Health Effects of Herbicides Spraying During The Second Vietnam War: Results of French-Vietnamese Joint Epidemiological Studies

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1. Introduction

Between 1961 and 1975, about 91 million kg of phenoxy herbicides heavily contaminated by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) were sprayed on the south of Vietnam[1]. As a consequence, a total of about 170 kg TCDD were dispersed over a surface of one million ha.

Epidemiological studies carried out in Vietnam in the early 80's have claimed that an increased risk for various health endpoints had been observed. These findings were related to the contamination of the Vietnamese environment or to direct exposure at the time of spraying. Among these, an increased risk of liver cancer has been reported[2]. This study has been criticized for methodological weaknesses[3]. Studies of hydatidiform moles have also been conducted[4] which were *i)* correlational studies, therefore of limited value for establishing causal relationship; *ii)* a case-control study, suggesting a strong association between maternal exposure and molar pregnancies, also suffering from some methododological drawbacks[5]. In addition, assessment of individual exposure in case-control studies has almost always been rough and an exposure index rarely constructed.

These findings clearly needed further research. A series of studies were consequently undertaken within a French-Vietnamese joint programme from 1989 up to 1993, on exposure index construction, liver cancer, and hydatidiform moles. The main results of these studies are summarized below.

2. Correlation between dioxin levels in adipose tissue and estimated exposure to Agent Orange in south Vietnamese residents[6]

Questionnaire-based exposure assessment leads to misclassification and consequently to statistical power loss or to bias. Furthermore, as a general case, dioxin analysis is too expensive to allow individual measurements at an epidemiological scale, *i.e.* hundreds of subjects, and sample collection raise ethical as well as practical issues (withdrawal of

either 100 ml venous blood or adipose tissue). These reasons prompted us to attempt to construct and validate a model for quantifying exposure to Agent Orange.

Adipose tissue samples from 27 Vietnamese males born before 1953 and undergoing abdominal surgery in Cho Rây Hospital, Hô Chi Minh City, were taken while anaesthetized, after obtaining subjects consent. These materials, sent frozen to France, were analyzed by mass spectrometry and gas chromatography for chlorodibenzo-*p*-dioxin (PCDD) and chlorodibenzofuran (PCDF) congeners content[7]. Each subject was interviewed according to a standardized questionnaire about his successive places of residence (at the village level) from birth until the study (1989) of which geographical coordinates were determined. Five subjects always had their main residence in Hô Chi Minh City and were considered as not exposed. The lists of spraying were then compiled from these coordinates and data from the US Air Force HERBS Tape database (dates, herbicide category, amounts sprayed, flight coordinates) within a radius of 10 kilometers from each place of residence.

The Agent Orange cumulative exposure index was calculated assuming that *i)* decreases in spatial exposure are inversely proportional to the distance between the point of impact and place of residence; *ii)* time of exposure decreases according to exponential first-order kinetics, with a TCDD half-life in the soil set at either 9 years[8] or 10-12 years[9,10]. Exposures were calculated by summing the durations of residence in each contaminated area. A second index was calculated to take into account the biological fate of TCDD in adipose tissue from the beginning of exposure until sampling, using biological half-lives of either 5, 6 or 7 yrs[11,12].

The mean TCDD level in adipose tissue was 7.8 ppt. For the group of 27 subjects, we found a Pearson correlation coefficient of 0.36 ($p = 0.07$) between the TCDD levels and the exposure index after log-transformation of both variables. When the analysis was restricted to the 22 subjects with a positive exposure index, the above coefficient rose to 0.50 ($p = 0.02$). In addition, no correlation was found between other PCDD/F congeners levels and exposure index (except for "other H7CDF", *i.e.* non-toxic H7CDF).

We conclude that despite some inaccuracies and limitations (*e.g.* study power), our method could be useful for future epidemiological studies in Vietnam.

3. Hepatocellular cancer risk factors in Vietnam[13]

A case-control study was conducted in 2 hospitals in Hanoi, both recruiting patients from the north of Vietnam. Cases were 152 male patients with a diagnosis of hepatocellular cancer (HCC) old enough to have been drafted during the second Vietnam war, *i.e.* born before January 1, 1953. Diagnostic criteria were clinical or echographic suspicion of HCC, and a raised titer of serum α-fetoprotein, above 500 ng/ml (ELISA Abbott test). Two hundred forty-one controls, also born before 1/1/1953, were chosen among patients admitted for surgery in the same hospitals during the same period for reasons other than neoplasm or liver disease. They were frequency-matched for age (±5 years), hospital and places of residence (greater Hanoi or provinces).

Patients interviews were conducted "blind" to case or control status, with some difficulties in one hospital. The questionnaire included standard questions about lifestyle and specific ones regarding occupational exposure to pesticides, stays in the south of Vietnam, with notion of locations, dates and direct contact with aerial herbicide sprayings during the

war. This information was used as a surrogate measure of potential exposure to Agent Orange.

Odds ratios (OR) adjusted for matching variables and other potential confounders were estimated using unconditional logistic regression, or exact non-parametric statistical inference when numbers were small.

In agreement with the fact that HCC etiology in this part of the world is dominated by the role of chronic infection with hepatitis B virus (HBV)[14], we found that positivity for hepatitis B surface antigen HBsAg was the main risk factor for HCC in our sample (OR = 61.7; 95% confidence interval 30-128). Five subjects (3 cases, 2 controls), all HBsAg negative, were found to have been infected by hepatitis C virus (OR = 38 among HBsAg negative subjects; 95% CI 2.8-1443). Alcohol consumption was associated with HCC and interacted with HBsAg positivity. Agricultural use of organophosphorous -but not organochlorine- pesticides (30 liters/year or more) was also a risk factor for HCC (OR = 4.7; 95% CI 1.1-20).

Overall, having stayed in the south of Vietnam after 1960 for military purposes did not carry an increased risk for HCC, nor did self reported direct or indirect contact with sprayings. However, there was a significant trend in risk with increasing duration of stay in the south ($p<0.01$). An increased risk for HCC was also observed in soldiers who stayed for more than 10 years there, after adjustment for matching variables, HBsAg and anti-HCV status and alcohol consumption (OR = 5.3; 95% IC 1.1-27.6).

The risk estimates of HCC associated with length of stay in the South were higher in the HBsAg positive than in the HBsAg negative group, although there was no statistical interaction between HBV and military service in the South. This points to the possible contribution of factors acting directly or concomitantly with HBV in the etiology of HCC. These factors might be aflatoxins (known liver carcinogens) and TCDD, in this case in agreement with both experimental data and recent findings from the Seveso accident[15].

4) Risk factors for gestational trophoblastic disease in Vietnam[16]

Hydatidiform mole (HM) is a significant complication of pregnancy and has been identified as the main risk factor for choriocarcinoma, a highly malignant cancer. The etiologic factors of this disease, or gestational trophoblastic disease (GTD), are yet poorly understood. Incidence rates vary throughout the world, with a high rate in Asia, although the respective contribution of ethnic and environmental factors is not elucidated. Late maternal age is the most firmly established risk factor for GTD[17].

A case-control study was conducted in the Obstetrical and Gynecological Hospital in Ho Chi Minh City, which is the main referral maternity hospital for the south of Vietnam. One hundred thirteen new cases with a histologically confirmed diagnosis of partial or complete HM or choriocarcinoma and admitted in this hospital between March and May 1990 were included in the study. The same number of controls were chosen among women admitted during the same period for gynecological or obstetrical surgery and matched with cases for age (±5 years) and place of residence (greater Ho Chi Minh City or province). Patients interviews were conducted "blind" to case or control status. In addition to standard questions on patient's lifestyle, gynecological and obstetrical history was recorded. Dietary habits were assessed in a semiquantitative way in order to provide

some indications on socio-economic status as well as on exposure to PCDD/F through food.

For each subject, an exposure index to Agent Orange was calculated using a detailed residential history as described above (section 2).

Odds ratios (OR) adjusted for matching variables and other potential confounders were estimated using unconditional logistic regression. A multivariate logistic regression was performed to examine confounding among variables found to be significant.

There was an increasing risk with numbers of previous pregnancies and previous live births. For the latter, the trend in risk is significant (p = 0.02). Having five or more meat dishes (both a nutritional and socioeconomic indicator) per week was associated with a decreased risk of GTD (OR = 0.4; 95% CI 0.2-0.9). Owning a television set, a socioeconomic indicator, was also associated with a decreased risk of GTD (OR = 0.5; 95% CI 0.2-0.9). As to occupational factors, we found that breeding pigs was associated with an increased risk of GTD (OR = 2.2; 95% CI 1.1-4.1), with a significant trend in risk with an increasing number of animals (p = 0.004).

Agent Orange cumulative exposure index was split into 4 classes for analysis. No excess risk was observed with either class, nor with exposure period, *e.g.* exposure *in utero*.

Literature results on the epidemiology of GTD are often conflicting[17, 18], *e.g.* risks associated with an increasing numbers of spontaneous or induced abortions. So are certain of our findings, *e.g.* that the risk increased with numbers of previous pregnancies and previous live births. Other results are in agreement with previously published literature. Overall, our findings suggest an influence of both economic status and chronic infections associated with contact with domestic animals.

5. Conclusion

The results of our HCC case-control study do not exclude an effect of contact with TCDD-contaminated Agent Orange on the incidence of HCC. On the other hand, our results in the GTD study do not support a relationship between the cumulative exposure index and an increased risk of GTD.

6. References

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