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Persistence of Dioxin TCDD in Southern Vietnam Soil and Water Environments and Maternal Exposure Pathways with Potential Consequences on Congenital Heart Disease Prevalence in Vietnam

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Abstract

Waterlogged soils and submerged sediments in wetlands and agricultural lands used for rice paddies and aquaculture have anaerobic conditions that slow and prevent the photo and microbial degradation of dioxin TCDD (2,3,7,8-tetracholorodibenzo-p-dioxin), enabling it to persist in environments for long periods. Over 1.6 million ha of land in southern Vietnam were sprayed with 2,4,5-T herbicides (e.g. Agent Orange) contaminated with dioxin TCDD during the Vietnam War (1961-1971); 45% of these ha received four or more spray flight missions. Dioxins are endocrine disrupters and may induce cardiovascular disease, growth, and developmental defects, diabetes, hormonal dysfunctions and disruptions, certain cancers, and chloracne. Outpatient screening clinic 2020 data on Vietnamese children suspected of congenital heart disease (CHD) showed the childhood CHD prevalence rate in Vietnam of 13.356/1000, significantly elevated compared to the Asian CHD prevalence rate of 3.531/1000. CHD prevalence rate differences between North Vietnam (2.541/1000) and south of the 17th parallel (10.809/1000) were significant. Vietnamese farmers, especially pregnant women whose occupations involve daily contact with soil and sediments where dioxin TCDD persists in the environment may be at risk of dioxin accumulation from dermal exposure and bioaccumulation via diet. There is an urgent need for funded longitudinal genetic and clinical studies to assess CHD and other organ system childhood malformations due to in utero TCDD exposure. We recommend an integrated research design involving 1) site-specific locations that received high volumes and multiple spray loads of herbicides during the Vietnam War; 2) soil sampling of submerged and waterlogged soils and sediments where TCDD may not have degraded; 3) production areas of agriculture, fisheries, and other aquatic products; 4) risk assessment dioxin levels in foods where TCDD is likely to bioaccumulate; 5) child-bearing age and pregnant women with potentially high sensitivity to long-term low dose exposure, and 6) men and women in occupations that are in daily contact with contaminated soil and sediments as part of their job routines.

Keywords

Agent Orange, 2,4,5-T Herbicides, Dioxin TCDD, Vietnam, Soils and Sediments, Congenital Heart Disease (CHD), Birth Prevalence, Saturated and Submerged Soils, Rice Paddies, Aquaculture, Rural Livelihoods, Bioaccumulation

1. Introduction

Dioxin contamination of soils and sediments in southern Vietnam is a continuing legacy of the Vietnam War (1961-1971) with implications for the present and future health of Vietnamese people and their children [1]-[11]. The dioxin TCDD (2,3,7,8-tetracholorodibenzo-p-dioxin), an unintended contaminant of chlorinated phenoxy pesticides formulated with 2,4,5-T (2,4,5-tricholorphenoxyacetic acids) has persisted in areas of known high use and concentration in southern Vietnam, south of the 17th parallel. Soil and sediment samples taken in 1999 from the Bien Hoa airbase showed dioxin concentrations as high as 1,164,699 pg/g (ppt) in soil dry matter [1]. In 2004, soil and sediment samples taken from surrounding areas of the Bien Hoa airbase continued to show high-risk levels of contamination up to 20.4 times higher for sediment and 46 times higher for soil compared to standard soil values [12]. Topsoil samples collected in 2017 from A-So airbase (Thua Thien Hue province, Vietnam) where Agent Orange with TCDD was stored and used during the Vietnam War revealed dioxin TCDD residuals remained at high levels more than fifty years later [7].

Dioxins are ecologically and biologically persistent chemicals with TCDD, the most toxic of all and the reference used to calculate TEQs (Toxic Equivalents) in assessments of human health risks associated with all dioxin-like compounds [10] [13] [14]. In rodent studies, TCDD is a known potent carcinogen, teratogen (factor causing malformation of an embryo), and immunotoxin [15]. The US National Toxicology Program and International Agency for Research on Cancer list dioxin and TCDD as endocrine disrupters and known human carcinogens [10]. A literature review by River and Foster [16] finds that TCDD and dioxin-like compounds are "potent modulators of immune and endocrine functions critical to the pathobiology of endometriosis". A 2008 study of the breast milk of lactating mothers who lived near former air force bases in southern Vietnam found dioxin levels to be 4-times higher than lactating mothers who lived in areas known not to have been sprayed with herbicides containing dioxin [8].

In infants and children, the possible association between dioxin and organ system malformations, specifically the cardiac system, is just beginning to be examined. Loffredo *et al.* [17] suggested an association between early pregnancy maternal exposures to herbicides and the occurrence of unique cardiovascular malformation in infants (**Figure 1**). Initial research into the possible association between herbicide exposure in utero and elevated resultant congenital anomalies in Vietnam was conducted by Giang *et al.* [18] on all infants delivered at the Da Nang Woman and Children's Hospital (DWCH) between 2015-2016 which serves both Da Nang city and several surrounding provinces in Central Vietnam. This study demonstrated an extremely high congenital anomaly prevalence rate (38.44/1000 births) with CHD being the most common anomaly found (52.3%) at a prevalence rate of 20.09/1000 births; higher than previously reported in the literature.

Re-analysis and reclassification of the original study's CHD data by pediatric cardiologists in 2021 and statistical comparison with recently published Liu *et al.* [19] CHD birth prevalence data demonstrated an overall congenital heart disease (CHD) birth prevalence rate of 14.712/1000 live births, significantly higher than the CHD Asian prevalence rate of 9.342/1000 reported by Liu *et al.* [19] from countries in the surrounding Asian regions [20]. These findings suggest that environmental persistence of the herbicide contaminant dioxin TCDD found in Central Vietnam could be an important variable associated with suspected elevated rates of CHD prevalence seen in other regions of Vietnam.

It is not surprising that dioxin TCDD concentrations in soils and sediments continue to be a high post-War period in the ten former airbases in southern Vietnam [10]. They were the sites that handled most of the storage and shipping

Figure 1. This Vietnamese child, born with congenital heart disease (CHD), needed heart surgery to live a healthy, normal life. Photo by VCF Heartbeat Vietnam.

of dioxin-laced herbicides and were staging areas for preparing and loading them for aerial spraying [10]. The Vietnam government and the United States have invested heavily in testing, cleanup, and remediation efforts at Da Nang and Bien Hoa airbases to reduce health risks to local populations [21] [22].

What is less apparent is the extent of residual dioxin TCDD contamination in the southern rural countryside that was repeatedly sprayed with dioxin TCDD contaminated 2,4,5-T herbicides used to defoliate vegetation, forests, and croplands. Further, we do not know much about the dioxin TCDD contaminant dose levels over time that could be associated with modern-day elevated negative health effects on infants, children, and childbearing age adults living in these areas. Stellman and Stellman [9] calculated that 3181 South Vietnam hamlets were sprayed affecting at minimum 2.1 million people and potentially as many as 4.8 million. They claim another 1430 hamlets were sprayed but population counts were not available.

This current study explores the possible association between CHD and thepersistence of dioxin TCDD at a countrywide level and in rural areas of southern Vietnam below the 17th parallel. One author (CBC), working with Vietnamese CHD children and with VinaCapital Foundation's NGO Heartbeat Vietnam (HBVN) since 2005 has noted that the rates of CHD he has encountered in Vietnam appear to be out of proportion to other similar "emerging" countries in the world he has worked in. HBVN has been dedicated to finding and repairing children with CHD and does yearly CHD outreach screening clinics in rural provinces. In 2020, HBVN screened 51,343 children in 86% of the provinces of Vietnam for CHD. In this study, using the 2020 HBVN CHD data, CHD prevalence rates are calculated for North, Central, and South Vietnam. As with the 2021 Giang *et al.* study [20], these CHD prevalence rates are then statistically compared to the results of the most recently published Liu *et al.* [19] meta-analysis and systemic review of childhood Asian CHD prevalence data based on 28 studies approximately 1,800,000 children and a rate of 3.532/1000 (95% CI 2.385 - 4.765) for statistical significance.

In this paper, we ask,

1) How does the incidence of CHD in Central and South Vietnam below the 17th parallel compare to North Vietnam which did not receive high concentrations of herbicides with TCDD contamination during the Vietnam War (1961-1971); and to surrounding LMIC Asian countries with no herbicide contamination?

2) Why might dioxin TCDD continue to persist in the southern Vietnam environment post-1971 applications of 2,4,5-T herbicides contaminated with 2,3,7,8 tetracholorodibenzo-p-dioxin (TCDD)? Specifically, what are the soil and sedimentation characteristics and processes that might retain dioxin TCDD in the southern Vietnamese environment for long periods of time?

3) What are the potential human exposure pathways for continuing impacts on human health? What are the living conditions, rural livelihoods, and agricul-

ture-fishery practices that bring rural households into daily contact with soil and sediments and might expose them to dioxin TCDD residues? And what changes in managing soil, sediments, and water resources at the household, commune, and larger landscape level might reduce human exposure to TCDD in daily lives (personal contact and dietary food chain) and decrease the harm to health it causes?

This knowledge is critical because there is growing evidence of dioxin bioaccumulation in animal and human fatty tissues throughout the southern Vietnam food web [3] [8] [22] [23]. Further, the teratogen and endocrine disrupter impacts of even low dose levels on pregnant women and their infants are not well understood. Vietnam has a relatively young female population with a current median age of 32.5 years (worldometer.com for 2020). In the 2021 Giang *et al.* study [20], women of childbearing age between 19 - 39 in 2015 made up more than 34% of the Vietnam female population with females between 25 - 29 years having birth rates of 135.1 births/1000. In 2021, that group's birth rate still remained high (133.1/1000 https://www.statista.com/statistics/1101017/vietnam-fertility-rate-by-age-group/) making them an extremely vulnerable population to potential environmental toxins during pregnancy.

The intent of this paper is to 1) report on calculated 2020 CHD prevalence rates in both South and Central Vietnam and compare them to contemporary published childhood CHD prevalence rates in surrounding LMIC Asian countries; 2) compare South and Central Vietnam CHD prevalence rates to North Vietnam rates above the 17th parallel which was not exposed to systematic herbicide applications; and 3) explore potential associations with the persistence of dioxin TCDD in the soil and water environment in southern Vietnam below the 17th parallel. Of specific interest are waterlogged and submerged soil and sediment characteristics in light of Vietnamese agricultural and fisheries occupations and the exposure pathways that inform potential long-term dioxin TCDD persistence in soil, sediments, and the food chain with implications for human health.

2. Methods and Materials

2.1. Data Collection to Assess Congenital Heart Disease (CHD) in Vietnam

Screening data on children suspected of CHD were collected in 2020 (full year) at HBVN provincial CHD outreach clinics encompassing 86% of the provinces of Vietnam. These screening clinics are conducted nearly every weekend at sites chosen by HBVN and staffed by volunteer MDs and RNs who have a background in CHD. Children were sent to the outreach clinics by physicians in the area for CHD screening; thus, every child screened was suspected of possible CHD either by a physical exam finding (e.g. murmur), family history of CHD, or parental concern about CHD. Children screened and suspected to have CHD all underwent a 2D echocardiogram/doppler examination of their heart performed by a trained Vietnamese pediatric cardiologist to either confirm or exclude a diagnosis of CHD (**Figure 2**). Overall, 51,343 children were screened for CHD and 687 children (1.34%) were found with confirmed CHD and were entered into the HBVN database. Data sets for each province were then extracted, classified into the 27 known CHD subtypes, and used to calculate CHD prevalence rates and 95% confidence intervals for North, Central, and South Vietnam and a combination of Central + South Vietnam. These CHD prevalence rates were then compared to the 2020 Liu *et al.* [19] published Asian childhood CHD prevalence rate for statistical significance. Statistical analyses were performed using Python (Version 3.4) and the significance level (two-tailed) was defined at $p < 0.05$. Data are presented as prevalence rate per 1000 live births with 95% confidence intervals.

For regional comparison of CHD prevalence rate purposes, the 17th parallel is the demarcation between northern provinces in Vietnam that did not receive herbicide applications during the Vietnam War and southern provinces below the 17th parallel where herbicides were routinely applied. Vietnam's regional definitions of North, Central, and South used in categorizing provincial prevalence rates are as follows: **North**, all provinces above the 17th parallel; **Centra**l, provinces below the 17th parallel Quang Tri, Thua Thien-Hue, Da Nang, Quang Nam, Quang Ngal, Kon Tum, Gia Lai, Binh Dinh, Phu Yen, Dak Lak, Khanh Hoa, Dak Nong, Lam Dong); and **South,** provinces Ninh Thuan, Binh Thuan, Binh Phuoc, Dong Nai, Tay Ninh, Binh Duong, Ba Ria-Vung Tau, Ho Chi Minh; Long An, Tien Giang, Ben Tre, Dong Thap, Vinh Long, Tra Vinh, An Giang, Can Tho, Hau Giang, Soc Trang, Kien Giang, Bac Lieu, Cau Mau.

2.2. Herbicides, Extent, and Patterns of Usage Data

Agent Orange [equal parts 2,4-D (dichloro phenoxy acetic acid) and 2,4,5-T

Figure 2. A young Vietnamese boy with congenital heart disease (CHD). Photo by VCF/Heartbeat Vietnam.

(trichlorophenoxyacetic acid)] was the herbicide used most frequently during the Vietnam War (1961-1971). However, Agents Purple, Pink, and Green formulations also included 2,4,5-T, and all four of these "rainbow" herbicides contained the by-product contaminant dioxin TCDD [10]. It has been estimated that over 19 million gallons (72 million liters) of herbicides with phenoxy and other chemical agents were dispersed in this ten-year period, of which 12 million gallons (45 million liters) were based in 2,4,5-T formulations [24]. Stellman *et al.* [24] [25] constructed a revised version of the US Department of Defense HERBS file by creating a relational database system (GIS) that linked records of spray flight paths, types of herbicide and gallons/liters applied, locations of military bases, movement of combat troops, and civilian population centers to estimate herbicide exposure across the southern Vietnam landscape. They systematically compared four different versions of herbicides files found at the US National Archives and the active file at the US Armed Services Center for Research of Unit Records (CRUR). See Stellman *et al.* [25] "A geographic information system for characterizing exposure to Agent Orange and other herbicides in Vietnam" for methodological details.

Stellman *et al.* [24] findings were published in *Nature* in 2003 as, "The extent and patterns of usage of Agent Orange and other herbicides in Vietnam." We have re-created two maps and a table in this paper based on data and graphics drawn from their published work. Spray patterns of Agent Orange and 2,4,5-T herbicides contaminated with TCDD, and Herbicide spray volumes in excess of 4800 liter and locations respectively are re-creations of maps from **Figure 3** of the Stellman *et al.* [24] paper. The estimated area and frequency of herbicides with 2,4,5-T and contaminant dioxin TCDD data in this paper are also based on data from Stellman *et al.* [24].

2.3. Vietnam Soil Characteristics

The dioxin TCDD aerially sprayed across a landscape can persist in soils and sediments by adsorption and adheres to the surface of soil aggregates high in clay [10]. TCDD has a strong affinity to adsorb to soils with concentrations of organic carbon; thus, the dioxin is more likely to be found in upper soil and sediment layers rather than in deeper levels [12]. Relationships among soil properties and processes vary at different spatial and temporal scales and are influenced by geomorphology, predominant vegetation, and land use [26] [27]. Soil ecosystems are open complex hierarchical systems with properties at small and large scales that influence what happens "up" and "down" scales [26]. At a micro-level, relationships between two soil properties may not hold at field or landscape levels. For example, relationships between soil carbon and microbial respiration could vary by clay content at the field level, micro-topography at a fine scale, and land use at the regional scale [27]. Another example of scale-dependent relationships is pesticide sorption as a combination of equilibrium and kinetic processes with the potential pesticide-soil interactions resulting in pesticide leaching in very

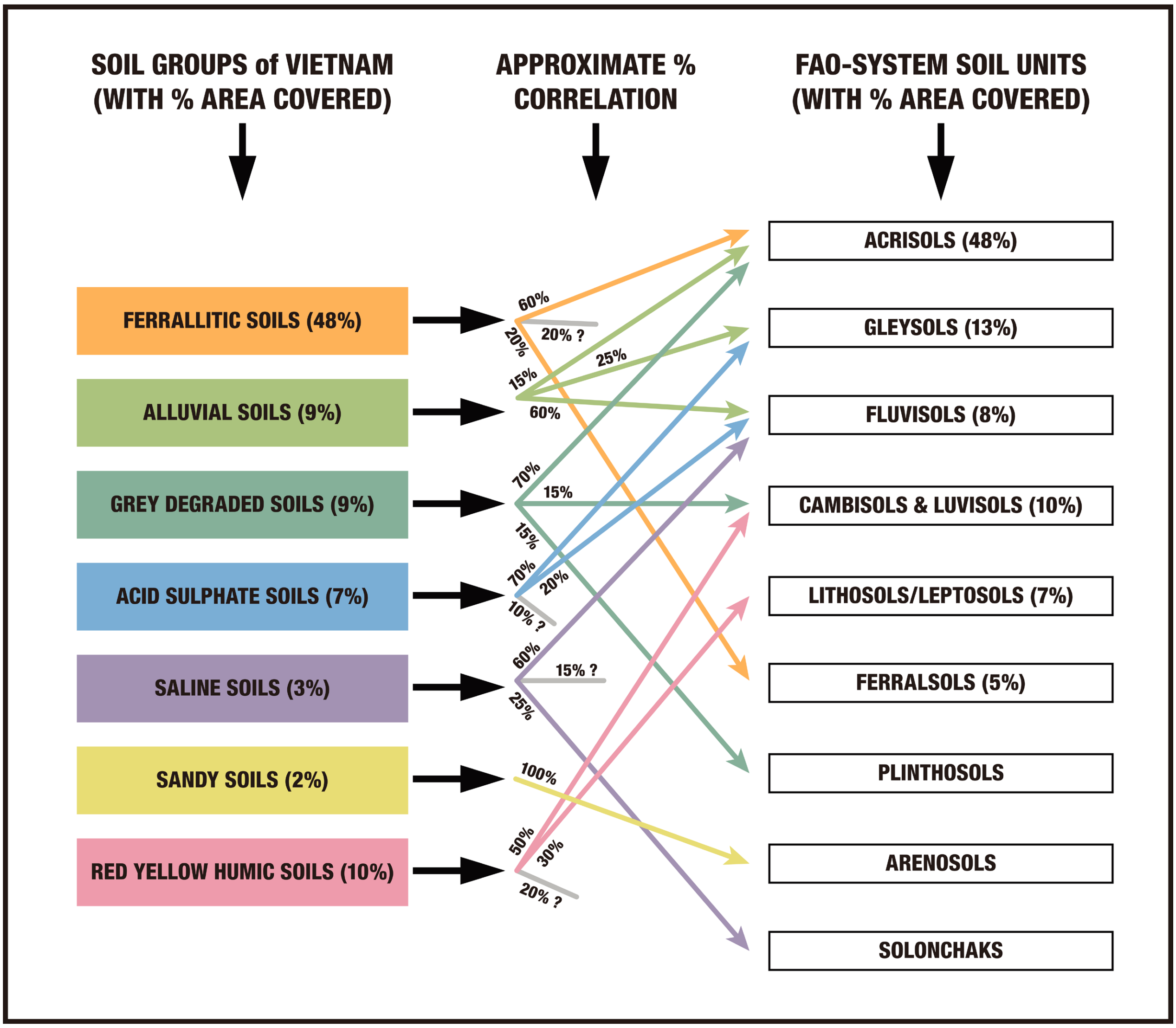


Figure 3. Adaptation of Sehgal 1989 correlation and classification of major Vietnam soils with UN FAO soil groups. Graphic design by Cruz Dragosavac.

small concentrations over very long periods of time [26].

Thus, the scale of exposure and soil characteristics, land use, climate, and local weather influence TCDD sorption-desorption-resorption processes and mobility via atmospheric conditions, wind action, precipitation and runoff rates, aerobic or anaerobic conditions, and combinations of natural and human-induced erosion and leakage [10] [12] [28]. Almost one-third of 33 million ha of Vietnamese soils are used for agriculture [29]. The main great soil groups primarily used for agriculture are shown in the left column of Figure 3. Rice and cash crops are dominant crop types grown in Grey Degraded soil, Alluvial soil, Acid Sulfate soil, Saline, and Sandy soil [29]. Cassava is primarily grown in Saline soil and Acid Sulfate soil. Coffee, rubber, cashew nut, coconut, tea, and pepper are mainly grown in Red Yellow soil and Ferralitic soils of the central high plateau, hills, and mountainous areas.

The Sehgal [30] correlation matrix and classification show the Soil Groups of Vietnam and corresponding Soil Units using the UN FAO System (**Figure 3**). According to research in 2008 by Pham Quang Ha [29] of the Institute for Agricultural Environment (IAE/VAAS) in Ha Noi, soil organic carbon (SOC) stocks in the upper layers are closely related to productivity in agriculture with higher stocks associated with higher crop productivity. Acid Sulfate soil was found to have as much as 7.3% organic carbon and a mean of 3.80%. Alluvial soil maximum SOC was slightly above 4%, with Fluvisols mean SOC at 1.99%. The mean SOC in Ferrasols was 2.22%; Saline soils had a maximum of 3.7% SOC and a mean of 1.72%; Acrisol SOC mean was 1.08%, and Sandy soil had the lowest mean SOC at.68% [29]. Southern Vietnam river basin soils are highly productive Fluvisols with the mean Mekong River SOC content of 2.81% and other southern river Fluvisols at 2.03%, both above the Vietnam average for Fluvisols. The Ha [29] study lists clay content of the major soil groups as follows: Red soil (49% - 55%), Acid Sulfate soil (42% - 57%), Saline soil (38-58%), Alluvial soil (33% - 41%) and Grey Degraded soil (11% - 18%).

Soil Formation, Submerged and Saturated Soils

Soil formation and characteristics are a function of parent materials, climate (e.g. precipitation and temperature), topography, vegetation, macro and microorganisms, time, and human activities [28] [31] [32]. Vietnam has three general physical geographic zones: lowlands (0 - 99 m above MSL); hilly lands (100 - 299 m above MSL); and mountains (>500 m above MSL) [30]. Located just above the equator between 8˚10'N and 23˚32'N, the country has a humid tropical climate with alternating wet and dry periods and high tropical temperatures. Cyclones and monsoon storms from southeast Asia over centuries have affected the different physical-chemical compositions of soils and how they weather. A changing climate, increasing variability in timing and rates of precipitation, drought, sea-level rise, saltwater intrusion into lowlands, and reduced Mekong River flows from upstream dams are continuing to affect soil characteristics and capacities to support agricultural crops.

Old Alluvium soils ~10 m above the Mekong Delta are characterized by floodplain sediment deposits the South China Sea left behind when it receded millions of years ago. These Old Alluvium floodplain terraces are mapped as Low-Humic Gley and Grey Podzolic soils primarily consisting of saturated clays with silt and fine sand [33]. Acrisols are Podzolic soils also called “Lateritic” because the clay content has low silica: alumina ratio. Some Grey Podzolics have base saturation levels above 50% and are called Ferric Luvisols (rather than Ferric Acrisols) [32]. The high carbon content of Acid Sulfate soil and Alluvium soil is the result of organic deposits left behind when the ancient sea receded [29]. Intense weathering and leaching of Old Alluvium, limestone, clay shale, basalts, and metamorphic rocks formed the Red-yellow soils and the Ferralsol soils of the highlands and Annamite Mountain Range.

A substantial portion of Vietnam’s lowlands and river valleys have soils and sediments that are underwater for long periods of time annually or are permanently submerged naturally or by engineered drainage works. The low-lying coastal region, valleys, and the river deltas are Alluvial soils encompassing Acrisols, Fluvisols, and Gleysols (**Figure 3**). Gleysols comprising 13% of Vietnam soils have distinctive gley horizons including a partially oxidized A horizon high in an organic matter [28]. Where the diffusion of oxygen is slow, the second horizon is streaked or mottled with manganese and iron deposits.

Submerged soils include dryland crop soils that may temporarily be saturated or waterlogged, paddy soils, and permanently submerged soils of marshes, wetlands, and lake, river, and ocean sediments [28]. Soils on the fringes of lakes and stream banks are seasonally waterlogged and frequently farmed to take advantage of fertile soil deposits and receding water levels. Waterlogging may be the result of flooding, a high water table, impermeable soil, or an impervious soil layer. A major characteristic of these submerged soils is that they are anaerobic, meaning oxygen is absent. The oxygen diffusion rate decreases as water saturates the soil, and within hours microorganisms in the soil use up any oxygen in the soil or water [28]. Oxygen sampling in flooded rice fields and submerged lake muds show an absence of oxygen, evidence that waterlogged soils and sediments have no oxygen present [28] and microbial activity is similarly low or non-existent.

Agriculturally, the deltas and river lowlands are the major agricultural production regions of Vietnam [30]. The Mekong River as well as South and Central region river deltas and Alluvial soils are very fertile and support two to three annual crops of rice, shrimp, and fish farming as well as a diversity of tropical fruits and vegetables making these areas Vietnam's breadbasket and a major source of export revenues.

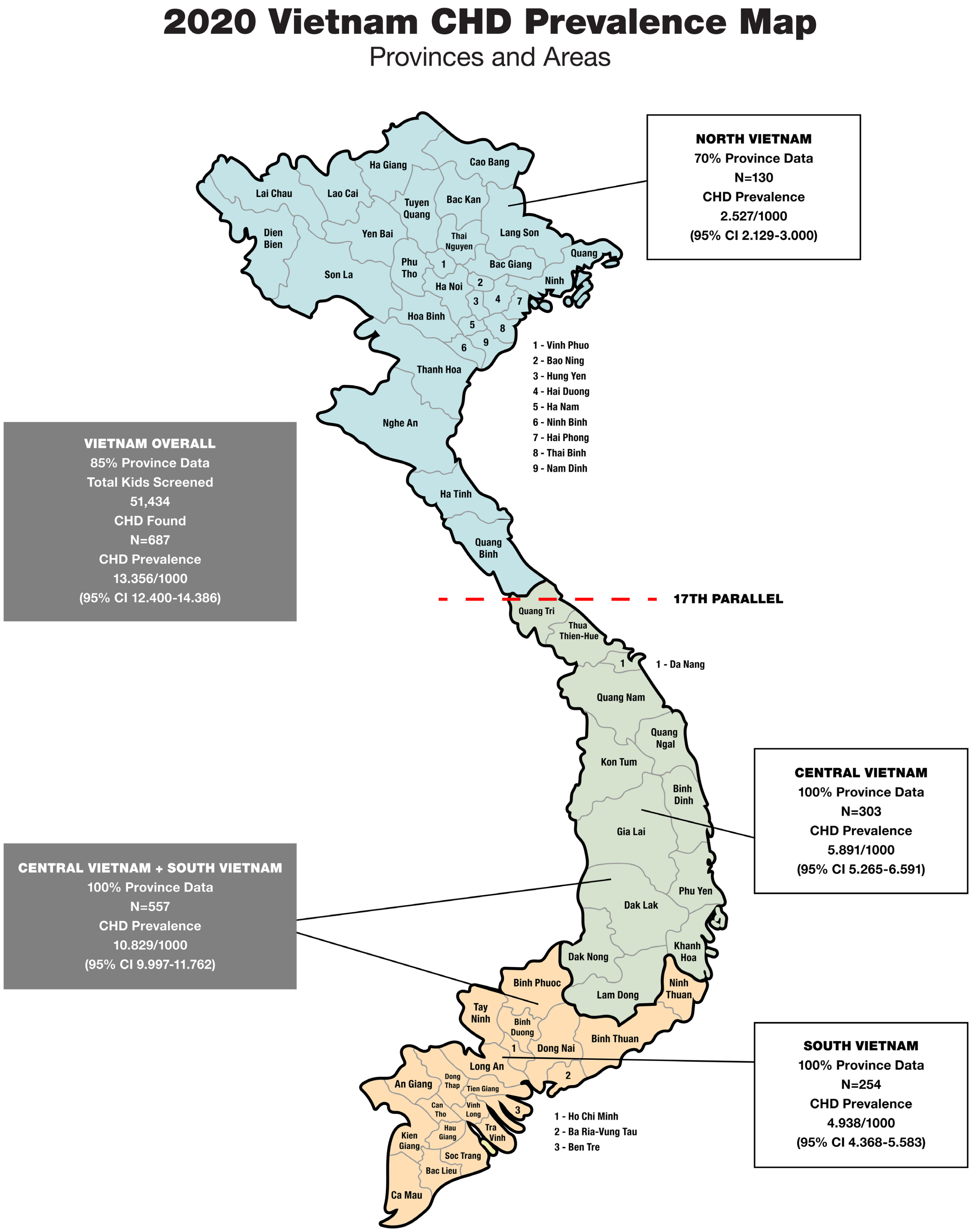
3. Results

3.1. Childhood CHD Prevalence Rates 2020 in Vietnam

The calculated HBVN CHD country-wide screening data for 2020 showed childhood CHD prevalence rates for the overall country of Vietnam at 13.356/1000 (95% CI 12.400 - 14.386) (**Table 1**, **Figure 4**). The calculated childhood CHD prevalence

Table 1. Heartbeat Vietnam Congenital Heart Disease 2020 CHD screening clinics prevalence data by region (total children screened = 51,434).

Vietnam Region	Percent (%) of Reporting Provinces	Number of CHD Patient Diagnosed	Calculated Prevalence Rate (per 1000)	95% Confidence Intervals (per 1000)
Vietnam Country	85.5%	687	13.356	12.400 - 14.386
North Vietnam	70%	130	2.527	2.129 - 3.000
Central Vietnam	100%	303	5.891	5.265 - 6.591
South Vietnam	100%	254	4.938	4.368 - 5.583
Central + South Vietnam	100%	557	10.829	9.997 - 11.762


2020 Vietnam CHD Prevalence Map

Provinces and Areas

NORTH VIETNAM
70% Province Data
N=130
CHD Prevalence
2.527/1000
(95% CI 2.129-3.000)

VIETNAM OVERALL
85% Province Data
Total Kids Screened
51,434
CHD Found
N=687
CHD Prevalence
13.356/1000
(95% CI 12.400-14.386)

CENTRAL VIETNAM
100% Province Data
N=303
CHD Prevalence
5.891/1000
(95% CI 5.265-6.591)

CENTRAL VIETNAM + SOUTH VIETNAM
100% Province Data
N=557
CHD Prevalence
10.829/1000
(95% CI 9.997-11.762)

SOUTH VIETNAM
100% Province Data
N=254
CHD Prevalence
4.938/1000
(95% CI 4.368-5.583)

1 - Vinh Phuo
2 - Bao Ning
3 - Hung Yen
4 - Hai Duong
5 - Ha Nam
6 - Ninh Binh
7 - Hai Phong
8 - Thai Binh
9 - Nam Dinh

1 - Da Nang

1 - Ho Chi Minh
2 - Ba Ria-Vung Tau
3 - Ben Tre

Figure 4. Vietnam childhood CHD (congenital heart disease) prevalence map 2020. Prevalence rates were calculated by comparing children found with CHD in the regions of North, Central, and South Vietnam as well as Central + South Vietnam to the total number of children screened country-wide in Vietnam for CHD in 2020. Graphic design by Cruz Dragosavac.

rates for regions North (2.527/1000 (95% CI 2.129 - 3.000)), Central (5.891/1000 (95% CI 5.265 - 6.591)), and South (4.938/1000 (95% CI 4.368 - 5.583)) revealed substantial variations among regions. Adding the Central and South regions together, the childhood CHD prevalence rate was 10.829/1000 (95% CI 9.997 - 11.762). Comparing these data to 2020 Asian childhood CHD prevalence rates published by Liu *et al* (3.531/1000 95% CI 3.016 - 4.765) [19] for statistical significance, we find that Central and South region individual rates as well as Central + South Vietnam combined rates had statistically significant elevated rates of childhood CHD ($p < 0.0001$) as compared to similar Asian low-middle income countries (LMIC).

Vietnam's southern regions below the 17th parallel that had been exposed to herbicides with TCDD were compared to North Vietnam which had no TCDD contaminated herbicide exposure during the Vietnam War to determine if there was any statistical significance to rates of childhood CHD prevalence in those areas that were sprayed with TCDD contaminated herbicides. This analysis revealed that the southern regions—Central, South, Central + South combined, had statistically significant elevated CHD prevalence rates ($p = 0.005$, $p = 0.004$, and $p = 0.01$ respectively) when compared to North Vietnam rates.

3.2. Herbicides, Extent, and Patterns of Use in Southern Vietnam

Approximately 76,000 m^3 of herbicide defoliants were sprayed over more than 10% of southern Vietnam by the United States (US) and the Republic of Vietnam military forces during the Vietnam War (1961-1971) [5] [24] [34]. It has been estimated that 65% of these herbicides used to defoliate large swaths of forests, mangroves, croplands, and military base perimeters contained 2,4,5-T (2,4,5-tricholorphenoxyacetic acid) and an unintended manufacturing by-product, the dioxin TCDD [10] [24] [25]. Stellman *et al.* [24] analyses of spray mission flight path records from 1962 - 1971 estimated that over 1.6 million hectares in Vietnam were sprayed with 2,4,5-T; and 728,288 ha were sprayed four or more times (**Table 2**).

The map in **Figure 5** shows the southern Vietnam geographic spray patterns

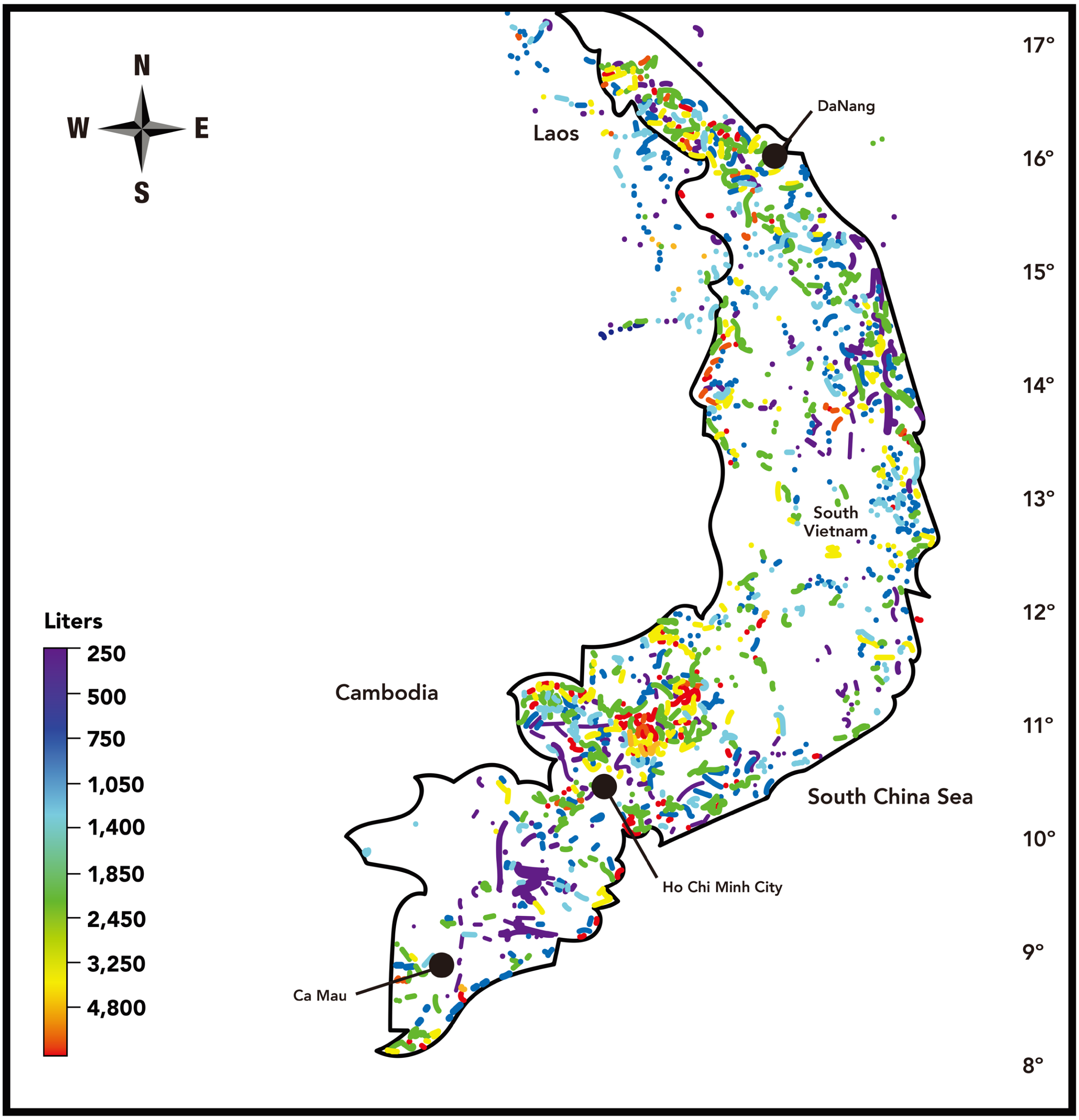
Table 2. 1962-1971 estimated area and frequency of herbicides with 2,4,5-T and contaminant dioxin TCDD. Spray frequency is the number of times spray mission flight paths overflew grids in Vietnam; GIS developed for herbicide exposure assessment.

Spray frequency	Hectares sprayed 2,4,5-T herbicides
1	343,426
2	332,249
3	275,770
4	236,232
5	153,192
6	119,127
7	75,062

Continued

8	51,371
9	32,988
10+	60,316
Total ha	**1,679,734**

Data from Stellman *et al.* 2003, Table 2.



Volumes of herbicide consisting of 2,4,5-T (Agent Orange) contaminated with the dioxin, TCDD sprayed by US military forces in RVN (South Vietnam), 1961-1971 (Stellman et al. 2003).

Figure 5. Spray patterns of Agent Orange and 2,4,5-T herbicides contaminated with TCDD. Graphic design by Cruz Dragosavac.

and volumes of 2,4,5-T herbicides that were contaminated with the dioxin TCDD below the 17th parallel.

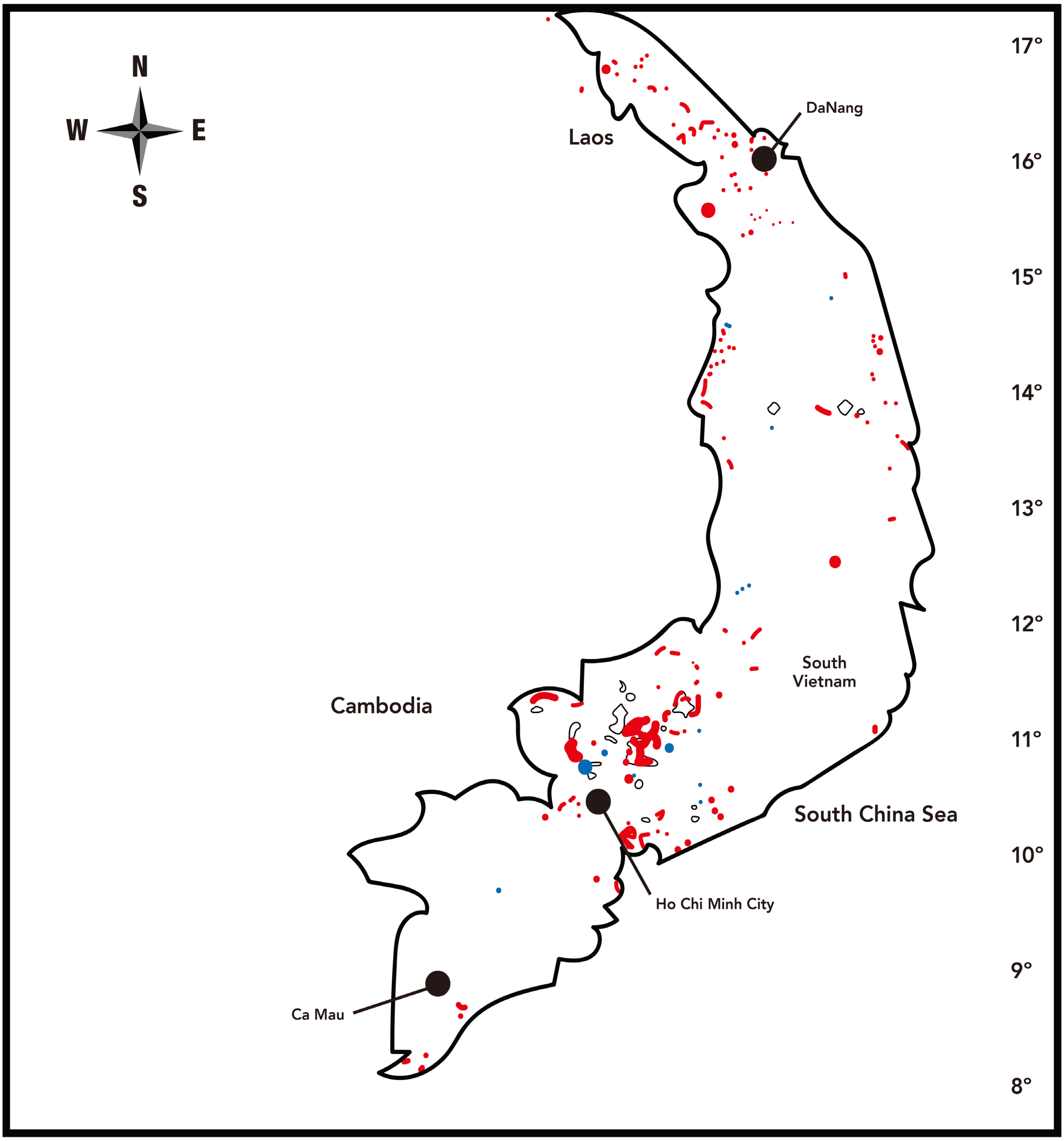
The Mekong Delta, especially vegetation and lands along the Bassac and main stem Mekong rivers and their tributaries, mangroves along with coastal areas, the Dong Thap (Plain of Reeds) agricultural area south of the Cambodia "parrots beak", and the countryside north and east of Ho Chi Minh City received 250 to 2450 liters of herbicides. Central Vietnam coastal areas, river deltas, and river valleys where tropical vegetation and agriculture thrived also received numerous spray applications and volumes in the 250 - 3250 liter range. Higher volumes of the contaminated herbicides (yellow and red colors on the map) concentrate north and west of Da Nang, along the Ho Chi Minh trail on both sides of Central Vietnam and Laos, the countryside north and east of Ho Chi Minh City, and surrounding Bien Hoa, and specific coastal locations. **Figure 6** offers a clearer picture of the locations in southern Vietnam that received herbicide volumes in excess of 4,800 liters. Many of these locations are associated with air force bases near Da Nang and Ho Chi Minh City.

3.3. Soils and Potential Persistence of Dioxin TCDD in Vietnam Agriculture and Environment

TCDD half-life can vary site-specifically based on environmental conditions such as frequency and concentrations of the chemical spray, climate and geography, soil characteristics, water management, acidic or alkaline pH, exposure to sunlight, availability of oxygen, and presence of bacteria [10] [35]. It is known to persist in the soils and sediments of military airbases, basecamps, and chemical storage areas for decades. TCDD persistence may also occur in landscapes that were heavily sprayed and areas where runoff and erosional processes moved TCDD contaminated soils and sediments to low places in the landscape where they are sheltered from photo volitation [3] [6] [10] [36] [37]. Further, there is general agreement that dioxin TCDD can be remobilized via soil and sediments by agricultural activities, natural erosion, road construction, and other infrastructure improvements. Dwernychuk *et al.* [3] suggest that sediment and soil disturbance could initiate a transfer of TCDD into local communities and the human food chain thereby increasing the risks to human health.

3.3.1. Submerged and Rice Paddy Soils

The Vietnam soils, rivers, and cities map (**Figure 7**) show that many of the lowlands are river valleys and deltas with Alluvial soils and Grey Degraded soils encompassing Fluvisols, Gleysols, Acrisols, and Plinthosols (**Figure 3**). The Mekong Delta, an alluvial plain 2 - 3 m (6 - 10 ft) above sea level, once a huge swamp of freshwater and saltwater marshes, is a dense network of canals, ditches, levee protected fields, and ponds (polders) where rice and shrimp are grown [38]. Freshwater wetlands are found in the Plain of Reeds, lands in the central delta among the many distributaries of the Mekong River, and valleys where rivers flow out of the Central Highlands to the sea. Acid Sulfate and Saline



Legend. Locations in RVN (South Vietnam) where herbicides were sprayed (1961-1971) in volumes greater than 4,800 liters (Stellman et al. 2003). Marker size denotes increasing in proportion to volume and color corresponds to herbicide codes:

● All herbicides with 2,4,5-T contaminated with dioxin, TCDD
● Herbicide Agent Blue (cacodylic acid, C2H2AsO2)
○ Herbicide Agent White (2,4-D and picloram)

Figure 6. Herbicide spray volumes in excess of 4800 liter and locations. Graphic design by Cruz Dragosavac.

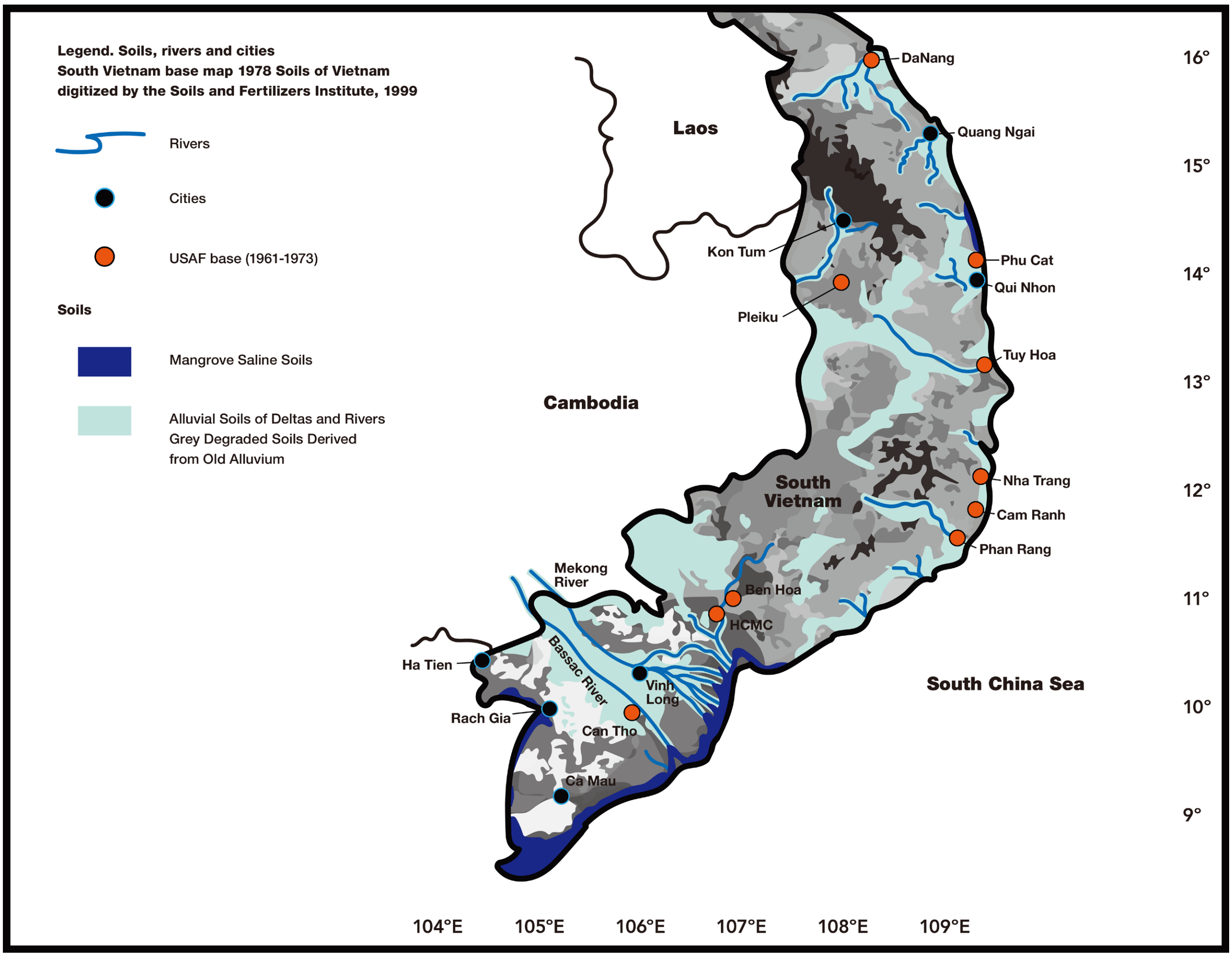


Figure 7. The soils of the valleys, low-lying coastal region and the river deltas are Alluvial and Grey Degraded soils. Agriculturally, the deltas and river lowlands are the major agricultural production regions of Vietnam (Sehgal 1989). Graphic design by Cruz Dragosavac.

soils with Gleysols and Fluvisols are found closer to coastal areas where waters with varying concentrations of salt affect the chemistry of submerged soils and the type of crops that can be grown. Water control projects and new rice genetics enabled Vietnam farmers to increase yields and expand production on more land over the last 50 years (1960-2010) so that by 2010 Vietnam was producing 4 - 5 million tons of rice annually and had become the #2 exporter of rice in the world [29].

Agriculturally, many lowlands and submerged soils have a long history of being "paddy" soils (**Figure 8**), meaning they are managed specifically for the wet cultivation of rice (*Oryza sativa* L.*)* [28]. This management entails the farmer levelling land and building levees or dikes to enclose the level land in order to maintain specific water levels to meet crop needs [38] [39]. Once the land is enclosed, a "puddling" process prepares the soil for rice growing. Land preparation entails one or two plowings and harrowings while the soil is submerged to create a soft soil bed for easy rice seedling transplant [28] [40] [41]. Puddling breaks down soil aggregates into micro-aggregates and individual particles and disperses them [41]. It also redistributes any soil and mineral deposits including iron and manganese just below the plow line resulting in an almost impervious structure that prevents water loss and nutrients from excessive percolation and reduces weeds in the rice field [40]. The rice is grown in 5 - 10 cm of standing water for 4 to 5 months, then drained dry for harvest; post-harvest the paddy is re-flooded within weeks or months depending on the season and next crop.

Fish are frequently grown in the flooded rice fields (**Figure 9**) and farmers

Figure 8. Wet cultivation of rice in "paddy" soils.

Figure 9. Farmers often use diversified intensified production systems that grow fish in rice paddies to recycle N and other nutrients and increase household income.

and their children wade in the muddy, sediment-rich waters with nets and spears or check traps to catch fish [42]. Many farms also have shallow dug ponds where fish and shellfish are raised. Small fishponds serve household food needs and larger ones for commercial production are found throughout Vietnamese lowlands [39].

Rice and fish are the main food crops for Vietnam households. Almost all rural households grow rice for family subsistence. The 1980s land reform laws enabled many rural households to produce surplus rice and other crops for markets turning rice production into a major export [39]. Although today a number of large-scale operations have reduced puddling intensity to save on energy and labor costs and more rapidly maximize the growing season, many small-scale farmers continue the puddling practice in their rice paddy fields [41]. While smallholders are more likely to use animals for plowing and larger-scale operations have moved to 5 hp power tillers, rice agriculture in Vietnam remains a hands-on (and feet), soil contact occupation. Thus, any contamination in paddy soils or fishpond sediments would inevitably bring farmers into contact via skin and/or hands with potential ingestion through food preparation and consumption activities.

3.3.2. Management and Uses of Soil and Water

The management of soil and water in lowlands via ditches, dikes, canals, and ponds is well suited to meeting household needs and diversified systems of agriculture of rice-fish (**Figure 9**); rice-livestock; and rice-vegetable-orchards [39] [42]. Taylor [42] reports that ponds in the sand dunes between the Mekong and Bassac rivers coastal belt were historically dug by households and communes as dry-season drinking water sources and used all year for watering animals, irrigating, and washing. Wading into these sedimented waters for routine washing could also expose humans to any contaminants attached to soil particles. Digging a ~3 m or larger pond and maintaining it requires "regular re-excavation to remove collapsed sand" since the walls can be unstable [42]. This is often done by hand. Taylor [42] further notes, that almost all wats (communal religious-educational compounds) in this region constructed large communal ponds for drinking and bathing as well as washing clothes and dishes.

The seasonally flooded coastal and inland plains with rice paddies range from shallow freshwater depressions and ponds, backwaters of rivers and streams to brackish salt marshes, melaleuca, and mangrove forests. Fluctuating wet and dry periods determine the extent to which soils and sediments are submerged in freshwater or saltwater and become Grey Degraded soils, Acid Sulphate, or Saline soils. Precipitation and flooding in the wet season flush saltwater ditches, canals, and rivers and temporarily turns salty waterways into freshwater flows. Transformations in hydrology with levees, sluice gates, and canals have converted heavily saline rivers and wetlands into freshwater zones for rice and vegetable crops. Local levees and canals are often manually maintained by pumping water in and out; removing of mud, silts, and sediments from ditches; and reinforcement of the dikes after heavy rains.

Soils and sediments found in low spots within this landscape are often waterlogged (Gley) anaerobic soils submerged in water for many months (**Figure 9**, **Figure 10**) temporarily or permanently and are devoid of oxygen [28]. These soils can become a sink for TCDD which is hydrophobic. The hydrophobicity of TCDD explains why residue studies on grasses and rice do not find TCDD in rice grains or grass and rice residues. TCDD in solution or emulsion is not available to be taken up by roots and systemically translocated throughout the rice or grass plant. Thus, Jensen *et al.* [43] findings that dioxin-TCDD residues on grasses had a half-life of 4 - 7 days; and were undetectable in rice grain are congruent with the properties of TCDD.

Although TCDD is not water soluble nor absorbed by plants, it can adhere (adsorb) to soil particles, sediments, leaf surfaces, and organic materials [4] [10]. These TCDD-sorbed particles may be ploughed into the soil more deeply or transported by water or farming practices to new locations on the landscape, as well as into streams, and deposited in wetlands, lakes, and ponds as sediments. There is additional evidence that soil colloids are mobile in subsurface environments and have the potential to be carriers of TCDD in shallow groundwater if not filtered out [4]. Fish and other aquatic animals, especially bottom feeders accumulate TCDD in their fatty tissues as they forage in TCDD contaminated sediments. When birds and larger predators feed on contaminated fish and snails, TCDD moves up the food chain bioaccumulating in each additional organism via their diets [44].

Figure 10. Water buffalo graze in anerobic rice paddy soils which are saturated and submerged in water for many months.

3.3.3. Ho Chi Minh Trail, Forested Slopes and River Valleys

Seventy-five percent of Vietnam is sloping land with more than half having slopes greater than 20˚ [45]. The uplands northeast of the Mekong Delta, where the Ho Chi Minh Trail runs through the southern foothills of the Annamite Cordillera have heavily forested slopes and river valleys with Old Alluvium terraces. The potential for severe soil erosion in humid tropical climates in this hilly landscape is especially high due to heavy rainfall [46]. A quantitative meta-analysis of 21 countries found that soil erosion was dramatically concentrated in tropical landscapes where the soil was without vegetation, *i.e.* bare land [46]. Landcover, rather than land use is a key factor in preventing soil erosion in sloping, hilly lands, and the high mountains regions. Herbicide defoliation of forests and forest fires throughout southern Vietnam exposed the Ferraliltic and Red-yellow soils in this landscape to severe erosion and left behind bare lands. The immediate effect would have been large-scale soil transport by wind and water runoff to lower positions in the landscape (valleys) which may or may not have led to TCDD photo or microbial decomposition.

Longer-term some defoliated forest stands have begun to recover with the emergence of weeds, bamboo, shrubs, and tree seedlings [33] [47]. In 2022, Tran *et al.* [47] published the first comprehensive study on changes in the species richness and diversity of microbial communities based on the concentration of TCDD in sandy/sandy loam soil in the Da Nang area and to the northwest (A Luoi and Quang Tri). They found that 50 years later, the complexity of vegetation from barren land to grassland to developing woodlands was negatively associated with the TCDD concentration gradient. Dramatic changes in bacterial community compositions on defoliated sites occurred over time. They concluded that dioxins significantly altered soil microbiomes and affected vegetation restoration trajectories that deviated from their original state.

3.4. TCDD Exposure Pathways

Assessments of human exposure to TCDD in the soil and sediment environment involve estimates of the exposure pathways, the magnitude, rates, and frequency or duration of exposure over time. Exposure pathways are determined by site-specific 1) environmental conditions—e.g. precipitation, temperature, sunlight (climate and season), type and systems of agriculture, soil characteristics, anaerobic (submerged) soils and sediments, topography; 2) potential for transport to new locations (e.g. soil erosion/ flooding contaminated soil and sediment transport and deposition); and 3) activities of the exposed individual/population at risk (e.g. occupations, time spent outside working with contaminated soil, sediments, and dust) [33]. Paustenback *et al.* [35] define a "complete" pathway as initiated when a chemical is released into an environment; transported by a medium such as soil, water, or air to which the chemical attaches; points where humans might potentially come in contact with the medium (e.g. soil) contaminated with the chemical; and an exposure route such as dermal, ingestion, and/or inhalation.

Human exposure to TCDD can come from direct dermal contact with dioxin-contaminated soils, sediments, and dust during daily agricultural activities such as preparing the soil, planting, and harvesting rice and aquaculture production and harvest (**Figure 11**, **Figure 12**). The highest levels of dioxin TCDD have

Figure 11. Vietnamese farmers (both men and women) work in saturated and waterlogged soils in their rice fields.

Figure 12. Fishers prepare ponds for the next aquaculture crop.

been found in soils, sediments, and the food chain, particularly dairy products, meat, fish, and shellfish [3] [5]. TCDD is lipophilic, meaning it dissolves in fatty tissues of fish and animals and can result in high concentrations of TCDD in animal and human fatty tissues and milk [2] [48]. Animal exposure can occur from burrowing and rooting in dioxin-contaminated soil and sediments and consuming contaminated soil and feedstocks. A second pathway to human exposure is indirect via diets, such as the consumption of contaminated bottom-feeding fish, prawns, shellfish, mollusks, and wetland species such as birds and animals that consume aquatic animals in the food chain [49]. TCDD accumulates in the fat of fish, animals, and humans within each species and becomes biomagnified as it moves up the food chain [23] [48].

Current Guidance on "Safe" Levels of Dioxin TEQ in Soils

The US Environmental Protection Agency (EPA) and current scientific guidance used to inform decisions about the magnitude of health risks in urban residential settings is no more than 1 ppb for dioxin TEQ (Toxic Equivalents) in soils [35] [50]. In 'safe' level assessments, the greatest exposure pathways in urban residential settings are soil ingestion (usually children under 6) and dermal contact. "These values are consistent with soil cleanup guidelines proposed and/or accepted by several public health agencies in the United States and other developed countries" [35]. US EPA suggests that the soil concentrations of 0.5 to 1 ppb should not lead to blood levels of TCDD markedly different from those in the general population. They find in TCDD experiments with rats and human cadaver skins using activated carbon, fly ash, and soil types with organic carbon above 10% that dermal uptake of TCDD is limited compared with absorption rate studies of contaminated soils with lower organic carbon content.

Experimental design assumptions underlying this "safe" level assessment apply to most urban residential locations in the United States and other developed countries. According to US EPA, "Exposure pathways from homegrown produce, livestock, eggs, dairy products or mother's milk were **not** (authors' emphasis) assessed in this risk assessment because these pathways are frequently absent or are not expected to appreciably contribute ….to estimated soil-related average daily dose in **urban residential settings** (authors' emphasis) where past contamination of soils is the primary concern"…however, if site-specific conditions indicate that these indirect exposure paths are substantial contributors … "then these urban resident soil clean up criteria may not be reasonable as a risk management tool" [35]. Thus, this guidance does not apply to site-specific factors such as locations where contaminated soils have appreciably changed the local fish concentration due to runoff, where cows or chickens are grazing on significantly contaminated soil, and where there continue to be significant ongoing sources of TCDD released into air or soil. This document claims that "…most urban areas have ordinances that prevent residents from raising livestock for consumption…" and suggests that livestock are not considered potential dietary sources of TCDD. Further, fish ingestion assumptions are based on the

US per capital fish ingestion rate and lifetime average daily doses based on < 12 fish meals per year of locally caught fish consumed (accumulation of contaminated sediments in water and bottom-feeding fish) and considered to be a low-risk exposure pathway.

US EPA is clear their analyses do not address agricultural or rural lands [35]. Yet, the nature of Vietnamese agriculture and fisheries occupations (with fish as a major protein source) as factors influencing potential exposure levels to TCDD is critical to Vietnam's risk assessments. A 2010 study that included fish farmers exposed to TCDD for more than 20 years near Da Nang, Vietnam found fishers had significantly higher mean serum TCDD concentrations (91.1 ppt) compared to those engaged in other occupations (10.8 ppt) [49]. Similarly, Bien Hoa fish farmers had higher mean serum TCDD concentrations (785.5 ppt) compared to those of different occupations (80.8 ppt) [49]. Two other factors in the Pham study [49] associated with submerged landscapes were found to be significant. Study participants who harvested aquatic vegetables or lotus from lakes and ponds had significantly higher serum TCDD concentrations controlling for other variables, and people who had properties flooded by monsoon rains had higher serum TCDD concentrations. Although this study was conducted near highly contaminated airbases, it reinforces the concern that Western thresholds may be insufficient to protect the health of Vietnamese farmers, and rural households who do not wear footwear, have homes with dirt floors and daily work the soil producing food [3] [35].

4. Discussion

The childhood CHD prevalence rate data calculated from the HBVN 2020 country-wide CHD screening clinics presented in this paper strongly suggest that herbicides with TCDD contamination may be associated with a higher prevalence of CHD in Vietnam where they were applied in the country during the Vietnam War. It is not possible to yet establish a 1:1 correlation that maternal exposure to residual TCDD will result in a child with CHD (or other congenital anomalies) but there is a growing body of data that suggests these associations need to be further studied. Indeed, recent advances in genomics suggest that, based on the data in our current study, it might be possible to conduct potential genetic linkage studies in the future to explore this TCDD/CHD association further. One potential future study, which could be conducted concurrently while children are being screened at HBVN clinics, would be to obtain DNA buccal swabs from parents whose child is found to have CHD as well as the CHD child and use non-CHD parents and non-CHD children as controls from the same HBVN screening clinics in areas where Vietnam soils and sediments are heavily contaminated with TCDD. The hypothesis to be explored would be that any detected chromosomal deletions/mutations found in the maternal (or potentially paternal parent) and the affected CHD child might be able to be linked to the expressed CHD their child is born with.

There are limitations to the childhood CHD prevalence rate data we are presenting. The data come from children either selected by physicians or their parents to be screened for CHD at the HBVN screening clinics. Children with more subtle (but still potentially pathological) CHD could easily be missed; thus, our current study potentially could under-represent the country-wide and regional CHD prevalence rates, especially in North Vietnam where only 70% of the provinces were screened in 2020. Further, given that we only had 70% of the CHD provincial data from North Vietnam, statistical significance when comparing North Vietnam to the other regions of Vietnam could potentially be altered. Finally, much of Vietnam remains quite rural so reaching potential patients with CHD remains quite a challenge for the HBVN CHD screening team.

While we recognize and acknowledge these limitations, the prevalence data disparities between North Vietnam and Central and South Vietnam cannot be ignored and the statistics suggest that these increased CHD prevalence rates are “not by chance alone” and suggest there is a potential association with residual dioxin TCDD in the soil. The implication of this study is that even after dioxin TCDD mitigation strategies have been employed in certain areas of Vietnam (Da Nang City and airbases in southern Vietnam), we still are lacking data to know if these have made any effect on lowering congenital anomaly rates (not just CHD) in Central and South Vietnam and suggests that on-going studies into this potential association are needed.

Dioxin TCDD is a known human carcinogen, an endocrine disrupter, and has developmental and reproductive effects [51] [52] [53]. It persists in the environment years longer than the herbicides it contaminates and accumulates in the fatty tissues of animals and humans at increasing concentrations as it moves up the food chain [3] [10] [23]. For many years there has been the assumption that the rainbow herbicides and their residues within days or a year or so degrade by photodegradation, evaporate into the atmosphere, or degrade by microbial action [10] [35] [43]. However, degradation is slowed considerably when TCDD contaminated soils and sediments are buried or sheltered from direct sunlight. Further, when TCDD attaches to colloidal organic or humic soil materials, it can be mobilized to a new location during periods of high water run-off [54]. The intersection of the heavy precipitation during the monsoon season and herbicide-defoliated fields, forests, and stream banks “naturally” erode these barren soils and transport them to the lowest spots in the landscape, fertile agricultural lowlands and valleys used for food production.

In high concentrations, we know that the TCDD residues in 2,3,5-T herbicide formulations have not degraded to safe levels over the last six decades. TCDD concentrations as high as 1000mg/kg have been reported in samples of sediment and the soil over 30 years after TCDD dioxin-contaminated herbicides were sprayed [5]. Dioxin (TCDD) half-life in soil more than an inch below the surface has been estimated at 20 to 100 years depending on soil type, landscape position, and other conditions [10] [35].

What we don't know is the persistence of TCDD in soil and sediments from numerous spray applications over the Vietnam countryside or the effect of daily low dose levels on rural farmers, especially pregnant women who come in direct contact with TCDD contaminated soils and sediments; and have diets where TCDD is bioaccumulating within their food chain. Although hot spots are known, there are very little systematic data on population exposures through residual contamination of soils or consumption of herbicidal chemicals taken up throughout the food chain [9].

There are reasons to be concerned that current "safe" level risk assessment recommendations set by developed countries based on urban residents' exposures may not be the best fit for rural Vietnamese whose occupations and agricultural activities may disturb contaminated buried soils and resuspend sediments in rice paddies, fishponds, and other crops grown in waterlogged and submerged soils. Although it may not be possible to totally eliminate dioxin-TCDD from the southern Vietnam environment, there are soil and sediment management strategies and agricultural practices that can reduce direct skin and body exposure and eliminate entry points for TCDD into the food chain. Rice paddy farming and fishing occur in submerged soils where sunlight seldom reaches and the potential for TCDD residues to persist is higher than for dryland farming where the practice is to turn over the soil and soil particles are exposed to sunlight.

Most Vietnamese diets have high levels of fish, shellfish, pork, and poultry; and even low concentrations of dioxin in fish and fat in meats can result in chronic dietary exposure and bioaccumulation. Once site-specific locations are identified as potential risk hazards, interventions might include changes in how and where fish are grown (*i.e.* raising fish in non-contaminated ponds), altering muddy environments where poultry and pigs are pastured, washing and peeling vegetables grown in contaminated soils [3] [5], and adoption of dry land rice production systems.

The World Health Organization (WHO) and developing countries such as Vietnam should consider TCDD risk assessments and contamination guidelines and standards that are more appropriate to the exposure pathways of rural places and people who are living close to the land [3]. The present total toxic equivalency factors (TEF) scheme and TEQ methodology are primarily intended for estimating exposure and risks via oral ingestion via dietary intake [13]. The direct application of the TEF/total toxic equivalency (TEQ) approach to abiotic matrices, such as soil and sediments may not well assess the risk to populations engaged in agriculture and other occupations that bring them daily into contact with contaminated soils and sediments.

5. Recommendations

In this paper, we have explored associations of CHD prevalence rates and potential TCDD persistence in soil and sediments in Vietnamese regions south of the

17th parallel where TCDD contaminated herbicides were heavily and repeatedly applied from 1961 to 1971. Currently available data are inadequate to test cause-effect relationships of health outcomes fifty years later. However, we are currently finding CHD disease patterns well above global rates of regions that were not systematically exposed to TCDD. This suggests there is an urgent need to fund longitudinal medical (screening) studies as well as genetic studies to assess both CHD and other congenital organ anomalies for the impacts of long-term low dose TCDD exposure in the vulnerable female reproductive population of Vietnam in these exposed areas.

Research design should incorporate intersecting variables including 1) site-specific locations that received high volumes and multiple spray loads of herbicides used during the Vietnam War; 2) soil characteristics including submerged and waterlogged soils and sediments where TCDD may not have degraded; 3) areas used for the production of agriculture, fisheries, and other aquatic products; 4) risk assessments of dioxin levels in food items where TCDD is likely to bioaccumulate; 5) child-bearing age and pregnant women and populations with potentially high sensitivity to long-term low dose exposure, and 6) men and women in occupations such as fisheries, agriculture, and construction that are in daily contact with contaminated soil and sediments as part of their job routines.

Traditional soil sampling methodologies that rely on discrete or composite sampling from a small number of data points must be updated to modern grid sampling methodologies to reliably estimate dioxin concentrations (TEQs) [55]. Incremental Sampling Methodology recently used in the characterization of soil and sediment and confirmation of remediation at Da Nang airbase demonstrated a much improved (decreased sampling variability) methodology utilizing decision units and sub-units, systematic random sampling, and independent replication of subsamples [55]. Less variability in TEQ findings will increase confidence in the type and extent of remediation needed. Current WHO and Vietnamese guidance for safe levels of dioxin TEQ in soil need to be re-evaluated in the context of developing countries' cultures and environmental conditions. Safe dose guidelines must be reviewed and revised to incorporate new scientific knowledge related to embryo development and genomic mutations associated with the effects of routine, long-term contact with TCDD contaminated soils and sediments by pregnant women, rural populations, and those in agricultural and fisheries occupations.

6. Conclusions

Vietnam is an agriculture-based economy with more than 70% of the population engaged in agriculture [45]. Most of the rural Vietnamese population live close to the land, with daily intimate contact with its soil, sediments, and water resources [3]. Agriculture, fishing, and forestry provide Vietnamese households with food security and livelihoods and are frequently pathways out of poverty [56]. If we better understood the geographic locations and soil conditions that retain low but potentially harmful concentrations of dioxin TCDD, systematic efforts could be made to reduce human exposure by direct contact with contaminated soil and prevent these compounds from entering and accumulating up the food chain. This has a huge potential "upside" for the public health of pregnant mothers and their offspring in affected areas of Vietnam. Early diagnosis of congenital anomalies is well known to result in better outcomes for the fetus. The ability to identify those at risk and then employ mitigation strategies remains of paramount importance to changing both CHD and other congenital anomaly prevalence rates in Vietnam in the future.

Hot-spot and superfund cleanup criteria, methodologies, and dioxin risk assessments in the United States and other developed countries have focused on the cleanup of residential and industrial sites and likely exposure scenarios [35] [57] [58]. Paustenbach *et al.* [35] are explicit that their work in urban residential settings and assumptions of soil exposure and pathways to human impact models do not apply to subsistence agriculture or large-scale agriculture, fisheries, grazing, and livestock raising practices in which farmers and their families may have chronic and direct skin contact with contaminated soils and in-direct pathways into the food chain [35]. The exposure pathways and health impacts on farmers and rural communities whose occupations and cultural practices entail daily contact over a lifetime with soil and sediments where dioxin-TCDD persists in the environment is a gap in the science that must be explored and better understood.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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