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Organochlorine Pesticides in Cow's Milk from Agricultural Region in Northwestern Spain

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During the past 30 years, the variety and usage of pesticides have increased in Spain and worldwide. Agricultural use of pesticides can be expected to result in residues in or food and feed. Since it is recognized that pesticides are needed to produce an economical food supply of high quality, the pesticide regulatory requirements are designed to ensure that the remaining residues do not constitute an unacceptable health risk. Several limited monitoring programs have received an extensive investigation in order to detect residues from organochlorine compounds in milk (Goursaud et al. 1972; Siyali and Stricker 1973; Tuinstra 1974; Goursaud 1976; Fries and Marrow 1976; Knoeppler 1976; Konrad and Gabrio 1977; Pozo Lora et al. 1977; Uhnak et al. 1978; Martinez Castro and Juarez 1979; Weilenmann 1979; Dogheim et al. 1988). This paper reports the findings of a pesticide residue study in cow's milk samples examined during the time period of one year between May of 1987 and March of 1988. The cow's milk samples destined to human consumption were collected from an agrarian area located at a geographic region of northwestern Spain. Analyses were conducted for DDT complex (DDT_s, DDD, DDE and isomers alpha, beta and gamma (lindane), aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide, alpha- and beta-endosulfan, metoxichlor and mirex.

MATERIALS AND METHODS

Cow's milk samples (10 samples) were weekly obtained from several farms, on Monday, over a eleven month period between May of 1987 and March of 1988. The collection period accounted 46 weeks, with a total of 460 milk samples. The samples were taken by inspectors of milk manufacture "Queserias El Esla". The farms selected as collection points were distributed into 10 routes which included all geographic area of spanish León Province, area mainly agrarian. The samples were immediately frozen and kept until analyzed. In each milk samples were performed both fat percentage and pesticide analyses. All milk samples contained 4% fat or less.

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HCB, α-HCH, β-HCH, γ-HCH (lindane), aldrin, dieldrin, endrin heptachlor, hepatchlor epoxide, α-endosulfan, β-endosulfan, o,p'-DDT, p,p'-DDT, p,p'-DDE, o,p'-DDE, p,p'-DDD, metoxichlor and mirex were extracted from the milk according to a method previously described (Suzuki et al. 1979) except that acetonitrile:n-hexane (20:80) replaced the mixture n-hexane:benzene: ethyl acetate (90:9.5:0.5) as eluant on a Florisil column.

After shaking and centrifugation (2000 g for min), 10 g of homogenized milk was extracted three times with n-hexane:acetonitrile:ethanol (20:5:1). The combined extracts were concentrated under a flow of nitrogen gas in a 30ºC water bath to approximately 5 mL. The concentrate was further purified by adsorption of a Florisil column and eluted with acetonitrile: hexane (20:80). The elution was evaporated under a flow of nitrogen gas in a 30ºC water bath and the residue resuspended in n-hexane (2 mL) for gas liquid chromatography analysis.

Gas liquid chromatography with electron capture detection was used for the identification and quantitation of organochlorine residues. A Perkin Elmer 8300 equipped with split/splitless injector, a temperature programmer and a nickel-63 electron capture detector was used. The electrone capture detector was operated at a temperature of 350ºC in the constant period mode. The chromatographic column was a 30 m x 0.53 mm i.d. (Supelco). This column was used to quantitate pesticide residues. The column carrier gas was nitrogen with a flow rate of 8 mL per min. The standardised chromatographic conditions were as follows: injector temperature, 250ºC; column temperature, isothermal at 190ºC for 2 min followed by temperature programming to 230ºC at 2ºC min^{-1}, the final temperature being maintained for 20 min. Splitless injection was used.

Retention times of peaks for residues and standards were measured from the solvent peak front and converted to retention times relative to aldrin.

The amount of residues in the injected aliquot was determined by comparing residue peaks with standard peaks within the linear response range of the electrone capture detector. Organochlorine residues were quantitated by integration of peak areas using a Waters 740 or a Perkin Elmer 360 data processor.

All solvents were of pesticide residue analysis grade and the purity of all reagents was carefully checked. Reference standards of the organochlorine compounds were purchased from commercial sources.

Residue levels of pesticides were corrected according to their recoveries and expressed as µg/kg milk fat (ppb).

Recovery experiments were realized as follows: one mL n-hexane solution containing chlorinated pesticides was added to each

sample of 10 g milk. Samples were fortified with the following pesticide concentrations (ppb): α-HCH 1.0, 2.0 and 6.0; β-HCH 1.5, 3.0 and 4.5; γ-HCH 1.0, 2.0 and 6.0; HCB 1.0, 2.5 and 6.0; p,p'-DDT 2.0, 4.0 and 6.0; p,p'-DDE 1.0, 2.0 and 6.5; p,p'-DDD 1.0, 2.0 and 6.5; o,p'-DDT 2.0, 4.0 and 6.0; o,p'-DDE 2.0, 4.0 and 6.0; aldrin 2.0, 3.5 and 6.5; dieldrin 2.0, 4.0 and 6.0; endrin 2.0, 4.0 and 6.0; heptachlor 2.0, 3.5 and 5.0; heptachlor epoxide 1.5, 3.0 and 4.5; α-endosulfan 1.5, 3.0 and 4.5; β-endosulfan 1.5, 3.0 and 4.5; metoxichlor 2.0, 4.0 and 6.0; mirex 2.0, 4.0 and 6.0. The three fortified samples and one unfortified sample from milk were subjected to the procedure described above. Results are tabulated in Table 1.

When a confirmation was considered necessary, a SPB-608 (30 m x 0.25 mm i.d.) (Supelco) and SE-30 (12 m x 0.25 mm i.d.) (S.G.E.) columns were used as confirmatory tests.

Table 1. Percentage recovery of chlorinated pesticides from fortified milk samples[a].

Pesticide	
α-HCH	97.3
β-HCH	90.5
γ-HCH (lindane)	97.2
HCB	98.1
p,p'-DDT	98.6
o,p'-DDT	97.2
p,p'-DDE	98.1
o,p'-DDE	97.6
p,p'-DDD	97.7
Aldrin	96.8
Dieldrin	93.6
Endrin	90.3
Heptachlor	95.2
Heptachlor epoxide	96.8
α-endosulfan	95.6
β-endosulfan	95.8
Metoxichlor	98.2
Mirex	99.3

[a]Each value is the average of 3 samples analyses

RESULTS AND DISCUSSION

Cow's milk samples were examined for residues of all organochlorine pesticides listed above.

Table 2 and 3 give the detected residues in 460 samples. All samples were contaminated with one or more of the investigated pesticides. Most of the milk samples were contaminated with

Table 2. Concentration of organochlorine pesticides in León cow's milk from May 1987 - March 1988

Levels (ppb)	Percentage of samples containing range of pesticide levels listed (May 1987 - March 1988)					
	α-HCH	β-HCH	γ-HCH	Total DDT	Aldrin + Dieldrin	HCB
None found	0	13	0	5	8	5
Trace	0	0	0	0	0	1
1 - 5	2	15	0	8	25	50
6 - 10	18	34	1	14	60	25
11 - 50	75	37	96	67	7	18
> 100	0.6	0	0	1	0	1
Total number of samples	460	460	460	460	460	460

Total DDT - represents its isomers and metabolites
Trace - trace amounts are less than 1 ppb

HCH isomers, DDT isomers, aldrin determined as the sum of aldrin and dieldrin and HCB. α-HCH and γ-HCH (lindane) were found in all analyzed samples whereas endrin, heptachlor, heptachlor epoxide, α-endosulfan, β-endosulfan, metoxichlor and mirex were not detected in any of the analyzed samples. The amounts of organochlorine residues detected in milk samples were compared with the legal limits established and published by the European Economic Community (EEC) (Table 4).

Table 3. Mean and maximum levels[a] and frequency (f) of detection of organochlorine pesticides in cow's milk samples from León, Spain (May 1987 to March 1988).

Pesticide	$\bar{x}$[b], ppm	Max., ppm	f
α-HCH	0.018	0.148	460
β-HCH	0.009	0.032	400
γ-HCH (lindane)	0.024	0.071	460
Total DDT[c]	0.021	0.164	439
Aldrin + Dieldrin	0.006	0.019	418
HCB	0.008	0.092	437
Endrin	-	-	0
Heptachlor	-	-	0
Heptachlor epoxide	-	-	0
α-endosulfan	-	-	0
β-endosulfan	-	-	0
Metoxichlor	-	-	0
Mirex	-	-	0

[a]All values are calculated on a fat basis and expressed as mg/kg, ppm.

[b]Each value is the average of 460 samples analyses.

[c]Represents its isomers and metabolites.

Taking into consideration the maximum residue limits (MRL_s) for organochlorines in milk on a lipid basis (Council Directive 86/363/EEC of 24 July 1986, O.J. no. L 221/43 of 7.8.86; Royal Decree 569/1990, BOE of 9 May 1990), the residues of α-HCH exceeded the allowed limit in only 3 milk samples (Table 4). The residues of β-HCH, γ-HCH (lindane), DDT isomers, aldrin and dieldrin, and HCB found were always below the MRL.

The results of this survey confirm that it is possible to obtain residue levels of pesticide in milk. Since domestic animals live in an environment where organochlorine pesticides have been, and continue to be used for insect and plant disease control, it is to be expected that their feed will be contaminated to some degree. Satisfactorily, the levels of pesticides found in the Province of León, northwestern Spain, are well within the established tolerances by the different Regulatory

Organizations (Codex Committee for Pesticide Residues (CCPR), 1984; Council Directive 86/363/EEC, 1986), and we hope that good agricultural practice will be maintained by these dairy farmers, in order to keep milk free of excessive contamination of pesticides. Nevertheless, pesticide residue monitoring programs should be implanted in all countries. During 1986, the EEC created a monitoring program of drug residues in tissues of animals (Council Directive 86/469/EEC of 16 September 1986, O.J. no. L 275/36 of 26.9.86). The present work shows the need to established such monitoring program in milk for human consumption in order to improve the food safety.

Table 4. Maximum residue limits[a] (MRL) of organochloride pesticides in milk compared with levels found in León Province (northwestern Spain).

Pesticide	MRL[a] mg/kg	No. of milk samples exceeding MRL
α-HCH	0.1	3
β-HCH	0.075	0
γ-HCH (lindane)	0.2	0
Total DDT (DDT$_S$, DDD, DDE and isomers)	1.0	0
Aldrin + Dieldrin	0.15	0
HCB	0.25	0

[a]MRL issued by the EEC (1986), (values are calculated on a fat basis).

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