Pergamon

PII: S0045-6535(96)00287-1

Chemosphere, Vol. 33, No. 8, pp. 1485–1494, 1996

0045-6535/96 $15.00+0.00

OCCURRENCE OF PERSISTENT ORGANOCHLORINE CONTAMINANTS IN HUMAN MILK COLLECTED IN SEVERAL REGIONS OF CZECH REPUBLIC

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(Received in Germany 27 April 1996; accepted 15 July 1996)

Abstract

The concentrations of persistent organochlorine contaminants including polychlorinated biphenyls (PCBs), DDT (its isomers and metabolites), hexachlorobenzene (HCB) and isomers of hexachlorocyclohexane (HCH) have been determined in human milk samples from the three regions in the Czech Republic. Results were generated by the congener-specific analyses (two-dimensional high resolution gas chromatography). The results are compared with the similar studies from other industrial countries (Norway, Great Britain, the Netherlands and Germany). Typical patterns of major PCBs contained in analyzed breast milk samples are presented. PCB No.153 was the dominant congener in all the samples. A good correlation was found between the sum of PCBs and PCB No.: 153 (r=0.99), constitutes approx. 30% of calculated PCBs content.

Keywords: PCB congeners, persistent chlorinated pesticides, congener-specific analysis, two-dimensional high resolution gas chromatography, human milk

Introduction

PCBs represent a widely investigated group of environmental pollutants. Hundreds of studies documented their accumulation in food chain caused by their lipophilicity and relative high resistance to degradation [1-3]. Particularly high concentrations of these compounds are found in fat-rich tissues of various biota, and in case of mammals they are excreted into milk during lactation. While the PCBs pattern in species at low trophic level is similar to that of exposure source (represented mostly by contaminated diet)[4], in warm-blooded organisms more extensive biodegradation occurs resulting in a reduction and/or elimination of some chlorobiphenyls (and also many organochlorine pesticides: OCPs). Because of the position of human beings on the top of the food chain, these changes are very pronounced both in samples of adipose tissue and breast milk and they yield a characteristic chromatographic profile consisting of the most persistent PCB congeners and some OCPs.

In former studies, the "total PCBs" were in most cases determined by GC utilising low resolution packed columns for a group separation of present analytes and the ECD (electron capture detector) for detection. The chromatographic patterns of analytes were then compared with the GC profile of the most relevant commercial PCBs mixture, on the basis of which the quantitation was carried out. This approach obviously could result in a significant bias of generated results. Congener-specific analysis which has been implemented over the last decade in connection with advances of high resolution GC columns provided basis for generation of more realistic data. Under these conditions, an improvement of data comparability was achieved and in addition to that, information necessary for the risk assessment based on quantitation of (even minor) PCBs possessing toxic potential is thus possible [5].

209 theoretically existing PCBs may be divided into several subgroups according to various criteria. For the purpose of regulation and to have a means for comparison of data from various laboratories, seven congeners, IUPAC Nos.: 28, 52, 101, 118, 138, 153 and 180 (major components of technical mixtures with medium to high persistency) were selected as "indicators" of contamination extent.

The aim of our study was to determine the levels of major persistent organochlorine contaminants occurring in human milk collected from mothers living in various regions of the Czech Republic. Results generated by congener-specific analysis are compared with relevant data reported in other European countries.

Experimental

Samples collection

Breast milk samples were collected from three regions of Czech Republic. The milk was expressed manually into the bottles and stored at 6°C until analysis. The age of mothers involved in this survey ranged from 18 to 30, all of them were healthy, giving birth to their first normal and healthy child. Questionnaire protocol prepared according to WHO methodology [6] was completed by mothers from Prague region. The questions were focused on the background data on mother such as age, weight, height, smoking and dietary habits, and as regards child, its sex, weight and length.

Analytical standards

PCB standards were obtained from Dr. Ehrenstorfer GmbH (Germany) in concentrations of 10 μg/ml in isooctane. Working standard mixture of the PCBs contained following congeners: (i) indicator PCBs: 28, 52, 101, 118, 138, 153, 180, (ii) other congeners commonly occurring in biotic samples: 31, 44, 66, 70, 74, 110, 128, 149, 163, 170 and (iii) some other mono- and di-ortho PCBs: 105, 114, 156, 167, 158.

Standard solutions of persistent chlorinated pesticides and their metabolites included: HCB, α-HCH, β-HCH, γ-HCH, o,p′-DDE, p,p′-DDE, o,p′-DDD, p,p′-DDD, o,p′-DDT, p,p′-DDT. They were also supplied by Dr. Ehrenstorfer GmbH (Germany), as solids (purity 98.7% and more). Working mixture of chlorinated aromatics in isooctane was prepared from these individual standards.

Methods

Fat collected from centrifuged cooled breast milk sample was mixed with anhydrous sodium sulphate and then the flowing powder was extracted with n-hexane. Gel permeation chromatography on Bio-Beads SX-3 (500 x 0.8cm ID stainless steel column, mobile phase chloroform, flow rate 0.6 $ml.min^{-1}$) was used for removing of the lipids [7].

Determination of PCB congeners and organochlorine pesticides was conducted using a two-dimensional high resolution GC-ECD [8]. The sample was analysed simultaneously on two parallel capillaries with different selectivities. The first one was NB-54, 5%phenyl-1%vinylmethylpolysiloxane phase. The second column was more polar column NB-1701, 7%phenyl-7%cyanopropylmethyl-polysiloxan (see Table 1). GC analyses were performed on a Hewlett Packard 5890 ser. II gas chromatograph equipped with split/splitless injector, electronically programmed pressure and two ^{63}Ni electron capture detectors (ECDs). GC chromatograph was operated under conditions summarised in Table 1. Hewlett Packard Chemstation software (HP 3365) was used for processing of data.

Table 1

GC conditions applied for PCB and OCP determination in human milk samples

columns consisting GC systems:	**NB-54, NB-1701** (supplied by Nordion, Finland)
length × I.D. × film thickness	50m × 0.20mm × 0.1μm (both)
injector temperature (°C)	250
detectors temperature (°C)	300
splitless period (min)	2.5
oven temperature program	60°C-2.5min, 30°C/min to 200°C, 1°C/min to 270°C
carrier gas	nitrogen
linear velocity (cm/s)	15.7
pressure program:	constant flow: 0.5 (ml/min) i.e. 130 kPa at 60°C

Results and Discussion

Serious pollution of food chains by PCBs occurred in the past in the Czech Republic. The main source of these contaminants in local human diet were milk, meat and eggs produced by farms where paints with high content of PCBs for coating of silos, sheds and other agricultural facilities were used (the composition of a commercial technical mixture Delor 106 that was contained in these paints corresponds to Aroclor 1254) [9]. Czech monitoring program established in the middle of 80ties revealed remarkably high levels of PCBs in

human milk. However, the content of contaminants used to be expressed at that time as "total PCBs" [10]. It should be noted that the comparability of generated data was rather poor, reflecting different "quantitation strategies" applied by individual laboratories. Implementation of congener-specific method at the beginning of 90ties made possible to get more information about the PCBs patterns in samples inspected within this project.

Table 2

Comparison of levels of indicator PCBs in human milk from Czech Republic (Prague subset) with the similar studies from Norway, Great Britain and the Netherlands

(PCBs expressed in the ng.g^{-1} in fat)

country [reference]	Norway [11] n=28#		Great Britain [12] n=32#		the Netherlands [13] n=19#		Czech Republic n=17#	
Congener PCB No.:	average (range)	median	average (range)	median	average (range)	median	average (range)	median
28	7.8 (nd-24.2)	22.1	31.5 (nd-188)	17.0	12.1 (0.2-188.6)	5.8	nd*	
52	**		26.2 (nd-154)	6.4	2.6 (nd-32.7)	1.5	nd*	
101	1.1 (nd-4.7)	nd	15.0 (nd-82)	4.9	1.5 (0.2-10.0)	1.1	nd*	
118	26.2 (9.6-56.7)	23.7	28.6 (5-197)	19.4	35.5 (9.7-94.0)	32.7	28.5 (7-59)	25
138	**86.8** (74.6-185.9)	**91.0**	**68.1** (18-261)	**56.4**	**129.9** (43.8-314.3)	**124.2**	**289.0***** (100-558)	**260**
153	**114.4** (49.6-259.4)	**9.9**	**85.9** (27-275)	**71.8**	**186.3** (59.9-475.7)	**174.7**	**379.0** (142-702)	**331**
180	**50.6** (9.7-108.3)	**46.0**	**74.9** (1-210)	**70.1**	**76.9** (2.5-418.8)	**71.3**	**240.2** (91-487)	**226**
Σ ind. PCB	**286.9**		**330.2**		**444.8**		**936.7**	

number of analysed samples

* below the limit of quantification i.e. < 5 ng.g^{-1} in fat

** not quantified due to the interference with the unknown peak

*** PCB 138 was coeluted with PCB 163 (expressed as PCB 138)

In Table 2 there are summarised our results together with similar data reported recently in Norway [11], Great Britain [12] and the Netherlands [13]. As can be seen, the sum of indicator PCBs was higher in local

samples compared to those of foreign origin while the content of lower chlorinated PCBs (congeners No. 28 and 52) as well as pentachlorobiphenyl No.101 were below the limit of determination (i.e. 5 $ng.g^{-1}$ in fat). This fact may be attributed to different contamination pattern of Czech diet and, consequently, different dietary exposure. As it was mentioned above, technical mixtures with prevailing content of hexachloro- and

Table 3

Comparison of levels of indicator PCBs in human milk from Czech Republic (Prague subset) with the data from Germany (Middle-Hesse) (*PCBs expressed in the $ng.g^{-1}$ in fat)*

Country [reference] years	Germany [17] 1984 /85 n=69#		Germany [17] 1990/91 n=68#		Czech Republic 1993/94 n=17#	
Congener PCB No.:	average (range)	median	average (range)	median	average (range)	median
28	0.5 (0.5-40)	5	17 (9-46)	18	nd*	
52	0.5 (0.5-60)	2	13 (6-44)	15	nd*	
101	15 (0.5-19)	21	14 (0.5-55)	17	nd*	
118	**		**		28.5 (7-59)	25
138	**250** (60-660)	**254**	**168** (65-669)	**184**	**289** *** (100-558)	**260**
153	**325** (70-750)	**324**	**240** (108-968)	**264**	**379** (142-702)	**331**
180	**160** (40-400)	**162**	**173** (75-1023)	**194**	**240** (91-487)	**226**
Σ ind. PCB	**751**		**625**		**937**	

number of analysed samples

* below the limit of quantification i.e. < 5 $ng.g^{-1}$ in fat

** not analysed in this study

*** PCB 138 was coeluted with PCB 163 (expressed as PCB 138)

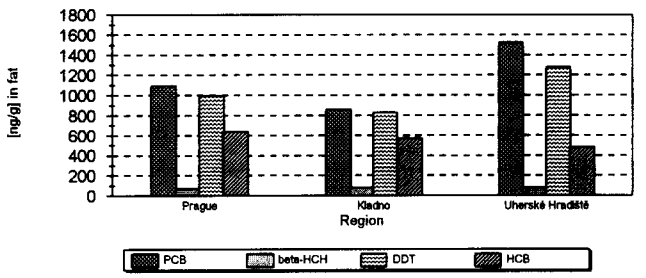
heptachlorobiphenyls were mostly the primary source of environmental pollution in our country. Regarding the composition of market basket of countries listed in Table 2, fish and fish products are consumed here in

Table 4

Content of persistent organochlorine contaminants in human milk - comparison of various regions in Czech Republic

(expressed in ng.g^{-1} in fat)

Region	PCB	β-HCH	DDT	HCB	#
Prague	1096	71	998	639	17
Kladno	860	79	832	570	12
Uherské Hradiště	1529	80	1283	482	7



PCB - sum of congeners Nos.: 28,66,70,74,105,118,138,153,156,170 and 180

β-HCH - hexachlorocyclohexane (beta isomer)

DDT - p,p′-DDT and p,p′-DDE

HCB - hexachlorobenzene

\# - number of analysed samples

higher rate. (The consumption statistics in Norway, United Kingdom, the Netherlands and former Czechoslovakia in the end of 80ties were as follows 41.1, 19.9, 9.2 and 6.8 kilograms of fish and fish products per capita and year, respectively [14].) Relatively high contents of PCBs were often reported in fish from Baltic and Northern Sea [15,16]. Since the biodegradation of PCBs in fish is very limited, higher intake of lower chlorinated PCBs (that are typically accumulated in these biota) via this commodity may be reflected in elevated levels in human milk.

In Table 3, our data are compared with those obtained in Germany - ten and five years ago [17]. The drop of sum of indicator congeners was recorded in the second set of samples, nevertheless the mean content of lower chlorinated PCBs (congeners No. 28 and 101) slightly raised. Unfortunately, in the quoted study there are not suggested reasons for these phenomenon.

Comparison of PCB levels in human milk was carried out in three different regions in the Czech republic: Prague represents industrial urban agglomeration, Kladno is a small city close to Prague and Uherské Hradiště is a locality characterised by high environmental burden of PCBs due to their outbreak from paints-

Table 5.

Contents of individual chlorobiphenyls in human milk samples from various regions in Czech Republic

(PCBs expressed in $ng.g^{-1}$ in fat, limit of quantification = 5 $ng.g^{-1}$ in fat)

	PCB 28	PCB 66	PCB 70	PCB 74	PCB 105	PCB 118	PCB138*	PCB 153	PCB 156	PCB 170	PCB 180
Prague (PCB total = 1096 $ng.g^{-1}$ in fat *) :**											
Average [ng/g]	**< 5.0**	**< 5.0**	**< 5.0**	**22.2**	**< 5.0**	**28.5**	**289.0**	**379.2**	**31.2**	**106.1**	**240.2**
Limit values [ng/g]	-	-	-	8.1-50.9	-	6.8-59.1	100.3-558	142.1-701.9	12.3-69.1	41.3-221.1	91.1-486.8
Contribution [%]**	< 0.5	< 0.5	< 0.5	2.0	< 0.5	2.6	26.4	34.6	2.8	9.7	21.9
Kladno (PCB total = 860 $ng.g^{-1}$ in fat *) :**											
Average [ng/g]	**13.6**	**5.6**	**10.7**	**15.1**	**6.2**	**32.9**	**204.0**	**273.3**	**32.6**	**93.5**	**172.2**
Limit values [ng/g]	6.1-23.5	< 5.0-8.9	6.4-24.7	6.4-27.1	< 5.0-11.3	18.5-46.2	138.1-304.5	174.2-426.1	20.7-60.2	63.2-171.1	115.5-323.9
Contribution [%]**	1.6	0.6	1.2	1.8	0.7	3.8	23.7	31.8	3.8	10.9	20.0
Uherské Hradiště (PCB total = 1529 $ng.g^{-1}$ in fat *) :**											
Average [ng/g]	**11.4**	**9.8**	**11.4**	**15.6**	**7.4**	**17.5**	**361.6**	**475.3**	**65.2**	**193.6**	**359.7**
Limit values [ng/g]	7.6-15.2	< 5.0-19.7	6.6-15.9	11.3-19.7	< 5.0-15.2	< 5.0-47.1	317.5-484.5	414.0-632.1	55.5-87.3	164.2-246.1	304.3-502.1
Contribution [%]**	0.7	0.6	0.7	1.0	0.5	1.1	23.7	31.1	4.3	12.7	23.5

* coelution of PCB No. 138 and 163 (expressed as PCB 138)

** contribution of individual congeners to the total PCB content calculated as sum of analysed congeners

*** sum of congeners Nos.: 28,66,70,74,105,118,138,153,156,170,180 - other congeners (see list of analytical standards in Experimental) were not found or were below the limit of quantification

producing plant and wide use of these paints in local agriculture facilities.

In Table 4 there are clearly illustrated increased levels of PCBs in the later locality with higher extent of pollution. As it was shown in another study [18] compared to typical "background" of Czech Republic, locally produced food crops are more contaminated and thus their consumption results in higher mothers burden. The levels of PCBs in Prague and Kladno were lower, no distinct differences were recorded between these regions. As to the chlorinated pesticides, the higher content of DDT (and its metabolite DDE) in Uherské Hradiště may be attributed to its wide use in this agricultural area in the past. Levels of HCB which may be formed in various combustion processes were slightly higher in industrial areas such as Prague and Kladno. The content of β-HCH was similar in all sets of examined samples.

Table 5 presents typical spectra of major PCBs contained in analysed samples. In accordance with results reported in many other studies [11,12,17,19]; PCB No.153 was the dominant congener in all our samples. A very good correlation was found between the sum of major PCBs and PCB No.153 (for set of 17 samples from Prague r = 0.99, correlation coefficient for other major congeners were r= 0.98 for PCB 180, and 0.93 for PCB 138, respectively). However, it should be noted that the extent of contamination characterised by the content of PCB 153 does not always correspond to the concentrations of planar PCBs that are considered to be mainly responsible for toxic effects in humans [11,13, 20].

Acknowledgements

The authors acknowledge Dr. J. Krečmerová, Dr. Z. Skulová Dr. Z. Zudová and Mgr. T. Zuckermann for the management of human milk samples collection and Dr. E. Vlnová and Dr. E. Vojtíšková for their assistance in samples analysis.

This work was supported by Grant Agency of Czech Republic, project No.: 13/93/0595.

References

1 J. Mes, W. H. Newsome and H. B. S. Conacher, Determination of Some Specific Isomers of Polychlorinated Biphenyl Congeners in Fatty Foods of the Canadian Diet, Food Addit. Contam. 6, 365-375, (1989).

2 J. T. Borlakoglu and R. R. Dils, PCBs in Human Tissues, Chemistry in Britain, September, 815-818,(1991).

3 W. F. Kutz, P. H. Wood and D. P. Bottimore, Organochlorine Pesticides and Polychlorinated Biphenyls in Human Adipose Tissue, Rev. Environ. Contam. Toxicol. 120, 1-83, (1991).

4 J. Sanchez, M. Solé and J. Albaigés, A Comparison of Distributions of PCB Congeners and other Chlorinated Compounds in Fishes from Coastal Areas and Remote Lakes, Intern. J. Environ. Anal. Chem. 50, 269-284, (1993).

5 H. Ahlborg, A. Hansberg and K. Kenn, Risk Assesment of Polychlorinated Biphenyls (PCB), The Nordic Council, Stockholm, Nord Report 26, (1992).

6 WHO/EURO: WHO Protokol for Second Round of Exposure Studies: Levels of PCBs, PCDDs and PCDFs in Human Milk, (1991).

7 J. Poustka, J. Hajšlová and K. Holadová, The Comparison of Elution Profiles of Polychlorinated Biphenyls and Fats in Various Gel Permeation Chromatographic Systems, Intern. J. Environ. Anal. Chem. 57, 83-89 (1994).

8 J. Hajšlová, R. Schoula, K. Holadová and J. Poustka, Analysis of PCBs in Biotic Matrices by Two-Dimensional GC/ECD, Intern. J. Environ. Anal. Chem. 60, 163-173 (1995).

9 J. Hajšlová, K. Holadová, V. Kocourek, J. Poustka, P. Cuhra and V. Raverdino, Determination of PCBs in Fatty Tissues by Means of Several Detection Techniques, Z. Lebensm. Unters. Forsch. 197, 562-569 (1993).

10 Official Methods of Analysis 2, method No.970.52, 15th edn, 1, 280 (1990).

11 H. R. Johansen, G. Becher, A. Polder and U. J. Skaare, Congener-Specific Determination of Polychlorinated Biphenyls and Organochlorine Pesticides in Human Milk from Norwegian Mothers Living in Oslo, J. Toxicol. Environ. Health 42, 157-171 (1994).

12 R. Duarte-Davidson, V. Burnett, K. S. Waterhouse and K. C. Jones, A Congener Specific Method for the Analysis of PCBs in Human Milk, Chemosphere 23, 119-131 (1991).

13 C. Koopman-Esseboom, M. Huisman, N. Weisglas-Kuperus, C. G. van der Paauw, L. G. M. Th. Tuinstra, E. R. Boersma and P. J. J. Sauer, PCB and Dioxin Levels in Plasma and Human Milk of 418 Dutch Women and their Infants, Predictive Value of PCB Congener Levels in Maternal Plasma for Fetal and Infant's Exposure to PCBs and Dioxins, Chemosphere 28, 1721-1732 (1994).

14 FAO, Fishery Statistics, Commodities, Food Balances Sheets, 77, 337 (1993).

15 Von R. Kruse und K. E. Krüger, Kongenere polychlorierte Biphenyle (PCBs) und chlorierte Kohlenwasserstoffe (CKWs) in Fischen, Krusten, Schalen- und Weichtieren und daraus hergestellten Erzeugnissen aus Nordatlantik, Nordsee, Ostsee und deutschen Binnengewässern, Arch. Lebensmittelhyg. 40, 99-104 (1989).

16 J. Falandysz, N. Yamashita, S. Tanabe, R. Tatsukawa, L. Rucińska and K. Skóra, Congener-specific Data on Polychlorinated Biphenyls in Tissues of Common Porpoise from Puck Bay, Baltic Sea, Arch. Environ. Contam. Toxicol. 26, 267-272 (1994).

17 S. Georgii, G. Bachour, J. Elmadfa and H. Brunn, PCB Congeners in Human Milk in Germany from 1984/85 and 1990/91, Bull. Environ. Contam. Toxicol. 54, 541-545 (1995).

18 M. Vávrová, PCBs in agricultural ecosystem, Thesis, Veterinary University, Brno (1994).

19 R. Duarte-Davidson, S. C. Wilson and K. C. Jones, PCBs and other Organochlorines in Human Tissue Samples from the Welsh Population: II-Milk, Environmental Pollution 84, 79-87 (1994).

20 E. Dewailly, J. P. Weber, S. Gingras and C. Laliberté, Coplanar PCBs in Human Milk in the Province of Quebec, Canada: Are they more toxic than Dioxin for Breast Fed Infants? Bull. Environ. Contam. Toxicol. 47, 491-498 (1991).