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Levels of PCDDs and PCDFs in Human Milk from Populations in Madrid and Paris

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Human breast milk represents a good substrate to examine the polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) background contamination of a population and also supplies important information about the exposure risk for nursing infants. Although adipose tissue and blood samples are commonly studied in the literature (Gonzalez et al., 1993; Huteau et al., 1990, Luotamo et al., 1993) milk Dioxin levels are more specifically of concern because of the facility of sampling and also the potential health damage which may be caused to the breastfed babies by those highly toxic synthetic chemicals.

The occurrence of dioxins in mother milk among the general population in all countries studied until now (Schecter et el., 1989; Startin et al., 1989; Frommberger et al., 1990; Schecter et al., 1990 a; Fürst et al., 1992; Dewailly et al., 1991) has confirmed a widespread pollution and intake of those compounds. Dietary and other sources such as contaminated chemicals, industrial activity, waste combustion and leaded car gasoline may be responsible for such a contamination, and also for the differences among levels found in different countries. Human contamination by PCDDs and PCDFs from the environment is clearly a multisource problem. Human milk as well as other human fluids studies up to know have only found the presence of 2,3,7,8 substituted PCDD or PCDF congeners present. The reason for this congener specific retention is usually explained by the metabolic conversion on the 2,3,7,and 8 lateral positions in combination with high binding affinities for the cytosolic receptor proteins.

Results presented in this study are limited to samples collected from populations in Paris (France) and Madrid (Spain). Both cities have a very high number of inhabitants, heavy traffic and nearby polluting industrial activities. The sets of mother milk samples from Madrid and Paris are of special interest because data are also available on PCDD/F human adipose tissues collected in the same areas from people exposed to a similar contamination. It is thus possible to compare dioxin and furan profiles in both kinds of samples. Analogies have been observed which tend to show a specificity in the dioxin distribution in human studied samples.

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The aims of this work are: **1).** To calculate the levels of the 2,3,7,8 substituted PCDD/F congeners in human milk from the Madrid and Paris population. 2) To compare our results with those published on data obtained in various countries with different levels of industrial development. 3) To relate dioxin and furan patterns in breast milk and adipose tissue samples from both countries, and 4) to estimate whether the intake of PCDD/F through mother lactation represents a potential hazard to nursing infants.

MATERIALS AND METHODS

Samples were collected in 1990 from 15 and 13 volunteering mothers from Paris (France) and Madrid (Spain) respectively after inquiring about their residence, number of pregnancies and present medication. The mother ages ranged between 25 and 40 years. All samples were collected in sterilised glass bottles and preserved at - 18°C before treatment and analysis.

Amounts between 50 to 75 g of samples were spiked with a nonane mixture containing 100 pg of each the seventeen $^{13}C_{12}$2,3,7,8-substituted PCDD/F congeners surrogate standards (Cambridge Isotope Laboratories), mixed to methanol and then submmitted to chloroform-hexane extraction. The chloroformic phase was separated and the methanol-water phase was reextracted twice with chloroform, and the two organic portions were combined. The organic phase was them evaporated using a rotavapor and allowed to come at constant weight under a gentle stream of pure nitrogen. Fat content was determined by weighting. Fat was then dissolved in hexane and removed with concentrated sulfuric acid. The hexane phase was rinsed, dried and concentrated before clean up on an activated alumina column as previously described (Jiménez et al., 1990).

HRGC/HRMS analyses were performed at 10,000 mass resolution on a VG 70-250SQ GC/MS system equipped with an Electron Impact only source. Acceleration voltage was 30 eV. The column used was a 60 m long, 0.25 mm i.d., 0.25 mm film thickness DB5 column (J&W). The column temperature was programmed from 90°C (hold 1 min) to 190°C at 25°C/min rate, then to 250°C (hold 7 min) at 3°C/min rate, then to 300°C at 4°C/min rate. Final temperature was maintained for 13 min. Helium was used as carrier gas. The 1,5 µl sample was splitless injected. Toxic isomers retention times, chromatographic windows, chromatographic resolution and mass spectrometry sensitivity were daily checked. The absolute detection limit was 10^{-14} g for 2,3,7,8 -TCDD. Quality control criteria were defined, among which isotopic retention times, ratios and extraction purification recoveries. The two major ions of the molecular ion cluster were monitored for each compound. Procedure blanks were carried for each set of 5 samples analysed. Milk samples were individually analysed. The detection limit was between 0.4 to 1 pg/g for tetra- to octa- dioxins and furans isomers.

Results are expressed in ppt (pg/g fat weight basis) for each individual 2378-substituted isomer and for the sum of all dioxin and furan congeners, and

converted in 2,3,7,8- TCDD Toxic equivalents (TEQs) using the NATO International toxic eqivalent factors (I-TEF) criteria (NATO, 1988).

RESULTS AND DISCUSSION

Mean levels of total PCDDs and PCDFs were 380,9 and 103,2 pg/g respectively in women breast milk from Paris, and 341,5 and 57,9 pg/g in the women breast milk from Madrid population. As shown in table I, these differences are mainly due to P_5CDF, H_7CDD and O_8CDD. The tetra, penta and heptafuran congeners were also more elevated in samples from Paris population. The differences found in the levels and congener distributions of people from both cities could be due to differences in local contamination and also in food consumption (more than 90 % of the PCDD/Fs daily intake is usually considered to derive from food (Ryan et al., 1985). Paris has important municipal waste incinerators (MWI) in its surrounding areas, while Madrid does not have any of them .On the other hand , Spanish and French diets are differents although both of them are included in the mediterranean diet. Present levels were lower than those found in our previous study carried on human adipose tissue sampled in the same areas (Huteau et al., 1990; González et al., 1992). We found then an average of 871,8 and 258,7 pg/g (fat weight basis) for total PCDDs and PCDFs in adipose tissues from people living in Paris, and 1608,3 and 203,4 pg/g (fat weight basis) , in adipose tissues from the population of Madrid.

The congeners which made the major contribution to total PCDD and PCDF levels in mother breast milk from Madrid were 123789-H_6CDF, 123678-H_6CDD, 1234678-H_7CDD, O_8CDF and O_8CDD, and the minor components were 12378-P_5CDF, 23478-P_5CDF, 123478-H_6CDD and 1234789-H_7CDF. The congeners most frequently found were 234678-H_6CDF, 123678-H_6CDD, 1234678-H_7CDD and O_8CDD. The major components in mother breast milk from Paris polpulation were 23478-P_5CDF, 1234678-H_7CDF O_8CDF and O_8CDD, and the minor component was 12378-P_5CDF. All isomers, except T_4CDD and T_4CDF were found in all investigated samples.

The PCDD/Fs levels found in breast milk from both populations are similar to those observed in other industrialized countries, in a WHO/EURO in a world-wide field study on contamination of breast milk with PCDD/Fs (Tarkowski and Yrjänheikki , 1989). The most remarkable differences in the WHO/EURO study were on the 23478-P_5CDF levels, as was found between French and Spanish breast milk. The Spanish breast milk contains significantly lower levels of 23478-P_5CDF than other samples from Western Europe ,including French milk samples The reason for this discrepancy is still unknown, but the fact that unleaded gasoline and municipal waste incinerator are much more common in Western Europe than in Spain would explain these differences.

The pattern of different group of congeners in both adipose tissues and mother milk of the two populations are represented in figure 1, and we have found great

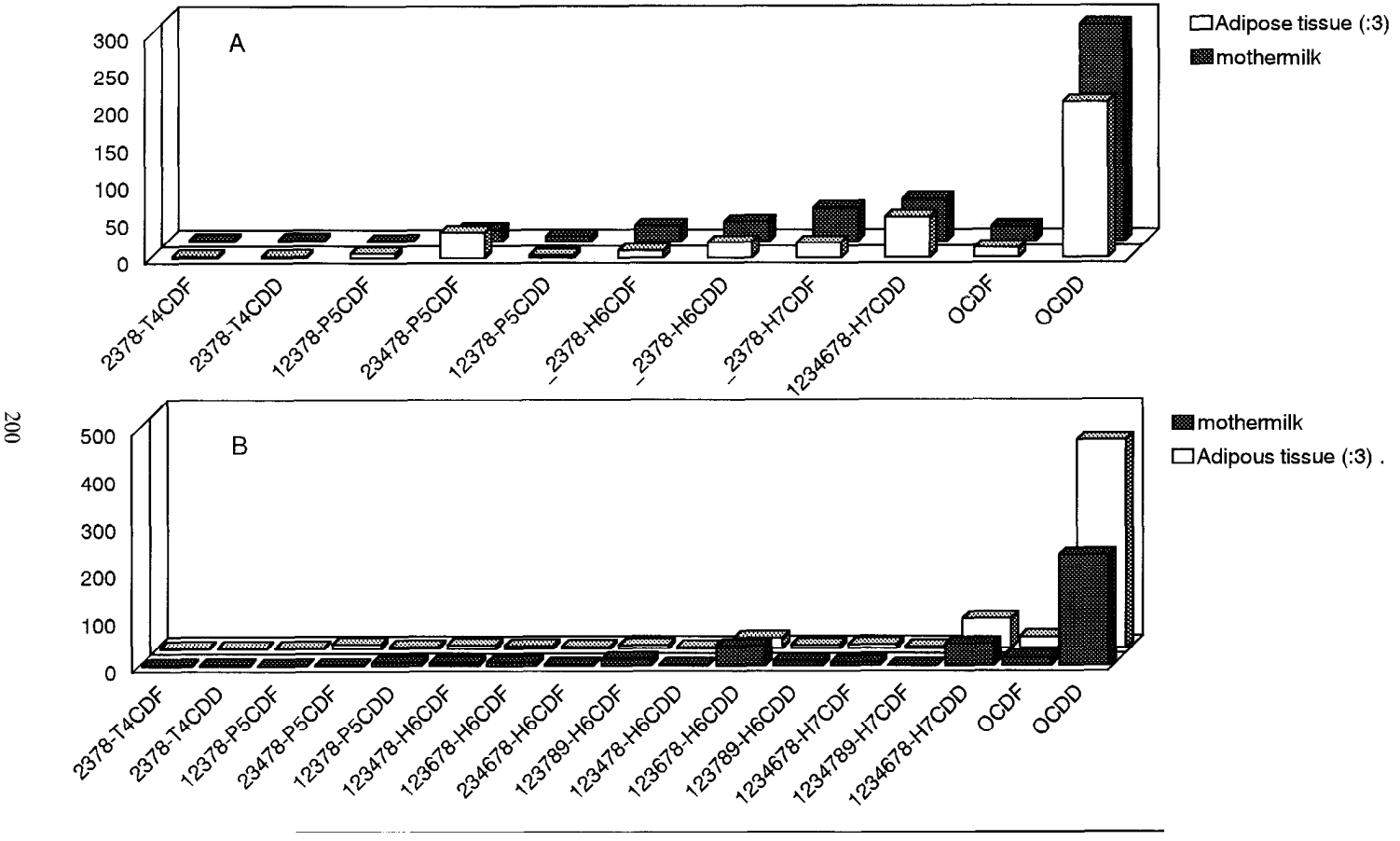

Figure 1. PCDD/F levels in human milk and adipose tissue from populations in Paris (A) and Madrid (B) in pg/g fat weight basis

Table 1. PCDD/F levels in human milk from Paris and Madrid populations (pg/g fat weight)

SAMPLE	FRANCE	SPAIN
	Mean±SD	Mean±SD
2378-T4CDF	1,8±0,8	1,02±1,2
2378-T4CDD	2,4±1,1	1,2±1,9
12378-P5CDF	0,5±0,4	0,7±0,9
23478-P5CDF	16,5±5,2	0,9±2,1
12378-P5CDD	6,6±1,8	6,7±8,6
Σ2378-HxCDF	20,4±23,1	30,0±9,6
Σ2378-HxCDD	25,9±7,0	53,6±14,6
Σ2378-HpCDF	45,0±34,9	7,2±7,54
1234678-HpCDD	56,0±22,1	46,0±31,7
OCDF	19,0±26,4	18,1±4,2
OCDD	290,0±129	234,0±44,8
ΣPCDDs	380,9±161	341,5±118,7
ΣPCDFs	103,2±90,8	57,9±25,5
ΣPCDDs+PCDFs	484,1±250,8	399,4±142,2
I-TEQ	20.1	13.31

similarities between the profiles of both types of samples in the two polpulations. PCDD levels were higher than PCDF, they increase progressively from tetra- to octa- chlorinated dioxin congeners, while PCDF levels were more uniform in their congener distribution.

The mean levels of PCDD/Fs expressed in I-TEQs on a fat weight basis were 20.1 ng/kg and 13.3 ng/kg respectively in mother milk samples from Paris and Madrid. Figure 2 shows the comparison of the I-TEQ found in both Paris and Madrid populations with those found in similar urban surveys from the published literature since 1986 (Fürst et al., 1992; Startin et al., 1989; Schecter et al., 1990a;

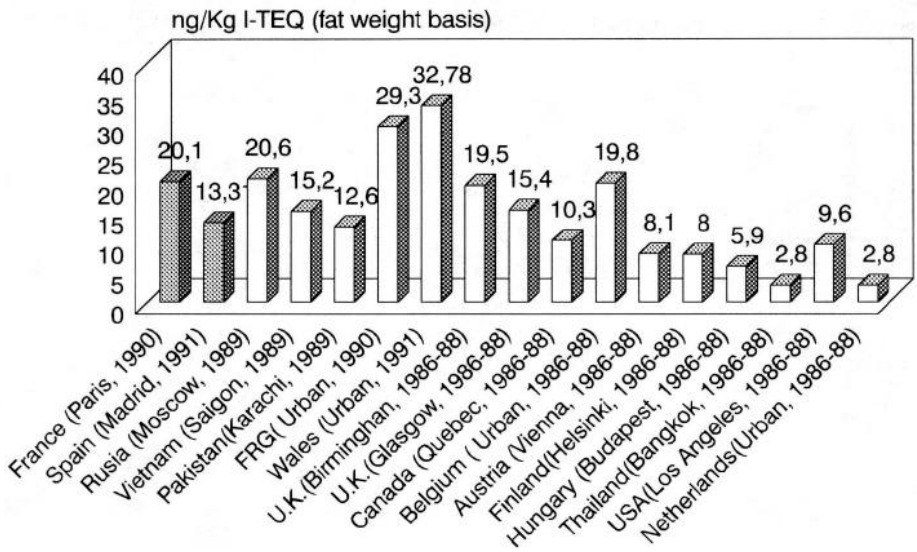

Figure 2. Total I-TEQ in breast milk from various countries

Tarkowski and Yrjänheikki, 1989 ; Schecter et al., 1990 b). The I-TEQ found in mother milk from people living in Paris and Madrid are similar to that measured in Vietnam Russia, England, Germany, Belgium and Netherlands. They are higher than in the USA, Nordic countries, Austria, Canada and Pakistan, and much higher than Netherlands, Thailand and Hungary (Budapest). Generally the more polluted industrial area is, the higher the PCDDs and PCDFs human breast milk levels are. Breast milk samples from countries under development actually show comparable PCDD/F pattern, although their levels are lower than those found in industrialized countries. Thus, up to a some extend, PCDD/Fs levels in breast milk seem to reflect the degree of industrialization of the country where the feeding mother live. On the other hand, the fact that levels found in rural are a lightly lower than levels found in urban and industrial zones (Dewailly et al., 1991; Schecter et al., 1990 b), reflects that PCDDs and PCDFs are generally distributed about everywhere in the environment as are PCBs and other organochlorine.

The AD1 (average daily intake) of an infant of 5 kg consuming 700 ml of milk (3.5 % of fat content) per day would be 98.48 and 65.21 pg TEQ/kg/day for breastfed Paris and Madrid babies. These values are higher than the 10 pg TEQ/Kg/day considered by WHO/EURO as TDI (tolerable daily intake) for general population.

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