POLYCHLORINATED BIPHENYLS (PCBs) IN HUMAN FOLLICULAR FLUID

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Introduction

In recent years, there has been increasing evidence that polychlorinated biphenyls (PCBs) can alter endocrine homeostatis, this resulting in toxic effects, particularly on the developing organism. In spite of the considerable amount of data obtained from animal studies, the role of these chemicals in determining endocrine-related diseases in humans, and possibly a decrease of fertility, is still controversial. The effort to establish a correlation between exposure to PCBs and such effects is furthermore complicated by the presence, in human tissues, of congeners with different structure-dependent endocrine activity. In fact, some PCB congeners exert dioxin-like activity by interacting with the aryl hydrocarbon receptor, others have been shown to have pleiotropic effects on estrogen and androgen receptor.[1)]

As exposure data concerning the human reproductive system are essential for further risk assessment, we thought it of interest to determine the PCB congener levels in follicular fluid. The follicular endocrine environment is in fact correlated with oocyte quality, with steroid hormones and estrogen receptors, all playing a pivotal role in reproduction. It is then plausible that any interference with the finely-tuned mechanisms of follicle growth and oocyte development may impair reproductive health.

Material and methods

Sampling procedure

Sampling of follicular fluid was performed in the year 2000 at a fertility clinic in Rome. Follicular fluid was aspirated from follicles of 12 randomly selected women (aged 28–32 years) undergoing multiple ovulation and oocyte retrieval for *in vitro* fertilization (IVF) according to a standard protocol. The medical history of the patients showed infertility due to tubal factors, no medications taken in the last seven months, and no other health problems. All subjects gave an informed consent.

Analytical assessment

Two pools were prepared (six follicular fluids each), each consisting of about 30 mL. They were kept frozen at -20 °C until extraction. After drying with anhydrous sodium sulphate, samples were transferred to pre-extracted Soxhlet thimbles. Then, the two pools were spiked with a mixture of twelve ^{13}C-labelled PCBs (1.25 ng each congener), and extracted for 16 hours with a mixture of dichloromethane and *n*-exane (50:50, v/v). Two reagent blanks, comprising the sodium sulphate treatment, were also prepared and extracted similarly to the samples. The sample and blank extracts were carefully reduced to dryness. The extracted lipid contents were then determined. After re-suspension in *n*-exane, extracts were slurried with silica gel impregnated with

concentrated sulphuric acid. The two extracts were then quantitatively transferred and eluted through two columns containing alternate layers of silica gel impregnated with sulphuric acid and potassium hydroxide, separated by neutral silica. After volume reduction, the extracts were chromatographed in Florisil columns, and then quantified. Quantification was performed by HRGC-HRMS with a VG 70 SE mass spectrometer, operating at 5000 resolution in the SIM mode.

Results and discussion

Table 1 shows the individual PCB concentrations found in the two pools, expressed in ng/g both on the wet weight and lipid based. The levels of PCBs 18, 28, 37, 47, 49, 51, 52, 60, and 66, although determined, were not reported due to their very low levels, often below the determination limit and generally interfered by analytical background. The low levels of these congeners can be justified by the faster enzymatic degradation usually observed in animals for the PCBs with low degree of chlorination.[2] However, their relative contribution to the total PCB estimates appears to account only for a very minor percentage.

Table 1. PCB congener concentrations (ng/g) in follicular fluid collected in the year 2000 from subjects of the Rome area.

PCB	SAMPLE 1[a]		SAMPLE 2[a]	
Congener [IUPAC number]	*Wet weight*	*Lipid based*	*Wet weight*	*Lipid based*
2,4,4',5-T_4CB [74]	<0.001	<3	<0.001	<3
3,3',4,4'-T_4CB [77]	—[b]	—[b]	—[b]	—[b]
3,4,4',5-T_4CB [81]	<0.001	<3	<0.001	<3
2,2',4,4',5-P_5CB [99]	≈0.007	23	≈0.008	27
2,2',4,5,5'-P_5CB [101]	<0.01	<3	<0.01	<3
2,3,3',4,4'-P_5CB [105]	<0.001	<3	<0.001	<3
2,3,4,4',5-P_5CB [114]	<0.001	<3	<0.001	<3
2,3',4,4',5-P_5CB [118]	0.023	77	0.021	70
2,3',4,4',5'-P_5CB [123]	<0.003	<10	<0.001	<3
3,3',4,4',5-P_5CB [126]	<0.001	<3	<0.001	<3
2,2',3,4,4',5'-H_6CB [138]	0.10	330	0.098	330
2,2',4,4',5,5'-H_6CB [153]	0.15	500	0.15	500
2,3,3',4,4',5-H_6CB [156]	0.0090	30	0.0077	26
2,3,3',4,4',5'-H_6CB [157]	0.0020	6.7	0.0021	7.0
2,3',4,4',5,5'-H_6CB [167]	0.0074	25	0.0066	22
3,3',4,4',5,5'-H_6CB [169]	<0.001	<3	<0.001	<3
2,2',3,3',4,4',5-H_7CB [170]	0.030	100	0.025	83
2,2',3,4,4',5,5'-H_7CB [180]	0.12	400	0.12	400
2,2',3,4,4',5',6-H_7CB [183]	0.015	50	0.013	43
2,2',3,4',5,5',6-H_7CB [187]	0.019	63	0.015	50
2,3,3',4,4',5,5'-H_7CB [189]	0.027	90	0.022	73
2,2',3,3',4,4',5,5'-O_8CB [194]	0.0068	23	0.0058	19

(*a*) Pool of specimens from six women. (*b*) Interference from background.

The results obtained show the PCB compositions of Samples 1 and 2 to be very similar (Table 1 and Figure 1). Only for PCB congener 77, the remarkable difference observed (results unreported) in the two determinations has been provisionally attributed to background interference, as often observed by other authors in the analysis of human tissues.[3)]

A comparison of our data with the results obtained by other authors[4)] from the assessment of the three most abundant PCB congeners (138, 153, and 180) in follicular fluid shows a good agreement in both congener concentrations and distribution patterns.

Although wet weight PCB levels here are lower than the mean concentrations commonly reported for serum and other human tissues, due to the low lipid content of follicular fluid (approximately 0.03 %), the main congener concentrations and distributions are basically the same of what has been found in other biological matrices, as blood and mother's milk, when expressed on a lipid basis. A comparison of concentrations (Figure 2) of the most abundant congeners in follicular fluid (as per our data), milk,[3)] and blood[5)] show a very good analogy among congener profiles.

Altough a direct influence of endocrine-disrupting pollutants, such as PCBs, on oocyte fertilization in IVF procedures could not be established,[6)] the presence of the observed concentrations in human follicular fluid could raise some concern as to possible negative effects on oocyte biological competence and reproductive health. In order to better characterize the burden of endocrine-disrupting pollutants in the follicular fluid, analyses of PCDDs and PCDFs and some chlorinated pesticides are in progress.

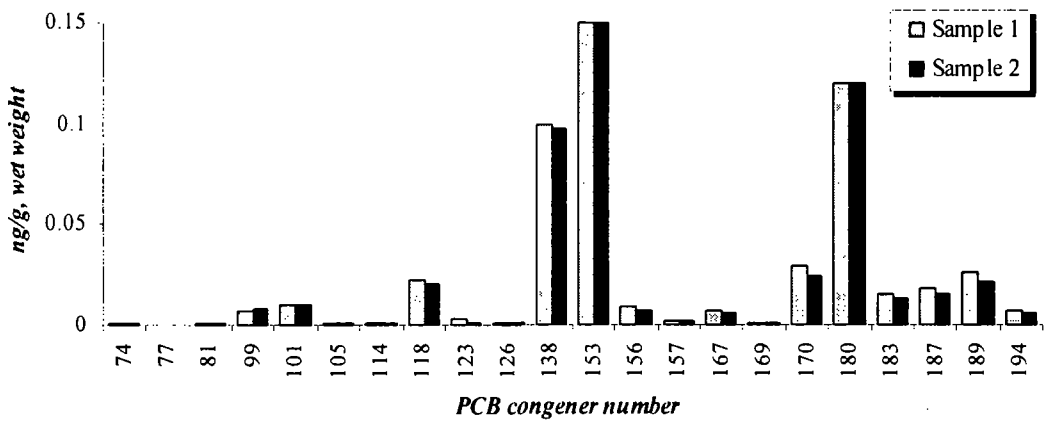

Figure 1. Congener profiles of the two samples of follicular fluid analyzed.

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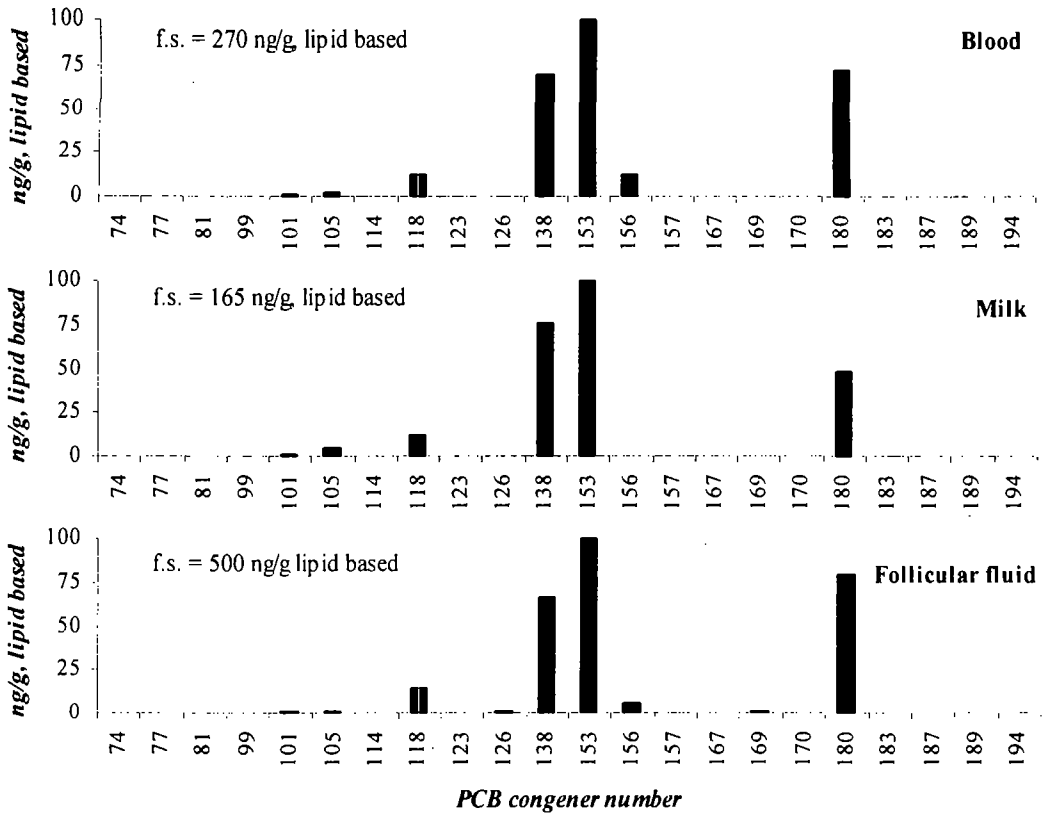

Figure 2. Comparison of congener profiles in blood,[5] human milk,[3] and follicular fluid (our data). For each matrix, congener concentration have been normalised with respect to the most abundant congener (PCB 153). Since data refer to different population groups and different countries, congener profiles are shown with the only purpose of comparison.

References

1) Bonefeld-Jørgensen E. C., Andersen H.R., Rasmussen T.H., Vinggaard A. M. (2001). Toxicology 158, 141-153.
2) Schrenk D.: Polychlorierte Biphnyle (PCB). In *Praktische Ummwltmedizin*, Springer Verlag, Heidelberg, 1994.
3) WHO European Centre for Environment and Health (Environment Health in Europe No. 3, 1996): Levels of PCBs, PCDDs and PCDFs in human milk. Second round of WHO-coordinated exposure study.
4) Pauwels A., Covaci A., Delbeke L., Punjabi U., and Schepens P.J.C. (1999). Chemosphere 39, 2433-2441.
5) Wuthe J., Piechotowski I., Päpke O., Zier B., Gabrio T., Krämer D., Kouros B., Schwenk M., Pfaff G. (1996). Chemosphere 32, 567-574.
6) Ensslen S.C., Riedel H.H., Bluthgen H., Heeschen W. (1990). Zentralb Gynakol 19, 1223-1226.