CURRENT ELEVATED DIOXIN LEVEL IN SERUM OF VIETNAMESE OLD MEN: PAST EXPOSURE OR CURRENT EXPOSURE

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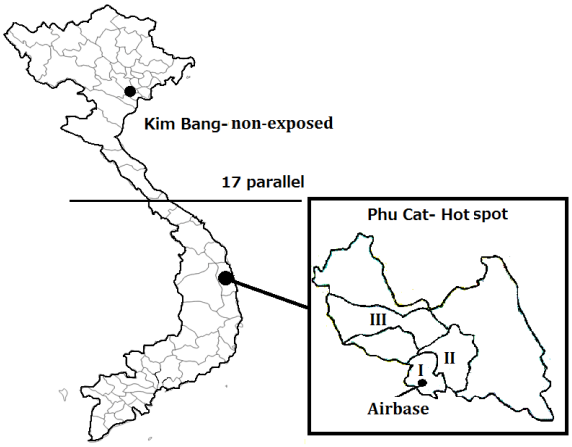
Introduction

During Vietnam War, US military sprayed 72 million liters of herbicides contaminated by 2,3,7,8 –Tetracholoro-dibenzo-p-Dioxin in the Southern Vietnam (1961-1971). Recently, Hatfield's theorized that former US Airbases are highly elevated by dioxin, which then known as hot spot areas such as former Airbases in Bien Hoa, Da Nang, Phu Cat[1]. A large study on level of dioxin in breast milk including 520 young mothers who was born after the war show that the higher level of dioxin is in hot spot, then sprayed area in comparison with non-exposed area[2]. However, there has not been any epidemiological study on Vietnamese old men, who ever experienced Vietnam War and continued to be exposed by dioxin presently. In this study, we assess the dioxin level in Vietnamese old men in a hot spot and a non-exposed area, then determine whether their elevated dioxin level caused by past exposure during herbicide spraying period or by current exposure.



Materials and methods

Between 2010 and 2011, we recruited men with age from 55-80 in Phu Cat district (n=97), which was known as hot spot and Kim Bang district (n=85) located in Northern Vietnam which was a non-exposed area. In Phu Cat district, three areas were separated depending on their proximity to the Airbase. The Airbase is inside the area I (Cat Tan and Ngo May communes), near the area II (Cat Tuong and Cat Trinh communes) and far from area III (Cat Hanh and Cat Lam communes) We collected 5ml serum to analyze dioxin by Shimadzu Techno-Research Inc., Kyoto, Japan. The analytical technique was described elsewhere[3]. Participants were interviewed about demographics and herbicide exposure history, especially where they were during herbicide spraying period (1961-1971) and after 1971, and whether they lived in defoliation area or was sprayed directly by herbicide. Dioxin data was transformed into $\log_{10}$ to improve normality. We used Student's t test and Chi-square test to compare between two groups. For more than two groups, we used ANOVA first and then Turkey's *Post hoc* test to find which pair has significant difference. We used multiple linear regressions to find which factor contributes to dioxin level including age, BMI, smoking, residency, area. Since serum concentration used in regression model was transformed into $\log_{10}$, regression coefficient was presented as 10^{β}, where β is regression coefficient. For categorical variables, this number describes fold change (increase if $10^{\beta}>1$ or decrease if $10^{\beta}<1$) of the dioxin concentration at each category compared with the reference category. Toxic equivalents (TEQ) were calculated using the international World Health Organization (WHO) toxicity equivalency factors (TEFs) 2005[4]. All statistical analyses were performed using the JMP@9 software package (SAS Institute, Japan)

Results and discussion

Table 1 shows the demographic characteristics of the two areas. The mean ages are similar with 68 year olds in the hot spot and 67 year olds in the non-exposed area. The height and weight is higher in the non-exposed area, but BMI is not different. In the non-exposed area, the rate of soldier is higher than the hot spot since many men in the North went to the South during Vietnam War. Habit of smoking and drinking alcohol is not significant difference between the two areas.

There are many congeners of dioxin, furan, and non-orthor PCBs below detection limit or non-detected (ND). In both areas, these congeners 2378-TeCDF, 12378-PeCDF, 123789-HxCDF, 234678-HxCDF, 1234789-HpCDF, OCDF, TeCB #81, TeCB #77 show very high percentage of ND (>70%). Other congeners 2378-TeCDD, 123478-HxCDD, 123789-HxCDD in the non-exposed area also show high percentage of ND (>80%). These congeners will not be shown in the results because of little contribution to TEQ dioxin.

Table1.Demographic characteristics of study participants.

	Hot spot (n=97)		Non-exposed (n=85)		
Continuous variables	**Mean**	**SD**	**Mean**	**SD**	**p Value**
Age (years)	68	6	67	5	0.9
Height (cm)	157	5	159	5	0.001
Weight (kg)	49	7	52	8	0.005
BMI (kg/m2)	20	2	21	3	0.1
Residency	65	11	58	10	0.001
Category variables	**N**	**%**	**N**	**%**	
Soldier	33	34	67	69	0.0001
Smoking	60	62	62	64	0.1
Alcohol	46	47	42	43	0.8

Table 2 shows the level of dioxin among the three groups in the non-exposed area.
Group A including men who stayed only in the Northern in their life time.
Group B including men who ever went to the Southern during herbicide spraying (1961-1971) and might stay there more after 1971
Group C including men who ever went to the Southern after 1971.
There is no significant difference of dioxin level among the three groups.

In the group B, we also asked if they ever lived in a defoliation area where the tree's leaves were fallen and/or was ever directly sprayed by herbicide. Then we compared between the two groups (yes and no) but found no difference.

Table2. Dioxin concentration among the three groups in non-exposed area

	Group A (n=35)		Group B (n=38)		Group C (n=12)	
Pg/g lipid	**Geomean**	**Median (interquartile)**	**Geomean**	**Median (interquartile)**	**Geomean**	**Median (interquartile)**
2378-TeCDD	1.5	1.5 (1.2-2)	1.4	1.4 (1.2-1.7)	1.6	1.6 (1-2.2)
12378-PeCDD	2.5	2.4 (1.5-4)	2.2	2.4 (1.5-2.9)	2.7	2.7 (1.5-4.6)
123678-HxCDD	4.5	4.1 (2.9-5.6)	4.3	4.5 (3-5.7)	4.8	4.6 (3.2-8.2)
1234678-HpCDD	6.8	5.8 (4.8-11)	6.5	6.6 (4.8-9.2)	6.1	6.6 (4.4-9.3)
OCDD	56.5	47 (33-96)	57.1	57 (36-88.8)	76	81 (45-138)
23478-PeCDF	7.4	7.1 (4.8-11)	6.6	6 (5.2-8.4)	8.2	7.4 (5.8-13)
123478-HxCDF	4.2	3.9 (2.8-5.6)	4.2	4.5 (2.9-5.5)	5.5	5.5 (3.2-9.7)
123678-HxCDF	4.9	5.3 (3.2-6.4)	4.6	4.7 (3.3-5.7)	5.5	5 (3.2-12.1)
1234678-HpCDF	3.7	3.7 (2.7-5.8)	4.0	4.1 (2.7-5.6)	5.1	5.1 (2.9-9.3)
PeCB #126	33.3	31 (18.5-57)	28.7	28 (15-51)	27.4	27.5 (16.8-43.4)
HxCB #169	26.3	23.5 (16-40)	21.2	22.8 (13.9-28.3)	24.5	21.5 (15.3-43)
TEQ pg/g lipid						
Total PCDFs	4.0	4.2 (2.6-5.2)	3.7	3.7 (3-4.4)	4.6	4 (3.5-7.6)
Total PCDDs	5.4	5.4 (3.6-7.8)	4.8	4.8 (4-6.1)	5.8	5.3 (3.9-8.3)
Total PCDD/Fs	9.6	9.7 (6.4-13.1)	8.6	8.6 (7.3-10.4)	10.4	10.1 (7.6-15.2)

Total Non-ortho PCBs	4.4	4 (2.6-7.3)	3.6	3.5 (2-5.8)	3.6	3.6 (2.6-6.2)
Total PCDD/Fs + non-orthor PCBs	14.5	13.5 (9.1-21.4)	12.6	12.5 (10.2-16)	14.2	13.8 (9.8-19.6)

Using multiple linear regression to adjust for other covariates including age, BMI, residency and smoking, we found that only smoking has negative association with some congeners (10^{β}, p value): 123678-HxCDD (0.75, p=0.03), 1234678-HpCDD (0.75, p=0.02), 123478-HxCDF (0.75, p=0.03), PCB126 (0.69, p=0.04), and TEQ total Non-orthor PCBs (0.7, p=0.03), TEQ PCDD/Fs+non-orthor PCBs (0.77, p=0.02).

Table 3 shows the dioxin level concentration among the three areas in hot spot. Most PCDDs congeners and non-orthor PCBs congeners are higher in the area (I) than that in the area (II) and the area (III). PCDFs congeners are not different among the three areas except for 23478-PeCDF. There is no difference betweet the area (II) and the area (III) in all congeners.

Table3. Dioxin concentration among the three areas in the hot spot

	Inside (I), n=39		Near (II), n=27		Far (III), n=31		p Value	
Pg/g lipid	Geo-mean	Median (interquartile)	Geo-mean	Median (interquartile)	Geo-mean	Median (interquartile)	I-II	I-III
2378-TeCDD	3.5	3.3 (1.9-5.6)	2.4	2.5 (1.8-3.4)	2.0	2.2 (1.4.2.9)		0.001
12378-PeCDD	10.8	10 (7.4-16)	7.4	7 (5-11)	6.7	6.6 (4.2-12)	0.05	0.005
123478-HxCDD	6.1	5.8 (3.7-8.3)	4.5	4.3 (3.5-5.7)	4.5	4.5 (2.9-6.1)	0.06	0.06
123678-HxCDD	24.1	22 (18-38)	15.1	15 (11-20)	17.0	16 (11-26)	0.005	0.04
123789-HxCDD	7.4	6.9 (5-10)	5.6	6.5 (3.6-7.3)	6.2	6 (3.6-11)		
1234678-HpCDD	33.4	32 (22-54)	21.2	21 (15-33)	30.1	28 (18-44)	0.02	
OCDD	370	340 (230-540)	294	300 (200-450)	286	270 (160-410)		
23478-PeCDF	15.5	15 (12-21)	12.2	12 (9.8-14)	11.5	11 (6.7-17)		0.04
123478-HxCDF	29.2	27 (20-44)	24.8	26 (16-34)	28.6	24 (16-52)		
123678-HxCDF	22.3	22 (14-34)	19.0	20 (13-26)	21.6	20 (13-40)		
1234678-HpCDF	41.6	42 (29-64)	36.1	38 (20-58)	45.0	42 (26-88)		
PeCB #126	52.8	44 (31-83)	25.7	24 (17.5-34)	28.5	27 (16.5-49)	0.001	0.001
HxCB #169	147	150 (86-220)	100	100 (68-130)	92	89 (66-140)	0.001	0.02
TEQ pg/g lipid								
Total PCDFs	11.5	10.9 (7.9-15.6)	9.6	9.6 (7.4-11.4)	10.1	9.6 (6.8-17.7)		
Total PCDDs	19.0	19.3 (12.8-27.7)	13.1	11.8 (9.7-17.5)	12.3	12.6 (8.4-19.9)	0.005	0.02
Total PCDD/Fs	31.0	29.8 (23.7-41.2)	23.0	23.2 (17.1-27.5)	22.9	19.9 (16.1-36.2)	0.04	0.05
Total Non-orthor PCBs	10.2	9.9 (5.8-14.1)	5.8	5.5 (4.2-6.5)	5.8	5.4 (4.3-5.4)	0.001	0.001
Total PCDD/Fs + non-orthor PCBs	41.7	41.7 (28.8-53.8)	29.2	28.6 (21.4-34.7)	28.9	24.9 (22.2-45.8)	0.01	0.01

Table 4 show dioxin concentration in three areas in the hot spot in reference to the non-exposed area after adjusted for age, BMI, smoking, residency. TEQ PCDD/Fs+PCBs of the area (I) is 3-fold while it is 2-fold in the area (II) or (III) in comparison with the non-exposed area. The overall R-square is the variation explained by model.

We also found that dioxin concentration have negative association with smoking after adjustion for age, BMI, residency. These are congeners (10^{β}, p value):

12378-PeCDD (0.8, 0.04), 123478-HxCDD (0.83, 0.03), 123678-HxCDD(0.83, 0.05), 1234678-HpCDD (0.73, 0.001), 123678-HxCDF (0.83, 0.02), and TEQ PCDDs (0.83, 0.03), TEQ PCDD/Fs (0.84, 0.03), TEQ PCDD/Fs+non-orthor PCBs (0.83, 0.02)

Table 4. Dioxin concentration in three areas in the hot spot in reference to the non-exposed area after adjusted for age, BMI, smoking, residency

	Inside (I)		Near(II)		Far(III)		Overall R2
Pg/g lipid	10^{β}	p value	10^{β}	p value	10^{β}	p value	
2378-TeCDD	2.3	0.0001	1.6		1.2		0.31
12378-PeCDD	4.4	0.0001	3.0	0.04	2.5		0.52
123478-HxCDD	2.2	0.0001	1.6		1.5		0.33
123678-HxCDD	5.3	0.0001	3.3	0.05	3.5	0.01	0.65
123789-HxCDD	2.5	0.0001	1.8		1.9		0.38
1234678-HpCDD	4.9	0.0001	3.2		4.3	0.0001	0.65
OCDD	6.1	0.0001	5.0	0.002	4.7		0.69
23478-PeCDF	2.1	0.001	1.6		1.5		0.32
123478-HxCDF	6.5	0.0001	5.4	0.0001	5.9	0.0001	0.75
123678-HxCDF	4.4	0.0001	3.7	0.003	3.9	0.0003	0.66
1234678-HpCDF	9.8	0.0001	8.2	0.0001	9.6	0.0001	0.76
PeCB #126	1.8	0.0001	0.9		1.0		0.15
HxCB #169	6.0	0.0001	4.0	0.004	3.4		0.65
TEQ pg/g lipid							
Total PCDFs	2.8	0.0001	2.3	0.03	2.3	0.04	0.56
Total PCDDs	3.5	0.0001	2.4		2.1		0.55
Total PCDD/Fs	3.2	0.0001	2.4	0.04	2.2		0.58
Total Non-orthor PCBs	2.6	0.0001	1.5		1.4		0.3
Total PCDD/Fs + non-orthor PCBs	3.0	0.0001	2.1		2.0		0.52

Note: β is regression coefficient based on $\log_{10}$(dioxin concentration)

This is the first epidemiological study about dioxin in serum of old men and their herbicide exposure in Vietnam. In non-exposed area, dioxin level is not different between men who were exposed by herbicide and who were not. This may be due to a long time of 40 years has passed since herbicide was last sprayed. Most dioxin congener levels are highest in the area (I) where Airbases were located, other area near Airbase (II and III) also show elevated, which mean current exposure cause elevated dioxin level. Men who worked in Phu Cat Airbase have high risk of dioxin exposure since two men working inside Airbase show highest level of TCDD (24 and 16 pg/g lipd).
In future studies, more information about food consumption, occupational exposure, and other potential factors needs to be collected to clarify their contribution to serum dioxin of Vietnamese men.

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