HUMAN EXPOSURE TO DIOXINS AND INFANT HEALTH RISK IN COMMUNITIES NEAR DA NANG AGENT ORANGE HOT SPOT

Minh NH[1]*, Son LK[1], Hue NTM[1], Nam VD[1], Thuong NV[1], Mo NT[1], Hung NX[1], Thuy NV[2], Loan TT[3], Hoai PM[3], Tuan NA[3],

[1] Dioxin Laboratory Project, Vietnam Environment Administration, Nr.556 Nguyen Van Cu, Ha Noi, Vietnam
[2] Center for Environment Monitoring, Vietnam Environment Administration, Nr.556 Nguyen Van Cu, Ha Noi, Vietnam
[3] Pollution Control Department, Vietnam Environment Administration, Nr.10 Ton That Thuyet, Hanoi, Vietnam

Introduction

Dioxins (Polychlorinated dibenzo-p-dioxins, PCDDs) and furans (polychlorinated dibenzofurans, PCDFs) are the most toxic chemical species among the persistent organic pollutants (POPs). Exposure of humans and animals to PCDD/Fs at certain levels may lead to negative health effects such as development of cancer, reproductive problems, immune alterations, disruption of hormones, etc[1,2]. Due to high lipophilicity and resistance to metabolisms, PCDD/Fs are bio-accumulated at high levels in fat tissues of animals. PCDD/Fs enter the human body mainly through the consumption of the contaminated animal-based foods. In human body, PCDD/Fs are highly concentrated in breast milk which is considered as an important route for excretion and primary source of intake for newborns. Sampling breast milk is convenient and noninvasive method therefore it is commonly used to assess the human body burden of PCDD/Fs as well as to evaluate risk of the exposure to infant and children health[3,4]. For such reason, since 1987 the World Health Organization (WHO) has used human breast milk as a matrix for global surveys to assess temporal trend and distribution of PCDD/Fs contamination[5].

In Vietnam, number of studies on PCDD/Fs contamination in human breast milk is very few. Kunisue et al. (2004) reported the PCDD/Fs and dl-PCBs WHO1998-TEQ (Toxic equivalent quantity) concentration of 13 pg/g lipid in breast milk of women residing near open dumping sites in Hanoi city[6]. Dwernychuk et al. (2002) revealed PCDD/Fs concentration from 2.99 – 21.9 pg/g lipid in breast milks of women living in areas of Aluoi Valley, Vietnam, where the United States (US) army sprayed the Agent Orange between 1965-1966[7].

Since 2007, the U.S. Congress has agreed a budget to carry out environmental health and remediation activities in Da Nang. A portion of this funding has been programmed to support health and social services for people with disabilities in the Da Nang area. The remainder has been allocated for environmental remediation at the Da Nang AO/Dioxin hot spot[8]. Since 2012, remediation activity using "In-Pile Thermal Desorption" technology for removal and destroy of PCDD/Fs in the contaminated soil has been started. It is initially estimated that about 61,600 m^3 of the dioxin contaminated materials will be cleaned up and implementation time of 4 years.

Because the remediation process is rather long, it is very important to monitor possible long-term impacts of relevant activities to the environment as well as to the local people. It is also necessary to understand the initial background of the human exposure to PCDD/Fs prior to and after completion of the remediation in order to provide insights into health risk of the local people. For these purposes, breast milk samples from primiparae who had resided in vicinities of Da Nang airbase for at least five years, were determined for PCDD/Fs residues. Besides, the daily intake of PCDD/Fs from breast milk was also estimated to assess potential health risk of the local infants.

Materials and methods

Sample collection

All mothers in lactation period from four wards namely Chinh Gian, An Khe, Khue Trung and Hoa Thuan Tay which are located near Da Nang military airbase were called for interview in 2011. Among those, twenty-seven breast milk samples were collected from primiparae who had continuously lived over five years in one of the four wards. The informed consents were obtained from all the donors. Questionnaires on sociodemographic

variables, years of residence, possible exposure through occupational contact and dietary aspects were recorded. Data on relevant information of sampling sites are given in Table 1. The participants in the study were 21–42 years of age, non-smokers and importantly, had lived in the respective areas for at least five years. Duration of breast feeding was mostly in the first stage of 0-24 weeks (average: 16 weeks). About 50-100 ml breast milk sample from each primipara was collected in chemically cleaned amber glass container and stored at -20oC until analysis. The breast milk samples from the four wards were analyzed separately to assess whether proximity to the AO/Dioxin contaminated sites may affect level of PCDD/Fs in mother milk.

Table 1: Information of breast milk donors living near Da Nang AO/Dioxin hot spot

Location	Age		Years of residence	Weeks of breast feeding	Food consumption[a]
Khue Trung, Cam Le ($n = 6$)	Mean	27	23	20	11%
	Range	21–31	10–30	8–36	
An Khe, Thanh Khe ($n = 7$)	Mean	27	22	14	50%
	Range	25–29	16–29	4–30	
Chinh Gian, Thanh Khe ($n = 7$)	Mean	31	21	14	No information
	Range	24–42	10–29	4–32	
Hoa Thuan Tay, Hai Chau ($n = 7$)	Mean	28	16	16	22%
	Range	25–31	5–28	4–32	

[a] Food originated near the Da Nang airbase.

Chemical analyses

Chemical analysis of PCDD/Fs was carried out based on the method 1613B (US EPA, 1994) using High resolution Gas chromatography coupled with High resolution Mass spectrometer (HRGC/HRMS). Prior to the chemical analysis, breast milk was freeze-dried and homogenized to obtain individual milk powder sample.

Samples extraction was done by pressurized liquid extraction system (PLE, FMS Co Ltd., USA) which combine three sample modules of 100 ml extraction cell. The extraction pressure was set to 1700 psi, the practical temperature was 120°C, and the number of the static cycle was two for all experiments. Five grams of milk powder sample was mixed with anhydrous sodiumsulphate and filled up to the extraction cell. Extractions were performed with n-hexane/dichloromethane/ethanol (5/2/1, v/v/v). The sample extracts were defatted with concentrated sulfuric acid and further purified and fractionated continuously on the multi-layer silica columns coupled with dual-layer activated carbon purchased from Supelco Co. (Supelco, 2004). Finally, the concentrated eluate of the sample was further evaporated with a gentle stream of nitrogen gas and spiked with 20 µL of injection standard. The quantifications were based on the isotope dilution mass spectrometry following the Method 1613B (US EPA., 1994)[9]. TEQ values were the lower-bound TEQ calculated based on the detected congeners and using the toxic equivalent factors (TEFs) issued by WHO in 2005[10].

The Wilcoxon-Mann-Whitney was used for assessing whether the contaminant levels between groups were significantly different. In this analysis, non-detectable levels were set to zero. The parameters used as independent variables are age, body mass index and consumption rate of local animal foods. Parameters with a p value less than 0.05 were considered as having significant relationship with contamination level. Calculations were performed using the statistical R software package version 3.0.

Results and discussion

Dioxins and furans in breast milks

Results of the mass concentration of PCDD/Fs and TEQ values in the breast milk samples are presented in Table 2. These values were obtained from 27 primiparae from four wards locating in vicinities of AO/Dioxin hot spot. Khue Trung ward (Cam Le district) is located in the south of Da Nang airbase, An Khe and Chinh Gian ward (Thanh Khe district) in the western and northern, and Hoa Thuan Tay (Hai Chau district) in the eastern side. Breast milk samples were collected at week 4-36 after delivery (mean: 16 week, Table 1). The mean lipid content of 27 breast milk samples was 4.3% (ranging from 2.1 - 9.3%).

The results indicated that, on mass basis, the PCDD/Fs mean concentration profile followed: Khue Trung (103 pg/g lipid) < Hoa Thuan Tay (162 pg/g lipid) < An Khe (175 pg/g lipid) < Chinh Gian (203 pg/g lipid). It is

interesting to observe that the PCDD/F concentrations in Khue Trung are significantly lower than those in An Khe (p = 0.032) and Chinh Gian (p = 0.021) which are located in the north of the airbase and close to the AO/Dioxin contaminated sites.

The mean PCDD/Fs-TEQ concentrations in milk samples from all these sites were ranged between 8.1 and 26 pg/g lipid. The profile of TEQ concentration was slightly different compared with those of PCDD/Fs mass concentration, showing Khue Trung < Hoa Thuan Tay < Chinh Gian < An Khe. Moreover, we also observed that the PCDD/Fs-TEQ concentrations follow similar trend of the PCDD/F mass concentration, showing significantly lower TEQ in Khue Trung compared to Hoa Thuan Tay (p = 0.032), Chinh Gian (p = 0.013) and An Khe (p = 0.0025). There is no significant difference among Chinh Gian, An Khe and Hoa Thuan Tay ward. Perhaps, the proximities of Chinh Gian, An Khe and Hoa Thuan Tay ward to the AO/Dioxin contaminated sites in the north of the airbase had caused elevated exposure of the mothers living in such communities.

Table 2: PCDD/Fs in breast milk samples of women living near Da Nang AO/Dioxin hot spot (pg/g lipid)

Congener	Khue Trung		An Khe		Chinh Gian		Hoa Thuan Tay	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
2,3,7,8-TCDD	1.9	0.75–2.4	14	3.0–43	5.1	1.5–24	7.6	2.4–27
1,2,3,7,8-PeCDD	2.5	1.5–3.6	6.3	4.9–9.1	5.1	2.8–9.1	5.2	1.8–14
1,2,3,4,7,8-HxCDD	5.4	3.4–11	2.4	0.28–3.9	2.5	1.3–3.6	2.7	1.3–7.3
1,2,3,6,7,8-HxCDD	0.84	0.40–1.5	15.4	8.3–21	13	4.2–24	11	2.5–32
1,2,3,7,8,9-HxCDD	0.86	0.12–1.7	2.0	0.46–3.4	2.1	1.6–3.2	2.0	0.77–4.4
1,2,3,4,6,7,8-HpCDD	5.4	3.4–11	11	5.7–20	14	4.9–26	11	2.8–27
OCDD	54	17–145	89	51–123	86	33–179	89	15–261
2,3,7,8-TCDF	0.68	0.46–0.89	1.1	0.72–1.9	2.7	1.1–5.8	1.8	1.4–2.7
1,2,3,7,8-PeCDF	0.59	0.45–0.69	0.88	0.63–1.3	2.6	1.1–5.9	1.4	1.0–1.9
2,3,4,7,8-PeCDF	4.3	2.5–6.3	6.8	4.6–9.4	8.5	4.4–12	5.4	2.7–8.9
1,2,3,4,7,8-HxCDF	8.9	5.5–14	10	5.1–27	23	10–49	8.2	3.4–11
1,2,3,6,7,8-HxCDF	5.6	3.4–8.6	6.1	3.4–17	14	5.7–26	5.7	2.8–7.6
1,2,3,7,8,9-HxCDF	0.75	0.38–1.7	0.89	0.43–1.4	1.8	0.91–3.8	1.2	0.75–1.6
2,3,4,6,7,8-HxCDF	0.38	0.28–0.47	0.62	0.22–1.5	2.1	1.1–3.2	1.7	1.1–243
1,2,3,4,6,7,8-HpCDF	5.4	2.4–9.7	5.3	3.3–13	16	6.6–50	5.8	3.2–9.8
1,2,3,4,7,8,9-HpCDF	4.5	0.63–8.6	1.6	0.41–4.3	2.2	1.0–5.4	1.2	0.77–1.8
OCDF	0.55	0.31–0.96	0.89	0.38–1.8	2.1	1.2–3.9	1.3	0.58–2.4
Total PCDD/Fs	103	58–227	175	117–237	203	90–292	162	49–414
PCDD/Fs-TEQ	8.1	4.3–12	26	14–51	19	8.7–45	18	6.8–51

In geographical comparison, it is important to note that the present study revealed much higher PCDD/Fs concentration and TEQ compared to a control cohort in Kim Bang (Ha Nam Province, Northern Vietnam), while comparable with those in women living near Da Nang airbase[11]. In the temporal comparison, the mean level of PCDD/Fs- WHO2005-TEQ in this study (18 pg/g lipid) was about a haft of those observed in Da Nang city in the early 1990s (37 pg/g lipid)[12]. However, because of differences between the two investigated cohorts, the observation of lower PCDD/Fs concentration in the present study may be insufficient to assess decreasing trend of the dioxin concentration in the human milk in Da Nang. Nevertheless, health concerns regarding to human exposure to dioxins in communities presently living near the AO/Dioxin hot spots are still remained.

Dietary habits are one of important factors which affect the population's exposure to PCDD/Fs[13,14]. Although the information was insufficient for the statistical test of significances, it is interesting to mention that An Khe had the highest percentage of the local food consumption and also had the highest TEQ levels found in women. The available information regarding consumption of foods originated from areas near the Da Nang airbase, especially fish and aquatic animals revealed slight relation with the PCDD/Fs level in the breast milk samples (Table 1).

Estimation of the infant's daily intake

The mean estimated daily intakes of TEQ (EDI-TEQ) for the local breast-fed infants were showed in Table 4. The lowest EDI-TEQ value was found in Khue Trung, Cam Le district (48.0 pg TEQ/kg bw/day) and the highest value was in An Khe, Thank Khe district (155.6 pg TEQ/kg bw/day). Range of EDI-TEQ in the present study was about 2 - 5 times higher than those in the control cohort of Kim Bang (24.5 pg TEQ/kg bw/day)[11], while was similar to the highest EDI-TEQ found in Wallonia, Belgium (120.08 pg TEQ/kg bw/day) and Czech Republic (152.01 pg TEQ/kg bw/day)[12]. The range of EDI-TEQ in the present study was much higher than the Tolerable Daily Intake (TDI) of 4 pg TEQ/kg bw/day proposed by The World Health Organization (WHO)[13]. In

Vietnam, breast milk usually serves as the main nutrient source for infants during the first state of newborns. Therefore, this result indicated the elevated risk of exposure of dioxins and furans not only to the mother living in the vicinities of Da Nang AO/Dioxin hotspot but also to their children.

Table 3: Estimated daily intake of TEQ for local breast-fed infants
(pg TEQ/kg bw/day)

EDI	mean	min	max
Khue Trung	48.0	29.9	83.8
An Khe	156	71.3	263
Chinh Gian	114	44.4	229
Hoa Thuan Tay	107	43.7	325

Acknowledgements

This study was implemented under the framework of the project for Establishment of Dioxin Laboratory in Vietnam (**AP-16657** and **BMGF-50799**) and the research grant **KHCN-33.01/11-15** from the Vietnam National Science and Technology Research Program KHCN-33/11-15 (Research and overcoming long-term consequences of Agent Orange/Dioxin on the environment and the human health in Vietnam). The authors would like to express sincerest thanks to all mothers who participated in this study and donated their milk samples.

References:

1. Colborn T, vom Saal FS, Soto AM. (1993); *Environ Health Perspect*. 101: 378–384.
2. Schecter A, Ryan JJ, Papke O. (1998); *Chemosphere*. 37: 1807–1816.
3. Kahn P, Gochfield M, Nygren M, Hansson M, Rappe C, Velez H. (1988); *J. Am Med Assoc*. 259: 1661-1667.
4. Schecter A, Dai LC, Thuy LTB, Quynh HT, Minh DQ, Can HD. (1995); *American Joumal of Public Health*. 85: 516-522.
5. Malisch R, Moy G. (2006); *Organohalogen Compd*. 68: 1627–1630.
6. Kunisue T, Watanabe M, Iwata H, Subramanian A, Monirith I, Minh TB, Baburajendran R, Tana TS, Viet PH, Prudente M, Tanabe S. (2004); *Arch Environ Contam Toxicol*. 47: 414–426.
7. Dwernychuk LW, Cau HD, Hatfield CT, BoivinTG, Hung TM, Dung PT, Thai ND. (2002); *Chemosphere*. 47: 117-137.
8. Sorenson KS, Chichakli RE, Chenevey PM, Montera JG, Diep TM, McNamee PJ, Boivin TG, Baker RS, Donovan F, Handler H. (2011); *Organohalogen Compoud*.
9. US EPA. Method 1613B – Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS; 1994.
10. Van den Berg M. et al. (2005); *Toxicol Sci.* 93: 223-41.
11. Tai PT, Nishijo M, Kido T, Nakagawa H, Maruzeni S, Naganuma R. (2011); *Environmental Science and Technology*. 45: 6625-6632.
12. Schecter A, Christiane FP, Peapke O, Ball M, Dai LC, Quynh HT, Phuong NTN, Beim A. (1991); *Chemosphere*. 23: 1903-1912.
13. Chao HR, Wang SL, Lee CC, Yu YK, Peapke O. (2004); *Food Chem Toxicol*. 42: 1299-1308.
14. Li J, Zhang L, Wu Y, Liu Y, Zhou P, Wen S. (2009); *Chemosphere*. 75: 1236-1242.
15. Ulaszewska MM, Zuccato E, Davoli E. (2011); *Chemosphere*. 83: 774-782.
16. Van Leeuwen FXR, Malisch R. (2002); *Organochalogen Compd*. 56: 311-316.