A CASE-CONTROL STUDY OF BIRTH DEFECTS AND CANCER

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Introduction:
Dioxin is an unwanted by-product of many industrial processes. It is a ubiquitous contaminant that persists in the environment and accumulates in the food chain[1]. Dioxin is a general term for an family of chemical compounds with an identical carbon-oxygen framework, of which 2,3,7,8 tetrachloro-dibenzo-*p*-dioxin (TCDD) has been identified as the most toxic member. TCDD is a chemically stable compound that also resists biological degradation and is insoluble in water, but highly soluble in fats and oils. The carcinogenicity of TCDD has been demonstrated in many animal species, and it is recognized as one of the most potent carcinogens, causing both common and uncommon tumors at multiple sites[2]. In 1997 the International Agency on Cancer Research (IARC) officially classified TCDD as a human carcinogen.[3] In a report draft released in May 2000, the Environmental Protection Agency (EPA) reassessed TCDD as a human carcinogen, a step above the previous ranking of probable carcinogen. Most populations in developing countries are exposed to environmental TCDD, its main source being food, with an estimated 98% of the exposure through the oral route with background level of serum TCDD generally in the range of nanograms per kilogram (parts per trillion) on a lipid basis.

In Vietnam, between 1962 and 1971, approximately 72 million liters of herbicides were used by the United States Air Force for destruction of crops and defoliation of vegetation. Agent Orange, an herbicide containing 2,4,5-T diluted in kerosene, constituted about 60% of the total volume of herbicides sprayed from airplanes, with an extra 6 million liters of herbicides applied in small scale from helicopters, riverboats, trucks, or backpacks[4]. The prevalence of exposure to TCDD contaminated herbicides in the Vietnam veteran population is unknown and study of TCDD in veterans is complicated by at least four factors. On one hand, the dioxin content of Agent Orange varied widely from sample to sample, ranging from less than 0.1 parts per million (ppm) to over 60 ppm[3]. Also, Agent Orange contained other contaminants, such as furans, that could also have a health impact. Further, the level of exposure depended on external factors such as changing weather patterns, accidental releases, and access to showers, etc. Finally, due to the lipophilic character of dioxins that prevents their excretion in the urine and causes their accumulation in body fat, individual characteristics such as body mass index (BMI) becomes an important consideration in exposure assessment.

Recently, several national and international occupational studies, with cohort sizes under 6,000, including the Ranch Hand study [5-6-7], have reported an increased overall cancer mortality. However, the investigators did not report statistically significant increases in any specific cancer type. This failure to find increased mortality at specific cancer sites may be due to the rarity of the cancers in cohorts of limited size and of the "relatively young" ages of the cohort. Of interest in the evaluation of TCDD and human carcinogenicity data are four cohort

studies of herbicide producers[8-9-10-11]. *These studies involve the highest known exposure to* TCDD in humans among all epidemiologic studies. An average increased risk of 1.4 for all cancers combined was seen in these cohort studies. Furthermore, statistically significant positive dose-response trends for all cancers combined were present in the largest cohort.

Recent findings in the area of dioxin toxicity have led to a better understanding of the mechanisms of toxic action. These chemicals exert their effects through interaction with a specific intracellular protein, the Ah receptor. The Ah receptor functions as a transcriptional enhancer, interacting with a number of other regulatory proteins. Dioxin appears to function as a hormone, initiating a cascade of events that is dependent upon the environment of each cell and tissue[12].

Neural tube defects are a group of malformations of the developing brain and spinal cord, the most common of which are anencephaly and spinal bifida. The etiology of neural tube defects is unknown, but suspected to be multifactorial in nature. The prevalence of spina bifida in the United States is 4.6 per 10,000 live births/stillbirths. A review of epidemiological studies on health outcomes and exposure to dioxin suggests that exposure may be associated with an increased risk of cancer and unfavorable outcomes of pregnancy. Among the Vietnamese population, a possible increase in birth defects after paternal exposure was identified by Sterling et al.[13] Unfortunately, in most studies inadequate vital data information and the small number of exposed pregnancies have hampered evaluation of congenital malformations recognized at birth in relation to exposure to dioxin compounds[14].

Human exposure to TCDD has been associated with toxic effects other than cancer[15], such as diabetes, cardiovascular disease, chloroacne etc. The majority of these effects have been reported among occupationally exposed groups, such as chemical production workers, pesticide users, and residents of areas contaminated with tainted waste oil, or fumes from hazardous waste incinerators[16]. Although in many countries the background exposure to TCDD seems to be decreasing[17], the health hazards associated with dioxin exposure will remain for several decades because of the slow elimination from the environment. The estimated half-lives of TCDD vary from a few hours on foliage to several days near the soil surface, to several years if absorbed in the soil. Given that the spraying of Agent Orange occurred in Vietnam between 1962 and 1971 the cohort of Vietnam veterans has had an average of 30 to 40 years time since exposure. This extended period of time improves the likelihood of detecting cancers with long latency periods and other adverse health effects in more than one generation of this national cohort. Acknowledging the limitations of retrospective quantitative exposure assessments, and accompanied by appropriate caveats, it is important to evaluate the relation between exposure to TCDD, cancer and birth defects.

Hypothesis and Study Objectives:

- To test the hypothesis that there is an association between an occupational exposure to dioxin (as one of the components of Agent Orange) during service in the Vietnam War and an increased prevalence of, or mortality from, cancer.
- To calculate age-specific cancer incidence from a cancer specific prevalence measure and compare to age/gender and cancer type specific SEER rates.
- To determine the association between the bivariate outcomes of cancer in the parent and/or birth defects in the child, by means of analyses relating tour duration, branch and dates of service, adjusting for known confounding variables.
- To describe the cancer distribution by primary cancer sites.

Study Population and Methodology:
The current project is embedded in an Environmental Protection Agency (EPA) funded study entitled "Reproductive Health, Serum Dioxin, and P450 Genes in Vietnam Veterans" that examines the reproductive health risks of military exposure to environmental toxicants. The project has acquired from the Departments of Defense (DoD) and Veterans Affairs, a computer file of the names, ages, and military ID numbers of all known U.S. Vietnam veterans (nearly 3 million).

Through an interagency agreement involving the VA, NIOSH, and the IRS, the project has obtained current addresses for the veterans. In order to minimize selection bias, a statistical sampling strategy was implemented using data from 40 different vital statistics centers (death, fetal death and birth certificates) to build a computerized list of identifiers (names, age, date of birth, state of residence etc) of "possible" veteran parents of offspring born with neural tube defects in any of the 50 US continental states between 1962-1995. After cross-linking the vital records listing with the computer file of Vietnam veteran's identifiers we have identified a sample of 15,000 veterans from all over the United States who are potentially parents of children with neural tube defects. Using Texas data, we have completed a pilot study in which 1,257 potential participants were mailed a letter to introduce the study and request his or her completion of a 4-page, self-administered survey to determined eligibility and willingness to participate in the study.

This study is designed to test the hypothesis that there may be an association between the presence of cancer in the veteran and birth defects on their offspring, and the level of exposure to Agent Orange during the Vietnam War, in a national sample of Vietnam veterans. In this case-control study, veterans will be eligible for the study if they served on active duty in Vietnam between 1962 and 1975 and have had at least one family pregnancy (regardless of disease status or birth defects). The study will have two phases. First, among alive eligible participants, prevalent cases will be ascertained by self-report of medically diagnosed cancer with follow-up confirmation using personal physician records; those veterans without cancer will serve as controls. Second, among deceased participants, cause of death will be ascertained through a National Death Index (NDI) search and case/control status will be assigned by ICD-10 mortality coding. An analogous approach will be taken with reported birth defects. An Exposure Index to Agent Orange (TCDD) (high / medium/ low) will be constructed based on responses to questions regarding branch and dates of service, occupational category, military rank, period and location of service.

Analysis: This case control study will be analyzed using multiple logistic regression. A model will be constructed to calculate the log odds of cancer against index level of exposure to Dioxin (Agent Orange). Using the presence or absence of a birth defect in the offspring of the veterans as a regression variable, controlling for available confounding variables such as age, race/ethnicity, smoking, family history of cancer, highest military rank, branch of military service among other variables. Cancer incidence data will be calculated from age specific prevalence measures using a two state deterministic model suggested by Hill, Forbes and Kozak[18]. Furthermore, for the mortality phase of the study a Standardized Mortality Ratio (SMR) will be calculated by gender, age and cancer type.

Preliminary Results:

After eight weeks following the conclusion of our pilot study, 375 surveys have been received, giving a response rate of 45%. A total of 25 cancer cases among eligible participants have been identified including, eight skin cancers, four prostate cancers, two leukemia's, one soft tissue sarcoma and one non-Hodgkin's lymphoma. Furthermore, thirty cases of children being born with Neural Tube Defects have been reported among eligible participants who have responded this far.

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