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Comparison of seven indicator PCBs and three coplanar PCBs in beef, pork, and chicken fat

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Abstract

The risk assessment of polychlorinated biphenyls (PCBs) is difficult since complex congeners were used in many industrial applications for a long period of time and the residue monitoring in foods of animal origin and the environment were not established in comparable systems. The relationships of determined concentrations in indicator PCB congeners (mono- and di-ortho PCBs) and coplanar PCB congeners (non-ortho PCBs) in livestock products are presented in this study. The concentrations of seven indicator PCBs were compared with three coplanar PCBs in beef, pork, and chicken fat. Distributions of concentration for the indicator PCBs in three different animal species were similar except for that of PCB-118 (2,3′,4,4′,5-pentachlorobiphenyl) in pork fat. The congeners with the highest concentration were PCB-138 (2,2′,3,4,4′,5′-hexachlorobiphenyl) in beef and pork fat and PCB-28 (2,4,4′-trichlorobiphenyl) in chicken fat. The bioaccumulation and metabolism of PCBs in animal species represent different congener profiles in livestock products. The percentage of the total concentration of three coplanar PCBs was about 2% of the total concentration of the seven indicator PCBs. Relatively high concentration of mono-ortho and di-ortho PCBs in fat samples of livestock products may be calculated with the concentration of coplanar PCBs that can be usually determined on a sequential procedure with dioxin analysis. Therefore, the relationship of the amounts between seven indicator PCBs and three coplanar PCBs may be useful to derive the composition and level of contaminants in beef, pork, and chicken.

Keywords: Indicator PCB; Coplanar PCB; Dioxin; Furan; Beef; Pork; Chicken

1. Introduction

Polychlorinated biphenyls (PCBs) were ubiquitously found in the environment and even in many kinds of food since these were widely used in various industrial applications (Hutzinger et al., 1974; Schecter and Li, 1997; Schecter et al., 1997; Abad et al., 2002; Lindström et al., 2002). Food contamination such as in the Yusho incident (1968) in Japan, Yu-Cheng incident (1979) in Taiwan, and animal feed incident in Belgium developed wide concern on the ill-effects of PCBs to human health (Covaci et al., 2002). The lipophilicity of PCBs results in their bioaccumulation primarily in fatty compartment of tissues and organs (Heinrich-Hirsch et al., 1997; Thomas et al., 1999).

The industrial application of PCBs started in the early 1930s, a couple of decades after Schmidt and Schultz described the first synthesis of PCBs in 1881 (Cairns and Siegmund, 1981). Since then, over 2 million tons of PCBs were produced as commercial mixtures of 60–90 congeners such as Aroclor in the United States

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and Great Britain, Kaneclor in Japan, Clophen in Germany, Fenclor in Italy, Pyralene in France, and Solvol in USSR. The patterns of congeners (individual chlorinated biphenyls) in environmental samples possibly reflect the type of contaminants. However, the congener compositions of environmental PCB residues do not completely represent the degree of commercial compositions. Due to metabolic breakdown, different species and specific congeners produced different congener patterns (Iseki et al., 2001; Lung et al., 2001; Traag et al., 2001). In addition, commercial PCBs usually contain trace amount of chlorinated naphthalenes and chlorinated dibenzofurans as impurities (Bowes et al., 1975; Albro and Parker, 1979). In the Belgian dioxin crisis in chicken and pork in 1999, due to the complexity of the PCBs, seven indicator (or marker) congeners were used to identify the composition of PCBs. These seven congeners were PCB-28 (2,4,4′-trichlorobiphenyl), PCB-52 (2,2′,5,5′-tetrachlorobiphenyl), PCB-101 (2,2′,4,5,5′-pentachlorobiphenyl), PCB-118 (2,3′,4,4′,5-pentachlorobiphenyl), PCB-138 (2,2′,3,4,4′,5′-hexachlorobiphenyl), PCB-153 (2,2′,4,4′,5,5′-hexachlorobiphenyl), and PCB-180 (2,2′,3,4,4′,5,5′-heptachlorobiphenyl), which were predominantly present in most PCB-mixtures and in environmental samples (Capel et al., 1985; Frignani et al., 2001). Approximately 50 kg of PCBs and 1 g of mostly furans were introduced into the food chain related to the Belgian accident (Bester et al., 2001; Covaci et al., 2002). Seven PCBs were analyzed from the probable contaminated chicken and pork products using relatively simple analytical procedure compared to dioxin analysis. Dioxins were analyzed when the concentration of PCBs exceeded the Belgian tolerance limit of 200 ng/g of fat weight for the sum of the seven indicator PCBs. The existence of indicator PCBs are important for the prediction of lipophilic contaminants although their toxicity is less than that of dioxin-like PCBs (so called coplanar PCBs). Indicator PCBs can vary with the contaminated source of PCBs. The maximum limit of PCBs in various countries was used for different congeners. For example, Belgium and the Netherlands use PCB-28, 52, 101, 118, 138, 153, and 180, Germany uses PCB-28, 52, 101, 138, 153, and 180, Italy uses tri- to octa-chlorinated biphenyls, and Sweden uses PCB-153 only for the maximum levels in foods (European Commission, 2000). The calculations for the concentration also vary with the use of either the sum of detectable congeners or specific congener. Therefore, the results are commonly expressed based on total PCBs calculated regardless of which Aroclor(s) was used as a reference standard. This implies that most results are not directly comparable. Most PCB congeners are present at much higher levels than the polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans (PCDDs/PCDFs) with the exception of the toxic coplanar PCB congeners. Fürst (2001) reported that the mean levels of seven indicator PCBs were about 450 times higher than the levels of three coplanar PCBs in dairy products. The coplanar PCBs are important due to their toxicity and the other PCBs are also noteworthy especially when they are found in higher concentration in food (Hong et al., 1992; Schecter et al., 1997).

The World Health Organization (WHO) reported toxic equivalent factor (TEF) for four non-ortho substituted PCBs and eight mono-ortho substituted PCBs in 1998 (WHO-ECEH, 1999). Three non-ortho coplanar PCBs, namely PCB-77 (3,3′,4,4′-tetrachlorobiphenyl, PCB-126 (3,3′,4,4′,5-pentachlorobiphenyl, and PCB-169 (3,3′,4,4′,5,5′-hexachlorobiphenyl) are isostereomers of the toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Miller, 1983). The structural similarities indicate that the toxicity and bioaccumulation properties are similar to dioxins. Therefore, the analyses of dioxins and coplanar PCBs have been performed in one process (Yang et al., 2002). However, the indicator PCBs have been analyzed separately or together with coplanar PCBs (Ramos et al., 1998; Bester et al., 2001; Covaci et al., 2002). The relationship between indicator and coplanar PCBs may be used to estimate the degree of contamination and toxicity. Multi-residue methods are needed for the simultaneous identification and quantification of these groups of compounds with different order of concentration in foodstuffs. PCBs and dioxins were analyzed together in meat samples through a sequential step of sample treatment in this study. The results of PCBs were only presented here to show the relationship of contamination levels of the seven indicator PCBs and three coplanar PCBs.

2. Materials and methods

2.1. Materials

PCB congeners (Wellington Laboratories, Canada), IUPAC No. 28, 52, 101, 118, 138, 153, and 180 as indicator PCBs and 77, 126, and 169 as coplanar PCBs were used to determine contamination of PCBs in beef, pork, and chicken. HPLC-grade acetonitrile, hexane, dichloromethane, and petroleum ether were obtained from J.T. Baker USA. Carbon-13 standards of dioxins (Cambridge Isotope Laboratories, USA) were used as internal standards. Randomly selected 53 beef, 66 pork, and 30 chicken samples were collected nationwide in the year 2001. The samples included domestic and imported products.

2.2. Sample preparation

Samples were processed using general procedures in EPA Method 1668 with EPA Method 8290 for several

modifications for sequential determination of PCBs and dioxins (Feil et al., 2000). The analytical procedure was developed in a sequential order for dioxins, coplanar PCBs, and indicator PCBs. Proper amount of fat was extracted from homogenized sample by oven drying under 80 °C (Bester et al., 2001; Covaci et al., 2002). The 5 g extracted fat was transferred into a glass bottle and 200 ml of hexane and the internal standards were added. About 100 g of 30% acid silica was added and mixed for 2 h to remove fat. A sodium sulfate column followed and the samples were concentrated to 1–2 ml by Speed Vac™ (Savant Co. USA). The solution was diluted with 3–5 ml of hexane and cleaned through a silica, alumina, and carbon column using Power-Prep™ (FMS Inc., USA) automated cleanup apparatus. The mono-ortho and di-ortho PCBs were collected during the second stage of alumina column with 50% dichloromethane/hexane and the non-ortho coplanar PCBs and dioxins were collected at the next stage of carbon column with toluene. Two of the collected fractions were combined and concentrated to dryness in a rotary evaporator followed by nitrogen blow-down. The extracts were re-dissolved in nonane to a final volume of 100 μl. The external method with calibration curves was used for the calculation of PCBs. The blank and QC samples were run for series of samples. The internal standards used only the carbon-13 of dioxins to avoid chromatographic interference in the tetra- and penta-CDD/CDF and the PCBs (Djien Liem, 1999).

2.3. Analysis by HR-GC/MS

PCBs were identified using a Hewlett Packard 6890 gas chromatograph connected to a Micromass Autospec-Ultima high resolution mass spectrometer (HR-GC/MS) equipped with a CTC A200S autosampler. The mass spectrometer operated on the selected ion recording (SIR) mode. The sample of 2 μl was injected and chromatographic separation was achieved with a DB-5MS fused silica capillary column (50 m × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific, USA). Injector and transfer line temperatures were 280 and 290 °C, respectively. The GC oven temperature was held for 1 min at 120 °C. After which, the temperature was increased to 220 °C at 10 °C/min followed by an increase to a final temperature of 280 °C at the rate of 4 °C/min. The final temperature was held for 30 min. The carrier gas was helium and the column flow rate was 1 ml/min. Peak identifications were made by retention time and mass on two major ions. The external method was used to quantify detected peak of PCBs. Recoveries of the internal standards were 66–115%. The results were calculated using the limit of detection (LOD) value for non-detected compounds or values below the LOD.

3. Results and discussion

The meat samples, which included domestic and imported products, were collected. Ten congeners were analyzed in this study. The seven indicator PCBs were mono-ortho and di-ortho congeners and the three coplanar PCBs were non-ortho congeners. The seven indicator PCBs were identified by the Belgian government during the dioxin crisis in chicken and pork in 1999. The three coplanar PCBs, which have relatively high TEF values, are chemically dioxin-like PCBs. The absolute concentration of PCBs was used for the quantification of the seven indicator PCBs. The three coplanar PCBs were determined both through absolute concentration and toxic equivalency (TEQ) with TEF. The areas of the reconstructed first and second abundant ions were used to quantify PCBs through a mass chromatogram. Congener specific or total PCBs may be used depending on which assessment is necessary, toxicological or chemical. The seven indicator PCBs are more likely to be used in chemical point of view for the amount of existing PCBs in a sample. The three coplanar PCBs are useful in toxicological estimation.

3.1. Patterns of the seven indicator PCBs in beef, pork, and chicken

The levels of the seven indicator PCBs in beef, pork, and chicken fat were similar in distribution in terms of low to high molecular weight. Fig. 1 shows the profiles of the seven indicator PCBs in beef, pork, and chicken. The average concentration of the 53 beef, 66 pork, and 30 chicken samples for the respective congeners decreased from tri-CB to penta-CB. This was later increased to hexa-CB and then decreased again to hepta-CB. Table 1 summarizes the concentrations determined in beef, pork, and chicken fat. The concentrations for the seven indicator PCBs were compared among the three species. The levels of PCB-28, PCB-52, and PCB-101 were higher in

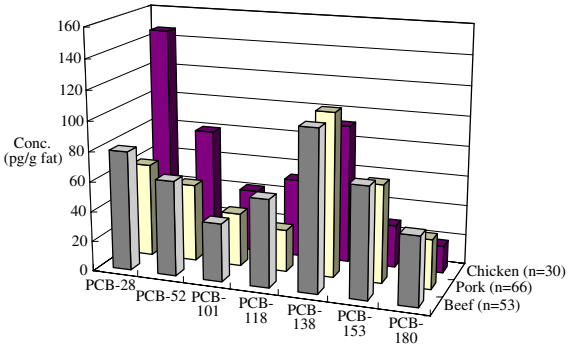

Fig. 1. Residual profiles of the seven indicator PCBs in beef, pork and chicken fat.

Table 1
Concentrations of the seven indicator PCBs in beef, pork and chicken (pg/g fat)

Congener	Beef ($n = 53$)			Pork ($n = 66$)			Chicken ($n = 30$)		
	Mean	Median	Min.–max.	Mean	Median	Min.–max.	Mean	Median	Min.–max.
PCB-28	79.64	50.17	5.78–603.59	62.26	36.19	0–603.81	146.87	64.50	5.61–800.01
PCB-52	63.24	35.08	2.10–522.50	51.53	31.78	1.02–439.83	80.06	28.0	4.13–718.81
PCB-101	38.64	32.48	0–227.14	35.44	24.22	0–150.99	41.77	13.72	2.69–291.32
PCB-118	58.14	39.0	7.30–255.74	27.96	20.12	1.71–144.62	52.31	37.39	3.56–165.0
PCB-138	106.35	71.0	0–1031.18	108.72	67.88	0–704.65	92.17	62.93	3.95–269.19
PCB-153	73.40	32.52	3.37–1458.32	65.08	31.82	0–701.32	28.30	19.71	2.93–100.76
PCB-180	45.67	24.23	1.97–1125.71	32.90	21.87	0–322.03	18.05	14.42	1.00–45.17
Sum of seven PCBs	465.08	284.48		383.89	233.88		459.53	240.67	

chicken than in beef and pork. The levels of PCB-118, PCB-153, and PCB-180 were higher in beef than in pork and chicken. The level of PCB-138 was slightly higher in pork than in beef and chicken. The total concentration of the seven indicator PCBs was in the following order: pork < chicken < beef. Winters et al. (1996a) reported differences in age and feeding patterns may affect to TEQ concentrations between animal classes. The concentrations of higher chlorinated congeners were much lower in chicken than in beef and pork. In chicken, PCB-28 and PCB-138 were found to be 146.87 and 92.17 pg/g of fat, respectively. The highest concentrations were 106.35 and 108.72 pg/g of fat for PCB-138 in beef and pork, respectively. This suggested that PCB-138 has a potentially higher bioaccumulation than the other PCBs in all three species. The bioaccumulated levels of PCBs in three species might be related to the life span of animals before they are slaughtered. Cattle have longer life span than pigs and chicken that allows for both more accumulation and metabolism of PCBs. The longer life span of cattle, compared with pork and chicken, may explain the higher levels of hexa-PCB and hepta-PCB. The shorter life span of chicken had higher levels of tri-PCB, tetra-PCB, and penta-PCB. However there is still a need to consider differences in the level of exposure. Covaci et al. (2002) reported a similar result that the lower chlorinated congeners had higher levels of PCBs in chicken than in pork. The level of PCB-28 was reported to be the highest in all the samples. It is probably because PCB-28 constituted high weight percentage of 8.71 and 6.52 of the total composition of Aroclor 1016 and Aroclor 1242, respectively (Schulz et al., 1989). Although, the distribution of PCBs in the samples does not exactly correspond to those of any commercial or standard mixtures. The contamination of animal feed in Belgium was related to a mixture of Aroclor 1254/1260 (Covaci et al., 2002). The patterns of PCB congeners were different in pork and chicken because of selective metabolism in livestock. It is difficult to determine the contaminated origin based on the congener profile of sample. The congener profiles of Aroclor 1016, 1242, 1248, 1254, and 1260 in literatures are not the same because of differences in the analytical conditions applied such as GC column and oven temperature (Capel et al., 1985; Manchester-Neesvig and Andren, 1989; Schulz et al., 1989). PCBs determined in samples from the environment, animal feeds, food, and human are changed by weathering, absorption, distribution, metabolism, accumulation, etc. Also, PCBs do not always undergo the same distribution and metabolism process.

3.2. Patterns of the three coplanar PCBs in beef, pork, and chicken

Three coplanar PCBs, PCB-77, PCB-126, and PCB-169 were analyzed from 53 beef, 66 pork, and 30 chicken fat samples. The concentrations and distribution of the three coplanar PCBs are shown in Table 2 and Fig. 2, respectively. The total concentrations of the three coplanar PCBs were 9.71, 7.13, and 9.30 pg/g fat for beef, pork, and chicken, respectively. PCB-77 was the highest level in pork and chicken and PCB-126 was the highest level in beef. The total TEQ of the three coplanar PCBs were 0.38, 0.20, and 0.25 pg/g fat for beef, pork, and chicken, respectively. The overall amount of the three coplanar PCBs to the seven indicator PCBs were 2.1%, 1.9%, and 2.0% for beef, pork, and chicken, respectively. This indicated that the relative amounts of dioxin-like non-ortho PCBs were small, however, their TEQ values were not negligible. PCBs were used as mixture of congeners and the combination of congeners depend on their source. WHO reported TEF values for 12 PCBs. Many studies have identified the three coplanar PCBs as the most toxic among those 12 PCBs. However, the existence of mono or di-ortho PCBs in foodstuffs is important because they may reflect the distribution and transformation of PCBs in our environmental variations and food chain metabolism. Di-ortho PCBs showed more neurochemical effects than dioxin-like coplanar PCBs (Seegal et al., 1991). There is limited data to compare the seven indicator PCBs and coplanar PCBs.

Table 2
Existence and TEQ values of three coplanar PCBs in beef, pork and chicken (pg/g fat)

Congener	Beef ($n = 53$)			Pork ($n = 66$)			Chicken ($n = 30$)		
	Mean	Median	Min.–max.	Mean	Median	Min.–max.	Mean	Median	Min.–max.
PCB-77	3.49	3.55	0–6.33	3.28	3.43	0–5.83	5.17	4.00	0–15.82
PCB-126	3.55	3.00	0–8.69	1.74	0[a]	0–6.63	2.35	2.65	0–5.47
PCB-169	2.67	2.15	0–5.92	2.10	2.00	0–6.97	1.78	2.00	0–3.81
Sum of three PCBs	9.71	8.70		7.13	5.43		9.30	8.65	
Total TEQ	0.38	0.32		0.20	0.05		0.25	0.28	

[a] The 34 samples were not detected. The lowest level detected was 2.05 pg/g fat.

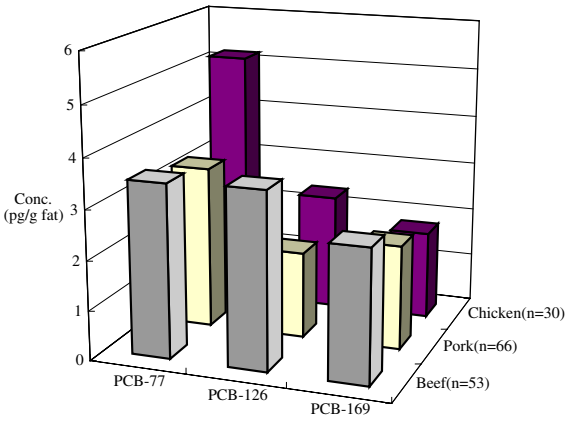

Fig. 2. Residual profiles of the three coplanar PCBs in beef, pork and chicken fat.

However, the concentrations of PCB-77 and PCB-118 in this study were ranged in the same order of magnitude to previous published data (Winters et al., 1996b; Ferrario et al., 1997; Lorber et al., 1998).

4. Conclusion

The concentrations of the seven indicator PCBs and three coplanar PCBs in beef, pork, and chicken fat were compared. The total concentrations of seven indicator PCBs were much higher than the concentration of three coplanar PCBs in all the three livestock products. The concentration of the seven indicator PCBs may be predicted with the concentration of the three coplanar PCBs based on the relationships presented in this study. The three coplanar PCBs are usually determined together with dioxins and furans in one analytical procedure. The concentration patterns of the seven indicator PCBs were different from standard Aroclor mixtures. The indicator may select any major components in the predictable samples. Aroclor 1016, 1242, 1248, 1254, and 1260 showed tri- to hexachlorinated biphenyls as principle components (Alford-Stevens, 1986). The samples used in this study could represent the general livestock products of beef, pork, and chicken that are in the market without any accidental contamination. This showed that the concentrations determined were the background levels in beef, pork, and chicken fat.

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