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Organochlorine Pesticide and Polychlorinated Biphenyl Residues in Human Milk from Rome (Italy) and Surroundings

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Organochlorine (OC) pesticides and polychlorinated biphenyls (PCBs) in human milk have been the subject of many studies. A comprehensive review of the literature, made by Jensen (1983), indicates numerous references around the world regarding their presences in this substrate.

Surveys were carried out in Italy by Paccagnella (1966) Cerutti (1975, 1976, 1978) and Ferioli (1982). These surveys are all eight years old with the exception of the latest work. Because of recent improvements in analitycal methodology, we believe an up-to-date study would provide additional information. Thus, this paper presents a survey of the levels of human milk contamination, in Rome and surroundings, by organochlorine pesticides and PCBs. This survey is a part of a larger monitoring program covering also geographical areas outside of Rome.

MATERIALS AND METHODS

Sixty-five human milk samples (average sample size, 20 ml) were collected between October 1983 and May 1985 from individual mothers from Rome (Italy) and surroundings. Milk was immediately transferred from the breast-pump to the residue-free bottle, to avoid prolonged contact with any plastic or rubber. Samples were stored at -20 °C until analysis.

All solvents were glass distilled and free of interfering substances. Florisil (Floridin, Co.) was activated at 130 °C overnight. Sodium sulphate anhydrous was purified by heating at 500 °C for two hours. Standard compounds were from the collection of this laboratory. All glassware was acetone and n-hexane prewashed in order to assure chemical cleanliness.

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Ten ml of each thawed and mixed milk sample were extracted with n-hexane + acetonitrile + ethyl alcohol = 20 + 5 + 1 ml, according to Suzuki (1979). The supernatant was removed after centrifugation and the extraction was repeated with two 20-ml portions of n-hexane.

The combined extracts were evaporated to dryness and then dissolved in 5 ml n-hexane. A 1.5 ml aliquot (equivalent to 3 g milk) was transferred to the top of a 2.5 g Florisil column (glass, 300 x 10 mm i.d.) and eluted with 50 ml of a mixture of ethyl acetate + benzene + n-hexane = 1 + 19 + 180 ml. The eluate was evaporated to dryness, diluted to a suitable volume with n-hexane and analyzed by gas chromatography with electron capture detector (GLC/ECD).

A VARIAN 3400 gas chromatograph, equipped with a 63Ni electron capture detector, was used under the following conditions: glass column (1.80 m x 4 mm i.d.) packed with 1.5% OV 17 - 1.95% QF 1 on 100-120 mesh Chromosorb W HP; temperatures: oven 205 °C, injector 220 °C, detector 300 °C; carrier gas Argon + Methane 5%, flow 55 ml/min. A Hewlett-Packard 3390 electronic integrator was used to calculate retention times and peak heights.

Twenty-five OC pesticides including HCB, α-HCH, lindane, quintozene, β-HCH, heptachlor, δ-HCH, aldrin, heptachlor epoxide, gamma-chlordane, o,p'-DDE, α-chlordane, α-endosulfan, p,p'-DDE, dieldrin, o,p'-TDE, kepone, endrin, o,p'-DDT, p,p'-TDE, β-endosulfan, p,p'-DDT, endosulfan sulphate, mirex, and methoxychlor can be separated in that order on the OV 17 - QF 1 column.

However, five of them do not show complete separation on this column, i.e. heptachlor is eluted between β-HCH and δ-HCH, o,p'-DDE between gamma-chlordane and α-chlordane, α-endosulfan too close to α-chlordane, kepone too close to endrin, and β-endosulfan too close to p,p'-TDE.

The sample solution was then analyzed using two other columns for separation of critical pairs and/or for confirmation of identity of the pesticides: i) glass column (1.80 m x 4 mm i.d.) packed with 5% OV 210 on 100-120 mesh Chromosorb W HP; temperatures: oven 190 °C, injector 220 °C, detector 300 °C; carrier gas Argon + Methane 5%, flow 50 ml/min. ii) glass column (1.50 m x 3 mm i.d.) packed with 3% XE 60 on 80-100 mesh Chromosorb W HP; temperatures: oven 160 °C, injector 220 °C, detector 300 °C; carrier gas Nitrogen, flow 60 ml/min.

By matching the retention times obtained for the sample solution and the standard compounds under the three GC

conditions it is possible to assigne identity to the above mentioned twenty-five OC pesticides.

The quantification of each compound was carried out by comparison of peak height in the sample with peak height of the same order obtained injecting a suitable volume of an external standard.

The limit of determination was 1 µg/kg whole milk for p,p'-DDT and compounds eluting before it on the OV 17 - QF 1 column, while was about 3 µg/kg whole milk for the others.

Experiments with milk samples spiked at levels ranging from 5 to 10 µg/kg gave recoveries greater than 80%, except for HCB which showed recoveries in the order of 70%. However, kepone was not recovered through the method.

After analysis for OC pesticides the sample solution was evaporated to dryness and treated with hot 5% KOH/ethyl alcohol. The sample solution was then extracted with 3 x 20 ml n-hexane and analyzed by GLC/ECD with the OV 17 - QF 1 column previously described.

PCBs were quantitated with reference to a Fenchlor 1254 and 1260 standard mixture (1:1) using the sum of the heights of four major peaks with gas chromatographic retention times greater than that of p,p'-DDE.

RESULTS AND DISCUSSION

Sixty-five human milk samples from individual donors from Rome (Italy) and surroundings were analyzed for OC pesticides and PCBs. The age of the mothers ranged from 20 to 37 years (median age: 27 years). Fifty per cent of the mothers were housewives,30% were employees, and 20% were workwomen. As to the parity, 50% of the donors were mothers nursing the first child, 25% nursing the second child, and 25% had had more than two children. The milk samples were collected within 10 days from the delivery (40%), within 20 days (15%), within 30 days (15%), and after 30 days (30%).

Table 1 reports the arithmetic mean, standard deviation and range values of OC pesticides and PCBs found; the results are expressed on whole milk basis. It can be seen that HCB, p,p'-DDE, p,p'-DDT and PCBs were present in almost all the samples analyzed, whereas lower percentage of positive results were observed for α-HCH, β-HCH and lindane. The major component in the milk samples was p,p'-DDE together with lesser amounts of the parent compound p,p'-DDT. The agricultural use of the

insecticide DDT was first severely restricted in Italy in 1973 and then banned in 1978. To evaluate the effectiveness of those regulatory actions we compared our results with those obtained in Italy by Cerutti (1976, 1978) in 1975-'77, even if samples of our and Cerutti's survey were from different Regions of Italy.

Table 1 - Residues of organochlorine pesticides and PCBs in human milk (µg/kg whole milk) in Rome and surroundings (1983-1985). (Results obtained in 65 subjects)

	Arithmetic mean	Standard deviation	Range	% of positive samples
HCB	5	3.6	1 - 13	100
α-HCH	2	0.5	1 - 2	27
β-HCH	5	3.9	1 - 13	67
lindane	T	--	---	27
p,p'-DDE	40	30.5	5 - 125	100
p,p'-DDT	7	4.0	1 - 22	93
PCBs	70	42.0	7 - 176	100

T : minor than 1 µg/kg whole milk

Our and Cerutti's data are presented in Table 2 arranged in arbitrary classes with the percentage of incidence.

As to p,p'-DDE and p,p'-DDT, it can be seen that a decrease of the average values has occurred over the years of banning. In our survey the ratio between p,p'-DDE and p,p'-DDT average values is 5.7 compared to 2.4 calculated from the Cerutti's data. Since p,p'-DDE levels reflect previous exposure, these results indicate the effectiveness of the regulatory actions adopted. Just for comparison it can be noted that a similar decrease of the p,p'-DDE to p,p'-DDT ratio after nine years of ban has been reported by Skaare (1981) for the Norwegian human milk. That indicates a similar effect of the same regulatory actions adopted in Norway and in Italy. Furthermore, the regulatory actions adopted in Italy have caused a disappearance of the higher values (say greater than 140 ppb and 24 ppb for p,p'-DDE and p,p'-DDT respectively) and more generally a shift of

the distribution toward lower values.

Table 2 - Comparison between results obtained in this survey and those found by Cerutti and Ferioli in former times (µg/kg whole milk).

	This survey			Cerutti's (1975-'77)	
	range	mean value	% of incidence	mean value	% of incidence
HCB	1-3	5	40	not investigated	
	4-6	(n=65)	34		
	7-9		13		
	10-12		--		
	13-15		13		
α-HCH	neg	2	--	6	62
	1-2	(n=18)	100	(n=22)	10
	>2		--		28
β-HCH	1-3	5	40	16	7
	4-6	(n=65)	30	(n=61)	11
	7-9		10		11
	10-12		10		15
	13-15		10		13
	>15		--		43
lindane	<1		100	11	6
	1-5		--	(n=57)	68
	6-10		--		9
	11-15		--		8
	16-20		--		2
	>20		--		7
p,p'-DDE	1-20	40	31	68	10
	21-40	(n=65)	36	(n=61)	25
	41-60		10		30
	61-80		12		10
	81-100		2		7
	101-120		7		3
	121-140		2		3
	>141		--		12
p,p'-DDT	1-4	7	30	28	--
	5-8	(n=65)	40	(n=61)	3
	9-12		22		16
	13-16		4		23
	17-20		2		17
	21-24		2		2
	>25		--		40

cont. Table 2

	This survey			Ferioli's (1982)	
	range	mean value	% of incidence	mean value	% of incidence
PCBs	1-20	70	6	67	5
	21-40	(n=65)	26	(n=61)	17
	41-60		18		28
	61-80		12		17
	81-100		16		11
	101-120		14		11
	121-140		--		11
	141-160		4		--
	161-180		4		--

A simple calculation based on the levels of p,p'-DDT found in human milk samples shows that the proposed Acceptable Daily Intake (ADI) of 0.02 mg/kg b.w. (FAO/WHO, 1985) was not exceeded by 3.5 kg infants ingesting 700 g of milk per day.

Hexachlorobenzene (HCB) was found in all the milk samples, despite the fact that it has not been ever allowed in Italy for agricultural uses. However HCB is recognized as a by-product of some industrial chlorination processes which are probably the main source of environmental contamination.

Alpha and beta isomers of HCH were banned in Italy since 1974 and our results show a slight decrease in the average values and a shift of the distribution toward lower values, in comparison to the Cerutti's data of 1975-1977. The lindane shows a similar decrease, although it has not been completely banned in Italy.

Due to the persistence and ubiquitous character of the OC pesticides, levels in human milk do not merely depend on the effect of regulatory actions of a single Country. However, the present survey provides sufficient indications of a trend toward a decrease of the levels of OC pesticides previously used in Italy and of the effectiveness of the regulatory actions adopted. The levels found mostly are in line, and sometimes lower, with the levels found in most of European countries (Jensen 1985).

As to the PCBs, our results compared with that by Ferioli (1982) do not indicate an appreciable decrease (see Table 2). However, the levels are in line with those of most industrialized countries as reported by

Weisenberg (1985) and Jensen (1985). As PCBs are very persistent compounds and very slowly metabolized in biological systems, it is probably too early to observe any effect on the levels in human milk since Italian and European restrictions of 1982.

A toxicological evaluation of the present PCB levels in Italian human milk is quite difficult, as PCBs are a mixture of compounds with different toxicological properties.

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