# EFFECTS OF DIOXINS EXPOSURE ON INFANT GROWTH IN A HOT SPOT AREA, VIETNAM

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## Introduction

In Vietnam, there are 3 airbases located in Bien Hoa, Da Nang and Phu Cat which were storages of the herbicides sprayed during the Vietnam war, and called "hot spot" of dioxin contamination. Previous studies shown that the levels of dioxins, especially 2,3,7,8-tetrachlorodibenzo-p-dioxin (TeCDD), were dramatically elevated in "hot spot". The residents around "hot spot" are highly risky to exposure of TeCDD, because of not only previous exposure but also resent exposure due to contaminated water from air base. Thanh Khe district in Danang city surround Danang airbase which is one of hot spot, and residents are suggested to be at health risk by recent exposure.

Fetus is known to be most sensitive to dioxin exposure, and infant body size at birth has been investigated as a good marker of fetal development. Growth in early period from the birth such as 1 month old is also affected by fetal condition. Previously, in Holland[1] and Japan[2,3], lower body size at birth was reported in the infants exposed to dioxin at back ground level. However, an effect of dioxin at high level has not been investigated in Vietnamese infants.

Therefore, to clarify the effects of dioxin exposure on fetal growth, infant body sizes at birth and 1 month after birth in Thanh Khe district were compared as those in control area.

## Materials and methods

*1. Study subjects and health survey*

Survey of newborns in Thanh Khe district hospital was started on July, 2008, and body size of 150 babies was measured until January, 2009. Son Tra district was selected as a control area and 80 infants born in Son Tra district hospital were recruited from November to December, 2009. When the infants became 1 month old, nurses of commune health center visited them, and followed up 147 infants (93%) in Thanh Khe and 80 infants (100%) in Son Tra. Infant size including weight, length (height), head circumference and body circumference were measured at birth in the hospital and postnatal 1 month at home. Ponderal index which is an obesity index was calculated to evaluate infant condition.

Breast milk samples were collected at postnatal 1 month and transferred to Japan for pretreatment and measurement of dioxin concentration of 17 isomers of polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Toxic equivalent of PCDDs (PCDDs TEQ), PCDFs (PCDFs TEQ) and PCDDs/PCDFs TEQ (total TEQ) were calculated by adding up the multiplying each congener concentration with its toxic equivalent factor (TEF) referred from the WHO 1997-TEF[4].

*2. Breast milk sample analysis*

About 10 g breast milk was used to extract fat content. A series of purification operations consisting of alkali digestion, hexane extraction and chromatography were carried out. The established method of analysis was described in details elsewhere[5]. The final extract were concentrated to 20 µl and analyzed by gas chromatography coupled to high-resolution mass spectrometry (HR-GC/MS, MStation-JMS700, JEOL, Japan)

**Results and discussion:**

*1. Dioxin exposure in Thanh Khe and Son Tra*

Both in Thanh Khe and Son Tra, dioxin isomers concentration of primipara were higher than those in other areas including a herbicide sprayed area during Vietnamese war and unsprayed areas in north Vietnam. However, TeCDD level of primipara in Thanh Khe was significantly higher than that in Son Tra (Table 1). Prevalence of mothers (primipara and multipara) with TeCDD in breast milk ≥3pg/gfat was higher (15.6%) in Thanh Khe as compared with that (5.0%) in Son Tra. Mother's age, residency and parity was related to the level of dioxins in breast milk both in Thanh Khe and Son Tra, but residency was the most strongly correlated to dioxin concentration of all isomers, suggesting dioxin contamination specific to their living area. By contrast, mother's age was the strongest factor related to dioxin concentration of breast milk in Son Tra, suggesting the source of dioxin is not specific in this area.

*2. Effects of infant growth*

Although no significant difference of weight, height, head circumference and abdominal circumference was observed, mean of head abdominal ratio (head circumference/abdominal circumference) at birth in boys was significantly lower in Thanh Khe than that in Son Tra district. The difference of head abdominal ratio between 2 areas was significant even after adjustment for gestational age. Moreover, in Thanh Khe, head abdominal ratio was significantly inversely correlated to HxCDD in boys after adjustment for gestational age.

In boys, weight and ponderal index at postnatal 1 month were significantly lower in Thanh Khe than those in Son Tra after adjustment for gestational age (Table 3). Head abdominal ratio was significantly higher in Thanh Khe than that in Son Tra (Table 3). Boys' weight at postnatal 1 month was significantly and inversely correlated with PeCDD, OCDD, PCDDs and total PCDDs/DFs in breast milk. In addition, increased weight during neonatal period from birth to 1 month old was inversely correlated with PeCDD, HxCDD, OCDD, PeCDF, HxCDF, HpCDD, PCDDs, PCDFs, and Total PCDDs/DFs in breast milk in boys. Significant inverse correlations between increased infant weight during neonatal period and PeCDF, HxCDF, PCFDs and PCDDs/DFs in breast milk were also observed in girls.

Therefore, infant growth at early period of their life might be influenced by maternal dioxin exposure to which infants are exposed during gestation.

Previously, effect of dioxins on birth weight has been reported in the infants born of the mothers who exposed to dioxins in other countries. Low birth weight was observed in Yu-Cheng children born of mothers exposed to very high levels of PCBs and dioxins from contaminated cooking oil. In Sweden, infants born to fishermen's wives who consumed contaminated fish had also lower birth weight than controls. In a Dutch study, inverse association of maternal plasma PCBs with birth weight and growth rate was reported. However, effects of dioxin exposure on infant growth have not been investigated in Vietnam where dioxins in environment and human are still high, especially in surrounding area of former herbicide storage airbase considered as "hot spot". Further follow-up survey is necessary to clarify health effects on subsequent generation in hot spot, Vietnam.

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Table 1 Geometrical means of dioxin concentrations in breast milk of primipara mothers living in 6 areas in Vietanam

| Congener (pg/g fat) | Th. Khe (n=43) | Son Tra (n=26) | Phu Cat (n=23) | Quang Tri (n=15) | Ha Tinh (n=15) | Kim Bang (n=19) |
|---|---|---|---|---|---|---|
| TeCDD | 2.0 | 1.4 | 1.7 | 1.1 | 0.7 | 0.5 |
| PeCDD | 4.7 | 4.9 | 5.2 | 3.0 | 1.4 | 1.7 |
| HxCDD1 | 2.2 | 2.2 | 2.3 | 1.7 | 0.6 | 0.7 |
| HxCDD2 | 8.6 | 8.7 | 8.7 | 8.7 | 1.6 | 1.6 |
| HxCDD3 | 2.7 | 2.7 | 3.2 | 2.0 | 0.5 | 0.7 |
| HpCDD | 11.3 | 10.5 | 14.9 | 17.1 | 1.7 | 2.8 |
| OCDD | 61.2 | 69.5 | 71.4 | 50.7 | 6.2 | 14.4 |
| TeCDF | 0.5 | 0.3 | 0.6 | 0.6 | 1.1 | 0.6 |
| PeCDF1 | 1.3 | 1.0 | 1.9 | 0.8 | 0.6 | 0.4 |
| PeCDF2 | 8.0 | 8.1 | 7.4 | 6.1 | 3.5 | 3.7 |
| HxCDF1 | 18.3 | 18.9 | 16.7 | 16.1 | 2.0 | 2.3 |
| HxCDF2 | 11.0 | 10.7 | 9.7 | 8.6 | 1.4 | 2.0 |
| HxCDF3 | 0.3 | 0.2 | 0.4 | 0.2 | 0.1 | 0.1 |
| HxCDF4 | 1.4 | 1.2 | 1.6 | 1.3 | 0.4 | 0.6 |
| HpCDF1 | 12.8 | 11.5 | 16.4 | 11.2 | 1.2 | 1.8 |
| HpCDF2 | 1.5 | 1.0 | 1.7 | 1.5 | 0.1 | 0.2 |
| OCDF | 0.6 | 0.5 | 1.0 | 0.2 | 0.1 | 0.3 |
| PCDDs TEQ | 8.3 | 7.9 | 8.5 | 5.7 | 2.5 | 2.6 |
| PCDFs TEQ | 7.5 | 7.4 | 5.4 | 4.8 | 1.6 | 1.7 |
| Total TEQ | 15.9 | 15.5 | 14.1 | 10.6 | 4.1 | 4.3 |

n: number of mothers

Table 2 Relationships of dioxins with age and residential period and parity of mothers

| | Thanh Khe | | | | | | Son Tra | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Congener | Mother age | | Residency | | Parity | | Mother age | | Residency | | Parity | |
| | Beta | p | Beta | p | Beta | p | Beta | p | Beta | p | Beta | p |
| TeCDD | 0.21 | 0.03 | 0.34 | 0.00 | −0.34 | 0.00 | 0.44 | 0.00 | 0.18 | 0.13 | −0.49 | 0.00 |
| PeCDD | 0.35 | 0.00 | 0.33 | 0.00 | −0.38 | 0.00 | 0.50 | 0.00 | 0.21 | 0.04 | −0.62 | 0.00 |
| HxCDD1 | 0.38 | 0.00 | 0.26 | 0.00 | −0.20 | 0.03 | 0.62 | 0.00 | 0.16 | 0.13 | −0.40 | 0.00 |
| HxCDD2 | 0.31 | 0.00 | 0.33 | 0.00 | −0.24 | 0.01 | 0.37 | 0.00 | 0.37 | 0.00 | −0.53 | 0.00 |
| HxCDD3 | 0.24 | 0.01 | 0.27 | 0.00 | −0.18 | 0.06 | 0.39 | 0.01 | 0.18 | 0.14 | −0.43 | 0.00 |
| HpCDD | 0.36 | 0.00 | 0.15 | 0.07 | −0.06 | 0.52 | 0.54 | 0.00 | 0.03 | 0.82 | −0.28 | 0.02 |
| OCDD | 0.40 | 0.00 | 0.21 | 0.01 | −0.09 | 0.29 | 0.28 | 0.04 | 0.33 | 0.01 | −0.32 | 0.01 |
| TeCDF | −0.11 | 0.29 | 0.33 | 0.00 | 0.07 | 0.49 | 0.19 | 0.21 | −0.04 | 0.77 | 0.02 | 0.88 |
| PeCDF1 | 0.06 | 0.56 | 0.29 | 0.00 | −0.11 | 0.26 | 0.36 | 0.02 | 0.04 | 0.77 | −0.21 | 0.10 |
| PeCDF2 | 0.27 | 0.00 | 0.37 | 0.00 | −0.36 | 0.00 | 0.47 | 0.00 | 0.24 | 0.03 | −0.60 | 0.00 |
| HxCDF1 | 0.14 | 0.15 | 0.37 | 0.00 | −0.15 | 0.11 | 0.33 | 0.01 | 0.26 | 0.03 | −0.53 | 0.00 |
| HxCDF2 | 0.14 | 0.16 | 0.38 | 0.00 | −0.14 | 0.12 | 0.34 | 0.01 | 0.30 | 0.01 | −0.44 | 0.00 |
| HxCDF3 | 0.10 | 0.32 | 0.10 | 0.24 | −0.12 | 0.23 | 0.50 | 0.00 | −0.23 | 0.06 | −0.45 | 0.00 |
| HxCDF4 | 0.22 | 0.03 | 0.22 | 0.01 | −0.15 | 0.11 | 0.50 | 0.00 | 0.00 | 0.98 | −0.35 | 0.01 |
| HpCDF1 | 0.08 | 0.41 | 0.29 | 0.00 | −0.07 | 0.46 | 0.36 | 0.01 | 0.08 | 0.51 | −0.39 | 0.00 |
| HpCDF2 | −0.12 | 0.25 | 0.22 | 0.01 | −0.07 | 0.51 | 0.12 | 0.45 | 0.12 | 0.38 | −0.25 | 0.07 |
| OCDF | 0.12 | 0.27 | 0.10 | 0.26 | −0.10 | 0.31 | 0.00 | 0.99 | −0.01 | 0.94 | 0.10 | 0.48 |
| PCDDs TEQ | 0.33 | 0.00 | 0.35 | 0.00 | −0.36 | 0.00 | 0.53 | 0.00 | 0.26 | 0.01 | −0.61 | 0.00 |
| PCDFs TEQ | 0.21 | 0.02 | 0.38 | 0.00 | −0.25 | 0.01 | 0.42 | 0.00 | 0.25 | 0.03 | −0.56 | 0.00 |
| Total TEQ | 0.28 | 0.00 | 0.37 | 0.00 | −0.32 | 0.00 | 0.50 | 0.00 | 0.27 | 0.01 | −0.62 | 0.00 |

Table 3 Comparisons of body size at postnatal 1 month between Thanh Khe and Son Tra area

| 1 month | Thanh Khe mean/adjusted mean# | SD/SE | Son Tra mean/adjusted mean | SD/SE | |
|---|---|---|---|---|---|
| Boys | | | | | |
| Weight | 4387.13 | 595.08 | 4592.22 | 538.72 | * |
| Weight# | 4403.07 | 66.42 | 4560.31 | 77.56 | |
| Length | 55.72 | 2.40 | 55.86 | 1.91 | |
| Length# | 55.81 | 0.26 | 55.74 | 0.30 | |
| Head | 37.15 | 1.36 | 37.16 | 1.52 | |
| Head# | 37.18 | 0.17 | 37.08 | 0.19 | |
| Abodomen | 38.26 | 2.54 | 37.46 | 2.56 | |
| Abodomen# | 38.28 | 0.30 | 37.39 | 0.35 | |
| Pondiral index | 2.50 | 0.41 | 2.64 | 0.29 | * |
| Pondiral index# | 2.53 | 0.03 | 2.64 | 0.04 | * |
| Head body ratio | 1.03 | 0.06 | 1.01 | 0.05 | * |
| Head body ratio# | 1.03 | 0.01 | 1.01 | 0.01 | * |
| Girls | | | | | |
| Weight | 4115.86 | 462.13 | 4149.50 | 470.96 | |
| Weight# | 4123.47 | 53.53 | 4126.46 | 91.23 | |
| Length | 54.74 | 2.39 | 55.79 | 3.02 | |
| Length# | 54.74 | 0.30 | 55.72 | 0.51 | |
| Head | 36.35 | 1.36 | 36.54 | 1.35 | |
| Head# | 36.34 | 0.16 | 36.57 | 0.28 | |
| Abodomen | 36.82 | 2.30 | 36.58 | 1.85 | |
| Abodomen# | 36.85 | 0.25 | 36.67 | 0.43 | |
| Pondiral index | 2.52 | 0.29 | 2.40 | 0.28 | |
| Pondiral index# | 2.52 | 0.03 | 2.40 | 0.06 | |
| Head body ratio | 1.01 | 0.06 | 1.00 | 0.03 | |
| Head body ratio# | 1.01 | 0.01 | 1.00 | 0.01 | |

#: mean adjused by gestational weeks, SD: standard deviation, SE: standard error

*: $p<0.05$ comparison between Thanh Khe and Son Tra