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HCH AND DDT RESIDUES IN HUMAN FAT IN THE POPULATION OF MURCIA (SPAIN)

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Abstract: In the Mediterranean province of Murcia (SE Spain) around 610,000 hectares of land are devoted to agriculture and organochlorine insecticide use has been common in the region for many years. To investigate HCH and DDT (isomers and metabolites) residues, a total of 231 fat samples were taken from the general population and analyzed. Organochlorine residues were found in 63.2% of the samples. The pattern of overspreading by these substances in this part of the world is similar to that described by different authors in other countries. p,p'-DDE, β-HCH and lindane residues, in this order, show the highest occurrence in the region and also the highest mean levels. Areas devoted to greenhouses, traditional dry farming of paprika pepper, and traditional horticultural cultivation are the most contaminated. Therefore, the past use of DDT and the actual employment of lindane seem to be reflected in the residents of these areas.

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INTRODUCTION

The excellent results obtained in the struggle against pests during recent decades has led to a considerable increase in agricultural production destined for human consumption. In this endeavor, the intensive use of organochlorinated pesticides, such as HCH (1,2,3,4,5,6-hexachlorocyclohexane) and DDT [2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane], have performed an important role. However, due to their lipophilic nature and high persistence they exhibit a great capacity to accumulate in biological fatty tissues where they frequently maintain their chemical properties, causing concern about their possible toxicity [2, 11, 12, 20]. Human beings are at the top of the food chain and are a clear example of bioaccumulation phenomena, as can be seen in human fat samples from all around the world (Tab. 1) [1, 4, 6, 12, 23].

In the Murcia region, due to geographical and climatological reasons, agriculture has been the main resource for decades, with the intensive use of insecticides, such as DDT in the past years and lindane more recently days [8, 19].

The aim of this study was to analyze the occurrence of these substances across the province, considering the four agricultural areas which can be distinguished based on their specialization imposed by the disponibility on water supply. These areas are:

1) Murcia (city) (intensive horticultural cultivation using traditional irrigation and high needs of pesticide);

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Table 1. Mean concentrations of the different compounds in human fat tissues from various countries (µg/g).

	Lindane	α-HCH	β-HCH	DDE	DDD	DDT	Ref.
Poland	0.074	0.016	0.228	5.745		0.537	15
Germany	0.009	0.001	0.011	0.170	0.001	0.016	11
Mexico	0.01	0.01	0.16	4.51	0.03	1.27	24
Greenland			0.132	3.874		0.178	3
Italy	0.128			7.35		0.83	7
Italy	0.104		0.213	0.395		0.064	5
Nairobi			0.034	3.26		2.49	12
Navarra			1.53	3.93		0.4	9
Catalonia	0.07		1.63	6.00	0.06	1.35	10
This paper	0.68	0.33	1.31	4.2	1.39	2.01	

2) Cartagena (extensive dry farming and greenhouse cultivation with high and moderate pesticide requirements, respectively);

3) Lorca (horticultural cultivation and dry farming with moderate and low pesticide requirements, respectively);

4) Yecla (viticulture with low pesticide needs).

MATERIALS AND METHODS

Sampling. Fatty abdominal tissue samples were removed from 231 patients undergoing abdominal surgery in four hospitals from the four representative geographical areas of the province of Murcia: Murcia, Cartagena, Lorca and Yecla.

Samples of 1-2 g each were frozen immediately and stored at -35ºC until analysis.

A questionnaire was completed by each patient aiming to relate the possible residue levels of the organochlorine insecticides with epidemiological data. Age, sex, cause for the surgical procedure, previous residency, work, prior contact with pesticides or accidental poisoning, metabolic diseases, and nutritional state were recorded. We excluded from the study subjects with a history of occupational or accidental exposure to pesticide. The population was selected according to the area of study, approximating age and sex among the groups. The mean age of the group was 49.6 ± 19.1 years, with 51.9% of women.

Extraction and analysis. Extraction and purification of the residues of organochlorine insecticides from the fat samples were carried out according to a method based on that proposed by [17]. Briefly, 0.15g of fat tissue were homogenized at high speed for 5 min, extracted with 15 ml of benzene, and filtered through anhydrous Na_2SO_4. The extract was dry concentrated to 5 ml, purified with Florisil® and eluated with 20 ml of 15% petroleum ether/ethilic ether. The eluates were dry concentrated and made up to 1 ml with hexane.

All solvents were of pesticide residue analysis grade.

The residues were quantified on a Carlo Erba gas chromatograph, HRGC 5300 Megaseries, equipped with a ^{63}Ni electron capture detector and a non-polar capillary molten silica RSL-200 (equivalent to SPB-5) column of 25 m length and 0.32 mm internal diameter. The system has a 42 vial automatic injector and coupled to a Spectra Physics recorder-integrator. Helium was used as carrier gas, at flow rate of 1ml/min and N_2 as detector make-up gas, at flow-rate of 60 ml/min. The injector and detector were maintained at 250 and 350ºC, respectively.

Injections were performed with the column oven at 70ºC. This temperature was held for 1 min and then programmed at 15º/min to 160ºC and 5ºC/min to 200ºC, which was held for 2 min, then at 3ºC/min to 280ºC.

Identification from retention times and quantification using external standard [16], allows the determination of the following organochlorine insecticides: 1) α-HCH, 2) β-HCH, 3) γ-HCH, 4) p,p'-DDT, 5) p,p'-DDE, 6) p,p'-DDD.

The detection limits and the recovery for individual compounds were: α-HCH 0.01 (mg/kg) and 99.95%; β-HCH 0.01 (mg/kg) and 95.82%; γ-HCH 0.01 (mg/kg) and 99.63%; p,p'-DDT 0.01 (mg/kg) and 85.35%; p,p'-DDD 0.01 (mg/kg) and 98.58%; p,p'-DDE 0.01 (mg/kg) and 98.86%;

For internal control of our measurements, pesticide identification was periodically confirmed by GC-MS, according to the method proposed by [21].

Table 2. Number of samples with each analyzed compound and their mean concentration expressed as mg/g of adipose tissue (ppm) in the whole population and in the different geographical areas.

	Whole population N=231			Murcia N=59 (traditional horticultural cultivation)		Cartagena N=59 (dry farming and greenhouse cultivation)		Yecla N=54 (viticulture)		Lorca N=59 (horticultural cultivation and dry farming)	
	n	%	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Lindane	55	23.8	0.68 ± 0.6	5	0.77 ± 0.2	25	0.96 ± 0.7	22	0.41 ± 0.2	3	0.22 ± 0.03
α-HCH	13	5.6	0.33 ± 0.36	0		6	0.43 ± 0.5	0		7	0.24 ± 0.09
β-HCH	45	19.5	1.31 ± 1.5	12	1.25 ± 0.5	23	1.67 ± 1.9	2	0.4 ± 0.2	8	0.59 ± 0.44
p,p'-DDE	89	38.5	4.12 ± 3.52	49	4.56 ± 3	30	3.57 ± 4.4	8	4.42 ± 2.5	2	0.68 ± 0.04
p,p'-DDD	21	9.9	1.39 ± 1.53	8	1.36 ± 2.3	7	1.61 ± 1	5	1.29 ± 0.6	1	0.58
p,p'-DDT	16	6.9	2.01 ± 1.18	2	1.5 ± 0.3	10	1.92 ± 1.3	3	2.61 ± 1.4	1	2.1

Table 3. Statistical relation between the number of samples with residues of the different compounds analyzed and their geographical origin.

	No. of samples with residues				Fisher's chi^2 test				Cramer's V
	Murcia N=59	Cartagena N=59	Yecla N=54	Lorca N=59	x^2	freedom degree	significance	verisimilitude reason	significance
Lindane	5	25	22	3	38.7926	3	0.00000	0.4098	0.0000
α-HCH	0	6	0	7	13.3511	3	0.00394	0.2404	0.00394
β-HCH	12	23	2	8	24.2219	3	0.00002	0.3238	0.00002
p,p'-DDE	49	30	8	2	96.7409	3	0.00000	0.6471	0.00000
p,p'-DDT	2	10	3	1	13.0005	3	0.00464	0.2372	0.00464

Statistical analysis. Mean values of organochlorine pesticide residues were calculated using basic statistics. To compare variability among residue levels, age and sex, the Pearson correlation coefficients and simple linear regression were calculated. To analyze the presence of residues in relationship with the geographical origin of the sample, the chi squared and the Cramer coefficient were applied, using the SPSS statistical package.

RESULTS

Our results show the presence of organochlorine residues in 146 samples, which accounts for 63.2% of the samples. The statistical data obtained for the residues of HCH and DDT are set out in Table 2. In the DDT group, p,p'-DDE residues were the most ubiquitous and showed the highest mean levels since p,p'-DDT and p,p'-DDD were found in a small number of samples, 16 and 21 respectively, generally accompanied by the detection of low p,p'-DDE levels. Lindane and β-HCH were found in a similar number of samples, four times more than α-HCH.

We did not find a statistical relationship between age or sex with the presence of each compound, or with its mean concentration. A statistically significant relation was only detected between the presence of each compound (except p,p'-DDD) and the geographical area (Tab. 3). Murcia and Cartagena were the areas showing the highest frequency of detection of the products (83% and 88% of samples, respectively) compared to Yecla and Lorca (53% and 25% respectively).

DISCUSSION

DDT group. Data on p,p'-DDE residues was not unexpected since p,p'-DDE is the most abundant and most persistent metabolite of the group, and is in accordance with other authors, both in Spain and in other countries [1, 4, 6, 12, 23] (Tab. 1). DDE residues may reflect the past use of DDT, which has been metabolized in the environment, and years later might be incorporated into the body through the diet [14].

The levels of p,p'-DDT were also similar to those reported by other investigators [1, 4, 12, 23] (Tab. 1), while p,p'-DDD levels were higher than those previously published by others, and generally accompanied by the detection of low p,p'-DDE levels. The short half-life of DDD in the human body after exposure to DDT [14] suggests an isolated and recent exposure to DDT in these cases.

The source of this exposure may either be direct, by a sudden misusage of this insecticide (banned for agricultural use in Spain since 1975), or indirect, through the consumption of foods imported from countries in which the use of this substance is legal, or from foods that are contaminated due to the DDT impurities of other authorized products.

HCH group. Lindane levels were higher than those found by other authors in similar studies [1, 4, 6, 12, 23] (Tab. 1) perhaps because of repeated exposures to this insecticide which is widely used in Spain in animal husbandry, horticultural cultivation, and for disinfecting soils.

Recorded β-HCH levels were similar to those found in other Spanish regions, and were higher than those described in other countries (Tab. 1); These β-HCH levels could be related to the use of lindane-containing pesticides, since this chemical arises from the impurities in technical lindane (generally about 2-3% of the lindane-containing pesticide products) and is much more persistent (it takes five times longer to be excreted from the human body than the other isomers) [14]. This results in a 10-30 times stronger ability of this isomer, compared to lindane, to accumulate in the human fat [13, 22]. The α-HCH levels were also higher than those previously recorded by others (Tab. 1).

In none of the samples were these levels in the range capable of inducing acute or subacute clinical disturbances, taking into account that the fatty deposits of these substances are quiescent from a toxicological point of view. Nevertheless, in the case of DDT and its metabolites, the risk of delayed toxicity may be present as they may act as "endocrine disrupters" if mobilized into the systemic flow stream [11].

The reason for the relationship established between the place of residence of the subjects and the presence of the different compounds may lie in the predominant agricultural practices of each geographical zone which may determine the type and quantity of the insecticides used. Murcia and Cartagena were the areas showing the highest frequency of detection of the products (83% and

88% of samples, respectively) compared to Yecla and Lorca (53% and 25% respectively).

The findings may reflect the intensive horticultural cultivation which has characterized the capital of the region (Murcia) for decades. These type of crops were associated with heavy insecticide use in past years, where small family-owned plots were the norm and pesticide use was difficult to control.

In Cartagena, on the other hand, it is probably the extensive cultivation of paprika pepper for many years which is responsive for the high levels of organochlorines recorded. Such compounds are frequently used on this crop, particularly lindane (together with β-HCH) because of its properties. Moreover, in this area during recent years, the expansion of greenhouse cultivation of solanacceae and other crops has led to the resurgence of traditional methods for pest control.

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