POLYCHLORINATED BIPHENYLS, HEXACHLOROBENZENE AND ORGANOCHLORINE PESTICIDE RESIDUES IN MILK FROM APULIA

COMPOSTI BIFENILICI ESACLOROBENZENE E PESTICIDI ORGANOCLORURATI IN CAMPIONI DI LATTE PRELEVATI DA ALCUNE AZIENDE AGRICOLE DELLA PUGLIA

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ABSTRACT

A survey was carried out on 450 milk samples (cow, buffalo, goat and sheep) from Apulian farms to determine the levels of polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB) and organochlorine pesticides (OCPs). These contaminants are highly undesirable in milk because it is widely consumed mainly by infants. Results showed that PCBs, 2,2-bis (4-chlorophenyl)-1,1-dichloroethylene (p,p'-DDE), HCB and hexachlorocyclohexane α isomer (α-HCH) were present in almost all of the samples analysed, while lindane (γ-HCH) was found only in cow milk sam-

RIASSUNTO

Al fine di determinare il livello di contaminazione da PCBs, POCs e HCB è stata condotta un'indagine su 450 campioni di latte (bovino, ovino, caprino e di bufala) provenienti da diverse aziende agricole pugliesi. La presenza di questi contaminanti nel latte non è desiderabile, poiché questo alimento è di largo consumo ed è un importante nutrimento soprattutto per i neonati. I risultati hanno evidenziato la presenza di PCBs, p, p'-DDE, esaclorobenzene (HCB) e esaclorocicloesano (α-HCH) in quasi tutti i campioni, mentre il lindano (γ-HCH) è stato osservato unica-

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ples. Among these pollutants, PCBs (16.7-66.7 μg/kg), HCB (3.58-7.57 μg/kg) and p,p'-DDE (7.38-20.96 μg/kg) were present at the highest concentrations. Results are expressed on a fat basis.

mente in alcuni campioni di latte bovino. Tra gli inquinanti determinati, i PCBs (16,7-66,7 μg/kg), l'HCB (3,58-7,57 μg/kg) e il p,p'-DDE (7,38-20,96 μg/kg) sono presenti a più alte concentrazioni.

INTRODUCTION

Polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB) and organochlorine pesticides (OCPs) are a very resistant group of toxic substances which in the past were produced in large amounts and used extensively in industry and agriculture. Because of their stability and indiscriminate use, they accumulate in the environment. Being lipophilic substances, they concentrate in the animal tissues richest in fat, reaching maximum levels in the most evolved species. In mammals the principal routes of elimination are biliary (PASCHAL *et al.*, 1974) and through the milk, so this product is often used as a biological indicator of the degree of environmental pollution. Milk is one of the most important substances where polychlorinated biphenyl compounds and organochlorine pesticides levels are continuously monitored (MUKHERJEE and MADHUBAN, 1993; EJOBI *et al.*, 1996; WALISZEWSKI *et al.*, 1996), because milk and its products play a central role in human nutrition. The present survey reports the results of PCBs, OCPs and HCB residues in milk destined for human consumption and collected from two agricultural areas of Apulia, in southern Italy.

MATERIALS AND METHODS

From January to May 1999, 196 cow milk samples (100 from the district of Bari and 96 from the district of Foggia), 142 sheep milk samples, 49 goat milk and 63 buffalo milk samples from farms in the district of Foggia were collected. The milk, collected in pre-cleaned universal bottles, was stored at 4°C and analysed 12-24 h after collection.

PCBs, OCPs (α-, β- and γ-HCH, p,p'-DDT, o,p'-DDT, p,p'-DDE, p,p'-DDD, o,p'-DDD), HCB, aldrin, heptachlor and heptachlor epoxide were extracted from the milk according to the method proposed by SUZUKI *et al.* (1979). Twenty millilitres of *n*-hexane, 5 mL of acetonitrile and 1 mL of ethanol were added to 10 mL of milk. The mixture was shaken vigorously and centrifuged at 2,000 rpm for 2 min. After separation of the *n*-hexane phase, the remaining solution was again extracted twice with 20 mL of *n*-hexane. The combined *n*-hexane phases, filtered through an anhydrous sodium sulphate layer into a Kuderna-Danish concentrator, were concentrated to 5 mL. The fat content was determined gravimetrically on an aliquot of 1 mL. To the remaining 4 mL of the *n*-hexane extract, 1 mL of concentrated H_2SO_4 was added for the clean up (MURPHY, 1972) and centrifuged. The supernatant was concentrated and transferred to a Florisil minicolumn (2.5 g Florisil, 60-100 mesh, Supelco, topped with ca 2 g anhydrous sodium sulphate) and heated at 650°C for 3 h. The pollutants were eluted from the column with 50 mL ethyl acetate-benzene-*n* hexane (1:19:80). The eluate was concentrated to 4 mL and 1.0 μL was injected into a 5300 Mega Series Carlo Erba HR gas chromatograph with an automatic injection system and an electron capture detector ECD-400, Ni^{63}

(temperature: 300°C). The GC was connected to an IBM PS/2 55SX PC equipped with a System Gold version 6.1 software program for integration purposes (Beckman). For all the analyses an SPB-608 Supelco fused-silica capillary column was used (length = 30 m, inside diameter 0.25 mm and film thickness 0.25 µm). Helium at a flow rate of 1 mL/min was used as gas carrier and nitrogen was the make-up gas at 60 mL/min. The inlet temperature was programmed from 50° to 180°C at a rate of 15°C/min, increased to 220°C at a rate of 4°C/min for 20 min and increased to 275°C at a rate of 5°C/min and held until the end of the run. PCBs were identified and quantitated by comparison with mixtures of Aroclor 1254 and 1260. The identity of the DDT group of compounds was confirmed by alkali conversion to their respective olefins and re-analysis by GLC. Analytical data for the DDT compounds were obtained by comparison between sample peak area and peak areas of external standards (mixture of OCPs from Supelco). Recoveries were determined by adding known amounts of PCBs, OCPs and HCB standards to blank samples. Results were found to be within 80-110%. The limits of quantification were from 0.1 to 0.4 ng/g for PCBs, OCPs and HCB. Quantification was done within the linear range of the detector. Non detected constituents were assigned a value of zero. Residues in 10% of the samples were confirmed by gas-liquid chromatography-mass spectrometry (Fisons MD 800, Milano, Italy). Concentrations are reported as µg/g on a fat weight basis.

RESULTS AND CONCLUSIONS

In all the milk samples examined the following organochlorine pesticides were absent: β-HCH, heptachlor, heptachlor-epoxide, aldrin, endrin, o,p'-DDT, p,p'-DDT, p,p'-DDD and o,p'-DDD. Lindane (γ-HCH), absent in the buffalo, sheep and goat samples, was found only in 6.6% of the cow milk samples from Foggia and in 25.7% of the cow milk samples from the district of Bari with very low mean values, equal to 4.31 µg/kg and 5.72 µg/kg respectively (Table 1).

α-HCH, was present in 98.4% of the cow milk samples collected from the district of Foggia and in 100% of the other samples analysed. Even if this pesticide was present in almost all the samples,

Table 1 - Concentration range and mean (µg/kg on lipid basis) value plus standard deviation in milk samples collected from the districts of Foggia and Bari.

	Goat milk (Foggia)	Sheep milk (Foggia)	Buffalo milk (Foggia)	Cow milk (Foggia)	Cow milk (Bari)
γ-HCH	ND	ND	ND	3.03-5.45	4.01-19.1
				4.31±0.66	5.72±3.24
α-HCH	0.21-1.18	0.31-3.24	0.30-1.73	0.52-6.16	0.31-3.26
	0.53±0.34	0.72±0.63	0.73±0.32	1.26±1.32	1.30±0.89
p,p'-DDE	2.28-17.69	2.04-25.0	3.88-15.91	1.94-43.35	2.96-53.23
	7.38±4.80	9.06±6.60	9.25±4.32	16.14±9.13	20.96±12.59
HCB	3.33-8.65	0.53-7.65	1.06-6.02	3.75-27.94	1.93-15.81
	6.03±1.76	4.55±1.47	3.58±1.24	7.57±5.02	5.42±3.10
PCBs	17.0-26.0	15.30-36.4	4.71-26.2	18.6-44.4	45.6-99.8
	20.2±2.4	20.2±5.0	16.7±6.2	34.5±7.4	66.7±17.3
% Lipids	3.7-5.7	6.0-8.6	7.0-8.8	3.1-4.3	2.5-5.5
	4.5±0.6	7.1±0.9	7.7±0.5	3.6±0.3	4.08±1.14

analytical data in all the different kinds of milk examined showed a modest level of contamination with mean values ranging from 0.53 µg/kg (goat milk) to 1.30 µg/kg (cow milk).

Concerning the DDTs, only p,p'-DDE was found in all the milk samples. Cow milk samples collected from Bari (20.96 µg/kg) had levels comparable to those from Foggia (av. 16.14 µg/kg); sheep milk samples (av. 9.06 µg/kg) and buffalo milk samples (av. 9.25 µg/kg) had similar levels, while lower concentrations were found in goat milk (av. 7.38 µg/kg). The complete absence of p,p'-DDT in the samples leads to the supposition that the modest amount of p,p'-DDE residues detected may have derived only from the metabolism of p,p'-DDT used in the past, rather than to further input.

Hexachlorobenzene (HCB) was detected in all the milk samples. Similar values were detected in the cow milk collected from Foggia (av. 7.57 µg/kg) and from Bari (av. 5.42 µg/kg) and in the goat (av. 6.03 µg/kg) and sheep milk (av. 4.55 µg/kg), while slightly lower levels were found in the buffalo milk (av. 3.58 µg/kg). The extensive presence of this substance in the environment is due mostly to the fact that, being a by-product of some chloride industrial processes, it is often present in widely used organic chloride compounds.

Polychlorinated biphenyls (PCBs) were found in all buffalo, goat and sheep milk samples. As for cow milk, all the samples from Bari and 92% of the samples from Foggia had PCBs. There were no differences in the mean levels of PCBs in the goat, sheep and buffalo milk, with mean values ranging from 16.7 to 20.2 µg/kg. The concentration in cow milk samples from Bari was two times higher (66.7 µg/kg) than in those from Foggia (34.5 µg/kg).

The results obtained from the survey are comparable to those found in other countries (Table 2), where DDT and HCH are still used on various crops. In Italy the use of pesticides in agriculture is regulated by a series of decrees. In particular, the ministerial decree of 14.08.1974 (G.U., 1974) prohibited the use of HCHs,

Table 2 - Polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB) and organochlorine pesticide residues (µg/kg on lipid basis) in cow milk from different countries.

Country	PCBs	α-HCH	γ-HCH	HCB	p,p'-DDE
France 1989[1]	-	3	24	-	-
Canada 1989[2]	15	-	-	-	8.6
India 1990[3]	-	16	200	-	-
Spain 1991[4]	-	18	24	8	21*
India 1993[5]	-	53	4	-	22
Mexico 1993[6]	-	55	26	25	28
Uganda 1993[7]	-	6	14	-	23
Italy 1991[8]	-	6	5-25**	6	5-14**
Italy 1992[8]	-	7	7	-	5-15**
Italy 1994[8]	-	5-20**	5	-	5-10**
Italy 2000[9]	51	1.3	5	7	18

[1] - VENANT *et al.*, 1991; [2] - FRANK and BRAUN, 1989; [3] - VERMA, 1990; [4] - DE LA RIVA and ANADÓN, 1991; [5] - MUKHERJEE and MADHUBAN, 1993; [6] - WALISZEWSKI *et al.*, 1996; [7] - EJOBI *et al.*, 1996; [8] - CANTONI *et al.*, 1994; [9] - present study.
* referred to ΣDDT.
** range.

while the ministerial decree of 11.10.1978 prohibited the use of DDT (G.U., 1978). Besides, the Ministry of Health with the decree of 22/1/98 (G.U., 2000) established maximum limits of HCH (milk: α-HCH = 4 μg/kg, β-HCH 3 = μg/kg, γ-HCH = 8 μg/kg fat basis) and DDT (milk: 40 μg/kg fat basis) residues allowed in milk and in other products of animal origin. The residue concentrations found in the milk samples analyzed in this study were lower than those limits. Nevertheless the samples were contaminated and because milk is one of the major routes through which these contaminants enter the human body, it is essential to monitor the presence of these compounds in milk at regular intervals.

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