LEVELS OF NON-ORTHO-SUBSTITUTED POLYCHLORINATED BIPHENYLS, DIBENZO-P-DIOXINS, AND DIBENZOFURANS IN HUMAN SERUM AND ADIPOSE TISSUE

D.G. Patterson, Jr.*, G.D. Todd, W.E. Turner, S.G. Isaacs, and L.L. Needham

Division of Environmental Health Laboratory Sciences, Center for Environmental Health and Injury Control, Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, Atlanta, Georgia 30333

ABSTRACT

We have determined non-ortho-substituted PCB levels as well as PCDD and PCDF levels in adipose tissue and serum collected in Georgia, USA. The U.S. levels are compared with reported levels in samples from Japan and Sweden.

KEYWORDS

Coplanar PCBs; Non-Ortho-PCBs; PCDDs; PCDFs; Adipose Tissue; Serum; Toxic Equivalents

INTRODUCTION

Polychlorinated biphenyls (PCBs) were produced in the United States under the trade name Aroclor. A variety of Aroclor mixtures, which contain varying amounts of the 209 possible PCB congeners, were produced. Because of the widespread use of these Aroclor mixtures, varying amounts of individual PCB congeners have been distributed throughout the environment and have subsequently bioaccumulated in the lipid stores in humans. A large part of Aroclor toxicity in animals has been associated with the non-ortho-chlorine-substituted biphenyls, which are present in the mixtures in very small amounts. It has been reported (1-3) that 3,3'4,4'-tetrachlorobiphenyl (PCB-77, IUPAC number, adopted from reference 4), 3,3',4,4'5-pentachlorobiphenyl (PCB-126), and 3,3',4,4',5,5'-hexachlorobiphenyl (PCB-169) produce the following toxic and biological effects in animals: body weight loss, thymic atrophy, dermal disorder, hepatic damage, teratogenicity, reproductive toxicity, immunotoxicity, high binding affinity to hepatic cytosolic receptor protein (Ah receptor) and high induction potency of 3-methylcholanthrene type (3-MC-type) hepatic microsomal enzymes. A high level of induction of 3-MC-type hepatic drug metabolizing enzymes in rats' hepatoma cell lines (5) has also been attributed to mono-ortho-chlorine-substituted PCBs 2,3,3',4,4'-(PCB-105), 2,3',4,4',5-(PCB-118), and 2,3,3',4,4',5-(PCB-156). Recent studies by Tanabe et al. (6,10), Kannan et al. (11,12), Miyata et al. (13), and Kashimoto et al. (14) have found levels of PCB-77, PCB-126, and PCB-169 up to several orders of magnitude higher than 2,3,7,8-tetrachlorodibenzo-p-dioxin (2378-TCDD) in human adipose tissue samples from Japan. Noren et al. (15) reported high levels of these congeners in pooled mothers' milk from Sweden and Patterson et al. (16) found them in pooled serum from the USA. We examined human adipose tissues and serum to determine the distribution of these PCBs among the various lipid stores of the human body.

EXPERIMENTAL

The laboratory procedures we used to analyze polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and PCBs in adipose tissue and serum have been previously described in detail (16-22) and only a brief description will be given here.

Sample Cleanup and Quantification

We spiked the adipose tissue (17,18,21) or serum (16,19,20,22) samples with carbon-13 labeled PCDDs, PCDFs,

and PCBs and extracted with organic solvents. The solvent extracts were then processed through a five-column cleanup procedure developed by Smith, Stallings and Johnson (23) and modified and semiautomated (18,24) by us for PCDD/PCDF/PCB analyses. The final extracts were quantified by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry for the PCDDs and PCDFs and by high-resolution gas chromatography/isotope-dilution low-resolution mass spectrometry for the PCBs.

Sample Procurement

Pooled Human Serum and Plasma. Serum from more than 240 donors at the Centers for Disease Control (CDC) blood bank in Atlanta, Georgia, was pooled and aliquotted into 100-g samples and stored at -60 °C until analysis. Plasma collected from donors in Canada by Dr. J.J. Ryan (Department of Health and Welfare, Canada) was pooled and aliquotted into 100-g samples for analysis. Pooled human plasma from Sweden, which was analyzed for PCDDs and PCDFs as part of the interlaboratory study sponsored by the World Health Organization-European office, was analyzed for PCBs in 100-g aliquots.

Preparation of Lipoprotein and Protein Serum Fractions.
Preparative ultracentrifugation was performed in two Beckman L-8-80 ultracentrifuges at 4° C by using 60 Ti rotors. Lipoprotein fractions (very low density lipoproteins (VLDL), d=1.006 g/mL; low density lipoproteins (LDL), d=1.006-1.063 g/mL; and high-density lipoprotein (HDL), d=1.063-1.21 g/mL) were separated sequentially by preparative ultracentrifugation (25). Chylomicrons were separated from VLDLs by centrifugation of the fractions with a density ≤ 1.006 g/mL at 10,000 for 20 minutes. Density fractions were recovered by tube slicing and then aspiration. Isopycnic density was adjusted by adding solid KBr to lipoprotein solutions. The mass of nonlipoprotein serum proteins was recovered in the fraction with a density > 1.21 g/mL. We ensured near quantitative recovery by thoroughly washing and scraping tubes into a measured amount of isotonic saline solution. Volumes of lipoprotein-containing fractions were measured from each ultracentrifugal run. Purity of the isolated lipoprotein solutions was established by cellulose acetate chromatography (26) before the solutions were pooled into common fractions. The total volume of the combined lipoprotein fractions and the fractions with a density > 1.21 g/mL after ultracentrifugal preparation was within 2% of the starting volume.

Collection of Adipose Tissues. We collected adipose tissues at autopsy from males and females according to a specific protocol designed to ensure a lack of cross-contamination or laboratory contamination of the samples. We collected adipose tissue from around the heart, kidney, omentum, abdominal wall, buttock, and (in females) breast.

RESULTS AND DISCUSSION

The levels for PCB-77, PCB-126, PCB-169, mono-ortho and di-ortho-PCBs, and 2378-TCDD in pooled human serum and in adipose tissues collected in Atlanta, Georgia, are given in Tables 1 and 2.

Table 1. Measured Levels[a] of 2378-TCDD, Non-Ortho, Mono-Ortho, and Di-Ortho PCBs in Pooled Human Serum.

	Serum Pool 1[b]			Serum Pool 2[c]		
	n[d]	Mean ± SD	CV	n[d]	Mean ± SD	CV
2378-TCDD	101	0.159 ± 0.0241	15.1	84	0.0165 ± 0.0027	16.7
33'44'(77)	3	0.481 ± 0.0762	15.8	2	0.234	--
33'44'5(126)	3	0.183 ± 0.0203	11.1	2	0.186	--
33'44'55'(169)	3	0.151 ± 0.0103	6.8	2	0.140	--
233'44'(105)	4	33.2 ± 1.8	5.4	--	NM	--
23'44'5(118)	4	366 ± 19.2	5.3	--	NM	--
233'44'5(156)	-	NM[e]	-	--	NM	--
22'344'5'(138)	4	583 ± 33.0	5.7	--	NM	--
22'44'55'(153)	4	690 ± 60.4	8.7	--	NM	--
22'344'55'(180)	4	466 ± 24.0	5.2	--	NM	--
Total PCBs	4	3,100 ± 270	8.7	--	NM	--

[a] Levels in ppt on a whole-weight basis. [b] Pooled human serum (total lipid = 475.3 mg/dL) from more than 240 donors. This pool has been spiked to a higher level with 2378-TCDD (see Table 3 for levels on a lipid adjusted basis). [c] This pool is of unspiked normal human serum from more than 200 donors. [d] The number of repeat analyses on the same pool. [e] NM = not measured.

Table 2. Measured Levels[a] of 2378-TCDD and Non-Ortho PCBs in Human Adipose Tissue Collected in 1985.

	ADIPOSE TISSUE[b]			ADIPOSE TISSUE[c]		
	n[d]	Mean ± SD	CV	n[d]	Mean ± SD	CV
2378-TCDD	6	9.0 ± 0.4	4.7	3	8.5 ± 0.3	3.4
33'44'(77)	2	9.6	-	4	ND	--
33'44'5(126)	4	139 ± 16	11.5	4	103 ± 16	15.7
33'44'55'(169)	4	44.5 ± 2.3	5.2	4	154 ± 5.2	3.4
TOTAL PCB-77,126,169[e]	-	193	-	-	257	--

[a] Levels in ppt on a lipid-adjusted basis. [b] Female (white), age 35 years. [c] Male (white), age 69 years. [d] The number of repeat analyses on the same sample. [e] The sum of means.

The only human levels reported in the literature with which we can compare these results are given in Table 3. In general, the levels of the non-ortho-substituted PCBs in the U.S. samples appear to be lower than in the Japanese samples. The levels should be compared with caution, however, because the U.S. and Japanese samples were not collected to represent a certain population group but were obtained from volunteer donors (Table 3). The observation of Tanabe et al. (6) that in the Japanese samples the concentration of the PCBs vary in the order di-ortho > mono-ortho > non-ortho congeners is also true in the pooled serum from the United States (Table 1). In the 53 samples from Japan (6-14), the non-ortho-substituted PCBs were up to several orders of magnitude higher in concentration than 2378-TCDD (Table 3). The non-ortho-substituted PCB levels were only 15 times higher than 2378-TCDD in the U.S. samples.

Table 3. Reported Levels[a] of PCDDs, PCDFs, and PCBs in Human Adipose Tissue, Mothers' Milk, and Serum.

	Tanabe (9) Kannan, (12) Adipose Tissue[b] Wet-Wt.	Miyata (13) Adipose Tissue[c], Wet-Wt.	Noren (15) Pooled Mothers' Milk[d] Lipid Basis	Kashimoto (14) Adipose Tissue[e] Lipid Basis	Patterson[f] Adipose Tissue[g] Lipid Basis	Patterson[f] Pooled Serum[h] Lipid Basis
2378-TCDDS	9.5 (6.4 -18)	1.2 (0.8, 1.7)	-	5.5 (0.8-13.8)	8.8 (8.5, 9.0)	--
Total PCDDs	424 (160-1,400)	970 (440, 1,500	-	1,486 (129-5,036)	--	--
Total PCDFs	65.6 (7-120)	84.5 (69, 100)	-	69.9 (12.7-158.2)	--	--
33'44'(77)	350 (94-860)	39 (21, 57)	30	--	4.8 (ND, 9.6)	104
33'44'5(126)	330 (120-730)	250 (160, 340)	100	--	121 (103, 139)	39.5
33'44'55'(169)	91 (36-200)	132 (74, 190)	50	--	99 (44.5, 154)	32.6
Total PCB-77,126,169	768 (268-1,790)	421 (407, 435)	180	1,529 (180-4,873)	225 (193,257)	176
Total PCBs[i]	1.02 (0.43-1.82)	1.16 (0.93, 1.4)	-	0.775 (0.14-1.51)	--	0.67

[a] Levels in parts per trillion. [b] Tissues collected in 1985 from 12 cancer patients in Matsuyama, Japan. [c] Tissues collected in 1986 from two accidental death victims in Osaka City, Japan. [d] Milk collected in 1988-1989 from Stockholm, Sweden. [e] Tissues collected in 1986-1987 from 39 accidental death victims in Osaka, Nara, and Okinawa prefectures in Japan. [f] Data from the present study. [g] Tissues collected in 1985 from two accidental death victims in Atlanta, Georgia. . (see Table 2). [h] Serum collected in 1988-1989 from Atlanta, Georgia. (see Table 1). [i] Levels in parts per million.

Tanabe (9) and Kannan (12) have calculated 2378-TCDD enzyme induction equivalents (based on AHH and EROD induction potencies in rat hepatoma cell lines) for the 12 adipose tissue samples that they examined from Japan using data from Sawyer and Safe (27). By using the same data, we compared the equivalents for the U.S. and Japanese samples (Table 4). The non-ortho-substituted PCB-126 shows much higher enzyme induction equivalents

than 2378-TCDD (10-25 times higher for the Japanese and 3-4 times higher for the U.S. samples).

Table 4. 2378-TCDD Enzyme Induction Equivalents[a] in Human Adipose Tissue for Non-Ortho-Substituted PCBs and 2378-TCDD.

	US ADIPOSE TISSUE[b]		US ADIPOSE TISSUE[c]		JAPANESE ADIPOSE TISSUE[d]	
	AHH[e]	EROD[e]	AHH	EROD	AHH	EROD
2378-TCDD	0.031	0.031	0.03	0.03	0.03	0.03
33'44'(77)	0.00007	0.00007	-	-	0.0025	0.0025
33'44'5(126)	0.13	0.33	0.095	0.24	0.3	0.76
33'44'55'(169)	0.00014	0.0010	0.00052	0.0034	0.0003	0.002

[a] See Reference (27). AHH = Aryl Hydrocarbon Hydroxylase. EROD = Ethoxyresorufin-o-Deethylase. [b] See footnote (b) in Table 2. [c] See footnote (c) in Table 2. [d] Mean of 12 tissues reported by Kannan et al. (12).

We have also examined non-ortho-substituted PCB and PCDD and PCDF levels in various subfractions of serum (HDL, LDL, VLDL, protein), serum from Canada and Sweden, and adipose tissues from various human anatomical sites. These data cannot be included in this short abstract but will be discussed in the conference presentation.

Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Service.

REFERENCES

1. A. Poland, E. Glover, Mol. Pharmacol., 13, 924-938, 1977.
2. S. Safe, CRC Crit. Rev. Toxicol., 13, 319-395, 1984.
3. B. Leece, M.A. Denomme, R. Towner, et al., J. Toxicol. Environ. Health, 16, 379-388, 1985.
4. K. Ballschmiter, M. Zell, Fresenius J. Anal. Chem., 302, 20-31, 1980.
5. A. Parkinson, S. Safe, L.W. Robertson, et al., J. Bio. Chem., 258, 5967, 1983.
6. S. Tanabe, N. Kannan, M Ono, R. Tatsukawa, Chemosphere, 18, 485-490, 1989.
7. S. Tanabe, Marine Pollution Bulletin, 20, 247-248, 1989.
8. S. Tanabe, N. Kannan, A. Subramanian, et al., Chemosphere, 16, 1965-1970, 1987a.
9. S. Tanabe, N. Kannan, A. Subramanian, et al., Environ. Pollution, 47, 147-163, 1987b.
10. S. Tanabe, N. Kannan, T. Wakimoto, R. Tatsukawa, Intern. J. Environ. Anal. Chem., 29, 199-213, 1987c.
11. N. Kannan, S. Tanabe, M. Ono, R. Tatsukawa, Arch. Environ. Contam. Toxicol., 18, 850-857, 1987.
12. N. Kannan, S. Tanabe, R. Tatsukawa, Arch. Environ. Health, 43, 11-14, 1988.
13. H. Miyata, K. Takayama, J. Ogaki, et al., Chemosphere, 18, 407-416, 1989.
14. T. Kashimoto, K. Takayama, M. Mimura, et al., Chemosphere, 19, 921-926, 1989.
15. K. Noren, A. Lunden, J. Sjovall, Presented at Dioxin-89, Toronto, Canada, September 17-22, 1989.
16. D.G. Patterson, Jr., C.R. Lapeza, Jr., E.R. Barnhart, D.F. Groce, V.W. Burse, Chemosphere, 19, 127-134, 1989b.
17. D.G. Patterson Jr., J.S. Holler, C.R. Lapeza Jr., et al., Anal. Chem., 58, 705-713, 1986.
18. C.R. Lapeza Jr., D.G. Patterson Jr., and J.A. Liddle, Anal. Chem., 58, 713-716, 1986.
19. D.G. Patterson Jr., L. Hampton, C.R. Lapeza Jr., et al., Anal. Chem., 59, 2000-2005, 1987.
20. D.G. Patterson Jr., P. Purst, L.O. Henderson, et al., Chemosphere, 19, 135-142, 1989a.
21. D.G. Patterson Jr., J.S. Holler, W.T. Belser, et al., Chemosphere, 16, 935-936, 1987b.
22. J.R. Akins, K. Waldrep, J.T. Bernert, Jr., Clin. Chem. Acta, 184, 219-226, 1989.
23. L.M. Smith, D.L. Stalling, J.L. Johnson, Anal. Chem., 56, 1830-1842, 1984.
24. W.E. Turner, S.G. Isaacs, D.G. Patterson, Jr., L.L. Needham, Method 7 in "Environmental Carcinogens - Methods of Analysis and Exposure Measurement: Volume 11 - Polychlorinated Dibenzo-p-dioxins, Dibenzofurans, and Biphenyls," C. Rappe and H.R. Buser, Eds., WHO, International Association for Research on Cancer, Lyon, France, 1990.
25. R.H. Havel, H.A. Eder, J.H. Bragdon, J. Clin. Invest., 34, 1334-1353, 1955.
26. Corning Electrophoresis System Manual. Medfield, MA: Corning Medical and Scientific, 1985.
27. T. Sawyer, S. Safe, Toxicol. Lett., 13, 87-94, 1982.