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Residual congener pattern of dioxins in human breast milk in southern Vietnam

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1. Background

Vietnam is thought to be the site of the world's largest and most significant dioxin contamination event (dioxins include polychlorinated dibenzo-*p*-dioxin [PCDD] and polychlorinated dibenzo-furan [PCDF]). From 1961 to 1971, tactical herbicides were sprayed over regions of the former Republic Vietnam (southern Vietnam) through the activities of Operation Ranch Hand, the US military code name for the spray mission during the Vietnam War. The objective of this operation was to defoliate the jungle canopy and destroy crops to deny opposing forces strategic cover and food (Stellman et al., 1988, 2003a, 2003b). The most widely used defoliants were 2,4-dichlorophenoxyacetic acids (2,4-D) and 2,4,5-trichlorophenoxyacetic acids (2,4,5-T) (Stellman et al., 1988, 2003a, 2003b; IOM, 2002). The best-known mixture was known as Agent Orange (AO), a 50:50 mixture of the aforementioned herbicides. Defoliants such as AO that contained 2,4,5-T were contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at mean levels estimated between 1.2 and 12.2 parts per million (ppm), whereas higher concentrations of probably 100 ppm were reported to exist in other defoliants (Stellman et al., 2003a).

In the middle phase of the war, local Vietnamese newspapers reported an increase in congenital malformations in sprayed areas, and the toxicological effects of the tactical herbicides on newborn babies created a global sensation. Although numerous studies were performed between the 1980s and 1990s to determine whether exposure to tactical herbicides in Vietnam may have increased the risk of children being born with birth defects, the results were often inconsistent

(Ngo et al., 2006; Schecter, 2006; Tawara et al., 2008). More recently, Hatfield Consultants (Hatfield) reported persistently high levels of TCDD in soil and biota from the Aluoi Valley in central Vietnam, as well as in human tissue samples, including whole blood and breast milk. Through a series of field validation studies, Hatfield highlighted the need for adequate measures to properly assess the extent and impact of contamination around the so-called "hot spot." The "Hatfield hot spot theory" refers to the former locations defined as US military installations where AO was spilled, applied by truck-mounted sprayers, and sprayed intensively during Operation Ranch Hand (Dwernychuk et al., 2002). Well-known examples of such hot spots are the locations of former bases of Operation Ranch Hand at Bien Hoa city in Dong Nai province and Da Nang city (a municipality of Vietnam). The TCDD level at Bien Hoa has been reported to be as high as 1.2 million parts per trillion (ppt) in soil, and reached 270 ppt in blood (Schecter et al., 2001). On the other hand, soils in areas that were sprayed aerially would not be expected to have the same quantities of residual AO as those in the hot spots, as a result of several years of tropical rains, erosion, and the rate of degradation of the chemicals (Dwernychuk, 2005). In this context, human exposure through food chain transfer of TCDD is expected to be highest in the locations identified as hot spots where extremely high concentrations of TCDD have a continued presence in the soil. For this reason, the pattern of TCDD contamination referred to in hot spots seems to serve as a model of contamination throughout southern Vietnam (Dwernychuk et al., 2002).

61 At least 2.1 million and possibly as many as 4.8 million Vietnamese would have been exposed to
62 AO and other defoliants (Stellman et al., 1988, 2003a, 2003b). This could be equivalent to as
63 much as one-fourth of the total population of the former Republic of Vietnam during the war.
64 In spite of such massive aerial applications throughout southern Vietnam, not much information
65 has been made available to date regarding the entire congener pattern derived from all 17 of the
66 2,3,7,8-substitued PCDD/DFs residues in the general population. Furthermore, in the current
67 situation where post-war third generation babies are being born in Vietnam 35 years after the end
68 of the war, it would be more significant to investigate whether a specific residual pattern of
69 PCDD/DF congeners in the general population in southern Vietnam can be observed, in
70 correlation with current TCDD levels in human tissues. From the viewpoint of public health,
71 southern Vietnam can provide a prime example of such an evaluation study, where a previously
72 uncharacterized time-dependent change of the residual pattern of PCDD/DF congeners in human
73 tissues can be interpreted.
74 Therefore, we focused on lactating Vietnamese mothers who were born after the war in an
75 aerially sprayed area and a non-sprayed area, and analyzed PCDD/DFs levels in breast milk
76 samples from each area. The congener pattern of the PCDD/DFs in each case was analyzed to
77 determine whether specificity in the conger pattern exists in breast milk samples obtained from
78 individuals residing in the sprayed area. As discussed in a related paper, PCDD/DF congeners in

breast milk should indicate the dioxin level in mother's fat stores before and during pregnancy (Tawara et al., 2009).

2. Materials and Methods

2.1. Study sample

We focused our study on the Cam Chinh (CC) commune located in the Cam Lo district of Quang Tri province and the Cam Phuc (CP) commune in the Cam Xuyen district of Ha Tinh province (Fig.1). During the war, herbicides were sprayed over CC but not over CP. These 2 communes had similar economic and social conditions, customs, ethnic groups, and health care systems. In September 2002 and July 2003, breast milk samples (10–20 mL) were collected from lactating primiparous and multiparous mothers aged 20–30 years from both communes. A total of 84 mothers in the CC commune and 72 mothers in the CP commune donated milk samples after providing their consent to participate in the study. Consent was provided by signatures on a Vietnamese document explaining the purpose of the study. To conduct this study, we obtained permission from the Medical Ethics Review Board of Kanazawa Medical University. All samples were frozen immediately after collection for transport to Japan. Samples donated from mothers born after the war (< 31 years old) were used for this study. A total of 59 samples were obtained from the CC commune and 66 samples from the CP commune. The average age of the lactating mothers for parity is shown in Table 1.

98

99 *2.2. Measurement of PCDD/DFs in breast milk*

100 Fat in breast milk was extracted from 10 mL of each sample. After a series of purification

101 operations (Tawara et al., 2003), the final extract was concentrated by evaporation to 20 μL.

102 Quantification was performed using a high resolution mass spectrometer (HRMS: JEOL

103 MStation-JMS700), operating in a selected ion monitoring (SIM) mode. A gas chromatograph

104 (GC: HP-6890 Hewlett-Packard, Palo Alto, CA) was equipped with an ENV-5MS column with

105 30 m × 0.25 mm i.d. of 0.25 μm film thickness (Kanto Chemical Co., Inc.). Regarding the

106 sensitivity of the HRMS, a detection limit of 0.02 pg/g lipid was achieved at a signal-to-noise

107 (S/N) ratio of 3. Quality control and quality assurance protocols for this study were carefully

108 implemented in accordance with the regulations stipulated by the Japanese Industrial Standards

109 (JIS). Eligibilities for the analyses of dioxins were certified using the reference milk powder

110 (CRM 607) provided by the European Commission. From a 10-mL breast-milk sample, 75–90%

111 of the 13C-2,3,7,8-substitued PCDD/DF congeners was recovered; this result agreed favorably

112 with the recovery range specified by the JIS. Concentration levels of dioxins were determined by

113 actual measurement values and presented as 2,3,7,8-TCDD toxic equivalents (TEQ) levels.

114 Calculation of TEQ based on World Health Organization (WHO) 1998 toxic equivalency facters

115 (TEFs) (Van den Berg et al., 1998).

116

2.3. Analytical methods

Data were statistically analyzed using the SPSS (ver. 11.0) software package for Windows (SPSS, Chicago IL). Logarithmic transformation of the measured values of PCDD/DFs was performed to improve normality. Differences in the levels of PCDD/DFs between milk samples from the CC and CP communes were evaluated using the Mann-Whitney U and unpaired *t* test. A significance level of $P < 0.05$ was used for all statistical tests.

We defined the congener pattern as "residue peculiarity in the body of all 17 of the 2,3,7,8-substituted congeners" designated by the level of each congener. To demonstrate this measure objectively, we attempted to demonstrate a relative position of the level of each congener with respect to the total concentration level of dioxins. To perform this analysis, standardization of the concentration data for each congener was implemented by assuming that the mean concentration of each congener (designated μ) in the CP commune has a value of 0. The Z-score was calculated to indicate how the number of standard deviations was above or below the mean (μ) with respect to each congener concentration of samples taken from the CC commune. The following formula was used for Z-score calculations:

$$z = (x - \mu)/\sigma$$

where,

z is the Z-score, x is the value of the concentration of each individual congener in the CC commune, μ is the mean concentration of each congener in the CP commune, and σ is the standard deviation of each congener concentration in the CP commune.

3. Results

3.1. Levels of PCDD/DFs in breast milk

Differences in the congener levels between the CC and CP communes were compared using the Man-Whitney test and Student *t* test. As the results were similar, only the results of the latter are presented in Table 2. Generally, the levels of each PCDD/DF congener in the milk samples from the CC commune were higher than in the milk samples from the CP commune. Tests of differences in concentration levels of dioxins between the communes revealed that there is a significant difference in the levels of all PCDD/DF congeners, with the exception of 2,3,7,8-tetrachrolodibenzofuran (TCDF). The TCDD level of 0.82 pg g^{-1} lipid was the lowest level of PCDD congeners identified in samples from the CC commune, yet the TCDD level found in the CC commune was significantly higher than that in the CP commune (0.54 pg g^{-1} lipid). When the PCDD congeners were listed according to concentration levels, the order was found to be very similar between the CC commune and CP commune samples. The most abundant PCDD congener was commonly 1,2,3,4,6,7,8,9-octachrolodibenzo-*p*-dioxin (OCDD) at a mean concentration level of 43.68 pg/g lipid for the CC commune samples and 5.35 pg g^{-1} lipid

for the CP commune samples. The next most abundant PCDD congener was 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD) at a mean concentration of 13.24 and 1.30 pg/g lipid, for the samples taken from the CC and CP communes, respectively. The next most abundant PCDD congeners were 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD) and 1,2,3,7,8- pentachlorodibenzo-*p*-dioxin (PeCDD). These congeners were found in samples from the CC commune at concentrations of 6.11 pg g^{-1} lipid and 2.28 pg g^{-1} lipid, respectively. The latter 2 congeners were detected in samples obtained from the CP commune at concentrations of 1.10 pg g^{-1} lipid and 1.15 pg g^{-1} lipid, respectively. These concentration levels are similar to that of HpCDD.

Unlike the PCDD congeners, specifically elevated concentrations of PCDF congeners were only found in the milk samples obtained from the CC commune. 1,2,3,4,7,8-hexachlorodibenzofuran (HxCDF) and 1,2,3,4,6,7,8-heptachlorodibenzofuran (HpCDF) were abundantly detected at mean concentration levels of 12.86 pg g^{-1} lipid and 10.72 pg g^{-1} lipid, respectively, followed by 1,2,3,6,7,8-HxCDF at a mean concentration level of 7.52 pg g^{-1} lipid, and 2,3,4,7,8-pentadibenzofuran (PeCDF) at 4.37 pg g^{-1} lipid. In contrast, PCDF congeners in breast milk samples obtained from the CP commune were not abundant, and the maximum level was 2.73 pg g^{-1} lipid for 2,3,4,7,8-PeCDF. The next most abundant PCDF congeners were 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF. The mean concentration levels were similarly below 2 pg g^{-1} lipid.

3.2. PCDD/DF congener patterns

All individual data points of congener concentrations of samples obtained from the CC commune were converted into Z-scores, and 95% confidence intervals were calculated for performing the extent of the score range for each congener. Fig. 2 shows that the deviations from the CP mean are different for the different congeners. The mean of the Z-scores for TCDD was 0.82 (95% confidence interval, 0.52 to 1.12), which is below 1 standard deviation. Examples of congeners with Z-scores below 1 include 1,2,3,7,8-PCDF (Z-score of 0.64, 95% confidence interval, 0.34 to 0.95) and 1,2,3,4,6,7,8,9-octachlorodibenzofuran (OCDF; Z-score of 0.98, 95% confidence interval, 0.69 to 1.27). In contrast, the means of the Z-scores for 1,2,3,6,7,8-HxCDD, HpCDD, OCDD, 1,2,3,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, and 1,2,3,47,8,9-HpCDF were greater than 3 standard deviations. These Z-scores were 3.12 (95% confidence interval, 2.89 to 3.36) for 1,2,3,6,7,8-HxCDD, 4.13 (95% confidence interval, 3.91 to 4.35) for HpCDD, 4.16 (95% confidence interval, 3.91 to 4.42) for OCDD, 3.13 (95% confidence interval, 2.87 to 3.40) for 1,2,3,6,7,8-HxCDF, 3.15 (95% confidence interval, 2.94 to 3.37) for 1,2,3,4,6,7,8-HpCDF, and 3.28 (95% confidence interval, 3.01 to 3.54) for 1,2,3,4,7,8,9-HpCDF. These results indicate that concentration levels of the congeners are reflected in the extent of deviation.

A dendrogram representing hierarchical clustering was drawn by cluster analysis based on Ward's method, to classify congeners in terms of the extent of deviation. The dendrogram was

rearranged without changing the position of each vertical line indicating joined clusters (Fig. 3). As shown in Fig. 3, the last vertical line, corresponding to the largest rescaled distance, was obtained for the high concentration group (Cluster A in Fig. 3) for the congeners with absolute values of the mean of the Z-scores with more than 2 standard deviations, and the low concentration group (Cluster B in Fig. 3) for the congeners with absolute values of the mean of the Z-scores under 2 standard deviations from more than 0. This result confirmed the success in determining the peculiarity of the congener pattern of PCDD/DFs in breast milk samples from the CC commune by cluster analysis based on the dioxin levels.

4. Discussion

4.1. Characteristics of the congener pattern in breast milk samples obtained from sprayed areas

Both the Mann-Whitney U test and Student *t* test for intergroup means indicated that the levels of PCDD/DFs in the herbicide-sprayed area were uniformly higher than those in the non-sprayed area except for TCDF. The deviation from the CP mean shown by the Z-scores on a per-congener basis, actually occurred variably with respect to individual congeners. This could lead the scenario that particular congeners show different increases and decreases in concentration levels with respect to the other congeners through specific exposure to PCDD/DFs in the CC commune. From this viewpoint, greater deviations of Z-scores may imply higher concentrations and more specificity of the congeners.

The cluster analysis classified the 9 types of higher (the hexa-, the hepta-, and the octa-) chlorinated PCDD/DFs as being grouped into Cluster A. Fig. 4 focuses on each constituent congener in the sub-clusters (Cluster 1–3 in Fig. 3) of Cluster A, which were obtained by cutting between the first and the second vertical line of the dendrogram, as shown by the dotted line in Fig. 3. It should be noted that these sub-clusters correspond to the magnitude of the deviation represented by the Z-scores. As indicated by Fig. 4, OCDD and HpCDD in cluster 1 have the greatest deviation, and both means of the Z-scores were greater than 4 standard deviations. Subsequently, cluster 2 which includes 5 congeners (1,2,3,6,7,8-HxCDD, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, and 1,2,3,4,7,8,9-HpCDF) has a mean deviation between 3 and 4 standard deviations. Cluster 3 including 1,2,3,4,7,8-HxCDD and 1,2,3,7,8,9-HxCDD, has a mean deviation between 2 and 3 standard deviations. In this context, OCDD and HpCDD appear to be the most significant congeners. This may indicate that there are additional specific exposure sources in the CC commune.

A similar residual pattern of PCDD/DF congeners determined was previously reported in an earlier study with placenta and breast milk of the residents of the 2 neighboring provinces of Quang Tri and Quang Binh. The congener patterns determined in breast milk samples from these 2 provinces were similar and included specifically high levels of 7 congeners: OCDD, HpCDD, 1,2,3,4,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,6,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, and 2,3,4,7,8- PCDF (Fenshin et al., 2008).

231 Schecter et al. (1991) reported measurements of PCDD/DF congeners in breast milk possibly
232 collected in 1980s from areas in Dong Nai province and Da Nang city in southern Vietnam, and
233 Hanoi, the capital of Vietnam. The concentration level of each congener in breast milk samples
234 from Dong Nai and Da Nang city, in addition to Hanoi as a control for PCDD/DFs levels in
235 breast milk in those days, is shown with respect to the CC commune in Table 3. It was found that
236 in addition to the elevated levels of TCDD, the levels of almost all of the congeners in breast
237 milk samples from Dong Nai and Da Nang were uniformly higher than the levels measured in
238 breast milk samples from Hanoi. Particularly, it is notable that the levels of 1,2,3,6,7,8-HxCDD,
239 HpCDD, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, and
240 1,2,3,4,7,8,9-HpCDF in samples obtained from the CC commune are higher than the levels in the
241 samples obtained from Hanoi. This tendency, with the exception of 1,2,3,4,7,8,9-HpCDF, was
242 also identified between samples obtained from Don Nai and Hanoi, and similarly between
243 samples obtained from Da Nang city and Hanoi. In addition, noticeable differences in the levels
244 of OCDD were also detected between samples obtained from Don Nai and Hanoi, and between
245 samples obtained from Da Nang city and Hanoi during the same time. It follows from these
246 occurrences that the congener pattern characterized by higher chlorinated PCDD/DFs of the
247 hexa-, the hepta-, the octa-chlorinated PCDD/DFs, had been already identified in the 1980s as a
248 unique congener in areas that were sprayed with tactical herbicides.
249

4.2. Present exposure sources of prevalent PCDD/DFs in sprayed areas

A notable report by Feshin et al. (2008) indicated that TCDD was absent in the placenta and breast milk of women from Quang Bin province, while a congener pattern was observed that was similar to the pattern identified in samples obtained from Quang Tri. Quang Bin province is a neighbor on the north of Quang Tri province, and was not subjected to battles or spraying of tactical herbicides during the war. Accordingly, the increase and decrease in PCDD/DF congeners other than TCDD seems to be distinguished as being different from the effects of tactical herbicides.

The principal source of the PCDD/DFs appears to be due to the production of chlorinated phenols (chlorophenols) (Hay, 1982). Methods for synthesizing chlorophenols varied, depending on the product required. Dioxins have not been detected in every sample of chlorophenol, and this may either be due to the method of synthesis of the phenol or to the number of chlorine atoms present in the chlorophenol (Rappe and Garå, 1978).

Pentachlorophenol (PCP) presents a specific congener profile with elevated levels of OCDD, 1,2,3,4,6,7,8-HpCDD, and HpCDF (Firestone et al., 1972; Kontsas et al., 1998). This is the same profile as one of the profiles determined by our cluster analysis. This congener profile for PCP has been found in serum from sawmill workers and PCP production workers (Collins et al., 2007; McLean et al., 2009). Additionally, such specificity was also found to be present in breast milk samples obtained from residents in certain areas of central China, where substantial amounts of

PCP (sodium pentachlorophenol) salts had been sprayed since the 1960s for control of snail-borne schistosomiasis (Schecter et al., 1994; Xiao et al., 2010). Based on these occurrences, it can be considered that the congener profiles distinguish the specifically high levels of higher chlorinated PCDD/DFs, appearing in human fluid and tissue samples as distinct fingerprints for indicating exposure to PCP.

We have no definitive evidence at this point that PCP contamination has occurred in Vietnam. As discussed above, however, the residual congener pattern that appears to be related to PCP was previously identified in the 1980s in samples from AO hot spots in southern Vietnam. Even after 20 years, this pattern was determined in Quang Tri province by our study, and by Feshin et al. (2008). Furthermore, Feshin et al. identified the same pattern in samples obtained from Quang Binh province, a non-sprayed region adjoining Quang Tri, while this pattern was not identified in samples obtained from Hanoi by Schecter et al. (1991), and from Ha Tinh province by our study. It is inferred from this view that contamination with PCP remains an isolated incident, which has existed for 20 years.

At this stage, the association of PCP contamination with tactical herbicides typified by AO cannot be stated positively. Rappe et al. (1978) detected 2,4-di and 2,4,6-trichlorophenol impurities in some AO samples by GC/MS analyses. Similar to PCP, these chlorophenols are formulated by the direct chlorination of phenol; this completely differs from the formulation of 2,4,5-trichlorophenol by the hydrolysis of chlorobenzene under strong alkaline conditions (Rappe

288 and Garå, 1978). Generally, TCDD is formed during the formulation of 2,4,5-trichlorophenol in
289 2,4,5-T production; this caused the situation where TCDD was the major dioxin-like contaminant
290 in AO (Hay, 1982; Dweyer and Flesch-Janys, 1995; IOM, 2002; Dwernychuk et al., 2002;
291 Dwernychuk, 2005; Schecter et al., 2006b). On the other hand, the chlorophenols, including PCP,
292 produced by sequential direct chlorination were found to contain a wide variety of PCDD/DFs
293 (Firestone, 1972, Rappe and Garå, 1978b; Kontsas et al., 1998). In this context, it may be
294 expected that certain of tactical herbicides have a unique congener pattern when compared with
295 pure 2,4,5-T formulations.
296 Saito et al. (2010) examined the association of the levels of PCDD/DF congeners in breast milk
297 and dietary intake in the CC commune and in the CP commune, and reported that dioxin
298 exposure was less affected by usual dietary intake in the CC commune than the CP commune. It
299 was suggested by Saito et al. (2010) that the PCDD/DFs in breast milk from the CC commune
300 were maintained at constant levels after past exposure even 35 years after the end of the war.
301 Regarding the levels of PCDD/DFs in the CP commune, we should not overlook the fact that the
302 total TEQs determined from the samples obtained from the CP commune (4.04 pg g^{-1} lipid TEQ)
303 are nearly identical to the lowest value (3.34 pg g^{-1} lipid TEQ) that was recorded in Fiji in the
304 WHO-coordinated exposure study in 2000 (Malisch and van Leeuwen, 2003). Other reported
305 examples of these PCDD/DFs levels were 3.92 pg g^{-1} lipid TEQ and 3.94 pg g^{-1} lipid TEQ in
306 Brazil and Philippines, respectively, as reported by Malisch and van Leeuwen (2003). These

findings imply that the concentration of PCDD/DFs in the CP commune is not extremely low. Hence, in relation to the observations of Saito et al. (2010), we consider that the levels and congener pattern of PCDD/DFs in the samples obtained from the CP commune are the normal concentrations of dioxins that the people of modern Vietnam are exposed to.

Quang Tri province was 1 of the 10 provinces that experienced the heaviest impact by Operation Ranch Hand (Black, 1993). It is estimated that 47% of the total AO sprayed in Vietnam was sprayed in Quang Tri over the course of 300 to 700 military spray missions. This amounts to a total estimated volume of 171,000 liters (Black, 1993).

Thus, further examination is needed to identify the exposure sources of the prevailing PCDD/DFs in southern Vietnam, while considering the time-dependent changes in the pattern of dioxin residues in human tissues.

5. Conclusion

This study evaluated residual condition of dioxins related to tactical herbicides aerially sprayed over the regions of southern Vietnam through Operation Ranch Hand, and determined specificity in the PCDD/DF congener in breast milk samples obtained from individuals residing in an area sprayed with tactical herbicides. The congener pattern is characterized by higher (the hexa-, the hepta-, and the octa-) chlorinated dioxins, which appears to be the same profile as that presented by PCP, rather than 2,4,5-T contaminated with 2,3,7,8-TCDD. At this stage there is no evidence

to support the association of PCP contamination with tactical herbicides typified by AO. A GC/MS study in the 1970s detected from some AO samples the chlorophenol impurities, formulated by the direct chlorination of phenol, like PCP. Given these occurrences, further examination is needed to identify the exposure sources of the prevailing dioxins in southern Vietnam.

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Highlights of the manuscript

This study evaluated residual condition of dioxins related to tactical herbicides aerially sprayed over the regions of southern Vietnam through Operation Ranch Hand.

Specificity in the PCDD/DF congener in milk samples obtained from individuals residing in a sprayed area was determined.

The specific congener pattern appears to be the same profile as that presented by PCP, rather than 2,4,5-T.

Certain tactical herbicides may have a unique congener pattern, when compared with pure 2,4,5-T formulations.

Abstract

This study evaluated residual congener patterns of dioxin/furan (= PCDD/DF) related to tactical herbicides aerially sprayed over the regions of southern Vietnam through Operation Ranch Hand. The study focused on Cam Chinh (CC) commune, Quang Tri province (an area sprayed with tactical herbicides), and the Cam Phuc (CP) commune, Ha Tinh province (a non-sprayed area). Breast milk samples for analysis were collected in September 2002 and July 2003 from lactating primiparous and multiparous mothers born after the war (< 31 years old). We found the levels of each congener in the CC commune were higher than in the CP commune, and determined specificity in the PCDD/DF congener pattern in CC commune samples by cluster analysis. The congener pattern is characterized by higher (the hexa-, the hepta-, and the octa-) chlorinated PCDD/DFs; this appears to be the same profile as that presented by pentachlorophenol (PCP), rather than 2,4,5-trichlorophenoxy acid (2,4,5-T) contaminated with 2,3,7,8-TCDD. A GC/MS study in the 1970s detected the chlorophenols 2,4-di and 2,4,6-trichlorophenol in some Agent Orange samples, which contained, like PCP, a wide variety of PCDD/DF congeners. In this context, it may be expected that certain tactical herbicides contaminated with various chlorophenol impurities, have a unique congener pattern when compared with pure 2,4,5-T formulations.

Key words: PCDD/DFs residual congener pattern breast milk Agent Orange Vietnam pentachlorophenol

Residual congener pattern of dioxins in human breast milk in southern Vietnam

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1. Background

Vietnam is thought to be the site of the world's largest and most significant dioxin contamination event (dioxins include polychlorinated dibenzo-*p*-dioxin [PCDD] and polychlorinated dibenzo-furan [PCDF]). From 1961 to 1971, tactical herbicides were sprayed over regions of the former Republic Vietnam (southern Vietnam) through the activities of Operation Ranch Hand, the US military code name for the spray mission during the Vietnam War. The objective of this operation was to defoliate the jungle canopy and destroy crops to deny opposing forces strategic cover and food (Stellman et al., 1988, 2003a, 2003b). The most widely used defoliants were 2,4-dichlorophenoxyacetic acids (2,4-D) and 2,4,5-trichlorophenoxyacetic acids (2,4,5-T) (Stellman et al., 1988, 2003a, 2003b; IOM, 2002). The best-known mixture was known as Agent Orange (AO), a 50:50 mixture of the aforementioned herbicides. Defoliants such as AO that contained 2,4,5-T were contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at mean levels estimated between 1.2 and 12.2 parts per million (ppm), whereas higher concentrations of probably 100 ppm were reported to exist in other defoliants (Stellman et al., 2003a).

In the middle phase of the war, local Vietnamese newspapers reported an increase in congenital malformations in sprayed areas, and the toxicological effects of the tactical herbicides on newborn babies created a global sensation. Although numerous studies were performed between the 1980s and 1990s to determine whether exposure to tactical herbicides in Vietnam may have increased the risk of children being born with birth defects, the results were often inconsistent

(Ngo et al., 2006; Schecter, 2006; Tawara et al., 2008). More recently, Hatfield Consultants (Hatfield) reported persistently high levels of TCDD in soil and biota from the Aluoi Valley in central Vietnam, as well as in human tissue samples, including whole blood and breast milk. Through a series of field validation studies, Hatfield highlighted the need for adequate measures to properly assess the extent and impact of contamination around the so-called "hot spot." The "Hatfield hot spot theory" refers to the former locations defined as US military installations where AO was spilled, applied by truck-mounted sprayers, and sprayed intensively during Operation Ranch Hand (Dwernychuk et al., 2002). Well-known examples of such hot spots are the locations of former bases of Operation Ranch Hand at Bien Hoa city in Dong Nai province and Da Nang city (a municipality of Vietnam). The TCDD level at Bien Hoa has been reported to be as high as 1.2 million parts per trillion (ppt) in soil, and reached 270 ppt in blood (Schecter et al., 2001). On the other hand, soils in areas that were sprayed aerially would not be expected to have the same quantities of residual AO as those in the hot spots, as a result of several years of tropical rains, erosion, and the rate of degradation of the chemicals (Dwernychuk, 2005). In this context, human exposure through food chain transfer of TCDD is expected to be highest in the locations identified as hot spots where extremely high concentrations of TCDD have a continued presence in the soil. For this reason, the pattern of TCDD contamination referred to in hot spots seems to serve as a model of contamination throughout southern Vietnam (Dwernychuk et al., 2002).

61 At least 2.1 million and possibly as many as 4.8 million Vietnamese would have been exposed to
62 AO and other defoliants (Stellman et al., 1988, 2003a, 2003b). This could be equivalent to as
63 much as one-fourth of the total population of the former Republic of Vietnam during the war.
64 In spite of such massive aerial applications throughout southern Vietnam, not much information
65 has been made available to date regarding the entire congener pattern derived from all 17 of the
66 2,3,7,8-substitued PCDD/DFs residues in the general population. Furthermore, in the current
67 situation where post-war third generation babies are being born in Vietnam 35 years after the end
68 of the war, it would be more significant to investigate whether a specific residual pattern of
69 PCDD/DF congeners in the general population in southern Vietnam can be observed, in
70 correlation with current TCDD levels in human tissues. From the viewpoint of public health,
71 southern Vietnam can provide a prime example of such an evaluation study, where a previously
72 uncharacterized time-dependent change of the residual pattern of PCDD/DF congeners in human
73 tissues can be interpreted.
74 Therefore, we focused on lactating Vietnamese mothers who were born after the war in an
75 aerially sprayed area and a non-sprayed area, and analyzed PCDD/DFs levels in breast milk
76 samples from each area. The congener pattern of the PCDD/DFs in each case was analyzed to
77 determine whether specificity in the conger pattern exists in breast milk samples obtained from
78 individuals residing in the sprayed area. As discussed in a related paper, PCDD/DF congeners in

breast milk should indicate the dioxin level in mother's fat stores before and during pregnancy (Tawara et al., 2009).

2. Materials and Methods

2.1. Study sample

We focused our study on the Cam Chinh (CC) commune located in the Cam Lo district of Quang Tri province and the Cam Phuc (CP) commune in the Cam Xuyen district of Ha Tinh province (Fig.1). During the war, herbicides were sprayed over CC but not over CP. These 2 communes had similar economic and social conditions, customs, ethnic groups, and health care systems. In September 2002 and July 2003, breast milk samples (10–20 mL) were collected from lactating primiparous and multiparous mothers aged 20–30 years from both communes. A total of 84 mothers in the CC commune and 72 mothers in the CP commune donated milk samples after providing their consent to participate in the study. Consent was provided by signatures on a Vietnamese document explaining the purpose of the study. To conduct this study, we obtained permission from the Medical Ethics Review Board of Kanazawa Medical University. All samples were frozen immediately after collection for transport to Japan. Samples donated from mothers born after the war (< 31 years old) were used for this study. A total of 59 samples were obtained from the CC commune and 66 samples from the CP commune. The average age of the lactating mothers for parity is shown in Table 1.

98

99 *2.2. Measurement of PCDD/DFs in breast milk*

100 Fat in breast milk was extracted from 10 mL of each sample. After a series of purification
101 operations (Tawara et al., 2003), the final extract was concentrated by evaporation to 20 μL.
102 Quantification was performed using a high resolution mass spectrometer (HRMS: JEOL
103 MStation-JMS700), operating in a selected ion monitoring (SIM) mode. A gas chromatograph
104 (GC: HP-6890 Hewlett-Packard, Palo Alto, CA) was equipped with an ENV-5MS column with
105 30 m × 0.25 mm i.d. of 0.25 μm film thickness (Kanto Chemical Co., Inc.). Regarding the
106 sensitivity of the HRMS, a detection limit of 0.02 pg/g lipid was achieved at a signal-to-noise
107 (S/N) ratio of 3. Quality control and quality assurance protocols for this study were carefully
108 implemented in accordance with the regulations stipulated by the Japanese Industrial Standards
109 (JIS). Eligibilities for the analyses of dioxins were certified using the reference milk powder
110 (CRM 607) provided by the European Commission. From a 10-mL breast-milk sample, 75–90%
111 of the 13C-2,3,7,8-substitued PCDD/DF congeners was recovered; this result agreed favorably
112 with the recovery range specified by the JIS. Concentration levels of dioxins were determined by
113 actual measurement values and presented as 2,3,7,8-TCDD toxic equivalents (TEQ) levels.
114 Calculation of TEQ based on World Health Organization (WHO) 1998 toxic equivalency facters
115 (TEFs) (Van den Berg et al., 1998).

116

2.3. Analytical methods

Data were statistically analyzed using the SPSS (ver. 11.0) software package for Windows (SPSS, Chicago IL). Logarithmic transformation of the measured values of PCDD/DFs was performed to improve normality. Differences in the levels of PCDD/DFs between milk samples from the CC and CP communes were evaluated using the Mann-Whitney U and unpaired *t* test. A significance level of $P < 0.05$ was used for all statistical tests.

We defined the congener pattern as "residue peculiarity in the body of all 17 of the 2,3,7,8-substituted congeners" designated by the level of each congener. To demonstrate this measure objectively, we attempted to demonstrate a relative position of the level of each congener with respect to the total concentration level of dioxins. To perform this analysis, standardization of the concentration data for each congener was implemented by assuming that the mean concentration of each congener (designated μ) in the CP commune has a value of 0. The Z-score was calculated to indicate how the number of standard deviations was above or below the mean (μ) with respect to each congener concentration of samples taken from the CC commune. The following formula was used for Z-score calculations:

$$z = (x - \mu)/\sigma$$

where,

z is the Z-score, x is the value of the concentration of each individual congener in the CC commune, μ is the mean concentration of each congener in the CP commune, and σ is the standard deviation of each congener concentration in the CP commune.

3. Results

3.1. Levels of PCDD/DFs in breast milk

Differences in the congener levels between the CC and CP communes were compared using the Man-Whitney test and Student *t* test. As the results were similar, only the results of the latter are presented in Table 2. Generally, the levels of each PCDD/DF congener in the milk samples from the CC commune were higher than in the milk samples from the CP commune. Tests of differences in concentration levels of dioxins between the communes revealed that there is a significant difference in the levels of all PCDD/DF congeners, with the exception of 2,3,7,8-tetrachrolodibenzofuran (TCDF). The TCDD level of 0.82 pg g^{-1} lipid was the lowest level of PCDD congeners identified in samples from the CC commune, yet the TCDD level found in the CC commune was significantly higher than that in the CP commune (0.54 pg g^{-1} lipid). When the PCDD congeners were listed according to concentration levels, the order was found to be very similar between the CC commune and CP commune samples. The most abundant PCDD congener was commonly 1,2,3,4,6,7,8,9-octachrolodibenzo-*p*-dioxin (OCDD) at a mean concentration level of 43.68 pg/g lipid for the CC commune samples and 5.35 pg g^{-1} lipid

for the CP commune samples. The next most abundant PCDD congener was 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD) at a mean concentration of 13.24 and 1.30 pg/g lipid, for the samples taken from the CC and CP communes, respectively. The next most abundant PCDD congeners were 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD) and 1,2,3,7,8- pentachlorodibenzo-*p*-dioxin (PeCDD). These congeners were found in samples from the CC commune at concentrations of 6.11 pg g^{-1} lipid and 2.28 pg g^{-1} lipid, respectively. The latter 2 congeners were detected in samples obtained from the CP commune at concentrations of 1.10 pg g^{-1} lipid and 1.15 pg g^{-1} lipid, respectively. These concentration levels are similar to that of HpCDD.

Unlike the PCDD congeners, specifically elevated concentrations of PCDF congeners were only found in the milk samples obtained from the CC commune. 1,2,3,4,7,8-hexachlorodibenzofuran (HxCDF) and 1,2,3,4,6,7,8-heptachlorodibenzofuran (HpCDF) were abundantly detected at mean concentration levels of 12.86 pg g^{-1} lipid and 10.72 pg g^{-1} lipid, respectively, followed by 1,2,3,6,7,8-HxCDF at a mean concentration level of 7.52 pg g^{-1} lipid, and 2,3,4,7,8-pentadibenzofuran (PeCDF) at 4.37 pg g^{-1} lipid. In contrast, PCDF congeners in breast milk samples obtained from the CP commune were not abundant, and the maximum level was 2.73 pg g^{-1} lipid for 2,3,4,7,8-PeCDF. The next most abundant PCDF congeners were 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF. The mean concentration levels were similarly below 2 pg g^{-1} lipid.

3.2. PCDD/DF congener patterns

All individual data points of congener concentrations of samples obtained from the CC commune were converted into Z-scores, and 95% confidence intervals were calculated for performing the extent of the score range for each congener. Fig. 2 shows that the deviations from the CP mean are different for the different congeners. The mean of the Z-scores for TCDD was 0.82 (95% confidence interval, 0.52 to 1.12), which is below 1 standard deviation. Examples of congeners with Z-scores below 1 include 1,2,3,7,8-PCDF (Z-score of 0.64, 95% confidence interval, 0.34 to 0.95) and 1,2,3,4,6,7,8,9-octachlorodibenzofuran (OCDF; Z-score of 0.98, 95% confidence interval, 0.69 to 1.27). In contrast, the means of the Z-scores for 1,2,3,6,7,8-HxCDD, HpCDD, OCDD, 1,2,3,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, and 1,2,3,47,8,9-HpCDF were greater than 3 standard deviations. These Z-scores were 3.12 (95% confidence interval, 2.89 to 3.36) for 1,2,3,6,7,8-HxCDD, 4.13 (95% confidence interval, 3.91 to 4.35) for HpCDD, 4.16 (95% confidence interval, 3.91 to 4.42) for OCDD, 3.13 (95% confidence interval, 2.87 to 3.40) for 1,2,3,6,7,8-HxCDF, 3.15 (95% confidence interval, 2.94 to 3.37) for 1,2,3,4,6,7,8-HpCDF, and 3.28 (95% confidence interval, 3.01 to 3.54) for 1,2,3,4,7,8,9-HpCDF. These results indicate that concentration levels of the congeners are reflected in the extent of deviation.

A dendrogram representing hierarchical clustering was drawn by cluster analysis based on Ward's method, to classify congeners in terms of the extent of deviation. The dendrogram was

rearranged without changing the position of each vertical line indicating joined clusters (Fig. 3). As shown in Fig. 3, the last vertical line, corresponding to the largest rescaled distance, was obtained for the high concentration group (Cluster A in Fig. 3) for the congeners with absolute values of the mean of the Z-scores with more than 2 standard deviations, and the low concentration group (Cluster B in Fig. 3) for the congeners with absolute values of the mean of the Z-scores under 2 standard deviations from more than 0. This result confirmed the success in determining the peculiarity of the congener pattern of PCDD/DFs in breast milk samples from the CC commune by cluster analysis based on the dioxin levels.

4. Discussion

4.1. Characteristics of the congener pattern in breast milk samples obtained from sprayed areas

Both the Mann-Whitney U test and Student *t* test for intergroup means indicated that the levels of PCDD/DFs in the herbicide-sprayed area were uniformly higher than those in the non-sprayed area except for TCDF. The deviation from the CP mean shown by the Z-scores on a per-congener basis, actually occurred variably with respect to individual congeners. This could lead the scenario that particular congeners show different increases and decreases in concentration levels with respect to the other congeners through specific exposure to PCDD/DFs in the CC commune. From this viewpoint, greater deviations of Z-scores may imply higher concentrations and more specificity of the congeners.

The cluster analysis classified the 9 types of higher (the hexa-, the hepta-, and the octa-) chlorinated PCDD/DFs as being grouped into Cluster A. Fig. 4 focuses on each constituent congener in the sub-clusters (Cluster 1–3 in Fig. 3) of Cluster A, which were obtained by cutting between the first and the second vertical line of the dendrogram, as shown by the dotted line in Fig. 3. It should be noted that these sub-clusters correspond to the magnitude of the deviation represented by the Z-scores. As indicated by Fig. 4, OCDD and HpCDD in cluster 1 have the greatest deviation, and both means of the Z-scores were greater than 4 standard deviations. Subsequently, cluster 2 which includes 5 congeners (1,2,3,6,7,8-HxCDD, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, and 1,2,3,4,7,8,9-HpCDF) has a mean deviation between 3 and 4 standard deviations. Cluster 3 including 1,2,3,4,7,8-HxCDD and 1,2,3,7,8,9-HxCDD, has a mean deviation between 2 and 3 standard deviations. In this context, OCDD and HpCDD appear to be the most significant congeners. This may indicate that there are additional specific exposure sources in the CC commune.

A similar residual pattern of PCDD/DF congeners determined was previously reported in an earlier study with placenta and breast milk of the residents of the 2 neighboring provinces of Quang Tri and Quang Binh. The congener patterns determined in breast milk samples from these 2 provinces were similar and included specifically high levels of 7 congeners: OCDD, HpCDD, 1,2,3,4,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,6,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, and 2,3,4,7,8- PCDF (Fenshin et al., 2008).

231 Schecter et al. (1991) reported measurements of PCDD/DF congeners in breast milk possibly
232 collected in 1980s from areas in Dong Nai province and Da Nang city in southern Vietnam, and
233 Hanoi, the capital of Vietnam. The concentration level of each congener in breast milk samples
234 from Dong Nai and Da Nang city, in addition to Hanoi as a control for PCDD/DFs levels in
235 breast milk in those days, is shown with respect to the CC commune in Table 3. It was found that
236 in addition to the elevated levels of TCDD, the levels of almost all of the congeners in breast
237 milk samples from Dong Nai and Da Nang were uniformly higher than the levels measured in
238 breast milk samples from Hanoi. Particularly, it is notable that the levels of 1,2,3,6,7,8-HxCDD,
239 HpCDD, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, and
240 1,2,3,4,7,8,9-HpCDF in samples obtained from the CC commune are higher than the levels in the
241 samples obtained from Hanoi. This tendency, with the exception of 1,2,3,4,7,8,9-HpCDF, was
242 also identified between samples obtained from Don Nai and Hanoi, and similarly between
243 samples obtained from Da Nang city and Hanoi. In addition, noticeable differences in the levels
244 of OCDD were also detected between samples obtained from Don Nai and Hanoi, and between
245 samples obtained from Da Nang city and Hanoi during the same time. It follows from these
246 occurrences that the congener pattern characterized by higher chlorinated PCDD/DFs of the
247 hexa-, the hepta-, the octa-chlorinated PCDD/DFs, had been already identified in the 1980s as a
248 unique congener in areas that were sprayed with tactical herbicides.
249

4.2. Present exposure sources of prevalent PCDD/DFs in sprayed areas

A notable report by Feshin et al. (2008) indicated that TCDD was absent in the placenta and breast milk of women from Quang Bin province, while a congener pattern was observed that was similar to the pattern identified in samples obtained from Quang Tri. Quang Bin province is a neighbor on the north of Quang Tri province, and was not subjected to battles or spraying of tactical herbicides during the war. Accordingly, the increase and decrease in PCDD/DF congeners other than TCDD seems to be distinguished as being different from the effects of tactical herbicides.

The principal source of the PCDD/DFs appears to be due to the production of chlorinated phenols (chlorophenols) (Hay, 1982). Methods for synthesizing chlorophenols varied, depending on the product required. Dioxins have not been detected in every sample of chlorophenol, and this may either be due to the method of synthesis of the phenol or to the number of chlorine atoms present in the chlorophenol (Rappe and Garå, 1978).

Pentachlorophenol (PCP) presents a specific congener profile with elevated levels of OCDD, 1,2,3,4,6,7,8-HpCDD, and HpCDF (Firestone et al., 1972; Kontsas et al., 1998). This is the same profile as one of the profiles determined by our cluster analysis. This congener profile for PCP has been found in serum from sawmill workers and PCP production workers (Collins et al., 2007; McLean et al., 2009). Additionally, such specificity was also found to be present in breast milk samples obtained from residents in certain areas of central China, where substantial amounts of

PCP (sodium pentachlorophenol) salts had been sprayed since the 1960s for control of snail-borne schistosomiasis (Schecter et al., 1994; Xiao et al., 2010). Based on these occurrences, it can be considered that the congener profiles distinguish the specifically high levels of higher chlorinated PCDD/DFs, appearing in human fluid and tissue samples as distinct fingerprints for indicating exposure to PCP.

We have no definitive evidence at this point that PCP contamination has occurred in Vietnam. As discussed above, however, the residual congener pattern that appears to be related to PCP was previously identified in the 1980s in samples from AO hot spots in southern Vietnam. Even after 20 years, this pattern was determined in Quang Tri province by our study, and by Feshin et al. (2008). Furthermore, Feshin et al. identified the same pattern in samples obtained from Quang Binh province, a non-sprayed region adjoining Quang Tri, while this pattern was not identified in samples obtained from Hanoi by Schecter et al. (1991), and from Ha Tinh province by our study. It is inferred from this view that contamination with PCP remains an isolated incident, which has existed for 20 years.

At this stage, the association of PCP contamination with tactical herbicides typified by AO cannot be stated positively. Rappe et al. (1978) detected 2,4-di and 2,4,6-trichlorophenol impurities in some AO samples by GC/MS analyses. Similar to PCP, these chlorophenols are formulated by the direct chlorination of phenol; this completely differs from the formulation of 2,4,5-trichlorophenol by the hydrolysis of chlorobenzene under strong alkaline conditions (Rappe

288 and Garå, 1978). Generally, TCDD is formed during the formulation of 2,4,5-trichlorophenol in
289 2,4,5-T production; this caused the situation where TCDD was the major dioxin-like contaminant
290 in AO (Hay, 1982; Dweyer and Flesch-Janys, 1995; IOM, 2002; Dwernychuk et al., 2002;
291 Dwernychuk, 2005; Schecter et al., 2006b). On the other hand, the chlorophenols, including PCP,
292 produced by sequential direct chlorination were found to contain a wide variety of PCDD/DFs
293 (Firestone, 1972, Rappe and Garå, 1978b; Kontsas et al., 1998). In this context, it may be
294 expected that certain of tactical herbicides have a unique congener pattern when compared with
295 pure 2,4,5-T formulations.
296 Saito et al. (2010) examined the association of the levels of PCDD/DF congeners in breast milk
297 and dietary intake in the CC commune and in the CP commune, and reported that dioxin
298 exposure was less affected by usual dietary intake in the CC commune than the CP commune. It
299 was suggested by Saito et al. (2010) that the PCDD/DFs in breast milk from the CC commune
300 were maintained at constant levels after past exposure even 35 years after the end of the war.
301 Regarding the levels of PCDD/DFs in the CP commune, we should not overlook the fact that the
302 total TEQs determined from the samples obtained from the CP commune (4.04 pg g^{-1} lipid TEQ)
303 are nearly identical to the lowest value (3.34 pg g^{-1} lipid TEQ) that was recorded in Fiji in the
304 WHO-coordinated exposure study in 2000 (Malisch and van Leeuwen, 2003). Other reported
305 examples of these PCDD/DFs levels were 3.92 pg g^{-1} lipid TEQ and 3.94 pg g^{-1} lipid TEQ in
306 Brazil and Philippines, respectively, as reported by Malisch and van Leeuwen (2003). These

findings imply that the concentration of PCDD/DFs in the CP commune is not extremely low. Hence, in relation to the observations of Saito et al. (2010), we consider that the levels and congener pattern of PCDD/DFs in the samples obtained from the CP commune are the normal concentrations of dioxins that the people of modern Vietnam are exposed to.

Quang Tri province was 1 of the 10 provinces that experienced the heaviest impact by Operation Ranch Hand (Black, 1993). It is estimated that 47% of the total AO sprayed in Vietnam was sprayed in Quang Tri over the course of 300 to 700 military spray missions. This amounts to a total estimated volume of 171,000 liters (Black, 1993).

Thus, further examination is needed to identify the exposure sources of the prevailing PCDD/DFs in southern Vietnam, while considering the time-dependent changes in the pattern of dioxin residues in human tissues.

5. Conclusion

This study evaluated residual condition of dioxins related to tactical herbicides aerially sprayed over the regions of southern Vietnam through Operation Ranch Hand, and determined specificity in the PCDD/DF congener in breast milk samples obtained from individuals residing in an area sprayed with tactical herbicides. The congener pattern is characterized by higher (the hexa-, the hepta-, and the octa-) chlorinated dioxins, which appears to be the same profile as that presented by PCP, rather than 2,4,5-T contaminated with 2,3,7,8-TCDD. At this stage there is no evidence

to support the association of PCP contamination with tactical herbicides typified by AO. A GC/MS study in the 1970s detected from some AO samples the chlorophenol impurities, formulated by the direct chlorination of phenol, like PCP. Given these occurrences, further examination is needed to identify the exposure sources of the prevailing dioxins in southern Vietnam.

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Table 1
Number and average age of the study population for parity

	primiparaous mothers			multiparous mothers		
	N	Mean[c]	SD[d]	N	Mean	SD
CC commune[a]	25	23.4	3.37	34	26.5	1.85
CP commune[b]	35	22.7	2.31	31	26.3	2.05

[a] Cam Chinh commune, Quang Tri province. [b] Cam Phuc commune Ha Tinh province. [c] average age. [d] standard deviation.

Table 2

Comparing PCDD/DF concentrations in breast milk samples collected in 2002 and 2003 from the CC commune and the CP commune in Vietnam

PCDDs/DFs (pg g^{-1} lipid)	CC[a] commune (N = 59) Mean[c]	(SD)[d]	CP[b] commune (N = 66) Mean	(SD)	
2,3,7,8-TCDD	0.82	(2.04)	0.54	(1.66)	**
1,2,3,7,8-PCDD	2.28	(1.89)	1.15	(1.74)	**
1,2,3,4,7,8-HxCDD	1.42	(2.37)	0.42	(1.84)	**
1,2,3,6,7,8-HxCDD	6.11	(1.83)	1.10	(1.73)	**
1,2,3,7,8,9-HxCDD	1.62	(2.19)	0.37	(1.90)	**
1,2,3,4,6,7,8-HpCDD	13.24	(1.79)	1.30	(1.75)	**
OCDD	43.68	(1.83)	5.35	(1.66)	**
2,3,7,8-TCDF	0.53	(1.74)	1.06	(1.47)	**
1,2,3,7,8-PCDF	0.66	(2.18)	0.46	(1.75)	**
2,3,4,7,8-PCDF	4.37	(1.89)	2.73	(1.54)	**
1,2,3,4,7,8-HxCDF	12.86	(2.12)	1.37	(2.29)	**
1,2,3,6,7,8-HxCDF	7.52	(2.13)	1.13	(1.83)	**
2,3,4,6,7,8-HxCDF	0.99	(2.27)	0.35	(1.86)	**
1,2,3,7,8,9-HxCDF	0.28	(2.91)	0.09	(1.99)	**
1,2,3,4,6,7,8-HpCDF	10.72	(2.21)	0.09	(2.19)	**
1,2,3,4,7,8,9-HpCDF	1.48	(2.83)	0.10	(2.31)	**
OCDF	0.23	(3.27)	0.10	(2.36)	**
ΣPCDDs (pg g^{-1} lipid TEQ)	4.30	(1.79)	1.89	(2.12)	**
ΣPCDFs (pg g^{-1} lipid TEQ)	4.66	(1.95)	2.15	(1.53)	**
ΣPCDDs/DFs (pg g^{-1} lipid TEQ)	8.96	(1.83)	4.04	(1.52)	**

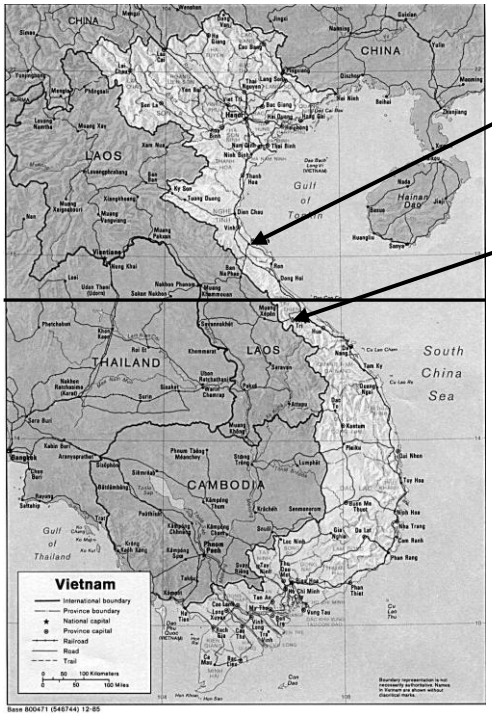
[a] Cam Chinh commune, Quang Tri province. [b] Cam Phuc commune, Ha Tinh province. [c] geometric mean. [d] geometric SD. ** $P < 0.001$.

Table 3
PCDD/DF concentrations in breast milk samples from three different areas in southern Vietnam and Hanoi.

PCDDs/DFs (pg g^{-1} lipid)	Da Nang[a] (Pool = 11)	Dong Nai[a] (Pool = 11)	Hanoi[a] (N = 30)	Quang Tri[b] (N = 59)
2,3,7,8-TCDD	5.6	10.0	2.1	0.82
1,2,3,7,8-PCDD	15.0	7.2	2.9	2.28
1,2,3,4,7,8-HxCDD	5.1	2.1	1.8	1.42
1,2,3,6,7,8-HxCDD	22.0	10.0	5.2	6.11
1,2,3,7,8,9-HxCDD	11.0	4.0	1.8	1.62
1,2,3,4,6,7,8-HpCDD	55.0	28.0	11.5	13.24
OCDD	292.0	119.0	78.3	43.68
2,3,7,8-TCDF	2.2	1.6	2.0	0.53
1,2,3,4,7,8-PCDF	4.1	1.0	1.0	0.66
2,3,4,7,8-PCDF	17.0	13.0	6.1	4.37
1,2,3,4,7,8-HxCDF	34.0	19.0	4.2	12.86
1,2,3,6,7,8-HxCDF	18.0	11.0	3.1	7.52
2,3,4,6,7,8-HxCDF	10.0	2.1	1.4	0.99
1,2,3,7,8,9-HxCDF	ND (0.5)	ND (0.5)	ND (0.5)	0.28
1,2,3,4,6,7,8-HpCDF	40.0	6.2	3.4	10.72
1,2,3,4,7,8,9-HpCDF	ND (0.5)	ND (0.5)	ND (0.5)	1.48
OCDF	7.4	0.9	2.1	0.23
ΣPCDDs (pg g^{-1} lipid TEQ)	25.0	19.1	6.0	4.30
ΣPCDFs (pg g^{-1} lipid TEQ)	15.6	10.0	4.3	4.66
ΣPCDDs/DFs (pg g^{-1} lipid TEQ)	40.6	29.1	10.3	8.96

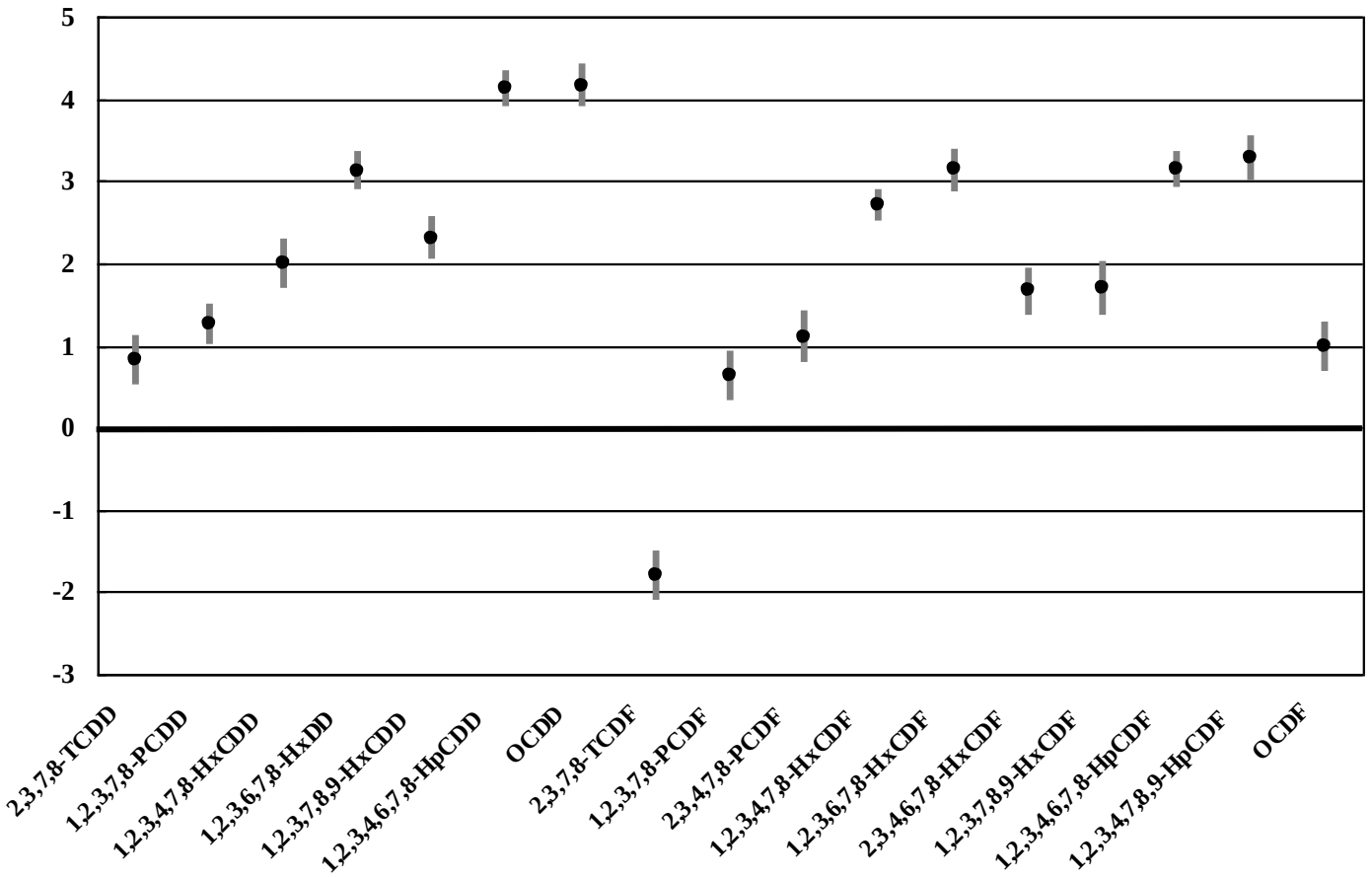
[a] Data for Da Nang, Dong Nai, and Hanoi referred to Schecter, et al. (1991). [b] Cam Chinh commune, Quang Tri province. EachTEQ value determined by Schecter, et al. (1991) was re-calculated for preparation of this table, using the WHO-1998 TEFs (Van den Berg et al., 1998).

Figure



Cam Phuc commune in the Cam Xuyen district, Ha Tinh province

Cam Chinh commune in Cam Lo district, Quang Tri province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-3
2,3,7,8-TCDD
1,2,3,7,8-PCDD
1,2,3,4,7,8-HxCDD
1,2,3,6,7,8-HxDD
1,2,3,7,8,9-HxCDD
1,2,3,4,6,7,8-HpCDD
OCDD
2,3,7,8-TCDF
1,2,3,7,8-PCDF
2,3,4,7,8-PCDF
1,2,3,4,7,8-HxCDF
1,2,3,6,7,8-HxCDF
2,3,4,6,7,8-HxCDF
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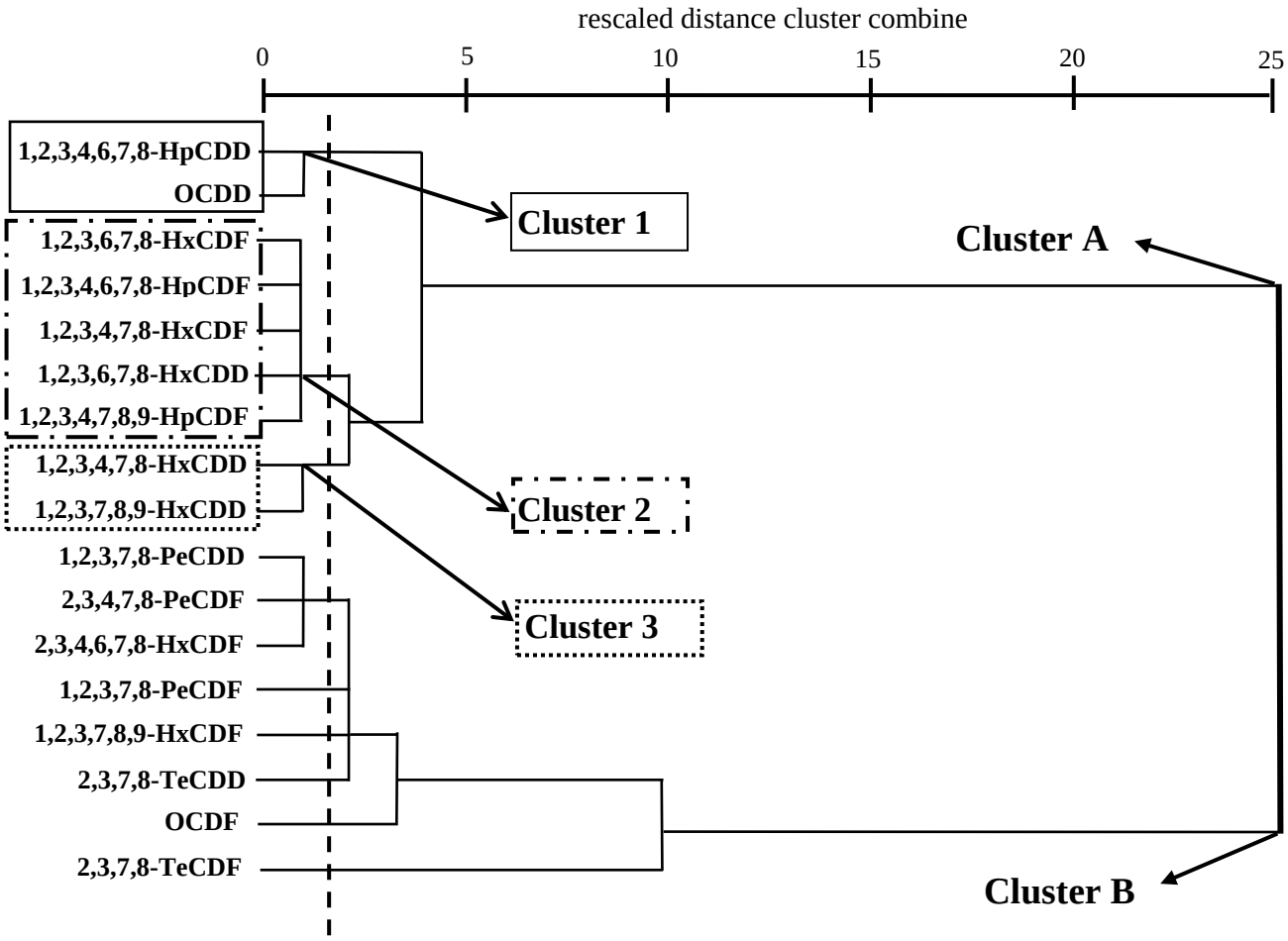
1,2,3,4,7,8,9-HpCDF
OCDF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OCDD
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1,2,3,4,6,7,8-HpCDF
1,2,3,4,7,8-HxCDF
1,2,3,6,7,8-HxCDD
1,2,3,4,7,8,9-HpCDF
1,2,3,4,7,8-HxCDD
1,2,3,7,8,9-HxCDD
1,2,3,7,8-PeCDD
2,3,4,7,8-PeCDF
2,3,4,6,7,8-HxCDF
1,2,3,7,8-PeCDF
1,2,3,7,8,9-HxCDF
2,3,7,8-TeCDD
OCDF
2,3,7,8-TeCDF
Cluster 1
Cluster 2
Cluster 3
Cluster A
Cluster B

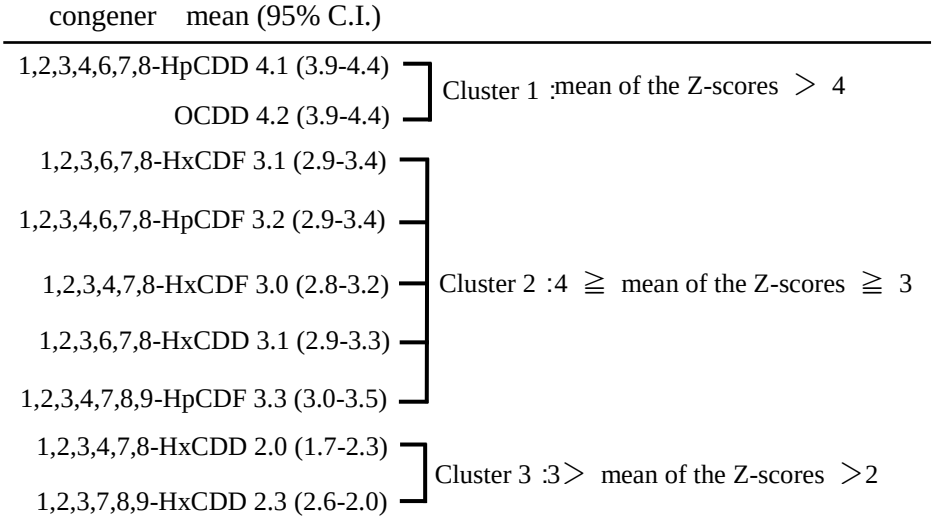
congener mean (95% C.I.)
1,2,3,4,6,7,8-HpCDD 4.1 (3.9-4.4)
OCDD 4.2 (3.9-4.4)
Cluster 1 : mean of the Z-scores ＞ 4
1,2,3,6,7,8-HxCDF 3.1 (2.9-3.4)
1,2,3,4,6,7,8-HpCDF 3.2 (2.9-3.4)
1,2,3,4,7,8-HxCDF 3.0 (2.8-3.2)
1,2,3,6,7,8-HxCDD 3.1 (2.9-3.3)
1,2,3,4,7,8,9-HpCDF 3.3 (3.0-3.5)
Cluster 2 : 4 ≧ mean of the Z-scores ≧ 3
1,2,3,4,7,8-HxCDD 2.0 (1.7-2.3)
1,2,3,7,8,9-HxCDD 2.3 (2.6-2.0)
Cluster 3 : 3＞ mean of the Z-scores ＞2

Figure Captions

Fig.1. Map of Vietnam showing study areas.

A solid line on the map expresses the used demilitarized zone (DMZ) of latitude 17 degrees north, a military boundary during the Vietnam War. The Cam Phuc commune represents a non-sprayed area. The Cam Chinh commune represents an area sprayed with tactical herbicides.

Fig. 2. Mean and 95% confidence interval for the Z-scores of all 17 of the 2,3,7,8-subsituted PCDD/DF congeners in breast milk samples from the Cam Chinh commune, Quang Tri province, an area sprayed with tactical herbicides.

All individual concentration data were converted into Z-scores to demonstrate a relative position of each congener level with an assumption that the mean concentration of each congener in the Cam Phuc commune, Ha Tinh province a non-sprayed area, has a value of 0. Refer to the text for details.

Fig. 3. Dendrogram of cluster analysis for PCDD/DFs in breast milk from CC commune.

Fig. 4. Characteristics of the sub-clusters of Cluster A referred in Figure 3.

Each constituent congener in the sub-clusters of Cluster A was obtained by cutting the dotted line in Figure 3, which distinguishes the first and the second vertical line of dendrogram. Refer to the text for details.