LEVELS OF PCBs, DDT, DDE AND DDD IN ITALIAN HUMAN BLOOD SAMPLES

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Introduction

The environmental contamination from polychlorinated biphenyls (PCBs) is effecting the exposure of the general population in a direct way through air inhalation, ingestion of particulate matter and dermal absorption and, most of all, in an indirect way through diet. Diet represents, in fact, the main way of human exposure to PCBs[1].

PCBs have potential teratogenic, carcinogenic, hormonal and immunological effects[2]. An association between endometriosis and high levels of PCB in plasma has also been reported[3]. Moreover, some congeners (PCB 105, PCB 118, PCB 153) have effects on thyroid hormones in animal models, although the PCB dose used in these experiments was an order of magnitude higher than the estimated human exposure. Humans are, however, exposed to a complex mixtures of PCB congeners[4].

In this study identification and quantification of 60 PCB congeners and 3 chlorinated pesticides in human whole blood samples are presented. The subjects examined in this pilot study were a small group of patients with possible endocrine-related problems and unknown specific exposure.

The aim of this study was to increase the present understanding about the distribution of the PCBs in human whole blood. The levels of DDT and metabolites were measured as well, since these compounds are consistently reported to contribute to the whole body burden of persistent chlorinated compounds, together with PCBs.

Materials and Methods

Samples of human blood were obtained from nine adult obese patients (seven women and two men) attending the endocrinological day hospital at the "Policlinico Umberto I", University of Rome La Sapienza; informed consent was obtained by each subject.

Blood samples were collected in vacutainer tubes and kept at +4°C until analysis. Each whole blood sample was mixed with a mixture of inert support (Extrelut, Merck) and magnesium sulfate in a 3/1 w/w ratio until a homogeneous matrix was obtained. Then it was spiked with labeled $^{13}C_{12}$ recovery standards (10 labeled PCB and 3 labeled pesticides). The sample matrix was then left air drying in the hood. The 2,5cm column was filled with this sample matrix and eluted with dichloromethane (150ml). The eluate was reduced to small volume, adsorbed in 1g of alumina and transferred into SFE thimbles.

Selective extraction was performed using supercritical fluid CO_2 extractor (SFE Hewlett-Packard 7680T). The extraction conditions were the same reported elsewhere except for the density value (0,7 g/ml) [5].
An analytical blank was associated to each three-samples batch and processed likewise. To limit the loss of the analytes due to evaporation, 1µl of *n*-tetradecane was added, as a keeper, to the extract. This fraction was concentrated and, after addition of a solution containing a syringe standard, directly analyzed by GC/LRMS.

Results and Discussion

The recoveries of the $^{13}C_{12}$ labeled internal standard added to the samples varied from 35% to 86% for the 10 different PCB congeners used as recovery standard. We did not determine the lipid content of the whole blood samples, therefore all concentrations are expressed on a whole blood basis.
Total levels of PCBs varied from 449 pg/g to 1794 pg/g whole blood. In Table 1, minimum and maximum for PCB are shown.
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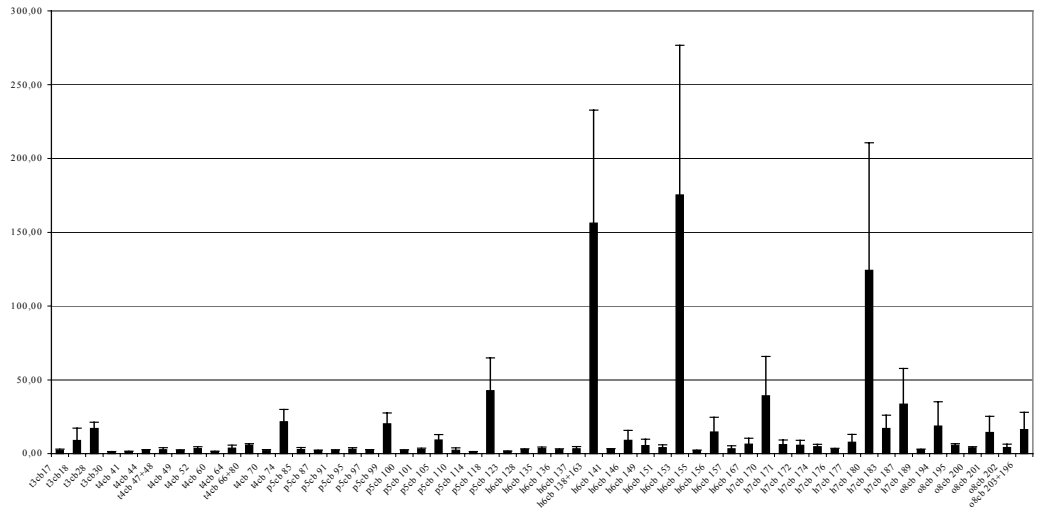
Table 1. Minimum and Maximum PCB levels in nine samples of humans blood (pg/g whole blood). The asterisks indicate the incidence of the blank sample (see text). The mono-ortho PCB congeners are reported in Italic.

PCB	min	max	PCB	min	max
t3cb17	< 1,27***	3,17	**h6cb 136**	< 1,81	< 4,38
t3cb18	< 1,46***	25	**h6cb 137**	< 1,96	7,22
t3cb28	ND	20,04**	**h6cb 138+163**	96,03*	320,03*
t3cb30	< 1,02	< 2,61	**h6cb 141**	< 2,21***	< 5,65
t4cb 41	< 1,25	< 3,44	**h6cb 146**	< 1,93	18,19*
t4cb 44	< 2,17	< 5,10	**h6cb 149**	< 1,48***	12,14**
t4cb 47+48	< 1,51	5,31	**h6cb 151**	1,82**	6,91**
t4cb 49	< 1,61	3,11**	**h6cb 153**	94,54*	407,81
t4cb 52	< 2,17***	4,68**	**h6cb 155**	< 1,42	< 3,48
t4cb 60	< 1,17	< 3,56	***h6cb 156***	*7,88*	*38,28*
t4cb 64	< 2,09	8,38	***h6cb 157***	< *1,76*	*8,1*
t4cb 66+80	3,44**	5,11	***h6cb 167***	*2,58*	*15,85*
t4cb 70	1,28**	2,44**	**h7cb 170**	18,24	101,96
t4cb 74	9,72**	41,3	**h7cb 171**	< 3,56	12,41
p5cb 85	< 1,9	6,17	**h7cb 172**	3,67	13,18
p5cb 87	< 1,66	< 2,53	**h7cb 174**	< 3,1***	< 9,05
p5cb 91	< 1,86	< 4,14	**h7cb 176**	< 2,17	< 6,21
p5cb 95	< 2,05***	< 5,52**	**h7cb 177**	2,74**	15,83**
p5cb 97	< 1,99	< 4,51	**h7cb 180**	59,99*	327,73
p5cb 99	6,66**	34,55	**h7cb 183**	10,73	36,96*
p5cb 100	< 1,82	< 4,08	**h7cb 187**	11,74**	85,41*
p5cb 101	< 1,76***	4,74**	***h7cb 189***	< *2,07*	< *6,33*
p5cb 105	*4,38*	*17,39*	**o8cb 194**	8,89	57,69
p5cb 110	< 1,1***	4,8**	**o8cb 195**	< 4,22	< 12,7
p5cb 114	< *1,02*	< *2,5*	**o8cb 200**	< 2,92	< 8,83
p5cb 118	*13,12***	*92,89*	**o8cb 201**	5,43	39,43
p5cb 123	< *1,3*	< *3,10*	**o8cb 202**	< 2,67	9,32
h6cb 128	< 2,03	< 5,29	**o8cb 203+196**	7,38	43,64
h6cb 135	< 2,33	< 5,61	**Tot PCB**	**449**	**1794**

The values reported in Table 1 need some analytical comments beforehand: some congeners are lower than limit of determination (LOD) in all the samples; in this case the variability is most likely due to analytical factors rather than to physiological ones. Moreover, in many cases the measurement is influenced by the blank level; the incidence of the blank sample on the reported value is flagged with one to three asterisks in the 5-125% range of incidence of the blank signal on the analyte; when the blank signal is higher than 1.25 times the sample, the reported value is

substituted by the ND (not determinable) acronym, according to a procedure described elsewhere[6]. The influence of blank is unavoidable when determining low levels of ubiquitarian pollutants. In reporting the data in Table 1, we have choosen to report in the minimum column the ND value when the data set relative to that congener included both determined values and ND values and to report the LOD value when the data set included the three possibilities (ND, LOD, determined values).
The TEQ associated to the 8 mono-ortho congeners (reported in Italic in Table 1) determined varied from 0,0035 to 0,0054 pgTE/g whole blood, computed using the WHO-TEF[10].
The PCB mean contamination profile is shown in Figure 1 together with the standard deviation. The most abundant congeners in all samples were PCB 138, PCB 153 and 180, in agreement with other studies on general population and people exposed through food, such as fish-eaters of Lake Michigan[7,4,8,9]. These congeners (138, 153, 180) accounted for more than 50% of the ΣPCB concentration in whole blood. In particular, the PCB 153 was predominant; this congener is associated with lipoproteins in the blood, according to Norén[11].
The concentrations of PCB in these samples were most often lower than those reported by studies performed in other countries.
Moreover in our previous study on Italian obese patients[12], the PCB levels in human blood were in the same range reported in the present paper.

Figure 1. Mean congener concentration (pg/g whole blood) in nine blood samples with variation bar representing one standard deviation.



Many years after DDT ban, the level of its metabolite DDE is still generally higher than the sum of all PCBs; this fact does not imply that there is human exposure directly to DDT, as DDE is present in food at higher concentrations than DDT; this is due to the higher persistence of DDE with respect to DDT. In Table 2 the levels of 3 chlorinated pesticides (DDE, DDD, DDT)are reported. Our data indicate that biomonitoring studies should ideally evaluate all major persistent chlorinated

pollutants. In fact, these compounds are consistently present in the same subjects; moreover, the available evidence[13] cannot exclude a potential for additive effects from the overall body burden of organohalogens.

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Table 2. Minimum and Maximum chlorinated pesticide levels in nine samples of humans blood (ng/g whole blood)

	min	**max**
DDE	0,38586	7,52738
DDD	<0,00237	0,01287
DDT	0,05465	0,1311

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