CASE-CONTROL STUDIES ON SOFT-TISSUE SARCOMA AND NON-HODGKIN LYMPHOMA IN VIETNAM

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BACKGROUND

During the Second Indochina War from 1961 to 1975, about 91 million kilograms of herbicides were sprayed on what was then South Vietnam. Not only was the general population directly exposed to these chemicals at the time of spraying, but indirect exposure has occurred since, through the persistence of several toxins in the ecosystem and particularly in the food chain. Three major types of herbicides were employed, labelled as Agents Orange, White, and Blue. Westing estimates that a person in South Vietnam between 1961 and 1978 had a one in ten chance of being sprayed by Agent Orange and a one in five chance of being sprayed by a herbicide (1). About 10% of former South Viet Nam was sprayed, with the region around Ho Chi Minh City (formerly Saigon) the most extensively covered.
There has been a considerable volume of research into the possible carcinogenic effects of exposures to chlorophenoxy herbicides (2). Much of the concern arises from the fact that chlorophenoxy herbicides may be contaminated with polychlorinated dioxins and furans, including 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) which is a potent animal carcinogen (2). A total of 170 kilograms of dioxin was applied to South Viet Nam between 1966 and 1969. Dioxin is persistent in the environment, with a half-life estimated between two years and five years (depending on the type of soil and climatic factors).

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An association between phenoxyherbicides and several types of cancer has been found in studies involving exposures during manufacture, use in agriculture or after industrial accidents (2-5). The tumours principally implicated are non-Hodgkin lymphomas and soft-tissue sarcoma. Other neoplasms have been associated less consistently. Studies of US veterans of the Viet Nam era have failed to confirm the findings from occupational studies. These studies merely regard as 'exposed' servicemen who had at some time been in Vietnam. Exposure levels to the general population of South Viet Nam in the areas subjected to spraying are considerably greater than in the US military personnel who were responsible for it (6). In Viet Nam, the first suggestion of a link between cancer and herbicide exposure was the observation by Tung (7) of an increase in relative frequency of liver cancer in Viet Duc Hospital, Hanoi. An association between liver cancer and exposure to Agent Orange was found in a recent case-control study in Vietnam (8).
The main objective of the two case-control studies on soft-tissue sarcoma (STS) and non-Hodgkin lymphoma (NHL), is to examine the association between environmental exposure to dioxins and occurrence of these neoplasms in the population of Viet Nam during the period 1993-95, i.e. some 20-30 years after the direct exposure to dioxins as a result of herbicide spraying during the war. We report in this paper the main features of the studies together with results from the pilot study.

DESIGN AND METHODS

Two hospital-based case-control studies are being conducted in South Viet Nam. The source population for the study is the whole population above age 25 residing in Southern Viet Nam (around 30 million people). The existence of only one referral centre for cancer with a catchment area extending to the whole South Viet Nam will allow the inclusion of the majority of newly diagnosed cases in South Viet Nam. 150 cases of STS, 150 cases of NHL and 600 hospital controls will be interviewed (blindly as to case status) over a three-year period. A pilot-tested questionnaire is used to collect information on suspected risk factors for STS and NHL, namely: demographic and anthropometric variables; detailed residential history; working history; extra-occupational exposure to pesticides including direct exposure during spraying operations; previous diseases (immunological and childhood); life-style factors (tobacco, alcohol); dietary factors; use of medicaments; exposure to ionising radiation (diagnostic or therapeutic X-rays). Adipose tissue samples will be collected for cases and controls. In addition, 20 millilitres blood will be taken and stored for later analyses of antibody titters to viral agents, micronutrients, and possibly analyses of PCDD's as technology develops.

ESTIMATION OF EXPOSURE TO TCDD AND OTHER POLYCHLORINATED COMPOUNDS

Essentially two methods of quantitating exposure in individuals are available: (i) direct measurement of dioxin in adipose tissue and (ii) estimation of exposure based on location of residence.

Direct measurement of polychlorinated dibenzodioxins and dibenzofuran levels in human tissue offers the most objective index of individual exposure. The analysis of polychlorinated compounds involves both the determination of levels of these compounds in adipose tissue and pattern recognition of the source of exposure. The ratio of 2,3,7,8-TCDD to 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF can serve as a specific marker for the exposure to Agent Orange.
Exposure will also be estimated by taking a careful residential history and comparing this to data on Agent Orange spraying by the US military (available through the 'HERBS' tapes). The methodology requires various assumptions concerning the environmental half-life of dioxin and the distance from the actual track of the spraying mission at which the individual was located. The application of this methodology to dose estimation of US veterans is described by Stellman (9). This method clearly has scope for considerable misclassification. However, a study in Viet Nam (10) of 27 residents suggested a correlation between dioxin level and an exposure index (similarly constructed).

CASE ASCERTAINMENT AND DIAGNOSTIC CONFIRMATION
All new cases referred to the Oncology Centre with STS or NHL during the period 1993-1995, will be identified. For all cases, a tissue sample for histological examination will be taken, and only those confirmed histopathologically will be retained in the case series. The case status will be ascertained by slide review of fixed tissue by two independent pathologists. The classification of cases will be based on morphological and immunocytochemical techniques.

THE PILOT STUDY
The pilot study involved 29 subjects (5 STS cases, 5 NHL cases and 19 controls) interviewed at the Oncology Centre. Blood and adipose tissue samples were collected for 27 subjects. The prevalence of exposure to herbicides during the war was 27% (8 subjects), a value similar to previous estimations (1). Characteristics of subjects and exposure to different suspect risk factors is shown in Table 1.

SUMMARY
The ecological effects of widespread spraying of phenoxy herbicides in Vietnam have been documented. Effects in humans have been less investigated. The pilot study indicated that a high prevalence of exposure to herbicides during the war. The two ongoing case-control studies which involve determination of dioxin in adipose tissue, validation of diagnosis of STS and NHL and validation of exposure to other chemicals and previous infections through analyses of blood samples will provide the first opportunity of evaluating effect of herbicide spraying in humans in S Vietnam.

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Table 1

Distribution of selected risk factors among 29 subjects in the pilot study.

Exposed to herbicides during spraying in the way	yes	8
	no	21
Served in armed forces in wartime	yes	11
	no	18
Lived or worked in a farm	yes	22
	no	7
Ever used DDT	yes	9
	no	20
Ever exposed to solvents in work	yes	5
	no	24
Smoking status.	Current smokers	16
	Ex-smokers	3
	Never smoked	10