Bull. Environ. Contam. Toxicol. (1995) 54:534–540

Environmental Contamination and Toxicology

Organochlorine Residues in the Adipose Tissue of the Population of Navarra (Spain)

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Received: 28 May 1994/Accepted: 26 August 1994

Organochlorine residues (OCR) in adipose tissue of the general population of Navarra Region (Northern Spain) have been determined as indicators of the environmental and food related exposition to these ubiquitous pollutants. The main organochlorine insecticides, hexachlorobenzene (HCB) and polychlorinated biphenyls (PCBs) have been identified and quantified in 86 individual samples. The results have been analysed in order to determine possible associations with individual parameters as sex, age, obesity or occupation, and compared with those previously obtained in some other near regions.

This work continues a series of previous studies on other Spanish populations that showed high mean levels of some residues, specially HCB (See Table 2)(To-Figueras et al. 1986; Camps et al. 1989; Gómez-Catalán et al. 1991 & 1993, Ferrer et al. 1992). HCB is a chemical with several possible sources and a potential risk to human health; its main toxic features in experimental animals are porphyria and cancer, and its porphyrinogenic potential in humans was demonstrated in an alimentary toxic outbreak in Turkey. Very recently a small community with high HCB concentrations in blood (living in an area with high HCB airborne levels, next to a chemical factory that produce chlorinated solvents) has been identified in Spain and a significant incidence excess of soft tissue sarcoma and thyroid cancer has been observed (Grimalt et al. 1994).

MATERIALS AND METHODS

Human adipose tissue samples (N=86) were removed from abdominal wall of patients submited to abdominal surgery from July to December 1991 at three regional hospitals. Documented residence in the area during the last ten years was the only selection criterion applied. Samples were stored at -30°C in glass containers until analyzed. Register forms including data about sample donors -age, sex, occupation, height, weight, pathologies, alcohol and tobacco habits, length of residence in the area, etc- were annexed.

About 0.5 g of frozen tissue was ground with anhydrous sodium suphate and extracted with hexane in a Soxhlet apparatus. Extracted lipid was determined gravimetrically in a fraction. A chemical clean-up with sulphuric acid was applied to extracts. When analyses by GC-MS were performed, a further clean-up by adsorbent column chromatography in silica was applied. Extracts were split in two fractions: the first for determination of major residues (HCB, DDE, ß-HCH) with aldrin as internal standard and the second for determination of PCBs and DDT with octacloronaphtalene as internal standard.

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Gas chromatography with electron capture detection (Hewlett-Packard 5890), a capillary SPB-5, 30m long, 0.32 mm ID, 0.30 um film thickness column (Supelco) and split-less injection was utilized for the quantification of residues.

All glassware was washed with detergent and distilled water and rinsed with ethanol and n-hexane. n-Hexane was pesticide residue analysis grade (Merck). Blank samples were run to check for possible contaminations. Assays with fortified chicken adipose tissue and fortified olive oil showed quantitative recovery of all the residues. Precision was determined by analysis of some sample replicates (N=5): the coefficients of variation were less than 10% for PCBs and less than 6% for HCB, ß-HCH, pp'-DDE and pp'-DDT. Interference between pp'-DDT and a minor PCB peak precluded an accurate quantification of DDT in some samples. Analytical standards were supplied by Supelco.

Identification was confirmed by GC-MS in some samples (HP-5971A), with EI ionization in SIM mode. Assignement of PCB peaks was based in the degree of chlorination as determined by MS and comparing the retention times with those of Aroclor peaks, and assuming the peak identification reported by Safe et al. (1985) and Schulz et al. (1989). Quantification of PCBs was done by calibration against Aroclor 1260 by two different methods as previously described (Gómez-Catalán et al. 1991); briefly, the first method (A) quantify whole PCBs by addition of peak areas in sample and Aroclor chromatograms, and the second method (B) quantify single peaks assuming the composition determined by Schulz et al (1989) for Aroclor 1260 and calculating individual response factors. Individual congeners have been designated by Ballschmiter numbers, although some incoherences have been reported (Guitart et al. 1993).

Statistical analysis of data was performed with the BMPD package (Statistical Software Inc.). When the distributions of frequencies were not normal, non-parametric tests or log-transformed variables were used.

RESULTS AND DISCUSSION

Table 1 summarizes the results of the whole sample population. Concentrations are expressed relative to extracted lipid (µg/g; ppm). The arithmetic mean ($\bar{x}$) overestimates the value of the central tendency because the distributions of frequencies have a marked asymmetry to the right originated by the presence of some individuals with high concentrations; therefore, the geometric mean (gm) is a better index.

Organochlorine residues were found in all samples, although DDT was not quantified in seven and PCBs in two because of some chromatographic interferences; lack of these values could introduce some bias in the results, particularly a slight overestimation of the pp'-DDT mean concentration because samples not quantified showed low range levels. Other residues as Lindane, DDD or dieldrin have not been included because of very low levels as has been shown in other Spanish populations (Gómez-Catalán et al, 1993). The more striking result was the high level of HCB when compared with the world-wide literature, but similar to concentrations determined in other regions of Spain and some European populations (Table 2). Considering the geometric means, HCB was the major OCR in the population of Navarra, displacing pp'-DDE, that is the residue with the highest average concentration in the majority of occidental populations investigated. This high incidence of HCB accumulation in Spain is very intriguing and possible origins have been previously discused elsewhere (Gómez-Catalán et al. 1993).

pp'-DDE is the major metabolite of DDT and a more persistent residue; the ratio DDE/DDT may be considered as an index of the oldness of the contamination by DDT. Organochlorinated persistent insecticides were prohibited in Spain in 1975 and, according

Table 1. Residues of organochlorine compounds in human fat (µg/g extracted lipid)

residue	$\bar{x}$	SD	SEM	gm	min	max	N
HCB	3.37	2.20	0.24	2.81	0.42	12.53	86
ß-HCH	1.53	0.92	0.10	1.18	0.01	5.25	86
pp'-DDE	3.93	4.00	0.43	2.60	0.22	22.00	86
pp'-DDT	0.40	0.43	0.05	0.25	0.01	2.19	79
PCBs (A)[1]	2.44	1.14	0.12	2.21	0.77	6.27	84
PCBs (B)[1]	2.36	1.19	0.13	2.12	0.76	6.78	84

x: arithmetic mean; SD: standard deviation;
SEM: standard error of the mean; gm: geometric mean
N: number of samples
[1]: whole PCBs quantified by methods A or B

Table 2. OCR levels in adipose tissue in some populations (arithmetic mean; µg/g extracted lipid or whole adipose tissue)

population	HCB	DDE	DDE/DDT	PCBs	ref
Navarra (Spain) 1991	3.37	3.93	10.7	2.44	this study
Barcelona (Spain) 1987-88	2.42	6.98	5.1	1.14	Gómez et al. 1991 & 93
Catalonia (Spain) 1985-88	2.99	6.00	5.3		Gómez et al. 1993
Zaragoza (Spain) 1988-89	2.95	2.96		1.52*	Ferrer et al. 1992
Ontario (Canada) 1983-84	0.01	2.6		2.1	Frank et al. 1988
Canada 1985	0.025	0.811	17	0.41	Mes et al. 1990
UK 1982-83	0.11	1.3	11	0.9	Abbott et al. 1985
Italy 1983-84	2.26	7.35	9	1.75	Focardi et al. 1986
Poland 1986	0.22	14.1	8		Szymczynski 1986

(*) Unpublished results

to that, succesive determinations in different populations have shown an increasing DDE/DDT ratio: in the present study this value was 10.7 (geometric mean), whereas in the Catalonian population in 1987-88 it was only 5.3. Furthermore, DDE mean level was lower than those determined previously in other Spanish areas but present data dont allow to discriminate if this is so because of a decrease in DDE accumulation or by a minor incidence of DDT+DDE contamination in the Navarra area (Table 2).

ß-HCH is a persistent by-product of technical grade lindane (ɣ-HCH), still widely used because it is effectively degraded in the environment. The levels of ß-HCH were relatively high but similar to those obtained in other Spanish populations and suggest a heavy use of lindane-based pesticides and perhaps with a high degree of ß-HCH impurities.

PCBs are used in industrial applications and therefore they have a different origin than OC insecticides, and different ways of entry into the environment. However, the environmental properties -persistence, liposolubility- of PCBs and OC insecticides are qualitatively similar. Our results show:

a) Quantified as a whole, their mean concentration was only slightly lower than those of

DDE and HCB, and in the same order as those found in other Spanish populations (Table 2).

b) The two quantification methods used produced almost identical results and an excellent correlation between them was found.

c) The chromatographic pattern was very reproducible among samples, with prevalence of highly chlorinated congeners and qualitatively very similar to Aroclor 1260. The major congeners were, expressed as mean percentage in mass±SD: #153 (19.4±4.4), #180 (13.0±2.3), #138 (9.2±2.7), #170+190 (8.0±2.0), #171+202+156 (5.5±1.7), #187 (5.3± 1.6), #178 (4.6±1.7), #118 (3.2±2.0, roughly approximate because of insufficient resolution with #149 and low concentration present in Aroclor 1260). The predominant homolog series were: Cl-7 (44%), Cl-6 (38%), Cl-8 (10%), Cl-5 (6%). The prevalence of the highly chlorinated homologs is a consequence of the increment of environmental persistence with the chlorination degree.

The more relevant aspects of statistical analysis are shown in table 3; The possible associations between residue concentrations and some other individual parameters -sex, occupation, residence subarea, obesity index, age- and correlations between residues themselves were investigated. Following the same order as in the table:

a) Only for HCB a significant sex-related difference was found, with higher mean concentration in women. Several population surveys have found contradictory results in OCR accumulation sex-related effects and no clear explanation has been proposed.
b) Differences between agrarian workers and the rest were no significant, but the agrarian sample was too scarce to take out definitive conclusions. Anyway, this result suggests that occupational exposure to OC pesticides is not significant nowadays.

c) Some significant associations were observed between HCB and DDT and geographic area, with lower concentrations in the Northern area. This might be related with the socio-economic characteristics: South and Central zones concentrate extensive agriculture and major industry, whereas the North is a mountainous area with cattle, forage and some light industry as main activities.

d) No correlation with obesity was found. Obesity increments the accumulated body burden, but not the concentration.

e) Residues of HCB, ß-HCH, DDE and DDT showed a positive and significant correlation with age; however, PCBs did not. Correlation with age is usually interpreted as a consequence of the long half lives of this residues in man and the relation between age and length of exposure. This relation may be true in the case of OC insecticides to which men have been exposed during the last 40-50 years; however, in the case of PCBs, general exposure began later and correlation with age only can be expected in young people. As in our sample only six individuals were under 30y old, this precluded the possible observation of correlation for PCBs. In a previous work we reported a correlation between PCBs and age in the population of Barcelona originated, as discussed there, by the lower levels in individuals under 35 years old (Gómez-Catalán et al. 1991).

f) OC residues, except PCBs, showed positive significant mutual correlation. This might be a consequence of the common correlation with age; to confirm or discard this, partial correlation analysis was done.

g) Partial correlation allows to determine the relationship between two variables having eliminated the influence of a third. Results indicate that a clear correlation between

Table 3. Summary of more relevant results of statistical analysis. When not indicated, no significant association was found (NS: $p>0.05$)

a) Residues vs Sex (1)

	males (N=37)	females (N=49)	
HCB	2.56±1.52 µg/g	3.98±2.43 µg/g	p=0.001
PCBs (A)	2.70±1.16	2.25±1.11	p=0.06

b) Residues vs Occupation (1)

	agrarian (N=17)	other (N=68)
	NO SIGNIFICANT	

c) Residues vs Subarea (2)

	North (N=17)	Central (N=54)	South (N=15)	
HCB	2.62±1.36(1)	3.24±2.02	4.68±3.03(2)	p=0.023(1-2)
DDT	0.21±0.19(1)	0.42±0.49(2)	0.41±0.34(2)	p=0.027(1-2)

d) Correlation Residues vs Obesity index (weight/height^2)(3)

NO SIGNIFICANT

e) Correlation Residues vs Age (3)

	HCB	HCH	DDE	DDT	PCBs(A)	PCBs(B)
r	0.52	0.46	0.39	0.39	0.12	0.19
p	<0.01	<0.01	<0.01	<0.01	0.37(NS)	0.11(NS)

f) Correlation Residues vs Residues (3)

	HCH	DDE	DDT	PCBs(A)	PCBs(B)
HCB	0.69(*)	0.48(*)	0.42(*)	0.00(NS)	0.07(NS)
HCH		0.44(*)	0.65(*)	0.04(NS)	0.04(NS)
DDE			0.71(*)	0.13(NS)	0.16(NS)
DDT				0.08(NS)	0.08(NS)
PCBs(A)					0.98(*)

g) Residues vs Residues and Age. Partial correlations (3)

	HCH/Age	DDE/Age	DDT/Age
HCB	0.58(*)/0.13	0.35(*)/0.42(*)	0.29(*)/0.41(*)
HCH		0.44(*)/0.56(*)	0.56(*)/0.55(*)
DDE			0.66(*)/0.23

(1) U Mann-Whitney Test. (2) ANOVA. (3) Correlation analysis, using log-transformed values

(*): signification level $p<0.01$

residues remains when the effect of age was corrected. The origin of this association is clear in the case of DDE-DDT because of their metabolic relation; for the rest, it may be explained by the common fate of OC residues in the environment and, specially, in the trophic chains. However, it is to some extent striking the strong correlation between HCB and ß-HCH; a possible metabolic relation between HCB and HCH isomers has been proposed by some authors (Gopalaswamy and Aiyar. 1984).

The lack of correlation between PCBs and the rest of residues might originate from quantitative differences in their main environmental and trophic pathways, caused by their different ways and periods of entry into the environment.

From the viewpoint of the toxic risk for human health, two aspects of our results could be emphasized:

a) The high levels of HCB, present in all the Spanish populations studied. An hypothetical association between HCB accumulation and high incidence of porphyria in Spain has been proposed (Enríquez de Salamanca et al. 1990). An epidemiological study in a small population environmentaly and/or occupationaly exposed to airborne HCB, with blood levels not too much higher than those found in 1985 in general population of Barcelona (Gómez-Catalán et al. 1987), has shown an excess of incidence for soft-tissue sarcoma and thyroid cancer (Grimalt et al. 1994).

b) Some PCB congeners can produce dioxine-like toxic effects. The toxicity of a complex mixture of PCBs can be expressed in terms of an equivalent quantity of 2,3,7,8-TCDD (toxic equivalents, TEQs) by addition of TEQs of individual congeners; these TEQs are calculated as product of congener concentration by a congener toxic equivalent factor (TEF). Safe (1990) has proposed TEFs for individual PCBs, PCDDs and PCDFs; using these values some authors have calculated the contribution of diferent residues to whole dioxin-like toxicity in adipose tissue or human milk (Duarte-Davidson et al. 1993, Dewailly et al. 1991). They found that major contributers were not PCDDs but three mono-ortho-PCBs (#118, #105, #156), and two non-ortho-PCBs (#126, #169).

Because of the very low levels and interference with other congeners we have not determined non-ortho-PCBs; mono-ortho-PCBs had the following mean approximate values: #118, 76 ng/g (TEQ 76 pg/g); #105, 39 ng/g (TEQ 39 pg/g); #156 (50% of the #156+171+202 peak, as determined by MS), 65 ng/g (TEQ 65 pg/g). As a whole, they amount a TEQ of 180 pg TCDD/g. However, this value must be regarded with caution because of: 1st) other PCBs, PCDFs and PCDDs have not been considered, 2nd) Safe's TEFs for PCBs have not received acceptance by the international agencies, and 3rd) the approximate kind of the quantification method used.

Acknowledgments. The authors thank Dr J.L. Castiella the assistence in collecting samples. This work has been supported by a grant from Escola de Medicina del Treball.

REFERENCES

Abbott DC, Goulding R, Holmes DC, Hoodless RA (1985) Organochlorine pesticide residues in human fat in the United Kingdom 1982-1983. Human Toxicol 4:435-45

Camps M, Planas J, Gómez-Catalán J, Sabroso M, To-Figueras J, Corbella J (1989) Organochlorine residues in human adipose tissue in Spain: Study of an agrarian area. Bull Environ Contam Toxicol 42:195-201

Dewailly E, Weber JP, Gingras S, Laliberté C (1991) Coplanar PCBs in human milk in the province of Québec, Canada: Are they more toxic than dioxin for brest fed infants? Bull Environ Contam Toxicol 47:491-498

Duarte-Davidson R, Harrad SJ, Allen S, Sewart SS, Jones KC (1993) The relative contribution of individual PCBs, PCDDs and PCDFs to Toxic Equivalent values derived for bulked human adipose tissue samples from Wales, United Kingdom. Arch Environ Contam Toxicol 24:100-107

Enríquez de Salamanca E, López-Miras A, Muñoz J, To-Figueras J, Conde C (1990) Is hexachlorobenzene overload related to porphyria cutanea tarda? A speculative hypothesis. Medical Hypothesis 33:69-71

Ferrer A, Bona MA, Castellano M, To-Figueras J, Brunet M (1992) Organochlorine residues in human adipose tissue of the population of Zaragoza (Spain). Bull Environ Contam Toxicol 48:561-566

Focardi S, Fossi C, Leonzio C, Romei R (1986) PCB congeners, hexachlorobenzene and

organochlorine insecticides in human fat in Italy. Bull Environ Contam Toxicol 36:644-50

Frank R, Rasper J, Smout MS, Braun HE (1988) Organochlorine residues in adipose tissues, blood and milk from Ontario residents, 1976-1985. Can J Public Health 79:150-8

Gómez-Catalán J, To-Figueras J, Planas J, Rodamilans M, Corbella J (1987) Pentachlorophenol and hexachlorobenzene in serum and urine of the population of Barcelona. Human Toxicol 6:397-400

Gómez-Catalán J, Sabroso M, To-Figueras J, Planas J, Corbella J (1991) PCB residues in the adipose tissue of the population of Barcelona (Spain). Bull Environ Contam Toxicol 47:504-7

Gómez-Catalán J, Planas J, To-Figueras J, Camps M, Corbella J (1993) Organochlorine pesticide residues in the population of Catalonia (Spain). Bull Environ Contam Toxicol 51:160-4

Gopalaswamy UV, Aiyar AS (1984) Biotransformation of lindane in the rat. Bull Environ Contam Toxicol 32:148-156

Grimalt JO, Sunyer J, Moreno V, Amaral OC, Sala M, Rosell A, Anto JM, Albaiges J (1994) Risk excess of soft-tissue sarcoma and thyroid cancer in a community exposed to airborne organochlorinated compound mixtures with a high hexachlorobenzene content. Int J Cancer 56:200-203

Guitart R, Puig P, Gómez-Catalán J (1993) Requirement for a standarized nomenclature criterium for PCBs: Computer-assisted assignement of correct congener denomination and numbering. Chemosphere 27:1451-9

Mes J, Marchand L, Davies DJ (1990) Organochlorine residues in adipose tissue of Canadians. Bull Environ Contam Toxicol 45:681-688

Safe S, Safe L, Mullin M (1985) Polychlorinated Biphenyls: Congener-specific analysis of a commercial mixture and a human milk extract. J Agric Food Chem 33:24-29

Safe S (1990) Polychlorinated biphenyls, dibenzofurans and related compounds: environmental and mechanistic considerations which support the development of Toxic Equivalency Factors (TEFs). Crit Rev Toxicol 21:51-88

Schulz DE, Petrick G, Duinker JC (1989) Complete characterization of Polychlorinated Biphenil congeners in commercial Aroclor and Clonphen mixtures by multidimensional gas chromatography-electron capture detection. Environ Sci Technol 23:852-9

Szymczynski GA, Waliszewski SM, Tuszewski M, Pyda P (1986) Chlorinated pesticide levels in human adipose tissue in the district of Poznan. J Environ Sci Health A 21:5-14

To-Figueras J, Rodamilans M, Gómez-Catalán J, Corbella J (1986) Hexachlorobenzene residues in the general population of Barcelona, Spain. In: Morris CR, Cabral JRP (ed) Hexachlorobenzene: Proceedings of an International Symposium. IARC Scientific Publications Vol 77. Lyon. p 147-48