



## Levels of polychlorinated dibenzodioxins and polychlorinated dibenzofurans in breast milk samples from three dioxin-contaminated hotspots of Vietnam



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### HIGHLIGHTS

- Former U.S. air bases are dioxin contamination hotspots in Vietnam.
- We determined breast milk dioxin levels in women living around three air bases.
- TCDD levels were high from women living close to Bien Hoa and Da Nang air bases.
- TCDD levels were not high from women living further from Phu Cat air base.
- The levels of other dioxins were not elevated from women living near air bases.

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### ABSTRACT

We determined polychlorinated dibenzodioxin (PCDD) and polychlorinated dibenzofuran (PCDF) levels in breast milk of 143 primiparae living around the three most dioxin-contaminated areas of Vietnam. The women sampled lived in the vicinity of former U.S. air bases at Bien Hoa (n = 51), Phu Cat (n = 23), and Da Nang (n = 69), which are known as dioxin hotspots. Breast milk samples from Bien Hoa City, where residents live very close to the air base, showed high levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), with 18% of the samples containing >5 pg TCDD/g lipid. However, Phu Cat residents lived far from the air base and their samples showed lower TCDD levels, with none containing >5 pg TCDD/g lipid. In Da Nang, TCDD levels in mothers from Thanh Khe (close to the air base, n = 43) were significantly higher than those in mothers from Son Tra (far from the air base, n = 26), but not other PCDD and PCDF (PCDD/F) congeners. Although TCDD levels in Bien Hoa were the highest among these hotspots, levels of other PCDD/F congeners as well as the geometric mean concentration of total PCDD/F level in Bien Hoa (9.3 pg toxic equivalents [TEQ]/g lipid) were significantly lower than the level observed in Phu Cat (14.1 pg TEQ/g lipid), Thanh Khe (14.3 pg TEQ/g lipid), and Son Tra (13.9 pg TEQ/g lipid). Our findings indicated that residents living close to former U.S. air bases were exposed to elevated levels of TCDD, but not of other PCDD/F congeners.

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### 1. Introduction

Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are widespread and persistent toxic chemicals in the environment. They are released into the environment through combustion or as by-products of chemical manufacturing processes. Due to

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their lipophilicity, they accumulate in fat tissue and enter the human body through the food chains. They can transfer from mothers to infants via breast milk and have long half-lives in the human body (Milbrath et al., 2009).

During Operation Ranch Hand (1962–1971), the U.S. army sprayed millions of liters of various herbicides in a region south of the former Demilitarized Zone at the 17th parallel in southern Vietnam. Agent Orange, a 50:50 mixture of n-butyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic (2,4,5-T), was the most widely used herbicide. The latter compound, 2,4,5-T, was contaminated to varying degrees with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which is the most toxic congener of the dioxin group. Other herbicides were also used in Vietnam, including Agent White (2,4-D; picloram), Agent Blue (cacodylic acid), Agent Purple (2,4-D; 2,4,5-T), Agent Green (2,4,5-T), and Agent Pink (2,4,5-T) (Stellman et al., 2003).

Baughman and Meselson (1973) detected high TCDD contamination in fish collected from heavily sprayed areas in southern Vietnam, suggesting that this compound had accumulated significantly in the food chains in sprayed areas. Breast milk samples collected in 1970 also showed an extremely high TCDD level of 1832 parts per trillion (ppt) in a sprayed area (Schecter et al., 1995). Although tropical rain, erosion, and chemical breakdown have reduced TCDD levels, this contaminant is still found at higher levels in sprayed areas than in unsprayed regions of northern Vietnam (Schecter et al., 1995; Tawara et al., 2011).

Dwernychuk et al. (2002) found elevated levels of dioxins in soil, fish, duck, pooled human blood, and breast milk in regions close to former U.S. air bases. They theorized that high dioxin concentrations remained in the soil at former U.S. air bases, where the herbicide was stored, used, and accidentally spilled (Dwernychuk, 2005). These areas are called dioxin hotspots, with the three most contaminated air bases located in Bien Hoa, Da Nang, and Phu Cat (Dwernychuk et al., 2006). Large numbers of 208-liter drums of Agent Orange, Agent White, and Agent Blue were recorded in Bien Hoa (98,000, 45,000, 16,000 drums), Da Nang (52,700, 29,000, 5000 drums), and Phu Cat (17,000, 9000, 2900 drums) (Young, 2009). The highest soil TCDD concentrations were recently reported as 259,000 ppt, 236,000 ppt, and 365,000 ppt at the Bien Hoa, Phu Cat, and Da Nang air bases, respectively (Office 33 and Hatfield Consultants, 2011); these levels are far higher than the standard for residential soil in some countries of less than 1000 pg toxic equivalent [TEQ]/g (U.S. Environmental Protection Agency, 2009).

The present human breast milk biomonitoring study was conducted to assess human exposure to dioxins in residents living near these hotspots. In a previous study, we found higher dioxin levels in the breast milk of mothers living around the dioxin hotspots at Da Nang and Phu Cat, as compared with other sprayed and unsprayed areas (Tai et al., 2011). In Da Nang, an inverse association between maternal dioxin exposure and the neurodevelopment of their offspring was identified, suggesting that dioxin exposure was associated with this health risk (Nishijo et al., 2014; Tai et al., 2013). Anh et al. (2014) also reported risk factors associated with increased breast milk dioxin levels in mothers living in the Thanh Khe district of Da Nang. In addition, Hue et al. (2014) reported high TCDD levels in mothers living in the area surrounding the Da Nang air base.

However, no recent study has investigated breast milk dioxin levels in mothers living around the Bien Hoa air base, the largest hotspot in Vietnam. Therefore, the present study measured breast milk dioxins in primipara mothers in Bien Hoa, as compared with those of primiparae living in other dioxin-contaminated areas.

## 2. Materials and methods

### 2.1. Study areas

Fig. 1 shows the locations of the dioxin hotspots investigated in the present study. The Bien Hoa air base is located in Bien Hoa City, an

industrial center in Dong Nai Province with many factories producing chemicals, paper, paints, and foodstuffs. Study participants were selected from communes (Tan Phong and Trung Dung) located within 4 km of the Bien Hoa air base. The Phu Cat air base is located in Phu Cat district, a rural area of Binh Dinh Province with no industrial zone nearby. There was no resident living within 5 km from Phu Cat airbase; therefore, study participants were selected from several communes (Cat Tuong, Cat Lam, Cat Hanh, Ngo May, and Cat Trinh), which are 5–15 km from the air base. The Da Nang air base is located in Da Nang City, an urban region of the South Central Coast of Vietnam. Study participants lived either in Thanh Khe district, located within 3 km of the air base, or in Son Tra district, which is located further from the air base (7–10 km).

### 2.2. Study participants

This study was approved by the Medical Ethics Committee of Kana-zawa University (Health Permission No. 89). Between 2008 and 2010, a total of 143 primiparae, including 51 primiparae in Bien Hoa, 23 primiparae in Phu Cat, 43 primiparae in Thanh Khe, and 26 primiparae in Son Tra agreed to participate in this study. We explained the purpose of study and then obtained written informed consent from every participant. The women were aged between 20 and 40 years. We collected each woman's breast milk between 4 and 16 weeks after childbirth. Furthermore, we interviewed them to collect demographic data, including age, residency, education level, employment, and income.

### 2.3. Dioxin analyses

We collected 10–20 mL breast milk from each mother. Each woman washed their hands and then expressed milk into a clean paper cup. The milk was immediately transferred into chemically cleaned containers and frozen on dry ice. The samples were transported by air to Japan, and stored at  $-30^{\circ}\text{C}$  until analysis.

Breast milk samples were analyzed in accordance with previously reported methods (Tai et al., 2011; Tawara et al., 2011). Briefly, lipids were extracted from 10 g breast milk by liquid extraction and spiked with 40–80 pg of seventeen  $^{13}\text{C}_{12}$ -labeled PCDD/F congeners, as an internal standard. PCDD/Fs were purified on a multi-layer silica gel column and separated by an active carbon-dispersed silica gel column. The final sample extract was evaporated to dryness under a nitrogen stream then re-dissolved by addition of 20  $\mu\text{L}$  of nonane containing 40 pg of  $^{13}\text{C}_{12}$ -1,2,3,4-TCDD and  $^{13}\text{C}_{12}$ -1,2,7,8-TCDF as external standards.

PCDD/Fs were quantified using a gas chromatograph (HP-6980, Hewlett-Packard, Palo Alto, CA) equipped with a high-resolution mass spectrometer (HRMS: JEOL MS station—JMS700). Analyses were performed in the selected ion-monitoring mode, and the resolution was maintained above 10,000. Seventeen PCDD/F congeners were calculated on a lipid basis and then converted to TEQ using the World Health Organization toxicity equivalency factors 2005 (Van den Berg et al., 2006). The recovery rate for all PCDD/F congeners typically ranged between 60% and 95%. The detection limits were determined at a signal-to-noise ratio of 3. Samples with undetectable congener concentrations were assigned a value equal to half the detection limit.

### 2.4. Statistical analyses

We used the JMP@9 software package (SAS Institute, Japan) and R Statistical Environment (R Development Core Team, 2013) to conduct statistical analyses. Dioxin concentrations were  $\log_{10}$  transformed to improve normality before using statistical tests. Analysis of variance (ANOVA) was used to compare dioxin levels between hotspots, followed by Tukey's post-hoc test to identify significant differences between individual study sites. Pearson correlation was used to calculate correlations between dioxin concentration and age or residency. Finally,

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