

ASSOCIATION OF HAVING BIRTH DEFECT CHILDREN WITH SERUM DIOXIN LEVELS IN VIETNAM

Tawara K¹, Nishijo M¹, Nakagawa H¹, Kido T², Naganuma R², Suzuki H², Hung TM³, Thom LTH³, Dung PT³, Nhu DD³

¹Department of Epidemiology and Public Health, Kanazawa Medical University, Uchinada-machi, Ishikawa Prefecture, 920-0293, Japan; ²Division of Health Sciences, Graduate School of Medical Science, Kanazawa University, Kanazawa-city, 920-0942, Japan, ³Division for Mitigation of the Consequences of the Chemicals used during the War on Human Health (10-80 Division), Vietnam Ministry Health, Ha Noi, Viet Nam

Abstract

In order to evaluate the PCDDs/DFs (DXNs) levels in Vietnam after an elapse of three decades after the war, comparative studies were introduced into a herbicide-sprayed commune and a non-sprayed commune. The study showed the current serum TCDD level of the residents in the herbicide sprayed area, was not so spectacular that it affected the isomer profiles. However we found out that the TCDD level was associated with an increase in the risk of having birth defect children.

Introduction

Nearly ~ 77 million liters of herbicides were sprayed on the former Republic of Vietnam between 1961 and 1971 for military purpose¹. Since 1970s, a number of studies have been conducted to determine whether exposure to herbicides in Vietnam may have increased the risk of having children with birth defects, though the results have often been inconsistent². After an elapse of three decades after the Vietnam War, an increased concern to this association still exists in conjunction with long term effects of herbicides/dioxins to postwar generations.

Materials and Methods

In order to evaluate the current levels of PCDDs/DFs (DXNs) in Vietnam, comparative studies were introduced into two communes; namely, CC commune in Quang Tri Province where herbicide mixture known as "Agent Orange (AO)" had been dispersed between 1962 and 1971, and CP commune in Ha Tinh Province which had never experienced military herbicide operation. Based on the personal health research conducted in each commune in 2001, blood samples of 3ml-8ml were collected in 2002 from normal men/ women of the 10s-60s, and fathers/ mothers with birth defect children of the 30s-60s in each commune. Analytical serum samples were basically pooled in 5 ml by 4 individual samples (1.25 ml for each sample) in the same age of the sample group. Quantitation was performed by a high resolution mass spectrometer (HRMS; JEOL MStation-JMS700) equipped with a gas chromatograph (HP-6890).

DXN levels between the two communes were compared for both men and women every sample group (normal group and parent group of birth defect children). Furthermore the analytical data were restricted to the ones of over 30 year subjects representing the war generation. Then, differences of DXN levels between the two sample groups in the same commune were examined for both men and women.

Logarithmic transformation of the variables was performed as needed to improve normality, and all values except for the number of samples and subjects' age in this paper, are presented as geometric ones.

Results and Discussion

Although we don't show the results in this paper, the significance test recognized that the concentration levels in almost all isomers of the CC commune were significantly higher than those of the CP commune every sample group. Especially significant differences in HxCDFs and HpCDFs were remarkably observed. Then the DXN levels between two sample groups restricted to the subjects over 30 years old each, were compared for each sex every commune. As a result, the concentration levels of all isomers of parent group of birth defect children in the CP commune needed not to be higher than those of normal group in the same commune (Table 1). As shown in Table 2, such a tendency was similarly observed in the CC commune's sample. However a statistically significant difference was recognized in the TCDD and 1,2,3,7,8,9-HxCDF levels between normal men and fathers with birth defect children ($P < 0.05$). With regard to TEQ, any statistically significant difference between two sample groups in each commune was not recognized from both men and women (Table 3).

Taking the results of significance tests into consideration, a logistic regression model was employed to examine the association of the probability of having birth defect children with the serum TCDD level. The presence or absence of birth defect child was used as a dependent variable, and sex of parent, age (10-y interval), and serum TCDD level applied to logarithmic transformation value were used as independent variables. As summarized in Table 4, the TCDD level was associated with an increase in the risk of having birth defect children.

In either sample group; normal group or parent group of birth defect children, significantly higher levels of HxCDFs and HpCDFs in the CC commune than those in the CP commune were equally recognized for both men and women. The HxCDFs and HpCDFs level proportions expressed in percentage of PCDDs/DFs-TEQ of the CC commune, ranged from 30 to 40%, and from 2.5 to 4%, respectively. That is, the HxCDFs and HpCDFs levels account for 40% or more of PCDDs/DFs-TEQ for both sample groups in the CC commune. Such spectacularly high levels of HxCDFs and HpCDFs commonly characterize the isomer profiles in the CC commune. In fact statistically significant level differences of these isomers between the two sample groups were not recognized for both men and women in the CC commune. It is thought from this view that spectacularly high level of HxCDFs and HpCDFs may reflect on-going main exposure sources of DXNs in the CC commune.

On the contrary, the TCDD levels of both fathers and mothers with birth defect children in the CC commune were not so remarkable that they affected the isomer profiles like HxCDFs and HpCDFs. Indeed, the TCDD level proportion expressed in percentage of PCDDs/DFs-TEQ of the fathers/ mothers of birth defect children in the CC commune reached 5% only. However the TCDD level proportion for the normal group in the CC commune did not complete even 2%, which was roughly equivalent to those of both two sample groups in the CP commune. In other words, the TCDD levels of the fathers/ mothers with birth defect children in the CC commune appear to be unusual even at low level, in comparison with other sample groups. In addition, statistically significant level differences between the two sample groups were recognized only for men in the CC commune, and such a tendency was also observed for women in the CC commune as well, though significant difference was not recognized. Accordingly it is thought that the residents in the CC commune of Quang Tri Province had been exposed to DXNs contained in AO in wartime, which may result in the TCDD level difference between normal men/ women and fathers/ mothers with birth defect children in the CC commune. From this view point, an association of having birth defect children with serum TCDD level is suggested.

A logistic regression model using logarithmic transformation value estimated a risk of having birth defect children in association with the serum TCDD level. The result indicated that the odds of having birth defect children in 10 pg/g-lipid of the serum TCDD level were 10.94 to 1. That is, it was recognized that the provability of having birth defect children in the case of 10 pg/g-lipid of the serum TCDD level is 10.94 times greater than the provability in the case of 1 pg/g-lipid of the serum TCDD level.

Due to the aerial applications and handling on AO for military installation, there exist two primary sources of TCDD contamination in Vietnam; contamination via spray mission by C-123 aircraft, and contamination on former US military installation where herbicides were stored, dispensed, and spilled³. Especially the sites assigned to the latter instance are given a nickname of "hot spots", in which many studies have been intervened to theorize the pattern of TCDD contamination as a model of "current" DXNs condition throughout the southern Vietnam⁴. However such sites as "hot spots" seem to be improper to modeling, because of their specific situation as former military installation. On the other hand, the areas contaminated with TCDD via aerial applications cover over 15% of the southern Vietnam⁵. Therefore this instance may be preferably proper to modeling the "current" isomer profiles in the southern Vietnam, and estimating a "current" risk of certain diseases such as birth defect in association with AO. Consequently it is suggested from a public health aspect that further researches with areal variation in the southern Vietnam may be necessary to discuss causation between TCDD level and development of birth defect.

Acknowledgments

We gratefully acknowledge co-operation of many Vietnamese and Japanese, too many to mention here. We are particularly grateful for the assistance of Mr Masahiro Watanabe & all other staffs at the AI Group of Nagoya Center of JEOL DATUM LTD., and Mr Kenji Matsuura and Mr Noriyuki Yahata at International Technical & Training Center, AI Technical Division of JEOL DATUM LTD. who co-operated with us to improve the GC/HRMS environment for measuring PCDDs/DFs in a small volume of human tissue samples.

References

1. Stellman JM, Stellman SD, Christians R, Weber T, Tomasallo C. *Nature* 2003; 422: 681.
2. Ngo AD, Taylor R, Christine LR, Nguyen TV. *Int J Epidemiol* 2006; 35:1220.
3. Dwernychuk LW, Hung TM, Bovin TG, Bruce GS, Dung PT, Son LK, Hatfield CT, Dung NT, Allan JA, Nhu DD, Thuc PV, Moats DJ, Borton L. *Organohalogen Comp* 2006; 86:312.
4. Dwernychuk LW, Hoang DC, Hatfield CT, Bovin TG, Hung TM, Dung PT, Thai ND. *Chemosphere* 2002; 47: 117.
5. Schecter A, Quynh HT, Pöpke O, Malisch R, Constable JD. *J Occup Environ Med* 2003; 45: 781.

Table 1 Comparison of PCDDs/DFs levels in serum samples between normal subjects and parents with birth defect children in a non-sprayed commune of Ha Tinh Province in Vietnam for each sex.

PCDDs/DFs (pg/g-lipid)	Fathers of BDC ¹ (44.0 ± 8.6YR)		Normal men (43.9 ± 10.4YR)		Mothers of BDC (40.0 ± 6.0YR)		Normal women (44.2 ± 8.2YR)	
	Mean	(SE)	Mean	(SE)	Mean	(SE)	Mean	(SE)
TCDD	0.15	(5.54)	0.15	(3.67)	0.19	(7.02)	0.24	(3.91)
PCDD	3.06	(1.45)	2.42	(3.43)	1.18	(5.58)	1.22	(3.94)
1,2,3,4,7,8-HxCDD	0.14	(7.47)	0.49	(3.88)	0.47	(6.84)	0.28	(5.55)
1,2,3,6,7,8-HxCDD	4.14	(1.53)	4.66	(1.46)	3.36	(1.18)	2.74	(4.46)
1,2,3,7,8,9-HxCDD	0.69	(7.52)	0.56	(4.26)	3.26	(1.54)	0.59	(4.29)
HpCDD	4.98	(1.66)	6.94	(1.53)	6.45	(1.39)	7.68	(1.26)
OCDD	27.5	(1.66)	44.77	(1.63)	24.02	(1.43)	47.19	(1.61)
TCDF	0.06	(8.98)	0.28	(3.59)	0.15	(7.86)	0.87	(4.14)
1,2,3,4,7,8-PCDF	0.14	(3.52)	0.14	(3.11)	0.14	(3.83)	0.26	(3.67)
2,3,4,7,8-PCDF	5.92	(1.17)	8.96	(1.64)	5.86	(1.28)	4.31	(4.58)
1,2,3,4,7,8-HxCDF	5.23	(1.22)	7.96	(1.31)	5.43	(1.35)	3.65	(4.55)
1,2,3,6,7,8-HxCDF	4.83	(1.31)	7.24	(1.40)	4.95	(1.39)	3.47	(4.14)
2,3,4,6,7,8-HxCDF	2.48	(1.81)	0.21	(9.54)	2.12	(2.02)	0.56	(6.35)
1,2,3,7,8,9-HxCDF	0.22	(6.04)	0.14	(1.88)	0.22	(6.17)	0.17	(2.09)
1,2,3,4,6,7,8-HpCDF	6.71	(1.45)	5.54	(4.27)	7.80	(1.35)	5.64	(3.53)
1,2,3,4,7,8,9-HpCDF	0.33	(2.23)	0.16	(3.03)	0.37	(2.40)	0.10	(1.44)
OCDF	0.98	(7.33)	0.31	(5.14)	2.54	(1.22)	0.19	(4.01)

¹: birth defect children

The age of all fathers and mothers of birth defect children was over 30 years old. Data shown in the table do not include the results of the subjects below 31 years old. MEAN = geometric mean. SD = geometric SD

Table 2 Comparison of PCDDs/DFs levels in serum samples between normal subjects and parents with birth defect children in a sprayed commune of Quang Tri Province in Vietnam for each sex.

PCDDs/DFs (pg/g-lipid)	Fathers of BDC ¹ (46.0 ± 6.6YR)		Normal men (44.4 ± 6.8YR)		Mothers of BDC (42.5 ± 6.3YR)		Normal women (47.7 ± 10.4YR)		
	Mean	(SE)	Mean	(SE)	Mean	(SE)	Mean	(SE)	
TCDD	1.59	(1.55)	0.36	(6.53)	*	1.45	(1.71)	0.44	(5.12)
PCDD	8.20	(1.13)	6.77	(1.44)		7.87	(1.39)	4.63	(4.10)
1,2,3,4,7,8-HxCDD	5.25	(1.42)	4.80	(1.62)		4.80	(1.52)	4.77	(1.21)
1,2,3,6,7,8-HxCDD	28.75	(1.36)	22.27	(1.70)		23.67	(1.66)	18.92	(1.53)

1,2,3,7,8,9-HxCDD	7.40 (1.23)	5.37 (5.05)	8.31 (1.55)	7.84 (1.35)
HpCDD	46.84 (137)	43.17 (2.09)	69.82 (1.42)	25.53 (1.60)
OCDD	247.01 (1.34)	224.44 (1.84)	381.65 (1.28)	284.32 (1.45)
TCDF	0.05 (4.07)	0.59 (5.90)	0.26 (5.65)	0.28 (4.05)
1,2,3,4,7,8-PCDF	0.93 (3.83)	0.38 (6.09)	1.90 (1.42)	0.79 (6.51)
2,3,4,7,8-PCDF	16.42 (1.32)	17.84 (1.61)	12.70 (1.34)	13.61 (1.37)
1,2,3,4,7,8-HxCDF	56.23 (1.56)	77.77 (1.66)	53.19 (1.64)	57.89 (1.40)
1,2,3,6,7,8-HxCDF	40.97 (1.55)	55.25 (1.66)	38.73 (1.67)	40.33 (1.44)
2,3,4,6,7,8-HxCDF	4.12 (1.28)	2.11 (7.83)	4.30 (1.29)	3.74 (1.48)
1,2,3,7,8,9-HxCDF	3.43 (1.60)	0.62 (5.20) *	2.88 (2.45)	0.81 (5.55)
1,2,3,4,6,7,8-HpCDF	79.80 (1.50)	120.31 (1.82)	96.05 (1.77)	100.56 (1.45)
1,2,3,4,7,8,9-HpCDF	8.10 (1.67)	11.27 (1.91)	7.90 (1.79)	8.73 (1.55)
OCDF	4.34 (1.54)	1.57 (9.90)	3.36 (1.73)	0.53 (6.45)

¹: birth defect children * : P < 0.05

The age of all fathers and mothers of birth defect children was over 30 years old. Data shown in the table do not include the results of the subjects below 31 years old. MEAN = geometric mean. SD = geometric SD

Table 3 Comparison of TEQ levels in serum samples between normal subjects and parents with birth defect children for each sex in a non-sprayed commune of Ha Tinh (HT) Province and a sprayed commune of Quang Tri (QT) Province in Vietnam.

(pg WHO-TEQ/g-lipid)	Fathers of BDC ¹ (46.0 ± 6.6YR)		Normal men (44.4 ± 6.8YR)		Mothers of BDC (42.5 ± 6.3YR)		Normal women (47.7 ± 10.4YR)	
	Mean	(SE)	Mean	(SE)	Mean	(SE)	Mean	(SE)
Non-sprayed commune in HT								
PCDDs	4.17	(1.28)	3.91	(1.97)	3.10	(1.93)	2.43	(2.57)
PCDFs	4.42	(1.20)	6.31	(1.54)	4.42	(1.29)	3.33	(3.88)
PCDDs/ DFs	8.59	(1.19)	10.22	(1.58)	7.52	(1.47)	5.76	(2.83)
Sprayed commune in QT								
PCDDs	14.64	(1.15)	11.74	(1.63)	14.10	(1.36)	10.51	(1.76)
PCDFs	19.92	(1.39)	24.36	(1.64)	17.80	(1.46)	18.48	(1.40)
PCDDs/ DFs	34.56	(1.27)	36.10	(1.61)	31.90	(1.33)	28.99	(1.47)

¹: birth defect children

The age of all fathers and mothers of birth defect children was over 30 years old. Data shown in the table do not include the results of the subjects below 31 years old. MEAN = geometric mean. SD = geometric SD

Table 4 Result of a logistic regression model after forcing Sex and age in the model

		B	SE	Significant level	Odds ratio	95% CI
Sex	Men/Women	0.049	0.829	0.953	1.05	0.21-5.33
Age group	40s.50s/30s			0.851		
Log ₁₀ TCDD	(x+1)/x	2.392	1.116	0.032	10.94	1.23-97.4