LEVELS OF PCBs IN HUMAN TISSUES OF THE INHABITANTS OF MADRID (SPAIN)

Fernández Mario Antonio[a], Mateo José Ramón[a], Ramos Juan José[a], Gómara Belén[a], Herrero Laura[a], González María José[a], Abad Esteban[b], Rivera Josep[b], Parera Jordi[b], Ábalos Manuela[b], Aragonés Nuria[c], Pérez Beatriz[c], Martínez Mercedes[d], García José Frutos[d] and BIOMADRID Group

[a] Dept. of Instrumental Analysis and Environmental Chemistry, Institute of General Organic Chemistry, CSIC. Juan de la Cierva, 3. 28006 Madrid (Spain). [b] Laboratory of Mass Spectrometry. Dept. of Ecotecnologies. Chemical and Environmental Research Institute of Barcelona, IIQAB-CSIC. Jordi Girona, 18-26. 08034 Barcelona (Spain). [c] Cancer Epidemiology Unit, National Center for Epidemiology, Carlos III Institute of Health. Sinesio Delgado, 6. 28029 Madrid. [d] Institute of Public Health of Madrid Community. Julián Camarillo, 4. 28037 Madrid (Spain).

Introduction

Polychlorinated biphenyls are a family of toxic and persistent organic pollutants (POPs) that are present in environmental samples at very low concentration levels. Although they were banned in the 70's in many developed countries, they have a long mean life due to their lipophilicity and resistance to degradation in the environment. So, they can be concentrated from it by living organisms, especially in food, that is the main via to humans. Levels in human serum can be correlated to the levels in human milk, and other tissues, reflecting the body burden of these contaminants. Human samples like serum, breast milk, and placenta are nondestructive matrices adequate for monitoring human exposure to PCBs indicating both parent and neonate body burdens. Although much is known about organochlorine contaminants in human tissues like serum and breast milk[1,2] data on the levels of these environmental pollutants in human serum or other tissues from Spanish population are relatively scarce, and there are only some data on populations exposed to some type of POPs [3,4] or studies on population with very low number of samples[5, 6].

The main objective of this study is to report the levels and accumulation profiles of PCBs in serum (maternal, paternal, and umbilical cord), placenta, and breast milk samples in the population living in Madrid (Spain) and compare the results with previous studies in other countries.

Material and methods.

The study design and the sampling collection were conducted by the Public Health authorities of the Community of Madrid and the Institute of Health Carlos III (Madrid, Spain). A total of 374 individual samples (135 maternal serum samples, 132 paternal serums and 107 umbilical cord serums), 79 placentas, and 95 breast milk samples were collected between October 2003 and May 2004, from volunteers living in two areas of the community of Madrid (Spain). Maternal and paternal blood samples from volunteers were obtained when the mothers were admitted to childbirth preparation classes in the public health system, and umbilical cord blood was taken from the umbilical cord vein by syringe just after childbirth. Placentas were obtained after delivery, and breast milk samples were obtained 3 weeks after delivery. The goals and requirements of the study were explained to all participants. Donors were asked to participate and to sign a consent form. Pregnancies were full-term, and no medical problems were detected during pregnancy. All mothers were healthy and primiparas and older than 15 years. The mean age of parents was 30.5 years. None of the donors reported any work related potential for exposure to PCBs. Once at the laboratory, serum samples were frozen at -20 ºC, and breast milk and placenta samples were freeze-dried and stored at room temperature until analysis. The sample collection was approved by the local committee of medical ethics.

Sample Treatment.

Ortho-PCBs. Serum samples were extracted and purified using a semiautomated solid-phase extraction and cleanup method described elsewhere[7]. Briefly, it consisted of a solid-phase extraction (SPE) of 0.5-1 mL of

serum sample, previously treated with 1 mL of formic acid and 50 μL of acetonitrile, using Oasis® HLB cartridges (Waters, Mildford, U.S.A). After loading of the sample into the cartridge and drying of the cartridge, the analytes eluted using 4 mL of toluene were directly passed through a multilayer column filled with 0.06 g of silica gel activated at 140 ºC for 48 h and 0.24 g of silica gel activated and modified with sulfuric acid (44%, w/w) and anhydrous sodium sulfate. This eluent was concentrated under nitrogen up to an appropriate volume for its analysis by GC-μECD.

A variation of the method previously published for food sample analyses[8] was used for breast milk and placenta samples by reducing the solvent and adsorbent amounts. Briefly, this consisted of matrix solid-phase dispersion (MSPD) of 0.5 g of freeze-dried breast milk and 2.0 g of placenta. Further cleanup and lipid removal was performed by using acid and basic impregnated silica gel multilayer columns, and *n*-hexane as elution solvent.

No-ortho PCBs. Pools of serum and milk were used to analyse coplanar PCBs. The preanalytical treatment for dioxin-like-PCBs was carried out using 40 mL of pooled serum samples or 40 g of pooled milk. The samples were added with labelled standards ($^{13}C_{12}$-PCBs) and liquid-liquid extracted twice. After gravimetically determination of lipid content and sulfuric treatment, the purification was automatically carried out in a Fluid Management System (FMS®) thorugh a multilayer column containing silica, alumina and carbon.

Instrumental Determination using GC-μECD and GC-ITD-MS/MS and GC-HRMS.
21 PCBs selected (28, 52, 95, 101, 105, 114, 118, 123, 132, 138, 149, 153, 156, 157, 167, 170, 180, 183, 189, and 194) , from tri- to octachlorinated congeners, were determined using an Agilent (Agilent, Palo Alto, CA) 6890 Series gas chromatograph equiped with a μECD. A J&W Scientific (USA) capillary column DB-5 (60 m length, 0,25 mm i.d., 0,25 μm film thickness). Oven temperatures was programmed from 80ºC (2 min) to 185ºC at a rate of 30ºC/min (3 min), then to 230ºC at a rate of 1.5ºC/min (15 min) and finally to 270ºC at a rate of 5ºC/min (25 min). Flow of nitrogen as carrier gas was 1.8 mL min^{-1}. Samples (1 μL) were injected in splitless mode (1 min) at 270ºC. Detector was set at 300ºC [9].

Confirmation of all the PCB congeners were carried out in a Varian Gas Chromatograph CP-3800 (Varian, CA, USA) coupled to an Ion Trap Mass Spectrometry Detector (Varian Saturn 2000), acquiring in MS/MS mode. Samples were injected in a programmable temperature vaporizing (PTV) in splitless mode (4 μL). A VF-5MS (FactorFour™, Varian, Palo Alto, CA, USA) capillary column (55 m length, 0.25 mm i.d. and 0.25 μm film thickness) was employed. Oven program was as follows: from 100ºC (2 min) to 200ºC (3º min) at a rate of 30ºC/min and then to 230ºC (15 min) at a rate of 3ºC min^{-1} and finally to 270ºC (15 min) at a 5ºC min^{-1} [10].

Coplanar PCBs (77, 81, 126 and 169) were determined by using an Agilent 6890N gas chromatograph coupled to a HRMS Micromass Autospec Ultima NT (Manchester, IK). A Capillary column DB-5 (40 m, 0,18 mm i.d., 0,18 μm) was programmed from 140ºC (1 min) to 200ºC (1 min) at a rate of 20ºC/min and then to 300ºC at a rate of 3ºC/min. 1 μL of sample was injected on spliless mode (1 min, 280ºC) and Helium at 35 cm/s was used as carrier gas. Mass Spcetrometer operated at 37 eV (EI+) in the SIM mode at approximately 10,000 resolving power (10% valley definition). Ion source temperature was 250ºC, electron beam current of 600 μA, and ion acceleration voltage of 8 kV. The electron bean energy was adjusted to maximise the response for the *m/z* 331 ion of PFK. All parameters have been described previously [11].

Both GC-MS determinations were based on the isotopic dilution technique by simultaneous detection at the corresponding retention time of the two most intense ions for each congener, both native and $^{13}C_{12}$-labelled.

Results and discussion

PCB congeners were analysed in maternal (M.S.), paternal (P.S.), umbilical cord serum (U.C.S.), breast milk (B.M.) and placenta (P.) PCBs (as sum of all *ortho*-PCB congeners) were detected in 100% of samples. Figure 1 shows the percentage of contribution of the most abundant congeners found in the different tissues analysed. In serum samples, the highest concentrations were found in congeners 153, 138, 101 and 180; for milk samples, the

most concentrated were congeners 153, 180, 138 and 170. However, in placentas, the most important congeners quantitatively speaking congeners were 52, 101, 153, 180 and 138. On the other hand, congeners 28, 52, 95, 114, 157 and 183 were detected in less than 30% of serum samples; in breast milk, congeners 28, 123, 167, 157 and 189 were detected in less than 50% of samples; and in placentas congeners 95, 114, 123, 156, 157, 167 and 189 were detected in less than 40% of samples. According to tissues, median of PCB concentrations were higher in placenta (7.55 ng g^{-1} f.w.), breast milk (5.69 ng g^{-1} f.w.) and serum samples (4.14 ng g^{-1} f.w.). Taking only in to account serum samples, their levels were very similar, being slightly higher in umbilical cord (4.76 ng g^{-1} f.w.) than in paternal (3.97 ng g^{-1-} f.w.) and maternal serums (3.83 ng g^{-1} f.w.).

Regarding to non-*ortho*-PCB congeners, the highest levels were always found for PCB 126, followed by PCB 169, PCB 77 and PCB 81, as is shown in figure 2.

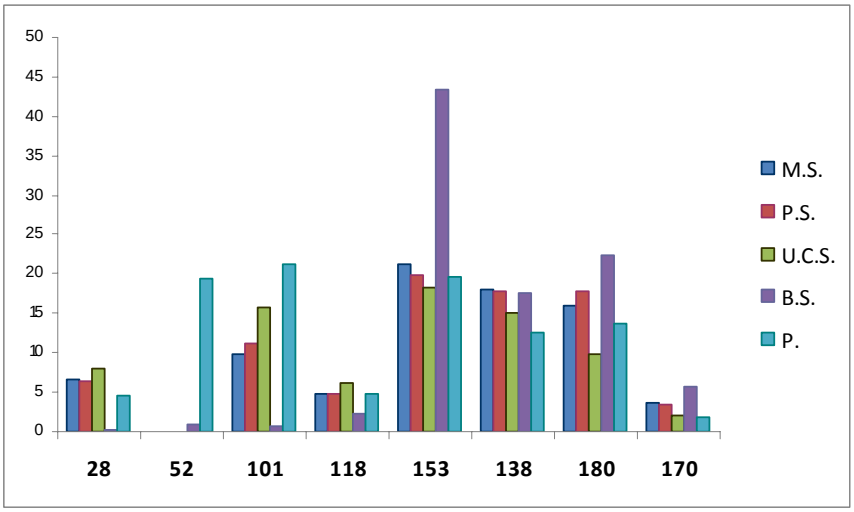

Figure 1. Contribution (%) of the most concentrated congeners of PCBs in the tissues studied.

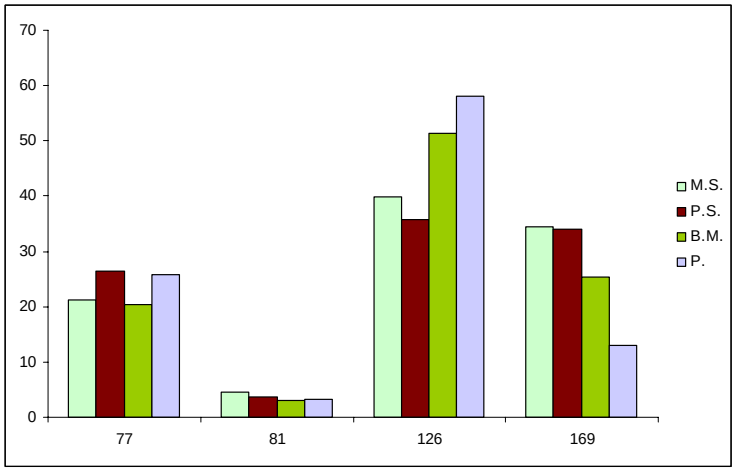

Figure 2. Contribution (%) of the non-*ortho* PCBs in the tissues studied.

Country	Tissue	PCBs ng g^{-1} l.w.	Non-*ortho*-*PCBs* ng g^{-1} l.w	Ref.
Japan	Breast milk	100-140	12.0-19.0	12
China	Breast milk	292.7		13
Poland	Breast milk	153.0		14
India	Breast milk	32.5		15
USA	Brest milk		30.89	16
Italia	Serum	4.11 ng mL^{-1} f.w.		17
China	Serum		12.85	18
China	Placenta	28.5		19

The table 1 shows some results of PCB congeners determined by other researchers in similar tissues in other countries. According to these data, levels of PCBs in Spain are in a similar range that those found by other authors in similar industrial countries. It is not easy to compare data of PCBs in placenta, because they are very scarce.

Acknowledgments

Authors thank to General Direction of Public Health and Institute of Public Health of the Community of Madrid and CSIC by the supporting of this work and FIS Project reference: PI040777.

References

1. Costopolou, D., Vassiliadou I.,Papadopoulos, A..,Makropoulos, V. and Leondiadis, L. *Chemosphere* 2006; 65: 1462.
2. Chen, J.-W., Wang, S.-L., Yu, H.-Y., Liao, P.-C. and Lee C.-C. *Chemosphere* 2006; 65: 1667.
3. Sala M, Sunyer J, Otero R, Santiago-Silva M, Camps C, Grimalt JO. *Occupational and Environmental Medicine* 1999; 56: 152.
4. Zumbado, M. Goethals, M., Álvarez-León, E.E., Luzardo, O.P., Cabrera, F., Serra-Majem, L., Domínguez-Boada, L. *Sci Total Environ* 2005; 339: 49.
5. Jiménez, B. Hernández, L.M., Eljarrat, E., Rivera, J. and González M.J.. *Chemosphere* 1996; 33: 2403.
6. Schuhmacher, M. Domingo, J.L., Llobet, J.M., Lindström, G. and Wingforset, H. *Chemosphere* 1999; 38: 1123.
7. Ramos J.J., Gómara, B., Ramos, L., and González, M.J. *J. Chromatogr A* 2007; 1152: 124.
8. Bordajandi, L.R., Gómez, G., Fernández, M.A., Abad, E., Rivera, J., González, M.J. *Chemosphere* 2003; 53: 163.
9. Gómara, B., Ramos, L. and González, M.J. *J Chromatogr B* 2002; 766: 279.
10. Gómara, B., Fernández, M.A., González, M.J. and Ramos, L. *J Sep Sci* 2006; 29: 123.
11. Abad E., Martínez K., Caixach J. and Rivera J. *Chemosphere* 2006; 63: 570.
12. T. Kunisue, M. Muraoka, M. Ohtake, A. Sudaryanto, N. Hung Minh, D. Ueno, Y. Higaki, M. Ochi, O. Tsydenova, S. Kamikawa, T. Tonegi, Y. Nakamura, H. Shimomura, J. Nagayama, S. Tanabe. *Chemosphere* 2006; 64: 1601.
13. G. Zhao, Y. Xu, G. Han, B. Ling. *Sci. Total Environ.* 2007; 378: 281.
14. K. Jaraczewska, J. Lulek, A. Covaci, S. Voorspoels, A. Kaluba-Skotarczak, K. Drews, and P. Schepens. *Sci. Total Environ.* 2006; 372: 20.
15. A. Subramanian, M.Ohtake, T. Kunisue, S. Tanabe. *Chemosphere* 2007; 68: 928.
16. A. Schecter, I. Kassis and O. Päpke. *Organohalogen Comp*. 1996; 30: 33.
17. Apostoli, P., Magoni, M., Bergonzi, R., Carasi, S., Indelicato, A., Scarcella, C., Donato, F. *Chemosphere* 2005; 61: 413.
18. Jing-Fang Hsu, Yue-Liang Guo, Shu-Yao Yang, Pao-Chi Liao. *Chemosphere* 2005; 61; 1231.
19. Chao, H.R., Wang, S.L., Lin, L.Y., Lee, W.J., Päpke, O. *Food Chem Toxicol.* 2007; 45: 259.