



Evaluation of Organochlorine Pesticide Residues in Human Urine from Rural Population in Sudan

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Abstract

This study was conducted to assess the prevalent effect of organochlorines insecticide residues on randomized population sample from El-Hosh Town community, South Gezira. 36 human urine samples such as 12 from male, 12 from female and 12 from children were collected from randomized population samples. Residues of organochlorine pesticides were extracted by hexane and analyzed by gas chromatography (GC). The results revealed that, only DDE was detected. DDE was detected in four out of 12 (33%) female and 3 out of 12 (25%) male urines in concentrations ranging between 4.64 – 5.31 ppm. No detectable residues of DDE were found in children. DDE concentrations in females (5.17 ppm) were higher than those in male urines (4.18 ppm). The results indicated that the DDE residues level were not associated with age or weight. Although the use of most of these compounds has been severely restricted more than thirty year ago, the presence of their residues indicate their characteristic of long term persistence.

Keywords: Residues of pesticide, Monitoring, Human Urine, Sudan.

1. Introduction

Persistent organochlorine pesticides (OCPs) have been used intensively in agriculture and public health for a relatively long period of time [1-2]. Humans therefore, absorb organochlorine compounds with food, via inhalation and skin [3]. Pesticide hazards are frequent and severe in developing countries where pesticide use is widespread. The extensive use of pesticide is known to lead to serious public health and environmental problems [4]. The organochlorine insecticides were introduced in the Sudan Gezira in 1945. Since then it was continuously used for the control of cotton pests, in increasing amounts, up to 1981. The total amount of DDT sprayed on the Gezira over the last thirty years of its use was estimated to be 10 million kgs [5]. Smaller amounts have been used in other cotton growing areas in the country, New Halfa, Blue Nile, Nuba Mountains and Tokar Delta Schemes. So the widespread applications of organochlorine in agriculture, public health and in and around homes can result in the accumulation of pesticides residues in the environment. Due to their resistance to be metabolized and their lipophilic characteristics, organochlorines (OCs) can spread out extensively. They tend to be accumulated in biological samples which have lipid serum, adipose tissue and breast milk [6]. The amount of fat in the subcutaneous fatty tissue is 80%, in serum 1% and 2%-4% in milk [7]. Lipophilic contamination levels in the subcutaneous fatty tissue are five times higher than milk and 100-350 times more than blood [8]. Developed countries have banned many organochlorine pesticides because of their

potential toxic effects to man and their impacts on the ecosystem [9]. To estimate the effect of certain government measures to ban the use of persistent toxic chemicals, most countries have conducted initial monitoring programs to determine organochlorine pesticides in human tissues [10,1]. The measurement of the level of OCPs in urine of human populations are good markers in determining the extent of exposure and in the evaluating the hazard [2,11].

2. Materials and Methods

2.1. Study of population and sampling

Urine samples were collected from a random sample of population. Thirty six urine samples were collected randomly from the general population in 2008. Twelve from males, twelve from females and twelve from children. The samples were kept in a plastic container and stored at 5° until analysis.

2.2. Chemical and analytical standard

Acetone, dichloromethane, n-hexane celite, alumina and potassium hydroxide anhydrous sodium sulphate (pesticide residue grade) were obtained from Panreac (Barcelona, Spain). Silica gel adsorbent was obtained from Sigma–Aldrich (St. Louis, MO, USA). Certified standards of Gamma-HCH (99.6% purity), Aldrin (99.0% purity) and Heptachlor epoxide (99.0% purity) DDT (99.2 purity %) were supplied by Dr. Ehrenstorfer (Augsburg, Germany). Individual stock standard solutions were prepared in acetone. Standard solutions for gas chromatographic (GC) analysis were prepared by suitable dilution of the stock standard solutions with n-hexane.

2.3. Extraction and clean up procedure

Samples were extracted in hexane by using the method described by Tewari and Sharma [12]. 21 ml of urine were measured and filtered and 15 ml of distilled water was added. The volume was measured and completed to 100 ml of distilled water and 10 g anhydrous Na₂SO₄. The mixture was transferred to separatory funnel and hexane (40 ml) was added and shaken well for 5 min. After separation of layers, extract was filtered and transferred again to separatory funnel and 10 ml of hexane was added and shaken well. After separation of layers the hexane layer collected. All extracts were mixed with two drop of 10% potassium hydroxide in order to convert DDT into DDE prior to analysis.

2.4. Condition chromatographic

Thin layer chromatography (TLC) plates were divided into longitudinal columns made by scraping the silica gel. About 20 µl of pesticide standard and samples extract were spotted. The plates were placed in a developing tank containing 100 ml n–heptane which was the best system for separation of the pesticides in the current investigation. When the solvent front approached 10 cm above the base line, the plates were removed from the tanks, allowed to dry at room temperature and after dryness the plates were visualized under short wave ultraviolet lamp at 254 nm.

The analysis was carried out by gas chromatography. Using a carlo Erba Fracto Vap 2101 equipped with electron capture detector (ECD). Glass column used was 3m x 0.25 mm id packed with 5% OV-210 on chromosorb WHP 80-100 mesh. Temperature of the injection block, oven and detector were 250, 190, 300°C respectively. Nitrogen carrier gas flow rate 60 ml/minute, Injection volume for standards and samples was 1µl.

3. Results and Discussion

3.1. Performance of the analytical method

The linear dynamic range, precision (as relative standard deviation) and sensitivity (as limit of detection) values for determination of Aldrin, DDE, gamma HCH and Heptachlor epoxide are reported in Table 1. Recovery percentages, accuracy, the limit of determination and coefficient of variation (CV%) were determined by spiking human urine at 0.01 ppb of pesticides tested.

Table 1: Figures of merit obtained for the used method.

Analyte	Linear range µg mL ⁻¹	Y = (a ± S _a) X + (b ± S _b)	R ²	LOD µg mL ⁻¹	LOQ µg mL ⁻¹	RSD (%) (n=5)
Gamma HCH	0.015-0.100	(8363.5±156.4801) X - (0.651±0.6520)	0.9995	0.003	0.009	3.6
Heptachlor epoxide	0.020-0.200	(20041±123.3099)X - (55.442±7.5006)	0.9997	0.001	0.003	4.3
DDE	0.025-0.200	(9373.5±166.4601) X - (0.875±0.8750)	0.9998	0.002	0.007	4.7
Aldrin	0.003-0.050	(19041±1136) X - 7.5006)	0.9999	0.001	0.004	4.3

a; slope a; b, Intercept; R, regression coefficient; LOD, limit of detection; LOQ, limit of quantification, RSD, relative standard deviation.

As can be seen from table 1, using the detector response for the pesticides tested was linear in the range of the concentrations studied (0.003 to 0.2 µg. ml⁻¹). Correlation coefficients (r) were almost around 0.999. Recoveries were found from 87 - 97 %, and the limit of quantification (LOQ) was determined.

3.2. Evaluation of pesticide residues in human urine from rural population in Sudan

The results presented in Table 2 showed that, out of 36 samples of human urine that were analyzed, DDE was detected in 4 out of 12 (33%) female and 3 out of 12 (25%) male. No detectable residues of DDE were found in children.

Table 2: Percentage of organochlorine pesticide residues in human urine

Human population	Number of samples analyzed	Number of samples have OCP residues	The percentage of samples have OCP residues
Female	12	4	33%
Male	12	3	25%
Children	12	0	0

The results of the random sampling on the basis of age and weight (Table 3) have been computed. DDE, the main metabolite of DDT, was detected in the urine of people from El-Hosh at relatively high frequency. In general DDE concentration in female (5.17 ppm) were higher than those in males (4.18 ppm). It is suspected that this is due to the past wide use of DDT and subsequent exposure through food chain and past domestic exposure in DDT used in combating malaria, since the women of this area spend more of their time in the home than do the men. This differences is in agreement with several authors [13-16] but in contrast to Nakata [17] and Stehr-Green [18] which showed that the organochlorine (OC) concentration in males were higher than those in females.

Table 3: Organochlorine pesticide residues in Human urine.

Serial No	Sex	Age	Weight (kgs)	Organochlorine pesticide residues (ppm)			
				Gamma HCH	Aldrin	Heptachlor epoxide	DDE
1	F	38	70	< LOD	< LOD	< LOD	5.149
2	F	39	82	< LOD	< LOD	< LOD	5.093
3	F	54	91	< LOD	< LOD	< LOD	5.132
4	F	30	68	< LOD	< LOD	< LOD	5.313
5	M	50	66	< LOD	< LOD	< LOD	4.885
6	M	60	76	< LOD	< LOD	< LOD	4.639
7	M	60	55	< LOD	< LOD	< LOD	4.858

F. female; M. male

The analysis of different ages from Fig. 1, indicated that, DDE residue levels are quite stable among distinct segment of the general population and was not associated with age. These findings are in contrast to Violante [19] and Bertram [20], who argued that, changes in human levels as a results of decreased chlorinated pesticide residues is more easily detected by monitoring younger than older subject, as the change is mainly dependent on the duration of exposure. The influence of age is less well defined, although it is generally acknowledged that, residue levels rise with increasing age, in general agreement with the data obtained from animals [21-22] found that, the level of DDT/DDE rapidly increased from about 5.5 to 11.5 ppm up to the age group 35 – 44 years but became stabilized at 9.0 ppm in the four subsequent age group (45 – 85 years old).

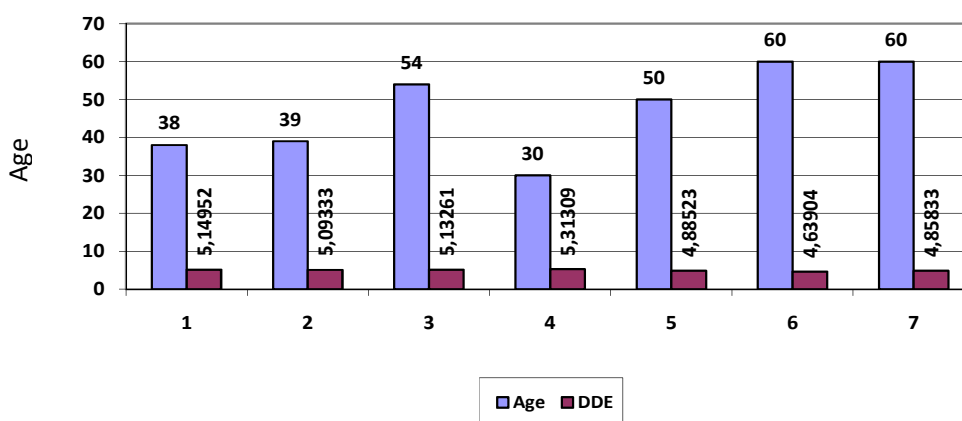


Figure 1: Relationship between organochlorine pesticides residues in human urine and age.

When results are grouped by age, it's observed, that people born within time of the official prohibition to use DDT (30 years old) show higher levels of DDE residues than the people born before. This may be attributed to dietary sources of exposure especially food of animal origin and also through water, outdoor and indoor air, dust and soil which result in the bioaccumulation of these chemicals in the human body. These results are consistent with Cruz [23] and Hanaoka [24]. So relatively higher concentration of OC residues associated with human in the older age group may be the result of comparatively longer period of exposure to food and environmental contaminant [25].

For weight, the result presented on Fig. 2 showed that, no direct relationship was found between concentration of DDE and the weight gain of the donors. This result coincide with those recorded by Polishuk [26] and Acker [27]. It's generally known that, the concentration of OCP increased with increasing in human weight. Heavier ones had higher pesticide residues. This is in agreement with those of Matuo [28] in Brazil, which may be explained in terms of fat content of the milk, while, a study conducted by Arrebola [29] found that, levels of HCB in adipose tissue were three times higher in women than in men.

Adipose tissue may act as a reservoir for the continuous release of chemicals that are therefore, present in blood and urine and may reflect historical exposure to pesticides that are not now in use. Residues of pesticides and their metabolites in various human tissues and fluids collected from general population are indicative of the total body burden of these pesticides and of past and present exposure [30]. Most members of the general population are not occupationally exposed to pesticides; their contact comes from other more covert sources. Since there was no occupational exposure, so the value measured represent back ground exposure caused by accumulation of DDT in the food chain – or via inhalation due to spraying at home.

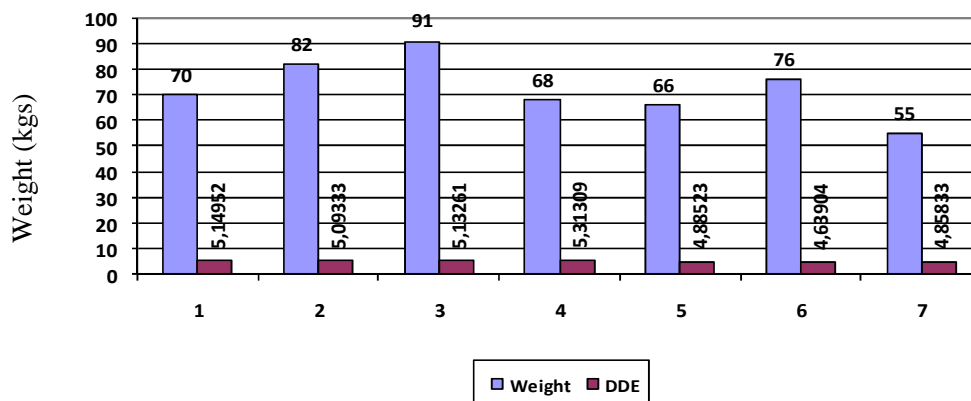


Figure 2: Relationship between organochlorine pesticide residues in human urine and body weight (kgs).

Naturally factors such as food habits, physiological state and life style must play an important role in determining the residue levels in man. The problem is that, currently there are no concrete data indicating the significance of any of them. For instance, the data from a residue survey of wild animals and some observations [31] along with simple logic suggest that, meat-eaters should accumulate more chlorinated pesticide residues than vegetarians. It is rather difficult to do an epidemiological evaluation on basis of the present findings, because of the small size of samples and absence of health and history of subject samples. Although DDT has been banned since 1981, the presence of pesticide metabolites in human urine samples is an indication of their long term persistence in human bodies and continued discrete elimination.

Conclusions

The study indicated the prevalence of some organochlorine pesticide residues including DDE in human urine, owing to their past use in agricultural and sanitary purposes and subsequent storage in adipose tissues. DDE was found at highest concentration in human urine. These ranged between 4.64 – 5.31 ppm. The design and implementation of appropriate epidemiological studies and their integration with monitoring of human, food and environmental samples would be a major step in assessing the risks of OC residues in food and controlling or eliminating them. -This study was carried in a very small area compared to the total area of Gezira scheme, which was the site of use of these chemicals. Therefore, more extensive survey is necessary before realistic evaluation of these contaminants could be made.

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