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Levels of Organochlorine Residues in Blood Plasma from Three Populations in Nicaragua

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The intensive use of organochlorinated pesticides in developing countries is a source of worry, because of the poisonous effect which these chemical compounds have on human beings. In spite of the fact that the use of DDT was prohibited in Nicaragua from 1980 on (DGTA, 1990), the Ministry of Health used this organochlorinate in vector control programs up to the year 1989 (CSUCA, 1991). At present retailing of DDT still continues in the unlicensed trading sector.

A previous work (Gupta et al., 1978) demonstrated that a good positive relationship exists between exposure to organochlorinated pesticides and the concentrations found in human blood. Up to now no investigation has been published in which data about organochlorine residues in the blood of populations exposed or not exposed in Nicaragua were presented. The aim of the present study was to determine the levels of organochlorine residues in blood plasma of two Nicaraguan populations and of a Danish population resident in Nicaragua.

MATERIALS AND METHODS

Blood samples were obtained from individuals chosen at random of the 30 to 40 years age group, of Danish and Nicaraguan nationalities and of the same age class for comparative purposes. All volunteers were grouped by the degree of exposure to organochlorinates into three groups. The first was composed of 23 danes (in Denmark the use of organochlorinates was discontinued twenty years ago) with a period of residence in Nicaragua of less than three years. The second consisted of 20 Nicaraguans who live in the capital of Managua where DDT has been used to control the propagation of health hazards. A third group was composed of 23 Nicaraguans who live in the rural area (El Viejo, Chinandega) where the organochlorinated pesticides are used intensively in and over a prolonged

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period of time. For each of the three groups 10 mL of blood were taken from each individual by venipuncture; the samples were preserved with EDTA at 10%, centrifuged at 8000 rpm for 5 min and the plasma was extracted and weighed. Approximately 5 g of plasma was shaken 10 seconds with 1 Ml 6M HCl, then with 2 mL isopropanol for 10 seconds more. An extraction was made with 5 mL hexane and 1 mL methylene chloride stirring for 10 min and centrifuging. By means of a Pasteur pipet the upper layer (organic phase) was carefully transferred to a tube with 3 mL 0.4M $KHCO_3$ in formaldehyde. The $KHCO_3$/formaldehyde would retain the phenols for eventual analysis later on. The upper organic layer which contained the neutral substances and blood fat was transferred and the extraction was repeated with 5 mL hexane. The combined organic phases were evaporated to dryness applying a gentle stream of nitrogen and the weight of the lipids determined. The lipids were dissolved in 4 mL heptane and mixed with 0.5 g H_2SO_4:silicagel 1:3 and the upper organic layer transferred to another tube. The mixture H_2SO_4:silicagel was extracted with 3 mL hexane, and the combined organic phases finally were evaporated almost to dryness by applying a gentle stream of nitrogen and then diluted to 2 mL with hexane to a concentration of 20 $ng \cdot L^{-1}$ HCB, used as internal standard (*Jensen, pers. comm*). This fungicide has never been used in Nicaragua and samples analyzed without internal standard did not show any HCB peak.

A Varian 3400 gas chromatograph equipped with a ^{63}Ni electron capture detector was used for the chromatographic analysis of the organochlorine residues. A volume of 3 μL was injected in "splitless" mode into a DB_5 30 m long capillary column, with an internal diameter of 0.32 mm and 0.25 μm film thickness, using hydrogen as carrier gas with a linear velocity of 55 cm/sec and nitrogen as make-up gas. The temperature program employed was 80°C (1 min), 4°C/min to 200°C, 3°C/min to 230°C, 15°C/min to 250°C (5 min). The temperatures of the detector and injector were 320°C and 170°C.

All the samples were analyzed for: α-BHC, ß-BHC, lindane, aldrin, heptachlor, heptachlor-epoxide, p,p'-DDE, p,p'-DDD, p,p'-DDT and toxaphene. The minimum detection limits for the residues analyzed were: 0.1 $ng \cdot g^{-1}$ for α-BHC; 0.4 $ng \cdot g^{-1}$ for ß-BHC, heptachlor, p,p'-DDD and p,p'-DDT; 0.2 $ng \cdot g^{-1}$ for lindane, aldrin, p,p'-DDE and heptachlor-epoxide; and 8 $ng \cdot g^{-1}$ for toxaphene. The recoveries exceeded 65 % for all the compounds analyzed.

RESULTS AND DISCUSSION

The levels of organochlorine residues detected in the three populations are presented in Tables 1-3. These show

the individual values, the means and the standard deviations of the organochlorinated pesticide residues on the basis of plasma and blood fat measurements. ß-BHC and lindane were detected in few individuals and at low

concentrations. Neither α-BHC, heptachlor, aldrin, heptachlor-epoxide nor toxaphene were detected in any of the populations.

A one-way analysis of variance (ANOVA) showed a significant difference between the three populations in total DDT concentration, both in plasma and in fat. Among the Danish population, as was expected, the lowest levels were found, since the use of DDT in Denmark was discontinued 20 years ago, and because the Danish volunteers who participated in the investigation had been residing in Nicaragua for less than three years.

The Nicaraguan population which lives in the capital showed a mean concentration of ΣDDT in plasma significantly lower than that of the rural population. DDT has supposedly been used in both areas for health hazard propagation control, so that the difference in concentration of ΣDDT in blood plasma would reflect the exposure of the rural population to DDT used in the agricultural activities of the area, or indeed also to the exposure to DDT and its metabolites through its accumulation in the food consumed by them. The question is whether the difference found owes its origin to the fact that DDT has continued to be used in agriculture since 1980, against the law, or rather that it points to intensive use in the years prior to its prohibition. In the Danish population, DDT was found in only 2 individuals (one of them has formerly been living in Spain for 12 years), the rest showed only DDE residues. In the Nicaraguan rural population 20 of 22 individuals had both DDT and DDE, and of the Nicaraguan capital population 11 of 20 had DDT residues.

In spite of the significant difference that existed between the three groups, the values found did not differ greatly from other values reported in the literature. Skaare et al. (1988) detected 10 ± 8 $ng \cdot g^{-1}$ of p,p'-DDE in serum of Norwegian women and 150 ± 75 $ng \cdot g^{-1}$ of p,p'-DDE in serum of immigrant women from the Third World, who live in Norway. In Yugoslavia, Krauthacker (1991) found a mean concentration of 6 $\mu g \cdot L^{-1}$ of ΣDDT in serum of women from rural areas; Pines et al. (1987) reported mean concentrations in serum of Israeli women of 74.0 $ng \cdot g^{-1}$ in 1975 to 23.7 $ng \cdot g^{-1}$ in 1985-86. Dale et al. (1966) showed, that there is no significant difference between the concentrations found in plasma and serum so these values can be directly compared.

Table 1. Concentrations of ß-BHC and ΣDDT in plasma of Danes who live in Nicaragua ($ng \cdot g^{-1}$).

No	Sex	ß-BHC	ΣDDT	% Fat
1	M	<DL	3.00	0.79
2	M	<DL	2.30	0.60
3	M	<DL	3.73	1.15
4	F	<DL	1.61	0.30
5	F	5.49	5.74	1.48
6	F	<DL	1.80	0.92
7	M	<DL	5.88	2.10
8	M	0.43	2.79	0.76
9	F	<DL	0.70	0.95
10	F	<DL	2.90	0.67
11	M	5.72	3.05	0.80
12	M	6.14	2.50	0.40
13	F	<DL	1.66	0.73
14	F	<DL	1.07	0.97
15	M	<DL	2.18	1.07
16	F	<DL	6.05	0.51
17	F	<DL	1.58	0.84
18	F	<DL	0.48	0.97
19	F	<DL	1.87	0.69
20	F	<DL	1.84	0.54
21	F	<DL	1.48	0.22
22	M	<DL	1.58	0.22
23	F	<DL	1.43	0.57
Range		<DL - 6.14	0.48 - 6.05	0.22 - 2.10
Mean		4.45*	2.49	0.79
SD		2.69*	1.55	0.42

Sex F: female; M: male,
ΣDDT: p,p'-DDE +p,p'-DDD + p,p'-DDT
* calculated from positive values
DL: detection limit

Table 2. Concentrations of ß-BHC, Lindane and ΣDDT in plasma of Nicaraguans who live in the capital ($ng \cdot g^{-1}$).

No	Sex	ß-BHC	lindane	ΣDDT	% Fat
1	F	<DL	<DL	7.55	0.77
2	M	<DL	<DL	13.98	0.94
3	M	<DL	<DL	6.52	1.15
4	M	<DL	<DL	11.14	0.84
5	M	<DL	<DL	21.91	1.56
6	M	<DL	<DL	7.91	0.79
7	M	<DL	<DL	6.33	0.84
8	F	0.43	0.96	8.23	1.26
9	M	<DL	<DL	9.76	1.11
10	M	<DL	<DL	7.82	1.14
11	F	<DL	<DL	10.16	0.90
12	F	<DL	<DL	12.80	0.88
13	F	<DL	<DL	14.15	0.86
14	F	<DL	<DL	5.43	0.77
15	F	0.29	<DL	16.71	1.04
16	M	0.38	<DL	22.92	1.67
17	M	<DL	<DL	18.14	1.21
18	M	0.27	<DL	27.16	0.77
19	M	<DL	<DL	9.95	0.86
20	M	<DL	<DL	10.55	1.67
Range		<LD-0.43	<LD-0.96	5.43-27.16	0.77-1.67
Mean		0.34*	0.96**	12.46	1.05
SD		0.08*		6.07	0.29

Sex F: female; M: male,
ΣDDT: p,p'-DDE + p,p'-DDD + p,p'-DDT
* calculated from positive values
** value found in one individual
DL: detection limit

Table 3. Concentrations of ΣDDT in plasma of Nicaraguans who live in the rural area ($ng \cdot g^{-1}$).

No	Sex	ΣDDT	% Fat
1	M	26.40	2.95
2	M	44.63	1.73
3	M	38.39	1.14
4	M	22.79	1.52
5	M	82.81	1.43
6	M	46.48	1.19
7	M	25.20	1.20
8	M	30.96	0.80
9	M	49.99	1.38
10	M	41.10	1.17
11	M	21.36	2.10
12	M	17.10	1.29
13	M	67.50	1.16
14	M	23.83	0.80
15	M	49.39	1.55
16	M	14.37	0.64
17	F	42.11	0.94
18	F	15.71	0.86
19	M	45.46	2.43
20	M	13.19	0.49
21	F	36.53	1.00
22	M	19.70	1.23
Range		13.19-82.81	0.49-2.43
Mean		35.23	1.32
SD		17.84	0.58

Sex F: female; M: male,
ΣDDT: p,p'-DDE + p,p'-DDD + p,p'-DDT
DL: detection limit

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