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Organochlorine Pollutants in Meats and Cow's Milk from Madrid (Spain)

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It is well known that the widespread use of organochlorine insecticides in agriculture and polychlorinated biphenyls (PCBs) in industry has produced serious problems to man due to their capacity to accumulate in food chains. The organochlorine compounds (OCs) are highly lipophilic and can accumulate in fat-rich foods, such as milk and meats; thus, it is not surprising that high levels of these compounds have been found in human breast milk and adipose tissue, given that milk and animal meats play a central role in human nutrition. Environmental contamination with OCs has decreased in Spain since the agricultural use of the organochlorine insecticides were first restricted and then banned in 1977, with the exception of γ-HCH, and the use of PCBs has been reduced in Spain; however, several recent studies indicate that pollution with OCs still does exit (González et al. 1991, Hernández et al. 1992).

In this study we have attempted: a) to estimate the levels of OCs in cow's milk and meat samples collected in Madrid (Spain) during 1990-91; b) to find out if the residue levels exceeded the limits recommended by the European Economic Community (EEC); c) to compare our results with those published in Spain and other countries.

MATERIALS AND METHODS

Cow's milk and meat samples were acquired at random in 1990 and 1991 in different markets in Madrid. The samples were frozen as soon as practicable and kept at -20°C until used for analysis. The analysis of the samples was carried out essentially as previously described by Tessari and Savage (1980) and Holt et al. (1986). Thus, samples of meat (10 g) were diced into 0.5-1 g pieces and ground in a glass mortar with washed sand and sodium sulfate anhydrous, whereas cow's milk samples (10 g) were dispersed in sodium sulphate anhydrous; both mixtures were extracted with hexane-acetone in a Soxhlet apparatus. The extracts were then evaporated by a purified Nitrogen flush whithout external heating and the amount of fat accurately weighed. OCs were isolated by a liquid-liquid partitioning

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process and purified chromatographically on a Florisil 60-100 mesh column; cleanup was achieved using 25% diethyl ether in hexane as eluate.

Gas chromatography analysis was done on a Hewlett-Packard 5890 GC equipped with Ni 63 Electron Capture Detector. A 30 m long capillary column covered with RSL-200 was used. Chromatographic conditions were as follow: detector 280°C; injector 300°C; temperature programme, isothermal phases at 180°C (1 min) and 250°C (30 min), with intermediate temperature increase rate of 2°C/min. Peaks were identified on the integrator by retention times; tolerance allowed was ± 0.05 min. A 50 m capillary column covered with BP-5 was used to confirm the identity of the organochlorine pollutants. The peaks were measured by area counts given by the integrator (minimum count 500). Quantitation was done comparing the peak areas in the sample with those in corresponding standards. PCBs were quantitated by comparing the peak areas from seven major peaks with those of Aroclor 1260. Recoveries of OCs ranged from 89-101%, except for HCB which showed recoveries of 80%, but the residue data in the tables were not adjusted on the basis of these recoveries. The detection limit was 1 ng/g for organochlorine insecticides and 10 ng/g for PCBs. Levels were expressed in ng/g (fat basis). For samples in which no residues could be detected, a value of one-half the reportable limit was assigned for statistical analysis.

RESULTS AND DISCUSSION

Results in ng/g fat basis from 30 analyzed cow's milk samples are given in Table 1. It can be seen that all the cow's milk samples contained PCBs; γ-HCH, heptachlor epoxide and p,p'DDE were present in the greater part of the samples analyzed, whereas a lower percentage of positive results were observed for α-HCH and ß-HCH. No residues of other chlorinated insecticides included in the analytical survey were detected (HCB, aldrin, dieldrin, heptachlor, dichlorobenzophenone, p,p'TDE and p,p'DDT).

The EEC (1986) established as maximum residue values in milk 150 ng/g fat basis for aldrin + dieldrin, 50 ng/g f.b. for chlordane, 1,000 ng/g f.b. for total DDT compounds, 100 ng/g f.b. for heptachlor + heptachlor epoxide and α-HCH, 250 ng/g f.b. for HCB, 75 ng/g f.b. for ß-HCH and 200 ng/g f.b. for γ-HCH. None of the mean levels of organochlorine insecticides investigated in this study exceeded the EEC recommendations. However, 3.3% of cow's milk samples contained amounts of heptachlor + heptachlor epoxide exceeding the EEC recommendations, as did 16.6% of samples for ß-HCH, 10% of samples for α-HCH, and none for aldrin + dieldrin, chlordane, total DDT compounds, HCB and γ-HCH.

In Table 2 the levels of organochlorine insecticides in cow's milk recorded in the present work were compared with those of previous Spanish investigations. When results from previous studies from 1972 and 1977 were compared to our 1990-91 data, significant reductions of the residue levels of organochlorine insecticides took place in milk samples, with the exception of heptachlor epoxide. However,

Table 1. Residues of organochlorine pesticides and PCBs in cow's milk (ng/g fat basis) in Madrid.

Residue	Mean	S.D.	Min.	Max.	Freq.
α-HCH	25.0	48.5	N.D.	170	27.6
ß-HCH	26.7	55.5	N.D.	195	48.3
γ-HCH	33.9	30.0	N.D.	120	89.6
H.ep.	18.7	43.2	N.D.	222	93.1
p,p'DDE	13.9	23.9	N.D.	97.5	72.4
PCBs	579	398	114	1794	100

Number of samples = 30; S.D. = Standard deviation; Min. = Minimum; Max = Maximum; Freq = Frecuency of positive samples; N.D. = Not detected; H. ep. = Heptachlor epoxide.

when comparing research from 1987 to our findings in 1990-91 this decrease is less evident. Thus, HCB, aldrin, dieldrin and total DDT compounds levels in our study are lower than levels reported by De la Riva and Anadón (1991), whereas α, ß, γ-HCH and heptachlor epoxide levels in our study are higher than those reported by De la Riva and Anadón. The comparison of the present investigation with those of previous surveys in Spain indicates the effectiveness of regulatory actions adopted, since the agricultural use of the organochlorine insecticides were first restricted and then banned in Spain in 1977, with the exception of γ-HCH.

Table 2 also shows chlorinated hydrocarbon residue levels in cow's milk samples of several nations. In comparison with published data, and with some exceptions (India, Israel 1976), levels of α, ß and γ-HCH are higher in Spanish milk samples than elsewhere; it may be because lindane is still commercially available in Spain. On the other hand, the levels we obtained for dieldrin and heptachlor epoxide are much lower than those recorded in Table 2; likewise, the total DDT compound levels in our study are lower than levels reported in other countries, reflecting that some countries are still consumers of p,p'DDT or that our values are older than other nations which restrictions put on the use of this compound. Finally, mean levels of PCBs in this work are higher than those reported by Greece and Canada; the use of PCBs has been reduced in Spain, but it is probably too early to observe the effect of restrictive measures on the concentrations in cow's milk, given that the ubiquity and the persistence of these compounds is great.

Results in ng/g fat basis from 36 analyzed lamb samples, 22 pork samples, 30 chicken samples and 41 cattle samples are given in Table 3. As is shown in this Table all the samples contained PCBs; organochlorine insecticide levels and percentage of positive results in lamb and chicken samples were higher than those found in pork and cattle samples. No residues of other chlorinated insecticides included in the analytical survey were detected (HCB, aldrin, dieldrin, heptachlor, dichlorobenzophenone, p,p'TDE and p,p'DDT).

The EEC (1986) established as maximum residue value for meat of 20 ng/g fat

Table 2. Organochlorine compounds in cow's milk in various countries. Mean (ng/g fat basis).

Country	Yr	N	α-HCH	ß-HCH	γ-HCH	H.ep.	Dld.	DDE	TDE	DDT	PCBs	Ref
Israel	76	20	N.A.	N.A.	265	167	178	420	346	215	N.A.	1
USA	76	500	N.A.	N.A.	10	50	90			10*	N.A.	2
Israel	83	30	N.A.	N.A.	27	62	51	56	33	15	N.A.	1
Grecia	83	¿?	11	6	11	0	0	N.A.	N.A.	30	0	3
Canada	86	1184	N.A.	N.A.	N.A.	N.A.	N.A.	8.6	N.A.	N.A.	15	4
Israel	86	33	N.A.	N.A.	13	51	18	55	32	0	N.A.	1
Hong-K.	87	310		9**		N.A.	N.A.	<10	0	10	N.A.	5
India	?	80	16	8.7	200	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	6
Spain	72	?	125	N.A.	100	N.A.	N.A.	150	N.A.	100	N.A.	7
Spain	77	329	200	N.A.	71	18	2	68	31	33	N.A.	8
Spain	87	460	18	9	24	0	6***		21*		N.A.	9
Spain	90	30	25	26	34	19	0	14	0	0	579	10

Yr = Year of carrying out; N = Number of samples; H.ep. = Heptachlor epoxide; Dld = Dieldrin; * = p,p'DDE + p,p'TDE + p,p'DDT; ** = α-HCH + γ-HCH; *** = Aldrin + Dieldrin; N.A. = Not analyzed; Ref = References; 1: Pines and Cucos (1988); 2: Wedberg et al. (1978); 3: Fytianos et al. (1985); 4: Frank and Braun (1989); 5: Man Hing Ip (1990); 6: Verma (1990); 7: Carrasco et al. (1976); 8: Pozo et al. (1977); 9: De la Riva and Anadón (1991); 10: This work.

Table 3. Residues of organochlorine pesticides and PCBs in meats (ng/g fat basis) in Madrid.

Cattle (41)					
Residue	Mean	S.D.	Min.	Max.	Freq.
α-HCH	5.7	7.8	N.D.	30	90.2
ß-HCH	3.4	10.4	N.D.	61	26.8
γ-HCH	1.5	3.2	N.D.	15	39.0
p,p'DDE	23.0	57.5	N.D.	340	68.3
PCBs	765	449	180	1660	100
Pork (22)					
Residue	Mean	S.D.	Min.	Max.	Freq.
α-HCH	0.8	1	N.D.	4	50
ß-HCH	1	2.4	N.D.	10	22.7
γ-HCH	0.6	1	N.D.	4	40.9
H.ep.	1.2	3.3	N.D.	11	13.6
p,p'DDE	4	6.1	N.D.	21	72.7
PCBs	787	740	100	2260	100
Lamb (36)					
Residue	Mean	S.D.	Min.	Max.	Freq.
α-HCH	25.1	115	N.D.	693	69.4
ß-HCH	44.7	190	N.D.	1150	61.1
γ-HCH	6.9	15.1	N.D.	60	52.8
p,p'DDE	54.1	94.2	N.D.	540	91.7
PCBs	1032	1013	180	5190	100
Chicken (30)					
Residue	Mean	S.D.	Min.	Max.	Freq.
α-HCH	31.9	90	N.D.	487	66.7
ß-HCH	49.9	152	N.D.	748	50
γ-HCH	59	115	N.D.	459	90
H.ep.	5.4	5.7	N.D.	22.8	93.3
p,p'DDE	39.1	51.6	4.4	58.4	100
PCBs	664	686	132	3268	100

( ) = Number of samples; S.D. = Standard deviation; Min. = Minimum; Max. = Maximum; Freq. = Frequency of positive samples; N.D. = Not detected; H.ep. = Heptachlor epoxide.

basis for aldrin + dieldrin, 5 ng/g f.b. for chlordane, heptachlor + heptachlor epoxide, HCB and α-HCH, 100 ng/g f.b. for total DDT compounds and γ-HCH (200 ng/g f.b. for γ-HCH in lamb) and 10 ng/g f.b. for ß-HCH. None of the

Table 4. Organochlorine insecticides in meats in various countries. Mean (ng/g fat basis).

Country	Yr	Sample	α-HCH	ß-HCH	γ-HCH	HCB	Hpt	Dld	DDE	TDE	DDT	Ref
USA	75	Various		0.5*	0.2	N.A.	N.A.	4	9	1	4	1
Nigeria	83	Cattle	0	10	0	0	N.A.	N.A.	30	40	10	2
Nigeria	83	Chicken	10	30	30	0	N.A.	N.A.	0	0	0	2
Iraq	83	Cattle	N.A.	N.A.	116	N.A.	124	101		875**		3
Iraq	83	Lamb	N.A.	N.A.	225	N.A.	67	67		1048**		3
Hong-K.	87	Various	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	10	10	20	4
Spain	72	Various	33	N.A.	70	N.A.	N.A.	N.A.	167	N.A.	186	5
Spain	?	Pork	40	N.A.	3	230	100	N.A.	20	N.A.	100	6
Spain	90	Cattle	5.7	3.4	1.5	0	0	0	23	0	0	7
Spain	90	Pork	0.8	1	0.6	0	0	0	4	0	0	7
Spain	90	Lamb	25	45	6.9	0	0	0	54	0	0	7
Spain	90	Chicken	32	50	59	0	0	0	39	0	0	7

Yr = Year of carrying out; Hpt = Heptachlor; Dld = Dieldrin; N.A. = Not analyzed; * = α + ß-HCH; ** = p,p'DDE + p,p'TDE + p,p'DDT; Ref = References; 1: Johnson and Manske (1977); 2: Atuma (1985); 3: Al-Omar et al. (1985); 4: Man Hing Ip (1990); 5: Carrasco et al. 1976; Pozo et al. (1982); 7: This work.

mean levels of organochlorine insecticides investigated in this study exceeded the EEC recommendations. However, 2.7% and 5.4% of lamb samples contained amounts of α and ß-HCH, respectively, exceeding the EEC recommendations, as did 3.3% and 10% of chicken samples.

In Table 4 the levels of organochlorine compounds in meats recorded in the present work were compared with those of other countries. The concentrations of organochlorine insecticides reported here are slightly higher than those reported for comparable species samples in Nigeria (Atuma 1985); however, they are lower than those reported for lamb and cattle in Iraq (Al-Omar et al. 1985); likewise, the total DDT compound levels in our study are lower than those estimated by Man Hing Ip (1990) in Hong Kong. The comparison of the results of this study with the data from the Spanish investigations show in Table 4 indicates that organochlorine insecticides are decreasing, although differences in samples exist and the results must be compared with caution.

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