Analytical Method and Results from the Analyses of USEPA Historical Food Samples for Dibenzo-p-Dioxins/-Furans/Coplanar PCBs

Joseph Ferrario[a], Christian Byrne[a], Aubry E. Dupuy, Jr.[a], Dwain L. Winters[b], Matthew Lorber[c], Susan Anderson[d]

[a] USEPA, OPPTS, Environmental Chemistry Laboratory, Building 1105, John C. Stennis Space Center, MS, USA 39529; [b] USEPA, Office of Prevention, Pesticides, and Toxic Substances, USEPA, 401 M St., SW, Washington, D.C. 20460; [c] USEPA, ORD, National Center for Environmental Assessment (8623D), 401 M St., SW, Washington, D.C. 20460; [d] Versar, Inc., 6850 Versar Center, Springfield, VA 22151

Introduction

As part of the USEPA's Dioxin Exposure Initiative (DEI)[1], fourteen historical food samples were analyzed for the presence of the 2,3,7,8-Cl substituted dibenzo-p-dioxins/-furans and selected coplanar PCBs. The samples consisted of variously prepared and preserved beef, pork, and poultry dating from 1908 through 1983. The samples were obtained from The U.S. Army Quartermaster Museum, The Smithsonian Institution, USDA Russsell Research Laboratory, and The Johnson Space Center. Due to limited sample availability, the various types of samples involved, and the need to achieve the lowest whole weight detection limits, an extraction procedure was employed to remove the lipid from the entire sample prior to sample workup. Once obtained, the desired amount of lipid was prepared for analyses using conventional sample preparation techniques. The analyses of the entire lipid content removed from more than 100 grams of sample resulted in whole weight detection limits of between 5-10 parts-per-quadrillion for the 2,3,7,8-TCDD for several of the samples. The method used for the analysis of these compounds in the variety of samples and the concentrations found expressed on a lipid adjusted basis are presented in this paper. The data from the analyses of these samples has been evaluated in a companion paper presented at this conference[2].

Material and Methods

Each sample container was photographed and then opened to examine the condition of the preserved food item and the state of the sample (solid meat, ground, freeze dried, etc.). The chicken samples contained chunks of meat and were ground using a meat grinder prior to extraction. In most cases the samples were homogenized with a Brinkmann Polytron® tissue homogenizer during the extraction procedure to avoid sample loss. Except for the chickens, the entire sample was extracted and a aliquot of the extract removed for lipid determination.

Each sample was weighed into a 250 ml Teflon® centrifuge tube (50-60 g/tube) or, if necessary, split into two or more tubes to provide enough room for the addition of solvent and proper homogenization. Samples were extracted with the tissue homogenizer using a mixture

of hexane/methylene chloride/2-isopropanol (5:3:2). The volume of extracting solvent used for each sample depended on the volume of sample and was generally 50 ml. A ratio of 1:1 sample: solvents (v/v) was used for the first extraction. Subsequent extractions used similar extraction volumes; however, the total volume of solvent used did not exceed a total of 250 ml for the three extractions, even if multiple tubes were used. Care was taken to ensure that the sample was completely homogenized which could take as long as 10 minutes depending on the coarseness of the sample. The crude homogenate was then centrifuged for 10 minutes at 2000 rpm to facilitate a clean separation. This resulted in a two or three phase separation depending on the water content of the sample. If water was present in the sample, the top layer consisted of hexane/methylene chloride, the middle layer consisted of 2-isopropanol/water, and the lower layer consisted of the extracted tissue. If the sample was dry, the upper layer contained the entire solvent mixture and the lower layer the extracted tissue. Following centrifugation the upper layer was removed with a pipet and percolated through 20 g of sodium sulfate into a 250 ml volumetric flask. Each sample was extracted three times, the extracts combined, and the volume adjusted to 250 ml. For samples that had low lipid content or consisted of less than 25 g total (whole weight), the final volume was 100 ml. The lipid was determined gravimetrically and a volume equivalent to approximately 5 g of lipid (when possible) was removed for subsequent preparation.

The samples were fortified with the ^{13}C-labeled surrogates and cleaned up using acid/base silica gel, alumina, and PX-21 carbon prior to analysis by high resolution mass spectrometry. The details regarding the clean-up and analytical procedures for the analyses of both the dioxins/furans and the coplanar PCBs are described elsewhere [3,4].

Results and Discussions

The results from the analyses of the samples are presented in Table 1 together with a description of each sample type, the sample origin, and the year the sample was packaged. The concentrations are expressed in parts per trillion (ppt) on a lipid adjusted basis. The amount of lipid analyzed for each sample and the whole weight are also presented. From these values the percent lipid and the concentration of each congener on a whole weight basis can be calculated. The limit of detection (LOD) for the dioxins/furans based on one gram in ppt are as follows: tetras - 0.5, pentas through heptas - 1.5, and octas - 10. The congener specific LOD for any sample can be determined on either a lipid or whole weight basis by dividing by the appropriate sample weight.

An examination of the sample weights, types, and associated lipid contents indicated the variability of the sample types involved in this study and the need for an extraction procedure that allowed for the extraction of the entire lipid content from each sample. This approach enhanced the probability of detecting low-level residues for these historical samples, especially samples with low lipid content where low detection limits are essential in order to produce meaningful data. Initial experiments with various solvents and combinations of solvents were not successful. Hexane, methylene chloride, and benzene alone and in combination were tested. These solvents were evaluated based on their ability to penetrate and disperse the tissue during the homogenization process and on their ability to cleanly separate from the tissue upon centrifugation. In most cases the solvents were not suitable and caused the ground tissue to become sticky and to coalesce into a mass minimizing contact with the solvent.

The solvent mixture used in this study (5:3:2, hexane/methylene chloride/2-isopropanol) completely penetrated the various tissues and allowed the formation of a uniformly dispersed

homogenate that clearly separated from the pelletized tissue when centrifuged. Another advantage of this solvent mixture was that, when the extracting tissues contained significant amounts of water, the 2-isopropanol combined with the intertissue water and formed a separate layer beneath the hexane/methylene chloride. The organic layer could then be removed from the aqueous phase. If the tissue was dry, the isopropanol could be removed from the extract during the solvent exchange step by azeotropic distillation with hexane prior to the subsequent clean-up [5]. This approach has been successfully employed for the analyses of other classes of compounds in tissues with high water content [6].

The effectiveness of this approach was evaluated by comparing the results from the analyses of control chicken tissue extracted using this lipid isolation method to the results obtained from previous analyses of the same tissue using the whole tissue extraction method. The breast and thigh meat from the control chicken were removed from the bone and combined to simulate the composition and consistency of the historical chicken sample. The lipid was extracted and analyzed as described herein and the results compared to those obtained from the analyses of adipose, thigh, and breast from the same chicken extracted using more conventional methods employing methylene chloride. An evaluation of the data demonstrated that the results were comparable to those from the analyses of adipose or thigh which contain relatively high lipid content. When the results were compared to those from the analyses of the breast tissue of relatively low lipid content, the advantage of using the present method was illustrated by the detection and quantitation of several congeners that were not detected in the previous analyses, even though approximately forty grams were analyzed. The analyses of samples fortified with natives and replicate samples showed that the precision and accuracy were better than ± 20 % for the majority of congeners and the results were reproducible with recoveries between 50-70%. These results demonstrate that extremely low detection limits expressed on a whole weight basis can be obtained from a wide variety of processed food items of different composition and lipid content by first extracting the lipid prior to the conventional processing procedures.

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Table 1: EPA Sample Results from Historical Food Samples; Concentration (ppt, lipid adjusted)

Sample Description	WW I Ration Meat + Bread	K Ration Beef + Pork Loaf	C Ration Dried Cream	Can Deviled Ham	Maritime Survival Bacon Bar	Astronaut Freeze Dried Beef	Astronaut Freeze Dried Bacon	Exp Food Raw Chicken	Exp Food Cooked Chicken	C Ration Pork Slices w/ juices	C Ration Beef Steak w/ gravy	Astronaut Thick Ham Slice	MRE Beef Slices w/ sauce	MRE Turkey w/ gravy
Source	Smithsonian	US Army	US Army	Smithsonian	Smithsonian	JSC	JSC	USDA	USDA	Versar	US Army	JSC	US Army	US Army
Year	1908	1945	1957	1968	1968	1971	1971	1977	1977	1979	1980	1982	1983	1983
Weight:														
Whole (g)	174.90	44.60	4.30	19.76	23.19	92.20	20.54	105.00	106.20	49.20	44.29	94.8	84.9	80.4
Lipid (g)	6.27	4.47	1.22	6.11	5.71	1.19	4.93	6.51	6.26	5.57	5.65	4.25	3.38	3.8
Congener														
2,3,7,8-TCDD	-	-	-	0.21	0.59	-	0.17	0.29	0.27	-	-	-	-	-
1,2,3,7,8-PeCDD	-	-	-	-	1.22	-	-	-	-	-	-	-	-	-
1,2,3,4,7,8-HxCDD	-	-	-	1.47	1.12	-	0.90	-	-	-	0.61	0.74	-	-
1,2,3,6,7,8-HxCDD	-	0.56	4.98	6.42	4.93	-	2.40	1.69	1.59	2.34	3.49	2.59	-	-
1,2,3,7,8,9-HxCDD	-	-	-	1.23	2.08	-	-	-	-	-	0.58	-	-	-
1,2,3,4,6,7,8-HpCDD	0.31	1.88	21.26	58.02	69.38	1.55	22.28	5.45	5.71	36.32	10.29	25.49	1.65	0.45
OCDD	1.68	7.67	95.87	363.13	313.89	8.04	135.07	21.75	20.00	409.68	26.58	180.74	9.94	3.45
2,3,7,8-TCDF	0.17	-	-	-	-	-	0.42	2.27	2.42	-	-	-	-	0.18
1,2,3,7,8-PeCDF	-	-	-	-	-	-	-	0.32	-	-	-	-	-	-
2,3,4,7,8-PeCDF	0.26	0.93	-	0.71	0.57	-	0.63	0.72	0.88	-	-	-	-	0.41
1,2,3,4,7,8-HxCDF	-	0.60	-	2.34	2.18	-	2.49	-	-	1.11	0.73	1.68	-	-
1,2,3,6,7,8-HxCDF	-	0.64	-	1.04	0.68	-	0.75	0.33	-	-	0.42	-	-	-
2,3,4,6,7,8-HxCDF	-	0.22	-	0.19	0.20	-	-	-	-	-	-	-	-	-
1,2,3,7,8,9-HxCDF	-	-	-	0.12	-	-	-	-	-	-	-	-	-	-
1,2,3,4,6,7,8-HpCDF	0.38	8.22	-	14.71	9.81	-	7.93	0.45	0.39	8.42	1.27	10.54	-	-
1,2,3,4,7,8,9-HpCDF	-	-	-	0.80	-	-	-	-	-	-	-	-	-	-
OCDF	-	0.48	-	4.39	3.44	0.64	1.44	0.64	1.38	6.23	-	2.75	-	-
PCB 77	2.32	5.45	1368.75	58.89	113.80	81.02	116.19	67.69	72.01	8.31	8.17	0.33	4.86	1.80
PCB 118	156.60	657.77	9313.43	2128.83	1247.78	2148.59	8280.41	5619.85	6845.94	119.72	943.38	205.48	493.87	388.47
PCB 105	73.14	231.64	3191.14	515.33	397.19	1037.71	1884.14	1454.50	1569.60	24.01	148.77	52.07	100.77	100.26
PCB 126	0.39	2.41	15.13	4.57	2.91	19.25	6.57	16.20	15.87	0.11	7.52	0.23	6.82	2.37
PCB 156	3.79	39.53	161.28	468.78	154.52	314.21	410.26	567.10	580.27	19.59	89.84	31.19	54.20	40.50
PCB 157	0.79	8.47	33.38	101.02	34.25	56.97	75.69	109.14	114.30	5.10	20.03	7.21	12.97	9.58
PCB 169	0.15	0.84	-	1.19	0.47	1.25	0.64	1.71	1.50	-	1.12	0.35	1.06	0.56

The concentrations in bold are below the LOQ.
The concentrations underlined exceed the QA limits for the ion ratios.