The relationship between dioxins exposure and risk of prostate cancer with steroid hormone and age in Vietnamese men

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HIGHLIGHTS

- In the hotspot group, sex hormone levels were significantly increased with age.
- Mean 3β-HSD activity levels were higher in the hotspot than the non-sprayed group.
- In the hotspot group, 3β-HSD activity levels were significantly increased with age.

GRAPHICAL ABSTRACT
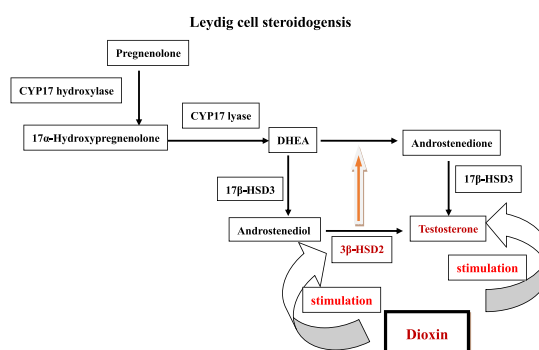
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ABSTRACT

Although Vietnam's massive herbicide exposure in 1960s and 1970s was clearly injurious to health, not all causal relationships have been clarified. We therefore explored associations among dioxins, steroid hormones, age and prostate cancer risk in men. We compared serum levels of dioxin, steroid hormones and prostate specific antigen (PSA) in men aged 56–81 years from herbicide-exposed hotspots ($n = 50$) with those from non-sprayed regions ($n = 48$). Mean serum levels of dioxin congeners in the hotspot group were 1.5–11.3 times higher than the non-sprayed group depending on specific compound. Levels of testosterone, estradiol and 3β-hydroxysteroid dehydrogenase (3β-HSD) activity in the hotspot group were also significantly higher than in non-sprayed group. Estradiol levels were significantly related to levels of several specific dioxin derivatives in both group. Significant positive correlations were also found between DHT and 1234678-HpCDD or 1234678-HpCDF; and between 3β-HSD activity and 123678-HxCDD, 123478-HxCDF, 123678-HxCDF, or HxCB#169. After adjusting for age,

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Testosterone
3β-Hydroxysteroid dehydrogenase activity
Hotspot

body mass index, and tobacco use, multiple linear regressions showed levels of dihydrotestosterone (DHT), estradiol, testosterone and 3β-HSD activity were not associated with dioxins in the two groups; however, levels of DHT, testosterone and 3β-HSD activity increased significantly with age in the hotspot group. The hotspot and non-sprayed groups did not significantly differ in PSA levels. But six of the hotspot subjects had PSA levels >3 ng/mL, 3 of whom were suspected to have prostate cancer (PC) after digital rectal examination. Our findings suggest that dioxin exposure can lead to increased levels of several sex steroid hormones with age. The correlation of dioxin with steroid hormone levels and prostate cancer risk should be studied further.

1. Introduction

Dioxin and dioxin-like compounds comprise the chemical class of polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs). As environmental pollutants, these highly toxic compounds have well-established harmful effects in animals and humans.

During Operation Ranch Hand (1962–1971), the United States Air Force sprayed approximately 107 million lbs. of herbicides in the south of the former Demilitarized Zone at the 17th parallel for defoliation and crop destruction. The best-known herbicide was Agent Orange (AO), which is 50:50 mixture of n-butyl esters of 2,4-dichlorophenoxyacetic acid (2,4D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5T); the 2,4,5T was also contaminated with varying levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Stellman et al., 2003). Dioxins can cause reproductive and developmental problems, damage the immune system, interfere with hormone signaling and cause cancer (WHO, 2010). In 1998, the U.S. National Academy of Sciences concluded that "limited/suggestive evidence" indicated an association between AO and prostate cancer (PC) (Institute of Medicine, 1998). In the largest study to date ($N = 13{,}144$), United States Vietnam veterans who reported exposure, AO was found to double the risk of developing PC, particularly its most aggressive form, compared with those who were not exposed (Chamie et al., 2008). Several other recent studies of Vietnam veterans also support the association of AO exposure to increased risk of PC (Ansbaugh et al., 2013; Yi and Ohrr, 2014). Li and Wang found that the PCB congener PCB-126 induced differential changes in androgen, cortisol, and aldosterone biosynthesis in human adrenocortical H295R cells (Li and Wang, 2005). As androgens are fundamental for the development and maintenance of the prostate gland and play a key role in PC biology, a long-standing hypothesis has been that circulating hormone levels affect PC risk (Lindstrom et al., 2010).

During the war, many Vietnamese people and Vietnam veterans were heavily exposed to dioxins, which is now manifesting as health problems, including skin disorders, liver damage and adverse reproductive effects (Constable and Hatch, 1985; Tamburro, 1992; Wolfe et al., 1995; Institute of Medicine, 2008). We had found breast milk and serum dioxins levels were significantly higher in residents of hotspot regions than in those from non-sprayed regions (Kido et al., 2014; Manh et al., 2014; Manh et al., 2015; Kido et al., 2016); and that salivary and serum adrenal steroid hormone levels in Vietnamese women and their children were associated with breast-milk dioxins levels (Kido et al., 2014; Kido et al., 2016). However, few studies have addressed effects of dioxins exposure on steroid hormone or PC in Vietnamese men (Sun et al., 2014; Sun et al., 2016). Here, we explored associations among dioxins, steroid hormones and PC risk with age in Vietnamese men.

2. Subjects and methods

2.1. Study areas

Phu Cat airbase, a dioxins hotspot located in Binh Dinh Province, was a former United States airbase during the Vietnam War (Manh et al., 2014).

The control region is a non-sprayed region in the Kim Bang district in Ha Nam Province, in the northern part of Vietnam. The control region was not sprayed with chemical herbicides during the war. Both the hotspot and the control region are rural and there is no industrial pollution nearby (Manh et al., 2014).

2.2. Study population

The study subjects included 50 and 48 men, from the dioxins hotspot and the non-sprayed region, respectively. All men aged over 50 residing in three areas located in and around the airbase were recruited by local hospital staff in 2009 and 2011. Ninety-seven men consented to participate. Among them, 50 subjects were selected at random for analysis of steroid hormone levels. In the non-sprayed region, all male residents over 50 were recruited similarly, and 85 men agreed to participate. Of those, 48 men were selected randomly. In August 2009 and August 2011, blood (10 mL each) was collected from the men at 8:00–10:30 a.m. Serum samples were obtained by centrifuge, then stored in a cooling box and frozen in dry ice for several days. All samples were transported to Japan for analysis. The serum samples were stored at −70 °C until analysis. Levels of PSA and steroid hormones were determined for all subjects; levels of dioxins congeners were determined for 48 men from the dioxins hotspot and 36 men from the non-sprayed region.

This study was approved by the Medical Ethics Committee of Kanazawa University.

2.3. Measurement of serum hormone estimation by LC-MS/MS

Serum steroid analysis was carried out by LC-MS/MS (API-4000 Applied Biosystems, MDS SciEx, Toronto, ON, Canada) as described previously (Kido et al., 2014). Briefly, serum (200 μL) was diluted with purified water to a volume of 1.0 mL, and cortisol-^{2}H$_4$ (1 ng), dihydroepiandrosterone (DHEA)-^{2}H$_4$ (100 pg), testosterone-2H$_3$ (100 pg), progesterone-^{13}C$_3$ (100 pg), estrone-^{13}C$_4$ (100 pg), and estradiol-^{13}C$_4$ (100 pg) were added as internal standards. After extraction with ethyl acetate, the extract was applied onto a cartridge column (C18) to removed impurities. The obtained purified fractions were derivatized with picolinic acid according to the procedure described by Yamashita et al. (Yamashita et al., 2009). The reaction mixture was then applied onto an interSept pharm cartridge column to remove excess reagents. Levels of nine hormones were simultaneously determined by LC-MS/MS. The lowest estimation levels per assay were 50 pg for cortisol; 50 pg for cortisone; 5 pg for DHEA; 1 pg for testosterone; 1 pg for dihydrotestosterone (DHT); 10 pg for progesterone; 10 pg for androstenedione (A-dione); 1.0 pg for estrone, and 0.5 pg for estradiol. Both the accuracy and precision in inter- and intra-day assays were within ± 20% of the lowest level and within ± 15% for concentrations other than the lowest concentration.

Enzyme activities were calculated from serum steroid levels using the formulas below:

$$5\alpha - \text{reductase activity} \, (\%) = \text{DHT/testosterone}$$

Table 1
Demographic characteristics of participants in the hotspot and non-sprayed regions.

Characteristics	Hotspot			Non-sprayed region		p value
Continuous variable	N	Mean ± SD / GM (GSD)		N	Mean ± SD / GM (GSD)	
Age (years)	50	68.0 ± 6.4		47	65.0 ± 4.8	0.010[a]
Height (cm)	50	156.8 ± 4.7		48	159.5 ± 5.4	0.009[b]
Weight (kg)	50	49.4 ± 8.3		48	52.6 ± 7.9	0.051[b]
BMI (kg/m^2)	50	20.1 ± 2.8		48	20.7 ± 2.6	0.276[b]
PSA (ng/mL)	50	1.0 (2.5)		48	0.7 (2.1)	0.054[b]
Categorical variables	No.	%		N	%	
Alcohol use (yes)	21	42		23	48	0.556[c]
Tobacco use (yes)	28	56		36	75	0.048[c]
Currently employed (yes)	33	66		23	48	0.071[c]
Farmer	26	79		10	43	0.001[c]
Worker	0	0		2	9	
Fisherman	0	0		1	4	
Teacher	0	0		0	0	
Other job	7	21		10	43	

BMI: body mass index; GM: geometric mean, GSD: geometric standard deviation.
PSA: prostate-specific antigen.
SD: standard deviation.
[a] Welch's *t*-test.
[b] Student's *t*-test.
[c] Chi squared test.

$$\text{CYP17—lyase activity} (\%)$$
$$= (\text{DHEA} + \text{testosterone} + \text{A—dione} + \text{DHT} + \text{estrone} + \text{estradiol})$$
$$/(\text{cortisol} + \text{cortisone})$$

$$3\beta\text{—hydroxysteroid dehydrogenase activity} (3\beta\text{—HSD}, \%)$$
$$= (\text{testosterone} + \text{DHT} + \text{estrone} + \text{estradiol})/\text{DHEA}$$

$$\text{Aromatase activity} (\%)$$
$$= (\text{estrone} + \text{estradiol}))/(\text{A—dione} + \text{testosterone}).$$

2.4. Measurement of dioxins isomers by GC-HRMS

The serum samples were spiked with a mixture of $^{13}C_{12}$-labelled PCDDs/PCDFs and PCBs as internal standards. PCB and dioxin were extracted with hexane-saturated sulfuric acid 3 times. Both PCB and PCDD/DF fractions was applied to HRGC-HRMS (HP-6980, Hewlett-Packard, Palo Alto, CA, USA) and non-ortho PCB and PCDD/F congeners were estimated. Selective ion monitoring mode was used for determination; resolution was kept at above 10,000. Recovery was 95%–104% for PCBs and 78%–89% for PCDD/Fs. Serum concentrations of dioxin, furan, and non-ortho PCB congeners below the limit of detection (LOD) were assigned a value equal to half the LOD.

A detailed description of the analytical method for dioxins can be found elsewhere (Manh et al., 2014).

2.5. Measurement of PSA in serum

Total serum PSA levels were determined by immunological method, using enzyme immune assay system (Tosoh; AIA-2000).

2.6. Statistical analysis

We compared baseline characteristics of the hotspot group and the non-sprayed group using two-sided Student's *t*-test, Welch's *t*-test or Wilcoxon rank sum test for continuous variables, and chi-squared test for categorical variables. Linear regression analysis was used to evaluate correlation between steroid hormones, dioxins and age in the two groups. Values for dioxins, DHT, DHEA, testosterone, and A-dione levels were log-transformed to improve normality. Backward elimination of multiple linear regression was used to evaluate relationships between hormone levels as dependent variables, and dioxin congeners, PCBs, age, body mass index (BMI), and tobacco use as independent variables. When the partial regression coefficient was significant ($p < 0.05$), the independent variable was considered a relevant factor. Subjects of the hotspot group and the non-sprayed group were combined since distributions of dioxin congeners between two groups were partially overlapped and considered as continuous variation. $p < 0.05$ was considered significant. All statistical analyses were performed using the JMP-9 software package (SAS Institute, Japan).

3. Results

3.1. Characteristics of the participants

Table 1 shows baseline characteristics in 50 men from the hotspot and 48 men from the non-sprayed region. The mean age of the hotspot group (68 years; 59–81 years) was significantly older than for the non-sprayed group (65 years; 56–77 years; $p = 0.010$). The mean height of the non-sprayed group was significantly greater (159.5 vs. 156.8 cm; $p = 0.009$), but BMI (20.7 vs. 20.1 kg/m^2; $p = 0.276$) and mean PSA levels did not differ significantly between the two groups. Weight differences (52.6 vs. 49.4 kg) were borderline significant ($p = 0.051$).

The frequency of alcohol use was similar in the two groups. In the non-sprayed cohort, tobacco use was reported by 75%, while only 56% of the hotspot cohort reported using tobacco. The percentage of farmers was 79% in the hotspot group and 43% in the non-sprayed group.

3.2. Serum steroid hormone levels

Table 2 shows the results of serum hormone levels of men from hotspot and non-sprayed regions. Levels of testosterone ($p = 0.003$) and estradiol ($p = 0.024$) were significantly higher in the hotspot than in the non-sprayed region. Levels of DHEA ($p = 0.047$) was a significantly lower in the hotspot than in the non-sprayed region, but cortisol, cortisone, progesterone, DHT, A-dione and estrone were not found statistically significant differences between the two regions studied as described previously (Sun et al., 2016).

On the other hand, 3β-HSD activity ($p = 0.002$) and aromatase activity ($p = 0.03$) calculated from the ratio of serum hormone levels as

Table 2
Levels or ratios of serum steroid hormone in males from the hotspot and non-sprayed regions.

Steroids/enzyme		Hotspot ($n = 50$) Mean ± SD/GM (GSD)	Non-sprayed region ($n = 48$) Mean ± SD/GM (GSD)	p value
Testosterone	(pg/mL)	6310 (1.4)	5395.1 (1.4)	0.003[a]
DHEA	(pg/mL)	1260 (1.6)	1513 (1.7)	0.047[a]
Estradiol	(pg/mL)	13 ± 4	11 ± 3	0.024[a]
5α-Reductase	%	10 ± 3	11 ± 3	0.310[b]
3β-HSD	%	666 ± 467	454 ± 297	0.002[b]
CYP17-lyase	%	26 ± 81	11 ± 7	0.105[b]
Aromatase	%	0.5 ± 0.2	0.6 ± 0.2	0.030[b]

SD: standard deviation, GM: geometric mean, GSD: geometric standard deviation.
5α-Reductase (%) = DHT / testosterone × 100.
3β-HSD (%) = (DHT + testosterone + estradiol + estrone) / DHEA × 100.
CYP17-lyase (%) = (DHT + testosterone + DHEA + A-dione + estradiol + estrone) / (cortisol + cortisone) × 100.
Aromatase (%) = (estradiol + estrone) / (testosterone + A-dione) × 100.
[a] Student's *t*-test.
[b] Wilcoxon rank sum test.

described in the experimental section, were statistically significant different between the two regions studied, but CYP17-lyase activity and 5a-reductase activity were not different between the two regions studied.

3.3. Correlations between age and steroid hormones

Fig. 1 shows correlations for steroid hormones and age between the hotspot and non-sprayed groups. As men age, serum androgen and
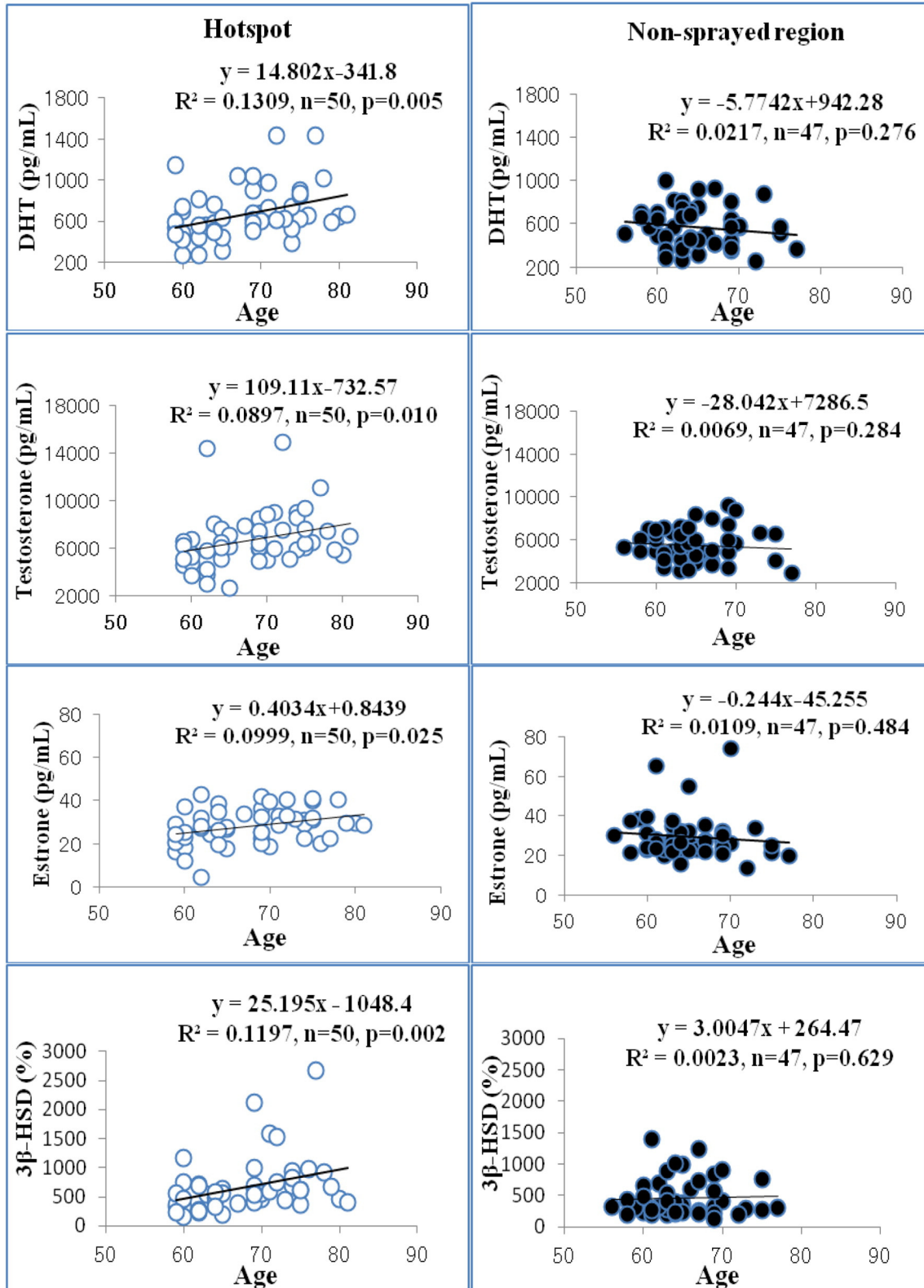

Fig. 1. Correlation among steroid hormone, 3β-HSD and age. $3\beta - HSD\ (\%) = (DHT + testosterone + estradiol + estrone)/DHEA \times 100$

estrogen levels gradually decline in a phenomenon known as andropause (Ferrini and Barrett, 1998; Ellis and Nyborg, 1999). Around age 40, androgen levels begin to decrease, and testosterone has been observed to decline at a rate of 0.4% per year (Myers and Meacham, 2003). However, mean testosterone ($p = 0.01$), DHT ($p = 0.005$), estrone ($p = 0.025$), and 3β-HSD activity ($p = 0.002$) significantly increased with age in the hotspot group, but not in the non-sprayed group. In contrast, estradiol, cortisol, cortisone, progesterone, A-dione, and DHEA levels displayed no association with age in either group (data not shown). The hotspot group showed a gradual rise in mean sex hormone levels with age (Fig. 1); however, the functional significance of androgen and estrogen in older men is still unclear.

3.4. Variance rate of PSA and PC

Six of the hotspot subjects had PSA levels >3 ng/mL, 3 of whom were suspected to have PC after digital rectal examination (DRE; Table 3).

3.5. Serum dioxins levels

Table 4 shows mean dioxins levels in the hotspot and non-sprayed groups. Mean levels of most dioxins, furans, and non-ortho PCBs were significantly higher in the hotspot group than in the non-sprayed group. However, >70% of the 2378-TeCDF,12378-PeCDF, 123789-HxCDF, 234678-HxCDF, 1234789-HpCDF, OCDF, TeCB#81, and TeCB#77 congeners were below detection limits or not detected (ND) in both groups, and are therefore not shown in Table 4.

3.6. Correlation of steroid hormones and dioxins

In both groups, significantly positive correlations were found between DHT and 1234678-HpCDD and 1234678-HpCDF; between 3β-HSD activity and 123678-HxCDD, 123478-HxCDF, 123678-HxCDF, and HxCB#169; and between estradiol and 12378-PeCDD, 123678-HxCDD, 1234678-HpCDD, 1234678-HpCDF (Table 5). In addition, we found significant negative correlations for both groups between DHEA and 123678-HxCDD or HxCB#169.

However, after using multiple linear regression, we found no significant correlation between DHEA, DHT, estradiol, 3β-HSD activity and any independent variables (dioxins levels, age, BMI, and tobacco use) in the two groups (Table 6). We also found no significant correlation between independent variables and other steroid hormones or enzyme activity in the two groups (data not shown). These findings implies that dioxins may effect steroid hormone levels with other factors in older men since multiple correlation coefficients in Table 6 were relatively low, although confirmation with further study is required.

4. Discussion

Dioxins can cause developmental problems in children, lead to reproductive and infertility problems in adults, result in miscarriages, damage the immune system, and interfere with hormones (WHO,

Table 3
Variance rate of PSA and DRE in the hotspot.

Subject (No.)	Age (Years)	Estrone (pg/mL)	Testosterone (pg/mL)	PSA (ng/mL) 2009	PSA (ng/mL) 2010	Variance (%)	DRE (+ −)
1	73	31.4	5039.5	7.5	8.7	16	−
2	64	38.6	5086.9	3.8	7.9	105	+
3	80	29.3	5400.6	3.2	3.0	−4.8	−
4	75	30.9	6018.8	3.4	5.1	51	+
5	74	30.9	8969.3	6.1	8.0	33	−
6	60	25.4	3728.1	4.5	6.4	43	+

Patients 2, 4, 6 were suspected of having prostate cancer. Following DREs.
DRE: digital rectal examination; PSA: prostate specific antigen; −: normality; +: abnormality.

Table 4
Serum dioxins levels in men from an herbicide-sprayed hotspot and men from a non-sprayed regions.

Dioxin congeners (pg/g lipid)	LOD (pg/g lipid)	Hotspot (n = 48) GM	Hotspot (n = 48) GSD	Non-sprayed region (n = 36) GM	Non-sprayed region (n = 36) GSD	Fold	p value
2378-TeCDD	0.01	2.6	2.1	1.4	1.5	1.8	0.000
12378-PeCDD	0.01	9.5	2.0	2.2	1.8	4.2	0.000
123478-HxCDD	0.02	5.7	1.8	2.6	1.6	2.2	0.000
123678-HxCDD	0.02	21.6	1.8	3.8	1.5	5.7	0.000
123789-HxCDD	0.02	7.6	2.0	2.7	1.6	2.8	0.000
1234678-HpCDD	0.02	33.2	2.0	6.2	1.7	5.4	0.000
OCDD	0.05	352.2	1.8	59.8	1.8	5.9	0.000
23478-PeCDF	0.01	14.4	1.8	6.4	1.5	2.3	0.000
123478-HxCDF	0.02	32.8	1.8	3.9	1.7	8.4	0.000
123678-HxCDF	0.02	24.9	1.8	4.4	1.6	5.7	0.000
1234678-HpCDF	0.02	47.4	2.0	4.2	1.9	11.3	0.000
PeCB#126	0.1	39.0	2.2	26.7	2.1	1.5	0.026
HxCB#169	0.1	117.0	2.0	19.1	1.7	6.1	0.000

LOD: limit of detection, GM: geometric mean, GSD: geometric standard deviation.

2010). A recent study of United States Vietnam War veterans found an increased risk of PC associated with AO exposure, and driven by increased risk of high-grade PC in men who underwent prostate biopsies (Ansbaugh et al., 2013). Current serum dioxin levels in residents of sprayed regions of Vietnam are much lower today, owing to wash-off by tropical rain and chemical breakdown over the past 45 years (Schecter et al., 1991; Manh et al., 2014). However, we found serum levels of dioxins, furans, and non-ortho PCBs were 1.5–11.3 times higher (depending on the specific compound) in the hotspot group than those in the non-sprayed group. Although dioxins is suspected to cause long-term endocrine disruption, very few epidemiologic studies have investigated its effect on steroid hormones and PC risk in Vietnamese men. This study aimed to determine whether dioxins effects on steroid hormone and PC risk were associated with age in men from an herbicide-exposed region.

Since PC was established as an androgen-dependent disease in 1941 (Huggins and Hodges, 1941), androgen-deprivation therapy has been accepted as a gold-standard treatment for PC. PC is common in men older than 50 years. The prostate is regulated by androgen and the role of androgens in the development of PC has been widely studied (Miller and O'Neill, 1990; Gann et al., 1996). Although positive family history is also a risk factor for PC, none of the subjects in this study reported that a family member had suffered PC. Aside from being androgen-dependent, the mechanism of PC development and progression remains largely unknown. Although steroid hormones, particularly androgens, are implicated in PC pathogenesis, most epidemiologic studies have shown no association, and the influence of steroid hormones on PC risk is poorly understood (Daniels et al., 2010).

Here we demonstrated that levels of testosterone and estradiol were significantly higher in the hotspot group than in the non-sprayed group, but levels of testosterone and estradiol were not associated with dioxins in these two groups, even after using multiple linear regression to adjust for age, BMI, and tobacco use. However, levels of DHT, testosterone and estrone significantly increased with age in the hotspot group only (Fig. 1). Although we previously showed that median PSA levels did not differ between the two groups, Six of the hotspot subjects had PSA levels >3 ng/mL, 3 of whom were suspected to have prostate cancer (PC) after digital rectal examination. Chamie et al. (2008) reported that mean PSA levels in men exposed to AO (3.1 ng/mL) did not significantly differ from those of unexposed men (1.8 ng/mL), but about twice as many exposed men suffered PC (239 vs. 124). Our results are nearly identical to those findings.

A cohort study of older men found that higher estrone levels were strongly associated with increased risk of PC (Daniels et al., 2010). Likewise, one prospective study found increased PC risk associated with

Table 5
Simple correlation coefficients among steroid hormone, 3β-HSD and dioxins congeners in men combined from herbicide-sprayed hotspots and unsprayed regions.

Dioxin congeners	Testosterone		DHT		DHEA		Estradiol		3β-HSD	
	r	p	r	p	r	p	r	p	r	p
12378-PeCDD	0.178	0.105	0.110	0.318	−0.157	0.155	0.244	0.025	0.175	0.111
123678-HxCDD	0.188	0.086	0.161	0.144	−0.250	0.022	0.223	0.041	0.235	0.031
1234678-HpCDD	0.179	0.104	0.216	0.049	−0.141	0.202	0.260	0.017	0.155	0.159
123478-HxCDF	0.182	0.098	0.183	0.096	−0.205	0.061	0.201	0.067	0.230	0.035
123678-HxCDF	0.154	0.163	0.147	0.183	−0.203	0.064	0.197	0.073	0.215	0.049
1234678-HpCDF	0.212	0.053	0.223	0.042	−0.141	0.201	0.230	0.036	0.196	0.075
HxCB#169	0.193	0.079	0.090	0.416	−0.223	0.042	0.181	0.099	0.232	0.034

r: Correlation coefficient, 3β-HSD (%) = (DHT + testosterone + estradiol + estrone) / DHEA × 100.

high levels of testosterone and low levels of sex hormone-binding globulin (Gann et al., 1996; Parsons et al., 2005). However, results from other prospective studies have not supported these findings (Heikkila et al., 1999; Chen et al., 2003; Stattin et al., 2004), and a meta-analysis of prospective studies found no statistically significant associations between risk and levels of testosterone and its metabolites (Eaton et al., 1999). These inconsistencies may reflect discrepancies between etiologically relevant hormones in younger men and measured levels in older men's sera, as male steroid hormone levels decline with age (Ellis and Nyborg, 1999). A recent study of older men found those with very low DHT levels tended to have slightly lower PC risk, but not significantly so (Muller et al., 2012). DHT is the primary effector androgen and is converted from testosterone by 5α-Reductase (Carson and Rittmaster, 2003). Most serum DHT is presumably derived from intracellular conversion of testosterone in the prostate, which might explain why serum DHT was unrelated to PC risk (Muller et al., 2012).

Prostate cancer distribution partially reflects changes in androgen production with age. Serum testosterone decreased in aged male mice lacking the aryl hydrocarbon receptor (Baba et al., 2008); this implies a mechanism by which dioxins causes PC by affecting aryl hydrocarbon receptors.

As shown Table 2 estradiol was significantly higher in the hotspot group than in the non-sprayed group, but it was not associated with dioxins, even after using multiple linear regression to adjust for age, BMI, and tobacco use. Estradiol has been reportedly associated with testosterone and DHT (Parsons et al., 2005). Levels of DHT and testosterone were significantly higher in the hotspot group than in the non-sprayed group, which could explain why we found no correlation between dioxins and estradiol in the two groups. Mean levels of DHEA were significantly lower in the hotspot group than in the non-sprayed group. However, after adjusting for age, BMI, and tobacco use, DHEA and dioxins were not significantly correlated. Our previous study found an inverse association of highly chlorinated dioxin congeners in maternal breast milk with DHEA levels in 3-year-old Vietnamese children (Kido et al., 2016). Steroidogenesis is more likely to be influenced by dioxin in children than in older men.

We calculated individual enzyme activities from serum steroid levels as ratio of 5α-reductase activity, CYP17-lyase activity, 3β-hydroxysteroid dehydrogenase activity (3β-HSD), and aromatase activity. The ratio of 5α-reductase activity (DHT/testosterone) did not significantly differ between the two groups. Levels of testosterone were significant higher in the hotspot group than in the non-sprayed group, possibly as DHT increases with elevation of testosterone only. In humans, the endoplasmic reticular cytochrome P450 with 17α–hydroxylase and 17–20 lyase (CYP17) is critical in the biosynthesis of steroid hormones and has become a valuable target in PC treatment (Moreira et al., 2008; Vasaitis et al., 2011; Pezaro et al., 2012). In this study, the ratio of CYP17-lyase activity did not significantly differ between these two groups. In contrast, DHT and testosterone were significantly higher in the hotspot group than in the non-sprayed group. CYP17 inhibitor (which inhibits the key enzyme that catalyzes biosynthesis of androgens from pregnane precursors) could reportedly prevent androgen production from all sources (Vasaitis et al., 2011). In humans, two 3β-HSD isozymes are encoded by the HSD3B1 (3β-HSD1) and HSD3B2 (3β-HSD2) genes. A recent study suggested that 3β-HSD1 is a valid target for the treatment of castration resistant PC (Chang et al., 2013). The percentage of 3β-HSD activity was significantly higher in the hotspot group than in the non-sprayed group. Baba et al. (2008) reported that 3β-HSD expression in testicular Leydig cells decreased in aged male mice lacking the aryl hydrocarbon receptor. (Baba et al., 2008). Although 3β-HSD activity was not associated with dioxins in the two groups, even after using multiple linear regression to adjust for age, BMI, and tobacco use, its activity significantly increased with age in the hotspot group only. This finding implies that dioxins may increase 3β-HSD activity in older men, although confirmation with further study is required.

In this study, we found dioxins/herbicide levels were significantly higher in the hotspot group, and percentage of aromatase activity was significantly lower in the non-sprayed group. Aromatase activity is decreased by the herbicide glyphosate (Gasnier et al., 2009).

Our study has several limitations. First, we only examined subjects for PC (by DRE) whose PSA > 3 ng/mL; all subjects were not examined in both regions. Furthermore, our study was based on relatively small numbers of Vietnamese men. Further studies will be needed with larger sample sizes, and prostate biopsy sampling and histological examinations to confirm our findings.

Table 6
Multiple regression analysis for steroid hormone and 3β-HSD in men combined from herbicide-sprayed hotspots and unsprayed regions.

Dioxin congeners	Testosterone			DHT			DHEA			Estradiol			3β-HSD		
	β	p	R^2	β	p	R^2	β	p	R^2	β	p	R^2	β	p	R^2
12378-PeCDD	0.075	0.510	0.115	0.006	0.959	0.099	−0.096	0.417	0.064	0.198	0.091	0.085	0.057	0.614	0.130
123678-HxCDD	0.086	0.462	0.116	0.044	0.705	0.101	−0.187	0.117	0.085	0.152	0.204	0.070	0.113	0.325	0.138
1234678-HpCDD	0.071	0.559	0.114	0.094	0.439	0.106	−0.040	0.746	0.057	0.182	0.142	0.076	−0.003	0.983	0.128
123478-HxCDF	0.080	0.493	0.115	0.076	0.518	0.104	−0.141	0.239	0.072	0.125	0.298	0.063	0.111	0.334	0.131
123678-HxCDF	0.038	0.748	0.111	0.030	0.801	0.100	−0.135	0.261	0.071	0.119	0.323	0.062	0.086	0.456	0.134
1234678-HpCDF	0.104	0.374	0.119	0.122	0.302	0.111	−0.061	0.616	0.059	0.161	0.183	0.072	0.060	0.608	0.124
HxCB#169	0.101	0.371	0.119	−0.003	0.976	0.099	−0.166	0.151	0.080	0.122	0.292	0.064	0.129	0.247	0.142

Multiple regression analysis was adjusted for age, body mass index (BMI), and tobacco use.
β: standardized coefficients, R^2: multiple correlation coefficient.
3β-HSD (%) = (DHT + testosterone + estradiol + estrone) / DHEA × 100.

5. Conclusion

Despite decreasing levels of dioxin in the environment, our findings indicate that dioxin exposure can lead to increases in several sex steroid hormones with age. The correlation of dioxin with steroid hormone levels and PC risk should to be clarified by further study.

Acknowledgments

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