Organochlorine Pesticides in Spanish Sterilized Milk and Associated Health Risks

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ABSTRACT

Organochlorine pesticides (α-HCH, β-HCH, lindane, δ-HCH, aldrin, dieldrin, heptachlor, heptachlor epoxide, clordane, pp'-DDE, op'-DDD, pp'-DDD, op'-DDT, pp'-DDT endrin and methoxychlor) were investigated in Spanish sterilized milks. Ninety percent of the milk samples were found to be contaminated. Thirty-two percent of the samples were contaminated by clordane and showed levels which exceeded the maximum residue limit permitted in the Directive 86/386/EC for organochlorines in milk as calculated on a lipid basis. Aldrin was only found in one sample, while 89.9% of the samples were contaminated by one or more HCH isomers. Mean heptachlor epoxide contamination (0.026 ppm) was approximately 10 times higher than contamination by heptachlor. There is no health risk involved in the consumption of using sterilized milk in Spain.

The investigation of organochlorine pesticides is of interest from a sanitary, ecological, economic, social and purely scientific point of view since they are biocides which are not easily eliminated from the environment. The pesticides enter the food chain, especially through fats, and present a potential health risk. These compounds modify the ecosystem and are capable of eliminating many species; they influence national and international trade, and their social impact lies in the ever increasing ecological interest in potential dangers to the world.

For these reasons, we decided to ascertain the qualitative and quantitative contamination of sterilized milk by organochlorine pesticides and any health risks which might be associated with their consumption.

MATERIALS AND METHODS

Samples

Two hundred and eight samples of sterilized milk (ultrahigh temperature process) from all parts of Spain were bought at ordinary commercial outlets.

Analytical procedures

Fat was extracted following the procedures of the Belgian Norm (5): shake 100 ml milk with 400 ml acetone and 100 ml petroleum ether for 2 min in a separating funnel. Discard the aqueous phase, add 100 ml petroleum ether, and shake again for 1 min. Wash the organic phase with 200 ml distilled water, discard the aqueous layer, and dry the solvent solutions over sodium sulphate anhydrous and evaporate in a flask using the rotary evaporator. The organochlorine pesticides were extracted and purified following the method of Bush et al. (9) adapted to the compounds in question: a column packed with florisor which had been activated at 200°C for 12 h, cooled to ambient temperature in a desiccator, and deactivated by adding 2% distilled water, and anhydrous sodium sulphate was used to remove any remaining water. The pesticides were extracted in two fractions, the first by eluting the column with hexane to obtain pp'-DDE, aldrin, and heptachlor and the second by eluting with a mixture of hexane and methylene chloride to obtain dieldrin, endrin, heptachlor epoxide, pp'-DDT, op'-DDT, pp'-DDD, op'-DDD, HCH group clordane and methoxychlor.

To study recovery, we used different proportions and quantities of solvents to those used by Bush et al. (9). Sixty milliliters hexane was used for the first fraction and 60 ml of a hexane/ethyl ether mixture (9:1 vol/vol) for the second. Both volumes are different to those mentioned in the method of Bush; 0.5 g of fat and 2 ml hexane were transferred to the column with 10 g florisor and 1 cm sodium sulphate for the two elutions. For every 5 samples, a column blank was carried out.

The organochlorine pesticides were determined by a Hewlett Packard 5890 gas chromatograph with a 63Ni electron capture detector. The experimental conditions were as follows: injection temperature 225°C, oven temperature 190°C, and detector temperature 225°C. The carrier gas was argon/methane 95:5 with a flow rate of 34 ml/min. The column was of neutral glass 2 m x 2-mm inside diameter. The packing was 5% QF on a Chromosorb of 80-100 mesh. The results were confirmed by using a packed column, 3% SE-30, on Chromosorb of 80-100 mesh of neutral glass 2 m x 2-mm inside diameter. The standardized chromatographic conditions were injection temperature 240°C, oven temperature 184°C, and detector temperature 225°C.

All the samples were analyzed in duplicate.

The pesticide standards used were from Supelco, Inc., Bellefonte, PA.

Quantification

A Hewlett Packard 3392 A integrator was used for quantification, and the calculation procedure was based on the external standard method.

Reproducibility

The reproducibility of the results in the experimental conditions of the equipment was tested by studying the results of 10 consecutive injections of pesticides standard solutions (0.005, 0.05, and 0.01 ppm) under the same conditions as the samples were tested.
**Detection and recovery limits of the method**

The criterion of the American Chemistry Society (1) was followed to calculate the detection limit. Detection limits were as follows: α-HCH, aldrin, and clordane 0.003 ppm; β-HCH, lindane, δ-HCH, dieldrin, heptachlor, heptachlor epoxide, pp'-DDE, op'-DDD, pp'-DDD, and pp'-DDT 0.001 ppm; op'-DDT 0.01 ppm.

Recoveries were determined by direct fortification of whole milk with solutions of known concentrations of each of the pesticides under study. A complete analysis was undertaken in accordance with the procedures described. A blank study was also made with the same milk (Table 1).

**TABLE 1. Pesticide recoveries in whole milk.**

<table>
<thead>
<tr>
<th>Organochlorine pesticides</th>
<th>Recoveries (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-HCH</td>
<td>72.80</td>
</tr>
<tr>
<td>β-HCH</td>
<td>31.26</td>
</tr>
<tr>
<td>Lindane</td>
<td>100.00</td>
</tr>
<tr>
<td>δ-HCH</td>
<td>100.00</td>
</tr>
<tr>
<td>Clordane</td>
<td>72.46</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>55.88</td>
</tr>
<tr>
<td>Hep. epoxide</td>
<td>88.80</td>
</tr>
<tr>
<td>Aldrin</td>
<td>76.01</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>100.00</td>
</tr>
<tr>
<td>pp'-DDE</td>
<td>85.80</td>
</tr>
<tr>
<td>op'-DDD</td>
<td>44.44</td>
</tr>
<tr>
<td>pp'-DDD</td>
<td>53.70</td>
</tr>
<tr>
<td>op'-DDT</td>
<td>0.00</td>
</tr>
<tr>
<td>pp'-DDT</td>
<td>38.52</td>
</tr>
<tr>
<td>Endrin</td>
<td>100.00</td>
</tr>
<tr>
<td>Metylchlor</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Contaminated with 3 ppm. Each value is the average of three samples analyses.

The response linearity of the detector used was tested under the same conditions for small concentration intervals of aldrin (0.2-2 ng), and the regression line was obtained. The correlation coefficient was \( r = 0.98 \).

**Cleaning the materials**

The glassware used in the preparation of standards and in the extraction of fats and pesticides was cleaned using the method of Garrido et al. (12).

**Distillation methods and purity tests**

The solvents used were distilled following the Pesticides Analytical Technique (3), using a glass column with Rasching rings in the presence of sodium hydroxide, calcium hydroxide, and activated carbon.

The purity of the solvents, distilled water and sodium anhydride was checked by gas chromatography (3).

**RESULTS AND DISCUSSION**

The results obtained for each pesticide are shown in Tables 2 and 3 and are explained below.

**Hexachlorocyclohexane**

The level of α-HCH in milk ranged between 0.003 and 1.292 ppm with a mean level of 0.043 ppm. Of the samples analyzed, 30.2% contained α-HCH, β-HCH was found in fewer samples. Lindane was present in 34.6% of the samples analyzed with an average concentration of 0.012 ppm. The most commonly found isomer was δ-HCH which had the highest mean concentration (0.128 ppm).

A comparison with previous studies of Spanish milk (16) shows that the level of HCH contamination has fallen, although it has not been totally eliminated. This improvement is undoubtedly due to the prohibition of HCH in phytosanitary products and insecticide fertilizers in 1976; however, it has not been banned for use as a disinfectant in cowsheds or in skin antiparasitical compounds, which remain the most important sources of contamination alongside persistent presence of the lindane in the environment.

The bibliography consulted shows no reference to δ-HCH in milk, although it is the most commonly found and most highly concentrated isomer of the four HCH isomers in Spanish milk.

The percentage of samples which exceeded the European Community (EC) limits (8) was 5.7% for α-HCH, 5.7% for β-HCH, and 1.4% for δ-HCH, all less than those found by Pozo et al. (16) and by Martinez et al. (14). The European Economic Community Directive did not mention δ-HCH, although levels of this HCH were also lower.
than for previous years, a fact also noted by Pines et al. (15) in a study of milks from Israel in the period 1976-1986.

**Aldrin and dieldrin**

Aldrin was only detected in one sample at a concentration of 0.003 ppm. Dieldrin was present in 54.3% of the samples analyzed with a mean concentration of 0.026 ppm, above that detected by Lauro et al. (13) in Italian pasteurized milks and by Frank et al. (11) in Ontario, although in this case the number of positive samples found was higher than in our studies.

A comparison with the studies of Pozo et al. (16) shows that dieldrin levels have increased by 92.5%, while those of aldrin have fallen by almost 100%.

The EC has established a maximum limit for aldrin + dieldrin in milk as 0.15 ppm, a figure which is exceeded in 1.9% of the samples analyzed.

Wedberg et al. (17) in Illinois found no samples which exceeded the limits laid down for dieldrin by the American Environmental Protection Agency and the Food and Drug Administration. Pines et al. (15) found no samples exceeding the limits of Food and Agricultural Organization/World Health Organization (6) in Israel.

**Heptachlor and heptachlor epoxide**

Seven and six-tenths percent of the samples studied showed contamination by heptachlor. Contamination by heptachlor epoxide was higher (30.7% of the samples) with an average value of 0.026 ppm. Frank et al. (11) found that 99% of the samples were contaminated.

The mean concentration of heptachlor epoxide was 10 times higher than levels for heptachlor contamination. Pozo et al. (16) found higher heptachlor levels in 1977 (0.066 ppm) than we did in 1990 (0.002 ppm). The contrary was true in the case of heptachlor epoxide.

The results show a 96% reduction in heptachlor levels in Spanish sterilized milk since 1977 and a 44% increase in heptachlor epoxide. The results for the latter compound may be due to the epoxidation of heptachlor in the environment (11) and the fall in heptachlor due to the restrictions placed on organochlorine pesticides.

The EC (8) recommends limits for heptachlor and heptachlor epoxide residues as 0.10 ppm, a figure exceeded by 8.6% of the milk samples analyzed.

**Chlordane**

Chlordane contamination ranged from 0.0004 and 1.292 ppm with a mean value of 0.067 ppm. The number of contaminated samples represents 47.5% of the samples analyzed, while Frank et al. (6) found that only 2% of the milk samples analyzed in Ontario were contaminated.

Spanish sterilized milk has suffered a considerable increase (98.8%) in chlordane contamination since the studies of Pozo et al. (16). Thirty-two percent of the samples exceed the limits of the EC Directive (8) which is set at 0.05 ppm. These results suggest that, despite its prohibition in 1977, chlordane is still widely used in Spanish agriculture and that greater control should be exercised.

**Endrin and methoxychlor**

Neither endrin nor methoxychlor was detected in the samples. Frank et al. (11) found similar results in a survey of milk in Ontario.

**DDT and metabolites**

The minimum level of pp'-DDE detected was 0.003 ppm and the maximum 1.484 ppm. Mean concentration was 0.024 ppm and this compound contaminated 27.4% of samples.

The mean concentration of op'-DDD was 0.015 ppm, ranging from 0.003 to 0.187 ppm. pp'-DDD contamination ranged from 0.002-0.187 ppm with a mean of 0.009 ppm. pp'-DDT contamination ranged from 0.021-0.157 ppm with a mean level of 0.006 ppm. No case of op'-DDT contamination was detected.

The mean value of DDT and its metabolites was 0.056 ppm, which is higher than those obtained by other authors (11, 13, 15); 58.6% of the samples showed contamination by DDT or one of its metabolites.

Contamination of Spanish sterilized milk by DDT and its metabolites has fallen since the investigations of Pozo et al. (16) and Martinez et al. (14). The mean value found for total DDT by Pozo et al. (16) was 0.153 ppm, which means that levels have fallen by 63%. The percentage of samples found to be contaminated by the above authors was 79 and 100%. This has fallen to 58.6% in our case. Frank et al. (11) also detected a fall of 90% in DDT total in the period from 1967 to 1983. This tendency was noted by Pines et al. (15).

The most widely found metabolite was pp'-DDD (28.3%) followed by pp'-DDE (27.4%). pp'-DDT contamination was detected in 8.6% of the samples, while no traces of op'-DDT were found. DDT metabolites were more commonly found than DDT itself, which agrees with the results obtained by Dogheim et al. (10) in Egypt. This is probably due to the rapid degradation of DDT into its metabolites in the environment.

Only one sample exceeded the limits of the EC Directive (8) for DDT total permitted (1 ppm). No such cases were found by Lauro et al. (13), Frank et al. (11), Pines et al. (15), and Dogheim et al. (10).

Pozo et al. (16) found that 1.2% of samples exceeded Food and Agricultural Organization/World Health Organization (2) limits of 1.25 ppm. Martinez et al. (14) found 16% of the samples they analyzed exceeded this limit. These data corroborate our findings of a qualitative and quantitative diminution of DDT in sterilized milks in Spain since earlier works.

In general, then, there has been a fall in organochlorine pesticides found in Spanish sterilized milk since previous studies, although chlordane, heptachlor epoxide and dieldrin levels have increased.

**Daily intake estimation**

We have examined the health risks which might be associated with the consumption of milk containing organochlorine pesticides in excess of the maximum levels recommended by the EC Directive (8). The average daily intake (ADI) of these pesticides in milk were calculated.
and compared with ADI data from the Food and Agricultural Organization/World Health Organization (4) (Table 4). This figure is based as the average daily consumption of milk in Spain, 343 ml per person per day (7) and according to the mean concentration of each pesticide. There is no ADI for α-HCH and β-HCH.

**TABLE 4. Average daily-intake and daily intake (DI) estimation of the organochlorine pesticides.**

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>ADI</th>
<th>DI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lindane</td>
<td>0.1</td>
<td>0.00014</td>
</tr>
<tr>
<td>Clordane</td>
<td>0.001</td>
<td>0.00073</td>
</tr>
<tr>
<td>Heptachlore total</td>
<td>0.0005</td>
<td>0.00031</td>
</tr>
<tr>
<td>Aldrin + dieldrin</td>
<td>0.0001</td>
<td>0.00029</td>
</tr>
<tr>
<td>DDT + metabolites</td>
<td>0.005</td>
<td>0.00061</td>
</tr>
</tbody>
</table>

\* ADI ppm (4).

Despite the high percentage of samples exceeding the EC limits as regards the maximum permitted levels of chlordane in milk, the average Spanish daily consumption of this pesticide in milk was 0.00073 ppm. This, in itself, represents little risk to health, although the possible long-term effects of pesticide accumulated in human fat tissue should not be forgotten.

At present levels, the consumption of Spanish sterilized milk does not pose a health risk for the consumer.

**REFERENCES**