

## ***Bioaccumulation Assessment of DDT Pesticide and its Metabolites in Pregnant Women Head Hair Regarding Age, Birth Rate and Fish Consumption Rate***

**Barghi, M.<sup>1\*</sup>, Bahramifar, N.<sup>2</sup>, Esmaili sari, A.<sup>3</sup>, Mirsanjari, M.M.<sup>4</sup>, Dahmardeh Behrooz, R.<sup>5</sup>**

1- M.Sc., Environmental Sciences, Tarbiat Modarres University, Nour-Iran

2- Assist. prof., Department of chemistry, Payame Nur University (sari) &  
Tarbiat Modarres University, Nour-Iran      nbahramifar@yahoo.com

3- Prof., Department of Environmental Sciences, Tarbiat Modarres University, Nour-Iran      esmaili@modares.ac.ir

4- Assist. prof., Department of Environmental Sciences, Malayer University, Malayer-Iran  
mmmsanjari@unipune.ernet.in

5-Academic staff, Department of Environmental Sciences, Zabol University      dahmardeh\_behrooz@yahoo.com

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### **Introduction**

Pesticides are widely used and large quantities of them are dispersed in the environment and are subsequently found in air, water, soil and food sources. According to previous surveys, Over 27,000 tones of pesticides were used in Iran during 2000-01. Beside the legal usage of some pesticides, other active ingredients are imported illegally some of which are banned in Iran including Persistent Organic Pollutants (POPs) which are carcinogenic and extremely harmful to humans and the environment. In addition, pregnant women exposure to pesticides in the environment is a major public health concern, since a majority of pesticides contain neurotoxicants. So fetus is subjected to a greater risk compared to adults, by toxic effects of these chemicals due to rapid state of growth and development of its brain.

It is likely most of the maternal exposures to environmental pesticides are subtle and result in little or no recognizable effects in pregnant women. Yet, serious concerns have been raised about their adverse effects on the fetus and their potential role in subsequent developmental, learning and behavioral difficulties in children. Moreover, chromosomal abnormalities and DNA damages have been observed in infants whose mothers have had antenatal exposure to pesticides.

Nowadays body fluids and tissues are commonly used to evaluate human exposure to some chemicals. However, all of them have some limitations. In this way hair has been identified as a suitable alternative and indicator of short (current) and long-term exposure to organochlorine compounds. The relatively high percentage of lipids in the hair (3.5-4%) and similar concentrations of persistent organic pollutants (reported relative to the amount of lipid) makes it a suitable matrix for analysis of persistent organic pollutants.

### **Materials and Methods**

#### *Sample collection and preparation*

The Pregnant women head hair samples (approximately 0.5 g each one), were collected from Oct- Dec 2007 from Noushahr (N = 19) and its surrounding villages (N = 16). Meanwhile, the questionnaires including dietary habits such as fish consumption rate, pregnant women's age and birth rate were filled by the pregnant women. The collected hair samples were washed by hair shampoo and cut into a 1 to 3 mm long pieces. 200 mg of hair pieces (with lengths of ~1 mm) from each sample were accurately weighted, spiked with 5 ng of internal standard (PCB 143) and overnight incubated at 40°C in 2 ml of 3 N HCl.

Liquid-liquid extraction with 2 × 3 ml hexan: DCM (4:1) was used. The combined fractions of organic solvent were purified on a cartridge filled from the bottom with 250 mg deactivated alumina (10% water),

500 mg of acidified silica and 250 mg anhydrous  $\text{Na}_2\text{SO}_4$ . The modified silica gel was prepared as follows: to 50 g of silica gel, 27 ml of concentrated sulphuric acid were added drop wise, while the mixture was stirred to ensure good homogeneity. The acidified silica was stirred for another 30 min following completion of acid addition. The cartridge was pre-washed with 2 ml of hexane: DCM (1:1, v/v) and 2 ml of hexane. The final elute was concentrated to approximately 50  $\mu\text{l}$  under a gentle nitrogen stream. After being transferred to vial, it was stored at  $-20^\circ\text{C}$ .

#### *Quantification and quality assurance*

Gas chromatographic (GC) analysis was performed using a Dani 1000 gas chromatograph equipped with  $^{63}\text{Ni}$  electron capture detector.

#### *Statistical analysis*

At first the data were tested for normality using Kolmogorov-Smirnov test. Some of them were found to be normal ( $\text{sig} > 0.05$ ), therefore parametric procedures (Independent-Samples T-test) were used to analyze them. Those data which were not normal were analyzed by Mann-Whitney U test. Tests were separated for each compound class to calculate the mean according to pregnant women's age, birth rate and fish consumption rate.

### **Results**

After data analysis, mean DDTs concentrations (the sum of 5 isomers) in Noushahr and Noushahr's surrounding villages were determined as 12.5 and 47.7 ng in 1 g of hair respectively. The concentration of each isomer is shown in table 1.

**Table 1: Mean DDTs Concentrations in Two Research Sites (ng in 1 g of hair)**

<i>o,p</i> -DDE	<i>p,p'</i> -DDE	<i>p,p'</i> -DDD	<i>o,p</i> -DDT	<i>p,p'</i> -DDT	Isomer type location
0.3	14	3.5	6.8	24	Noushahr's villages
0.9	5.7	4.3	0.6	1.2	Noushahr

The Mann-Whitney U test showed a significant difference between the mean concentrations of DDTs (the sum of 5 isomers), *p,p'*-DDE, *p,p'*-DDT and *o,p*-DDT in Noushahr and Noushahr's surrounding village samples ( $P < 0.05$ ).

In order to find the relation between age and DDTs concentration in the samples, they were separated into two groups of women aged over and below 25. The Mann-Whitney U test results indicated that there was no significant difference between those groups. The second variable in this survey was the birth rate. Thus, the samples were again separated into two groups of women who expected their first child and those who expected their second child. Results did not show any difference between those two groups, too. Nevertheless, the DDTs concentrations in the samples of both sites were lower in women who expected their second child.

The next parameter was fish consumption rate. To achieve this aim, the samples were divided in two groups: women who eat fish 4 times or less in one month and women who eat fish more than 4 times in one month. As a result, statistical analysis has shown no significant difference between these two groups, but the mean DDTs concentrations were higher in women who eat more fish (Table 2).

**Table 2: Mean DDTs Concentrations in Women Head hair Samples in Noushahr and Noushahr's Surrounding Villages with Respect to Age, Birth rate and Fish Consumption Rate (ng in 1 g of hair)**

P.Value		Number		DDTs concentration				
Village	city	Village	city	Village		City		parameter
				Range	mean	Range	mean	
0.47	0.13	8	7	11.4–90.8	(9.2)34.9	3.9–54.6	(7.8)19.6	Age
		11	9	8.5–305.3	(26.6)58.4	3.4–13.7	(1.32)7.4	Less than 25 years 25 or more
0.51	0.33	9	5	8.5–107.5	(11.7)37.2	3.9–13.7	(1.8)7.6	Fish consumption rate
		10	11	8.9–305.3	(28.5)58.7	3.4–54.6	(4.8)15	4 times or less in one month More than 4 times in one month
0.29	0.18	10	11	8.6–305.3	(26)62.9	3.9–54.6	(5.2)16.3	Birth rate
		6	8	8.5–90.8	(9.4)28.7	3.37–13.7	(1.5)6.7	0 1

### Discussion and Conclusion

According the results of this survey, DDTs concentrations in samples of Noushahr's surrounding villages were significantly higher than Noushahr's samples. The reason could be the direct usage of this pesticide in farms near the villages where the samples were collected from. This pesticide may have entered pregnant women's body by respiration in pollutant areas. The other way for DDTs to enter maternal bodies could be consumption of the products produced at these polluted farms.

The  $p, p' - DDE / p, p' - DDT$  ratio provides information regarding the accumulation of  $p, p' - DDT$  in the past because industrial DDT is composed of 77%  $p, p' - DDT$ , 15%  $o, p - DDT$ , 4%  $p, p' - DDE$  and some other trace impurities and  $p, p' - DDT$  is metabolized into  $p, p' - DDE$  and other metabolites in human body. A ratio less than 5 indicates a recent exposure to parent DDT. The lower this value the more recent the exposure is to parent DDT. In this survey the ratio obtained from Noushahr and its villages were respectively 6.5 and 1. These results indicated a more current consumption of DDT in pregnant women of Noushahr's surrounding villages.

Another reason for the higher ratio in Nuoshahr samples could be more oily food consumption by townspeople. Because these kinds of food have high concentration level of  $p, p' - DDE$ . So more consumption of these foods by townspeople results in more accumulation of  $p, p' - DDE$  in their bodies.

In addition, higher  $p, p' - DDE / DDTs$  ratio suggests older exposure to DDT. So, higher ratios in Noushahr samples indicate an older exposure in comparison with village samples.

Over all, this survey shows that DDTs concentrations in Noushahr surrounding villages were significantly higher in comparison with Noushahr samples. Also the ratios show recent exposure of DDTs in Noushahr surrounding villages. Finally the DDTs concentrations in the pregnant women who had expected their first child and ate more fish were higher than the others.

In order to improve the present situation, it is recommended to determine a new method for regular monitoring of DDT pesticides in human body especially in rural areas close to large farms, and to control the kind of pesticides farmers use more in their farms.

### Key words

DDT, Hair, Gas Chromatography, Noushahr, Pregnant women