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Effect of Several Kitchen Treatments on Hexachlorocyclohexane Residues in Ovine Meat

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The insecticide lindane (γ-hexachlorocyclohexane, γ-HCH), the only organochlorine pesticide still legal in Spain, is used in agriculture and gardening (seed and soil protection), forestry (wood and timber protection), animal husbandry, and human medicine. Its production involves the formation of several stereoisomers, such as α-, β–, and δ-isomers of HCH. The β-HCH, due to its lipophilic properties, can bioaccumulate in man and animals up to 10-30 times more than the other HCH isomers and amounts to 90% of HCH residues in human adipose tissues (Ruiter 1985).

The 4th Annual Report on Carcinogens (1985) documented experiences in which HCH was reported as a probable carcinogen to man, and this group of compounds had been previously proben carcinogen is to mice (IARC 1979). The IARC also reported 30 human cases of aplasic anemia after exposure to α-HCH and lindane.

The FAO/WHO (1984, 85) established an acceptable daily intake (ADI) of 10 µg/kg body weight for lindane, and Hapke (1983) proposed ADIs of 5 and 1 µg/kg body weight for α– and β-HCH, respectively. The European Community and Spanish regulations for ovine meat set an maximum residue level (MRL) of 2 ppm of lindane, 0.2 ppm of α-HCH, and 0.1 ppm of β-HCH on a fat basis.

Several reports on the effect of technological and culinary processes on the chlorinated contamination of foods showed that a certain degree of decrease can be attained (Liska et al. 1967; Ritchey et al. 1969; Kubacki and Lipowska 1980; Jan and Malnersic 1982; Melkebeke et al. 1983; González and Visweswariah 1984). Additionally, Ohlinger et al. (1982) proposed studies on the fate of HCH isomers and metabolites since they are often present in animal products as a consequence of biological and microbial actions.

However, most of the research has been conducted with fortified samples in which the residues may not have the same behaviour as in naturally-contaminated samples. Therefore, the present study is intended to evaluate several kitchen treatments (grilling, roasting, and cooking) on naturally HCH-contaminated ovine meat. Additionally, because Spain is the second largest producer of ovine meat in the European Community and ranks third in ovine meat consumption, we hope these data will contribute to the general information on current levels of HCH and the estimation of daily HCH intakes.

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MATERIALS AND METHODS

The study was conducted with 75 samples belonging to three commercial pieces of the ovine carcasses (chop, leg and lap). Samples were purchased in local shops and markets of Zaragoza, Spain. Each commercial piece was subjected to a different culinary treatment as previously described (Conchello et al. 1993). Chop samples were grilled without oil or dressing and reached an internal temperature of 85°C. Leg samples were roasted in a conventional electric oven and reached an internal temperature of 100±1°C. Lap samples were subjected to 130°C for 10 min in a pressure cooker.

Levels of α–, β–, and γ–HCH residues were determined. Extraction and cleanup of residues were performed using the method by Telling et al. (1977) as described in Conchello et al. (1992). This method is based on a fat extraction using acetone:hexane (1:4) and further cleanup on a column of alumina. Pesticides are eluted with hexane. The eluate is concentrated and analyzed by gas-liquid chromatography. The fat content of samples was determined in order to express the results on a fat basis.

As it was impossible to obtain samples without HCH residues and due to the absence of international reference material, a recovery study was performed at the cleanup step using two hexane solutions at 1 and 10 ppb of α–, β–, and γ–HCH. Percentages of recovery in four assays (table 1) ranged from 80 to 100% and were considered satisfactory (Mestres 1990).

Table 1. Recovery of two HCH solutions at 1 ppb (1 μg/L) and 10 ppb (10 μg/L).

Pesticide	1-ppb Solution %Recov.±Std. Error (n=4)	10-ppb Solution %Recov.±Std. Error (n=4)
α–HCH	85 ± 5	94 ± 4
Lindane	86 ± 7	92 ± 3
β–HCH	83 ± 6	91 ± 4

Analysis was performed with an HP-5890 gas chromatograph fitted with an electron-capture detector (^{63}Ni). Three packed columns with different stationary phases namely, 1.5% SP-2250/1.95% SP-2401, 5% QF-1, and 10% DC-200, were used for identification and quantitation of residues. Optimum operation parameters were determined for each column employed (table 2). Considering retention times relative to aldrin, the column with mixed phases was chosen for quali-quantitative analysis as it presented the best resolution for the three isomers studied. The other columns were used for confirmatory analysis.

Table 2. Operation parameters of the three packed columns employed.

Parameter	A	B	C
T (°C) Column	200	195	210
T (°C) Inlet	250	250	250
T(°C) Detector	300	300	300
Carrier Gas	Ar/CH_4 or N_2	N_2	Ar/CH_4
Flow (mL/min)	50 - 52	30	58

A: Column 1.5 % SP-2250 + 1.95 % SP-2401
B: Column 5 % QF-1; C: Column 10 % DC-200

Quantification was done by the external standard technique using a multilevel calibration table (1, 10, and 50 ppb). The detection limit of the HCH isomers studied was 4 ppb (μg/kg on fat basis).

Results obtained were statistically analyzed by Wilcoxon's nonparametric test and chi-square (χ_2) test.

RESULTS AND DISCUSSION

Table 3 shows results in μg/kg fat basis obtained for both raw and cooked chop, leg, and lap samples as well as the variation of the HCH content due to the culinary treatment. Statistical results of Wilcoxon's test are also shown. Lindane was present in all treated pieces except for one leg sample. Raw chop showed a mean value of 75 μg lindane/kg on fat basis, while grilled chop averaged 50 μg lindane/kg, that means a 33% reduction ($p < 0.01$) was found. Roasted leg samples yielded the highest percentage of lindane reduction (39%) since the mean concentration dropped from 80 ppb in raw samples to 49 ppb in roasted ones. Cooking treatment of lap samples yielded the lowest lindane losses (17%), from 75 ppb in fresh to 62 ppb after cooking.

Table 3. Effect of culinary treatment on the HCH content in ovine meat samples. (Wilcoxon's test). Results expressed as ppb (μg/kg on a fat basis).

		GRILLING		ROASTING		COOKING	
		Raw	Grilled	Raw	Roasted	Raw	Cooked
γ-HCH	Mean (ppb)	75	50	80	49	75	62
	Standard dev.	145.8	113.4	161.9	94.1	142.5	122.5
	Maximum	488	440	519	329	454	463
	% Reduction	33%		39%		17%	
	Wilcoxon (p)	**$p < 0.01$**		$p > 0.05$		$p > 0.05$	
α-HCH	Mean (ppb)	24	22	24	21	25	23
	Standard dev.	38.1	32.0	39.0	23.6	41.4	30.5
	Maximum	159	129	144	100	155	124
	% Reduction	8%		12%		8%	
	Wilcoxon (p)	$p > 0.05$		$p > 0.05$		$p > 0.05$	
β-HCH	Mean (ppb)	11	14	11	15	14	14
	Standard dev.	13.5	9.9	15.5	12.9	18.9	8.4
	Maximum	54	40	50	55	71	29
	% Increase	27%		36%		0%	
	Wilcoxon (p)	$p > 0.05$		$p > 0.05$		$p > 0.05$	

Although the three treatments produced losses ranging from 17% to 39%, only grilling caused a significant reduction ($p < 0.01$) since the frequency of samples that showed either a decrease or an increase in the lindane level varied among treatments (table 4). Thus, 29 out of the 75 samples (this is 39%) showed a higher lindane level after its culinary treatment; the grill treatment was the most efficient since it produced the highest number of samples (68%) in which the lindane content decreased, followed by cooking (60%) and roast treatment (56%). However, the chi-square test did not detect significant differences ($p < 0.05$) in the number of treated samples showing a lindane reduction after the culinary treatment. Therefore, the variability between treated samples in which the lindane content increased and those showing decreased content could explain the non significant differences obtained when Wilcoxon´s nonparametric test was applied.

Table 4. Effect of culinary treatment on organochlorine residues.(χ^2 test).

Culinary process	α-HCH		β-HCH		Lindane	
	I	D	I	D	I	D
Grilling	36	64	56	44	32	68
Roasting	52	48	64	36	44	56
Cooking	40	60	68	32	40	60
TOTAL	42.67	57.33	62.67	37.33	38.67	61.33
χ^2	p = 0.4923		p = 0.6710		p = 0.6747	

I: Percentage of samples in which the residue level increased.
D: Percentage of samples in which the residue level decreased.

Several authors have reported a decrease of lindane residue level in chicken and bovine meat samples after different heat treatments. Thus, Liska et al. (1967) reported a reduction of 60% lindane on a whole chicken basis after pressure cooking for 1-3 hr, and Ritchey et al. (1972) obtained a much lower percentage of reduction in samples after frying and roasting. Morgan et al. (1972) compared the levels of γ-HCH in raw and cooked chicken tissue and observed a lindane reduction of 60% after pressure cooking, and Gonzalez and Visweswariah (1984) noted that ordinary cooking during 30 min also caused a significant reduction on the lindane content.

Mirna and Coretti (1979) analysed rabbit meat after hot-smoking (2 hr at 60°C) and found a lindane reduction of 12%. They also analysed rabbit meat samples after cooking (1.5 hr at 100°C) and found this treatment as the most effective in eliminating γ-HCH since 65% of the initial contamination was removed. This high losses of γ-HCH were mainly caused by the volatility of this compound. Similarly, Jan and Malnersic (1982) found a reduction in the γ-HCH level in cooked beef meat (2 hr at 115°C). As reported by Kubacki and Lipowska (1980) in canned meat products, a decrease of the initial lindane contamination by about 25% was achieved after pasteurization at 80°C and commercial sterilisation at 120°C. Similar percentage of reduction (27%) was reported by Garcar et al. (1987) in meat products subjected to sterilization. Recently, Ariño (1991) reported a nonsignificant reduction (4%) in the γ-HCH content during the processing of pork bologna. Most of the authors, who found significant γ-HCH losses in meat and meat products, attribute these losses to the volatility of this compound and to the elimination with the fat rendering. This is not likely to happen in meat products filled into artificial, plastic casing.

Our results agree with those obtained by the mentioned authors, although it cannot be supported statistically except for the grill treatment. Therefore we cannot assure that the three culinary treatments studied produced an uniform decrease in the γ-HCH content, mainly because of the high variability observed in the samples. Hovewer, we only studied the effect of treatments on a fat basis, not on a whole or dry-matter basis, so the total pesticide burden may have also decreased by rendering of fat during treatments.

The α- isomer of HCH showed the least reduction by the three kitchen treatments studied. Roasting treatment caused the highest α-HCH reduction (12%) from 24 ppb in fresh to 21 ppb after treatment (table 3). Contamination levels after grill and cooking amounted to 22 ppb and 23 ppb, which means a 8% reduction as compared to raw chop (24 ppb) and lap (25 ppb) samples, respectively.

Additionally, the percentage of detection of α-HCH in grilled chop samples decreased slightly when compared with the raw ones (88% versus 84%), whereas the presence remained similar in leg and lap samples (84% and 76%). None of the three treatments studied yielded significant differences in the α-HCH levels in chop, leg and lap samples as compared by Wilcoxon's test ($p > 0.05$) (table 3). The previously mentioned variability observed in the frequency of samples in which the lindane content changed was also demonstrated for α-HCH (table 4). Thus, 32 out of the 75 samples (43%) experienced an increase of the α-HCH content, being the highest percentage of samples with decreased levels caused by grill (64%), followed by cooking (60%) and roasting (48%). The chi-square test did not indicate significant differences ($p > 0.05$) among treatments, as it was observed with lindane. Our results suggest that there exists a general reduction of mean α-HCH levels after treatment although the individual effects are very heterogeneous.

Gonzalez and Visweswariah (1984) reported a reduction in the α-HCH content in chicken samples after cooking for 30 min. Ohlinger et al. (1982) obtained significant reduction of α-HCH content in beef samples after roasting and microwave treatment, while cooking had a smaller effect. Ariño (1991) did not find any significant reduction of α-HCH in pork bologna after heat treatment.

β-HCH content averaged 14 ppb in grilled chop, 15 ppb in roasted leg, and 14 ppb in cooked lap. When comparing these values with those of raw samples (11 ppb, 11 ppb, and 14 ppb, respectively), it seems that the culinary treatments did not cause a reduction in the β-HCH contamination. Thus, treated chop and leg samples showed higher β-HCH content than that of raw samples, with an increase around 30-40%, whereas after cooking the levels remained unchanged (table 3). The percentage of detection of β-HCH increased after the culinary treatment from 72% (raw) to 88% (treated) in chop and lap, and from 60% to 80% in leg. However, Wilcoxon's test (table 3) indicated that these differences were not significant.

Table 4 shows the percentage of samples in which the β-HCH content increased after treatment. The highest percentage corresponds to cooking treatment (68%), followed by roasting (64%) and grill (56%), although the chi-square test did not show any significant difference among treatments ($p > 0.05$). These results are in good agreement with Gonzalez and Visweswariah (1984) who reported an increase in the β-HCH content in chicken meat after cooking. On the other hand, Ohlinger et al. (1982) observed a significant reduction in the β-HCH content after roasting and microwave treatment. Overall there was a general decrease of α- and γ-HCH in contrast to the increase observed for β-HCH (table 5). Chi-square test shows that only cooking treatment has a qualitative effect on the three HCH isomers and this effect is different from the other two treatments studied ($p < 0.05$).

Table 5. Comparison of culinary treatment on β-HCH and α+γ-HCH.(χ^2 test).

Culinary process	α+γ-HCH I	α+γ-HCH D	β-HCH I	β-HCH D	Test $\chi2$ p
Grilling	34	66	56	44	0.0682
Roasting	48	52	64	36	0.1904
Cooking	40	60	68	32	0.0222

I: Percentage of samples in which the residue level increased.
D: Percentage of samples in which the residue level decreased.

Table 6 shows the total distribution of HCH isomers in both raw and treated samples to evaluate the relative proportion of each compound. β-HCH is the only isomer whose relative proportion increased noticeably (from 10% to 15% in chop, from 9% to 18% in leg, and from 12% to 14% in lap). As discussed previously, there was an increase of 30-40% of β-HCH levels so that some of the lindane lost was likely converted to the β-HCH isomer. Gonzalez and Visweswariah (1984) reported that heat treatments promoted β-HCH isomerization since cooking yielded higher relative proportion of this isomer while that of α–, δ– and γ-HCH decreased. This aspect is important to the human health since β-HCH is the more hazardous isomer of HCH to man. Jan and Malnersic (1982) observed γ-HCH isomerization to α-HCH in beef samples cooked for 2 hr at 115°C and indicated that the fat content plays an important role on the stability of γ-HCH in meat during cooking. Additionally, Van Rethergem (1976) reported that the heat treatment and the homogenising of milk slightly increased the extractability of organochlorine pesticides though HCH isomerizations could not be ruled out.

According to data available from the Meat Annual Report (1990) the ovine meat consumption in Spain during 1988 was 4.3 kg/person. Considering the mean fat content of the analysed samples (20%) and the mean lindane levels detected in all treated samples, the subsequent daily lindane intake reached 0.11-0.15 μg lindane/day. This value is far below the ADI established by the FAO/WHO (1984, 85) since it only represents 0.02% of that acceptable intake. Thus, the risks to human health by consuming ovine meat contaminated by lindane is considerably low. Hapke (1983) proposed an ADI for α– and β-HCH of 5 and 1 μg of pesticide/kg body weight, respectively. Calculated daily intakes for an adult of 70 kg by consuming ovine meat amount to 0.05 μg of α-HCH and 0.03-0.04 μg of β-HCH, representing 0.01% and 0.04-0.06% of the ADI recommended by Hapke

Table 6.- Relative percentage of HCH isomers as affected by the culinary treatment.

SAMPLE	α-HCH	Lindane	β-HCH	Total
Raw chop	22	68	10	100
Grilled chop	23	62	15	100
Raw leg	21	70	9	100
Roasted leg	25	57	18	100
Raw lap	22	66	12	100
Cooked lap	23	63	14	100

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