46. Dioxins in human breast milk in Vietnam

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Abstract

Several decades after herbicide spraying ceased, dioxin concentrations in both the environment and humans remain elevated in the sprayed areas of South Vietnam. Human breast milk is a good internal exposure indicator for estimating an individual’s dioxin level. The concentrations of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and toxic equivalents (TEQs) in human breast milk in hot spots and sprayed areas were found to be significantly higher than those in the unsprayed areas. Likewise, dioxin concentrations in the breast milk of multiparae were lower than those of primiparae in the hot spots, and the sprayed and unsprayed areas, respectively. Dioxin exposure as a result of usual dietary intake was less affected in the sprayed area than in the non-sprayed area, thus suggesting that past exposure rather than present dietary intake may affect current dioxin concentrations in breast milk in the sprayed area. As far as adverse health effects in humans are concerned, the visual acuity of both eyes for people in the sprayed area was significantly lower than that for people in the non-sprayed area on the basis of the contrast acuity test. Cortisol and cortisone levels in the saliva of primiparae in the hot-spot area were significantly higher than those in the non-exposed area. The correlation between salivary cortisol and cortisone levels and the PCDD + PCDF TEQ levels of breast milk in the combination of hot spot and non-exposed area was significant according to the curve (bell type), whereas the curve for estradiol levels and dioxin exposure was U-shaped.

Keywords: epidemiological study, dioxin exposure, visual acuity, sister chromatid exchange, steroid hormone
Key facts

- At Bien Hoa, a former USA Air Force base, the highest concentrations of 2,3,7,8-tetra-chlorinated dibenzo-p-dioxin (TCDD) were 1,164,699 pg/g in soil and 177 pg/g in sediment samples. Together with other former military airbases, such as Phu Cat and Da Nang, these airbases are considered to be ‘hot spots’ for dioxin contamination in Vietnam.
- Food frequency questionnaire for Vietnamese is a tool to estimate the habitual nutrient intake of the Vietnamese population. The food frequency questionnaire is a standard tool in nutritional epidemiology and calculates the intake of nutrients.
- Cortisol is a corticosteroid hormone produced by the zona fasciculata of the adrenal cortex, which is part of the adrenal gland.
- Estradiol is a hormone produced from androgen in the ovary, adrenal cortex, and brain which has a critical impact on reproductive and sexual function and also affects other organs and the bones. Stimulation of estradiol metabolism may therefore not be the only antiestrogenic effect of TCDD.

Summary points

- Several decades after herbicide spraying ceased, dioxin concentrations in the environment and in humans remain elevated in the sprayed areas of South Vietnam.
- Dioxins are lipophilic compounds that have a tendency to accumulate in the fatty tissues of animals and humans.
- Human breast milk is good internal exposure indicator for estimating an individual’s dioxin level.
- The polychlorinated dibenzo-p-dioxin (PCDD)/polychlorinated dibenzofuran (PCDF) concentrations and toxic equivalents (TEQs) in the hot spots and sprayed areas are significantly higher than those in the unsprayed areas.
- Breastfeeding has been found to be an important route of dioxin release in nursing mothers, with dioxin concentrations in the breast milk of multiparae being lower than those in primiparae in the hot spots and the sprayed and unsprayed areas.
- Dioxin exposure resulting from usual dietary intake is less affected in the sprayed area than in the non-sprayed area, thus meaning that past exposure rather than present dietary intake may have a larger effect on current dioxin concentrations in breast milk in the sprayed areas in Vietnam.
- The cortisol and cortisone levels in the saliva of primiparae in the hot-spot area are significantly higher than those in primiparae from the non-exposed area.
- The correlation between salivary cortisol and cortisone levels and the PCDD + PCDF TEQ levels of breast milk in the combination of hot-spot and non-exposed area is significant according to the curve (bell-type), whereas the curve between estradiol levels and dioxin exposure is U-shaped.
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Abbreviations

2,4,5-T 2,4,5-trichlorophenoxyacetic acid
ACTH Adrenocorticotropic hormone
AHR Aryl hydrocarbon receptor
AHRR Aryl hydrocarbon receptor repressor
CYP Cytochrome P450
GIS Geographic information system
PCDDs Polychlorinated dibenzo-p-dioxins
PCDFs Polychlorinated dibenzofurans
SCE Sister chromatid exchange
TCDD 2,3,7,8-tetra-chlorinated dibenzo-p-dioxin
TEQ Toxic equivalent
β Standard partial regression coefficient

46.1 Introduction

46.1.1 Vietnam War

During the Vietnam War (1961-1971), the United States military carried out an operation codenamed Ranch Hand that involved spraying over 19.5 million gallons of herbicide onto the land to defoliate large areas of forest and crops in Vietnam, Laos and Cambodia in order to deny their use by opposition forces (Stellman et al., 2003). Agent Orange, a 50:50 mixture of the herbicides 2,4,5-T and 2,4-dichlorophenoxyacetic acid, accounted for two thirds of the herbicides used. The defoliant 2,4,5-T was, however, contaminated with an extremely toxic substance, namely TCDD, which is known to have adverse effects on human health.

46.1.2 What are dioxin and its congeners?

In general, dioxins refer to PCDDs, PCDFs and coplanar polychlorinated biphenyls, which are lipophilic compounds that bind to sediment and organic matter in the environment and which have a tendency to accumulate in the fatty tissues of animals and human beings.

46.2 Dioxin in human breast milk

46.2.1 Origin of dioxin

It has been reported that the largest source of dioxin contaminants is dietary intake, which accounts for over 90% of total exposure (Liem et al., 2000). As dioxins are lipid soluble and tend to accumulate in adipose tissue, such as seafood, meat, dairy products and eggs, the highest concentrations accumulate in human tissue via the food chain. These studies also suggest that
human intake of dioxins has been decreasing for several decades (Bilau et al., 2008; James et al., 2003; Mato et al., 2007). Moreover, several studies have reported positive correlations between the intake of fish and dairy products and dioxin concentrations in breast milk, which are specific factors influencing the accumulation of dioxin in humans.

46.2.2 Exposure index

It is very important to select breast milk as a human specimen since it is relatively easy to determine the individual exposure level of dioxins. In contrast, it is very difficult to analyze dioxins in blood due to the large amounts needed. Human breast milk is therefore a good internal exposure indicator for estimating an individual's dioxin levels.

46.2.3 Previous studies in Vietnam

Schecter et al.’s study of dioxin contamination in food eaten by Vietnamese people (2003) focussed on Agent Orange in the 30 to 40 years that have passed since the herbicides were sprayed. This study found a total TEQ of 286-343 ng/kg wet weight for ducks, 0.35-48 ng/kg wet weight for chickens, and 0.19-66 ng/kg wet weight for fish, compared with normal TCDD levels in food of less than 0.1 ng/kg. Moreover, previous studies have reported the presence of dioxin in soil, food, human blood and breast milk samples from southern Vietnam even though the Agent Orange contamination occurred 30-40 years prior to sampling (Dwernychuk et al., 2002; Mai et al., 2007; Schecter et al., 2006). There is therefore a potential for continual dioxin exposure as a result of the consumption of fish and meat from contaminated areas.

Human breast milk samples have been randomly collected in various areas in Vietnam since 1970. The samples collected in 1970 contained strikingly high TCDD concentrations ranging from 333 to 1,832 pg/g lipid. Samples collected in subsequent years contained lower TCDD concentrations (133-266 pg/g lipid in 1973; 2.1-11 pg/g lipid in 1985-1988; 1.4-19 pg/g lipid in 1999). However, these levels were much higher than the concentration of 2 pg/g lipid found in the unsprayed areas.

From an epidemiological point of view, Tai et al. (2011) collected breast milk samples from mothers living in geographically widespread areas in Vietnam, including the hot spots and the sprayed and unsprayed areas, estimated current dioxin exposure levels of Vietnamese nursing mothers, and clarified the factors that contribute to the elevated dioxin concentrations in breast milk of mothers living in contaminated areas. Breast milk samples were collected from 520 nursing mothers residing in these areas in order to quantify the dioxin levels. The total toxic equivalents of 2,3,7,8-substituted PCDDs/PCDFs in breast milk of mothers living in the hot spots and the sprayed and unsprayed areas were 14.10, 10.89, and 4.09 pg/g lipid, respectively, for primiparae, and 11.48, 7.56, and 2.84 pg/g lipid, respectively, for multiparae, thus highlighting the significant differences in the values for the three areas. Dioxin levels in the hot spots were highly correlated with the residency of mothers after adjustment for their age and parity.
46.2.4 Primiparae and multiparae

Breastfeeding has been found to be an important route of dioxin release in nursing mothers. Thus, dioxin concentrations in the breast milk of multiparae were lower than those for primiparae in general populations (LaKind et al., 2001; Uehara et al., 2006; Wittsiepe et al., 2007). The PCDD/PCDF TEQ ratios for multiparae and primiparae were 0.81, 0.69, and 0.69 in the hot spots and the sprayed and unsprayed areas, respectively. When this ratio was calculated only for those mothers living in Thanh Khe district, the location closest to an airbase, it increased even further to 0.89. The high ratio in the hot spots may reflect a high intake of dioxins relative to their elimination through breastfeeding in multiparae (Table 46.1). It is also consistent with the above assessment that current dioxin exposure in the hot spots remains elevated.

46.2.5 Congener pattern of dioxins in human breast milk

Tawara et al. (2011) have investigated the residual congener pattern of dioxins in human breast milk in southern Vietnam. Breast milk samples for analysis were collected in 2002 and 2003 from lactating primiparous and multiparous mothers born after the war (<31 years old) in Cam Chinh commune, Quang Tri province (an area sprayed with tactical herbicides), and the Cam Phuc commune, Ha Tinh province (a non-sprayed area). The levels of each congener in the Cam Chinh commune were higher than in the Cam Phuc commune. The specificity of the

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Table 46.1. Categorization of 2,3,7,8-tetra-chlorinated dibenzo-p-dioxin (TCDD) concentration and polychlorinated dibenzo-p-dioxins (PCDD)/polychlorinated dibenzofurans (PCDF) toxic equivalents (TEQs) in the breast milk of mothers from the hot spots and the sprayed and unsprayed areas in Vietnam (with permission from ACS Publications).
PCDD/DF congener pattern in Cam Chinh commune samples was subsequently determined by cluster analysis (Figure 46.1) and found to be characterized by higher (hexa-, hepta-, and octa-) chlorinated PCDD/DFs. The profile was therefore similar to that presented by pentachlorophenol rather than 2,4,5-T contaminated with TCDD. A gas chromatograph and mass spectrometer study in the 1970s detected the chlorophenols 2,4-di- and 2,4,6-trichlorophenol in some Agent Orange samples, which, like pentachlorophenol, contained a wide variety of PCDD/DF congeners. In this context, it is likely that certain tactical herbicides contaminated with various chlorophenol impurities have a unique congener pattern when compared with pure 2,4,5-T formulations.

46.2.6 A geographic information system study

In environmental health research, there is a recognized need to develop methodologies to carry out epidemiological research. A GIS is one means of improving the study of dioxins in Vietnam in order to estimate people's herbicide exposure. A GIS can also combine both extensive and intensive databases on the dispersal of herbicides, the location of US army military units and bases, and the location of civilian population centers in Vietnam, thereby providing a unique basis for the integration and improvement of epidemiological studies (Stellman et al., 2003; Viel et al., 2008). However, despite the fact that it can efficiently integrate records of where herbicides were used, GIS-based exposure assessment in epidemiological studies of the environment is still relatively uncommon.

Figure 46.1. Cluster analysis dendrogram for polychlorinated dibenzo-p-dioxins (PCDD)/polychlorinated dibenzofurans (PCDF) in breast milk from Cam Chinh commune (with permission from Elsevier).
Very few studies have applied a GIS to the study of dioxin levels in soil and breast milk in Vietnam. Nhu et al. (2009) determined the correlation between dioxin concentration in soil, sediment and breast milk in a herbicide-sprayed area in Southern Vietnam, namely Cam Chinh commune in, Quang Tri province. Quang Tri province was sprayed with herbicide 150 times at a rate of 6,602 l/hectare and borders the demilitarized zone along the 17th parallel that once divided North and South Vietnam. The concentration of PCDDs and PCDFs in soil, sediment and breast milk samples was determined and the data analyzed using the geostatistical Kriging method. Initially, breast milk samples were collected from lactating females aged between 20 and 40 years of age at Quang Tri province in 2002-2003; these samples were analyzed at the Kanazawa Medical University, Japan. The location of all these women's homes was verified using the global positioning system. The global positioning system data were coded in terms of latitude (Y) and longitude (X) and the position of the houses located on the map (Figure 46.2). Soil and sediment samples were taken from the same area (Figure 46.2) and also analyzed by the Japanese laboratory. These data were used to create a surface contour map of dioxins for this area using the Geostatistical Analyst program, one of the extension systems of ArcGIS. The black points in Figure 46.3 indicate the dioxin levels in soil and the white points the dioxin levels in breast milk. The larger black points (high dioxin level) are surrounded by dark colors, whereas the smaller black points (low dioxin level) are surrounded by lighter colors. Similarly to Figure 46.3, the black points on the Kriging map for PCDFs (Figure 46.4) indicate dioxin levels in soil and the white points dioxin levels in breast milk. However, the location of the black points is different.
from those in Figure 46.3 and the dark color is also different: in this case it is not around the high dioxin levels in soil. This means that the distribution patterns of PCDDs and PCDFs in soil are different and thus that there are no significant correlations between the estimated dioxin levels in soil obtained by the Kriging method and those in breast milk. Consequently, the possibility remains that another exposure route, such as exposure to herbicides used during the Vietnam War, might affect dioxin levels in breast milk, although more soil data are needed to make more reliable geographical estimations.

46.2.7 Dioxin in breast milk and food group intake in Vietnam

Although animal meat has previously been examined as a possible dioxin source in Vietnam, the relationship between the consumption of different foods and the concentration of dioxin
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Figure 46.4. Estimated distribution of polychlorinated dibenzofurans (PCDF) from soil samples, as determined using the Kriging method (with permission from The Japanese Society for Hygiene).

in breast milk remains unclear. Furthermore, nutritional investigations in herbicide-sprayed areas in Vietnam have proved inconclusive. The main dietary source of dioxins in Japan is fish, whereas the main sources in Europe and the United States are meat and dairy products, thus reflecting differences in dietary habits. As a result, more detailed information is needed to elucidate the role of routine dietary intake on human exposure to dioxins 30-40 years after Agent Orange use in Vietnam was discontinued. Such information may also prove useful in discussing the prevention of exposure to dioxin via the diet. Saito et al. (2010) have attempted to clarify the association between dioxin concentrations in breast milk and food group intake in herbicide-sprayed and non-sprayed areas in Vietnam and also to investigate the specific factors influencing dioxin levels in breast milk. This survey was conducted in August of 2007. A total of 80 mothers from the sprayed area and 42 mothers from the non-sprayed area participated in the study, and had the concentration of PCDDs and PCDFs in their breast milk determined.
Furthermore, all participants completed a questionnaire to obtain information regarding their personal characteristics and usual dietary intake. Dietary intake was assessed by a food frequency questionnaire for Vietnamese developed by Kusama \textit{et al.} (2005). Table 46.2 shows the TEQ levels for PCDDs and PCDFs and Total (PCDDs + PCDFs) in breast milk, which were significantly higher in the sprayed area than in the non-sprayed area. Table 46.3 summarizes the median energy, nutrient and food group intake per day (with 25 and 75 percentiles) in the sprayed and non-sprayed areas and a comparison of the difference between the two. Mean energy intake did not differ significantly between the sprayed and non-sprayed areas (1,854 and 1,793 kcal/day, respectively). However, lipid intake was higher in the non-sprayed area than in the sprayed area ($P=0.036$). The intake of fruit and fruit juice was highest in both areas, followed by cereals, meat and meat products, dark green vegetables, sugars, confectioneries, and soft drinks. The intake of pulses ($P<0.001$), yellow vegetables ($P<0.011$), fruit and fruit juice ($P=0.044$), and alcoholic beverages ($P<0.001$) was higher in the sprayed area than in the non-sprayed area. In no instance was the intake of any food significantly higher in the non-sprayed area than in the sprayed area.

Multiple linear regression analysis was used to determine which food groups were significantly correlated with breast milk PCDDs, PCDFs and TEQ-Total as dependent variables, with subject characteristics (area, age, BMI and monthly income of husband) and food group intake as independent variables. To reduce the number of variables in the multivariate model, prognostic factors were included in the model by way of a mixed-direction stepwise analysis. A multiple linear regression analysis involving all subjects (sprayed and non-sprayed areas) found that area was the only variable associated with PCDDs ($\beta=0.610$, adjusted $R^2=0.367$), PCDFs ($\beta=0.643$, adjusted $R^2=0.408$) and TEQ-Total ($\beta=0.647$, adjusted $R^2=0.413$). In the sprayed area, the associations between food group intake and PCDDs (adjusted $R^2=0.072$), PCDFs (adjusted $R^2=0.052$), and TEQ-Total (adjusted $R^2=0.026$) concentrations showed that the adjusted $R^2$ values of regression were small (Table 46.4). In the non-sprayed area, the adjusted $R^2$ values of regression were higher than in the sprayed area for all models with PCDDs (adjusted $R^2=0.216$), PCDFs (adjusted $R^2=0.296$) and TEQ-Total (adjusted $R^2=0.182$) as a dependent variable (Table

| Table 46.2. Comparison of dioxin concentrations [pg toxic equivalent/g fat] in breast milk between herbicide-sprayed and non-sprayed areas (with permission from The Japanese Society for Hygiene).\(^1\) |
|---------------------------------|-----------------|-----------------|-----------------|
| **Dioxins**                     | **Sprayed area (n=80)** | **Non sprayed area (n=42)** | **P-value**     |
| TEQ-PCDDs                       | 4.54 (2.95-6.39) |
| TEQ-PCDFs\(^2\)                | 5.06 (3.37-7.91) |
| TEQ-total                       | 10.13 (6.46-14.20) |
|                                | 1.88 (1.55-2.34) |
|                                | 1.99 (1.40-2.39) |
|                                | 3.80 (2.97-4.81) |
|                                | <0.001\(^a\)     |
|                                | <0.001\(^b\)     |
|                                | <0.001\(^b\)     |

\(^1\) Data are shown at median (25-75\(^{th}\) percentile).
\(^2\) Data of TEQ-PCDFs are log-transformed to improve normality.
\(^a\) t-test.
\(^b\) Wilcoxon signed rank test.
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Table 46.3. Dietary intake from food frequency questionnaire in herbicide-sprayed and non-sprayed areas (with permission from The Japanese Society for Hygiene).  

| Dietary intake | Sprayed area (n=80) | Non sprayed area (n=42) | P-value  

| Energy | 1,854 (1,440-2,423) | 1,793 (1,389-2,225) | 0.620  

| Protein | 87.2 (78.5-92.9) | 87.6 (83.3-95.7) | 0.227  

| Lipid | 52.8 (45.3-60.0) | 56.7 (52.3-61.7) | 0.036  

| Carbohydrate | 290.4 (270.4-312.4) | 276.9 (269.1-294.7) | 0.059  

| Fiber | 12.8 (10.9-16.0) | 15.0 (12.4-19.4) | 0.018  

| Cereals | 255.2 (205.5-330.6) | 239.9 (185.6-324.6) | 0.435  

| Potatoes and starches | 31.0 (10.5-66.0) | 40.8 (25.6-70.5) | 0.070  

| Nuts and seeds | 25.0 (15.1-26.2) | 25.0 (12.8-25.0) | 0.984  

| Pulses | 0 (0.0) | 0 (0.0) | <0.001  

| Soybean products | 15.1 (7.6-30.6) | 15.1 (7.6-30.2) | 0.617  

| Dark green vegetables | 175.4 (126.8-254.0) | 197.2 (120.8-299.7) | 0.309  

| Yellow vegetables | 45.3 (26.8-69.3) | 32.0 (13.8-43.9) | 0.011  

| Other vegetables | 126.8 (86.1-207.1) | 94.0 (57.9-179.0) | 0.063  

| Fruit and fruit juice | 504.7 (402.0-694.1) | 459.7 (290.1-604.3) | 0.044  

| Fats | 6.3 (2.7-12.0) | 7.2 (1.7-10.8) | 0.436  

| Vegetable oils | 16.1 (10.5-21.6) | 15.0 (8.9-23.0) | 0.663  

| Meat and meat products | 206.0 (130.1-316.7) | 262.1 (172.0-372.3) | 0.125  

| Fish and fish products | 52.6 (33.4-84.6) | 59.6 (30.7-109.0) | 0.777  

| Shellfish and shellfish products | 14.0 (5.1-31.0) | 13.9 (5.0-28.3) | 0.944  

| Eggs | 30.2 (16.3-49.4) | 33.4 (12.5-76.0) | 0.668  

| Milk and dairy products | 0.6 (0.7) | 0 (0.0) | 0.984  

| Sugars, confectioneries, soft drink | 159.5 (122.3-222.8) | 162.1 (112.9-183.9) | 0.236  

| Alcohol beverages | 0.4 (0.7) | 0 (0.0) | <0.001  

| Seasonings | 41.3 (28.2-54.1) | 44.2 (30.1-57.0) | 0.508  

1 Data are shown at median (25-75th percentile). Nutrient intake is adjusted for energy intake by the residual method.
2 Units of energy data is kcal/day and others is g/day.
3 Wilcoxon signed rank test.

46.4). In the non-sprayed area, all models showed an association between BMI and TEQ-PCDDs, PCDFs, and TEQ-Total (β=0.333, 0.413, and 0.349, respectively). PCDDs were most strongly associated with intake of yellow vegetables (β=0.442) and PCDFs with intake of shellfish and shellfish products (β=0.350), whereas no association was found between TEQ-Total and any factor other than BMI. It was therefore concluded that dioxin exposure was less affected by usual dietary intake in the sprayed area than in the non-sprayed area in Vietnam. Likewise, it was
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Table 46.4. Stepwise multiple linear regression of toxic equivalent (TEQ)-PCDDs, TEQ-polychlorinated dibenzo-p-dioxins (PCDF), TEQ-Total levels in breast milk and food group intake in herbicide-sprayed and non-sprayed areas (with permission from The Japanese Society for Hygiene).

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<td>TEQ-Total</td>
<td>0.182</td>
</tr>
<tr>
<td>Constant</td>
<td>&lt;0.0001</td>
<td>TEQ-Total</td>
<td>0.627</td>
</tr>
<tr>
<td>Nuts and seeds</td>
<td>-0.197</td>
<td>0.080</td>
<td>0.349</td>
</tr>
</tbody>
</table>

All data are log-transformed for analysis. Variables: age, BMI, cereals, potatoes and starches, nuts and seeds, pulses, soybean products, dark green vegetables, yellow vegetables, other vegetables, fruit and fruit juice, fats, vegetable oils, meat and products, fish and products, shellfish and products, eggs, milk and dairy products, sugars, confectioneries, soft drink, alcohol beverages, seasonings.

suggested that past exposure rather than present dietary intake may have a greater effect on current dioxin concentrations in breast milk in the sprayed area. Indeed, this study suggests that present dioxin concentrations in breast milk were a result of continuous past exposure even after 30-40 years had passed.
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46.3 Human health effects

Epidemiological studies have been conducted in herbicide-sprayed and non-sprayed areas since 2002 to clarify the adverse health effects induced by the dioxins present in herbicides sprayed during the Vietnam War. We have previously reported significantly higher dioxin levels in serum, breast milk and adipose tissues in inhabitants of sprayed areas than those in non-sprayed areas, although no significant differences were found regarding early indicators of adverse health effects such as liver or thyroid function and immunological activity.

46.3.1 Visual acuity

Dioxins have similar characteristics, especially lipid-solubility, as organic solvents, some of which are known to readily induce neurological dysfunction. In light of this, the visual acuities of 83 women from a sprayed area and 68 women from a non-sprayed area were determined using the contrast acuity test (CAT 2000, Neitz, Japan) by Kido et al. (2006). The visual acuity of both eyes of those subjects from the sprayed area were significantly lower than those from the non-sprayed area under all conditions, with a change of contrast from 100 to 2.5%, except for 2.5% contrast in the left eye ($P<0.01$). (Figure 46.5) The simple relationship between dioxin levels and visual acuity was found to be significant for both eyes ($P<0.05$). A multiple regression analysis was subsequently performed using contrast acuity as an objective variable and area, age, delivery number, and dioxin levels as explanatory variables. Only the area factor was found to be significant ($P<0.01$; Table 46.5). These results did not show a clear relationship between current dioxin levels and visual acuities in the inhabitants of these areas in Vietnam. However,

![Figure 46.5. Comparison of the visual acuity of the right eyes of lactating mothers from herbicide-sprayed and non-sprayed areas.](image-url)
the herbicides sprayed during the war may still be one of the main risk factors accounting for the difference in visual acuities amongst lactating mothers in the two areas as they are considered to be involved in one of the factors that differentiate the two areas.

### 46.3.2 Sister chromatid exchange

Horikawa *et al.* (2008) investigated the genetic effects of dioxins in the sprayed and non-sprayed areas by checking SCE. Thus, peripheral blood samples were collected from individual women (51 in Quang Tri province and 20 in Ha Tinh province) and the SCE values of those from the sprayed area were found to be significantly higher than those from the non-sprayed area. Furthermore, individual log-transformed SCE values were found to be strongly correlated with the log-transformed TEQ values ($r=0.463$; Figure 46.6 and 46.7). This report was the first to suggest the genotoxicity of internal dioxins in Vietnamese people exposed during the Vietnam War. Although many animal or *in vitro* studies have shown that exposure to dioxins increases SCE values (Rowland *et al.*, 2007), no similar studies have shown a correlation between internal dioxin levels and SCE values in humans. Dioxins are considered to express their toxicity via the AHR pathway, with exposure to dioxins leading to negative feedback by expression of an AHRR. It should be noted, however, that a substantial number of individuals in the sprayed area have similarly low SCE and TEQ values to those in the non-sprayed area. The AHR pathway involves various factors including AHR, AHRR, and AHR nuclear trans-locator, most of which have several genotypes. Of these, the AHRR genotypes are considered to be involved in susceptibility to dioxins. A genotyping study of genes involved in the AHR pathway may help to explain the different susceptibility of individuals to dioxins.

Table 46.5. Relationship between acuity and area, age, delivery number, and dioxin levels in breast milk.

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Acuity of right eye (contrast 100% $n=130$)</th>
<th>Acuity of left eye (contrast 100% $n=133$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>$P$-value</td>
</tr>
<tr>
<td>Area (Sprayed area: 1, Non-sprayed area: 2)</td>
<td>0.321</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Age</td>
<td>-0.203</td>
<td></td>
</tr>
<tr>
<td>Number of delivery</td>
<td>0.153</td>
<td></td>
</tr>
<tr>
<td>Dioxin level</td>
<td>-0.015</td>
<td></td>
</tr>
<tr>
<td>Multiple correlation coefficient</td>
<td>0.378</td>
<td></td>
</tr>
</tbody>
</table>

$^1 \beta$: Standardized partial regression coefficient.
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Figure 46.6. Comparison of individual sister chromatid exchange (SCE) values from the Quang Tri (QT-high), Quang Tri (QT-middle), and Ha Tinh groups (HT).

Figure 46.7. Correlation between log-transformed sister chromatid exchange (SCE) and toxic equivalent (TEQ) values.
46.3.3 Steroid hormone

Hormone levels are known to be altered by low-dose exposure to TCDD, with fetal death occurring after high-dose exposure. Li *et al.* (2005) have demonstrated that the biosynthesis of androgens, cortisol, and aldosterone is altered by dioxin-like PCB126 in human adrenocortical H295R cells.

In light of this, Nhu *et al.* (2010, 2011) determined the effect of dioxin exposure on adrenal steroids in saliva, and dioxin levels in breast milk, of primiparae in an Agent Orange/dioxin hot spot in comparison with a non-exposed area in Vietnam. Cortisol and cortisone levels in the saliva of primiparas in the hot-spot area were found to be significantly higher than those in the non-exposed area. The correlation between cortisol and cortisone and the PCDD + PCDF TEQ in the combination of hot-spot and non-exposed area was significant according to the curve (bell-type, inverted U type) (Figure 46.8). The curve between estradiol levels and dioxin exposure was U-shaped (Figure 46.9). These results showed correlations between the salivary estradiol level and PCDF or PCDD + PCDF TEQ in the combination of hot-spot and non-exposed areas. However, the plots of progesterone and testosterone levels in saliva, and dioxin in breast milk, showed no correlations between progesterone or testosterone levels and PCDD, PCDF, or PCDD + PCDF TEQs in either area.

Previous studies have shown that endocrine disruption often follows similar curves (U- or inverted U-shaped; Crews *et al.*, 2006; Welshons *et al.*, 2006). The combined effect of these multiple actions of endocrine disrupters can, however, be difficult to interpret and is often misinterpreted as variability in the assay system. Indeed, all hormones exert non-linear actions on their targets, thus implying that the combined effect of these multiple non-linear dose-responses is not predictable.

![Figure 46.8. Correlation between cortisol in saliva and dioxin (TEQ PCDDs + PCDFs) in breast milk of primiparae (with permission from Taylor & Francis).](image-url)

\[ y = -26.656x^2 + 823.94x - 2261.4 \]

\[ r = 0.601 \]

\[ P < 0.01 \]
Dioxins in human breast milk in Vietnam

Contrasting results were found for estradiol, where the observed reduction in estradiol levels was likely due to the inhibitory effects of dioxin on CYP17 and 17,20-lyase activity in the ovary rather than on aromatase activity. In this respect, Moran et al. (2003) have reported that the molecular target for endocrine disruption of human luteinizing granulosa cells by dioxin is CYP17, which specifically decreases the supply of androgens for estradiol synthesis, rather than aromatase. Furthermore, Li et al. (2005) have demonstrated that dioxin-like PCB126 diminishes androstenedione production as well as CYP17 mRNA and 17α-hydroxylase and 17,20-lyase activity (CYP17) in human adrenocortical H295R cells, whereas dioxin-like PCB126 stimulates cortisol biosynthesis by CYP11B1 induction and controls CYP21B by suppressing c-AMP-induced CYP21B expression. Furthermore, a high concentration of PCB126 might sensitize ACTH regulation in adrenocortical cells by increasing ACTH receptor levels. Such complex and multiple effects of dioxins on adrenal steroid levels seem to result in non-linear curves. Likewise, both dioxin and PCB were found to accumulate in the adrenal gland and may therefore alter adrenal steroid synthesis. TCDD has been implicated in the decreased bioactivity of ACTH, the primary secretagogue for corticosteroid production. ACTH increases the concentration of cholesterol in the inner mitochondrial membrane and also stimulates the main rate-limiting step in cortisol synthesis during which cholesterol is converted to pregnenolone in a reaction catalyzed by cytochrome P450scc, which initiates steroid biosynthesis.

The salivary gland contains 11α-hydroxysteroid dehydrogenase, which converts most of the cortisol in blood plasma to cortisone as the hormone passes through this gland. Dioxin might therefore stimulate this enzyme in the salivary gland, thereby increasing the rate of conversion of cortisol into cortisone. The salivary cortisol levels showed significant correlation with cortisone
levels in blood. Saliva sample can be collected non-invasively. Therefore, analysis of steroid hormone in saliva will be developed as a useful indicator for epidemiological studies.

46.3.4 On children

Body measurements (body height, body weight, head circumstance, and chest circumstance) were compared for children aged 4 to 16 weeks from both sprayed and non-sprayed areas. No significant differences were found between the two areas. The relationship between dioxin in mothers’ breast milk and each body measurement was also studied, and body weight and chest measurement ($P<0.05$) were found to exhibit statistically significant negative correlations with Total PCDDs/PCDFs. The results of a multiple regression analysis with body weight or chest measurement of the subjects as dependent variables and TEQ PCDDs/PCDFs in breast milk (geometric mean) and age of infants as explanatory variables showed significant relations between Total PCDDs/PCDFs and body weight or chest measurement ($P<0.01$) in 43 subjects.

In a related study undertaken in 2011, we collected saliva from children aged 1 and 3 years and analyzed the dioxin levels in their mothers’ breast milk together with salivary hormones such as cortisol, cortisone and dehydroepiandrosterone. The results of this study will be published in due course.

References


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T. Kido et al.


