ESTIMATION OF SOME CARCINOGENIC PESTICIDES
OVERLOAD IN HUMAN ADIPOSE TISSUE

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ABSTRACT

Polychlorinated biphenyls (PCBs) are potentially hazardous compounds in the environmental pollution for human beings persistent. The high lipophilicity and the resistance to biodegradation of PCBs allow the bioaccumulation of these chemicals in the fatty tissues of organisms.To test the hypothesis that the amount and quality of organochlorine residues in adipose tissue related to the tumor incidence, the PCBs body burden in patients was studied. The objective of this study was to determine the levels of PCBs in the adipose tissue of different patients lived in Delta Egypt especially Al-Dakhelia, Al-Garbia, and Damietta which were the most probably used pesticides. For this purpose, levels of eight major persistent PCB congeners (IUPAC Numbers: 101, 118, 138, 153,170, 180, 183, 187) and the DDT metabolite p, p' DDE, p, p' DDD and p, p' DDT ,HCH were measured in 25 patients adipose tissue samples by gas chromatography with electron capture detection (GC-ECD). The concentrations of PCB 28, PCB 52 and PCB 101 were found to be higher than those found in industrialized countries.
Also breast cancer women showed higher median

values of (138, 153 and 180) with respect to brain, lung, liver, and prostate cancer men. A positive correlation (P <0.05) was found between the age of donors and the tissue levels of PCBs 28,118,138, 153,170,180,183,187 and 188, and also their is a positive correlation of both p, p' DDT and DDE with age in females.

The results are compared with the similar studies from other countries, the higher level of these compounds detected in this study seems to be similar to results obtained in other countries.

Key words: human adipose tissue, pollution, polychlorinated biphenyl, congeners, isomer.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are among the most prevalent environmental pollutants, being detected in almost all living organisms PCBs are stored in muscle and fat of animals and humans, but they can also reach other compartments such as brain, liver and lung [1,2]

The (PCBs) are a group of presistance contaminants of environmental and toxicological importance. Residues of these xenobiotics have been identified throughout the world. Many studies have examined their distribution in organism [3]

The various congeners differ markedly in persistence and toxicity [4, 5, and 6]. These chemicals bio-accumulate in the food chain, because of their relative insolubility in water and high solubility in fats, PCBs are accumulated in fatty tissues of exposed animals and human [7] .The [8] suspects that PCBs are probable human carcinogens. [9, 10]

Most of the organochlorinated. biphenyl (PCBs) have been shown to exhibit estrogenic or antiestrogenic activities in biological tests [11] Moreover the main metabolites of DDT, 4, 4'-DDE, and the fungicide vinclozoline have been shown to exhibit antiandrogenic effects for the developing human fetus crossing the placenta. [12]

The pattern of chlorine substitution is also important in the activation of the hepatic microsomal system Mixed Function Oxidase

(MFO) [13]. This enzymatic system may transform xenobiotics, such as the polycyclic aromatic hydrocarbon, into metabolites that are more toxic than their parent compounds [14].

The enzymes ,also known as cytochromeP-450-mediated monooxygenases, constitute an important and highly variable group of enzymes which metabolize a wide variety of endogenous and exogenous lipophilic compounds-including steroid, fatty acid, prostaglandine, vitamins, polycyclic aromatic carcinogens, organochlorine insecticides, as well as polychlorinated and polybrominated biphenyls [15].

The major route of human exposure to PCBs is ingestion, mainly of fish and shellfish surface soils, drinking water and groundwater, indoor air, and workplaces [12]. The detection of PCBs in blood, breast milk, and adipose tissue samples from the general population indicates widespread exposure to PCBs from environmental sources [16].

The majority of the 209 PCB congeners are inactive, however, around a quarter of this number are biologically active. This group is subdivided into the following :3- methylchlolantreen (3-MC) inducer of cytochrome P-448 enzyme, phenobarbital-type(PB) inducer of cytochrome P-450 enzyme, and mixed type inducers of both cytochrome P-448 and P-450 system [17]. The Toxic Equivalency Factor TEF [18], based on the activity of single PCB congeners relative to that of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin, (2, 3, 7, 8-TCDD) was used to evaluate the toxicological risk associated to the presence of PCBs in humans. [19, 20]

The aim of this study was to determine total PCB and some carcinogenic pesticides in samples of human adipose tissue from different localities. The influence of habits, age sex and previous nutritional food present habits occupation on the tissue levels of PCBs was also investigated.

MATERIALS AND METHODS

Twenty-five human adipose tissues (10 women and15 men aged from 27 to 83 year) were taken during surgical operations from different patients with breast ,brain, lung, liver and prostate cancer in

tumor marker units in different hospitals at Delta zone especially Al-Dakhelia, Al-Garbia, and Damietta which were the most probably used pesticides. Adipose tissue samples were kept frozen at-80°C in glass containers until analysis.

Analysis of polychlorinated biphenyls:

Total PCBs were extracted according to[21,22,23] Samples were digested in 1 N KOH / ethanol solution for 1 h and the resulting extract was transferred to hexane. The hexane layer concentrated was cleaned in a 1.5-g silica gel packed in a glass column 10 mm i.d. =20 cm . The eluate from the silica gel column was concentrated to 6 ml. A 3-ml aliquot of this extract was reserved for the determination of PCB isomers. The remaining 3 ml was run on a column 5 mm i.d. =20 cm packed with 125 mg of activated carbon to separate non-ortho coplanar PCB congeners from the other isomers. An initial fraction eluted with 100 ml of 20% dichloromethane in hexane contained PCBs with ortho chlorines and other xenobiotics. A second fraction eluted with 100 ml of benzene / ethyl acetate 50:50 containing non-ortho chlorine-substituted coplanar PCBs was micro-concentrated and the residues were transferred to 5 ml of hexane. This hexane extract was treated with 5 ml of 10% fuming sulfuric acid and rinsed in distillated water. Organochlorine compounds were analyzed by gas chromatography with a Perkin-Elmer Auto system gas chromatograph fitted with a 63Ni electron capture detector. The capillary column, SBP-5 Supelco, was 30 m long, 0.25 mm i.d. with a 0.25-mm thick coating. Helium was used as the carrier gas. Injection was set at split less mode, injector temperature was 270 °C, and the column was programmed from 120 to280°C at 58/ min and maintained at 280°C for 10 min. Detector temperature was 300°C and gas make-up was argon / methane 95:5 . The concentrations of individually resolved peaks of PCB isomers were summated to obtain total PCB concentrations.

A mixture of Aroclor 1260 was used as external standards and for calibration, recovery evaluation and confirmation. For coplanar PCB analysis, injection was set at Split less mode, injector temperature was 200°C and detector temperature was 290°C. The column was programmed from 150°C for 1 min to 180°C at 258°C / min and to

280°C at 258 °C /min and maintained at 280°C for 10 min. Single congeners, PCB-77, PCB-126 and PCB-169 supplied by Dr Ehrenstorfer GmbH, were used as external standards and for calibration, recovery evaluation and confirmation . Blanks were determined before analysis of samples and their maximum values were subtracted from the results.

The different sets of data were examined for statistical differences by the Mann–Whitney U-test [24]. Spearman rank correlation was calculated to measure association between residues.

In order to test differences between subgroups, Kruskal –Wallis nonparametric ANOVA test was applied. The relationship between PCB levels and age of donors was evaluated by a simple linear correlation. Differences were considered to be significant when $P<0.05$.

RESULTS

Levels of Organochlorine Pesticides and PCBs

The levels of PCB congeners found in adipose tissue samples are presented in Tables (1&2). PCBs 138,153, 170, 180 and 187 were found in all samples analyzed, PCBs118, 183 and 188 were found in more than 90%of samples. The mean level of total PCBs (summation of mean levels found for individual congeners) found in samples amounted to 0.56mg-1 of adipose tissue. The compound found at the highest concentration was PCB 180, followed by the congeners 153 and 138. From the mean concentration of the mono-o- congener PCB118 and based on the toxic equivalency factor of this congener (0.0001) relative to the strongest compound, 2,3,7,8-tetrachlorodibenzo-p- -dioxin (2,3,7,8-TCDD)[18] .

Among organochlorines, DDTs concentrations were found to be the highest in the range of 0.8 to 1110 ng.g-1, with a mean value of 324 ng.g-1 on lipid weight basis, The order of concentrations of DDT compounds was p,p'-DDE >p, p'-DDT > p,p'-DDD.

The mean concentrations of p, p'-DDE, p, p'-DDT and p, p'-DDD were 122 (range; 1.3 - 1110 ng. g-I), 2.6 (0.8 – 16 ng.g-1) and 3.4 ng.g-1 (0.4 - 18 ng.g-I), respectively.

Next to DDTs, PCBs were greater in adipose tissues. Average

PCB concentration was 73.35 ng.g-1 (0.8-369 ng.g-1).

HCHs concentrations varied between 2 and 821 ng.g-1, with an average of 8.2 ng.g-1 on lipid weight basis as showed in Tables (3&4). Among four HCH isomers α- and β-HCH were detected in all the samples. However, α − and γ -HCH was detected only at trace amounts. B -HCH was the most predominant isomer with a composition of total HCHs.

The mean age of donors was 55 years (range 26- 83 years) table (5). The correlations between the age of donors and the tissue levels of PCB congeners are presented in Fig. (1). A positive correlation (P <0.05)was found between the age of donors and the tissue levels of PCBs 28,118,138, 153,170,180,183, 187 and 188,while no significant correlation was found between age and tissue levels of PCBs 152 and 101 (Fig.1).

Table (1): Details of sample information in the present study of PCB:

	Age	Sex	PCB 101	PCB 118	PCB 138	PCB 153	PCB 170	PCB 180	PCB 183	PCB 187	Total
1	27	M	2	19	154	141	31	76	5	23	451
2	33	M	0	68	112	91	77	232	6	41	627
3	42	M	3	57	23	81	37	221	4	16	442
4	61	M	4	16	31	77	44	66	5	10	281
5	65	M	7	18	46	108	126	178	18	29	530
6	66	M	0	44	221	276	119	154	22	22	858
7	67	M	3	84	176	165	21	84	34	34	601
8	70	M	5	17	29	133	95	97	11	22	409
9	71	M	8	10	31	94	77	63	9	16	308
10	75	M	6	61	55	161	20	154	34	14	505
11	77	M	8	22	84	212	66	87	6	80	565
12	81	M	7	18	31	339	45	56	7	56	559
13	82	M	0	78	98	92	37	254	41	69	669
14	83	M	0	45	76	151	99	342	38	80	831
15	86	M	4	92	46	369	132	167	22	75	934
Average male	65.7		3.8	43.2	78.8	166	68.4	148.7	17.4	39.1	885
16	35	F	5	21	119	99	22	267	15	46	294
17	45	F	6	74	201	67	24	228	6	16	622
18	46	F	0	12	132	115	28	191	9	11	498
19	64	F	0	23	227	97	31	342	11	54	785
20	64	F	8	42	45	88	73	50	24	8	338
21	66	F	8	9	58	341	84	225	37	55	817
22	67	F	7	19	211	215	66	194	18	19	749
23	71	F	4	45	36	197	25	99	25	14	445
2425	75	F	3	24	62	112	32	74	6	66	329
	75	F	0.8	23	42	85	141	65	9	42	407.8
Average female	60.8		4.2	29.2	113.3	101.9	52.6	173.5	16	33.1	5284.8

Table (2): Concentration of PCB congeners (ng /g weight) in subcutaneous
adipose tissue.

% of P CB	Range	median	Mean± SD	IUPAC n°=30	Congener
2.9	0- 8 (8)	5	4.41+3.18	101	22`455` pentachlorobiphenyl
5.1	9-92 (83)	23	36.3+25.8	118	23` 44` 5 pentachlorobiphenyl
11.1	31-227 (196)	62	91.4+65.08	138	22`   344` 5` Hexachlorobiphenyl
16.9	67-369 (302)	115	166.7+98.5	153	22`   44` 55` Hexachlorobiphenyl
7.0	20-141 (121)	45	58.9+38.1	170	22`33`44`5 Heptachlorobiphenyl
15.7	50-342 (292)	167	168.1+98.1	180	22`   344` 55` Heptachlorobiphenyl
2.3	6-41 (35)	15	18.6+12.4	183	22`   344` 5` 6 Heptachlorobiphenyl
4.1	8-80 (72)	46	42.4+26.6	187	22`   34` 55` 6 Heptachlorobiphenyl

Table (3): Details of sample information in the present study (DDT& HCH)

No	Age	Sex	P,P-DDE	P,PDDD	DDT - P,P	α-HCH	β-HCH	γ-HCH	Total
1	27	M	9	ND	ND	ND	ND	ND	9
2	33	M	228	ND	ND	ND	ND	ND	228
3	42	M	323	ND	4.1	224	ND	3.3	554
4	61	M	112	ND	1.1	ND	ND	1.5	114
5	65	M	60	5.2	ND	ND	ND	ND	65
6	66	M	2.5	4	0.9	ND	ND	6.1	12.5
7	67	M	223	2.9	4.3	ND	4	ND	234
8	70	M	141	ND	ND	ND	ND	ND	141
9	71	M	6	18	ND	ND	18	7.2	49
10	75	M	136	2.3	16	ND	6	ND	160
11	77	M	34	ND	7	ND	ND	2.1	43
12	81	M	110	9	ND	2	ND	ND	121
13	82	M	203	3.1	2.1	ND	28	4.2	240
14	83	M	241	ND	3.2	ND	3.1	ND	247
15	86	M	33	4.5	ND	2.2	ND	6.1	45.5
Average male	65.7		124	3.8	2.5	15.2	3.9	2.1	158.3
16	35	F	18	ND	ND	ND	ND	ND	18
17	45	F	33	4.3	ND	ND	ND	ND	37
18	46	F	342	ND	ND	821	ND	2.3	1165
19	64	F	1.3	ND	ND	ND	ND	ND	1.3
20	64	F	221	1.6	0.8	5	31	ND	259.4
21	66	F	49	0.4	4	ND	ND	5.1	58.5
22	67	F	371	ND	8	ND	ND	7.3	386
23	71	F	1110	16	25	ND	ND	ND	1151
24	75	F	787	5	8	ND	69	ND	869
25	75	F	11	2.1	3.3	139	37	25	217
Average female	60.8		294.3	2.94	3.9	9.65	13.7	3.96	413.8

Table (4): Concentration of DDT & HCH(ng /g weight) in subsutaneous adipose tissue.

Female no=10		Male no=15		
Mean ± S.D	Median	Mean ±S.D	Median	
91.77 + 3.6	0.0	16.2 + 2.97	0.0	αHCH
11.11 + 2.4	0.0	4.21 + 8.41	0.0	ßHCH
1.55 + 2.65	0.0	4.2 + 8.6	0.5	ýHCH
232 + 387	221	121 + 104	111	DDE
1.33 + 1.87	1	3.4 + 4.9	2.5	DDD
2.55 + 3.35	1	2.6 + 4.3	1.0	DDT

Table (5): Average age of donors

Average year		no	Sex
Range	Median		
26 -87	65.78	15	Male
35- 75	60.11	10	Female
26-87	63	25	Total

Fig (1): Correlation between age and PCB
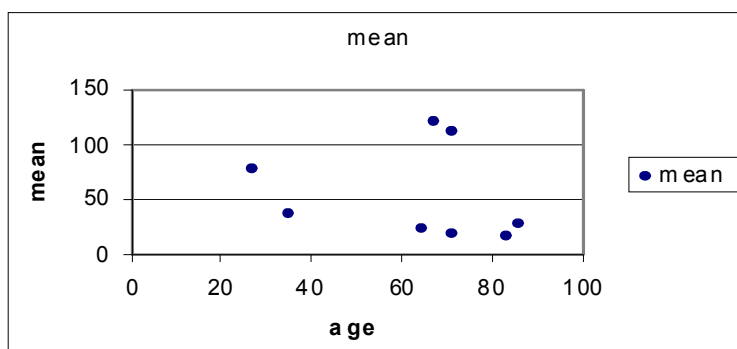
DISCUSSION

Congeners PCB 153, PCB 180, and PCB 138 contain six to seven chlorine atoms that are components of commercial PCB mixtures. These congeners have high persistency capacity in living organisms and they are commonly detected in human adipose tissue as major PCB compounds PCB 153 is the most diffuse PCB congener in the environment, and its found in high concentration in those species which are at the top of the food chain, also the literature points out PCB-153 as the most common congener in the environment because it is very persistent and slowly degradable [25, 26, 27, 7]

Women showed higher median values with respect to men, however, such differences were not statistically significant. This result is in accordance with finding of [28] they found that no significant difference between the PCBs in Sienese Italy males and females. The most abundant congeners included pentachlorobiphenyl(PCB-118) , (PCB-138)and (PCB-153) hexachlorobiphenyls, and (PCB-170), (PCB-180)and(PCB-187) heptachlorobiphenyls., [29]

The levels of eight PCBs in human adipose tissue found in the present study are compared with levels obtained in some other countries (table, 1). The concentrations of PCB 28, PCB 52 and PCB 101 were found to be higher than those found in industrialized countries

The following congeners are listed in order of prevalence:
PCB-153 > PCB-180 >PCB-138 > PCB-170>PCB-118>PCB-187.

The general pattern of the main classes of isomers was as follows: hexa-> hepta-> octa> penta-chlorobiphenyls;

As mentioned before, three PCB congeners were dominating (PCB 153, PCB 180, and PCB 138). The higher level of these three PCB compounds compared to other PCB compounds detected in this study seems to be similar to results obtained in other countries.

In Table (2), previous results of [30] are compared with the results from the present study. The subjects in the two studies had been living in Ankara during the same time period. The three PCB compounds (IUPAC Nos. 138, 153, and180) were also major components.

Elevated levels of polychlorinated biphenyls, bis (4-chlorophenyl) -1,1 dichloroethene, and bis(4-chlorophenyl)-1,1,1 trichloroethane were found in fat samples from women with cancer, compared with those who had benign breast disease. These results, although preliminary, suggest a role for environmentally derived suspect carcinogens in the genesis of mammary carcinoma[31,32].

p, p' DDE is the most abundant pesticide residue with concentration ranging from 1.3 to1110 ng/g lipids; the average was 231 ng/g. In several other studies, much higher 5-10 times concentrations have been found [28].In other study in korea, the mean concentration of DDE was found to be about twice as high as that obtained in this study and in Italy a some what higher concentration was measured 395 ng/g in average [33] DDE accounted for 79% of total pesticide concentration of males and 46% of females. DDT has a half life of over 60 year in the environment [11]) and its metabolite DDE is also very persistent [34, 35]

Unlike p, p'-DDT, p, p'-DDE is stable in organisms as well as in the environment
The ratio of p, p'-DDE to p, p'-DDT in human adipose tissues was calculated based on the values reported for a few countries .In general, it is known that the ratio of p, p'-DDE to p, p'-DDT in human tissues tend to increase with time [36]. In other words, the higher ratio of p, p'-DDE to p, p'-DDT suggested the prohibition on the use of DDT[37]. The ratio of p, p'-DDE to p, p'-DDT in human adipose tissues in USA and UK, where the use of DDT has been prohibited since the 1970, have increased with time. On the other hand, in India, the ratio was not changed over time. Similarly, South Vietnamese samples showed lower p, p'-DDE to p, p'-DDT ratio.

The average DDE/DDT ratio was 23. In previous studies the ratio has been much lower approximately 2-3 in the 1960 and 10-20 in 1980 [38, 39]. Only DDE showed significant age correlation in females uncorrected P=0.048. [33, 40] found a positive correlation of both p, p' DDT and DDE with age.

CONCLUSIONS

Epidemiological and toxicological data suggest that many of deaths by pesticides might be prevented if (a) the use of pesticides most toxic to humans was restricted, (b) pesticides could be safely stored in rural communities, and (c) the accessibility and quality of care for poisoning could be improved.

This study shows that, the exposure to PCBs seems to continue in Egypt in spite of the fact that it was carried out on the limited number of subjects and because of the selected population results are not representative for whole Egyptian population.

Further research needs to be conducted in highly industrialized and polluted areas in Egypt and several environmental sources have to be studied (air, drinking water, sediment, foods, fish, human milk, adipose tissue).

In addition, studies should focus on the toxicological implications (for instance, the effects on fertility) on humans and wild life, which could be made possible by measuring exposure levels in these different sources.

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