Determination of PCBs and Dioxins in Rat Tissues

Kathy Boggess, Janet Paper, John Wagner and Robert E. Smith
Midwest Research Institute, 425 Volker Blvd., Kansas City, MO 64110
and Diane Overstreet
NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709

Introduction

Methods based on high resolution gas chromatography coupled to high resolution mass spectrometry (HRGC/HRMS) and isotope dilution were evaluated to determine trace levels of 2,3,7,8 TCDD, 2,3,4,7,8 PeCDF, 1,2,3,7,8 PeCDD, 3,3'4,4',5-PeCB (PCB 126), and 2,2',4,4',5,5'-HxCB (PCB 153). The method evaluation focused on spike recovery experiments from composite quality control pools of rat whole blood, serum, liver, lung, and mesenteric fat fortified with the native analytes over a concentration range from 1 pg/g or part- per- trillion (ppt) up to 200 pg/g. Unspiked matrices also were analyzed to establish background for the quality control tissues. The sample size was equivalent to the amount of tissue harvested from a 13 week old Sprague-Dawley rat. The 1 pg/g spike level for the mesenteric fat matrix yielded a final extract concentration of 50 femtogram per microliter (fg/µl). The HRGC/HRMS calibration range was extended a factor of 10 lower than specified in most environmental methods for dioxin and furan analyses to achieve the trace level detection limits required for the study. The objective of the method evaluation study was to establish method performance for determination of these analytes in subsequent animal studies. Because PCB 153 was to be spiked at least 1000 times higher than the other analytes in subsequent animal dosing experiments, this analyte was spiked at ng/g concentrations for method evaluation.

Materials and Methods

To ~ 2.5 mL of whole blood and serum, ~ 1 g of lung and mesenteric fat, and ~ 5 g liver was added 200 pg of $^{13}C_{12}$ internal quantitation standards (IQS); then native analytes were added. The study design for each matrix consisted of two unspiked samples, three replicates at the 1 pg/g spike level and three replicates at the 200 pg/g spike level in order to provide precision information at both ends of the concentration range specified. Four additional spike levels were evaluated at 2, 10, 40, and 100 pg/g to establish method performance over the linear range anticipated in the subsequent dosed animal studies.

The lung, liver, and mesenteric fat matrices were extracted using a tissuemizer®. The samples were weighed into 50 mL culture tubes and extracted three times with 10 mL methylene choride. The extracts were dried with Na_2SO_4 and solvent exchanged to hexane. The whole blood and serum were extracted by adding 2.5 mL of saturated aqueous NH_4SO_4, 2.5 mL ethanol, and 2.5 mL hexane. The mixture was placed on a rotary extractor for 30 minutes. The hexane was recovered and the extraction was repeated two times. The hexane was dried with Na_2SO_4 and volume reduced to 1 mL under nitrogen. The hexane extracts from each matrix were put through a series of

chromatography cleanup procedures. The extracts were first put on a column packed with 1 g neutral silica plus 8 g sulfuric acid modified silica that had been pre-washed with hexane. The analytes were eluted off the column with hexane. The samples were then split into two portions. One split was concentrated to 1.0 mL and analyzed for ng/g or parts per billion concentrations of PCB 153 with no further cleanup. The extract was solvent exchanged to nonane and spiked with $^{13}C_{12}$-PCB 138 as a recovery standard at 100 pg/µl extract concentration and analyzed by HRGC/HRMS.

The remaining split of the extract was put through additional cleanup for pg/g analysis of TCDD, PeCDF, PeCDD and PCB 126. Two additional chromatography cleanup columns, neutral alumina, and AX-21 carbon, were used to separate these analytes from biological interferences and the higher spike concentration of PCB 153. The extracts were put on a column packed with 4 g Na_2SO_4, 4 g neutral alumina, and another 4 g Na_2SO_4. Interfering compounds were removed in the first fraction, 8% CH_2Cl_2:hexane (v/v). The analytes of interest were collected in the second fraction, 20ml 60:40 CH_2Cl_2:hexane (v/v). This fraction was concentrated to ~2mL and applied to a column packed with AX-21 Carbon on Celite. The carbon column was eluted with 6 ml of hexane 4 ml of 1:1 CH_2Cl_2:hexane (1:1), followed by 1 mL of 75:20:5 CH_2Cl_2:CH_3OH:benzene (v/v/v) to remove non-planar biological interferences and traces of PCB 153 not removed from the alumina column. The columns then were inverted and eluted with 40 mL of toluene. This fraction contained PCB 126 along with TCDD, PeCDF, and PeCDD. The toluene fractions were evaporated under nitrogen to dryness and then spiked with 10 µL of a 10 pg/µl $^{13}C_{12}$-1,2,3,4 recovery standard in nonane. The samples were analyzed by HRGC/HRMS using a DB-5 GC column and a Fisons Autospec Ultima mass spectrometer operated in the selected ion monitoring mode with mass resolution >10,000. The isotope dilution technique described in the U.S. EPA method 1613 [1] was used for quantitation. Instrument calibration standards were prepared and analyzed at a concentration 10 times lower than the range specified in the EPA method.

Results and Discussion

The accuracy and precision results for the lowest spike level shown in Table 1 verified performance of the method to the low parts per trillion detection required for small tissue sample sizes. The blank matrices analyzed with the spiked matrices showed response for some of the target analytes at concentrations estimated less than 1 pg/g. Although the measured responses for the peaks were above the instrument noise, the detection of these analytes in the unspiked matrices were estimated as "trace" concentrations because the responses were beneath the lowest instrument calibration standard. Trace amounts of 2,3,7,8 TCDD, 2,3,4,7,8 PeCDF , and 1,2,3,7,8-PeCDD (0.19,0.27, and 0.32 pg/g, respectively) were found in the unspiked whole blood. PCB 126 (5.63 pg/g) and PCB 153 (0.53 ng/g) were detected significantly above instrument background as shown in Figures 1 and 2.

In the unspiked control rat tissues less than 0.2 pg/g 2,3,7,8 TCDD was detected in liver and 0.37 pg/g was detected in mesenteric fat. PCB 153 was the most prevalent analyte detected in the control tissues with 0.53 ng/g in whole blood, 0.88 ng/g in serum, 3.7 ng/g in liver, 12 ng/g in fat, and 1.2 ng/g in lungs.

References

1 U.S. EPA (1994) Method 1613: Tetra-Through Octachlorinated Dioxins and Furans by isotope Dilution HRGC/HRMS. EPA 821-B-94-005.

Table 1. Method Recoveries (%) for Spiked Tissue Matrices

	1 ppt Mean	1 ppt RSD	10 ppt Mean	10 ppt RSD	200 ppt Mean	200 ppt RSD
Whole Blood:						
2,3,7,8 TCDD	90.2	7.0	80.9	4.2	80.3	0.7
2,3,4,7,8 PeCDF	60.4	13.4	71.0	5.8	72.3	6.4
1,2,3,7,8 PeCDD	57.4	12.8	75.4	2.7	78.3	1.5
PCB 126	-	-	75.4	6.2	68	0.0
Serum						
2,3,7,8 TCDD	110	7.5	81.4	1.0	84.0	16.5
2,3,4,7,8 PeCDF	-	-	84.8	9.8	84.5	16.1
1,2,3,7,8 PeCDD	127	10.4	82	1.7	84.5	12.4
PCB 126	77.2	17.5	69.2	8.2	76.7	15.2
Fat						
2,3,7,8 TCDD	108	6.8	65.1	1.9	74.9	6.9
2,3,4,7,8 PeCDF	-	-	78.2	14.3	73.6	10.6
1,2,3,7,8 PeCDD	82.7	12.1	73.3	7.6	69.3	6.9

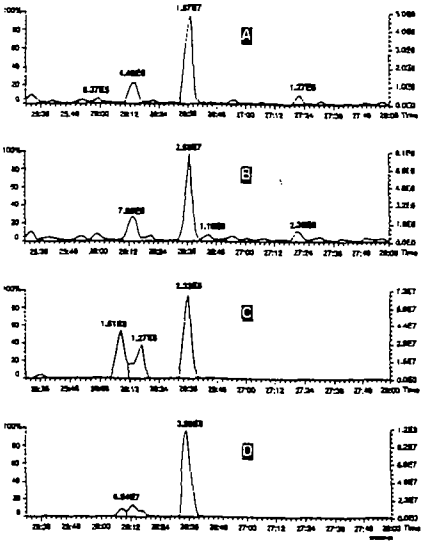

Figure 1 a-d. PCB 126 in Whole Blood (a) mass 323.8834 (b) mass 325.8804
(c) $^{13}C_{12}$-PCB 126 mass 335.9242 (d) $^{13}C_{12}$-PCB 126 mass 337.9219

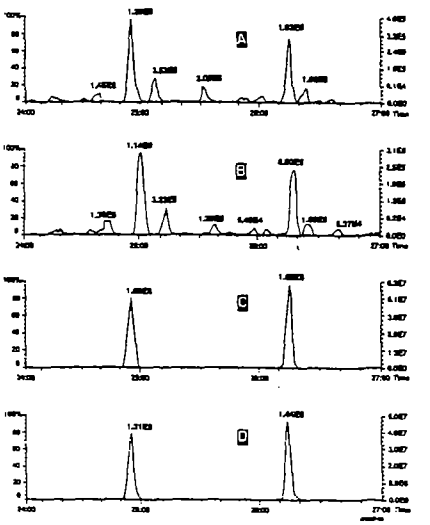

Figure 2 a - d. Detection of PCB 153 in Whole Blood (a) mass 359.8415 (b) mass 361.8385
(c) $^{13}C_{12}$-PCB 138 mass 371.8811 (d) $^{13}C_{12}$-PCB 138 mass 373.8781