

## EVALUATION OF *p,p'*-DDE, *p,p'*-DDT AND POLYCHLOROBIPHENYLS (PCBs) LEVELS IN SAMPLES OF HUMAN MILK FROM ROME, FLORENCE AND THE SURROUNDING AREAS

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**Summary.** - Sixty-five samples of human milk obtained from individual donors living in Rome and surrounding areas between 1982 and 1984, and 28 samples from Florence and surrounding areas obtained during 1985 were analyzed for residues of *p,p'*-DDE, *p,p'*-DDT and PCBs. Levels of *p,p'*-DDE were between 5 and 126 ppb ( $\mu\text{g/kg}$  of milk), with an average value of 45 ppb (median 34). Levels of *p,p'*-DDT ranged from 1 to 79 ppb, with an average value of 10 ppb (median 7). PCBs were found at levels ranging from 7 to 304 ppb, with an average value of 74 ppb (median 66). As to the *p,p'*-DDT, when compared to the data previously obtained in Italy during 1975-77, the present findings show a decrease of the average value and a lower incidence of samples with higher values. These effects are less pronounced for the *p,p'*-DDE. No relevant variation was observed in the levels of PCBs in comparison with the data obtained in Italy during 1981-82.

**Riassunto** (Valutazione dei livelli di *p,p'*-DDE, *p,p'*-DDT e policlorobifenili (PCB) in campioni di latte umano provenienti dalla zona di Roma e di Firenze). - Sessantacinque campioni di latte umano, prelevati nel periodo 1982-84 da singole nutrici residenti in Roma e provincia, e 28 campioni, prelevati nel 1985 da nutrici residenti in Firenze e provincia, sono stati analizzati per la determinazione di *p,p'*-DDE, *p,p'*-DDT e PCB. Il *p,p'*-DDE è risultato presente in concentrazioni varianti da 5 a 126 ppb ( $\mu\text{g/kg}$  di latte), con un valore medio di 45 ppb (mediana 34). Il *p,p'*-DDT è risultato presente in concentrazioni da 1 a 79 ppb con un valore medio di 10 ppb (mediana 7). I PCB, infine, variavano in un intervallo di concentrazione di 7-304 ppb, con un valore medio di 74 ppb (mediana 66). Per quanto riguarda il *p,p'*-DDT, i dati attuali, confrontati con quelli ricavati nel periodo 1975-77 in Italia, mostrano una diminuzione del valore medio e una minore incidenza di campioni con valori elevati. Questi effetti sono meno marcati per quanto riguarda il *p,p'*-DDE. Nessuna sostanziale variazione nei livelli di PCB è stata osservata in confronto con i dati rilevati nel 1981-82.

### Introduction

A good index to evaluate the degree of human exposure to persistent pollution agents is given by the amounts of these substances in human milk and adipose tissue. Human milk is an excellent indicator of bioconcentration of substances with high environmental persistence since breastfeeding mothers are at the top of the nutritional chain.

This study focuses in particular on organochlorinated pesticides (OCs) and on industrial chemicals such as polychlorobiphenyls (PCBs) both for their persistence in the environment and their capacity to bioaccumulate in organisms.

Studying human milk contamination is important also from a health standpoint as lipophilic contaminants - which are gradually ingested with the diet and accumulate in adipose tissue - are greatly mobilized during breastfeeding. Moreover, human milk is all the more important as it is the only food taken in the first months of a baby's life. Besides, the weight of intaken milk reaches proportions in relation to the baby's body weight of 20-30%, never again surpassed in a person's lifetime.

Several studies have been published throughout the world on the presence of DDT and other organochlorinated compounds in human milk samples, such as in Germany [1-3], Sweden [4], USA [5-7], United Kingdom [8], the Netherlands [9], Poland [10], Belgium [11], Russia [12], Canada [13], Japan [14, 15], Yugoslavia [16], Argentina [17], Norway [18] and Israel [19].

In addition, Jensen published two important reviews summing up the results found in the literature on this subject [20, 21].

Researchers in Italy have long studied the levels of OCs and PCBs in human milk. Among these the work of Pacagnella *et al.* on human milk and cow milk published in 1966 [22], Cerutti *et al.* published in 1975-78 on colostrum [23] and human milk [24, 25] and Ferioli *et al.* of 1982 on PCB in human milk [26].

With the exception of Ferioli's paper that deals exclusively with PCBs, the results of the other two works were obtained approximately 10 years before the commencement of this research. It therefore seemed necessary not only to update but also to expand the existing knowledge regarding levels of PCBs and pesticides of the DDT group present as contaminants in milk of Italian women, so as to be able to verify their levels of contamination.

In Italy, in fact, various organochlorinated pesticides, DDT in particular, have first been severely restricted and later banned altogether in the seventies as in most other European countries [27-31].

In this paper we report the DDT, DDE (the main DDT metabolite), and PCB levels contained in 93 samples of human milk taken at the Ospedale Nuovo Regina Margherita of Rome in the period 1982-84 and at the Ospedale Meyer of Florence in 1985.

Part of the data presented herein are already contained in two previous publications by the same authors [32, 33].

## Experimental

### Sample preparation

Samples were frozen at -25 °C immediately after collection. At analysis, they were thawed and homogenized by immersion in ultrasound bath for 15 min.

### Extraction

The Suzuki *et al.* method [34] was used for extraction, with slight modifications. We verified that this method, under our conditions, yields a recovery of more than 80% for the compounds studied.

Ten ml of milk were placed in a centrifugation tube with a PTFE-lined screw cap. Twenty ml of *n*-hexane were added plus 5 ml of acetonitrile and 1 ml of ethanol. The tube was then shaken for 2 min and centrifuged at 2000 rpm for another 2 min. The hexanic phase was filtered through an anhydrous sodium sulfate column and collected in a 100 ml Erlenmeyer flask. Hence, the aqueous phase was extracted with *n*-hexane (2 x 20 ml). The hexanic phases were then combined and concentrated at 5 ml. Thus, 1.5 ml of hexane solution were removed (equal to 3 ml of milk) and purified by chromatography on a Florisil column.

### Florisil column chromatography

Florisil, 2.5 g (60-100 mesh) PR grade from Floridin Co. (activated at 130 °C for one night), was poured into a glass column (300 mm x 10 mm i.d., with tap of PTFE). Later, 2 g of anhydrous sodium sulfate were added. The column was washed with *n*-hexane (3 x 5 ml) and the liquid discarded. Hexanic solution (1.5 ml) from the extraction was placed in the column. The OCs and PCBs were eluted

with 50 ml of ethyl acetate + benzene + *n*-hexane (180 + 19 + 1) mixture. The eluate was concentrated to dryness and the residue redissolved with 1 ml of *iso*-octane.

### Gas chromatographic analysis

#### Conditions:

- Varian 3700 gas chromatograph with electron capture detector (GC/ECD);

- glass column, 180 cm x 4 mm i.d., packed with 1.5% OV-17 + 1.95% QF-1 on Chromosorb W HP 100-120 mesh;

- temperatures: injector, 220 °C; oven, 205 °C; detector, 300 °C;

- carrier gas, Ar-5% methane; flow, 45 ml/min.

*iso*-Octane solution (1-5 µl) from the Florisil column chromatography was injected. Then the organochlorinated pesticide residues were identified and determined for comparison with standard solutions.

### Glass capillary gas chromatography

The sample extracts - already analyzed on packed column - were analyzed again on capillary column for a qualitative check-up.

#### Conditions:

- Dani 3900 gas chromatograph with electron capture detector;

- fused silica column; 50 m x 0.32 mm coated with OV-1; film thickness, 0.17 µm;

- temperatures: injector, 220 °C; oven, 180 °C; detector, 250 °C;

- carrier gas: nitrogen, 2.5 ml/min; auxiliary: nitrogen, 55 ml/min;

- splitter, 1:20.

### PCBs determination

After GC/ECD analysis for organochlorinated pesticides the solution was concentrated to dryness and 20 ml of 5% KOH solution in ethanol were added. The container was heated over boiling water (80 °C) for 30 min. After cooling to room temperature, the solution was diluted with 50 ml distilled water and extracted with 3 x 50 ml *n*-hexane. The resulting hexanic phases were concentrated to dryness and the residue redissolved with 1 ml *iso*-octane.

For PCBs determination, 1-5 µl of this solution were analyzed in GC/ECD with OV-17 + QF-1 column under the described conditions.

For quantitative and qualitative comparison, an Arochlor 1254 and 1260 (1:1) mixture was used as standard. For the determination we used the ratio between the sum of the heights of five significant peaks present in the standard and the corresponding ones in the sample.

## Results and discussion

Of the analyzed human milk samples, sixty-five samples, taken between 1982 and 1984, were made available by the Ospedale Nuovo Regina Margherita in Rome. All

the samples (size between 20 and 40 ml) were from single donors. About 50% of the donors resided in Rome and the other 50% in the province of Rome.

The ages of the donors ranged from 20 to 37 (median age 27). Fifty percent of the mothers were housewives, 30% office workers and 20% factory workers. Fifty percent of the mothers had delivered their first child, 25% their second and 25% more than 2 children. Forty percent of the samples were collected within 10 days from delivery, 15% within 20 days, 15% within 30 days and 30% after 30 days.

Another group of 28 samples of human milk, taken in 1985, were made available by the Ospedale Meyer of Florence. These samples also came from single breast-feeders. In this group, the donor's ages ranged from 25 to 35 years old (median: 26). Sixty percent of the mothers lived in Florence, whereas 40% came from surrounding areas. Sixty-seven percent of the mothers had delivered their first child, 27% their second and 6% their third. All multiparous mothers had breastfed their previous children.

Figures 1 and 2 show the age and occupation distribution for all donors included in the study.

As to the analytical methodology adopted for the determination of persistent organochlorinated compounds, extraction and purification were carried out according to

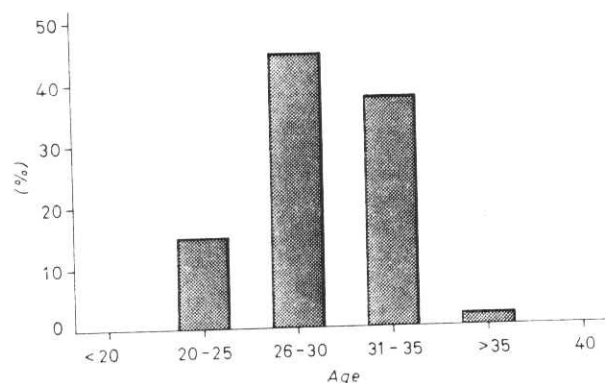


Fig. 1. - Percent of all the donors (n = 93) according to age.

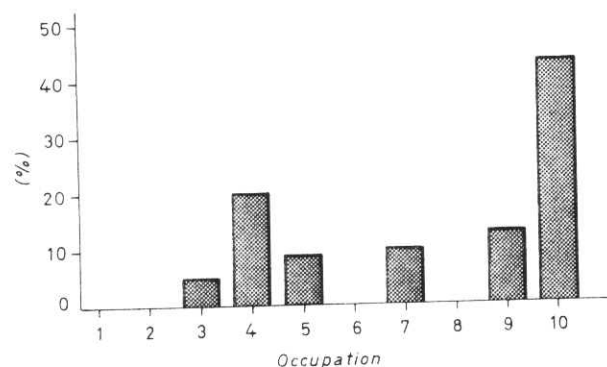


Fig. 2. - Percent distribution of all the donors (n = 93) according to occupation. 1) unemployed; 2) farm workers; 3) industry workers; 4) employeres; 5)dealers; 6) craftwomen; 7) professionals; 8) nurses; 9) teachers; 10) housewives.

the Suzuki *et al.* method [34]. Instead, the GC/ECD analysis on multiple gas chromatographic columns was developed in our laboratory.

Chromatograms of the analyzed samples revealed that the most significant single component peaks were those of DDE and DDT. This study reports only the results of these two compounds, as they are representative of environmental contamination. These compounds were present at such concentrations, that it was possible to reliably establish their identity under the conditions adopted.

Peaks with retention times corresponding to other organochlorinated compounds were also identified. These peaks were in the part per billion order (ppb =  $\mu\text{g/kg}$ ), and therefore, their identity could not be established with certainty.

Table 1 reports the average, standard deviation, median, range of values and percentage of the samples with measurable residues. As can be seen, there is a high percentage of samples with measurable residues for all 3 compounds considered. DDE (average 45, range 5-126 ppb) was the predominant single component accompanied by smaller quantities of the parent compound DDT (average 10, range 1-79 ppb).

To be able to compare our results with those obtained in the period 1975-77 by Cerutti *et al.* [24, 25], the latter - which were originally expressed by the authors on the milk-fat basis - were transformed by means of the percentage of fat reported in the same study, so as to express them in term of milk's weight. Table 2 shows the value distributions of the results obtained in this study as well as in Cerutti's [24, 25] according to arbitrary value classes. Even with the precautions suggested by the limited number of samples considered in both studies, we noticed that the average values of DDE and DDT found in this study are lower than those calculated from the results reported by Cerutti *et al.* [24, 25].

Particularly noteworthy in our results is the lowering or disappearance of higher values, i.e. higher than 140 ppb and 24 ppb for DDE and DDT, respectively. In general terms, an overall trend towards lower values is noticed in the distribution.

In this study the ratio between average DDE and DDT values was about 4.5, whereas in Cerutti's it was about 2.4. Since DDE is a product of the transformation of DDT, the higher its levels the older the origin of contamination. Therefore, an increase of the DDE/DDT ratio indicates that the contamination we measure today derives mainly from DDT released into the environment in the past. This indirectly proves the usefulness of restriction and banning of DDT which were issued in previous years. A similar variation of the DDE/DDT ratio over a 9-year ban period for the use of DDT was reported by Skaare [18] in human milk in Norway, also serving as an indicator of similar consequences from restrictive measures taken both in Norway and Italy.

Because these compounds are persistent and widespread, the levels present in human milk do not depend exclusively on the effect of measures taken in a single

country. This study, however, sufficiently confirms the downward trend of DDE and DDT levels, proving the effectiveness restrictive measures may have on the environment.

On the other hand, DDE values measured are, with the exception of Greece, of the same order of magnitude as those found in other EEC countries (Table 3), while DDT values are among the lowest.

A health evaluation of the values found for DDT is possible if we use the acceptable daily intake (ADI) as a reference. The ADI has been recently elaborated by the Joint FAO/WHO Meeting on Pesticide Residues [35]. After

examining the existing literature, FAO/WHO established the ADI value at 0.02 mg/kg b.w. If we assume the average weight of a child to be 3.5 kg and the average daily intake of milk in the first three months of a child's life to be 700 g, we calculate an average daily intake of 0.002 mg/kg b.w. which is 10 times lower than the ADI proposed by FAO/WHO. For DDE an analogous evaluation is not possible since no ADI has been established.

PCBs were found in 84% of the samples at concentrations ranging from 7 to 304 ppb with an average value of 74 ppb and a median value of 66 ppb (Table 1). These values are not very different from those found by Ferioli *et*

Table 1. - Statistical parameters derived from values ( $\mu\text{g/kg}$  of milk) of residues of *p,p'*-DDE, *p,p'*-DDT and PCBs found in human milk samples from Rome and Florence during the period 1982-1985

	Samples analyzed (no.)	Mean	Standard deviation	Median	Range	Positive samples (%)
<i>p,p'</i> - DDE	93	45 (n = 87)	32.2	34	5-126	93
<i>p,p'</i> - DDT	93	10 (n = 83)	11.4	7	1-79	89
PCB	61	74 (n = 51)	52.3	66	7-304	84

Table 2. - Comparison between the distribution of values ( $\mu\text{g/kg}$  of milk) obtained in this survey and that of values reported by Cerutti *et al.* [24, 25] and by Ferioli *et al.* [26], arranged according to arbitrary classes

	This survey			Cerutti <i>et al.</i> (1975-1977)	
	Range	Mean	Samples (%)	Mean	Samples (%)
<i>p,p'</i> -DDE	1 - 20		26		10
	21 - 40		34		25
	41 - 60		10		30
	61 - 80	45	14	68	10
	81 - 100	(n = 87)	7	(n = 61)	7
	101 - 120		7		3
	121 - 140		2		3
	> 140		0		12
<i>p,p'</i> -DDT	1 - 4		24		0
	5 - 8		40		3
	9 - 12		18		16
	13 - 16	10	8	28	22
	17 - 20	(n = 83)	6	(n = 61)	17
	21 - 24		2		2
	> 24		2		40
PCBs				Ferioli (1982)	
	1 - 20		6		5
	21 - 40		26		17
	41 - 60		16		28
	61 - 80	74	12	67	17
	81 - 100	(n = 51)	16	(n = 61)	11
	101 - 120		16		11
	121 - 140		0		11
	141 - 160		2		0
	161 - 180		4		0
	> 180		2		0

Table 3. - Comparison among levels of residues ( $\mu\text{g/kg}$  of milk) of *p,p'*-DDE, *p,p'*-DDT and PCBs founds in human milk in some EEC countries

Country	<i>p,p'</i> -DDE		<i>p,p'</i> -DDT		PCBs
	Mean	Range	Mean	Range	Mean
Italy (a) (1982-85)	45	5-126	10	1-79	74
Belgium (b) (1976)	59	2-150	47	20-75	n.r.
Denmark (b) (1982)	28	24-31	35	n.r.	n.r.
FRG (b) (1973-74)	65	15-230	32	2-190	133
Greece (b) (1974-75)	258	n.r.	86	n.r.	n.r.
The Netherlands (b) (1979)	24	n.r.	67	n.r.	n.r.
United Kingdom (b) (1979-80)	41	1-210	3	1-40	n.r.

(a): this survey; (b): data from Jensen [21]; n.r.: not reported

al. [26] in 1982. To date, no ADI has been established for this class of compounds. From our results we calculate an average daily intake, under the previously described conditions, of 0.007 mg/kg b.w., which is similar to those found in other EEC countries [21] such as Belgium (0.008), Denmark (0.010), West Germany (0.011), the Netherlands (0.003) and United Kingdom (0.003).

Both for DDT, DDE and PCB no attempt has been made to evaluate the results according to parameters such as origin and place of residence, age, parity and occupation, due to the fact that for many of these samples such

information was not available and also because the number of samples for each class was in some cases very limited.

Hence, although this study offers useful information for a comparison with previous investigations, it is clearly not to be considered final since an analysis of a larger number of samples including many more geographic areas is needed to render it more representative.

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