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

42   (Ngo et al., 2006; Schecter, 2006; Tawara et al., 2008). More recently, Hatfield Consultants

43   (Hatfield) reported persistently high levels of TCDD in soil and biota from the Aluoi Valley in

44   central Vietnam, as well as in human tissue samples, including whole blood and breast milk.

45   Through a series of field validation studies, Hatfield highlighted the need for adequate measures

46   to properly assess the extent and impact of contamination around the so-called "hot spot."

47   The "Hatfield hot spot theory" refers to the former locations defined as US military installations

48   where AO was spilled, applied by truck-mounted sprayers, and sprayed intensively during

49   Operation Ranch Hand (Dwernychuk et al., 2002). Well-known examples of such hot spots are

50   the locations of former bases of Operation Ranch Hand at Bien Hoa city in Dong Nai province

51   and Da Nang city (a municipality of Vietnam). The TCDD level at Bien Hoa has been reported to

52   be as high as 1.2 million parts per trillion (ppt) in soil, and reached 270 ppt in blood (Schecter et

53   al., 2001). On the other hand, soils in areas that were sprayed aerially would not be expected to

54   have the same quantities of residual AO as those in the hot spots, as a result of several years of

55   tropical rains, erosion, and the rate of degradation of the chemicals (Dwernychuk, 2005). In this

56   context, human exposure through food chain transfer of TCDD is expected to be highest in the

57   locations identified as hot spots where extremely high concentrations of TCDD have a continued

58   presence in the soil. For this reason, the pattern of TCDD contamination referred to in hot spots

59   seems to serve as a model of contamination throughout southern Vietnam (Dwernychuk et al.,

60   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

79   breast milk should indicate the dioxin level in mother's fat stores before and during pregnancy

80   (Tawara et al., 2009).

81

82   **2. Materials and Methods**

83   *2.1. Study sample*

84   We focused our study on the Cam Chinh (CC) commune located in the Cam Lo district of Quang

85   Tri province and the Cam Phuc (CP) commune in the Cam Xuyen district of Ha Tinh province

86   (Fig.1). During the war, herbicides were sprayed over CC but not over CP. These 2 communes

87   had similar economic and social conditions, customs, ethnic groups, and health care systems.

88   In September 2002 and July 2003, breast milk samples (10–20 mL) were collected from lactating

89   primiparous and multiparous mothers aged 20–30 years from both communes. A total of 84

90   mothers in the CC commune and 72 mothers in the CP commune donated milk samples after

91   providing their consent to participate in the study. Consent was provided by signatures on a

92   Vietnamese document explaining the purpose of the study. To conduct this study, we obtained

93   permission from the Medical Ethics Review Board of Kanazawa Medical University. All samples

94   were frozen immediately after collection for transport to Japan. Samples donated from mothers

95   born after the war (< 31 years old) were used for this study. A total of 59 samples were obtained

96   from the CC commune and 66 samples from the CP commune. The average age of the lactating

97   mothers for parity is shown in Table 1.

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2.2. Measurement of PCDD/DFs in breast milk

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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117   *2.3. Analytical methods*

118   Data were statistically analyzed using the SPSS (ver. 11.0) software package for Windows (SPSS,

119   Chicago IL). Logarithmic transformation of the measured values of PCDD/DFs was performed to

120   improve normality. Differences in the levels of PCDD/DFs between milk samples from the CC

121   and CP communes were evaluated using the Mann-Whitney U and unpaired *t* test. A significance

122   level of $P < 0.05$ was used for all statistical tests.

123   We defined the congener pattern as "residue peculiarity in the body of all 17 of the

124   2,3,7,8-substituted congeners" designated by the level of each congener. To demonstrate this

125   measure objectively, we attempted to demonstrate a relative position of the level of each

126   congener with respect to the total concentration level of dioxins. To perform this analysis,

127   standardization of the concentration data for each congener was implemented by assuming that

128   the mean concentration of each congener (designated μ) in the CP commune has a value of 0. The

129   Z-score was calculated to indicate how the number of standard deviations was above or below the

130   mean (μ) with respect to each congener concentration of samples taken from the CC commune.

131   The following formula was used for Z-score calculations:

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

134

135   where,

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136   z is the Z-score, x is the value of the concentration of each individual congener in the CC

137   commune, μ is the mean concentration of each congener in the CP commune, and σ is the

138   standard deviation of each congener concentration in the CP commune.

139

140   **3. Results**

141   *3.1. Levels of PCDD/DFs in breast milk*

142   Differences in the congener levels between the CC and CP communes were compared using the

143   Man-Whitney test and Student *t* test. As the results were similar, only the results of the latter are

144   presented in Table 2. Generally, the levels of each PCDD/DF congener in the milk samples from

145   the CC commune were higher than in the milk samples from the CP commune. Tests of

146   differences in concentration levels of dioxins between the communes revealed that there is a

147   significant difference in the levels of all PCDD/DF congeners, with the exception of

148   2,3,7,8-tetrachrolodibenzofuran (TCDF). The TCDD level of 0.82 pg g^{-1} lipid was the lowest

149   level of PCDD congeners identified in samples from the CC commune, yet the TCDD level

150   found in the CC commune was significantly higher than that in the CP commune (0.54 pg g^{-1}

151   lipid). When the PCDD congeners were listed according to concentration levels, the order was

152   found to be very similar between the CC commune and CP commune samples. The most

153   abundant PCDD congener was commonly 1,2,3,4,6,7,8,9-octachrolodibenzo-*p*-dioxin (OCDD) at

154   a mean concentration level of 43.68 pg/g lipid for the CC commune samples and 5.35 pg g^{-1} lipid

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155   for the CP commune samples. The next most abundant PCDD congener was

156   1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD) at a mean concentration of 13.24 and 1.30

157   pg/g lipid, for the samples taken from the CC and CP communes, respectively. The next most

158   abundant PCDD congeners were 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD) and

159   1,2,3,7,8- pentachlorodibenzo-*p*-dioxin (PeCDD). These congeners were found in samples from

160   the CC commune at concentrations of 6.11 pg g^{-1} lipid and 2.28 pg g^{-1} lipid, respectively. The

161   latter 2 congeners were detected in samples obtained from the CP commune at concentrations of

162   1.10 pg g^{-1} lipid and 1.15 pg g^{-1} lipid, respectively. These concentration levels are similar to that

163   of HpCDD.

164   Unlike the PCDD congeners, specifically elevated concentrations of PCDF congeners were only

165   found in the milk samples obtained from the CC commune. 1,2,3,4,7,8-hexachlorodibenzofuran

166   (HxCDF) and 1,2,3,4,6,7,8-heptachlorodibenzofuran (HpCDF) were abundantly detected at mean

167   concentration levels of 12.86 pg g^{-1} lipid and 10.72 pg g^{-1} lipid, respectively, followed by

168   1,2,3,6,7,8-HxCDF at a mean concentration level of 7.52 pg g^{-1} lipid, and

169   2,3,4,7,8-pentadibenzofuran (PeCDF) at 4.37 pg g^{-1} lipid. In contrast, PCDF congeners in breast

170   milk samples obtained from the CP commune were not abundant, and the maximum level was

171   2.73 pg g^{-1} lipid for 2,3,4,7,8-PeCDF. The next most abundant PCDF congeners were

172   1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF. The mean concentration levels were similarly below

173   2 pg g^{-1} lipid.

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175   *3.2. PCDD/DF congener patterns*

176   All individual data points of congener concentrations of samples obtained from the CC commune

177   were converted into Z-scores, and 95% confidence intervals were calculated for performing the

178   extent of the score range for each congener. Fig. 2 shows that the deviations from the CP mean

179   are different for the different congeners. The mean of the Z-scores for TCDD was 0.82 (95%

180   confidence interval, 0.52 to 1.12), which is below 1 standard deviation. Examples of congeners

181   with Z-scores below 1 include 1,2,3,7,8-PCDF (Z-score of 0.64, 95% confidence interval, 0.34 to

182   0.95) and 1,2,3,4,6,7,8,9-octachlorodibenzofuran (OCDF; Z-score of 0.98, 95% confidence

183   interval, 0.69 to 1.27). In contrast, the means of the Z-scores for 1,2,3,6,7,8-HxCDD, HpCDD,

184   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

185   standard deviations. These Z-scores were 3.12 (95% confidence interval, 2.89 to 3.36) for

186   1,2,3,6,7,8-HxCDD, 4.13 (95% confidence interval, 3.91 to 4.35) for HpCDD, 4.16 (95%

187   confidence interval, 3.91 to 4.42) for OCDD, 3.13 (95% confidence interval, 2.87 to 3.40) for

188   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

189   3.28 (95% confidence interval, 3.01 to 3.54) for 1,2,3,4,7,8,9-HpCDF. These results indicate that

190   concentration levels of the congeners are reflected in the extent of deviation.

191   A dendrogram representing hierarchical clustering was drawn by cluster analysis based on

192   Ward's method, to classify congeners in terms of the extent of deviation. The dendrogram was

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193   rearranged without changing the position of each vertical line indicating joined clusters (Fig. 3).

194   As shown in Fig. 3, the last vertical line, corresponding to the largest rescaled distance, was

195   obtained for the high concentration group (Cluster A in Fig. 3) for the congeners with absolute

196   values of the mean of the Z-scores with more than 2 standard deviations, and the low

197   concentration group (Cluster B in Fig. 3) for the congeners with absolute values of the mean of

198   the Z-scores under 2 standard deviations from more than 0. This result confirmed the success in

199   determining the peculiarity of the congener pattern of PCDD/DFs in breast milk samples from

200   the CC commune by cluster analysis based on the dioxin levels.

201

202   **4. Discussion**

203   *4.1. Characteristics of the congener pattern in breast milk samples obtained from sprayed areas*

204   Both the Mann-Whitney U test and Student *t* test for intergroup means indicated that the levels of

205   PCDD/DFs in the herbicide-sprayed area were uniformly higher than those in the non-sprayed

206   area except for TCDF. The deviation from the CP mean shown by the Z-scores on a per-congener

207   basis, actually occurred variably with respect to individual congeners. This could lead the

208   scenario that particular congeners show different increases and decreases in concentration levels

209   with respect to the other congeners through specific exposure to PCDD/DFs in the CC commune.

210   From this viewpoint, greater deviations of Z-scores may imply higher concentrations and more

211   specificity of the congeners.

212   The cluster analysis classified the 9 types of higher (the hexa-, the hepta-, and the octa-)

213   chlorinated PCDD/DFs as being grouped into Cluster A. Fig. 4 focuses on each constituent

214   congener in the sub-clusters (Cluster 1–3 in Fig. 3) of Cluster A, which were obtained by cutting

215   between the first and the second vertical line of the dendrogram, as shown by the dotted line in

216   Fig. 3. It should be noted that these sub-clusters correspond to the magnitude of the deviation

217   represented by the Z-scores. As indicated by Fig. 4, OCDD and HpCDD in cluster 1 have the

218   greatest deviation, and both means of the Z-scores were greater than 4 standard deviations.

219   Subsequently, cluster 2 which includes 5 congeners (1,2,3,6,7,8-HxCDD, 1,2,3,4,7,8-HxCDF,

220   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

221   between 3 and 4 standard deviations. Cluster 3 including 1,2,3,4,7,8-HxCDD and

222   1,2,3,7,8,9-HxCDD, has a mean deviation between 2 and 3 standard deviations. In this context,

223   OCDD and HpCDD appear to be the most significant congeners. This may indicate that there are

224   additional specific exposure sources in the CC commune.

225   A similar residual pattern of PCDD/DF congeners determined was previously reported in an

226   earlier study with placenta and breast milk of the residents of the 2 neighboring provinces of

227   Quang Tri and Quang Binh. The congener patterns determined in breast milk samples from these

228   2 provinces were similar and included specifically high levels of 7 congeners: OCDD, HpCDD,

229   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

230   2,3,4,7,8- PCDF (Fenshin et al., 2008).

13

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

250   *4.2. Present exposure sources of prevalent PCDD/DFs in sprayed areas*

251   A notable report by Feshin et al. (2008) indicated that TCDD was absent in the placenta and

252   breast milk of women from Quang Bin province, while a congener pattern was observed that was

253   similar to the pattern identified in samples obtained from Quang Tri. Quang Bin province is a

254   neighbor on the north of Quang Tri province, and was not subjected to battles or spraying of

255   tactical herbicides during the war. Accordingly, the increase and decrease in PCDD/DF

256   congeners other than TCDD seems to be distinguished as being different from the effects of

257   tactical herbicides.

258   The principal source of the PCDD/DFs appears to be due to the production of chlorinated phenols

259   (chlorophenols) (Hay, 1982). Methods for synthesizing chlorophenols varied, depending on the

260   product required. Dioxins have not been detected in every sample of chlorophenol, and this may

261   either be due to the method of synthesis of the phenol or to the number of chlorine atoms present

262   in the chlorophenol (Rappe and Garå, 1978).

263   Pentachlorophenol (PCP) presents a specific congener profile with elevated levels of OCDD,

264   1,2,3,4,6,7,8-HpCDD, and HpCDF (Firestone et al., 1972; Kontsas et al., 1998). This is the same

265   profile as one of the profiles determined by our cluster analysis. This congener profile for PCP

266   has been found in serum from sawmill workers and PCP production workers (Collins et al., 2007;

267   McLean et al., 2009). Additionally, such specificity was also found to be present in breast milk

268   samples obtained from residents in certain areas of central China, where substantial amounts of

269 PCP (sodium pentachlorophenol) salts had been sprayed since the 1960s for control of

270 snail-borne schistosomiasis (Schecter et al., 1994; Xiao et al., 2010). Based on these occurrences,

271 it can be considered that the congener profiles distinguish the specifically high levels of higher

272 chlorinated PCDD/DFs, appearing in human fluid and tissue samples as distinct fingerprints for

273 indicating exposure to PCP.

274 We have no definitive evidence at this point that PCP contamination has occurred in Vietnam. As

275 discussed above, however, the residual congener pattern that appears to be related to PCP was

276 previously identified in the 1980s in samples from AO hot spots in southern Vietnam. Even after

277 20 years, this pattern was determined in Quang Tri province by our study, and by Feshin et al.

278 (2008). Furthermore, Feshin et al. identified the same pattern in samples obtained from Quang

279 Binh province, a non-sprayed region adjoining Quang Tri, while this pattern was not identified in

280 samples obtained from Hanoi by Schecter et al. (1991), and from Ha Tinh province by our study.

281 It is inferred from this view that contamination with PCP remains an isolated incident, which has

282 existed for 20 years.

283 At this stage, the association of PCP contamination with tactical herbicides typified by AO

284 cannot be stated positively. Rappe et al. (1978) detected 2,4-di and 2,4,6-trichlorophenol

285 impurities in some AO samples by GC/MS analyses. Similar to PCP, these chlorophenols are

286 formulated by the direct chlorination of phenol; this completely differs from the formulation of

287 2,4,5-trichlorophenol by the hydrolysis of chlorobenzene under strong alkaline conditions (Rappe

16

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

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307 findings imply that the concentration of PCDD/DFs in the CP commune is not extremely low.

308 Hence, in relation to the observations of Saito et al. (2010), we consider that the levels and

309 congener pattern of PCDD/DFs in the samples obtained from the CP commune are the normal

310 concentrations of dioxins that the people of modern Vietnam are exposed to.

311 Quang Tri province was 1 of the 10 provinces that experienced the heaviest impact by Operation

312 Ranch Hand (Black, 1993). It is estimated that 47% of the total AO sprayed in Vietnam was

313 sprayed in Quang Tri over the course of 300 to 700 military spray missions. This amounts to a

314 total estimated volume of 171,000 liters (Black, 1993).

315 Thus, further examination is needed to identify the exposure sources of the prevailing PCDD/DFs

316 in southern Vietnam, while considering the time-dependent changes in the pattern of dioxin

317 residues in human tissues.

318

319 **5. Conclusion**

320 This study evaluated residual condition of dioxins related to tactical herbicides aerially sprayed

321 over the regions of southern Vietnam through Operation Ranch Hand, and determined specificity

322 in the PCDD/DF congener in breast milk samples obtained from individuals residing in an area

323 sprayed with tactical herbicides. The congener pattern is characterized by higher (the hexa-, the

324 hepta-, and the octa-) chlorinated dioxins, which appears to be the same profile as that presented

325 by PCP, rather than 2,4,5-T contaminated with 2,3,7,8-TCDD. At this stage there is no evidence

18

326   to support the association of PCP contamination with tactical herbicides typified by AO. A

327   GC/MS study in the 1970s detected from some AO samples the chlorophenol impurities,

328   formulated by the direct chlorination of phenol, like PCP. Given these occurrences, further

329   examination is needed to identify the exposure sources of the prevailing dioxins in southern

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331

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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

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

2   Vietnam

3

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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

3

42   (Ngo et al., 2006; Schecter, 2006; Tawara et al., 2008). More recently, Hatfield Consultants

43   (Hatfield) reported persistently high levels of TCDD in soil and biota from the Aluoi Valley in

44   central Vietnam, as well as in human tissue samples, including whole blood and breast milk.

45   Through a series of field validation studies, Hatfield highlighted the need for adequate measures

46   to properly assess the extent and impact of contamination around the so-called "hot spot."

47   The "Hatfield hot spot theory" refers to the former locations defined as US military installations

48   where AO was spilled, applied by truck-mounted sprayers, and sprayed intensively during

49   Operation Ranch Hand (Dwernychuk et al., 2002). Well-known examples of such hot spots are

50   the locations of former bases of Operation Ranch Hand at Bien Hoa city in Dong Nai province

51   and Da Nang city (a municipality of Vietnam). The TCDD level at Bien Hoa has been reported to

52   be as high as 1.2 million parts per trillion (ppt) in soil, and reached 270 ppt in blood (Schecter et

53   al., 2001). On the other hand, soils in areas that were sprayed aerially would not be expected to

54   have the same quantities of residual AO as those in the hot spots, as a result of several years of

55   tropical rains, erosion, and the rate of degradation of the chemicals (Dwernychuk, 2005). In this

56   context, human exposure through food chain transfer of TCDD is expected to be highest in the

57   locations identified as hot spots where extremely high concentrations of TCDD have a continued

58   presence in the soil. For this reason, the pattern of TCDD contamination referred to in hot spots

59   seems to serve as a model of contamination throughout southern Vietnam (Dwernychuk et al.,

60   2002).

4

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

79   breast milk should indicate the dioxin level in mother's fat stores before and during pregnancy

80   (Tawara et al., 2009).

81

82   **2. Materials and Methods**

83   *2.1. Study sample*

84   We focused our study on the Cam Chinh (CC) commune located in the Cam Lo district of Quang

85   Tri province and the Cam Phuc (CP) commune in the Cam Xuyen district of Ha Tinh province

86   (Fig.1). During the war, herbicides were sprayed over CC but not over CP. These 2 communes

87   had similar economic and social conditions, customs, ethnic groups, and health care systems.

88   In September 2002 and July 2003, breast milk samples (10–20 mL) were collected from lactating

89   primiparous and multiparous mothers aged 20–30 years from both communes. A total of 84

90   mothers in the CC commune and 72 mothers in the CP commune donated milk samples after

91   providing their consent to participate in the study. Consent was provided by signatures on a

92   Vietnamese document explaining the purpose of the study. To conduct this study, we obtained

93   permission from the Medical Ethics Review Board of Kanazawa Medical University. All samples

94   were frozen immediately after collection for transport to Japan. Samples donated from mothers

95   born after the war (< 31 years old) were used for this study. A total of 59 samples were obtained

96   from the CC commune and 66 samples from the CP commune. The average age of the lactating

97   mothers for parity is shown in Table 1.

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2.2. Measurement of PCDD/DFs in breast milk

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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117 *2.3. Analytical methods*

118 Data were statistically analyzed using the SPSS (ver. 11.0) software package for Windows (SPSS,

119 Chicago IL). Logarithmic transformation of the measured values of PCDD/DFs was performed to

120 improve normality. Differences in the levels of PCDD/DFs between milk samples from the CC

121 and CP communes were evaluated using the Mann-Whitney U and unpaired *t* test. A significance

122 level of $P < 0.05$ was used for all statistical tests.

123 We defined the congener pattern as "residue peculiarity in the body of all 17 of the

124 2,3,7,8-substituted congeners" designated by the level of each congener. To demonstrate this

125 measure objectively, we attempted to demonstrate a relative position of the level of each

126 congener with respect to the total concentration level of dioxins. To perform this analysis,

127 standardization of the concentration data for each congener was implemented by assuming that

128 the mean concentration of each congener (designated μ) in the CP commune has a value of 0. The

129 Z-score was calculated to indicate how the number of standard deviations was above or below the

130 mean (μ) with respect to each congener concentration of samples taken from the CC commune.

131 The following formula was used for Z-score calculations:

132

133 $z = (x - \mu)/\sigma$

134

135 where,

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136   z is the Z-score, x is the value of the concentration of each individual congener in the CC

137   commune, μ is the mean concentration of each congener in the CP commune, and σ is the

138   standard deviation of each congener concentration in the CP commune.

139

140   **3. Results**

141   *3.1. Levels of PCDD/DFs in breast milk*

142   Differences in the congener levels between the CC and CP communes were compared using the

143   Man-Whitney test and Student *t* test. As the results were similar, only the results of the latter are

144   presented in Table 2. Generally, the levels of each PCDD/DF congener in the milk samples from

145   the CC commune were higher than in the milk samples from the CP commune. Tests of

146   differences in concentration levels of dioxins between the communes revealed that there is a

147   significant difference in the levels of all PCDD/DF congeners, with the exception of

148   2,3,7,8-tetrachrolodibenzofuran (TCDF). The TCDD level of 0.82 pg g^{-1} lipid was the lowest

149   level of PCDD congeners identified in samples from the CC commune, yet the TCDD level

150   found in the CC commune was significantly higher than that in the CP commune (0.54 pg g^{-1}

151   lipid). When the PCDD congeners were listed according to concentration levels, the order was

152   found to be very similar between the CC commune and CP commune samples. The most

153   abundant PCDD congener was commonly 1,2,3,4,6,7,8,9-octachrolodibenzo-*p*-dioxin (OCDD) at

154   a mean concentration level of 43.68 pg/g lipid for the CC commune samples and 5.35 pg g^{-1} lipid

9

155   for the CP commune samples. The next most abundant PCDD congener was

156   1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD) at a mean concentration of 13.24 and 1.30

157   pg/g lipid, for the samples taken from the CC and CP communes, respectively. The next most

158   abundant PCDD congeners were 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD) and

159   1,2,3,7,8- pentachlorodibenzo-*p*-dioxin (PeCDD). These congeners were found in samples from

160   the CC commune at concentrations of 6.11 pg g^{-1} lipid and 2.28 pg g^{-1} lipid, respectively. The

161   latter 2 congeners were detected in samples obtained from the CP commune at concentrations of

162   1.10 pg g^{-1} lipid and 1.15 pg g^{-1} lipid, respectively. These concentration levels are similar to that

163   of HpCDD.

164   Unlike the PCDD congeners, specifically elevated concentrations of PCDF congeners were only

165   found in the milk samples obtained from the CC commune. 1,2,3,4,7,8-hexachlorodibenzofuran

166   (HxCDF) and 1,2,3,4,6,7,8-heptachlorodibenzofuran (HpCDF) were abundantly detected at mean

167   concentration levels of 12.86 pg g^{-1} lipid and 10.72 pg g^{-1} lipid, respectively, followed by

168   1,2,3,6,7,8-HxCDF at a mean concentration level of 7.52 pg g^{-1} lipid, and

169   2,3,4,7,8-pentadibenzofuran (PeCDF) at 4.37 pg g^{-1} lipid. In contrast, PCDF congeners in breast

170   milk samples obtained from the CP commune were not abundant, and the maximum level was

171   2.73 pg g^{-1} lipid for 2,3,4,7,8-PeCDF. The next most abundant PCDF congeners were

172   1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF. The mean concentration levels were similarly below

173   2 pg g^{-1} lipid.

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174

3.2. PCDD/DF congener patterns

176   All individual data points of congener concentrations of samples obtained from the CC commune

177   were converted into Z-scores, and 95% confidence intervals were calculated for performing the

178   extent of the score range for each congener. Fig. 2 shows that the deviations from the CP mean

179   are different for the different congeners. The mean of the Z-scores for TCDD was 0.82 (95%

180   confidence interval, 0.52 to 1.12), which is below 1 standard deviation. Examples of congeners

181   with Z-scores below 1 include 1,2,3,7,8-PCDF (Z-score of 0.64, 95% confidence interval, 0.34 to

182   0.95) and 1,2,3,4,6,7,8,9-octachlorodibenzofuran (OCDF; Z-score of 0.98, 95% confidence

183   interval, 0.69 to 1.27). In contrast, the means of the Z-scores for 1,2,3,6,7,8-HxCDD, HpCDD,

184   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

185   standard deviations. These Z-scores were 3.12 (95% confidence interval, 2.89 to 3.36) for

186   1,2,3,6,7,8-HxCDD, 4.13 (95% confidence interval, 3.91 to 4.35) for HpCDD, 4.16 (95%

187   confidence interval, 3.91 to 4.42) for OCDD, 3.13 (95% confidence interval, 2.87 to 3.40) for

188   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

189   3.28 (95% confidence interval, 3.01 to 3.54) for 1,2,3,4,7,8,9-HpCDF. These results indicate that

190   concentration levels of the congeners are reflected in the extent of deviation.

191   A dendrogram representing hierarchical clustering was drawn by cluster analysis based on

192   Ward's method, to classify congeners in terms of the extent of deviation. The dendrogram was

11

193   rearranged without changing the position of each vertical line indicating joined clusters (Fig. 3).

194   As shown in Fig. 3, the last vertical line, corresponding to the largest rescaled distance, was

195   obtained for the high concentration group (Cluster A in Fig. 3) for the congeners with absolute

196   values of the mean of the Z-scores with more than 2 standard deviations, and the low

197   concentration group (Cluster B in Fig. 3) for the congeners with absolute values of the mean of

198   the Z-scores under 2 standard deviations from more than 0. This result confirmed the success in

199   determining the peculiarity of the congener pattern of PCDD/DFs in breast milk samples from

200   the CC commune by cluster analysis based on the dioxin levels.

201

202   **4. Discussion**

203   *4.1. Characteristics of the congener pattern in breast milk samples obtained from sprayed areas*

204   Both the Mann-Whitney U test and Student *t* test for intergroup means indicated that the levels of

205   PCDD/DFs in the herbicide-sprayed area were uniformly higher than those in the non-sprayed

206   area except for TCDF. The deviation from the CP mean shown by the Z-scores on a per-congener

207   basis, actually occurred variably with respect to individual congeners. This could lead the

208   scenario that particular congeners show different increases and decreases in concentration levels

209   with respect to the other congeners through specific exposure to PCDD/DFs in the CC commune.

210   From this viewpoint, greater deviations of Z-scores may imply higher concentrations and more

211   specificity of the congeners.

12

212 The cluster analysis classified the 9 types of higher (the hexa-, the hepta-, and the octa-)

213 chlorinated PCDD/DFs as being grouped into Cluster A. Fig. 4 focuses on each constituent

214 congener in the sub-clusters (Cluster 1–3 in Fig. 3) of Cluster A, which were obtained by cutting

215 between the first and the second vertical line of the dendrogram, as shown by the dotted line in

216 Fig. 3. It should be noted that these sub-clusters correspond to the magnitude of the deviation

217 represented by the Z-scores. As indicated by Fig. 4, OCDD and HpCDD in cluster 1 have the

218 greatest deviation, and both means of the Z-scores were greater than 4 standard deviations.

219 Subsequently, cluster 2 which includes 5 congeners (1,2,3,6,7,8-HxCDD, 1,2,3,4,7,8-HxCDF,

220 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

221 between 3 and 4 standard deviations. Cluster 3 including 1,2,3,4,7,8-HxCDD and

222 1,2,3,7,8,9-HxCDD, has a mean deviation between 2 and 3 standard deviations. In this context,

223 OCDD and HpCDD appear to be the most significant congeners. This may indicate that there are

224 additional specific exposure sources in the CC commune.

225 A similar residual pattern of PCDD/DF congeners determined was previously reported in an

226 earlier study with placenta and breast milk of the residents of the 2 neighboring provinces of

227 Quang Tri and Quang Binh. The congener patterns determined in breast milk samples from these

228 2 provinces were similar and included specifically high levels of 7 congeners: OCDD, HpCDD,

229 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

230 2,3,4,7,8- PCDF (Fenshin et al., 2008).

13

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

250 *4.2. Present exposure sources of prevalent PCDD/DFs in sprayed areas*

251 A notable report by Feshin et al. (2008) indicated that TCDD was absent in the placenta and

252 breast milk of women from Quang Bin province, while a congener pattern was observed that was

253 similar to the pattern identified in samples obtained from Quang Tri. Quang Bin province is a

254 neighbor on the north of Quang Tri province, and was not subjected to battles or spraying of

255 tactical herbicides during the war. Accordingly, the increase and decrease in PCDD/DF

256 congeners other than TCDD seems to be distinguished as being different from the effects of

257 tactical herbicides.

258 The principal source of the PCDD/DFs appears to be due to the production of chlorinated phenols

259 (chlorophenols) (Hay, 1982). Methods for synthesizing chlorophenols varied, depending on the

260 product required. Dioxins have not been detected in every sample of chlorophenol, and this may

261 either be due to the method of synthesis of the phenol or to the number of chlorine atoms present

262 in the chlorophenol (Rappe and Garå, 1978).

263 Pentachlorophenol (PCP) presents a specific congener profile with elevated levels of OCDD,

264 1,2,3,4,6,7,8-HpCDD, and HpCDF (Firestone et al., 1972; Kontsas et al., 1998). This is the same

265 profile as one of the profiles determined by our cluster analysis. This congener profile for PCP

266 has been found in serum from sawmill workers and PCP production workers (Collins et al., 2007;

267 McLean et al., 2009). Additionally, such specificity was also found to be present in breast milk

268 samples obtained from residents in certain areas of central China, where substantial amounts of

269   PCP (sodium pentachlorophenol) salts had been sprayed since the 1960s for control of

270   snail-borne schistosomiasis (Schecter et al., 1994; Xiao et al., 2010). Based on these occurrences,

271   it can be considered that the congener profiles distinguish the specifically high levels of higher

272   chlorinated PCDD/DFs, appearing in human fluid and tissue samples as distinct fingerprints for

273   indicating exposure to PCP.

274   We have no definitive evidence at this point that PCP contamination has occurred in Vietnam. As

275   discussed above, however, the residual congener pattern that appears to be related to PCP was

276   previously identified in the 1980s in samples from AO hot spots in southern Vietnam. Even after

277   20 years, this pattern was determined in Quang Tri province by our study, and by Feshin et al.

278   (2008). Furthermore, Feshin et al. identified the same pattern in samples obtained from Quang

279   Binh province, a non-sprayed region adjoining Quang Tri, while this pattern was not identified in

280   samples obtained from Hanoi by Schecter et al. (1991), and from Ha Tinh province by our study.

281   It is inferred from this view that contamination with PCP remains an isolated incident, which has

282   existed for 20 years.

283   At this stage, the association of PCP contamination with tactical herbicides typified by AO

284   cannot be stated positively. Rappe et al. (1978) detected 2,4-di and 2,4,6-trichlorophenol

285   impurities in some AO samples by GC/MS analyses. Similar to PCP, these chlorophenols are

286   formulated by the direct chlorination of phenol; this completely differs from the formulation of

287   2,4,5-trichlorophenol by the hydrolysis of chlorobenzene under strong alkaline conditions (Rappe

16

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

17

307   findings imply that the concentration of PCDD/DFs in the CP commune is not extremely low.

308   Hence, in relation to the observations of Saito et al. (2010), we consider that the levels and

309   congener pattern of PCDD/DFs in the samples obtained from the CP commune are the normal

310   concentrations of dioxins that the people of modern Vietnam are exposed to.

311   Quang Tri province was 1 of the 10 provinces that experienced the heaviest impact by Operation

312   Ranch Hand (Black, 1993). It is estimated that 47% of the total AO sprayed in Vietnam was

313   sprayed in Quang Tri over the course of 300 to 700 military spray missions. This amounts to a

314   total estimated volume of 171,000 liters (Black, 1993).

315   Thus, further examination is needed to identify the exposure sources of the prevailing PCDD/DFs

316   in southern Vietnam, while considering the time-dependent changes in the pattern of dioxin

317   residues in human tissues.

318

319   **5. Conclusion**

320   This study evaluated residual condition of dioxins related to tactical herbicides aerially sprayed

321   over the regions of southern Vietnam through Operation Ranch Hand, and determined specificity

322   in the PCDD/DF congener in breast milk samples obtained from individuals residing in an area

323   sprayed with tactical herbicides. The congener pattern is characterized by higher (the hexa-, the

324   hepta-, and the octa-) chlorinated dioxins, which appears to be the same profile as that presented

325   by PCP, rather than 2,4,5-T contaminated with 2,3,7,8-TCDD. At this stage there is no evidence

326   to support the association of PCP contamination with tactical herbicides typified by AO. A

327   GC/MS study in the 1970s detected from some AO samples the chlorophenol impurities,

328   formulated by the direct chlorination of phenol, like PCP. Given these occurrences, further

329   examination is needed to identify the exposure sources of the prevailing dioxins in southern

330   Vietnam.

331

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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)		CP[b] commune (N = 66)		
	Mean[c]	(SD)[d]	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. ** $P < 0.001$.
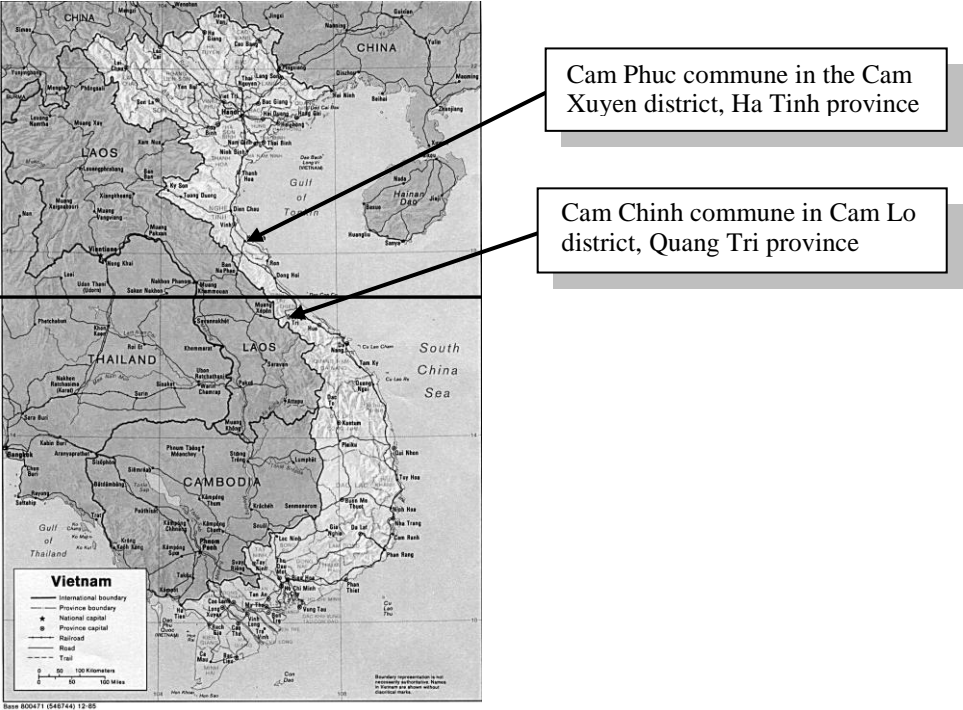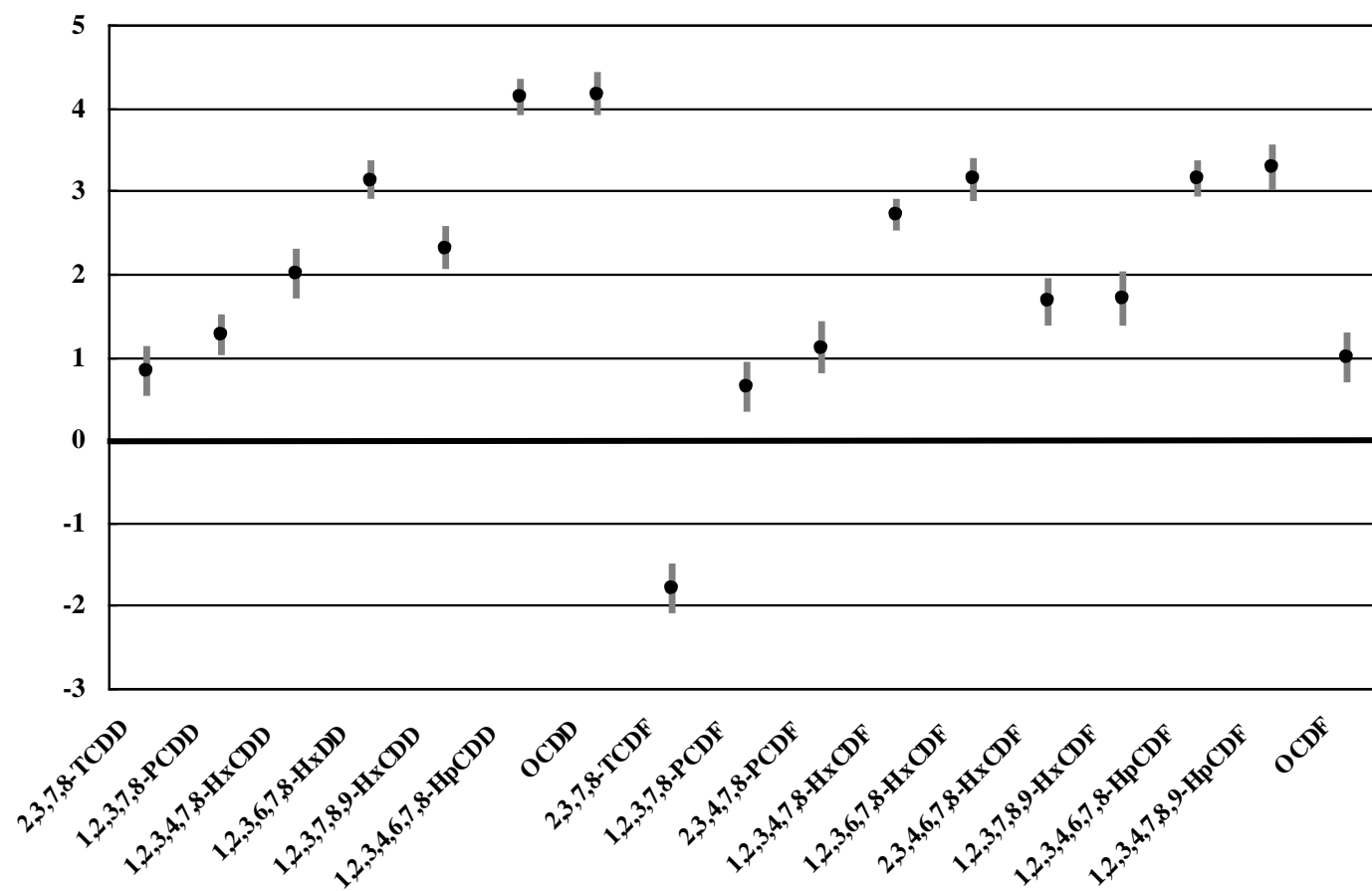
Table 3

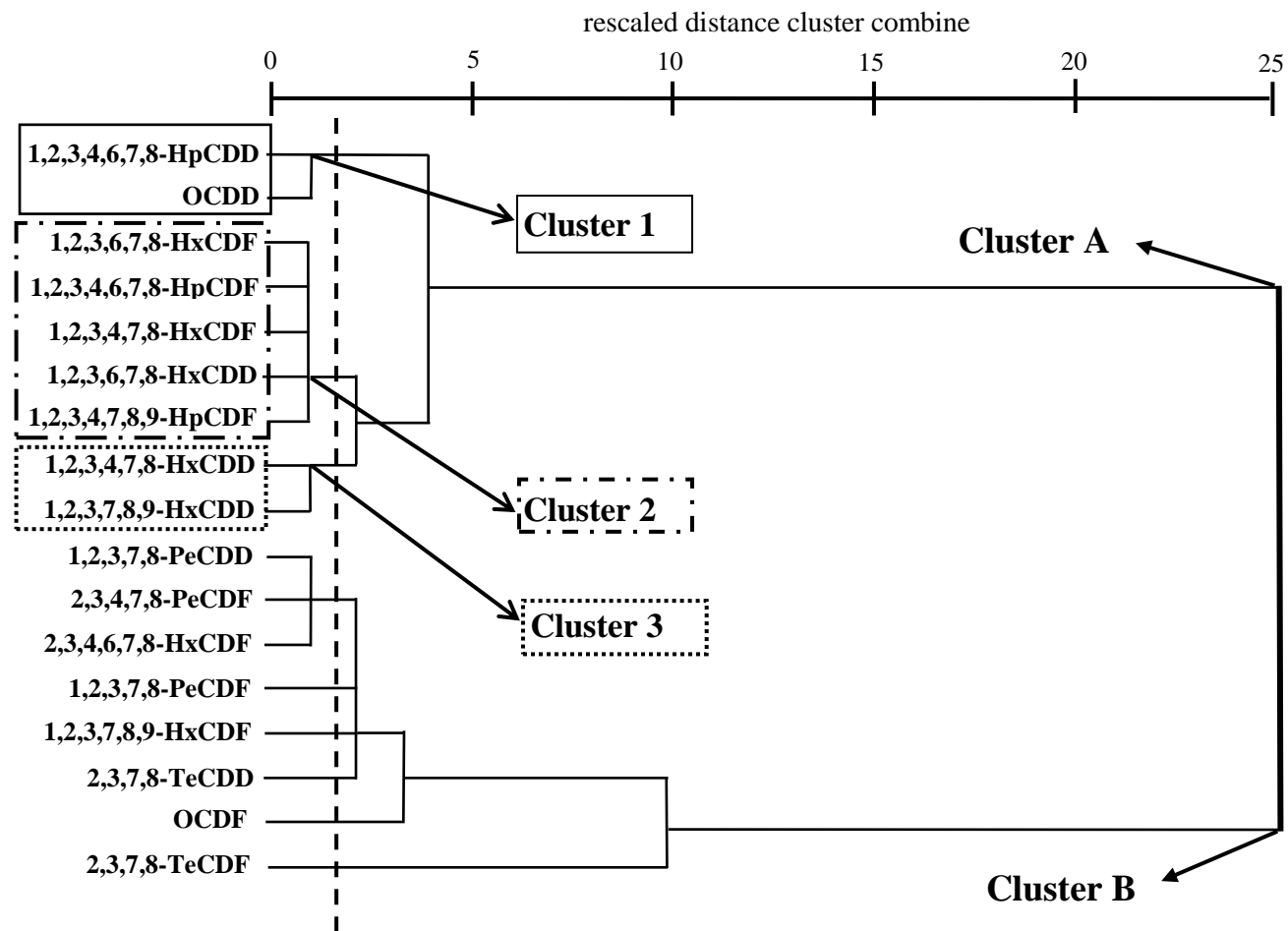PCDD/DF concentrations in breast milk samples from three different areas in southern Vietnam and Hanoi.

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

rescaled distance cluster combine

0   5   10   15   20   25

1,2,3,4,6,7,8-HpCDD
OCDD
→ Cluster 1

1,2,3,6,7,8-HxCDF
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
→ Cluster 2

1,2,3,4,7,8-HxCDD
1,2,3,7,8,9-HxCDD
→ Cluster 3

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 A

Cluster B

congener	mean (95% C.I.)	
1,2,3,4,6,7,8-HpCDD	4.1 (3.9-4.4)	Cluster 1 : mean of the Z-scores $>$ 4
OCDD	4.2 (3.9-4.4)	
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)	Cluster 2 : 4 $\geqq$ mean of the Z-scores $\geqq$ 3
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)	
1,2,3,4,7,8-HxCDD	2.0 (1.7-2.3)	Cluster 3 : 3 $>$ mean of the Z-scores $>$ 2
1,2,3,7,8,9-HxCDD	2.3 (2.6-2.0)	

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.