Asian Journal of Chemistry Vol. 21, No. 8 (2009), 6301-6311

Serum Concentrations of Organochlorine Pesticides in Non-Vocationally Exposed Population of Harbin City, China†

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This work reports the method for determining organochlorine pesticides in human serum and evaluate the accumulation level of organochlorine pesticides in non-vocationally exposed residents lived at Harbin city, P.R. China. The blood samples were treated with ultrasonic, purified by C_{18} solid phase extraction column and tested with the capillary columns gas chromatograph equipped with ECD detector. The recovery of ten kinds of organochlorine pesticides in human serum were 84.2-92.1 %. The relative standard deviation of the method was 2.61-14.4 %, linearity range at 0.2-50.0 μg/L. The range of detection limit were 0.02-0.05 ng/mL and the correlation coefficient were $r > 0.999$. Of the 220 people investigated, 60 % were detected with different serum levels of organochlorine pesticides (range: 0.012-32.107 μg/L). The detection rate was 58.1 and 61.7 % in male and female, respectively. The serum level of accumulated organochlorine pesticides of non-vocationally exposed residents in Harbin were higher in female than that in male. However, both of them are in lower level of accumulated organochlorine pesticides.

Key Words: Organochlorine pesticides, Serum, Gas chromatography, Incretion interferential substance.

INTRODUCTION

Organochlorine pesticides as persistent organic pollutants are aexterior, semivolatile and toxic chemicals which may cause hazards to the environment and human health. Organochlorine pesticides chronically consist in environment and it is very difficult to degrade them through physical, chemical or biological ways, whereas, easy to accumulate in organisms through the food chain[1-3]. Generally, the residue of organochlorine pesticides and metabolites have a low level in the environment. However, concentration in organism would be increased by thousands times highly in blood or fat through food chain accumulation[4]. By disturbing hormone's secretion, synthesis, transportation and combination as well as the biological effects of

†This work was supported by China's 11th Five-year Scientific and Technical Plan (2006BDA27B05).

organisms, organochlorine pesticides are chemical contaminants with character of causing abnormal incretion leading to the changes or damages of reproduction, development and behaviour[5-7]. Although the production of organochlorine pesticides were inhibited all over the world in the early of 1970s. The incidence of hormone-dependent organ cancers, such as galactophore, ovary, prostate and spermary cancer, have evidently moved up recently by 2-3 times, which were likely to be related to environmental chemical contaminations[8,9]. Thus, it is very important to know how to be accumulation and consequence supervene harms of organochlorine[10-14]. Thereby, the content in blood and fat in human body can indicate the exposing level to the pollutant so that we may determine the relations between exposing level and risk of cancer occurrence.

In the study, temperature-programmed capillary columns gas chromatography with electron capture detector was used to determine the residue of organochlorine pesticides in blood of non-vocational exposed residents of Harbin, P.R. China.

EXPERIMENTAL

Gas chromatograph GC-2010 with electron capture detector and GC-solution chromatograph workstation were purchased from Shimadzu Corporation (Japan). XK96-A vortex oscillator was bought from JiangSu (China). Ultrasonic wave oscillator and TD-4A centrifuge came from YingTai instrument plant (ChangSha, China). HSE-12 solide phase extractor, C_{18} solid phase extraction column and HGC-12 Nitrogen purging instrument came from HengAo corporation (Tianjin, China). Acetone, *n*-hexane, methanol were guaranteed reagent (GR) grade. Standard materials of organochlorine pesticides (purity: ≥ 99 %): heptachlor, Aldrin, β-chlordane, α-chlordane, dieldrin, *p,p′*-DDE, Endrin, *o,p′*-DDT, *p,p′*-DDD and *p,p′*-DDT were bought from National Center for Standard Part (Beijing, China). The blank serum (The blank serum is that there are not the organochlorine pesticides in the serum) was bought from Changzheng company (Shanghai, China). Preparation of standard solution: dissolve 1.0 mg standard organochlorine pesticides into 100 mL volumetric flasks respectively with *n*-hexane to a final concentration of 0.1 μg/mL in each stock solution. The stock solutions were diluted into different concentrations using *n*-hexane.

Chromatograph column was DB-5 quartz elastic capillary column, 30 m × 0.25 mm × 0.25 μm and highly pure nitrogen was used as carrying gas. The column temperature increasing procedure: the initial temperature of 185 ºC was held for 1 min and then increased to 230 ºC at a rate of 5 ºC/min and held for 2 min. The temperature of vapourizer and detector was 260 and 300 ºC, respectively; tail-blowing gas flow rate: 30 mL/min; column flow rate: 1 mL/min; column pressure: 120 Kpa, the split ratio was 50:5. Injection volume was 1 μL.

General procedure: Three hundred residents have been chosen randomly for investigation from October 2006 to May 2007 from the Second Affiliated Hospital of Harbin Medical University, Harbin, P.R. China. All were investigated to have

requirements of 21-60 years old, be lived in Harbin for over 20 years, except from exposing experience to organochlorine pesticides and others relating diseases of vocations. A totally of 220 qualified residents, 115 female and 105 male, aging from 21 to 60, with the average age of 48.2, were chosen from 300 residents. Regarding the occupation of the subjects, they are 86 civil servants, 54 workers, 21 teachers and 59 retired persons. However, they are 57 of them smoke cigarette and 109 drink alcohol. 21 Persons had high blood pressure and 17 female underwent abortion. One hundred and fifty four subjects are Harbin permanent residents and 66 are immigrant.The volunteers were divided into 4 groups according to age, 21-30, 31-40, 41-50 and 51-60, respectively. Five mL of vein blood were donated by each volunteer, poured into 10 mL test glass tubes containing EDTA anticoagulant and then kept them at -20 ºC until analysis. Blood sample was centrifuged (2500 rpm/min, 20 min), 2.0 mL of serum were transferred to a 50 mL colorimetric flask. After added 10 mL of acetone, the blood sample was mixed with vortex oscillator, extracted for 0.5 h with ultrasonic oscillator, laid aside over night and the supernatant was removed after centrifugation (3000 rpm/min, 10 min). The residue was added 10 mL of acetone, centrifuged and the supernatant was also collected. The same procedure was repeated twice. All the supernatant was transferred into the rotary evaporator until residue was less 1 mL under water bath at 65 ºC, 0.1 Mpa vacuum. 2 mL of ethyl acetate-cyclohexane (1:1, v/v) were added to extracte the organochlorine pesticide. The same procedure was repeated for three times. The ethyl acetate-cyclohexane top layer was transferred into an injection vial, evaporated to dryness under a gentle stream of nitrogen and then redissolved in 1 mL *n*-hexane. The dissolved solution was purified on the C_{18} solid phase extraction column which was activated with 1 mL of methanol and 1 mL of deionized water. After organochlorine pesticides were retained on the sorbent and subsequently eluted by 1 mL of *n*-hexane. The eluate was collected in Eppendorf tubes and evaporated to dryness under a steam of nitrogen and resuspended in 100 mL of *n*-hexane. 1 μL of each solution was injected onto the GC column.

Detection method: Ten varieties of organochlorine pesticide stock solutions were mixed with blank serum respectively to prepare 6 mixed standard solutions with concentration that the concentration gradient was from 0.5 to 10 μg/L, respectively. The samples prepared and chromatograph condition were the same procedure as sample collection and preparation mentioned above. Injection volume of each sample was 1 μL. The results were analysized by GC solution chromatograph workstation software taking the peak area as the x-coordinate and the concentration as the y-coordinate. The calibrations were made.

One microliter of sample solution was injected into chromatographic column under the same as the procedure mentioned above. The relative retention time for each sample was determined and external standard method were used for quantitive analysis.

The data were expressed mean ± SE Statistical analysis were performed using SPSS version 10.0 (Harbin, China). Differences between means were analyzed for significance using the one-way ANOVA test. Differences were considered significant at $p < 0{\cdot}05$.

RESULTS AND DISCUSSION

The chromatographic conditions used in the developed method provided an excellent separation within *ca.* 20 min. Fig. 1 shows the standard chromatogram of ten organochlorine pesticides.

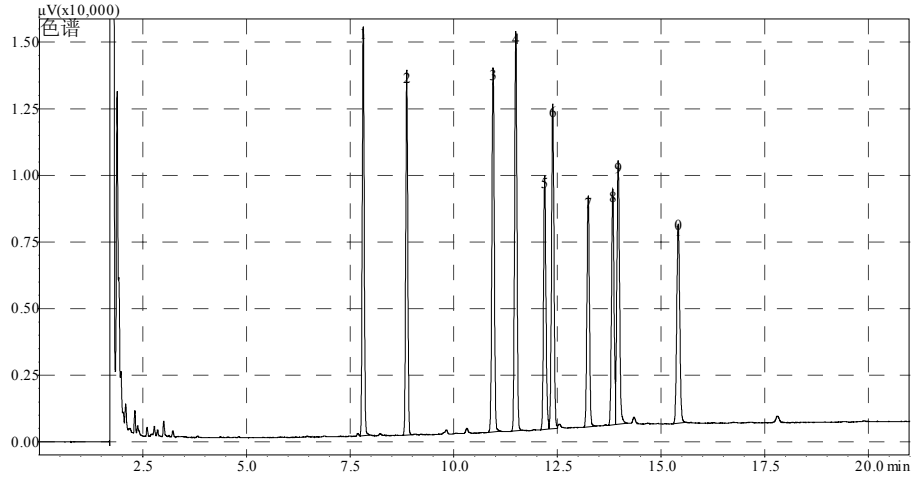

Fig. 1. Standard chromatogram of ten organochlorine pesticides
1. Heptachlor; 2. Aldrin; 3. β-Chlordane; 4. α-Chlordane; 5. Dieldrin; 6. *p,p′*-DDE; 7. Endrin; 8. *o,p′*-DDT; 9. *p,p′*-DDD; 10. *p,p′*-DDT

Fig. 2 shows the chromatogram of blood samples. The concerned elements were completely separated from endogenous substances in the blood samples.

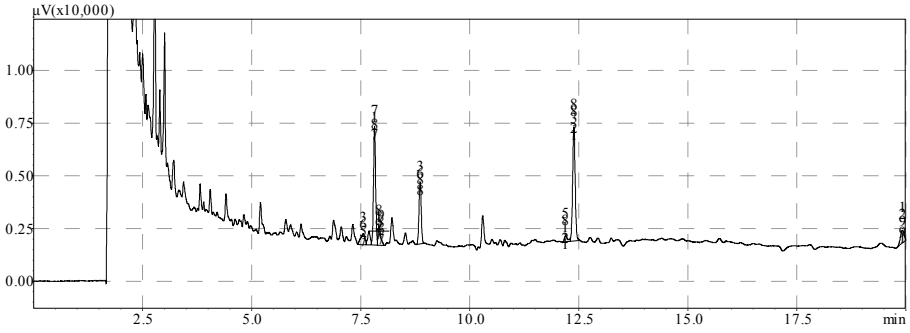

Fig. 2. Chromatogram of blood samples
1. Heptachlor 7.817; 2. Aldrin 8.863; 3. *p,p′*-DDE 12.388

Under the chromatographic conditions in this study, based on the method of setting up standard curve as described above, linearity data are summarized in Table-1. The samples from mixed standard of 10 organochlorine pesticides presented favourable linear relationship within the concentration range of 0.2-50.0 μg/L (Table-1). A satisfactory linearity is observed, as indicated by the correlation coefficients that range from 0.9991 to 0.9999. Due to different chemical structure and electro-negativation, the responding value and minimum detection limit of different element displayed on ECD were different.

TABLE-1
THE EQUATION, THE CORRELATION COEFFICIENT AND
THE DETECTION LIMIT OF TEN ORGANOCHLORINE PESTICIDES

Compounds	Equation	Correlation coefficient (r)	Detection limit (μg/L)
Heptachlor	y = 1273.27x + 434.90	0.9999	0.02
Aldrin	y = 979.41x - 53.64	0.9996	0.03
β-Chlordane	y = 1218.70x + 229.41	0.9997	0.03
α-Chlordane	y = 1481.46x + 71.64	0.9991	0.02
Dieldrin	y = 885.89x + 187.86	0.9997	0.04
p,p'-DDE	y = 1081.97x + 102.74	0.9997	0.03
Endrin	y = 895.41x + 73.62	0.9998	0.03
o,p'-DDT	y = 780.54x - 47.81	0.9997	0.03
p,p'-DDD	y = 937.04x - 21.34	0.9996	0.05
p,p'-DDT	y = 831.82x + 8.50	0.9998	0.04

The precision and accuracy studies were conducted by spiking the blank serum samples,with three known concentrations: 5.0, 20.0 and 40.0 μg/L. The recovery was determined by 6 replicates of samples. The precision of instrument was also calculated by 6 replicates injection of organochlorine pesticide at 0.5 μg/L. Precision and accuracy data are presented in Tables 2 and 3.

Tables 2 and 3 showed that the relative standard deviations (RSD) of residue were 2.61-14.4 %, precisions were 5.80-12.6 % and the recovery rate were 84.2-92.1 % ± 6.61, which were in conformity with the international analyzing quality requirements of WHO/UNEP AND AOA.

Samples analysis of investigated population: We divided the investigated volunteers into 4 groups according to age, 21-30, 31-40, 41-50, 51-60, respectively. The serum samples were treated according to the method as described above. The concentrations of 10 organochlorine pesticides were determined under the optimal separation conditions, with the detection rate of organochlorine pesticides of all age groups as listed in Table-4.

In Table-4, serum organochlorine pesticides were tested positive in 132 persons from 220 subjects and the detection rate was 60.0 %. Heptachlor, aldrin, dieldrin and endrin were detected in serum from 43 persons, with detection rate 19.5 %. β-Chlordane and α-chlordane were not detected in the serum. The serum samples

TABLE-2
EXPERIMENT ON THE PRECISION OF 10 ORGANOCHLORINE PESTICIDES (n = 6)

Compounds	Adding level (μg/L)	Mean $\overline{X} \pm S$ (μg/L)	RSD (%)	Instruments RSD (%)
Heptachlor	5.0	4.57 ± 0.52	11.3	10.1
	20.0	16.6 ± 1.38	8.32	
	40.0	34.2 ± 0.89	2.61	
Aldrin	5.0	4.34 ± 0.22	5.06	10.6
	20.0	17.2 ± 0.82	4.79	
	40.0	33.8 ± 1.80	5.33	
β-Chlordane	5.0	4.27 ± 0.17	4.03	6.67
	20.0	18.2 ± 1.13	6.21	
	40.0	34.0 ± 1.32	3.89	
α-Chlordane	5.0	4.05 ± 0.42	10.3	5.80
	20.0	17.2 ± 0.82	4.76	
	40.0	34.3 ± 1.72	5.02	
Dieldrin	5.0	4.04 ± 0.49	12.2	9.50
	20.0	18.5 ± 0.79	4.26	
	40.0	34.5 ± 1.30	3.78	
p,p'-DDE	5.0	5.07 ± 0.54	10.6	12.4
	20.0	18.0 ± 1.03	5.71	
	40.0	34.6 ± 1.71	4.93	
Endrin	5.0	4.79 ± 0.69	14.4	10.4
	20.0	17.7 ± 0.95	5.40	
	40.0	34.6 ± 1.86	5.39	
o,p'-DDT	5.0	4.30 ± 0.43	10.0	10.4
	20.0	17.6 ± 0.90	5.14	
	40.0	33.7 ± 1.97	5.85	
p,p'-DDD	5.0	4.38 ± 0.15	3.38	11.1
	20.0	17.8 ± 0.63	3.52	
	40.0	34.5 ± 1.66	4.82	
p,p'-DDT	5.0	4.80 ± 0.45	9.31	9.30
	20.0	18.0 ± 0.90	5.00	
	40.0	34.8 ± 1.99	5.71	

of 125 were detected to have DDT and the detection rate was 56.8 %. In addition, there was a positive correlation between age and serum organochlorine pesticides level. With the growing up of age, the content of organochlorine pesticides in human body increased.

Table-5 showed that the concentration range of organochlorine pesticides and its means. As shown in Table-5, the lowest concentration of 0.012 μg/L occurred in dieldrin and aldrin. The highest concentration was 32.1 and 29.3 μg/L in *o,p′*-DDT and *p,p′*-DDT, respectively.

The correlation between gender and serum organochlorine pesticides level was listed in Table-6.

TABLE-3
AVERAGE RECOVERY RATE OF 10 ORGANOCHLORINE PESTICIDES WITH DIFFERENT CONCENTRATIONS (n = 6)

Compounds	Concentrations (μg/L)	Recovery rate (%)						$\bar{X} \pm S$ (%)
Heptachlor	5.0	105.3	82.6	77.7	99.8	93.1	90.1	86.7 ± 7.73
	20.0	88.6	94.0	77.8	75.7	80.2	82.7	
	40.0	84.2	84.6	82.7	86.9	86.3	89.0	
Aldrin	5.0	87.4	80.7	90.7	83.6	86.2	92.5	85.7 ± 4.38
	20.0	87.0	86.9	84.0	77.4	88.6	90.8	
	40.0	76.7	83.6	88.1	85.9	82.7	89.2	
β-Chlordane	5.0	82.7	84.3	86.5	80.7	89.0	89.2	87.1 ± 4.90
	20.0	95.7	97.1	87.2	82.5	90.3	93.7	
	40.0	84.2	82.4	81.3	86.7	84.6	90.5	
α-Chlordane	5.0	71.0	79.9	91.7	71.6	85.2	86.5	84.2 ± 6.06
	20.0	85.8	83.9	86.9	79.4	89.5	90.8	
	40.0	78.9	83.4	85.6	87.5	87.1	91.7	
Dieldrin	5.0	71.7	79.4	95.0	68.3	85.0	85.3	86.5 ± 7.74
	20.0	92.6	93.9	92.1	85.0	94.6	96.3	
	40.0	82.9	86.2	83.7	87.5	85.0	91.9	
p,p'-DDE	5.0	111.1	88.5	91.8	111.3	95.2	110.8	92.1 ± 9.48
	20.0	93.1	94.4	86.2	81.9	88.7	94.6	
	40.0	78.6	85.6	87.7	88.0	90.0	90.0	
Endrin	5.0	81.5	95.8	115.9	80.0	101.5	99.9	90.2 ± 9.15
	20.0	82.9	84.7	90.3	84.7	92.3	94.6	
	40.0	87.5	86.7	80.5	88.0	82.4	93.8	
o,p'-DDT	5.0	80.3	86.5	94.8	71.8	92.9	89.8	86.0 ± 6.11
	20.0	86.6	89.9	87.2	80.1	89.2	93.6	
	40.0	84.1	81.2	80.5	86.2	80.4	93.2	
p,p'-DDD	5.0	88.8	86.4	87.4	83.1	92.1	87.5	87.7 ± 3.46
	20.0	86.3	87.8	89.0	85.8	93.1	92.7	
	40.0	80.3	84.5	86.1	87.0	87.0	93.2	
p,p'-DDT	5.0	107.0	92.8	80.7	96.4	101.8	96.7	91.0 ± 7.13
	20.0	93.0	95.4	86.4	83.5	88.8	92.5	
	40.0	80.4	83.0	86.3	91.6	88.0	93.4	

TABLE-4
DETECTION RATE OF ORGANOCHLORINE PESTICIDES OF ALL AGE GROUPS

Age	Quantity	Detection rate (%)							
		Heptachlor	Aldrin	Dieldrin	*p,p'*-DDE	Endrin	*o,p'*-DDT	*p,p'*-DDD	*p,p'*-DDT
21-30	49	6.1	4.1	6.1	6.1	2.0	8.2	4.1	8.2
31-40	51	7.8	1.9	1.9	7.8	1.9	9.8	11.7	15.6
41-50	68	4.4	5.9	5.9	13.2	2.9	13.2	13.2	16.2
51-60	52	5.8	7.7	7.7	16.2	5.8	19.2	32.7	30.8

TABLE-5
CONCENTRATION OF ORGANOCHLORINE PESTICIDES IN 220 BLOOD SAMPLES (μg/L)

Compounds	Means	Geometric means	Range
Heptachlor	0.243	0.325	0.046-0.720
Aldrin	0.063	0.072	0.012-0.104
Dieldrin	0.117	0.086	0.012-0.398
p,p'-DDE	1.106	0.092	0.723-8.290
Endrin	0.049	0.037	0.026-0.082
o,p'-DDT	6.570	5.620	1.260-32.107
p,p'-DDD	2.890	2.771	0.970-17.820
p,p'-DDT	4.530	4.043	0.820-29.301

TABLE-6
DETECTION RATE OF ORGANOCHLORINE PESTICIDES IN MALE AND FEMALE

Gender	Quantity	Detection rate (%)							
		Heptachlor	Aldrin	Dieldrin	*p,p'*-DDE	Endrin	*o,p'*-DDT	*p,p'*-DDD	*p,p'*-DDT
Male	105	4.8	3.8	4.8	11.4	2.9	13.3	11.4	14.3
Female	115	7.0	6.1	6.1	13.9	3.5	12.2	16.5	20.0

Table-6 showed that the detection rate in female was higher than that of male.

Organochlorine pesticides are organic compounds with properties of stability, long half-life, slow-degrading, being heat-resistant and acid-resistant, undissolved in water, strong lipophilic and easy to dissolve in organic solvent[4]. Therefore, they may exist for a long time at ecosystem such as water, soil, *etc*. Because of globally transferring with the property of semi-volatility at the symbol of saturated steam pressure, they degrade slowly in the environment. They accumulate in human food chain, causing harms to biology with high alimentation level[5-7]. When entered a living body, they tend to combine with protein and/or fat of the blood and form stable compounds, which cause great problem to the extraction and clean up by blood. It is hard to extract organochlorine pesticides from blood sample by using organic solvent such as ligarine, *n*-hexane, *etc*. Generally the recovery rate is below 50 %. However, acetone was taken as a solvent in this study. Blood specimens were extracted by ultrasonic and kept still overnight for concentrating. By changing the polarity of extracting solvent, samples were re-extracted with acetate-cyclohexane for further concentrating. After the sedimentation of polar substance, higher extracting efficiency was obtained. The samples were purified by C_{18} solid phase extraction column and eluted with *n*-hexane, which helps to eliminate any other interferential substances. Thus, a simple, fast, exact and reliable method to inspect and test organochlorine pesticides in human blood was established and the levels of accumulation of organic pesticides of non-vocationally exposed population in Harbin were monitored and analyzed.

Organochlorine pesticides show the estrogen-like effect and if enter in the body, it will interfere with the release, transport, metabolism of the endocrine substances and activate or inhibit the endocrine system. Thus, the body was out of control and the homeostasis was undermined. Organochlorine pesticides not only increase the risk of breast cancer in women, but also stimulate breast cancer MCF-7 cell proliferation and the expression of P53[15]. Moreover, organochlorine pesticides are hepatotoxicity because they could easily pass through the cell membrane, where they play as a ligand and combine to the transcription factor aryl hydrocarbon receptor[16]. Research on the relationship between serum concentrations of organochlorine pesticides and their isomers with galactophore cancer shows that the detection rate of organochlorine pesticides in latex gets to 87 % high, mostly DDT, which has relation with the concentration potential and metabolizing of organochlorine pesticides in organism with higher level of food chain[17]. The excreting ability of organochlorine *p,p′*-DDT in body is relatively weak. Daily excretion only equals to 1 % of what was taken. Therefore, organochlorine *p,p′*-DDT showed a comparatively strong accumulating effect in organism of higher level in food chain.

Results in this study showed that the detection rate of organochlorine DDT in the selected population was the highest, which is 56.8 %. All the four organochlorine pesticides were detectable (Table-5) and the geometric means are: *o,p′*-DDT > *p,p′*-DDT > *p,p′*-DDD > *p,p′*-DDE. Detection rate of female is 62.6 %, with the highest content of concentration 32.1 μg/L, which was almost in conformity with the results of other reports[18,19].

In this study, organochlorine pesticides, such as heptachlor, aldrin, dieldrin and endrin, were found in the serum of normal population. The detection rate of heptachlor was up to 5.9 % and the rate in female was 7.0 % which is higher than that in male and its highest content was 0.72 μg/L, geometric mean was 0.325 μg/L; the minimum detection rate for endrin was 3.2 % and the highest content was 0.082 μg/L, 3.5 % in female and 2.9 % in male. So more concerns should be paid to the long-term health effects of the accumulation of organochlorine pesticides in female.

It is also found that there was a positive correlation between age and serum organochlorine pesticides level. The content of organochlorine pesticides in human body has the trend of increasing along with the growing up of age (Table-4), which may be related to the accumulating property and long half-life of organochlorine pesticides. Organochlorine pesticides enter into body through food chain. They are easy to combine with fat or fatty tissues. The content of concentration will increase along with the growing up of age, which is more significant in female. Previous studies also supported present results[20,21]. Thus, it may be presumed that age is an indicator for predicting the exposure level of organochlorine pesticides and evaluating the impact on health in female.

DDT was once largely produced and used from 1960s to 1980s in China, total accumulative quantity about 400,000 tons, up to 20 % of international production. Though DDT was forbidden to use in China at the middle of 1980s, compared to

developed countries, we still prolonged using them for 10 more years[22,23]. DDT mainly accumulates in fatty tissue of human body, while not so much in blood. The content of DDT in blood is about 1/100 of that in fatty tissue[24,25]. However, it is not easy to obtain human body's fatty tissue, whereas, blood may reflect the contaminant, that is why we selected blood samples in this experiment. Detection rate of DDT in non-vocational exposed population in Henan China during the period of 1979-1980 was 96.34 % with average content of 60.3 μg/L, which was evidently higher than that of developed countries at the same period. It was also higher than the results of present study, which showed that the forbidden use of organochlorine pesticides has had effect in China.

There has been many research work are reported on serum concentrations of organochlorine pesticides and their relationship with diseases such as cancer. DDT was mainly studied, while there is limited information on heptachlor, chlordane, aldrin, dieldrin, endrin, *etc.*, which are persistent organic pollutants that are forbidden to use as described in 'Stockholm Treaty'.

Conclusion

In this study, we provided a fast detecting method to determine organochlorine pesticides in blood sample as well as the basic data indicating that they accumulated in human body.

ACKNOWLEDGEMENT

The authors thank the Second Affiliated Hospital of Harbin Medical University for providing the blood samples.

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(*Received*: 10 December 2008; *Accepted*: 28 May 2009) AJC-7612