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REGULAR ARTICLE

Association between dioxin concentrations in breast milk and food group intake in Vietnam

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Abstract

Objectives To clarify the association between dioxin concentrations in breast milk and food group intake in herbicide-sprayed and nonsprayed areas in Vietnam.

Methods This survey was conducted in August 2007 in sprayed and nonsprayed areas, respectively. The interviews were performed using a questionnaire to obtain information on personal characteristics and usual dietary intake. Eighty mothers of sprayed area and 42 mothers of nonsprayed area participated in the study. Breast milk was analyzed for concentration of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs).

Results Multiple regression analysis showed that location (sprayed or nonsprayed area) has the highest association with the toxic equivalents (TEQ)-PCDDs, TEQ-PCDFs, and TEQ-Total rather than other factors. In the sprayed area, the adjusted R^2 values of regression were approximately 0.1. On the other hand, the adjusted R^2 values in the nonsprayed areas were higher than those in the sprayed area, i.e., between 0.2 and 0.3, and showed that there were significant associations with body mass index (BMI) in all models.

Conclusions Dioxin exposure was less affected by usual dietary intake in the sprayed area than in the nonsprayed area in Vietnam. It was clear that past exposure rather than present dietary intake may affect present dioxin concentrations in breast milk in the sprayed area in Vietnam. This study suggests that present dioxin concentrations in breast milk were maintained by continuous past exposure even after 30–40 years had passed.

Keywords Dioxin · Breast milk · Food · Dietary intake · Vietnam

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Introduction

During the Vietnam War, herbicide was sprayed over forests and villages in Central and Southern Vietnam to defoliate the vegetation between 1961 and 1971. The primary mixture used was Agent Orange, which contained 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) [1]. In general, dioxins refer to polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar polychlorinated biphenyls (co-PCBs), 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.

It has been reported that the largest source of dioxin contaminants is dietary intake, which accounts for more than

90% of total exposure [2]. Because dioxins are lipid soluble and tend to accumulate in adipose tissue, such as seafood, meat, dairy products, and eggs, the highest concentrations are accumulated in human tissue through the food chain. These studies also suggest that human intake of dioxin has been decreasing for several decades [2–6]. Moreover, several studies have reported positive correlations between the intake of fish and dairy products and dioxin concentrations in breast milk, which are the specific factors influencing the accumulation of dioxin in humans [7–9]. There have, however, been few studies in Vietnam, in spite of the fact that a great deal of herbicide was sprayed there.

The 2003 study of dioxin contamination in food eaten by the Vietnamese people carried out by Schecter et al. [10] was the first document focusing on Agent Orange in the 30–40 years that have passed since the herbicides were sprayed. That study showed a total TEQ for ducks from 286 to 343 ppt wet weight, for chickens from 0.35 to 48 ppt wet weight, and for fish from 0.19 to 66 ppt wet weight, against usual TCDD levels in food of less than 0.1 ppt. Moreover, previous studies have reported dioxin in samples of soil, food, human blood, and breast milk in southern Vietnam, even though the Agent Orange contamination occurred 30–40 years before sampling [11–13]. Therefore, there is potential for continuous dioxin exposure through the consumption of fish and meat from contaminated areas.

Previously in Vietnam animal meat was examined; however, the relationship between the consumption of different foods in the diet and the concentration of dioxin in breast milk remains unclear. In addition, nutritional investigations in herbicide-sprayed areas in Vietnam have been inadequate. In Japan the main source of dietary intake of dioxins is fish, while the main sources in Europe and the USA are meat and dairy products, reflecting differences in dietary habits [14]. For these reasons, more detailed information is needed to elucidate the role of routine dietary intake in human exposure to dioxin 30–40 years after Agent Orange use was discontinued in Vietnam. Such information may also prove useful in discussing the prevention of exposure to dioxin through dietary intake. The purpose of this study is to clarify the association between dioxin concentrations in breast milk and food group intake in herbicide-sprayed and nonsprayed areas in Vietnam and also to investigate the specific factors influencing dioxin levels in breast milk.

Materials and methods

Study population

Study areas were designated in the north-central area of Vietnam, namely the Cam Chinh commune in the Cam Lo district of Quang Tri province, where herbicides were sprayed during the war, and the Cam Phuc commune in the Cam Xuyen district of Ha Tinh province, which was not sprayed with herbicides during the war. These two communes were once separated by the demilitarized zone (DMZ), the 17th parallel that divided the country during the Vietnam War. A large number of herbicides were sprayed on Cam Chinh commune on the southern side of the DMZ, but they were not sprayed on Cam Phuc commune on the northern side.

In this study, participants were selected from a 2002–2003 survey of the long-term effects of dioxin on human health. Briefly, this investigation sought to clarify the relationship between dioxins and ecological and human health in Vietnam. Ninety lactating mothers in a sprayed area and 72 lactating mothers in a nonsprayed area were recruited for the investigation in September 2002 and July 2003. Participants were between 20 and 30 years old and provided 10 to 20 ml breast milk. Samples were analyzed for dioxin concentrations. The methods and results of that study were reported previously [15–17].

This survey was conducted in August of 2007 at local district health centers in sprayed and nonsprayed areas, respectively. The interviews were performed using a questionnaire to obtain information on personal characteristics and usual dietary intake by Vietnamese researchers specially trained for this study and who belonged to the 10-80 Division of the Vietnam Ministry of Health.

Measurements

Information on age, family size, number of children, years of residence, monthly income of husband, level of education, occupation, and smoking status were obtained by the original questionnaire. Body height and weight were measured and body mass index (BMI) was calculated. Dietary intake was assessed by a food frequency questionnaire (FFQ) for Vietnamese. This questionnaire was developed by Kusama et al. [18] as a tool to estimate the habitual nutrient intake of the Vietnamese population. The FFQ is a standard tool in nutritional epidemiology and calculates the intake of nutrients. The intake of calories and nutrients were computed by multiplying the frequency of intake for each food item by the nutrient content of the specified portion size. The reproducibility and validity of this FFQ were established by using 24-h dietary recalls (24HRs) and repeated FFQ. This FFQ consisted of a 116-item food list (foods and dishes) and questions about breakfast and ingredients for dishes eaten at lunch and dinner, and includes foods and dishes, consumption frequency, and portion size. Question responses were given on a scale from 1 to 10 for frequency of consumption: never, less than once per month, 2–3 times per month, 1–2 times per week, 3–4 times per week, 5–6 times per week, once per day, 2–3 times per day, 4–5 times per day, and more than 6 times per day. Portion size was categorized into three sizes: small (approximately half of the median size), median, and large (1.5 times the median size). Participants were asked about the average consumption frequency and portion size of each of the food items listed in the questionnaire using a book with full-sized photographs of all food items at the median size for the FFQ interview to improve the accuracy of the estimation of portion size.

Data analysis

Analysis focused on dioxin concentrations in breast milk in years 2002 and 2003 as a dependent variable. Independent variables were food group intake and subject characteristics. Concentrations of dioxins were presented as toxic equivalent (TEQ) levels. Calculation of TEQ was based on World Health Organization (WHO) 1998 toxic equivalency factors (TEFs) [19].

For each individual, dietary intake was calculated by the Vietnam EIYOKUN dietary assessment system. Each nutrient intake was used to calculate the energy-adjusted nutrient intake as the residual from the regression, with nutrient intake as the dependent variable and energy as the independent variable.

Median with 25 and 75 percentiles of dioxin concentrations in breast milk, intake of nutrient and food groups, and subject characteristics were calculated, and differences between herbicide-sprayed and nonsprayed areas were assessed by Student *t* test, Welch's test, and Wilcoxon signed-rank test. Chi-square was used to compare differences in proportion of education levels, occupation, and smoking status between these areas. The correlation between dioxin concentrations and characteristic data and food group intake were examined using Spearman's correlation coefficients. Before calculating the Pearson correlation coefficient, the distribution of all data was carefully checked, and if any data proved unsuitable for normal distribution, values were log-transformed to improve normality. To identify the major sources of different food group intake contributing to dioxin concentrations in breast milk, stepwise multiple regression analysis of food group intake for dietary habits was used to seek the most significant combination of variables. Data analyses were carried out using JMP®6 software (SAS Institute, Japan), and the statistical level for significant difference was set at 0.05.

Ethical approvals

The purpose of the present study was explained thoroughly, and written informed consent was obtained from each participant through their local people's authorities committee. All data were transformed to codes in the analysis process for individuals and they were not identified. To conduct this survey, we obtained permission from the Medical Ethics Committee of Kanazawa University (permission no: Health-89).

Results

The study population consisted of 122 mothers, namely 80 mothers (participation rate 88.9%) in herbicide-sprayed area and 42 mothers (participation rate 58.3%) in nonsprayed area. Table 1 presents the characteristics of subjects in herbicide-sprayed and nonsprayed areas. Median age was statistically different between the sprayed and nonsprayed areas ($P = 0.016$) at 32.0 and 30.0 years, respectively. Median height (149.9 versus 152.3 cm; $P = 0.005$) and body weight (44.0 versus 45.0 kg; $P = 0.005$) were also statistically different between the two areas. However, median BMI was 19.0 and 19.3 kg/m^2, respectively, showing no statistical difference between the two areas. A statistically significant difference in education between the two groups was shown, but there were no significant differences in occupation or smoking status.

Table 2 shows the TEQ levels of PCDDs, PCDFs, and total (PCDDs + PCDFs) in breast milk in sprayed and nonsprayed areas. All dioxin concentrations values were log-transformed to improve normality. There were statistical differences ($P < 0.001$) in TEQ-PCDDs, TEQ-PCDFs, and TEQ-Total between sprayed and nonsprayed areas.

Table 3 summarizes the median intake (with 25 and 75 percentiles) of energy and nutrient and food group per day in sprayed and nonsprayed areas and a comparison of the difference between the two areas. Mean energy intake was not statistically different between sprayed and nonsprayed areas, measuring 1,854 and 1,793 kcal/day, respectively. Intake of lipid ($P = 0.036$) in the nonsprayed area was higher than in the nonsprayed area. In the two areas, intake of fruit and fruit juice was the highest; next came cereals, then meat and meat products, dark-green vegetables, sugars, confectioneries, and soft drinks. Intake of pulses ($P < 0.001$), yellow vegetables ($P < 0.011$), fruit and fruit juice ($P = 0.044$), and alcoholic beverages ($P < 0.001$) in the sprayed area were higher than in the nonsprayed area. On the other hand, there were no cases of significantly higher intake amounts in the nonsprayed area over the sprayed area.

The food group intake that correlated statistically with dioxin concentrations in breast milk in the sprayed area is shown in Table 4. In the sprayed area, no statistical correlations were found between intake of any food group intake and dioxin concentrations in breast milk. In contrast, in the

Table 1 Characteristics of subjects in herbicide-sprayed and nonsprayed areas

Item	Sprayed area ($n = 80$)	Nonsprayed area ($n = 42$)	P value
Age (years)	32.0 (28.0–36.8)	30.0 (27.0–32.0)	0.016[a]
Height (cm)	149.9 (147.6–154.3)	152.3 (149.5–155.0)	0.005[a]
Weight (kg)	44.0 (40.0–46.4)	45.0 (42.8–50.3)	0.005[b]
BMI (kg/m^2)	19.0 (17.8–20.9)	19.3 (18.4–20.8)	0.268[a]
Family size (people)	5.0 (4.0–6.0)	4.0 (4.0–5.0)	0.001[a]
Number of children (people)	2.0 (2.0–3.0)	2.0 (2.0–2.3)	0.073[a]
Residence period (years)	29.0 (18.8–33.0)	27.5 (15.8–32.0)	0.301[a]
Monthly income of husband ($\times 10^4$ VND)	100 (76–150)	100 (50–150)	0.067[a]
Education level			
<Elementary school	23 (28.8%)	23 (54.8%)	0.006[c]
>Junior high school	57 (71.3%)	19 (45.2%)	
Occupation			
Farmer	70 (87.5%)	36 (85.7%)	0.783[c]
Other	10 (12.5%)	6 (14.3%)	
Smoking status			
Current	3 (3.8%)	0 (0%)	–
Never	77 (96.3%)	41 (100%)	

Data are shown as median (25th–75th percentile) or number (%)

BMI body mass index

[a] Wilcoxon signed rank test

[b] Welch's test

[c] Chi-square

Table 2 Comparison of dioxin concentrations (pgTEQ/gFat) in breast milk between herbicide-sprayed and nonsprayed areas

Dioxins	Sprayed area ($n = 80$)	Nonsprayed area ($n = 42$)	P value
TEQ-PCDDs	4.54 (2.95–6.39)	1.88 (1.55–2.34)	<0.001[a]
TEQ-PCDFs	5.06 (3.37–7.91)	1.99 (1.40–2.39)	<0.001[b]
TEQ-Total	10.13 (6.46–14.20)	3.80 (2.97–4.81)	<0.001[b]

Data are shown as median (25th–75th percentile). Data of TEQ-PCDFs are log-transformed to improve normality

[a] *t* test

[b] Wilcoxon signed-rank test

nonsprayed area positive statistical correlations were shown between intake amounts of fats ($r = 0.35$, $P = 0.023$), shellfish and shellfish products ($r = 0.31$, $P = 0.048$), milk and dairy products ($r = 0.31$, $P = 0.043$), and seasonings ($r = 0.39$, $P = 0.011$) and breast milk TEQ-PCDFs. Moreover, soybean product ($r = 0.32$, $P = 0.039$) and seasoning ($r = 0.32$, $P = 0.042$) intake were positively correlated with breast milk TEQ-Total. However, no food group intake was significantly correlated with breast milk TEQ-PCDDs.

Multiple linear regression analysis was used to determine which food groups were significantly correlated with breast milk TEQ-PCDDs, TEQ-PCDFs, and TEQ-Total as a dependent variable. The analysis focused on breast milk TEQ-PCDDs, TEQ-PCDFs, and TEQ-Total. Independent variables were subject characteristics (area, age, BMI, and monthly income of husband) and food group intake. To reduce the number of variables in the multivariate model, prognostic factors were included in the model by mixed-direction stepwise analysis. Stepwise criteria were that the variable could enter the equation when its F statistic probability was greater than 2.0.

Table 5 presents the multiple linear regression in all subjects (sprayed and nonsprayed areas). Multiple linear regression analysis found that area was the only variable associated with TEQ-PCDDs ($\beta = 0.610$, adjusted $R^2 = 0.367$), TEQ-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 TEQ-PCDDs (adjusted $R^2 = 0.072$), TEQ-PCDFs (adjusted $R^2 = 0.052$), and TEQ-Total (adjusted $R^2 = 0.026$) concentrations showed that adjusted R^2 values of regression were small (Table 6).

In the nonsprayed area the adjusted R^2 values of regression were higher than in the sprayed area for all models using TEQ-PCDDs (adjusted $R^2 = 0.216$), TEQ-PCDFs (adjusted $R^2 = 0.296$), and TEQ-Total (adjusted $R^2 = 0.182$) as a dependent variable (Table 6). In the nonsprayed area all models showed an association between BMI and TEQ-PCDDs, TEQ-PCDFs, and TEQ-Total ($\beta = 0.333, 0.413, 0.349$). TEQ-PCDDs was most highly associated with intake of yellow vegetables ($\beta = 0.442$), and TEQ-PCDFs was associated with intake of shellfish and shellfish products ($\beta = 0.350$), while no association

Table 3 Dietary intake from FFQ in herbicide-sprayed and nonsprayed areas

Dietary intake	Sprayed area ($n = 80$)	Nonsprayed area ($n = 42$)	P value[a]
Energy	1854 (1440–2423)	1793 (1389–2225)	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 (0–27.7)	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 (0–7.1)	0 (0–18.8)	0.984
Sugars, confectioneries, soft drink	159.5 (122.3–222.8)	162.1 (112.9–183.9)	0.236
Alcohol beverages	0.4 (0–7.1)	0 (0–0)	<0.001
Seasonings	41.3 (28.2–54.1)	44.2 (30.1–57.0)	0.508

Data are shown as median (25th–75th percentile). Units of energy data are kcal/day; others are in g/day. Nutrient intake is adjusted for energy intake by the residual method

[a] Wilcoxon signed-rank test

was found between TEQ-Total and any factor except BMI.

Discussion

This study adopted the concentration data for PCDDs and PCDFs in breast milk measured in years 2002 and 2003. Consequently, the lapse of time between data collection and this study is 4–5 years. Normally, coincident measure of dioxin concentrations and dietary survey are required. Nevertheless, the half-life of dioxin in human body has been estimated to be as long as 7.5 years [20]; therefore, the decrease in dioxin concentrations in subject breast milk at the time of data collection and the present study can be expected to exhibit no great difference. In addition, the economic-social and food distribution systems in the study areas have not undergone great change during the 5 years between data collection and the present study. Furthermore, the FFQ estimates long-term intake for usual dietary habit. Hence, we consider it possible to clarify the association between dioxin concentrations in 2002–2003 and present dietary intake in spite of the limitations of this method.

Correlation coefficients between dioxin concentrations in breast milk and food group intake were shown by FFQ nutrition survey in sprayed and nonsprayed areas in Vietnam. Although the mean value and range of dioxin concentrations in foods were different among countries, previous studies have reported that the main source of dietary intake of dioxin is adipose tissue and fish [2–4]. This process of biological condensation explains the high accumulation of dioxin in animals.

However, low dioxin exposure was seen in these studies under normal conditions, making it difficult to compare this study with previous studies due to the fact that our research areas were sprayed by herbicides, including dioxin, during the Vietnam War. There were no statistical correlations between dioxin concentrations and the intake of each food group in the sprayed area. In contrast, in the nonsprayed area, there were statistical relationships between the intake of fat, shellfish and shellfish products, milk and dairy

Table 4 Spearman correlation coefficients between dioxin concentrations in breast milk and food group intake

Food group	Sprayed area (n = 80)						Nonsprayed area (n = 42)					
	TEQ-PCDDs		TEQ-PCDFs		TEQ-Total		TEQ-PCDDs		TEQ-PCDFs		TEQ-Total	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
Cereals	−0.02	0.877	−0.12	0.281	−0.10	0.362	−0.08	0.606	0.16	0.304	0.05	0.745
Potatoes and starches	0.09	0.438	0.06	0.626	0.07	0.522	−0.01	0.949	0.16	0.314	0.07	0.673
Nuts and seeds	−0.14	0.211	−0.14	0.217	−0.15	0.185	0.19	0.226	0.18	0.250	0.20	0.207
Pulses	0.02	0.832	0.06	0.573	0.05	0.641	0.20	0.208	0.19	0.238	0.20	0.203
Soybean products	0.01	0.901	0.03	0.803	0.02	0.848	0.27	0.079	0.28	0.076	0.32	0.039
Dark-green vegetables	0.02	0.866	0.07	0.534	0.07	0.552	0.10	0.545	0.06	0.722	0.08	0.618
Yellow vegetables	−0.11	0.311	−0.16	0.148	−0.15	0.176	0.17	0.275	0.26	0.097	0.23	0.149
Other vegetables	0.05	0.690	0.10	0.359	0.11	0.354	0.02	0.923	0.09	0.583	0.07	0.653
Fruit and fruit juice	−0.05	0.648	−0.07	0.520	−0.07	0.538	0.00	0.996	0.20	0.213	0.11	0.484
Fats	−0.10	0.401	−0.12	0.275	−0.12	0.270	0.15	0.346	0.35	0.023	0.27	0.079
Vegetable oils	−0.07	0.537	−0.05	0.652	−0.06	0.608	0.06	0.723	0.30	0.051	0.21	0.188
Meat and meat products	−0.02	0.854	−0.01	0.949	−0.01	0.908	−0.04	0.786	0.13	0.418	0.06	0.693
Fish and fish products	0.03	0.807	0.14	0.222	0.10	0.397	0.19	0.237	0.10	0.549	0.15	0.344
Shellfish and shellfish products	0.00	0.989	−0.05	0.674	−0.03	0.801	0.07	0.681	0.31	0.048	0.23	0.148
Eggs	0.14	0.202	−0.02	0.882	0.06	0.586	−0.16	0.320	0.12	0.441	−0.03	0.838
Milk and dairy products	0.14	0.207	0.07	0.532	0.10	0.365	0.12	0.446	0.31	0.043	0.24	0.122
Sugars, confectioneries, soft drinks	−0.02	0.837	−0.06	0.572	−0.06	0.569	0.06	0.727	0.15	0.358	0.13	0.419
Alcohol beverages	0.11	0.324	0.05	0.651	0.09	0.446	0.05	0.754	0.15	0.335	0.10	0.531
Seasonings	−0.05	0.658	−0.07	0.520	−0.07	0.522	0.18	0.246	0.39	0.011	0.32	0.042

All data are log-transformed to improve normality

r correlation coefficients

Table 5 Stepwise multiple linear regression of TEQ-PCDDs, TEQ-PCDFs, and TEQ-Total levels in breast milk and food group intake in all subjects (n = 122)

Variables	Standardized coefficients	P value	Adjusted R^2
TEQ-PCDDs			
Constant		<0.0001	0.367
Area	0.610	<0.0001	
TEQ-PCDFs			
Constant		<0.0001	0.408
Area	0.643	<0.0001	
TEQ-Total			
Constant		<0.0001	0.413
Area	0.647	<0.0001	

All data are log-transformed for analysis. Variables: area code (1 for sprayed area, 0 for nonsprayed area), age, BMI, monthly income of husband, 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

products, and seasonings with TEQ-PCDFs in breast milk, although the correlation coefficient was small, and the intake of soybean products and seasoning with TEQ-Total in breast milk. However, all correlation coefficients were approximately 0.3; therefore, these results do not show a clear relation between dioxin concentrations in breast milk and food group intake.

The difference in correlations between dioxin concentrations in breast milk and food group intake in the two areas is attributed to the presence or absence of exposure to herbicide. In the sprayed area, although there is dietary exposure to dioxins in the sprayed and nonsprayed areas similar to studies by Huisuman et al. [7] and Takekuma et al. [8], past exposure may act as a stronger factor than present dietary intake in the sprayed area. Hence, it is implied that there was no statistical association between dioxin concentrations in breast milk and present dietary intake.

Multiple regression analysis showed that location (sprayed or nonsprayed area) made the highest contribution to dioxin concentrations in breast milk. It is noted that difference of location is a stronger factor than present dietary intake; that is, it is the strongest contributor to dioxin concentrations in breast milk, regardless of type. This indication stems from the fact that there was no statistical correlation between breast milk dioxin concentrations in simple correlation coefficients, and the fact that the economic-social systems of both areas are similar.

Incidentally, because the average age of participants in this study was 31.8 ± 5.5 years, it is suggested that present

Table 6 Stepwise multiple linear regression of TEQ-PCDDs, TEQ-PCDFs, and TEQ-Total levels in breast milk and food group intake in herbicide-sprayed and nonsprayed areas

Variables	Standardized coefficients	*P* value	Adjusted R^2
Sprayed area ($n = 80$)			
TEQ-PCDDs			
Constant		<0.0001	0.072
Fats	−0.308	0.038	
Eggs	0.262	0.064	
Alcohol beverages	0.231	0.055	
Nuts and seeds	−0.206	0.073	
TEQ-PCDFs			
Constant		0.000	0.052
Fats	−0.204	0.097	
Fish and products	0.167	0.135	
Alcohol beverages	0.159	0.188	
Nuts and seeds	−0.145	0.199	
TEQ-Total			
Constant		<0.0001	0.026
Nuts and seeds	−0.197	0.080	
Nonsprayed area ($n = 42$)			
TEQ-PCDDs			
Constant		0.636	0.216
Yellow vegetables	0.442	0.011	
BMI	0.333	0.025	
Sugars, confectioneries, soft drinks	−0.285	0.083	
Soybean products	0.238	0.116	
TEQ-PCDFs			
Constant		0.009	0.296
BMI	0.413	0.005	
Shellfish and products	0.350	0.020	
Seasonings	0.269	0.074	
Pulses	0.231	0.096	
TEQ-Total			
Constant		0.627	0.182
BMI	0.349	0.022	
Yellow vegetables	0.271	0.073	
Soybean products	0.267	0.085	

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 drinks, alcohol beverages, seasonings

dioxin accumulation in human tissue was influenced by breast-feeding by mothers who were exposed directly to a herbicide, and that dioxin concentrations in breast milk were influenced by intake of foods with high concentrations of dioxin, even if almost all subjects were born after 1977, well after the cessation of herbicide spraying. Schecter et al. [21] reported that serum dioxin concentrations in the sprayed area were higher than those in the nonsprayed area in a comparison of children who were born in the sprayed area and who moved to the sprayed area from the nonsprayed area after the spraying of herbicide. Furthermore, Tada [22] suggested that dioxin concentrations in adults might affect those in infants. For these reasons, it is implied that dioxin concentrations in breast milk are not influenced by present dietary intake.

Multiple regression analysis distinguishes area, given the fact that the adjusted R^2 values of regression were small, namely approximately 0.1, though some associations were statistically significant in sprayed area. We could not explain the influencing factor in dioxin concentrations in breast milk. In contrast, the adjusted R^2 values in the nonsprayed area were higher than those of the sprayed area, that is, between 0.2 and 0.3, and there were differences between the two areas. Although standardized coefficients were different for each model, there were associations with BMI in all models in the nonsprayed area. Body burdens for lipophilic chemicals are dependent on the weight and body fat of an animal. Dioxins are all highly lipophilic, resulting in their partitioning into fatty tissues [23]. One physiologically based pharmacokinetic model accounts for changes in BMI over time, with higher BMI being related to longer half-life for TCDD [24]. The results of the present study are consistent with these previous studies.

In consequence it was made clear that past exposure rather than present dietary intake affects present dioxin concentrations in breast milk in the sprayed area in Vietnam. In contrast, it is suggested that present dietary intake and BMI might affect those in the nonsprayed area. Therefore, dioxin exposure was less affected by usual dietary intake in the sprayed area than in the nonsprayed area in Vietnam. However, in order to clarify the relationship, more detailed information on background dioxin exposure, namely food distribution systems, waste disposal methods, and pesticide use, are needed. Furthermore, past accumulation of dioxin and reduction of dioxin concentrations in the body are issues to be considered. In addition, continued study is necessary due to the great potential for continuous exposure to dioxins through contamination of foods in high-exposure areas, called "hot spots," in spite of the fact that this study did not indicate any relationship between dioxin concentrations in breast milk and dietary intake.

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Appendix

See Table 7.

Table 7 List of food items (part 1) and seasoning and cooking methods (part 2) for the food frequency questionnaire for Vietnamese adults

Part 1

Cereals

1 Steamed plain rice
2 Bread (French type)
3 Bread (sandwich type)
4 Bread (French type) with pork meat
5 Bread (French type) with canned fish
6 Bread (French type) with pemicant (smoke-dried pork, shaved)
7 Bread (French type) with fried egg
8 Sandwich
9 Rice noodle (thick type) with pork
10 Rice noodle with beef and pork
11 Rice noodle with crab
12 Rice noodle with duck and bamboo shoots
13 Rice noodle with pork, fish, squid, and shrimp
14 Rice noodle with pork and pork products
15 Rice noodle with crab and pork products
16 Rice noodle (flat type) with pork
17 Rice noodle with beef
18 Rice noodle with chicken
19 Rice noodle with beef curry
20 Fried rice noodle with pork and entrails
21 Rice noodle and Chinese noodle with pork
22 Rice noodle with pork and pork entrails
23 Soup of noodle
24 Rice with pork skin
25 Rice with omelet
26 Rice with grilled pork
27 Pork and vegetable rolled cake
28 Fried rice with vegetable
29 Rice gruel with pork blood
30 Rice gruel with pork entrails
31 Glutinous rice with black bean
32 Glutinous rice with mung bean
33 Glutinous rice with peanut
34 Glutinous rice with meat

Vegetables and potatoes

35 Amaranth, Jute potherb
36 Swamp cabbage, Sweet potato leaves
37 Mustard green, Chinese cabbage
38 Malabar nightshade
39 Crown-daisy
40 Chinese leek
41 Wort, India penny
42 Cabbage
43 French bean
44 Green pepper
45 Tomato
46 Carrot
47 Ash gourd, wax gourd
48 Bitter gourd
49 Pumpkin squash
50 Gourd
51 Cucumber
52 Broccoli
53 White potato
54 Chinese yam
55 Sweet potato

Meat

56 Pork lean
57 Pork upper leg
58 Pork medium fat
59 Pork rib
60 Pork lower leg
61 Pork fat
62 Beef
63 Chicken
64 Duck
65 Pork blood

Fish and shellfish

66 Scad, anchovy
67 Snake head fish
68 Mullet
69 Goby
70 Tuna
71 Fat fish
72 Tilapia
73 Mackerel
74 Shrimp
75 Squid
76 Fresh water crab

Eggs

77 Hen egg
78 Duck egg

Tofu (soybean curd)

79 Tofu (soybean curd) fried
80 Tofu (soybean curd) raw

Dairy foods

81 Fresh milk without sugar
82 Fresh milk with sugar
83 Soybean milk
84 Milk powder, whole
85 Skim milk
86 Yogurt
87 Condensed milk

Fruit

88 Dragon fruit
89 Banana
90 Papaya
91 Pomelo
92 Longan
93 Orange
94 Water melon
95 Pear
96 Grape
97 Guava
98 Apple

Beverages

99 Bear
100 Soft drink
101 Fruit shake juice
102 Lemon juice
103 Green leaves juice
104 Orange juice
105 Coconut juice with kernel
106 Coffee
107 Instant coffee
108 Instant coffee with milk and sugar

Sweets and dessert

109 Glutinous rice cake with mung bean and pork fat
110 Steamed rice cake
111 Baked sweet Vietnamese style
112 Sponge cake
113 Sweetened maize, banana, yam, and sweet potato
114 Sweetened bean and glutinous soup
115 Sweetened bean soup
116 Tofu (soybean curd) with sweet syrup

Part 2

Cooking methods for dishes

1 Boiled with seasoning
2 Fried
3 Sauteed
4 Boiled
5 Soup
6 Grilled
7 Salted
8 Salad

Table seasoning

1 Fish sauce
2 Salted
3 Soybean sauce

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