LEVELS OF PCDDs AND PCDFs IN SERUM SAMPLES OF NON EXPOSED INDIVIDUALS LIVING IN MADRID (SPAIN).

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Introduction and objectives.

In order to evaluate the extend of human exposure to PCDDs and PCDFs, measurements of these xenobiotics in human tissues need to be conducted.
The extend of such exposure to toxic chemicals as PCDDs, PCDFs and PCBs in previous years was mostly studied using adipose tissue. This measurements have been demostrated to be very effective by several authors[1,2,3]. In recent years, the development of the analytical techniques has made possible determinations in blood, so that the method of preference are measurements in blood[4,5].
After having examined PCDD and PCDF levels in adipose tissue from individuals living in Madrid (Spain)[6] this study was designed to measure the blood serum levels of PCDDs and PCDFs from individuals living in Madrid (Spain) as an estimate of exposure to these xenobiotics in this population.

Material and methods.

Samples.
Blood samples were collected in Madrid in 1993 by the Red Cross from 11 donors with no known occupational exposure to dioxin and related compounds. Serum was separated from the cell blood fraction by the current method used in the Hospital and kept frozen until analysed. Age of donors was between 19 and 55 years.
Experimental.
Amounts of about 200 grams of serum were taken for analysis. A standard solution consisting of 16 ^{13}C-labelled PCDDs and PCDFs 2,3,7,8-substituted and 3 co-planar PCBs (#77, 126 and 169) was added to each sample and allowed to equilibrate for a period of 30 minutes.
The extraction and lipid removal was carried out using a liquid-liquid extraction procedure described in detail by Patterson et al.[4]. The sample extract was evaporated to a small amount and then loaded onto the first chromatography column for part I of a two-part sample clean-up procedure. In part I of the clean-up procedure the sample was applied to a series of adsorbents contained in two tandem columns. The first column contained small amounts of 40% cesium silicate and 40% sulfuric acid impregnated silica gel. The efluent from this column flows directly onto the second one which contained activated Florisil®. In part II of the procedure, after a change of solvent to hexane, the sample was applied to a third column containing 0.25 grams of 18% Carbopack C dispersed on Celite 545®. Planar analytes like PCDDs, PCDFs and co-planar PCBs were eluted from this column with toluene as a single fraction. The sample extract, once dried was reconstituted to 10 μl with n-nonane containing ^{13}C labelled 1234-TCDD and 123678-HxCDD as an external extandard just before analysis by HRGC-HRMS. A blank sample was runned every 3 serum samples in order to check any contamination throught the analytical run.

Analytical determination.
Resolution and quantification of PCDDs, PCDFs an co-planar PCBs were performed by HRGC-HRMS using an VG AutoSpec Ultima (VG Analytical, Manchester, U.K.) coupled to a Hewlet Packard 5890 Series II Gas Chromatograph. A fused silica capillary DB-5 column (60m, 0.25 mm. i.d., 0.25 μm film thickness, J&W Scientific, U.S.A.) was used using Helium as carrier gas at a column head pressure of 150 KPa. A minimum resolution of 10.000 was used when operating with the HRMS instrument.

Results and discussion.
Table 1 presents PCDD and PCDF results for all the samples studied corresponding to medium levels (arithmetic means) of each 2,3,7,8-substituted isomer analysed. Total levels found for PCDDs were 515.29 ppt. OCDD which is usually the congener found in the largest amount in human tissues for most countries[7], provides almost 68% of the total PCDD/F but only 2.5% of the toxicity using current toxicity estimates. From Table 1 it can be observed that some congeners, for example the OCDD, exhibited high S.D. values. This reflects a high variability in levels between the different samples studied.

Table 1. Leveles of PCDD and PCDF 2,3,7,8-substituted (ppt, pg/g fat weight) found in the serum samples analysed. (S.D. = Standard deviation; + = Number of positives, n=11).

Isomers	Average	S.D.	Range	+
2,3,7,8-T_4CDD	1.52	1.19	0.61-3.9	9
2,3,7,8-T_4CDF	4.66	3.84	0.76-11.51	9
1,2,3,7,8-P_5CDF	1.44	1.01	0.54-3.43	9
2,3,4,7,8-P_5CDF	6.98	2.12	2.45-9.59	10
1,2,3,7,8-P_5CDD	4.09	0.91	2.25-5.71	9
1,2,3,4,7,8-H_6CDF	5.80	1.11	4.84-8.23	10
1,2,3,6,7,8-H_6CDF	5.06	0.82	3.90-6.47	10
2,3,4,7,8,9,-H_6CDF	2.60	1.24	1.18-4.95	9
1,2,3,7,8,9-H_6CDF	1.83	1.68	0.14-4.85	5
1,2,3,4,7,8-H_6CDD	2.75	1.18	1.55-5.10	11
1,2,3,6,7,8-H_6CDD	32.63	12.42	18.83-57.21	10
1,2,3,7,8,9-H_6CDD	5.81	2.67	2.07-11.44	9
1,2,3,4,6,7,8-H_7CDF	12.79	3.25	7.46-17.97	11
1,2,3,4,7,8,9-H_7CDF	5.00	4.24	0.88-12.97	9
1,2,3,4,6,7,8-H_7CDD	71.46	34.73	32.53-137.16	11
OCDF	20.57	11.08	0.88-38.62	9
OCDD	397.03	173.62	117.05-690.06	11

Total levels for PCDFs were found to be 66.73 ppt, being the OCDF the major contributor to total levels. It is important to point out the high leveles exhibited by the 2,3,4,7,8-PnCDF.
It is also important to note that some of the congeners were present in all the samples studied. This is the case for OCDD, 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8-HpCDF and 123478-HxCDD. The congener found less abundant was the 1,2,3,7,8,9-HxCDF, which is consistent with observations reported by other authors[8]. Of the dibenzofurans, 1,2,3,4,7,8-HxCDF and OCDF were elevated. This is consistent with the finding reported by Schecter[9] that 1,2,3,4,7,8-HxCDF and OCDF are the PCDF congeners present in the highest amounts in the Na-PCP. This could be an explanation about the possible source of contamination in the samples studied.

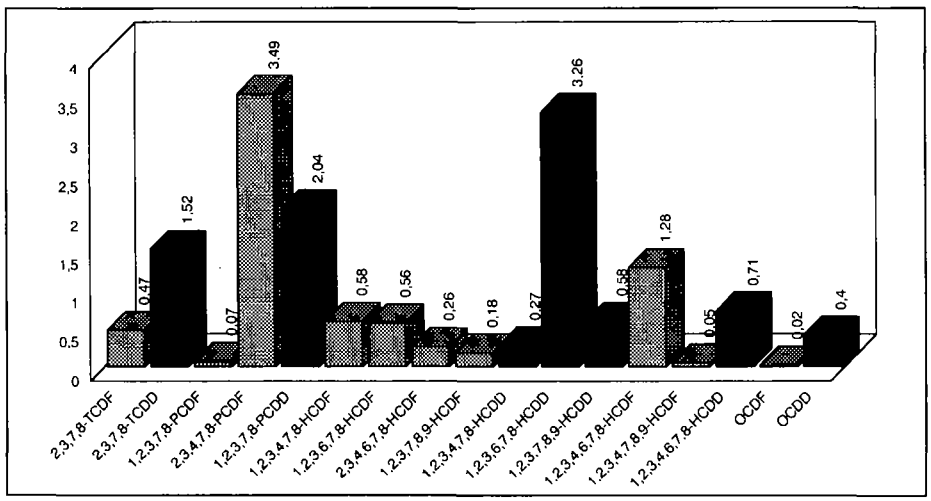

Figure 1. I-TEq values for PCDDs and PCDFs.

In order to better visualize the contribution of each isomer to the total I-TEQ, calculated I-TEQ[10] values are represented in Figure 1. Total I-TEQ were 8.78 ppt for PCDDs and slightly lower for PCDFs, with a value of 6.96 ppt on a lipid weight basis. It can be seen that the contribution from congeners with the highest levels does not corresponds with the highest I-TEQ values. It is remarcable the high values exhibited by 2,3,4,7,8-PnCDF with 3.49 ppt, 1,2,3,6,7,8-HxCDD with 3.26 ppt and 1,2,3,7,8-PnCDD with 2.04 ppt.
A larger portion of the toxicity is contributed by the 5- and 6- chlorinated dioxin and dibenzofuran congeners. The toxic 2,3,4,7,8-PnCDF makes a substantial contribution to dioxin toxicity here, providing almost 22% of the total dioxin toxic equivalents. Although total dioxins contribute 88.5% and dibenzofurans 11.5% to total dioxin levels, they each contribute almost 50% of the dioxin toxic equivalents, as reported by Schecter in the specimens from Ho Chi Minh City[7].
The congener 2,3,7,8-TCDD exhibited I-TEQ levels quite similar to the 1,2,3,4,6,7,8-HpCDF with values of 1.52 and 1.28 ppt respectively. I-TEQ values for the remaining isomers where below 1 ppt. The congeners with the lowest I-TEQ values were the 1,2,3,4,7,8-HpCDF and OCDF.
The larger contribution of the PCDDs relative to PCDFs can be noted in both PCDD/F levels and I-TEQ values.

Data found in this study for total PCDDs (515.29 ppt) and total PCDFs (66.73 ppt) are in good agreement with those reported by Papke[11] for blood samples taken in 1992 with values of 581.7 ppt for total PCDDs and 66.3 ppt for total PCDFs. When comparing total PCDD/F I-TEQ values, data in this study are slightly lower than those reported by Papke which corresponded to I-TEQ values of 26.00 ppt.

These data could allow us to find some specific source of exposure regarding the PCDD/F pattern. It should be pointed that in this study it was firstly assumed that the background contamination of the specimens studied results mainly from food consumption. However, agents as a high traffic, cigarrette smoke and other sources of PCDDs and PCDFs must not be eliminated when understanding the origin of the contamination of the studied individuals.

The value found for the ratio of 123478-HxCDD/123789-HxCDD/123678-HxCDD was 0.1/0.52/1 which is in good agreement with the typical ratio of PCP and NaPCP sources which values use to be 0.1/0.1-0.3/1 as reported by Miles et al.[12]. This finding contributes to explain the PCP and NaPCP as a possible source of contamination in the samples studied. The ratio between 123678-HxCDD/12378-PnCDD/123678-HxCDD was also investigated and the values found were 30.58/3.53/1. This value do not fit with any of the typical ratios corresponding to combustion sources as reported by some authors[13,14]. Hovewer incineration exposure should also be considered as a possible contributor to the source of contamination.

Linear regresion and correlation analyses were performed to determine the degree of linear association (r) between the total PCDD/F I-TEQ versus age. The intercept (a), slope (b) and correlation coefficient for the linear regression for I-TEQ values versus age shown in Table 2.

Table 2. Linear regression for Data.

Y = A + B * X		
Parameter	Value	S.D.
A	6.73	2.09
B	0.20	0.05
R = 0.79 S.D. = 2.14 N=11 P=0.003		

Data were analysed by multiple linear regresion techniques (Statgraphics) with the total I-TEQ concentration as the dependent variable and age as independent variable. In Figure 2 the total PCDD/PCDF I-TEQ values found at each individual are plotted againts age. The correlation coefficient (r) found was 0.79 for a $p<0.01$. This observation is similar to those reported by Pleβ et al.[15]

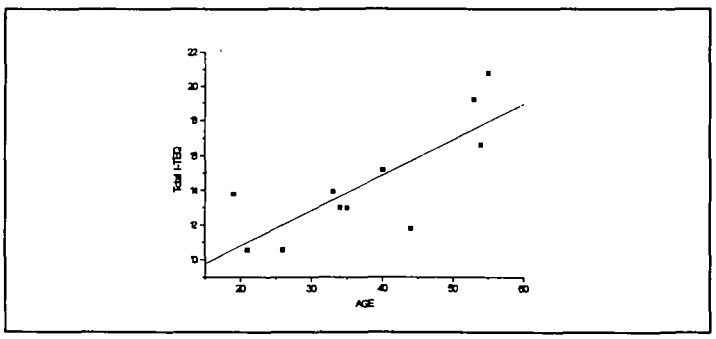

Figure 2. Association between Total I-TEQ concentration and age.

In this study data corresponding to body-mass-index were not available, so that we consider that this data and more sample analysis would be needed if we want to stablish an equation to predict new values as those authors did. But at least in this case it is quite clear that a correlation exits between age and total I-TEQ values.

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