Bull. Environ. Contam. Toxicol. (1989) 42:195–201

Bulletin of Environmental Contamination and Toxicology

Organochlorine Residues in Human Adipose Tissue in Spain: Study of an Agrarian Area

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The environmental pollution by persistent organochlorine residues has received much attention in the last years because of its possible effects on wildlife and human health (Bertram et al. 1985; Splinder 1983). These residues -organochlorine insecticides, hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs) and, in minor levels, polychlorinated dibenzofurans (PCDFs) and dibenzodioxins (PCDDs)- are accumulated in lipid-rich tissues. Their concentrations in adipose tissues of human populations are the best indices in determining the extent of exposure and in evaluating the hazard.

In a previous study on the urban population of Barcelona (Spain) during the years 1982-83, high levels of DDE, DDT, ß-HCH and HCB were determined (To-Figueras et al. 1985); the mean concentration of HCB (5.6 ppm) was among the highest reported in the bibliography. Recently the incidence of HCB in Barcelona has been confirmed by serum determinations (Gómez-Catalán et al. 1987). Several sources have been proposed to explain the origin of HCB in the environment: use as a fungicide, by-product of industrial chlorination procedures and contaminant of some pesticide formulations (Tobin 1986).

In the present paper we have investigated the levels of organochlorine residues -with special concern on HCB- in human adipose tissues from an agrarian area, located at 130 km from Barcelona, mainly devoted to fruit-trees and cereal culture. Results obtained will form part of an up-to-date report on organochlorine pollution in Spain, including several populations of different geographical and socioeconomic characteristics, that will make it possible to identify the sources and trends of this contamination.

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MATERIALS AND METHODS

Human abdominal adipose tissues (87 samples) were obtained from autopsies performed at the Judicial District of Lleida between October 1985 and March 1987. No selection criteria were applied. The samples were stored at -30ºC in glass containers until analyzed. Register forms including data about the sample donors -age, sex, occupation, cause of death and length of residence in the area- were annexed.

For the analysis about 0.3g of fresh tissue was homogenized and dehydrated with anhydrous sodium sulfate and extracted twice with hexane. An aliquot of the extract was evaporated under nitrogen stream until constant weight to determine lipid content. Clean up was performed with sulphuric acid (Veierov and Aharonson 1978) for the determination of all residues except for dieldrin. For the analysis of dieldrin an aliquot of the extract was cleaned by partition with acetonitril and elution through florisil (A.O.A.C. 1980).

Gas-liquid chromatography with electron capture detection (ECD-GLC) was utilized for the identification and quantification of residues. A Varian 3700 apparatus, equipped with a glass packed column (2.5% OV17, 1.95% OV-210 on Supelcoport 100/200) and a fused silica capillary column (SPB-5, 30m long, 0.32mm ID) was used. Aldrin was added as internal standard. Determination of PCBs was based in comparison with a standard reference solution of Aroclor 1260. Residue levels are reported as mg/kg extracted fat (ppm).

All solvents were of pesticide residue analysis grade and the purity of all reagents was carefully checked. Standards of the chlorinated hydrocarbons were purchased from commercial sources.

RESULTS AND DISCUSSION

Results are summarized in the Table 1. The arithmetic mean ($\bar{x}$) overestimates the value of the central tendency of the distributions of concentrations because of their assimetry to the right (e.g., Figure 1 shows the distribution histogram of HCB). The median (md) and the mean of the log transformed concentrations (geometric mean, gm) are better indices. Statistical analyses of data (correlations and comparisons among subpopulations) have been done with the log transformed distributions because they are closer to the normal distribution.

Organochlorine residues were found in all samples, except for DDD, it was only determined in 54, being at

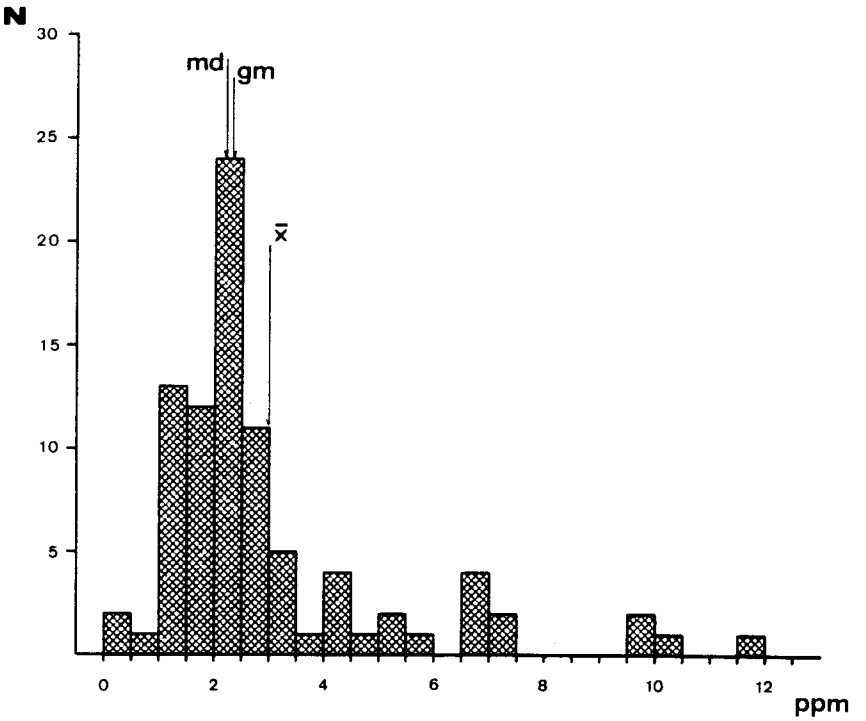

Figure 1. Distribution of HCB residues in human adipose tissue (ppm, mg/kg extracted lipid).
x: arithmetic mean; md: median; gm: geometric mean.

Table 1. Residues of organochlorine compounds in human fat(mg/kg extracted lipid).

	x	SD	SEM	gm	N
HCB	2.99	2.24	0.24	2.37	87
pp'-DDE	6.27	5.67	0.61	4.28	87
lindane	0.083	0.050	0.005	0.069	87
ß-HCH	3.06	5.18	0.56	1.70	87
pp'-DDD	0.079	0.079	0.011	0.048	54
pp'-DDT	1.50	0.89	0.09	1.27	87
dieldrin	0.072	0.068	0.007	0.055	87
PCBs	1.68	0.84	0.23		14
age	48.0	22.1	2.4		86

x: arithmetic mean; SD: standard deviation;
SEM: standard error of the mean; gm: geometric mean
N: number of samples.

levels in the remaining too low to be quantitatively evaluated. PCBs are only investigated in 14 samples; peak patterns are very repetitive and closely similar to that of Aroclor 1260, used as reference. Because of the intrinsic limitations of this method of quantification and the small number of samples determined, the mean value obtained for PCBs must be

considered as a rough estimate.

pp'-DDE had the highest average concentration as in the majority of occidental populations investigated, followed by HCB, ß-HCH and pp'-DDT. HCB and ß-HCH concentrations were very high when compared with world-wide literature (Abbott et al. 1985; Robinson et al. 1986); HCB mean values of the same order were determined in Italian and German populations (Focardi et al. 1986; Bertram et al. 1986).

Experimental and epidemiological evidences suggest that the ratio DDE/DDT increases with time after exposure or after use restriction of DDT, because of the faster elimination kinetics of DDT. In our population DDT and DDE correlated significantly ($r=0.486$, $p<0.01$) and the ratio DDE/DDT was 3.37, smaller than those found in other European countries in recent years (Abbott et al. 1985); this fact may be related with the later prohibition of DDT use in Spain (agrarian use of DDT was forbidden after 1977).

Concentrations of HCB, pp'-DDE, ß-HCH, pp'-DDD and pp'-DDT correlated positively with the age ($p<0.01$), whilst those of lindane and dieldrin do not. This common correlation with age could explain the existence of strong correlations between residues not directly related as HCB, ß-HCH and DDE.

The levels of residues were higher in female than in male individuals, except for dieldrin (Table 2) but difference was only significant for ß-HCH ($p<0.025$). Bias in the population (N males=71, N females=16) made it difficult to reach conclusions; however these results are in concordance with those obtained by other authors and are indicatives of the existence of some sex-linked factor in the accumulation of organochlorine residues.

Owing to the characteristics of the area studied and the forensic origin of the samples, a part of the tissues do not proceed from resident individuals but from visitors, mainly dead in traffic casualties. We have divided samples in two groups: a)resident population, with documented residence in the area for more than six years, and b) temporary population, those that do not satisfy the former requirement. Results are summarized in Table 3. Analyses of these results showed that mean levels of the three majoritary residues (HCB, DDE and ß-HCH) were higher in the resident population, being difference very significant for HCB ($p<0.001$) and ß-HCH ($p<0.025$). These difference can only partialy be explained by the mean age gap between the two populations and suggest a special incidence of HCB and ß-HCH pollution in this area.

Table 2. Residues of organochlorine compounds in male and female populations (mg/kg ext. lipid).

	gm		N		t
	male	female	male	female	
HCB	2.24	3.07	71	16	1.59
pp'-DDE	4.13	5.05	71	16	0.78
lindane	0.068	0.075	71	16	0.55
ß-HCH	1.51	2.82	71	16	2.37
pp'-DDD	0.044	0.060	40	14	0.97
pp'-DDT	1.25	1.35	71	16	0.46
dieldrin	0.056	0.051	71	16	0.45
age	47.7*	49.3*	70	16	0.26

gm: geometric mean; *: arithmetic mean
t: t value of the Student test

Table 3. Organochlorine residues in adipose tissue of "resident" and "temporary" subpopulations mg/kg extracted lipid).

	x		gm		N		t
	resid	temp	resid	temp	resid	temp	
HCB	3.58	2.06	2.93	1.70	53	34	3.62
pp'-DDE	6.84	5.37	4.77	3.63	53	34	1.35
lindane	0.081	0.085	0.067	0.073	53	34	0.58
ß-HCH	3.74	2.01	2.06	1.26	53	34	2.37
pp'-DDD	0.079	0.079	0.048	0.047	36	18	0.10
pp'-DDT	1.40	1.66	1.18	1.43	53	34	1.51
dieldrin	0.075	0.068	0.054	0.037	53	34	0.43
age	51.5	42.4			53	33	1.86

t: t value of the Student test

Higher levels of organochlorine residues on agrarian populations than in the general ones have been reported in other countries, and have been attributed to a heavier labor and environmental exposure (Greer et al. 1980). Incidence of ß-HCH reflect the use of lindane-containing pesticides, still marketed in Spain. Lindane has a short half life in the environment -this explains its low accumulation and the no correlation with age- whereas the ß isomer of HCH, present as an impurity in technical lindane, is a persistent residue. HCB high concentrations are difficult to interpret because of its diverse and not well known sources. Special incidence in agrarian areas could be indicative of a pesticide use-linked origin. HCB has never been massively used as fungicide in Spain and currently there is only one HCB containing formulation marketed, applied only as a seeds preservative; therefore HCB contamination might be caused by impurities of other pesticides (Tobin, 1986)

The mean concentration of HCB in the resident

population is lower than that of the urban population of Barcelona in years 1982-83 (To-Figueras et al. 1986). Direct comparison of these two studies in order to elucidate the origin of HCB contamination and its differential incidence on urban and rural areas is not possible because of the time elapsed between them.

Studies on some populations of Spain -including that of Barcelona- and on organochlorine residues in foods are currently in course. They will permit us to reach more precise conclusions on organochlorine exposure and accumulation in humans.

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Received January 12, 1988; accepted July 16, 1988.