

ORGANOCHLORINE PESTICIDE RESIDUES IN CHEESES FROM LEÓN, SPAIN

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The purpose of this paper was to know the residual levels of 9 organochlorine insecticides (lindane, heptachlor epoxide, aldrin, endrin, dieldrin, o,p'-TDE, p,p'-TDE, p,p'-DDE and p,p'-DDT) in cheese samples collected in León, in the north-west of Spain, in 1993 and 1994. The highest individual concentration detected was, in both years, for DDT: 0.9048 ppm (brand no. 6) in 1993 and 0.8581 ppm (brand no. 5) in 1994. The organochlorine pesticide mean load ranged from 0.0197 ppm to 0.3114 ppm in 1993, and from 0.0416 ppm to 0.3000 ppm in 1994. The most frequently detected pesticides were heptachlor epoxide (77.1%) in 1993 and lindane (62.5%) in 1994. None of the samples analyzed exceeded the maximum levels allowed by the present legislation.

Keywords: Organochlorine pesticides; residues; cheese

INTRODUCTION

As a result of a widespread use in the past, some chlorinated hydrocarbon pesticides were distributed worldwide as environmental pollutants. At present, the use of organochlorine pesticides is banned or very restricted in the developed countries due to their toxicity and high capacity of accumulation in living organisms. Residual levels of organochlorine pesticides are still found in a considerable portion of biological samples [1-3], mainly due to three factors: high persistence, extensive use in many developing countries, and restricted use in many developed countries.

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The EU legislation [4] allows the use of lindane and, severely restricted, endrin and aldrin. Nevertheless, each member country in EU can have additional rules, and thus in Spain only lindane can be used without restrictions for applications on some crops, in timber preservation, for individual treatment of damaged trees and as ectoparasiticide in domestic animals. On the other hand, the *DIRECCIÓN General de Salud Pública of the Ministerio de Sanidad y Consumo* allows the occasional use of any of these compounds if resistance to other insecticides usually used appears [5].

Milk (cow's milk, principally) is one of the most important media where organochlorine residue levels are monitored. On the other hand, milk and its derivative products have a special place in human diet and it is important to know both if organochlorine pesticide residues are present in this type of samples and if the detected residue levels are below the maximum levels allowed by the legislation established by the EU [6] and by the FAO/WHO [7].

MATERIAL AND METHODS

A total of ninety-two samples of cheese were obtained from different supermarkets in León, in the North-west of Spain (see Figure 1). These samples belonged to six different brands of cheese (numbered 1 to 6) and 16 samples were collected for each one of them, except for number 4 in which case the last sampling was not carried out due to distribution problems. The brands numbered 1, 2, and 3 were elaborated in the north of the province, in a mountainous area with few culture lands. The other three brands (numbered 4, 5, and 6) were elaborated in the southern area of the province, where culture

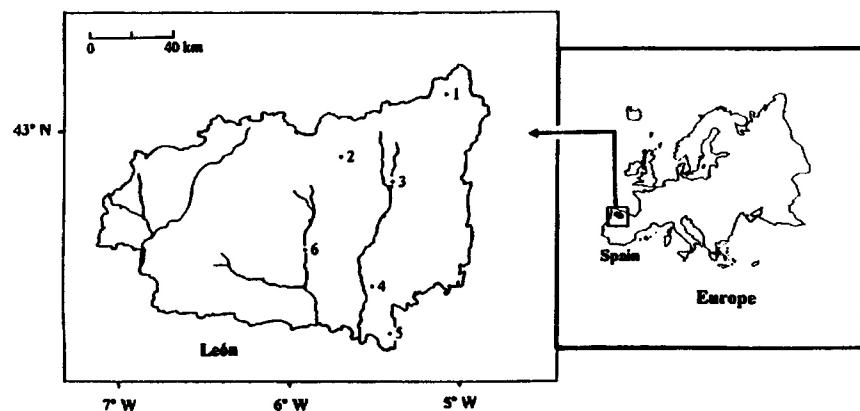


FIGURE 1 Map of León showing the sampling locations 1, 2, 3, 4, 5 and 6, corresponding to the manufacture areas.

lands are numerous. All the cheeses utilized for this study were elaborated with milk mainly collected in the influence area of the production place (see Figure 1). Number 1 and 6 were made with cow milk; number 3 with cow and sheep milk and number 2, 4, and 5 with cow, sheep and goat milk. The proportion of the several types of milk were not shown in the cheese labels and so, only the percentage of fat for any kind of cheese was taken into account owing to the fact that organochlorine insecticides are accumulated in fat. In this way, the number 1 had the highest content, 31%; the numbers 2, 3, 4, and 5 had the same percentage, 25%, and a 22% corresponded to the number 6.

Sampling was made in four different times (July and October of 1993 and in January and April of 1994) with intervals of three months, in order to detect possible seasonal variations in contamination. All samples were stored at -20°C until they were analysed.

Organochlorine residues were extracted from cheese samples according to the method proposed in 1990 by the AOAC [8] and 25 g of each sample were used. Clean-up of the extracts was also carried out by using the AOAC method and all the reagents were suitable for pesticide residue analysis and subjected to general purity tests.

Identification and quantification of residues were made by gas chromatography, with electron capture detection. A standard solution of nine organochlorine pesticides (lindane, heptachlor epoxide, aldrin, endrin, dieldrin, o,p'-TDE, p,p'-TDE, p,p'-DDE and p,p'-DDT) was used to identify and quantify the residue levels. The pesticides were purchased from Dr. Ehrenstorfer GmbH, Scharlau (Augsburg, Germany). These insecticides are contained in the standard hexane solution at the following concentrations (in ppm): 0.090 (lindane), 0.062 (heptachlor epoxide), 0.060 (aldrin), 0.081 (endrin), 0.048 (dieldrin), 0.075 (o,p'-TDE), 0.072 (p,p'-TDE), 0.095 (p,p'-DDE) and 0.165 (p,p'-DDT).

The peaks were identified by comparison of retention times with those of the standard and tolerance allowed was $\pm 5\%$ of retention times. The detection limit was established in $0.0005\text{ }\mu\text{g/mL}$ for dieldrin and o,p'-TDE; $0.001\text{ }\mu\text{g/mL}$ for lindane, heptachlor epoxide, aldrin, endrin and p,p'-TDE and $0.005\text{ }\mu\text{g/mL}$ for p,p'-DDE and p,p'-DDT. Residue concentrations in samples were calculated by comparing the peak areas in the sample with those showed by the mixed standard solution.

Recoveries of organochlorine pesticides from fortified samples by this method were: 80.0% for dieldrin, 81.3% for aldrin, 83.5% for endrin, 85% for o,p'-TDE, 86.3% for DDT, 89.1% for p,p'-TDE, 90.2% for heptachlor epoxide, and 100% for p,p'-DDE and lindane. Residue levels detected were corrected according to their recoveries.

A Hewlett-Packard gas chromatograph equipped with a ^{63}Ni electron capture detector was used. The carrier gas was argon-methane 5% (v/v) at a flow rate of 25 mL/min and sample volume injected was 3 μL . Two columns were utilized:

Column a) Glass, 185 cm long and 0.4 cm internal diameter containing a 10% coating of DC-200 on 80-100 mesh Chromosorb WHP [8]. A second glass column b) packed with 1.95% QF-1/1.5% OV-17 on 100/120 mesh Chromosorb W(AW/OMCS) was used to confirm the identity of the organochlorine residues when necessary. The operating temperatures were: inlet, 240 °C (column a) and 220 °C (column b); detector, 300 °C; column (a) 220 °C (21 minutes) and 230 °C (10 minutes); column (b) 180 °C (15 minutes) and 190 °C (20 minutes), with an intermediate temperature increase, in both cases, of 10 °C/min.

The influence of the locations where cheeses were produced in residual levels detected for each pesticide and in the total residues was analysed for statistical significance by using the analysis of variance (ANOVA), when possible. In the same way the influence of sampling period was analysed. When the results were significant, Duncan test was used to evaluate differences between data sets and a $P \leq 0.05$ was taken as the level of significance for all analyses. The STATGRAPHICS program [9] was employed to carry out the statistical tests.

RESULTS AND DISCUSSION

The mean concentrations (in ppm/fat basis), ranges and incidence percentages of organochlorine residues obtained in the 92 cheese samples analyzed are shown in Tables I to VI. These tables have been elaborated taking into account the year (1993 and 1994, when samples were collected).

In three samples no insecticide was detected; one of them belonged to the brand no. 1 and the other two to the brand no. 6. Heptachlor epoxide and lindane were found in 58 and 57 cheese samples, respectively, and they were the most frequently detected pesticides. p,p'-DDE was present in 30 samples, being the organochlorine compound belonging to the DDT group most frequently seen.

Taking into account the brands and the year of sampling, heptachlor epoxide was the most detected pesticide in the brands 2, 3, 4, 5 (in 1993) and 6 (in 1993). Lindane was also detected at the highest percentage in the brands 1 (in 1993), 3 (in 1993, together with heptachlor epoxide), and 4, 5, and 6 (in 1994). In some samples the presence of aldrin is detected, in spite of this insecticide is enzymatically oxidized to dieldrin. Other authors [1,3] have also detected aldrin in milk samples and this fact is reported in the *Monograph on*

TABLE I Mean, incidence (%) and range (ppm) of organochlorine pesticides in cheese brand no. 1

	1993	1994	Total (1993, 1994)
Lindane	0.0557 (75%) 0.0028–0.0899	0.0533 (25%) 0.0460–0.0607	0.0551 (50%) 0.0028–0.0899
Heptachlor epoxide	0.0378 (25%) 0.0129–0.0627	0.0045 (12.5%) —	0.0267 (18.75%) 0.0045–0.0627
DDE	—	0.1825 (37.5%) 0.0913–0.2581	0.1825 (18.75%) 0.0913–0.2581
o,p'-TDE	—	0.0127 (62.5%) 0.0091–0.0157	0.0127 (31.25%) 0.0091–0.0157
p,p'-TDE	—	0.0059 (12.5%) —	0.0059 (6.25%) —
Aldrin	0.0141 (37.5%) 0.0073–0.0205	0.0030 (12.5%) —	0.0113 (25%) 0.0030–0.0205
Endrin	—	0.0123 (12.5%) —	0.0123 (6.25%) —
Mean Load	0.0565	0.0929	0.0747

TABLE II Mean, incidence (%) and range (ppm) of organochlorine pesticides in cheese brand no. 2

	1993	1994	Total (1993, 1994)
Lindane	0.0140 (50%) 0.0097–0.0188	0.0292 (37.5%) 0.0017–0.0490	0.0205 (43.75%) 0.0017–0.0490
Heptachlor epoxide	0.0132 (87.5%) 0.0021–0.0376	0.0045 (37.5%) 0.0030–0.0052	0.0106 (62.5%) 0.0021–0.0376
DDE	0.0166 (37.5%) 0.0056–0.0377	0.0221 (37.5%) 0.0184–0.0251	0.0194 (37.5%) 0.0056–0.0377
o,p'-TDE	0.0071 (37.5%) 0.0035–0.0112	—	0.0071 (18.75%) 0.0035–0.0112
p,p'-TDE	—	0.0082 (37.5%) 0.0021–0.0191	0.0082 (18.75%) 0.0021–0.0191
DDT	0.0069 (12.5%) —	0.0390 (25%) 0.0079–0.0701	0.0283 (18.75%) 0.0069–0.0701
Aldrin	0.0033 (37.5%) 0.0019–0.0056	0.0044 (37.5%) 0.0011–0.0069	0.0038 (37.5%) 0.0011–0.0069
Dieldrin	0.0025 (12.5%) —	0.0039 (25%) 0.0031–0.0047	0.0035 (18.75%) 0.0025–0.0047
Mean Load	0.0299	0.0416	0.0353

TABLE III Mean, incidence (%) and range (ppm) of organochlorine pesticides in cheese brand no. 3

	1993	1994	Total (1993, 1994)
Lindane	0.0076 (75%) 0.0017-0.0155	0.0154 (37.5%) 0.0018-0.0273	0.0102 (56.25%) 0.0017-0.0273
Heptachlor epoxide	0.0120 (75%) 0.0022-0.0381	0.0045 (50%) 0.0013-0.0069	0.0090 (62.5%) 0.0013-0.0381
DDE	0.0064 (37.5%) 0.0057-0.0075	0.0308 (50%) 0.0157-0.0462	0.0203 (43.75%) 0.0057-0.0462
o,p'-TDE	0.0029 (37.5%) 0.0009-0.0064	0.0156 (12.5%) —	0.0061 (25%) 0.0009-0.0156
p,p'-TDE	—	0.0113 (25%) 0.0022-0.0204	0.0113 (12.5%) 0.0022-0.0204
DDT	—	0.0812 (25%) 0.0052-0.1572	0.0812 (12.5%) 0.0052-0.1572
Dieldrin	0.0029 (12.5%) —	0.0036 (50%) 0.0012-0.0056	0.0034 (31.25%) 0.0012-0.0056
Aldrin	—	0.0049 (12.5%) —	0.0049 (6.25%) —
Endrin	—	0.0110 (12.5%) —	0.0110 (6.25%) —
Mean Load	0.0197	0.0767	0.0482

Residues and Contaminants in Milk and Milk Products, published in 1991 by the International Dairy Federation [10].

DDT and analogs were generally found at low percentages and in brand no. 1 DDT was not detected in any sample. Nevertheless, the highest mean and individual concentrations were determined for pesticides of this group. So in both years, the highest individual concentration was for DDT: 0.9048 ppm (brand no. 6) in 1993 and 0.8581 ppm (brand no. 5) in 1994. In relation to the organochlorine pesticide mean load, it ranged in 1993 from 0.0197 ppm (brand no. 3) to 0.3114 ppm (brand no. 6), and from 0.0416 ppm (brand no. 2) to 0.3000 ppm (brand no. 5) in 1994.

Organochlorine residue levels detected in cheese samples were compared to the maximum residue limit recommended by the Codex Committee FAO/WHO for Pesticide Residues and also to the Maximum Residue Limits for Chlorinated Pesticides proposed by the European Union.

The Maximum Residue Limits for Chlorinated Pesticides proposed by the European Union in cheese are (expressed in mg/kg on fat basis) 0.2 for lindane, 0.15 for dieldrin (incl. aldrin), 1 for Σ DDT, 0.02 for endrin, and 0.1 for

TABLE IV Mean, incidence (%) and range (ppm) of organochlorine pesticides in cheese brand no. 4

	1993	1994	Total (1993, 1994)
Lindane	0.0050 (62.5%) 0.0038-0.0060	0.0153 (100%) 0.0113-0.0193	0.0096 (75%) 0.0038-0.0193
Heptachlor epoxide	0.0266 (100%) 0.0019-0.0769	0.0077 (100%) 0.0024-0.0172	0.0203 (100%) 0.0019-0.0769
DDE	0.0180 (37.5%) 0.0076-0.0232	0.0458 (25%) —	0.0249 (33.33%) 0.0076-0.0458
o,p'-TDE	0.0111 (37.5%) 0.0008-0.0170	0.0120 (75%) 0.0041-0.0183	0.0115 (50%) 0.0008-0.0183
p,p'-TDE	—	0.0266 (25%) —	0.0266 (8.33%) —
DDT	0.0058 (12.5%) —	0.1039 (25%) —	0.0548 (16.67%) 0.0058-0.1039
Aldrin	0.0036 (12.5%) —	—	0.0036 (8.33%) —
Dieldrin	—	0.0063 (25%) —	0.0063 (8.33%) —
Mean Load	0.0418	0.0777	0.0538

TABLE V Mean, incidence (%) and range (ppm) of organochlorine pesticides in cheese brand no. 5

	1993	1994	Total (1993, 1994)
Lindane	0.0088 (87.5%) 0.0018-0.0275	0.0339 (87.5%) 0.0154-0.0460	0.0213 (87.5%) 0.0018-0.0460
Heptachlor epoxide	0.0120 (100%) 0.0021-0.0395	0.0092 (37.5%) 0.0077-0.0115	0.0112 (68.75%) 0.0021-0.0395
DDE	0.0018 (12.5%) —	0.0180 (62.5%) 0.0082-0.0314	0.0153 (37.5%) 0.0018-0.0314
o,p'-TDE	—	0.0048 (62.5%) 0.0009-0.0141	0.0048 (31.25%) 0.0009-0.0141
p,p'-TDE	—	0.0222 (12.5%) —	0.0222 (6.25%) —
DDT	—	0.3995 (62.5%) 0.0579-0.8581	0.3995 (31.25%) 0.0579-0.8581
Dieldrin	—	0.0013 (12.5%) —	0.0013 (6.25%) —
Mean Load	0.0216	0.3000	0.1600

TABLE VI Mean, incidence (%) and range (ppm) of organochlorine pesticides in cheese brand no. 6

	1993	1994	Total (1993, 1994)
Lindane	0.0073 (37.5%) 0.0050-0.0102	0.0253 (87.5%) 0.0156-0.0537	0.0199 (62.5%) 0.0050-0.0537
Heptachlor epoxide	0.0283 (75%) 0.0063-0.0497	0.0207 (75%) 0.0042-0.0782	0.0245 (75%) 0.0042-0.0782
DDE	0.1876 (25%) 0.0261-0.3492	0.0121 (25%) 0.0099-0.0142	0.0998 (25%) 0.0099-0.3492
o,p'-TDE	0.0296 (12.5%) —	0.0260 (37.5%) 0.0239-0.0294	0.0269 (25%) 0.0239-0.0296
p,p'-TDE	0.1762 (25%) 0.0056-0.3467	0.0262 (37.5%) 0.0042-0.0411	0.0862 (31.25%) 0.0042-0.3467
DDT	0.9048 (12.5%) —	0.2131 (25%) 0.1762-0.2501	0.4437 (18.75%) 0.1762-0.9048
Aldrin	0.0055 (12.5%) —	0.0113 (50%) 0.0064-0.0187	0.0101 (31.25%) 0.0055-0.0187
Dieldrin	0.0046 (25%) 0.0032-0.0059	0.0063 (50%) 0.0026-0.0100	0.0057 (37.5%) 0.0026-0.0100
Mean Load	0.3114	0.1223	0.2034

heptachlor (incl. epoxide). These values are similar to those proposed by the Codex Committee on Pesticide Residues FAO/WHO in milk (mg/kg on fat basis): 0.25 for lindane, 0.15 for heptachlor (incl. epoxide), 0.15 for aldrin (incl. dieldrin), and 1.25 for DDT (incl. metabolites).

In any case the maximum residue limits established by the organisms above mentioned were exceeded by the detected concentrations in cheese samples analyzed in this study. The comparison of the results obtained with the maximum levels allowed by EU for cheese samples can be seen in Figure 2.

We have not found any references reporting organochlorine residue levels in cheese. Losada *et al.* [3] have published in 1996 a paper studying organochlorine residues in milk samples. The samples had been collected in 1992 in several farms located in the southern area of the province of León, near the factories where cheese brands no. 4, 5 and 6 were manufactured. In the study, lindane was the most detected pesticide (66.7%) and the highest mean residue level was for p,p'-DDE (0.005 ppm/wet weight). Heptachlor epoxide was only found in 20.5% of the samples and DDT and related compounds in 23%.

Hernández *et al.* [11] carried out a study in human breast milk collected in Madrid. These authors found 100% incidence percentage for DDT and

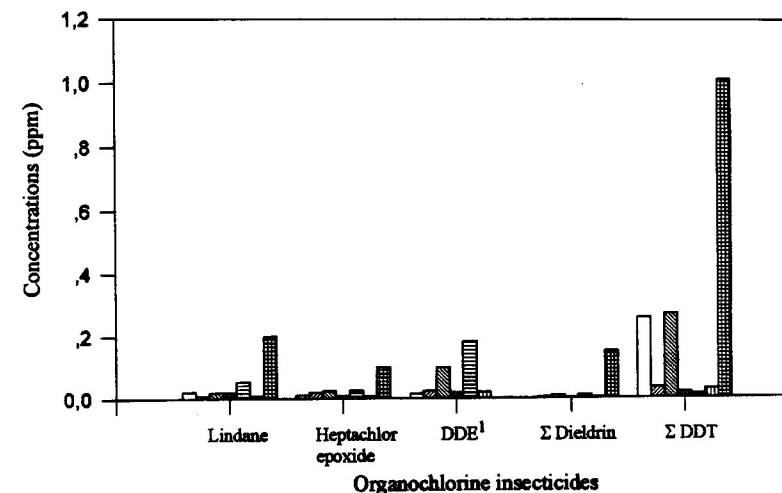


FIGURE 2 Mean levels (in ppm/fat basis) of organochlorine pesticides in the six cheese brands: 1 (white), 2 (diagonal lines), 3 (cross-hatch), 4 (solid black), 5 (horizontal lines), 6 (vertical lines) and Maximum Residue Limits by the EU (hatched).

¹Not established value.

related compounds and 68.6% for lindane. The lindane incidence percentage in this study was very similar (62.0%) but the incidence for DDT and related compounds was lower (59.9%).

In order to evaluate the influence of the cheese brand and date of sampling on the total load, a two-way analysis of variance (ANOVA) was used. No significant differences were found in relation to sampling period (spring, summer, autumn, winter), but the total load showed significant differences between the brand no. 6 (\bar{x} = 0.2034 ppm) and the brands no. 2 (\bar{x} = 0.0353 ppm), no. 3 (\bar{x} = 0.0482 ppm) and no. 4 (\bar{x} = 0.0538 ppm). When samples were grouped according to the year of sampling (1993, 1994) the one-way ANOVA analysis indicated that there were only significant differences in the total load for cheese samples in brand no. 5 (\bar{x} = 0.0216 ppm in 1993 and \bar{x} = 0.3000 ppm in 1994). In 1993 significant differences were found between the total load of cheese no. 6 and all the others, but in 1994 there were no significant differences.

Finally, in order to evaluate the influence of geographical area on the total load the samples were grouped taking into account the location of the factories: samples from north area (brands no. 1, 2, and 3) and from south area (brands no. 4, 5, and 6). The ANOVA analysis indicated that there were no significant differences between different periods of sampling but it showed significant differences in pesticide load between the two geographical areas.

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