EXPOSURE ASSESSMENT OF PESTICIDES FOR TAIWANESE NEONATE

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Introduction

Pesticides are widely and globally used. It is especially important for the tropical and subtropical region because vectors are proliferating in the warm and humid climate. Taiwan is located in the subtropical region and the pesticides use per hectare is very high. The ubiquitous presence of pesticides in the environment poses great public health concerns.

Four groups of synthetic pesticides are commonly used in Taiwan- organochlorines, organophosphates, carbamates, and pyrethrins [1]. Most of the organochlorines have been banned because of the properties of high persistence and bioaccumulation. Others also have been regulated in order to safeguard the public health. However, the residues of pesticide in food and vegetables, soil, and water were commonly reported as a result of intensive use for agricultural and public health purposes. Multiple pesticides detected in single crop were not uncommon. Our previous study also found that several metabolites were positively detected in urine specimens of Taiwanese workers [2]. Pesticides are neurotoxins and some of them are reported as endocrine disruptors. Exposures to pesticides are mainly from the ingestion of foods with pesticide residues and less from drinking water or inhalation for general population. The exposure of pregnant women is of particular concern. However, few studies have assessed the total amount of maternal and fetal exposure to pesticides. Researches have reported that neonatal exposure to certain insecticides during a period that coincides with peaks in the rapid growth of the brain leading to permanent changes in the cholinergic system and behaviour in the animals as adults [3-6]. Disturbances of the critical period may lead to long-lasting consequences of developmental, learning and behavioral difficulties in children [7-9]. The question arises whether the pesticides circulating in an pregnant women's body pass through the placenta, eliciting possible neurotoxic effects on developing fetuses. It is expected that the placenta is an effective barrier against fetal exposure to certain harmful proteins and also protects the developing embryo against some xenobiotics circulating in maternal blood that adversely affect fetal development. But recent studies identified 2,3,7,8-TCDD, PCBs, bisphenol A, NP, octylphenol, and phthalates in cord blood [10-12], indicating that almost all harmful chemicals in maternal circulation can pass to a fetus. Thus, it is worth knowing at what extent pesticides across the placenta and whether the fetal chemical levels will be higher than the maternal plasma levels. Since organophosphates pesticides are intensively used in Taiwan and they are readily breakdown in human body, the transplacetal absorption of organophosphates metabolite also needs to study. The purposes of this study are to determine prenatal exposure levels of pesticide in Taiwan and to determine the level of placental protection against various pesticides and their metabolites.

Materials and methods

The Ethics Committee of the Veteran General Hospital, Taipei (VGH) approved the study. Before delivery, the expectant mother gave their written informed consent. All of subjects filled the self-administered questionnaires. However, not all of subjects have been successfully collected the maternal venous blood, umbilical venous and arterial blood simultaneously. The umbilical venous and arterial blood samples were separately collected in a 10mL glass EDTA Vacutainer upon delivery at hospital and mother′ s blood was obtained by venous puncture before delivery. All samples were immediately chill transported to the laboratory. They were kept frozen until analysis. For parent pesticides analysis, the samples were pretreated and liquid-liquid extracted with hexane as described by Corrion et al. (2005) [13]. For organophosphate metabolites analysis, this study followed the procedures described by Bravo et al. (2002) [14]. The metabolites were derivatized

using 1-chloro-3-iodopropane before quantification. A chromatograph (Thermo, USA) equipped with a Trace DSQ mass spectrometer was employed for determination and a capillary column (J& W DB-5MS, 30m ×0.25mm, 0.25μm thickness, Agilent, USA) was employed for chromatography. The spectrometer was set at electron ionization mode and was operated in selected ion monitoring (SIM) mode at + 70eV. The studied parent pesticides included propoxur, DDT, chlorpyrifos, diazinon, malathion, and cypermethrin. Six organophosphate metabolites were analyzed, which were dimethylphosphate (DMP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethylphosphate (DEP), diethyl thiophosphate (DETP), diethyl dithiophosphate (DEDTP).

Results and discussion

The parent pesticides and their respective classes are presented in Table 1 along with the target and qualifier ion(s) and their expected retention times used for quantification of analyte of interest. Similar data are shown in Table 2 for the organophosphate metabolites.

Determination of twenty-one pairs of samples, including maternal venous blood (MVB), umbilical venous (UVB) and arterial blood (UAB), were completed (Table 3). DDT was not positively detected for all of samples. In contrast, propoxur had the highest detection rate for MVB, UVB and UAB. 87.5% in MVB, 52.4% in UVB and 38.1% in UAB were positively detected for propoxur. The second one was cypermethrin; the detection rates were 28.8%, 4.8% and 9.5% for MVB, UVB and UAB, respectively. Of the mother-fetus pairs analyzed, the detection rate was highest in MVB, followed by UVB and UAB. The high frequency of detection of propoxur and cypermethrin than other pesticides might be explained by the widely use of pests control (mosquitoes and cockroaches) in households. The concentrations of the pesticides were also shown in the table; the highest concentration (0.594μg/mL) was detected for malathion in the MVB. Except propoxur, the median concentrations of other pesticides were below the detection limit.

For the organophosphate metabolites, DEDTP was 100% positively detected in MVB; it was also highly detected in UVB (90.5%) and UAB (85.7%). DETP and DEP were also frequently detected (Table 4). On the contrary, DMDTP and DMTP were relatively low detected. To our surprise, the lowest detection rate appeared at DMP. Further confirmation is needed. The high frequency of detection of organophosphate metabolites as well as the higher concentrations of metabolites than the parent pesticide demonstrated the widely use of organophosphate pesticides in Taiwan and the readily breakdown of the pesticides in human body. Whyatt and Barr (2001) found DETP in 100% of meconiam samples studied in New York[15]. It was consistent with our findings – the presence of prenatal exposure to organophosphate metabolites. We are continuing to analyze more samples in order to give a more detail profile of prenatal pesticide exposure. From this result, the prenatal exposure to multiple pesticides is confirmed. This study explored the prenatal exposure of pesticides exposure and found the transplacental absorption of pesticides and their metabolites. Pregnancy involves the transfer of lipids and lipoproteins from maternal tissues through the placenta to the developing fetus, a process which results in transfer of dissolved xenobiotics through the placenta and their presence in tissues of the fetus. By this mechanism, prenatal exposure to xenobiotics occurs. Exposure to pesticides can be via ingestion of contaminated foods and drinking water, dermal absorption or inhalation. In addition to the dietary intake from food residue of pesticides, this study addresses home pesticides might be a high exposure risk among pregnant women. Corrective measures to prevent pregnant women exposure are necessary. Meanwhile, assessment of the health outcome in the infant of prenatal exposure to pesticides is also needed.

Acknowledgments

The authors would like to thank the National Science Council of the Republic of China, Taiwan, for financially supporting this research under contract no. NSC 95-2314-B-010-063.

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Table 1 GC-MS Retention time (RT) with target and qualifier ions for the parent pesticides.

Parent Pesticide	Target Ion (m/z)	Qualifier Ion (m/z)	RT (min)
*1,4 DCB	150	115,152	6.15
Carbamate			
Propoxur	110	152	17.16
Organochlorines			
p-p'-DDT	235	165	29.32
Organophosphates			
Chlorpyrifos	197	314, 97	23.28
Diazinon	137	179, 304	19.87
Malathion	173	93, 127	23.08
Pyrethroids			
Cypermethrin	163	181, 209	34.66

* 1,4-DCB served as internal standard.

Table 2 GC-MS Retention time (RT) with target and qualifier ions for organophosphate (OP) metabolites.

OP metabolites	Abbreviation	Target ions (m/z)	Qualifier Ion (m/z)	RT (min)
Dimethylphosphate	DMP	127	109, 167	8.82
Dimethyl thiophosphate	DMTP	99	195, 127	10.34
Dimethyl dithiophosphate	DMDTP	110	142, 169	11.02
Diethylphosphate	DEP	158	125, 93	9.82
Diethyl thiophosphate	DETP	138	170, 110	11.13
Diethyl dithiophosphate	DEDTP	186	121, 97	11.68

Table 3 Concentration of parent pesticides in maternal and cord blood.

Parent pesticide	Maternal venous blood			Umbilical venous blood			Umbilical Arterial blood		
	Positive detection (%)	Median (μg/mL)	Range (μg/mL)	Positive detection (%)	Median (μg/mL)	Range (μg/mL)	Positive detection (%)	Median (μg/mL)	Range (μg/mL)
Propoxur	87.5	0.026	<LOD~0.031	52.4	0.025	<LOD~0.027	38.1	<LOD	<LOD~0.026

p,p' -DDT	-	<LOD	-	-	<LOD	-	-	<LOD	-
Diazinon	9.5	<LO D	<LOD~0.033	4.8	<LOD	<LOD~0.020	4.8	<LOD	<LOD~0.036
Malathion	19.0	<LOD	<LOD~0.594	14.3	<LOD	<LOD~0.110	-	<LOD	-
Chlorpyrifos	19.0	<LOD	<LOD~0.033	4.8	<LOD	<LOD~0.025	-	<LOD	-
Cypermethrin	28.6	<LOD	<LOD~0.027	4.8	<LOD	<LOD~0.068	9.5	<LOD	<LOD~0.017

Table 4 Concentration of organophosphate metabolites in maternal and cord blood.

OP metabolite	Maternal venous blood			Umbilical venous blood			Umbilical Arterial blood		
	Positive detection (%)	Median (μg/mL)	Range (μg/mL)	Positive detection (%)	Median (μg/mL)	Range (μg/mL)	Positive detection (%)	Median (μg/mL)	Range (μg/mL)
DMP	-	<LOD	-	-	<LOD	-	-	<LOD	-
DMTP	28.6	<LOD	<LOD~0.319	14.3	<LOD	<LOD~0.287	19.0	<LOD	<LOD~0.267
DMDTP	14.3	<LOD	<LOD~0.168	47.6	<LOD	<LOD~0.253	47.6	<LOD	<LOD~0.178
DEP	76.2	0.120	<LOD~0.143	87.5	0.1 23	<LOD~0.162	76.2	0.122	<LOD~0.157
DETP	76.2	0.168	<LOD~0.195	87.5	0.172	<LOD~0.252	71.4	0.164	<LOD~0.187
DEDTP	100.0	0.140	0.131~0.180	90.5	0.147	<LOD~0.191	85.7	0.141	<LOD~0.181