WOMEN EXPOSURE TO PERSISTENT LIPOPHILIC ORGANOCHLORINES

Patricia Araque[2], Fátima Olea[1], Mª José Lopez[2], Isabel Cerrillo[2], Mariana F Fernández[2], Margarita Jiménez[1], Marta Castillo[2], Ana Rivas[2], Rolando Ramírez[2], José Manuel Molina-Molina[2]

[1]Nutrition Dpt. University of Granada.
[2]Lab. Medical Investigations, Hospital Clínico, University of Granada.

Introduction

The consequences of the use of pesticides in health and agriculture are contradictory: the benefits offered by the control of parasites that affect plant and human health must be balanced against the effects of these "biocide" chemical compounds on animal species and humans [1]. Legislation exists to prohibit or restrict the utilisation of some products but these are repeatedly detected in analyses of adipose tissue, serum, and milk from animal and human populations[2-4]. The concentrations in blood depend on both the daily intake and the mobilisation of organochlorine molecules accumulated in adipose tissue, which serves as a reservoir. The distribution of lipid content between adipose tissue and serum is of the order of 200 to 1, with 90 % corresponding to the first of these compartments and 0.4 % to the second[5,6]. As a result, all of these compounds have a long half-life. Mobilization of fat during weight loss allows these chemicals to re-enter the circulation and pass to serum. Reports of contamination of adipose tissue, serum and human milk by certain organochlorine pesticides prompted the present analysis of samples from these three sources. The identification and quantification of these organochlorine molecules is important because they have estrogenic effects and may act as endocrine disrupters.

Material and methods

472 women volunteers aged from 18 to 70 years were selected. The following samples were stored at -70ºC immediately after collection: 4-8 ml of milk from 72 women aged from 18-35 years; and 400 adipose tissue and 200 blood serum samples from women aged from 35 to 70 years, gathered during surgical treatment at Granada University Hospital and Almería Hospital.

To 2 - 4 mL of serum was added half of the same volume of methanol and the solution was shaken for 5 minutes. The extraction of organochlorines was performed using 10 ml ethyl ether/hexane (1:1 v/v) and centrifugation for 15 minutes at 3000 rpm. The organic phase was obtained and the extraction procedure was repeated twice more. Three organic residues were collected. These residues were concentrated to a volume of 1 ml. To this residue, 0.5 ml of concentrated sulphuric acid was added and centrifuged for 10 min. at 3000 rpm. The acid residue was extracted twice more with the addition of 1 ml hexane. The three organic phases were collected and dried. The dry residue was re-dissolved in 1 ml of hexane and cleaned up.

For the analysis of adipose tissue, a Pyrex glass chromatography column (6mm) was filled with 2 g of Alumina Merck 90 (70-230 mesh nº1097); 0.2g of the adipose tissue sample was weighed and homogenised with 2mL hexane and then eluted in the column with 20 mL hexane and the eluate was concentrated to a final volume of 1 mL. The extract obtained was dried under a nitrogen stream and the dry residue was purified by Sep-Pak

Four to eight ml of milk from each woman were mixed in a glass jar by shaking. To this mixture of 4-8 ml of milk, half of the same volume of methanol was added and the solution was shaken for 5 min;

0.1 g of sodium oxalate was then added and shaken. The extraction was performed using 10 ml ethyl ether/hexane (1:1 v/v). The extract was centrifuged for 15 min at 3000 rpm. The organic phase was obtained and the extraction procedure was repeated twice more. The three organic residues were collected. These residues were concentrated at low pressure to a volume of 1 ml. To this residue, 0.5 ml of concentrated sulphuric acid was added and centrifuged for 10 min at 3000 rpm. The acid residue was extracted twice more with the addition of 1 ml hexane. The three organic phases were collected and dried under a flow of nitrogen. The dry residue was re-dissolved in 1ml of hexane and cleaned-up.

The organic extracts obtained from the extractions were purified with the use of silica Sep-Pak (Wat 051900) after prior treatment of the cartridge with 2 ml hexane. The extract was eluted with 10 ml hexane and then with 10 ml hexane: methanol: isopropanol (45:40:15; v/v/v). Both eluates were collected and dried under nitrogen stream. The dry residue was dissolved in 1 ml hexane, labelled with the p-p´dichlorobenzophenone internal standard and analyzed with GC/ECD. The results were confirmed using GC/MS

Results and Discussion

The presence of 17 organochlorine molecules was determined by gas chromatography with electron capture detector. The presence of these products was confirmed by mass spectrometry. The mean values of the organochlorine pesticides measured in the adipose tissue and blood serum samples are shown in Tables 1 and 2, according to the age-range of the women (18-35 years; 35-70 years). Adipose tissue clearly acts as a reservoir for liposoluble compounds. In the women aged 18-35 years the most frequently found pesticides in fat and serum were HCB, and p,p‘DDE followed by lindane (fat) and aldrin (serum). Ordered by concentrations, HCB and p,p‘DDE were again those with the highest levels in fat; however, endosulphan II showed the highest level in serum. Among the older women (35-70 years) the most frequent pesticide found was p,p‘DDE both in fat and serum. Ordered by concentrations p,p‘DDE again showed the highest concentration.

Interestingly, DDTs were found significantly more frequently and at higher concentration in older than younger women, while the contrary occurs for younger women who showed higher concentrations of DDE metabolites. This suggests a primary exposure to the parent compounds in older women and secondary exposure to the metabolites in the younger women.

Table 1. Mean values of organochlorine pesticides determined in 72 adipose tissue and serum samples: Women aged 18-35 years
*Values expressed in ng/g of lipid. **Values expressed in ng/mL of serum

	Adipose tissue (N=72)			Serum (N=72)		
	Mean*	S.D	% Frequency	Mean**	S.D	% Frequency
o,p´DDT	5.93	16.9	20.9	1.52	2.25	54.1
p,p´DDT	6.82	20.3	13.5	5.04	5.95	50.0
p,p´DDD	44.85	89.4	25.4	7.21	16.6	23.6
p,p´DDE	1971.4	923.3	98.5	29.3	24.5	98.6
Metoxyclor	15.6	101.3	4.50	0.77	3.46	4.53
Lindane	61.2	88.8	53.7	0.41	0.79	36.1
HCB	1290.4	1057.9	100	22.2	23.1	100
Aldrin	38.7	128.1	10.4	6.64	8.03	83.3
Endrin	6.81	36.9	5.97	-	-	-
Dieldrin	121.0	719.4	2.99	3.24	6.85	52.8

E-I	11.3	49.5	14.9	0.71	0.71	61.1
E-II	14.6	29.9	28.3	38.5	49.4	59.7
E. ether	1.89	4.50	37.3	6.42	10.6	37.3
E. lactona	7.35	14.7	40.3	1.10	2.61	12.7
E. diol	5.18	18.6	20.9	0.34	0.71	16.3
E. sulphate	4.29	11.5	17.9	5.33	14.5	26.4

Table 2 Mean values of organochlorine pesticides determined in adipose tissue and serum samples. Women aged 35-70 years.
*Values expressed in ng/g of lipid; **Values expressed in ng/mL of serum.

	Adipose tissue (N=400)			**Serum (N=200)**		
	Mean*	**SD**	**%Frequency**	**Mean****	**SD**	**%Frequency**
0,p´DDT	15.54	11.62	18.70	0.52	0.91	23.5
p,p´DDT	39.10	31.09	23.09	2.47	2.55	76.5
o,p'DDD	215.13	170.63	20.78	3.41	1.82	3.5
p,p'DDE	504.87	571.32	97.92	8.3	12.8	100
Metoxychlor	31.71	53.21	4.15	0.49	0.01	1.0
Lindane	41.29	92.62	42.26	0.95	1.79	46.0
Aldrin	35.52	53.46	30.48	1.43	1.98	44.5
Endrin	44.50	16.92	12.70	0.34	0.72	9.0
Dieldrin	14.92	12.62	22.40	0.83	0.91	33.0
E-I	13.21	54.99	20.32	0.81	1.18	35.5
E-II	21.80	11.37	10.85	0.95	3.74	9.5
E-eter	3.49	10.27	51.96	1.44	1.95	73.0
E-lactona	7.09	23.59	14.78	0.22	0.44	17.0
E-diol	13.84	42.13	28.86	4.39	15.8	32.5
E-sulfate	80.00	187.74	10.85	0.70	1.21	24.0

The results for maternal milk show that human milk is an elimination route for xenobiotics and a contamination source for the breast-feeding baby (Table 3). Most of the human milk samples contained p,p'DDT, and p,p'DDE and HCB were detected in 100% of the samples.

Table 3. Mean values of organochlorine pesticides determined in 72 milk samples. Women aged 17-35 years.

	Mean	**SD**	**% Frequency**
o,p'DDT	0.31	0.92	34
p,p'DDT	2.78	6.62	77
o,p'DDD	8.51	11.5	56
p,p'DDE	28.8	29.2	100
Methoxychlor	0.48	2.20	25
Lindane	0.73	1.54	70

HCB	14.3	30.3	100
Aldrin	2.60	3.87	93
Dieldrin	0.54	0.57	68.5
E-I	0.12	0.15	63
E-II	2.45	5.37	30
E-ether	2.70	5.49	98
E-lactone	0.13	0.24	73
E-diol	0.09	0.18	50
E-sulphate	1.61	9.01	18

Values expressed in ng/mL of milk

Limits on exposure to these widespread organochlorine molecules must be established. The risk is not confined to human breastfeeding and any living being can be similarly contaminated by pesticides. Long-term follow up of cohorts with defined exposure levels, with considerations of numerous biological parameters, will provide valuable information on potential late effects.

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