# PORYCHLORINATED-DIBENZO-P-DIOXINS / FURANS AND PREGNANCY OUTCOME AMONG VIETNAMESE WOMEN LIVING IN A HERBICIDE SPRAYED AREA

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## Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic isomer of the dioxins and related compounds, and several animal studies have demonstrated that maternal exposure to TCDD affects fetal development, such as reducing litter size and birth weight. In humans, high rate of spontaneous abortion was observed in women of Seveso, Italy, although no relationship to TCDD in serum was detected shortly after accident. In Vietnamese and Ranch Hand soldiers who have been exposed to herbicide including dioxins sprayed during the war, the increase of birth defects in their infants, and the risk of pregnancy outcome, such as increase of spontaneous abortion and still birth have been reported in the comparisons with controls (Dung *et al*. 2007, Quang *et al*. 2007). However, there are few reports about relationships between pregnancy outcome and dioxin exposure levels in the residents living in a sprayed area.

Since 2002, we have conducted a case control study on dioxin levels and health effects among the residents in the herbicide sprayed area during the war from 1962 to 1971, Cam Chinh Commune in Quang Tri Province, Vietnam and found that dioxin levels in serum and breast milk in the sprayed area were significantly higher than those in non-sprayed area, Cam Phuc Commune in Ha Tinh Province. In the 2003 survey, we recruited mother-and-infant pairs living in Cam Chinh Commune, the sprayed area, and reported that chest circumstance of their infant was inversely related to the increase of total TEQ of dioxins in breast milk (Maruzeni, 2005).

Based on these findings, we examined the relationship between pregnancy outcome, such as spontaneous abortion and still birth, and dioxins isomers in maternal breast milk as an indicator of maternal exposure in the subjects examined in 2003.

## Materials and Methods

***Subjects*** Fifty-one mothers living in Cam Chinh Commune where herbicide was sprayed during the war, 36 mothers living in Cam Phuc Commune, non-sprayed area, and 75 women who delivered their infants in the hospitals in Toyama and Kanazawa, Japan were included in this study. Mothers who had enough breast milk to provide samples, and who were interested in the level of dioxin in their own breast milk, were recruited in Vietnam and Japan. Mean age of Japanese mothers was 29.8 years old, which was significantly older than that of Vietnamese mothers. Also, the rate of primipara in Vietnamese mothers was significantly lower than that in Japanese mothers.

***Data collection*** Information regarding parity, clinical history of pregnancy, and infant build were obtained via a questionnaire filled out by maternity nurses. Breast milk samples were collected into polypropylene tubes and frozen at -20°C until analysis. After fat extraction from 10 g of breast milk, $^{13}$C-2,3,7,8-substituted PCDDs/PCDFs as internal standards were added to the fat extract. A series of purification operations consisting of alkali digestion, hexane extraction and chromatography on both a multi-layered column of silica gel and a single-layer column of activated carbon dispersed on silica gel, were performed to separate and collect PCDDs/PCDFs by the methods described in our previous paper (Tawara *et al*. 2003). Quantitative analysis was performed by gas chromatography coupled to high-resolution mass spectrometry (HRMS, HR-GC/MS; Mat ation-JMS700, JEOL, Japan). The gas chromatograph (HP-6980) was mounted with an ENV-5 column, and attached to the HRMS unit. In addition to respective concentration of 17 isomers, toxic equivalents (TEQ) of

PCDDs, PCDFs and total dioxins were calculated for each sample, with reference to the international Toxicity Equivalent Factor (TEF) of WHO-TEF in 1997 (Van den Berg *et al*. 1998). All concentrations of each isomer and TEQs were determined in a lipid base.

**Results and Discussion**

Comparisons of the mean concentrations per 1 g milk fat of the 7 substituted PCDDs and the 10 PCDF isomers, and the TEQ-PCDDs, TEQ-PCDFs and TEQ-Total between Vietnamese and Japanese in primipara and multipara were shown in Table 1. Generally, PCDD, HxCDDs, OCDD, and PCDF in Vietnamese were lower, and HxCDFs, HpCDFs, and OCDF were higher than that those in Japanese. Moreover, three TEQs were significantly lower in Vietnamese. In comparisons of dioxin levels between sprayed and control areas in Vietnam, almost isomers in sprayed area were significantly higher than those in control area, except TCDD and TCDF (Table 2). Although TCDF in Vietnamese was lower than that in Japanese, the level of TCDD in the sprayed area was comparatively higher, especially in primipara women (Table 2). These findings suggested that total dioxin exposure level was not so high in the present days, but comparatively higher percentage of TCDD, which is characteristic of "Agent orange" exposure, was recognized in the herbicide sprayed area in Vietnam.

The numbers of pregnancy and delivery in the past time of mothers in sprayed area were significantly higher than those in control areas and Japanese (Table 3). The rate of abortion/stillbirth in the sprayed area was lower than those in Japanese, but it was significantly higher than that in control area in Vietnam (Table 3).

To clarify the effect of dioxin exposure on pregnancy outcome after adjustment for confounding factors, we computed regression coefficients and estimated odds ratio for the relationship between dioxin isomers in breast milk and experience of abortion/stillbirth in the past history, using logistic regression analysis. The results in dioxin sprayed area and all Vietnamese areas were shown in Table 4. Experience of abortion/stillbirth was significantly related to the increase of TCDD in breast milk, and the risk ratio (odds ratio) of abortion/stillbirth was 89.6 in the sprayed area and 175.3 in all Vietnamese areas. PCDD and 1,2,3,6,7,8-HxCDD also significantly increased the risk of abortion/stillbirth both in the sprayed area and all areas. PCDDs-TEQ and Total-TEQ showed significantly increased risk of abortion/stillbirth, because these three dioxin isomers with high TEF, contributed to the increase of TEQ of PCDDs and Total PCDDs/Fs. However, in furan isomers, only 2,3,4,7,8-PCDF showed increased risk ratio in the sprayed area. In addition, there were no significant relationships between dioxins isomers in breast milk and abortion/stillbirth in Japanese mothers. These findings suggested that pregnancy outcome might be influenced by maternal exposure to dioxins, especially TCDD.

**References**


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Table 1 Comparisons of dioxin concentrations in breast milk between Vietnamese and Japanese mothers

| | Primipara | | | | | Multipara | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Japanese(N=40) | | Vienames(N=27) | | | Japanese(N=35) | | Vienames(N=60) | | |
| | Mean | ( SD ) | Mean | ( SD ) | | Mean | ( SD ) | Mean | ( SD ) | |
| 2,3,7,8-TCDD | 0.92 | ( 2.11 ) | 0.91 | ( 1.91 ) | | 0.7 | ( 2.27 ) | 0.59 | ( 2.07 ) | |
| 1,2,3,7,8-PCDD | 4.92 | ( 1.63 ) | 2.09 | ( 1.81 ) | -** | 3.35 | ( 1.75 ) | 1.41 | ( 2.22 ) | -** |
| 1,2,3,4,7,8-HxCDD | 1.69 | ( 2.11 ) | 0.99 | ( 2.37 ) | -** | 1.2 | ( 1.66 ) | 0.71 | ( 3.16 ) | -** |
| 1,2,3,6,7,8-HxCDD | 16.59 | ( 1.6 ) | 3.82 | ( 2.57 ) | -** | 10.96 | ( 1.89 ) | 2.74 | ( 2.99 ) | -** |
| 1,2,3,7,8,9-HxCDD | 2.52 | ( 1.82 ) | 1 | ( 2.6 ) | -* | 1.44 | ( 2.39 ) | 0.76 | ( 3 ) | -* |
| 1,2,3,4,6,7,8-OCDD | 8.43 | ( 1.74 ) | 5.69 | ( 3.53 ) | | 5.28 | ( 1.79 ) | 4.81 | ( 3.92 ) | |
| 2,3,7,8-TCDF | 64.04 | ( 1.82 ) | 18.46 | ( 3.46 ) | -** | 50.2 | ( 1.57 ) | 18.47 | ( 3.31 ) | -** |
| 1,2,3,7,8-PCDF | 1.03 | ( 1.91 ) | 0.81 | ( 2 ) | | 1.01 | ( 1.73 ) | 0.68 | ( 1.94 ) | -** |
| 2,3,4,7,8-PCDF | 0.26 | ( 4.79 ) | 0.69 | ( 1.86 ) | ** | 0.35 | ( 1.78 ) | 0.47 | ( 2.23 ) | |
| 1,2,3,4,7,8-HxCDF | 9.61 | ( 1.68 ) | 4.74 | ( 1.69 ) | -** | 6.74 | ( 1.75 ) | 3.03 | ( 1.83 ) | -** |
| 1,2,3,6,7,8-HxCDF | 2.67 | ( 1.64 ) | 5.75 | ( 3.43 ) | ** | 1.84 | ( 1.88 ) | 4.05 | ( 4.38 ) | ** |
| 2,3,4,6,7,8-HxCDF | 2.77 | ( 1.67 ) | 3.63 | ( 3.01 ) | | 1.81 | ( 1.76 ) | 2.98 | ( 3.4 ) | ** |
| 1,2,3,7,8,9-HxCDF | 1.75 | ( 2.24 ) | 0.79 | ( 2.35 ) | -** | 1.18 | ( 1.86 ) | 0.53 | ( 2.64 ) | -** |
| 1,2,3,4,6,7,8-OCDF | 0.01 | ( 17.1 ) | 0.15 | ( 3.67 ) | ** | 0.02 | ( 15 ) | 0.16 | ( 3.13 ) | ** |
| 1,2,3,4,7,8,9-OCDF | 1.51 | ( 1.79 ) | 3.6 | ( 3.84 ) | ** | 1.04 | ( 2.41 ) | 3.23 | ( 5.09 ) | ** |
| PCDDs -TEQ | 0.03 | ( 13.8 ) | 0.42 | ( 4.57 ) | ** | 0.05 | ( 16.2 ) | 0.46 | ( 5.06 ) | ** |
| PCDFs –TEQ | 0.01 | ( 21.2 ) | 0.11 | ( 2.82 ) | ** | 0.02 | ( 20 ) | 0.18 | ( 3.22 ) | ** |
| PCDDs/DFs -TEQ | 8.21 | ( 1.61 ) | 3.82 | ( 1.82 ) | -** | 5.59 | ( 1.78 ) | 2.65 | ( 2.08 ) | -** |
| | 5.76 | ( 1.66 ) | 3.86 | ( 1.93 ) | -** | 4.07 | ( 1.71 ) | 2.75 | ( 2.09 ) | -** |
| | 14.06 | ( 1.6 ) | 7.75 | ( 1.84 ) | -** | 9.72 | ( 1.72 ) | 5.49 | ( 2.03 ) | -** |

**: P<0.01 : significantly higher, -*:P<0.05, -**: P<0.01 : significantly lower as compared with Japanese

Table 2 Comparisons of dioxin concentrations in breast milk between in sprayed and control areas in Vietnam

| | Primiparae | | | | | Multiparae | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control area(N=13 | | Sprayed area(N=14) | | | Control area(N=27 | | Sprayed area(N=37) | | |
| | Mean | ( SD ) | Mean | ( SD ) | | Mean | ( SD ) | Mean | ( SD ) | |
| 2,3,7,8-TCDD | 0.75 | ( 1.4 ) | 1.09 | ( 2.26 ) | | 0.47 | ( 1.91 ) | 0.68 | ( 2.12 ) | |
| 1,2,3,7,8-PCDD | 1.43 | ( 1.38 ) | 2.96 | ( 1.78 ) | *, -## | 0.86 | ( 2.06 ) | 1.92 | ( 1.98 ) | **, -## |
| 1,2,3,4,7,8-HxCDD | 0.58 | ( 1.43 ) | 1.63 | ( 2.46 ) | ** | 0.3 | ( 2.16 ) | 1.21 | ( 2.79 ) | ** |
| 1,2,3,6,7,8-HxCDD | 1.6 | ( 1.42 ) | 8.56 | ( 1.59 ) | **, -## | 0.88 | ( 1.88 ) | 5.56 | ( 1.85 ) | **, -## |
| 1,2,3,7,8,9-HxCDD | 0.47 | ( 1.81 ) | 2.02 | ( 1.87 ) | ** | 0.29 | ( 1.83 ) | 1.4 | ( 2.41 ) | ** |
| 1,2,3,4,6,7,8-OCDD | 1.72 | ( 1.47 ) | 17.3 | ( 1.71 ) | ## | 1.07 | ( 2.1 ) | 12.25 | ( 1.86 ) | **, ## |
| 2,3,7,8-TCDF | 6.41 | ( 2.01 ) | 49.26 | ( 2 ) | ** | 4.96 | ( 1.7 ) | 41.82 | ( 1.85 ) | ** |
| 1,2,3,7,8-PCDF | 1.2 | ( 1.48 ) | 0.56 | ( 2.07 ) | -# | 1.22 | ( 1.44 ) | 0.48 | ( 1.72 ) | -**, -## |
| 2,3,4,7,8-PCDF | 0.6 | ( 1.85 ) | 0.79 | ( 1.85 ) | **, -# | 0.37 | ( 1.84 ) | 0.55 | ( 2.39 ) | # |
| 1,2,3,4,7,8-HxCDF | 3.53 | ( 1.42 ) | 6.24 | ( 1.69 ) | **, -## | 2.28 | ( 1.56 ) | 3.61 | ( 1.88 ) | **, -## |
| 1,2,3,6,7,8-HxCDF | 1.88 | ( 1.84 ) | 16.25 | ( 1.7 ) | **, ## | 0.79 | ( 2.02 ) | 11.18 | ( 2.04 ) | **, ## |
| 2,3,4,6,7,8-HxCDF | 1.41 | ( 1.62 ) | 8.72 | ( 2 ) | **, ## | 0.82 | ( 1.74 ) | 6.66 | ( 2.08 ) | **, ## |
| 1,2,3,7,8,9-HxCDF | 0.44 | ( 1.72 ) | 1.34 | ( 2.1 ) | ** | 0.27 | ( 1.84 ) | 0.81 | ( 2.52 ) | **, -# |
| 1,2,3,4,6,7,8-OCDF | 0.09 | ( 3.24 ) | 0.24 | ( 3.58 ) | **, ## | 0.08 | ( 1.92 ) | 0.23 | ( 3.3 ) | **, ## |
| 1,2,3,4,7,8,9-OCDF | 1.07 | ( 1.87 ) | 11.09 | ( 1.93 ) | **, ## | 0.53 | ( 2.07 ) | 9.92 | ( 2.24 ) | **, ## |
| PCDDs -TEQ | 0.11 | ( 2.45 ) | 1.43 | ( 2 ) | **, ## | 0.09 | ( 1.68 ) | 1.23 | ( 3.26 ) | **, ## |
| PCDFs –TEQ | 0.09 | ( 2.64 ) | 0.14 | ( 2.97 ) | ## | 0.11 | ( 1.75 ) | 0.24 | ( 3.81 ) | **, ## |
| PCDDs/DFs -TEQ | 2.51 | ( 1.32 ) | 5.63 | ( 1.74 ) | **, -## | 1.56 | ( 1.82 ) | 3.7 | ( 1.83 ) | **, -## |
| | 2.36 | ( 1.41 ) | 6.11 | ( 1.7 ) | ** | 1.52 | ( 1.52 ) | 3.98 | ( 1.91 ) | ** |
| | 4.91 | ( 1.32 ) | 11.85 | ( 1.69 ) | ** | 3.13 | ( 1.59 ) | 7.77 | ( 1.83 ) | ** |

**: P<0.01: significantly higher, -*:P<0.05, -**: P<0.0 : significantly lower as compared with controls in Vietnam
##:P<0.01: significantly higher, - #:P<0.05, - ##: P<0.01: significantly lower as compared with Japanese

Table 3 Comparisons of the number of pregnancy and child birth between Vietnamese and Japanese mothers

| | | Japanese | Vietnamese | | |
|---|---|---|---|---|---|
| | | | Control area | Sprayed area | |
| | | N=75 | N=36 | N=51 | |
| Age | mean (SD) | 29.8 ( 4 ) | 25.6 ( 3.9 ) | 27.9 ( 5.3 ) | * |
| Primiparae | n (%) | 40 ( 53.3 ) | 13 ( 36.1 ) | 14 ( 27.5 ) | # |
| Number of pregnancy | mean (SD) | 1.3 ( 1.1 ) | 1.9 ( 0.8 ) | 2.2 ( 0.95 ) | *, # |
| Number of delivery | mean (SD) | 1.1 ( 1 ) | 1.8 ( 0.7 ) | 2.1 ( 0.8 ) | *, # |
| Abortion/Still birth | n (%) | 17 ( 22.7 ) | 3 ( 8.3 ) | 7 ( 13.7 ) | -# |
| Sex ratio of infant | Number of boys (%) | 19 ( 50 ) | 19 ( 50 ) | 20 ( 40 ) | |
| Birth weight of infant | mean (SD) | 3075 ( 441 ) | 2886 ( 307 ) | 2910 ( 336 ) | -# |

*:P<0.05: significantly higher as compared with Vietnamese mothers in control area
#:P,0.05: significantly higher, -#:P,0.05: significantly lower as compared with Japanese mothers

Table 4 Logistic regression coefficients and estimated odds ratios for the relationships between dioxin in breast milk and pregnancy outcome after adjustment of age and parity

| | Dioxin sprayed area (N=51) | | | | | All area (N=87) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | B | SE | Wald | Exp(B) | 95.0%CI | B | SE | Wald | Exp(B) | 95.0%CI |
| 2,3,7,8-TCDD | 4.49 | 2.12 | 4.5 | 89.6 | ( 1.41, 5707 ) | 5.17 | 1.9 | 7.4 | 175.3 | ( 4.24, 7253 ) |
| 1,2,3,7,8-PCDD | 3.22 | 1.57 | 4.22 | 24.9 | ( 1.16, 539 ) | 3.38 | 1.43 | 5.63 | 29.5 | ( 1.80, 489 ) |
| 1,2,3,4,7,8- | 3.43 | 1.84 | 3.47 | 30.7 | ( 0.84, 1130 ) | 3.89 | 1.64 | 5.63 | 49.3 | ( 1.97, 1230 ) |
| 1,2,3,6,7,8- | 3.81 | 1.89 | 4.07 | 45.1 | ( 1.11, 1827 ) | 3.58 | 1.53 | 5.46 | 35.8 | ( 1.78, 721 ) |
| 1,2,3,7,8,9- | 2.54 | 1.53 | 2.78 | 12.7 | ( 0.64, 252 ) | 2.33 | 1.32 | 3.11 | 10.3 | ( 0.77, 137 ) |
| 1,2,3,4,6,7,8- | 1.14 | 1.64 | 0.48 | 3.1 | ( 0.13, 78.3 ) | 0.83 | 1.34 | 0.39 | 2.3 | ( 0.17, 31.7 ) |
| OCDD | 1.1 | 1.54 | 0.51 | 3 | ( 0.15, 61.3 ) | 0.82 | 1.33 | 0.38 | 2.26 | ( 0.17, 30.7 ) |
| 2,3,7,8-TCDF | -1.38 | 1.73 | 0.64 | 0.3 | ( 0.01, 7.5 ) | -1.39 | 1.64 | 0.72 | 0.25 | ( 0.01, 6.2 ) |
| 1,2,3,7,8-PCDF | -0.28 | 1.15 | 0.06 | 0.8 | ( 0.08, 7.1 ) | 0.1 | 1.02 | 0.01 | 1.1 | ( 0.15, 8.2 ) |
| 2,3,4,7,8-PCDF | 3.78 | 1.78 | 4.52 | 43.9 | ( 1.35, 1437 ) | 5.01 | 1.84 | 7.38 | 149.2 | ( 4.03, 5537 ) |
| 1,2,3,4,7,8- | 2.69 | 1.64 | 2.7 | 14.6 | ( 0.60, 360 ) | 2.69 | 1.36 | 3.88 | 14.7 | ( 1.01, 213 ) |
| 1,2,3,6,7,8- | 2.78 | 1.58 | 3.07 | 16 | ( 0.72, 358 ) | 3.3 | 1.49 | 4.89 | 27.1 | ( 1.45, 505 ) |
| 2,3,4,6,7,8- | 0.7 | 1.12 | 0.39 | 2 | ( 0.22, 18.1 ) | 0.88 | 1.04 | 0.72 | 2.4 | ( 0.31, 18.6 ) |
| 1,2,3,7,8,9- | 0.33 | 0.83 | 0.16 | 1.4 | ( 0.27, 7.1 ) | 0.19 | 0.78 | 0.06 | 1.2 | ( 0.26, 5.6 ) |
| 1,2,3,4,6,7,8- | 1.03 | 1.35 | 0.58 | 2.8 | ( 0.20, 39.6 ) | 1.24 | 1.14 | 1.2 | 3.47 | ( 0.37, 32.1 ) |
| 1,2,3,4,7,8,9- | 0.53 | 0.94 | 0.32 | 1.7 | ( 0.27, 10.8 ) | 0.34 | 0.86 | 0.16 | 1.4 | ( 0.26, 7.5 ) |
| OCDF | 0.2 | 0.72 | 0.08 | 1.2 | ( 0.30, 5.0 ) | 0.2 | 0.68 | 0.09 | 1.2 | ( 0.32, 4.7 ) |
| PCDDs -TEQ | 3.8 | 1.77 | 4.61 | 44.9 | ( 1.39, 144 ) | 4.23 | 1.63 | 6.75 | 68.7 | ( 2.82, 1671 ) |
| PCDFs –TEQ | 3.27 | 1.71 | 3.66 | 26.3 | ( 0.92, 751 ) | 4.29 | 1.75 | 6.03 | 73.1 | ( 2.38, 2244 ) |
| PCDDs/DFs - | 3.78 | 1.82 | 4.33 | 43.7 | ( 1.24, 153 ) | 4.64 | 1.77 | 6.86 | 103.2 | ( 3.22, 3315 ) |

B: regression coefficient, SE: standard error, Exp(B): odds ratio of (x+1)/x, CI: confidence interval