., Mitchum, R.K. Method 8290: an analytical protocol for the multimedia characterization of polychlorinated dibenzodioxins and dibenzofurans by high-resolution gas chromatography/high-resolution mass spectrometry. *Chemosphere* **1989**, 16, 1–6, pp 119–131.
2. Albro, P.W., Corbett, B.J. Extraction and clean-up of animal tissues for subsequent determination of mixtures of chlorinated dibenzo-*p*-dioxins and dibenzofuran. *Chemosphere* **1977**, 7, pp 381–385.
3. Chang, R.R., Jarman, W.M., Hennings, J.A. Sample cleanup by solid-phase extraction for the ultratrace determination of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in biological samples. *Anal. Chem.* **1993**, 65, 18, pp 2420–2427.
4. Patterson, D.G. Jr., Hampton, L., Lapeza, C.R. Jr., Belser, W.T., Green, V., Alexander, L., Needham, L.L. High-resolution gas chromatographic/high-resolution mass spectrometric analysis of human serum on a whole-weight and lipid basis for 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin. *Anal. Chem.* **1987**, 59, 15, pp 2000–2005.
5. Turner, W.E., DiPietro, E.S., Cash, T.P., McClure, P.C., Patterson, D.G., Jr., Shirkhan, H. An improved SPE extraction and automated sample cleanup method for serum PCDDs, PCDFs, and coplanar PCBs, in *14th Int. Symp. on Chlorinated Dioxins and Related Compounds, Organohalogen Compounds*. Fiedler, H., Hutzinger, O., Clement, R., Sakai, S., Eds. Kyoto, Japan, **1994**, 19, pp 31–36
6. Papke, O., Ball, M., Lis, A. Potential occupational exposure of municipal waste incinerator workers with PCDD/PCDF. *Chemosphere* **1993**, 27, 1–3, pp 203–209.
7. Papke, O., Ball, M., Lis, A. PCDD/PCDF in humans, a 1993-update of background data. *Chemosphere* **1994**, 29, 9–11, pp 2355–2360.
8. Schecter, A., McGee, H., Stanley, J., Boggess, K. Dioxin, dibenzofuran, and PCB, including coplanar PCB levels in the blood of Vietnam veterans in the Michigan agent orange study. *Chemosphere* **1992**, 25, 1–2, pp 205–208.
9. Schecter, A., Papke, O., Ball, M., Cau, H.D., Dai, L.C., Ming, N.Q., Quynh, H.T., Phuong, N.N.T., Phiet, P.H., Chi, H.K., Vo, D.T., Constable, J.D., Spencer, J. Dioxin and dibenzofuran levels in blood and adipose tissue of Vietnamese from various locations in Vietnam in proximity to agent orange spraying. *Chemosphere* **1992**, 25, 7–10, pp 1123–1128.
10. Schecter, A., Papke, O., Ball, M., Grachev, M., Beim, A., Koptug, V., Cau, H.D., Dai, L.C., Quynh, H.T., Phuong N.N.T., Chi, H.K. Dioxin and dibenzofuran levels in human blood samples from Guam, Russia, Germany, Vietnam and the USA. *Chemosphere* **1992**, 25, 7–10, pp 1129–1134.
11. Schecter, A., Ryan, J.J., Constable, J.D. Partitioning of dioxin and dibenzofuran congeners between plasma and cell fractions of blood from 10 adult male patients. *Chemosphere* **1992**, 25, 12, pp 2017–2022.
12. Schecter, A., Ryan, J.J., Papke, O. Elevated dioxin blood levels in Russian chemical workers and their children following maternal exposure. *Chemosphere* **1994**, 29, 9–11, pp 2361–2370.
13. Folch, J., Lees, M., Sloane Stanley, G.H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem*. **1957**, 236, pp 497–509.