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ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BIPHENYLS IN HUMAN BREAST MILK IN THE SUBURBS OF HUE CITY, VIET NAM: PRELIMINARY RESULTS

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SUMMARY

The organochlorine compounds (OCs) such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) can accumulate in breast milk through the food chain. Human milk is a suitable bio-monitoring source to assess the burden of disease by these compounds in humans. In this study, human breast milk samples were collected from 30 lactating mothers who were farmers, and 10 samples from lactating mothers who were not farmers living in suburban communes of Hue city, during 2010. Questionnaires on lifestyle factors, dietary aspects, past disease, family history, occupation, and past and current exposure to pesticides were asked. The concentrations of OCPs as dichlorodiphenyltrichloroethane and its metabolites (DDTs), hexachlorocyclohexane isomers (HCHs) and polychlorinated biphenyls congeners (PCBs) were quantified by gas chromatoghraphy with micro electron capture detector (GC-μECD). Results are reported on milk fat basis. DDTs and HCHs were detected in all breast milk samples. PCBs were detected in some breast milk samples. There was no significant difference in levels of OCPs and PCBs between lactating mothers who were farmers or not farmers. The levels of DDTs, HCHs and PCBs in the breast milk samples were found to correlate positively with the age of the mothers.

***Keywords**: OCs, DDTs, HCHs, PCBs, Human breast milk, Vietnam*

1. Introduction

There are some plant protecting chemicals as organochlorine compounds (OCs), two of them are DDT (diclodiphenyltricloetan) and HCH (hexacloxyclohexan). They belong to a group of 12 persistent organic pollutants (POP_s:), which are the most dangerous chemicals for environmental and human health. DDT & HCH are toxic for the nervous system and immunization system. They produce materials causing cancer and hormone and reproductive disorders. A study from the American oncology institute showed that OCs are a cause of the increase in rate of some types of cancer in farmers

such as liver cancer, spleen cancer, malignant tumours of the skin, leukemia, stomach cancer, brain cancer, etc. Many current studies confirmed that mothers with contact or without contact with OCs had an excess of DDT and HCH in their breast milk. DDT & HCH are called endocrine disruptors. They are accumulated in the fatty tissue of humans and excreted through breast milk. Their excretion is very slow. Therefore, breast milk is used as bio- monitoring to assess the accumulation of DDT and HCH in human. As a result of this, DDT and HCH can be estimated in breast- fed babies

Worldwide, there is much research on assessing the accumulation of OCs in the environment, as well as the analysis and evaluation of their presence in breast milk, with the aim of defining the origin of morbidity and fatality in humans by these chemicals. However, there are few studies on this problem in Vietnam. This study was conducted in some suburban communes of Hue city. Its objective is to assess the content of OCs in breast milk.

2. Methods

2.1. Sampling

Forty mothers' breast milk samples were taken from three suburban communes of Hue city, Vietnam including Thuy Xuan (n=13), Huong Long (n=13) and Thuy Duong (n = 14). The samples were selected at random. Questionnaires were given to each mother to obtain information about their age, weight, height and occupation (table 1). The samples were stored at -20^{0}C until they were analysed.

Table 1. *Characteristics of the mothers participating in the study*

Characteristics / **Commune**		**Age**	**Height (cm)**	**Occupation**
Thuy Xuan	Mean	26	157	Farmer 76.9%
(n = 13)	Range	18-35	147-158	Others 23.1%
Huong Long	Mean	29.4	155	Farmer 76.9%
(n = 13)	Range	20-38	152-163	Others 23.1%
Thuy Duong	Mean	28.9	157	Farmer 71.4
(n = 14)	Range	19-40	153-167	Others 28.6
Overall	Mean	28.3	156	Farmer 75.0%
(n = 40)	Range	18-40	152-167	Others 25.0%

2.2. Sample analysis

The analysis procedure was conducted using the revised methods of Minh et al (2004) and Ulla Raab et al (2008).

Sample extraction:

Approximately 10g of breast milk samples were added onto 10 g pre-cleaned diatomite earth (Merck, Damstadt, Germany) packed in a glass column and extracted by 200 ml diethyl ether at a flow rate of 1 ml/min. The extract was concentrated to 8 ml by vacuum evaporator. One-fifth of the concentrated extract was used for fat content determination by the gravimetric method. The remaining extract was purified on chromatogaphic mini-column packed with 2g activated florisil and 1cm length of activated anhydrous sodium sulfate top side. The lipid in the purified-extract was removed by concentrated sulfiric acid treatment. The lipid removed extract was evaporated to 1 ml under a gentle stream of nitrogen and was ready for gas chromatography.

Gas chromatographic conditions:

The separation and detection of OCs was performed by the GC system (Agilent 7890 A) equipped with an auto-injection system (Agilent 7683B), micro-electron capture detector (μ-ECD) and HP5-MS capillary column (5% phenyl methyl siloxane phase, 30 m x 0,25 mm I.D. x 0,25 μm film thickness). Nitrogen was used as the gas carrier at a flow rate of 1.5 ml/min and make-up gas in the detector at 5 ml/min. 1 μl of the final extract was injected into injector operated at 285^0C and splitless mode. The temperature of the detector was 300^0C. The column oven temperature was programmed from 90^0C (held for 2 min) to 150^0C at a rate of 30^0C/min, to 204^0C (held for 3 min) at a rate of 3^0C/min, and finally to 280^0C (held for 10 min) at a rate of 8^0C/min.

2.3. Quality assurance

Multi-level calibration curves of OCs were created for quantification with good linearity ($r>0.999$). Method limits of quantification (LOQ) for OCs ranged between 0.04 and 0.18 ng/ml. The recovery of method was determined based on the analysis of spiked sample ranged between 80 and 137% for all compounds. The blanks were done in parallel and did not show the presence of OCs on their chromatograms.

2.4. Statistical analysis

Statistical treatment of the results was performed with Excel software. The Mann-Whitney U-test was used for comparisons of OCs level in breast milk with age, occupation, BMI and area. A P value < 0.05 was regarded as statistically significant.

3. Results and discussion

Table 2 showed contents of OCs (DDT, HCHs and PCB) in breast milk samples

in some suburban communes of Hue city. Table 3 summarizes the results of published studies on OCs in human breast milk in the world. The correlation between the content of OCs in breast milk and age, occupation and geographical factors was presented on table 4. Generally, DDTs and HCHs were found out in all studied milk samples while congeners of PCBs were only seen in some milk samples. In term of correlation, there was a relationship between the content of DDTs, HCHs and PCBs in breast milk and the age of donors ($p<0.05$). However, there was no the relationship between the content of OCs in breast milk and occupation, BMI and residence of donors ($p>0.05$).

Table 2. *Concentration (ng/g lipid wt) of organochlorine pesticides & PCB in human breast milk in three communes in Hue city*

	Thuy Xuan (n = 13)		**Huong Long (n = 13)**		**Thuy Duong (n = 14)**		**Overall (n = 40)**	
	Mean	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
Lipid, %	1.6	0.8 - 3.8	1.8	0.4 - 6.3	2.8	0.8 - 6.1	2.1	0.4 - 6.3
α-HCH	21	7.0 - 34	33	4.2 - 104	16	4.4 - 38	23	4.2 - 104
β-HCH	22	3.5 - 68	25	3.0 - 103	20	4.1 - 97	22	3.0 - 103
γ-HCH	33	10 - 59	41	6.1 - 107	23	6.3 - 54	32	6.1 - 107
∑ HCHs	76	21 - 149	99	13 - 248	59	27 - 189	78	13 - 248
p,p'-DDE	53	2.3 - 130	43	5.4 - 100	25	6.3 - 68	40	2.3 - 130
o,p' DDT	7.9	1.8 - 15	15	2.0 - 31	11	2.3 - 18	11	2.0 - 31
p,p' DDT	29	0.8 - 91	48	7.7 - 202	29	1.3 - 86	35	0.8 - 202
∑ DDTs	89	4.9 - 171	106	18 - 284	65	23 - 130	87	18 - 284
PCB 28	<DL	-	<DL	-	1.0	< DL - 1.0	0.3	<DL - 1
PCB 52	65	<DL - 65	40	<DL - 70	24	<DL - 24	43	<DL - 65
PCB 101	<DL	-	<DL	-	1.2	<DL - 1.2	0.4	<DL - 1.2
PCB 118	<DL	-	0.5	<DL - 0.5	16	<DL - 32	5.5	<DL - 32
PCB 153	<DL	-	0.2	<DL - 0.2	1.4	<DL - 2.2	0.5	<DL - 2.2
PCB138	<DL	-	<DL	-	0.7	<DL - 0.8	0.2	<DL - 0.8
PCB 180	13	0.7 - 27	14	1.0 - 27	19	5.3 - 48	15	0.7 - 48
∑ PCBs	78	0.7 - 92	55	1.0 - 98	63	5.3 - 109	65	0.7 - 108

DL: Detection limit of the method (PCB 28: 0.02 ppb, PCB 52: 0.05 ppb, PCB

101: 0.05 ppb, PCB 118: 0.11 ppb, PCB 153: 0.09 ppb, PCB 138: 0.11 ppb and PCB 180: 0.17 ppb)

Table 3. *Comparison of mean concentrations (ng/g lipid) of OCPs and PCBs in breast milk from different countries or regions.*

Country	Region	Year	n	Σ HCHs	Σ DDTs	Σ PCBs	Reference
Vietnam	Hue	2010	40	78	86.9	24.2	This study
	Hochiminh	2004	44	13.5	2300	14	Minh et al; 2004
	Hanoi	2004	42	58	2100	74	Minh et al; 2004
Indonesia	Jakarta	2001	16	14	630	33	Sudaryanto et al; 2006
Malaysia		2003	17	230	1600	80	Sudaryanto et al; 2005
Cambodia		2000	49	5.2	1500	25	Kunisue et al; 2003
The Philippine		2000	12	4.7	190	72	Kunisue et al; 2002
China	Hong Kong	1999	132	950	2870	42	Wong et al; 2002
	Guangzhou	2000	54	1110	3550	33	Wong et al; 2002
Australia		1995	60	350	1200	500	Quinsey et al; 1995
USA	Massachusetts	2004	38	19	65	-	Jonhson-estrepo et al; 2007
UK		1997-1998	168	103	470	-	Harris et al;1999
Japan		1998	49	210	290	200	Konishi et al; 2001
Russia		1996-1997	140	280	1040	380	Polder et al;2003
Sweden		1997	40	-	143	324	Noren & Meironyte; 2000
Germany		1995-1997	246	40	240	550	Schade and Heinzow; 1998

Ukraine		1993-1994	197	730	2700	594	Gladen et al; 1999

- Data not available

Table 4. *Correlation between age, occupation, areas factors and organochlorine pesticides concentrations in breast milk from the mothers participating in the study*

	Correlation coefficient (R)			p-value (two-tailed)		
	∑ HCHs	∑ DDTs	∑ PCBs	∑ HCHs	∑ DDTs	∑ PCBs
Age	0.567	0.431	0.328	0.034	0.013	0.026
Occupation	0.119	0.160	0.186	0.911	0.420	0.186
BMI	0.202	0.220	0.449	0.130	0.120	0.114
Areas	0.102	0.148	0.113	0.442	0.75	0.546

** Correlation is significant at the 0.05 level (2-tailed)*

3.1. HCHs

All breast milk samples contained an excess of HCH with its isomers as α-HCH, β-HCH and γ-HCH. Among them, the average content of γ-HCH (32.3ng/g lipid) was higher than that of α-HCH (23.3ng/g lipid) and β-HCH (22.4ng/g lipid). The total content of HCHs in breast milk of our study was higher than that of Nguyen Hung Minh et al in Hanoi (with 58 ng/g lipid β-HCH) and in Ho Chi Minh city (13.5 ng/g lipid β-HCH). Table 3 showed that total content of HCH in breast milk in this study was lower than that in studies in countries such as China Japan , Malaysia , and . However, the total content of HCHs in the study was higher than that in studies in Indonesia, Cambodia , the Philippines and the USA .

Although HCHs use has been forbidden in Vietnam since 1993, they were detected in biological samples and sediments in Thua Thien Hue province and other regions in the country. . Classic research has proved that HCHs, transferred to humans through food and water accumulated consistently in fatty tissue. Consequently, table 4 shows the relationship between the age of mothers and the contents of HCHs in their milk.

3.2. DDTs

Like HCH, the presence of P,P'-DDE, O,P' DDT and P,P' DDT in all breast milk samples showed that DDT has still been used in medicine and agriculture although it's use has been forbidden in Vietnam since 1995. The average contents of P,P'-DDE, O,P'-DDT và P,P'-DDT and DDTs in breast milk were 40.3; 11.4; 35.2 and 86.9 ng/g lipid,

respectively. Among them, the content of P,P'-DDE was higher than that of O,P'-DDT and P,P'-DDT. This result is much lower than that of Nguyen Hung Minh et al in Hanoi and Ho Chi Minh city (the content of DDTs was 2100 and 2300ng/g lipid, respectively). Environment and life conditions may explain for these differences. The content of DDT and its metabolites in breast milk in the study were the same as those of Jonhson-Restrepo et al in America (65ng/g lipid). However, they was lower than in other studies in Cambodia (1500ng/g lipid), The Philippines (190ng/g lipid), Malaysia, (1600ng/g lipid), Indonesia (630ng/g lipid) and developed countries such as Japan (290ng/g lipid), Germany (240ng/g lipid), Australia (1200ng/g lipid), the UK (470ng/g lipid), Russia (1040ng/g lipid), and the Ukraine (2700ng/g lipid) (Table 4).

3.3. PCBs

Among PCB congeners, PCB 180 was detected in all samples with its contents ranging between 7.7- 48.3 ng/g lipid. PCB 28 did not present in any sample. PCB 52 and PCB 101 were seen only some samples. Among them, the average contents of PCB 152 was the highest, 27.73 ng/g lipid with fluctuated interval between 5,52-56,32 ng/g lipid. The content of PCB 52 was the lowest, 7.0 ng/g lipid. The total average content of PCBs in 40 samples was 24.2ng/g lipid. This content was similar to that of studies in some Asian countries such as Indonesia (33ng/g lipid), Cambodia (25ng/g lipd), and China (33ng/g lipd). However, it was much lower than in studies in high income countries such as Australia (500ng/g lipid), Japan (200ng/g lipd), Germany (550ng/g lipid), the Ukraine (594ng/g lipid), and Sweden (324ng/g lipid) (Table 4)

Studies have proved that high contents of HCHs, DDTs and PCBs in breast milk may negatively affect the development of breast fed babies (Dahmardeh Behrooz et al; 2009). Medical documents indicated that HCHs, DDTs and PCBs disordered endocrine and resisted estrogen (Annika Smeds et al., 2001; A. Polder et al., 2009). Therefore the content of HCHs, DDTs and PCBs in breast milk is one good indicator to assess the risk to human health. In terms of lactating mothers, the contents of OCs in breast milk is the relationship with many factors such as age of mother, the number of their children, food, the contents of lipids in their body and other environmental factors (Kamila Jaraczewska et al., 2006).

4. Conclusions

All breast milk samples in the study are detected OCPs and PCBs. This is a problem for public health in the study settings. DDTs may be a main cause of health problems for breastfeeding mothers. Although the small sample size is not representative for the whole population of the study setting, the results of this study are initial and important evidence for detecting the infection of OCPs and PCBs in breast milk in the suburbs of Hue city.

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