# THE EFFECTS OF AGENT ORANGE/DIOXIN ON PREGNANCY OUTCOMES IN A SPRAYED AREA IN VIET NAM

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## Abstract

Sixty one percent of defoliant herbicides sprayed by the US military in southern Vietnam from 1962 to 1971 was Agent Orange, a 50:50 mixture of 2,4,5-T and 2,4-D. The defoliant 2,4,5-T was contaminated with a very toxic form of dioxin (TCDD) known to have adverse effects on human healh. There has been insufficient information to demonstrate an association between exposure to TCDD and pregnancy failures. The objective of this study was to determine whether an association exists between exposure to AO/dioxin among women living in a sprayed area and adverse pregnancy outcomes including spontaneous abortion, stillbirth, preterm delivery, and birth defects. A retrospective cohort study was implemented with 963 households in the sprayed site and 903 households in the non-sprayed site. The logistic regression analysis, adjusted for age at pregnancy, gravidity, alcohol ingestion, smoking, hormonal contraceptive use, and levels of pesticides used, shows nearly 3 fold greater risk of adverse pregnancy outcome among women living in the sprayed area compared with women living in the non-sprayed area. A well-designed follow-up study should be implemented to better understand the risk of adverse pregnancy outcomes in Agent Orange/dioxin contaminated areas.

## Introduction

It was estimated that southern Vietnam has been contaminated by 160 to 600 kg of dioxin as a result of over 80 million liters of defoliant herbicides were sprayed by the US military over a large area of forests and crops of southern Viet Nam from 1962 to 1971 [1, 2]. Substantial experimental animal research suggests that exposure to TCDD causes several reproductive and developmental failures; however, the evidence is less clear on maternal and paternal effects of TCDD exposure on other outcomes such as spontaneous abortion, stillbirth, preterm delivery, and birth defects [3]. Likewise, epidemiological studies have provided insufficient information to demonstrate whether an association exists between exposure to TCDD and adverse pregnancy outcomes because of limitation in defining exposure to AO/Dioxin, adjusting for confounding factors, and inconsistent results among studies.[3]

Results of a recent investigation demonstrates the apparent food chain transfer of TCDD from contaminated soil to cultured fish pond sediments to fish and duck tissues, then to humans as measured in whole blood and breast milk, and the investigators hypothesize that a principal route of exposure is the transfer of dioxin from soil into river sediment, then into fish, and from fish consumption into.[4, 5] Almost all of the epidemiological studies in Viet Nam state that the rates of these abnormal birth outcomes were elevated among AO/TCDD exposed people who are veterans or inhabitants of sprayed areas. [6] However, these studies had some limitations. First, exposure estimates were based entirely on ecological data of spraying mission as recorded by the US military, and no analytic work was done to improve exposure estimates. Second, confounding factors for abnormal birth outcomes were poorly controlled, and no multivariate analysis was conducted. Third, these studies have rarely been published in international science journals.

The purpose of this study is to determine whether associations exist between exposure to AO/Dioxin among women living in a sprayed area and adverse pregnancy outcomes including spontaneous abortion, stillbirth, preterm delivery, and birth defects. This study attemps to overcome the limitations of previous studies of Vietnamese population by using logistic regression to control for confounding factors.

## Materials and Method

Cam Chinh commune (CC) in Cam Lo district, Quang Tri province, an area sprayed with herbicides during the war, was selected as a case site. Cam Phuc (CP) commune in Cam Xuyen district, Ha Tinh province, which was not a sprayed area, was selected as the control site. Both the CC and CP communes are located in the central part of Vietnam, and have similar demographic and socioeconomic characteristics.

An environmental assessment was implemented to determine whether current TCDD contamination exists in both areas. Guided by a digitalized map of the spraying missions that was based on US military records and anecdotal stories of local inhabitants, six soil samples and four sediment samples were taken in areas of CC commune that were sprayed with AO/dioxin and were near to former military bases. Soil samples were collected at 0 to 10 cm depth stratum by a stainless steel soil corer, replicating methods used by Hatfield Consultants, Inc in A Luoi valley. Six animal tissue samples including fat, liver, and muscle of chickens, ducks and cultured fish were also obtained. All of these samples were analyzed for TCDD at the VECAC, a laboratory belonging to the 10-80 Division of the Vietnam Ministry of Health.

A retrospective cohort study was implemented with 963 households in CC commune and 903 households in CP commune selected to participate. These households were selected from population registration records by the Commune Peoples Committees. Each woman in a household who indicated that she had a pregnancy history was interviewed by a highly trained nurse-interviewer who was working in the local district health center. Information collected during the interview included personal habits related to smoking, alcohol ingestion, and contraceptive drug use; history of pesticide exposures; number of pregnancies; age at each pregnancy; and history of adverse pregnant outcomes. Adverse pregnancy outcomes consisting of birth defect, spontaneous abortion, stillbirth, and preterm delivery were reffered to the March of Dimes (Bloom,1981; Herz-Picciotto and Samuels, 1988; Kline et al.,1989; Bryce, 1991; CDC,2000).

Data were entered into computers using EPI INFO 6.04 software and converted to Stata 9.0 software for analysis. The crude cumulative incidence rates of adverse pregnancy outcomes (adverse pregnancy outcomes per 1,000 pregnancies per year) and their respective standard errors were calculated by Stata software 9.0, and compared between two geographic areas. Because US military started spraying herbicides in 1962, pregnancies occuring before 1962 were excluded from analysis. Rates were presented separately for each kind of adverse outcome, maternal age stratum, and time period (years). In order to control potential confounding factors, multivariate logistic regression was used to estimate odds ratios (ORs) between exposed and non-exposed communes adjusted for maternal age at pregnancy, gravidity, levels of agricultural pesticide usage, smoking, alcohol ingestion, and hormonal contraceptive use.

## Results and Discussion

Levels of 2,3,7,8-TCDD range from Non-Detectable (ND) to 13.98 pg/g, and total-TEQs range from 0 to 14.18. In these soil samples, TCDD was responsible for a high proportion (84.45% - 99.86%) of the total I-TEQ. Other dioxin and furan congeners were detected at lower levels, and might have resulted from the use of other herbicides. The 60% of soil samples collected in Cam Chinh commune had TCDD levels that exceeded US EPA residential guidelines for Region III (4.3pg/g) and Region IX (3.9pg/g), so these TCDD levels would trigger recommendations for more detailed risk assessment if reported in those regions of the US. Three soil and sediment samples as well as animal tissues collected in the control area, Cam Phuc commune, had no detectable 2,3,7,8-TCDD.

Data were gathered on a total of 1,866 women representing 5,600 pregnancies occuring between1962 and 2001 . The exposed commune (CC) included 963 women representing 2,731 pregnancies, and the non-exposed commune (CP) included 903 women representing 2,869 pregnancies. A total of 573 adverse pregnancy outcomes were reported from 1962 to 2001, including 163 in the non-exposed commune and 410 in the exposed commune. The mean age of pregnancies among women in the exposed commune (28.6 ± 6.52) was slightly but significantly lower than that among women in the non-exposed commune (29.0 ± 6.62), and pregnancies among women aged 18-20 years were more common in the exposed commune (8.26% vs 6.15%; $p<0.01$). The mean number of pregnancies per woman in the exposed commune was slightly smaller than that among women in the non-exposed commune (4.6±2.2 vs 4.8±2; $p=0.0002$), and the high order pregnancies(>= 4 pregnancies) were more common in the non-exposed commune (72.9% vs 65.6%; $p<0.01$). The exposed commune had higher proportions of women who drank alcohol (3.3% vs 1.2%; $p<0.01$) and smoked cigarettes (18.5% vs 0.5%; $p<0.01$) than the non-exposed commune. In contrast, women in the non-exposed commune used contraceptive

drugs more frequently than women in the exposed commune (5.8% vs 3.7%; p=0.0002). Many more women in the non-exposed commune were involved in spraying pesticides on their family farms than women in the exposed commune (70% vs 19%; p<0.01). The mean number of times in life years of spraying pesticides among women in the non-exposed commune was also much greater than the time for women in the exposed commune (93.4±102 vs 14±6.4; p<0.01). However, four outliers of spraying times (> 1,000 times in life years) was seen in the non-exposed area.

Crude analysis show a greater than two fold increased risk of adverse pregnancy outcome among women living in the sprayed area, Cam Chinh commune (IRR=2.4, p<0.01) compared with women living in the non-sprayed areas, Cam Phuc commune. A substantial risk difference is also illustrated between the two groups (2.8/1000). The backward selection method was used to identify the best models. It is illustrated that in the full model and all reduced models, which included the variables for smoking and pesticide usage, the incidence rate of adverse pregnancy outcomes in the sprayed commune is at least three times higher than in the non-sprayed commune; the difference is statistically significant with p<0.01. In almost all the other models, pregnancy failures were nearly three times higher in the sprayed commune as compared to the non-sprayed commune (OR=2.8 or 2.9, p<0.01). Overall, incidence rates of adverse pregnancy outcomes in the sprayed commune increased after the 1962-1965 period, and the highest incidence rate was found in the period of 1986-1990 (36.3/1000 pregnancy-year). In contrast, incidence rates of adverse pregnancy outcomes in non-sprayed commune were much lower and appeared fairly stable over time with the greatest in crease after 1990 (13.2 – 13.9/ 1000 pregnancy-year). The incidence rates of adverse pregnancy outcomes among women in the sprayed commune were statistically higher than that among women in non-sprayed commune for all periods of time since 1965 ($RR_{5\text{-year}}$= 1.82-3.7), and the risk difference reached maximum in the period of 1986-1990 (73.4%).

*Logistic Regression Models in comparison for incidence rates of pregnancy failures between two communes*

| N | Logistic regression model | OR | CI 95% | P value |
|---|---|---|---|---|
| 1 | Commune<br>- adjusted for age of pregnancy, number of pregnancies, alcohol ingestion, smoking, contraceptive drugs, pesticide usage level. | 3.3 | 2.3 – 4.7 | <0.01 |
| 2 | Commune<br>- adjusted for age of pregnancy, number of pregnancies, alcohol ingestion, smoking, contraceptive drugs. | 2.8 | 2.3–3.5 | <0.01 |
| 3 | Commune<br>- adjusted for age of pregnancy, number of pregnancies, alcohol ingestion, smoking. | 2.82 | 2.3–3.4 | <0.01 |
| 4 | Commune<br>- adjusted for age of pregnancy, number of pregnancies, alcohol ingestion. | 2.8 | 2.3–3.4 | <0.01 |
| 5 | Commune<br>- adjusted for age of pregnancy, number of pregnancies. | 2.7 | 2.2–3.3 | <0.01 |
| 6 | Commune<br>- adjusted for age of pregnancy | 2.7 | 2.2–3.2 | <0.01 |

The incidence rate of spontaneous abortion among women in the sprayed commune was 3.3 times higher than that among women in the non-sprayed commune (CI 2.1-5.3, p<0.01); similarly, the incidence rate of birth defects in the sprayed commune was 3.6 times higher than that in the non-sprayed commune (CI 2.1-6.3, p<0.01). The incidence rate of stillbirth in sprayed commune was moderately elevated in comparison with that in the non-sprayed commune (OR=1.8, CI 0.2-17.8, p=0.6), but the difference was not statistically significant. Only a few cases of preterm delivery were reported, so rates were not calculated. Spontaneous abortion was the most problem, dominating the adverse pregnancy outcomes in both the sprayed and the non-sprayed communes (54% and 59.7%). Respectively, the second most common problem was birth defects (37.7% and 30.0%).

This study has a number of limitations. First, although this study assessed environmental dioxin exposure in the sprayed commune without longitudinal data, this assessment could not demonstrate the full sequence of exposures and outcomes. In addition, the number of samples analyzed was small and might not be representative of environmental contamination in our research communes. All environmental and animal tissue samples were analyzed at the laboratory of 10-80 Division, the Ministry of Health, which had limited equipment and machines of low resolution, so the quality assurance (QA) and quality control (QC) of the analytical processes were lacking in sensitivity and precision. Second, the study relied on self-report by respondents, and it is impossible to validate independently the reliability of interviewees' answers. Sensitive or stigmatized events, such as birth defects or number of pregnancies before getting married, might be underreported. The study may also suffer from information bias and recall bias. Respondents may report incorrect information regarding types of adverse pregnancy outcomes, as well as the context in which the adverse outcomes occurred. Recall bias might be more likely to occur with older respondents in remembering the exact timing of distant events. Third, occupational trends, nutritional status, income and educational status could not be analyzed as potential confounders due to lack of reliable data.

In spite of its limitations, this study improves upon previous studies implemented in Vietnam because it combines environmental risk assessment with an epidemiological survey, and confounding factors are controlled for in the multivariate regression analyses. Some new conclusions can be drawn from the results of this study. First, although Operation Ranch Hand, in which US military sprayed large amounts of herbicides and defoliants in southern Vietnam, ended over 30 years ago, slightly elevated levels of 2,3,7,8-TCDD are still found in some soils and sediment in the sprayed area. Second, findings from this study support the hypothesis that exposure to Agent Orange is associated with a significant increase in the risk of adverse pregnancy outcomes, including spontaneous abortion, stillbirth, and birth defects. No association between Agent Orange exposure and preterm delivery was found. Maternal smoking and pesticide use may influence the risk of adverse pregnancy outcomes among the study population .However, this study could not determine whether the biological mechanism of adverse pregnancy outcomes is attributable to maternal or paternal factors. Finally, a well-designed follow-up study should be implemented in an Agent Orange/dioxin contaminated hot spot in Vietnam to determine the relationship between Agent Orange exposure and adverse pregnancy outcomes.

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