Uploaded to the VFC Website

November 2014

This Document has been provided to you courtesy of Veterans-For-Change!

Feel free to pass to any veteran who might be able to use this information!

For thousands more files like this and hundreds of links to useful information, and hundreds of "Frequently Asked Questions, please go to:

Veterans-For-Change

If Veterans don't help Veterans, who will?

Note: VFC is not liable for source information in this document, it is merely provided as a courtesy to our members & subscribers.

Chemosphere 118 (2015) 277–283

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Predictors for dioxin accumulation in residents living in Da Nang and Bien Hoa, Vietnam, many years after Agent Orange use

Diem T. Pham [a,*], Hang M. Nguyen [b], Thomas G. Boivin [c], Anna Zajacova [d], Snehalata V. Huzurbazar [e], Harold L. Bergman [a]

[a] Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82071, USA
[b] Office of National Steering Committee 33, The Vietnamese Ministry of Natural Resources and Environment, Ha Noi, Vietnam
[c] Hatfield Consultants Partnership, North Vancouver, British Columbia V7P 0A3, Canada
[d] Department of Sociology, University of Wyoming, Laramie, WY 82071, USA
[e] Department of Statistics, University of Wyoming, Laramie, WY 82071, USA

HIGHLIGHTS

- Blood dioxin levels were measured from residents in Bien Hoa and Da Nang, Vietnam.
- Blood dioxin levels were related to individual and environmental risk factors.
- Fish farming was associated with higher blood dioxin levels at both locations.
- Blood dioxin levels were positively correlated with living on flooded property.
- Da Nang dioxin sites are being cleaned up so exposure should decrease.

ARTICLE INFO

Article history:
Received 7 March 2014
Received in revised form 2 September 2014
Accepted 10 September 2014

Handling Editor: Andreas Sjodin

Keywords:
TCDD
Dioxin
Agent Orange
Risk factors
Blood concentration
Vietnam

ABSTRACT

Agent Orange (AO) was the main defoliant used by the US in Vietnam from 1961 to 1971; AO was contaminated with dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, or TCDD). Three major dioxin "hot spots" remain from previous AO storage and use at former US bases at Bien Hoa, Da Nang, and Phu Cat, posing potential health risks for Vietnamese living on or near these hot spots. We evaluated potential risk factors contributing to serum TCDD levels in Vietnamese residents at and near contaminated sites in Da Nang and Bien Hoa, Vietnam. We used multiple linear regression to analyze possible associations of blood dioxin concentrations with demographic, socioeconomic, lifestyle, and dietary risk factors for residents living on or near these hot spots. For the Da Nang study, fish farming on the site, living on property flooded from monsoon rains, and age were among the factors showing significant positive associations with serum TCDD concentrations. For the Bien Hoa study, fish farmers working at this site and their immediate family members had significantly higher serum TCDD concentrations. Our results suggest that water-related activities, especially fish-farming, at the hot spots increased the risk of exposure to dioxin.
© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

High levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) have been found in soils and aquatic sediments in several areas of southern Vietnam that were contaminated during the Vietnam War in 1961–1971 via spraying, handling and/or spillage of herbicides, mainly Agent Orange (Dwernychuk et al., 2002; Mai et al., 2007; Nhu et al., 2009). Agent Orange herbicide used by the US military in Vietnam was a 50:50 mixture of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), which was contaminated with TCDD during the manufacturing process. TCDD is a member of a class of chemicals known commonly as "dioxins", and it is considered to be the most toxic compound in this chemical group (Van den Berg et al., 2006). TCDD (hereafter sometimes referred to as dioxin) is associated with numerous health effects in adults including soft-tissue sarcoma, non-Hodgkin's lymphoma, chronic lymphocytic leukemia and also birth defects such as spina bifida in newborns (IOM, 2009).

* Corresponding author at: Zoology and Physiology Department (3166), University of Wyoming, 1000 E. University Ave., Laramie, WY 82071, USA. Tel.: +1 3073991170.

E-mail addresses: dpham@uwyo.edu (D.T. Pham), nguyen.my.hang@gmail.com (H.M. Nguyen), tboivin@hatfieldgroup.com (T.G. Boivin), azajacov@uwyo.edu (A. Zajacova), lata@uwyo.edu (S.V. Huzurbazar), bergman@uwyo.edu (H.L. Bergman).

http://dx.doi.org/10.1016/j.chemosphere.2014.09.064
0045-6535/© 2014 Elsevier Ltd. All rights reserved.

Additionally, elevated dioxin levels in food items collected near contaminated sites have also been reported (Hatfield Consultants, 1998; Schecter et al., 2003; Hatfield Consultants and Office 33, 2009, 2011). Even more worrisome, high levels of dioxin in blood and breast milk samples have also been found in Vietnamese residents living on or near these contaminated sites (Schecter et al., 1995; Dwernychuk et al., 2002; Hatfield Consultants and Office 33, 2009, 2011; Saito et al., 2010). Despite the elevated dioxin levels measured in human blood, breast milk, and environmental media in Vietnam, little research has been conducted to study specific risk factors contributing to dioxin exposure. A study by Saito et al. (2010) found that previous exposure was the major contributor to high levels of breast milk dioxin in females living in sprayed areas of Quang Tri Province, Vietnam. However, dioxin found on surface soils in sprayed areas is degraded by ultraviolet light; sprayed areas are, therefore, no longer a main concern for dioxin contamination (NRC, 2003; Dwernychuk, 2005). Compared to the sprayed areas, the areas where quantities of Agent Orange were stored, handled, and spilled have much higher dioxin concentrations in soils and in nearby aquatic sediments and are referred to as dioxin "hot spots" (Dwernychuk, 2005). Among these dioxin hot spots, the former US military bases at Bien Hoa, Da Nang, and Phu Cat are sites of major concern (Dwernychuk, 2005). Thus, it is important to investigate potential risk factors contributing to dioxin accumulation in residents living on or near these hot spots, so as to manage and remediate these and other dioxin contaminated sites to reduce human exposure.

The current study is part of an extensive reassessment of dioxin contamination and human exposure at Da Nang and Bien Hoa recently completed by Hatfield Consultants and the Vietnam Office of National Steering Committee 33 (Office 33) (Hatfield Consultants and Office 33, 2009, 2011). Although Hatfield Consultants and Office 33 (2009, 2011) and Boivin et al. (2011) already presented and partially analyzed serum dioxin data from the Da Nang and Bien Hoa studies, we extend their analyses using multiple linear regression. The objective of the current study is to evaluate potential demographic, socioeconomic, health, lifestyle, and dietary risk factors contributing to dioxin exposure and accumulation in residents who are living on or near the Da Nang and Bien Hoa dioxin hot spots.

2. Materials and methods

2.1. Study populations

This study is a part of three investigations conducted by Hatfield Consultants and the Government of Vietnam to determine the extent and level of dioxin contamination in the environment and the exposed human populations on and around former US military bases in Da Nang and Bien Hoa. Details of the 2006 and 2009 studies in Da Nang and the 2010 study in Bien Hoa are reported by Hatfield Consultants and Office 33 (2009, 2011).

Individuals participating in these studies were all at least 18 years old. Eligible individuals were selected from the population living on or around the former US military bases at Da Nang (now the Da Nang International Airport) and Bien Hoa (now a Vietnamese Military Airbase). For the Da Nang studies conducted in 2006 and 2009, the sub-populations included in the study were selected either randomly or non-randomly as follows. Sub-populations from which samples were selected randomly included: (1) An Khe Ward, Thanh Khe District (2009 study) located near the west side of the Da Nang Airport and also close to the Pacer Ivy Area, where Agent Orange drums were cleaned, re-labeled, and repacked for shipping the Agent Orange out of Vietnam in 1972 (Young, 2009); (2) Khue Trung Ward, Cam Le District (2009 study) is a low-lying wetland located south of the Da Nang Airport; (3) Thuan Tay Ward, Hai Chau District (2009 study) is near the eastern boundary of the Da Nang Airport; (4) Chinh Gian Ward, Thanh Khe District (2006 study) is a former wetland connected to Sen Lake near the north boundary of the Airport; and (5) Thuan Phuoc Ward, Hai Chau District (2006 study) was the control area located 5 km away from the Airport. Sub-populations from which samples were selected non-randomly included (1) Sen Lake workers and their family members (2006 and 2009 studies) who were chosen non-randomly because they had been in contact with the contaminated Sen Lakes inside the Airport; and (2) West Airbase workers and their family members (2006 and 2009 studies) who were chosen non-randomly because they had been in contact with lakes in the western perimeter of the Airport. The total sample size for these studies was 186 participants. For the Bien Hoa study conducted in 2010, Bien Hoa Airbase workers were chosen randomly and resulted in 42 total participants.

2.2. Questionnaires

All individuals participating in the survey and who provided blood samples did so voluntarily, and signed consent forms prior to sampling. Questionnaires and consent forms were in the Vietnamese language. Staff members from Office 33, the Ministry of Health and the local health department interviewed each volunteer donor in Vietnamese with guidance from Hatfield personnel. Surveys included information on participants' age, sex, smoking habits, general health, residency, work history, food consumption patterns, and general awareness about dioxins.

Demographic and socioeconomic variables including age, gender, and education were collected from questionnaire surveys and used in both the Da Nang and Bien Hoa analyses presented here. Age was determined in years at the time of sampling. Education levels were self-reported using multiple grade categories, but since the sample sizes for both Bien Hoa and Da Nang were small, this education variable was combined into 2 categories of "high school graduate and below" and "any postsecondary education".

Additionally, weight, height, current smoking status, and residency were included in this study. Weight (in kilograms) and height (in centimeters) information were self-reported, and were combined to calculate body mass index (BMI) as weight (kg) divided by height in meters squared (m^2), to obtain an estimate of participants' body fat. Current smoking status was recoded as a dichotomous (yes/no) categorical variable and residency information was collected by asking participants about their duration (in years) of living at current ward addresses.

2.3. Measurement of serum dioxin concentrations

To measure the blood serum dioxin concentration, each respondent was asked to donate his or her blood sample, with signed consent forms obtained in advance. Under the supervision of Hatfield personnel, trained medical staff from the local health departments in Da Nang and in medical clinics at the Bien Hoa Airbase collected participants' blood samples. The samples were then shipped to AXYS Analytical Services in Sidney, British Columbia, Canada for analysis using High Resolution Gas Chromatography Mass Spectrometry (HR GC/MS) (Hatfield Consultants and Office 33, 2009, 2011). These analyses provided concentrations of 17 PCDD and PCDF compounds in pg/g or parts per trillion (ppt) (lipid weight basis) and also the dioxin Toxicity Equivalent Quotients (TEQ) using Toxic Equivalency Factor (TEF) values from the WHO 2005 scale (WHO, 2011). The 2006 Da Nang dioxin concentrations were measured from whole blood samples and the 2009 Da Nang and 2010 Bien Hoa dioxin concentrations were measured from serum samples as reported by Hatfield Consultants and Office 33 (2009, 2011). Notably, although TCDD levels were measured in different tissues (serum samples and whole blood samples), results from studies conducted by other researchers comparing TCDD measures from whole blood and serum samples on a lipid-adjusted basis are highly correlated (Patterson et al., 1988; Schecter et al., 1991).

2.4. Data analysis

Descriptive statistics were calculated for all variables collected from the surveys for the Da Nang and Bien Hoa studies. Serum TCDD concentrations and TEQ values for both studies were highly skewed based on tests of normality and were, therefore, natural-log-transformed. Furthermore, bivariate analyses, specifically, Spearman rank test and ANOVA were also used to test the associations and distributions of serum TCDD concentrations and TEQ values with each variable in both studies. Additionally, to deal with Da Nang and Bien Hoa data sets with missing survey questionnaire responses that were randomly distributed in the data set, multiple imputation methods were applied to fill in each missing response with a set of plausible responses (Berglund, 2010). Then, multiple linear regression (MLR) was used as the main analytical method to assess how well the variation in serum TCDD concentrations can be explained when using a set of explanatory variables. Since a great deal of information was collected from each survey questionnaire, the following variable selection procedures were applied to both Da Nang and Bien Hoa datasets to choose variables for inclusion in the final models. First, forward stepwise variable selection and backward-elimination procedures were applied to select the variables with *p*-values smaller than the SAS software's default criterion of 0.05. To test the stability of their significance, selected variables resulting from a previous step were then added individually into the MLR models, which already included demographic factors. Variables selected from both steps were then combined in the final model along with demographic factors if the selected variables satisfied one of the following conditions: (1) they were found to be associated with tissue dioxin concentrations from previous published research; or (2) they were related to dietary habits, since one of the major pathways of dioxin accumulation in adults is associated with contaminated food consumption (NRC, 2003). Statistical analyses in this study were conducted using SAS version 9.2 software (SAS Institute Inc., Cary, NC, USA) and R version 3.1.0 (R Core Team, 2014) with the psych (Revelle, 2014), ggplot2 (Wickham, 2009), plyr (Wickham, 2011), and reshape2 (Wickham, 2007) packages.

2.4.1. Da Nang study

Data from the Da Nang study came from two different years, 45 participants from 2006, 111 from 2009 and 15 from both 2006 and 2009. To combine the data from the two years, we performed a paired *t*-test to test whether the differences in serum dioxin levels of the 15 participants in 2006 and 2009 are statistically significant. The result from this test allowed us to include the 2009 values for serum dioxin concentrations and, thus, drop out blood dioxin concentrations for the 2006 samples from these 15 re-sampled participants in the model for the Da Nang study ($t(14) = -1.073$, $p = 0.301$). Additionally, 31 participants were excluded from the analysis presented in this study because they either refused to allow blood samples to be taken or did not appear at the medical clinic to provide samples. Thus, the total number of participants included in the Da Nang MLR model was 140.

A Spearman rank test was performed to determine whether TCDD and TEQ values were correlated. Results from this test showed a highly positive association between the values for TCDD and TEQ ($r(138) = 0.866$, $p < 0.0001$). Thus, for the purposes of this study, only the concentrations of TCDD in serum samples were included in the analysis as a dependent variable.

Dichotomous independent variables used as explanatory variables for serum dioxin concentrations besides the demographic and socioeconomic variables in the Da Nang model were as follows: (1) for dietary factors: eating fish caught from ponds and lakes in the Da Nang Airport and eating fish muscle, fat and/or organs; (2) for lifestyle variables: working on or being inside the Da Nang Airport, buying (and presumably consuming) ducks from local markets, flood water entered participants' properties, harvesting lotus or other aquatic vegetables growing in ponds/lakes in the Da Nang Airport, living in Thanh Khe District, and working as fish farmers or being members of fish farmers' immediate families; (3) additionally, current smoking status was also included in the MLR analysis in this model.

2.4.2. Bien Hoa study

The total sample population for the Bien Hoa study was 42 participants. Similar to the Da Nang study, TCDD concentration in serum samples was significantly correlated to TEQ values based on the Spearman rank test ($r(40) = 0.99$, $p < 0.0001$). Thus, the dependent variable for this Bien Hoa model is the serum TCDD concentrations.

For the Bien Hoa study, we considered fewer explanatory variables in the model due to the smaller sample size ($n = 42$). Besides demographic, socioeconomic and health factors, the following dichotomous (yes/no) independent variables were included in the MLR model: eating birds at least a few times per week; eating fish caught from ponds and lakes in the Bien Hoa Airbase; eating fish muscle, fat and/or organs; raising and harvesting ducks inside the Bien Hoa Airbase; fish-farmers and their immediate family members; and personally handling or working with Agent Orange and/or other defoliants.

3. Results

The characteristics of participants included in the Da Nang study are shown in Table 1. Of the 140 participants in the Da Nang study, 56% were male, 79% were high school graduates or below, and 36% were currently smoking. The average age of Da Nang participants was 39.6 years (SD = 12.4 years) and they had an average BMI of 21.6 kg m^{-2} (SD = 3.0 kg m^{-2}). The average number of years residing at their current ward address was 22.1 years with a wide range of values (SD = 13.6 years). About one-third (37%) of the participants reported either working at or being inside the Da Nang Airport, while 16% were fish farmers or members of their immediate families. Sixty-nine (69) percent bought ducks from local markets. As for residence location, 42% of participants lived in Thanh Khe District and approximately 42% of participants had floodwater enter their property during monsoon rains.

Table 1 also shows characteristics of the 42 Bien Hoa participants. The average age of the sample population was 47.1 years (SD = 3.2 years) with an average BMI of 22.2 kg m^{-2} (SD = 2.0 kg m^{-2}). A majority (88%) of Bien Hoa participants were male, and 62% had an educational level of postsecondary. Their average total years of residency was 17.1 years (SD = 6.5 years). All participants in the Bien Hoa study were Airbase workers. Almost half (47%) of the participants raised and harvested ducks from ponds and lakes on the Bien Hoa Airbase. Fourteen (14) percent of Bien Hoa participants were fish farmers or members of farmers' immediate families.

3.1. Descriptive statistics for serum dioxin concentrations

Bien Hoa participants had higher geometric mean serum TCDD concentrations (82.431 ppt lipid weight basis) than Da Nang participants (11.913 ppt) as shown in Table 2. Similar to the serum TCDD concentrations, the Bien Hoa participants' mean serum TEQ

Table 1
Characteristics of participants in Da Nang ($n = 140$) and Bien Hoa ($n = 42$) studies.

Characteristic	Da Nang			Bien Hoa		
	Missing[a] (n)	Mean ± SD	n (%)	Missing[a] (n)	Mean ± SD	n (%)
Age (years)	0	39.6 ± 12.4		0	47.1 ± 3.2	
Body mass index (kg m^{-2})	6	21.6 ± 3.03		0	22.2 ± 2.0	
Residency (years)	1	22.1 ± 13.6		0	17.1 ± 6.5	
Male	0		79 (56.4)	0		37 (88.1)
Education	0			0		
High school graduate or below			111 (79.3)			16 (38.1)
Any postsecondary			29 (20.7)			26 (61.9)
Currently smoking	3		49 (35.8)	0		16 (38.1)
Fish farmers or immediate families	0		23 (16.4)	0		6 (14.3)
Eating fish muscle, fat and/or organs	1		70 (50.4)	0		23 (54.8)
Ever worked on or been inside Da Nang Airport	0		52 (37.1)			
Buying ducks from local markets	15		86 (68.8)			
Flood water enters home	1		58 (41.7)			
Harvesting lotus or other aquatic vegetables from Da Nang Airport	1		25 (18.0)			
Living in Thanh Khe district	0		59 (42.1)			
Ever eaten fish caught from ponds/lakes in Da Nang Airport	1		44 (31.6)			
Ever eaten fish caught from Bien Hoa Airbase				1		37 (90.2)
Eating birds at least a few times per week				2		20 (50.0)
Raising, harvesting ducks from ponds/lakes in Bien Hoa Airbase				2		19 (47.5)
Personally handling or working with Agent Orange and/or other defoliants				0		10 (23.8)

[a] Each number represents the number of participants with missing survey responses and for whom plausible responses were estimated using multiple imputation methods (Berglund, 2010).

Table 2
Serum TCDD concentrations and TEQ[a] values in pg/g lipid for participants in Da Nang, Bien Hoa, and National Health and Nutrition Examination Survey (NHANES) 2003–2004[b] studies.

	Da Nang ($n = 140$)	Bien Hoa ($n = 42$)	NHANES ($n = 719$)
2,3,7,8-TCDD			
Geometric mean	11.913	82.431	–[c]
Range	0.005–1340	13.7–1970	–[d]
50th Percentile	8.520	67.75	–[c]
75th Percentile	35.575	132.50	2.9
95th Percentile	212.400	1004.35	5.3
TEQ			
Geometric mean	54.107	197.9	14.1
Range	7.75–1410	19.3–2020	–[d]
50th Percentile	50.60	82.85	14.1
75th Percentile	79.15	143.25	22.4
95th Percentile	293.15	1043.35	39.0

[a] TEQ values were calculated from 2005 WHO TEF values (WHO, 2011). In the Da Nang and Bien Hoa studies, no detection = ½ detection limit value.
[b] NHANES data are extracted from Chen et al. (2013).
[c] These values are not calculated due to high proportions of results were below limit of detection.
[d] Not available.

values were approximately 3 times higher (197.9 ppt) than Da Nang participants (54.107 ppt). Complete values of dioxin congeners for Da Nang and Bien Hoa studies are summarized and presented in Supplementary Table 1.

3.2. Regression results for serum dioxin concentration

The results of multiple linear regression analyses for natural-log-transformed TCDD concentrations with demographic characteristics, lifestyles and dietary habits as explanatory variables for the Da Nang study are shown in Table 3. Older age was associated with significantly higher serum dioxin concentration ($e^{\beta} = 1.04$, 95% confidence interval (CI): 1.02, 1.061; $p = 0.0001$). Participants whose property was flooded during monsoon rains had significantly higher serum TCDD concentrations ($e^{\beta} = 1.71$, 95% CI: 1.058, 2.763; $p = 0.0288$). Participants who had worked on or been inside the Da Nang Airport had significantly higher serum dioxin concentrations ($e^{\beta} = 1.817$, 95% CI: 1.027, 3.215; $p = 0.0404$). Fish farmers working on the Da Nang Airport and their immediate family members were weakly associated with higher serum TCDD concentrations ($e^{\beta} = 2.65$, 95% CI: 0.98, 7.166; $p = 0.0548$).

MLR results for the Bien Hoa study (Table 4) suggest that raising and harvesting ducks inside the Bien Hoa Airbase was weakly associated with serum dioxin concentrations ($e^{\beta} = 1.9$, 95% CI: 0.949, 3.807; $p = 0.069$). But fish farmers working inside the Bien Hoa Airbase and their immediate family members had significantly higher serum dioxin concentrations than other participants in the sample population ($e^{\beta} = 3.748$, 95% CI: 1.287, 10.918; $p = 0.017$).

4. Discussion

Participants in this study were exposed to TCDD for a long period of time at both sites, with mean residencies of 22.1 and 17.1 years on or near the Da Nang and Bien Hoa sites, respectively. Though living on or near the Bien Hoa Airbase site for shorter average lengths of time, Bien Hoa participants had substantially higher geometric mean serum TCDD concentrations than Da Nang participants (Table 2). This difference in serum TCDD levels at the two sites was likely due to a number of factors including differences in exposure levels and pathways. In addition, samples from populations at the two sites were drawn differently with participants selected for the Bien Hoa study being limited to individuals who worked on the Bien Hoa site.

Given the differences in how study populations were drawn and the potential differences in exposure levels at the Da Nang and Bien Hoa sites, issues discussed here will focus on an evaluation of exposure pathways associated with reported serum dioxin concentrations in the Da Nang and Bien Hoa study populations. Results from the Da Nang MLR model (Table 3) showed that four demographic and lifestyle variables contributed significantly to increased serum TCDD concentrations in study participants: (1) living on property where flood water entered during monsoon rains, (2) working on or been inside the Airport, (3) age at sampling, and (4) fish farmers or their immediate families. For the Bien Hoa study (Table 4), due in part to the smaller sample size, only fish farmers or their immediate family members had a significant positive association with serum TCDD concentrations.

Table 3
Multiple linear regression model effects for natural-log-transformed serum TCDD concentrations for the Da Nang study ($n = 140$).

	Estimate (e^{β})	95% CI	p-Value
Age at sampling (years)	1.04	(1.02, 1.061)	0.0001
Body mass index (kg m^{-2})	1.028	(0.955, 1.107)	0.4592
Residency (years)	0.997	(0.98, 1.014)	0.7488
Male	1.126	(0.68, 1.863)	0.6418
Education[a]	1.325	(0.738, 2.379)	0.3427
Currently smoking	1.173	(0.708, 1.943)	0.5337
Ever eaten figh caught from ponds/lakes in the Da Nang Airport	1.171	(0.615, 2.228)	0.6281
Eating fish muscles, fat and/or organs	0.743	(0.472, 1.171)	0.1988
Ever worked on or been inside Da Nang Airport	1.817	(1.027, 3.215)	0.0404
Buying ducks from the local markets	0.786	(0.495, 1.247)	0.3027
Flood water entered home	1.71	(1.058, 2.763)	0.0288
Harvesting lotus or other aquatic vegetables from ponds/lakes in the Da Nang Airport	2.091	(0.815, 5.36)	0.1236
Living in Thanh Khe district	1.491	(0.855, 2.599)	0.1575
Fish farmers or immediate family members	2.65	(0.98, 7.166)	0.0548
Average adjusted R^2	0.447		

[a] "High School Graduate and Below" is the omitted reference group.

Table 4
Multiple linear regression model effects for natural-log-transformed serum TCDD concentrations for participants in the Bien Hoa study ($n = 42$).

	Estimate (e^{β})	95% CI	p-Value
Age at sampling (years)	0.949	(0.86, 1.047)	0.280
Body mass index (kg m^{-2})	1.021	(0.871, 1.197)	0.792
Residency (years)	0.986	(0.932, 1.043)	0.609
Male	1.348	(0.542, 3.352)	0.506
Education[a]	0.779	(0.409, 1.484)	0.432
Currently smoking	0.805	(0.44, 1.472)	0.467
Eating fish caught from ponds/lakes in the Bien Hoa Airbase	1.396	(0.506, 3.855)	0.505
Eating fish muscles, fat and/or organs	0.878	(0.436, 1.768)	0.706
Eating birds at least a few times per week	0.574	(0.302, 1.089)	0.086
Raising, harvesting ducks inside the Bien Hoa Airbase	1.9	(0.949, 3.807)	0.069
Handling or working directly with Agent Orange and/or other defoliants	1.231	(0.507, 2.988)	0.634
Fish farmers or immediate family members	3.748	(1.287, 10.918)	0.017
Average adjusted R^2	0.494		

[a] "High School Graduate and Below" is the omitted reference group.

To further assess the extent of dioxin contamination in Da Nang and Bien Hoa participants, we compared their mean serum lipid-adjusted TCDD and TEQ values to the National Health and Nutrition Examination Survey (NHANES) 2003–2004 data (Chen et al., 2013). Residents living on or near Da Nang and Bien Hoa sites have much higher serum TCDD levels and TEQ values as compared to this reference population (Table 2). This indicates a high priority for follow-up risk assessment studies to be conducted at Da Nang and Bien Hoa.

Working on or having been inside the Da Nang Airport was also a significant contributor to serum dioxin concentrations in study participants (Table 3). Hatfield Consultants and Office 33 (2009) reported very high soil dioxin concentrations (as high as 13,400 ppt dry weight) on the Da Nang Airport grounds, particularly at the former Mixing and Loading, and Storage Areas. Garabrant et al. (2009) also suggested that historic exposures contributing to serum dioxin concentrations included activities such as working at dioxin-contaminated sites in Midland and Saginaw, Michigan.

Results from our study also suggest that water-related activities at contaminated ponds and lakes inside the dioxin hot spots are an important source of exposure to dioxin for Da Nang and Bien Hoa participants. For instance, in both studies, fish farming activities were significantly associated with elevated serum dioxin concentrations (Tables 3 and 4). Moreover, Hatfield and Office 33 (2009, 2011) reported high TCDD concentrations in fish caught from these contaminated ponds and lakes inside both Da Nang and Bien Hoa sites. This association is likely due to consumption of contaminated fish as well as contact with contaminated water and sediments (Schecter et al., 2001). However, eating fish caught from ponds/lakes in both Da Nang and Bien Hoa studies was not significantly associated with higher serum TCDD levels due to the following limitations in our study: (1) we did not have sufficient information related to the frequency and the amount of fish consumption in these participants, and (2) our survey questions did not specify whether this dietary habit was a current or past practice. On the other hand, as expected from our MLR models, consumption of lotus or other aquatic vegetables harvested from ponds/lakes in the Da Nang Airport was not significantly associated with increasing the risk of exposure to dioxin in these participants since they have been reported to have low dioxin levels (NRC, 2003; Schecter et al., 2003). Nonetheless, these water-related activities at the Da Nang and Bien Hoa sites, as well as possible consumption of fish, lotus or ducks from these sites, are important sources of dioxin exposure as previously reported by Schecter et al. (2003), Hatfield Consultants and Office 33 (2009, 2011), and Tran et al. (2010).

In addition to water-related activities at contaminated ponds and lakes inside the dioxin hot spots, living at the areas where floodwaters enter homes located near dioxin contaminated sites also increases the risk of exposure to dioxin in residents living at these areas. Historically, these participants were more likely to have been exposed to higher TCDD contamination in soils. This result suggests that one of the potential mechanisms of dioxin spreading in these areas is via water-mediated transport of contaminated soils (Quynh et al., 1989; Hatfield and Office 33, 2009, 2011).

Though the effect of residency on increasing serum TCDD levels in our MLR models was not statistically significant, it is important to note that those who moved to or started working on the Da Nang site more recently (i.e. 5 years) have lower serum TCDD concentrations ($t(134.7) = 2.756$, $p = 0.007$). Additionally, in the Da Nang study, participants living closer to the Airport have higher serum TCDD levels than those living 5 kilometers (km) away from the Airport (Supplementary Figs. 3a and 3b). We were unable to conduct similar analyses on the Bien Hoa study because of the following reasons: (1) the sample population was limited to only Bien Hoa Airbase workers and (2) there are only two participants who moved to their current addresses 5 years ago. These participants, however, are fish farmers with extremely high serum TCDD concentrations; therefore using them in such an analysis might bias the result.

Our study also suggests that age of participants was also associated with elevated serum TCDD concentrations in the MLR model for the Da Nang study. We illustrate the distributions of serum TCDD concentrations by age of participants in the Da Nang and Bien Hoa studies in Supplementary Figs. 1 and 2. Similar results to ours were also reported in Quynh et al. (1989), Burns et al. (2010), and Chen et al. (2013). This age-TCDD relationship is likely associated with a longer exposure period and/or it may also reflect a longer TCDD half-life in older individuals (Wolfe et al., 1994).

There are several limitations in our study that prevented maximum exploration of the data. To be able to include more explanatory variables in the MLR model, we would have needed a larger sample size. This obstacle restricted the number of explanatory variables included in the Bien Hoa MLR model compared to the number included in the Da Nang model, which had a larger sample size. Additionally, one of the requirements to apply the MLR model is approximately even distribution of each answer category in dichotomous categorical variables. For example, in the Da Nang data sets, only 6 out of 140 participants reported that they raised or harvested ducks from the Da Nang Airbase. This variable was, therefore, not included in the Da Nang model.

5. Conclusions

We were able to find statistically significant and practically important associations between demographic and lifestyle variables and serum TCDD concentrations. Results from both Da Nang and Bien Hoa studies show that contributions to serum TCDD levels in people residing on or near these sites included activities such as working or being on the sites, as well as fish-farming on contaminated ponds and lakes. Equally important, our findings also show that local environmental conditions such as flooding during monsoon rains where these residents were living also increased the risk of exposure to TCDD residues. Although our study found several risk factors related to dioxin accumulation in residents living on or near Da Nang and Bien Hoa sites, we acknowledge that there might be some other major unidentified pathways of exposure to dioxin in these populations since our MLR models explained only small proportions of the overall variation in TCDD levels.

The findings in this study suggest a high priority for a follow-up risk assessment for residents living on or near these dioxin contaminated hot spots. Additionally, to reduce human exposure to dioxin effectively, not only public educational programs but also strategies for environmental remediation of dioxin contamination or removal of TCDD should be implemented at these sites (Tran et al., 2013). We note, however, that the Da Nang Airport is currently being cleaned up with funding by the US Government (USAID, 2013). Bien Hoa is also targeted for cleanup in the next few years (Boivin et al., 2011).

Acknowledgements

This research was funded in part by the University of Wyoming's Helga Otto Haub School of Environment and Natural Resources, the Social Justice Research Center and the International Programs Office. The Vietnamese Ministry of Natural Resources and Environment (Office 33) and Hatfield Consultants provided access to original data for this analysis. The original studies conducted by Hatfield Consultants and Office 33 were funded by the Ford Foundation.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2014.09.064.

References

Berglund, P.A., 2010. An introduction to multiple imputation of complex sample data using SAS® v9.2. SAS. In: Proceedings from the SAS Global Forum 2010, 11–14 April, 2010, Seattle, Washington, US.

Boivin, T.G., Nguyen, M.H., Son, L.K., Bruce, B.S., McNamee, P.J., Thang, V.C., et al., 2011. Agent Orange dioxin contamination in the environment and food chain at key hotspots in Vietnam: Da Nang, Bien Hoa and Phu Cat. In: Proceedings of the 31st International Symposium on Halogenated Persistent Organic Pollutants, 21–25 August 2011, Brussels, Belgium.

Burns, C.J., Collins, J.J., Humphrey, N., Bodner, M., Aylward, L.L., McBride, D., 2010. Correlates of serum dioxin to self-reported exposure factors. Environ. Res. 110, 131–136.

Chen, Q., Jiang, X., Hedgeman, E., Knutson, K., Gillespie, B., Hong, B., et al., 2013. Estimation of age- and sex-specific background human serum concentrations of PCDDs, PCDFs, and PCBs in the UMDES and NHANES populations. Chemosphere 91, 817–823.

Dwernychuk, L.W., 2005. Dioxin hot spots in Vietnam. Chemosphere 60, 998–999.

Dwernychuk, L.W., Cau, H.D., Hatfield, C.T., Boivin, T.G., Hung, T.M., Dung, P.T., et al., 2002. Dioxin reservoirs in southern Viet Nam – a legacy of Agent Orange. Chemosphere 47, 117–137.

Garabrant, D.H., Franzblau, A., Lepkowski, J., Gillespie, B.W., Adriaens, P., Demond, A., et al., 2009. The University of Michigan dioxin exposure study: predictors of human serum dioxin concentrations in Midland and Saginaw, Michigan. Environ. Health. Perspect. 117, 818–824.

Hatfield Consultants, 1998. Preliminary Assessment of Environmental Impacts Related to Spraying of Agent Orange Herbicide During the Viet Nam War, <http://www.hatfieldgroup.com/UserFiles/File/AgentOrangeReports/CIDA614/default.htm> (accessed 06.03.12).

Hatfield Consultants and Office of the National Steering Committee 33 Vietnam Ministry of Natural Resources and Environment (Office 33), 2009. Comprehensive Assessment of Dioxin Contamination in Da Nang Airport, Vietnam: Environmental Levels, Human Exposure and Options for Mitigating Impacts, <http://www.hatfieldgroup.com/services/contaminantagentorange/agentorangereports.aspx> (accessed 26.02.12).

Hatfield Consultants and Office of the National Steering Committee 33 Vietnam Ministry of Natural Resources and Environment (Office 33), 2011. Environmental and Human Health Assessment of Dioxin Contamination at Bien Hoa Airbase, Viet Nam. <http://www.hatfieldgroup.com/services/contaminantagentorange/agentorangereports.aspx> (accessed 26.02.12).

IOM (Institute of Medicine), 2009. Veterans and Agent Orange: Update 2008. The National Academies Press, Washington, DC.

Mai, T.A., Doan, T.V., Tarradellas, J., Felippe de Alencastro, L., Grandjean, D., 2007. Dioxin contamination in soils of Southern Vietnam. Chemosphere 67, 1802–1807.

Nhu, D.D., Kido, T., Naganuma, R., Sawano, N., Tawara, K., Nishijo, M., et al., 2009. A GIS study of dioxin contamination in a Vietnamese region sprayed with herbicide. Environ. Health Prev. Med. 14, 353–360. http://dx.doi.org/10.1007/s12199-009-0107-8 [Online 1 September 2009].

NRC (National Research Council), 2003. Dioxins and Dioxin-Like Compounds in the Food Supply: Strategies to Decrease Exposure. The National Academies Press, Washington, DC.

Patterson, D.G., Needham, L.L., Pirkle, J.L., Roberts, D.W., Bagby, J., Garrett, W.A., et al., 1988. Correlation between serum and adipose tissue levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in 50 persons from Missouri. Arch. Environ. Contam. Toxicol. 17, 139–143.

Quynh, H.T., Dai, L.C., Thom, L.T.H., 1989. Effects of geographical conditions, soil movement and other variables on the distribution of 2,3,7,8-TCDD levels in adipose tissues from Vietnam: preliminary observations. Chemosphere 18, 967–974.

R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, <http://www.R-project.org/>.

Revelle, W., 2014. Psych: Procedures for Personality and Psychological Research, Northwestern University, Evanston, Illinois, USA, <http://CRAN.R-project.org/package=psychVersion=1.4.4>.

Saito, K., Nhu, D.D., Suzuki, H., Kido, T., Naganuma, R., Sakakibara, C., et al., 2010. Association between dioxin concentrations in breast milk and food group intake in Vietnam. Environ. Health Prev. Med. 15, 48–56. http://dx.doi.org/10.1007/s12199-009-0106-9 (Online 15 September 2009).

Schecter, A., Papke, O., Ball, M., Ryan, J., 1991. Partitioning of dioxins and dibenzofurans: whole blood, blood plasma and adipose tissue. Chemosphere 23, 1913–1919.

Schecter, A., Le, C.D., Thuy, L.T.B., Quynh, H.T., Minh, D.Q., Cau, H.D., et al., 1995. Agent Orange and the Vietnamese: the persistence of elevated dioxin levels in human tissues. Am. J. Public Health 85, 516–522.

Schecter, A., Le, C.D., Päpke, O., Prange, J., Constable, J.D., Matsuda, M., et al., 2001. Recent dioxin contamination from Agent Orange in residents of southern Vietnam city. J. Occup. Environ. Med. 43, 435–443.

Schecter, A., Quynh, H.T., Pavuk, M., Päpke, O., Malisch, R., Constable, J.D., 2003. Food as a source of dioxin exposure in the residents of Bien Hoa City, Vietnam. J. Occup. Environ. Med. 45, 781–788.

Tran, T.T.H., Le, V.A., Nguyen, N.B., Tenkate, T., 2010. Environmental health risk assessment of dioxin exposure through foods in a dioxin hot spot – Bien Hoa City, Vietnam. Int. J. Environ. Res. Public Health 7, 2395–2406. http://dx.doi.org/10.3390/ijerph7052395.

Tran, T.T.H., Nguyen, N.B., Le, V.A., 2013. New strategy toward dioxin risk reduction for local residents surrounding severe dioxin hotspots in Vietnam. Global Health Action 6, 1–10. http://dx.doi.org/10.3402/gha.v6i0.21105.

USAID (The United States Agency for International Development), 2013. Progress Reports: Environmental Remediation of Dioxin Contamination at Da Nang Airport, <http://www.usaid.gov/vietnam/progress-reports-environmental-remediation-dioxin-contamination-danang-airport> (accessed 23.04.13).

Van den Berg, M., Birnbaum, L.S., Denison, M., Vito, M.D., Farland, W., Feeley, M., et al., 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol. Sci. 93, 223–241. http://dx.doi.org/10.1093/toxsci/kfl055 (Online 7 July 2006).

WHO (World Health Organization), 2011. 2005 Re-evaluation of Human and Mammalian Toxic Equivalency Factors (TEFs), World Health Organization Headquarters, Geneva, Switzerland, <http://www.who.int/foodsafety/chem/tef_update/en/index.html> (accessed 28.03.12).

Wickham, H., 2007. Reshaping data with the reshape package. J. Stat. Softw. 21 (12), 1–20. <http://www.jstatsoft.org/v21/i12/>.

Wickham, H., 2009. ggplot2: Elegant Graphics for Data Analysis, Springer, New York.

Wickham, H., 2011. The split-apply-combine strategy for data analysis. J. Stat. Softw. 40 (1), 1–29. <http://www.jstatsoft.org/v40/i01/>.

Wolfe, W., Michalek, J., Miner, J., Pirkle, J., Caudill, S., Patterson, D., et al., 1994. Determinants of TCDD half-life in veterans of operation ranch hand. J. Toxicol. Environ. Health 41, 481–488.

Young, A., 2009. The History, Use, Disposition and Environmental Fate of Agent Orange. Springer, New York. 123pp.