Exposure to Agent Orange and Occurrence of Soft-Tissue Sarcomas or Non-Hodgkin Lymphomas: An Ongoing Study in Vietnam

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Agent Orange was the most common herbicide used in the Second Indochina War in the course of military operations in the former South Vietnam. Agent Orange is contaminated by the carcinogen 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) in mean concentrations of 2 mg/kg. After much dispute of a causal association between exposure to herbicides containing TCDD and occurrence of soft-tissue sarcoma and non-Hodgkin lymphoma, two simultaneous case–control studies were set up in Vietnam to examine possible relationships. Subject recruitment is ongoing, with target numbers of 150 cases of soft-tissue sarcoma and 150 cases of non-Hodgkin lymphoma and diagnoses at the Cancer Center at Ho Chi Minh City, Vietnam. Two hospital controls are matched to each case. As in other studies of cancer in persons occupationally or otherwise exposed to herbicides and their contaminants, evaluation of past exposure of the recruited subjects is among the most complicated issues. Because accurate records are usually unavailable, surrogate measures of likely exposure are often calculated. As a first approach in our studies we used the Stellman and Stellman exposure index. The index is based on matching subjects' history of residence and the information on times and locations of Agent Orange spraying recorded on HERBS tape by the U.S. Army and taking into account the distance from the spraying as well as environmental and biologic half-life of TCDD. The exposure index is calculated in two centers, New York and Hanoi, with slightly different assumptions. In addition, samples of body tissues from the subjects (20 ml blood, 2 g adipose tissue, and tumor sections in paraffin blocks) are taken and stored. Their future analysis will provide additional source of exposure assessment. Strengths and weaknesses of both exposure measures are discussed in this paper. — *Environ Health Perspect* 106(Suppl 2):671–678 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/671-678kramarova/abstract.html*

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Background

Agent Orange

During the Second Indochina War (1961–1975), nearly 10% of the territory of former South Vietnam was sprayed with phenoxy herbicides, 34% of these areas more than once. The most extensively affected region was around Ho Chi Minh City (formerly Saigon) (*1*). Approximately 72 million liters of these chemicals were used by the U.S. Air Force for destruction of rice and crops and defoliation of vegetation (*2*). The most widespread chemical used was Agent Orange, which constituted about 60% of the volume of herbicides sprayed from airplanes. A further 6 million liters of herbicides was applied in small-scale spraying operations (from helicopters, riverboats, trucks, or backpacks) (*3*).

Phenoxy herbicides are derivatives (salts or esters) of phenoxyacetic acid. Agent Orange is a 1:1 mixture of *n*-butyl esters of 2,4,5-trichlorophenoxyacetic acid (2,4,5,-T) and 2,4-dichlorophenoxyacetic acid (2,4-D) (*2*). Both 2,4-D and 2,4,5-T are structurally similar to indole acetic acid, a natural plant hormone (auxin), and have similar courses of action (*4*). Their mixture has a more efficient herbicide effect than either chemical alone (*5*).

2,4,5-T is commonly contaminated by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), one of the most toxic manmade chemicals. TCDD is produced during synthesis of 2,4,5,-T (*4*). The degree of contamination is dependent on the temperature and pressure of the reaction (*6*). Although synthesis of 2,4-D does not produce TCDD (*7*), commercially manufactured 2,4-D might become contaminated if it is manufactured in the same production facilities as 2,4,5-T. The mean concentration of TCDD in Agent Orange was 2 mg/kg (2 ppm); individual lot concentrations ranged from 0.05 to about 30 mg/kg (*8*). Thus, an estimated total of approximately 230 kg of TCDD was deposited in South Vietnam.

Toxicity and Carcinogenicity

TCDD is a chlorinated aromatic compound that is chemically stable, insoluble in water and highly soluble in fats and oils, and that resists biologic degradation (*9*). Other higher chlorinated congeners are usually also present as contaminants. Their toxicity varies quantitatively, but not qualitatively; TCDD is the most toxic. The toxic equivalents of the congeners are expressed relative to the toxicity of TCDD (for which the toxic equivalent is set arbitrarily at 1.0), and are inversely related to the degree of chlorination (*10*).

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Abbreviations used: CI, confidence interval; 2,4-D, dichlorophenoxyacetic acid; IARC, International Agency for Research on Cancer; SMR, standard mortality ratio; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; UTM, universal transverse mercator.

Knowledge of dioxin pharmacokinetics and the factors inducing variations in dioxin metabolism in humans is still insufficient. Phenoxy herbicides are absorbed mainly via the digestive system in humans (*11*). However, TCDD is incompletely absorbed (*12*). The liver and fat are major repositories of TCDD in humans (*13*). The phenoxyacetic acids do not undergo notable transformation prior to renal excretion in mammals. Almost all of the 2,4-D is excreted, the principal route being urine; in humans excretion may continue over several days. Similar patterns have been noted for 2,4,5,-T, although the elimination and tissue distribution of both 2,4-D and 2,4,5-T are dose dependent (*12*). Elimination of TCDD occurs via excretion of metabolites in the bile and urine and passively through the gut wall. Metabolism is slow: The biological half-life of individual TCDD is several years in humans and is strongly dependent on the individual rate of TCDD metabolism (*14*).

The studies of 2,4-D in animals do not provide sufficient evidence for its carcinogenicity. The International Agency for Research on Cancer (IARC) (*15*) and the World Health Organization (*7*) do not consider these studies sufficient to prove the chemical is carcinogenic. A decision regarding the carcinogenicity of 2,4,5-T has not been made and may depend on evidence for carcinogenicity of TCDD, its contaminant.

Most countries have assessed the human cancer risk of TCDD by extrapolating from animal data. The 1600-fold variation for acceptable intakes estimated by U.S., Canadian, and European scientists indicates a large scientific uncertainty about TCDD toxicity (*16*). Carcinogenicity of TCDD has been demonstrated in many animal species and it is one of the most potent carcinogens, causing both common and uncommon tumors at multiple sites (*10*). In many countries TCDD is listed as a chemical known to cause cancer. IARC recently classified TCDD as a group 1 carcinogen, defined as "carcinogenic to humans" (*17*).

Association of Phenoxy Herbicides with Soft-Tissue Sarcomas and Non-Hodgkin Lymphomas

Soft-tissue sarcomas and non-Hodgkin lymphomas have been associated with the exposure to phenoxy herbicides since the late 1970s, after a series of reports from Sweden (*18–23*). In these case–control studies, a significantly increased risk was associated with occupational exposure to phenoxy herbicides (farmers or manufacturers) for both cancer types. These findings prompted further case–control studies in New Zealand (*24–26*), Italy (*27*), the United States (*28–31*), Australia (*32*), and Sweden (*33*). A slightly increased relative risk of soft-tissue sarcomas was observed in most of these studies for subjects exposed to herbicides (*24,27,32*) although the odds ratios were nonsignificant. A similarly weak association was observed in the studies of malignant lymphomas.

The associations were also examined in several large cohort studies of occupationally (*34–36*) or accidentally (*37*) exposed workers. The combined results, based on published information, show an increased risk for non-Hodgkin lymphomas (standard mortality ratio [SMR] = 2.6; 95% confidence interval [CI] = 1.3, 4.7) and soft-tissue sarcomas (SMR = 4.7; the CI could not be calculated from available data) (*17,38*).

The common difficulty for evaluation and interpretation of these studies is the limited accuracy of exposure data. In an IARC nested case–control study (*39*), a dose–response relationship evaluation was based on crude classification of exposure levels: nonexposed, low, medium, and high. The relative risk increased with the level of exposure in both disease groups; significantly for soft-tissue sarcomas and nonsignificantly for non-Hodgkin lymphomas.

Rationale of the Ongoing Case–Control Studies in Vietnam

Two simultaneous case–control studies are ongoing in Vietnam; their purpose is to evaluate an association between exposure to herbicides sprayed during the war and occurrence of soft-tissue sarcoma or non-Hodgkin lymphoma in the resident population.

Subjects

Cases are diagnosed at the Cancer Center in Ho Chi Minh City, the referral center for almost the entire target population of approximately 30 million people 25 years of age and older living in South Vietnam. Study recruitment started in 1993, and to date 800 subjects have been recruited. The complete study will include 150 cases of soft-tissue sarcomas, 150 cases of non-Hodgkin lymphomas, and two controls for each case (total 900 subjects). Diagnosis of all cases is based on histopathologic review by independent pathologists in Vietnam and Europe. Two hospital controls are matched to each case by sex, age (± 5 years), and place of residence (Ho Chi Minh City or elsewhere).

One of the two matched controls is a cancer patient. Eligibility criteria exclude cancers (apart from the cancers under study) that in 1992 (the planning phase of the study) were thought likely to be positively associated with exposure to phenoxy herbicides: liver cancer, cancer of respiratory organs, prostate cancer, thyroid and endocrine tumors, Hodgkin's disease, leukemias, and myeloma.

The second control is a hospital patient free of cancer, recruited from among patients attending the Cancer Center. These patients suffer from a variety of conditions including benign tumors and lymphadenopathy. About half of these noncancer controls are patients referred to the Cancer Center for suspected malignancy but not confirmed by pathologic examination. Informed consent is obtained from all recruited subjects.

Both sets of controls are recruited within the Cancer Center to ensure that the controls are from the same overall catchment population as the cases. However, it seems probable that the catchment population for different cancers may vary, with patients who require specialized facilities being referred from longer distances. This variation could be particularly marked for the noncancer control group, who are most likely referred for diagnostic purposes rather than treatment. For this reason we stratified patients and potential controls into two broad groups—those residing in Ho Chi Minh City and those residing elsewhere—and matched them on this variable. Because the exposure of interest is related to place of residence, any further stratification would certainly have resulted in over matching.

A second reason for recruiting controls from the Cancer Center is that all subjects are expected to give a 20-ml sample of blood and 2 g of adipose tissue. The latter requirement means, in effect, that the recruited controls are those under-going some form of surgical diagnostic or therapeutic procedure.

Finally, use of other cancer controls may be particularly useful in reducing interviewer bias or recall bias by subjects (*40,41*). Control groups should include individuals with conditions whose occurrence is unrelated to the determinant under study (*42*). This was the reason for exclusion of certain cancers possibly related to

dioxin. It is important that such exclusions do not bias the control group in the opposite direction so the remaining cancers are less likely to be exposed to dioxin than the general population, even as a consequence of confounding (*43*). We have no particular reason to believe that this will be so, and the use of a mixture of cancer types in the control group should minimize such bias. The more obvious confounders of an association between dioxin and cancer (for example, occupation) will in any event be examined in the analysis.

The recent IARC evaluation (*17*) suggests that TCDD has an overall carcinogenic effect on all cancers combined. If this is indeed so, the comparison between soft-tissue sarcoma and non-Hodgkin lymphoma cases and other cancers will in fact also measure any additional risk specific to these two cancer types.

Exposure Assessment

Self-Reports. In a direct interview, information is requested on demographic and anthropometric variables, detailed occupational history, previous diseases and use of medications, lifestyle and dietary factors, exposure to ionizing radiation, and history of occupational or domestic exposure to chemicals. The subjects are asked to recall the frequency and amounts of chemicals used, their purpose (herbicides, fungicides, or insecticides), and their brand names.

Particularly extensive data are collected on the lifetime residence history of the subjects. Each subject reports the names of all towns or villages lived in from birth until the date of recruitment. The place of residence includes name of province, district, subdistrict, and village. The starting and ending dates of residence in each location are also recorded. The knowledge of the detailed residence history is used for calculation of an exposure index for each subject, as described below. In addition, the questionnaire asks about the subject's personal experience of herbicides sprayed from airplanes during the war in each place of residence. Results of spraying as experienced by the subjects are also recorded.

Questions on military service during the war are also asked, including the period, branch, rank, and location of service.

Exposure Index. The exposure index proposed by Stellman and Stellman (*44*) was calculated for each recruited subject from the information on time–space location of his or her places of residence and from information recorded by the U.S. Army on the distribution of the herbicides in Vietnam during the war, known as the HERBS tape.

HERBS Tape

HERBS tape is an objective and valuable source of information on the patterns and extent of herbicide usage in Vietnam. It contains 17,000 records describing 6475 separate missions to spray herbicides from airplanes. The information recorded includes date of the mission (day, month, year), combat tactical zone (one of four geographic regions of the former South Vietnam, denoted I, II, III, or IV), mission number, agent used (Orange, White, Blue), number of gallons used, type of mission (defoliation, crop destruction, etc.), area sprayed (in hectares), leg designator for the mission, and geographical coordinator of mission leg (*44*).

A mission normally consisted of several legs, each representing a continuous spray route, which might have included changes of flight direction. Each HERBS tape record refers to a single coordinate point on the map of Vietnam—for each leg there are at least two records (i.e., start and end point of the leg if no changes of flight direction were planned). The coordinates are expressed in the universal transverse mercator (UTM) system, which is a rectangular grid ruled off in 100,000 m subgrids.

Although the HERBS tape only stores information for the spraying missions carried out after June 1965, it contains almost complete information on the distribution of Agent Orange in Vietnam. Limited amounts (less than 1.1 million liters) of other dioxin-contaminated herbicides, notably Agents Pink and Purple (active ingredient 2,4,5-T) were disseminated from 1960 to 1964 (*8*). HERBS tape records count 42.4 million liters of Agent Orange as being expended (*8*).

For more than 80% of the missions, the calculated ratio of volume to area sprayed (an approximation of concentration) is nearly constant. Fifteen percent of the different volume/area ratios occur among missions in the lowest volume category (less than 2000) liters and 5% of the missions dispensed (less than 400 liters of herbicides) (*44*).

Calculation of Exposure Index

Each place of residence reported by a subject is matched with a grid cell of a UTM map of South Vietnam (1:250,000 scale) and converted into geographic coordinates in the UTM system. When the name of a village is not found on the map, the coordinates of the closest town are used as surrogates. The times and places of residence are matched with times and places of herbicide spraying.

The calculation of the exposure index was proposed by Stellman and Stellman in 1986 (*44*) for evaluation of exposure of American veterans who served in Vietnam. One discrete and three continuous exposure indices (E_1 to E_3) were proposed. Their continuous indices are calculated in this present study and the indices gradually include more complex assumptions about the extent of exposure received.

Exposure indices are calculated independently in Hanoi (Committee 10–80 [National Committee for Investigation of the Consequences of the Chemicals Used during the Vietnam War]) and in New York by S. Stellman's group. In both centers the calculations are based on the original Stellman and Stellman paper (*44*), although there are some differences in the assumptions and algorithms used for calculations.

Index E_1 represents a summation of all exposures occurring on all occasions when the dates of the residence of a subject coincided with the dates of missions to a particular locale, calculated from the initial concentration of the herbicides, weighted by a reciprocal of the distance of the place of residence from the spraying track. The distance was actually calculated as a continuous variable from the established coordinates of the leg's vertices as recorded on HERBS tape rather than from center points or average locations of a spray track.

In New York index E_2 was calculated by cumulating the total amount of exposure resulting from all spraying missions that had occurred in the particular place of residence before or during the individual subject's time of residence, taking into account the initial concentration and the environmental decay of TCDD. The exposure derived from residual herbicides remaining in the place of residence since previous spraying missions was therefore also considered. The half-life of dioxin in the soil was assumed to be 1 year. All exposures are cumulated over all reported places of residence to produce index E_2.

In Hanoi, calculation of index E_2 takes into account the initial concentration of the herbicides sprayed and the biologic fate of dioxin in adipose tissue from the beginning of exposure until sampling (when the subject was recruited). The biologic half-life is assumed to be 6 years. All spraying missions in the particular place of residence

during the individual's time of residence are counted and cumulated over all reported places of residence.

The New York E_3 index is a combination of indices E_1 and E_2. It combines the environmental persistence of the cumulative dose of dioxin in the soil around the place of residence with the distance of the residence from spraying missions, the reciprocal of which is used as a weighing factor for the dose, as before. Again, exposures are cumulated over all places of residence. To test the exposure measures on the American veterans, index E_3 seemed to best approximate the exposure (*44*).

Hanoi E_3 also combines indices E_1 and E_2, taking into account biologic half-life of the cumulative dose of dioxin and the distance of the residence from spraying missions. A test of this index is reported by Verger and colleagues (*45*).

TCDD Levels in Body Tissues. Biologic samples (2 g adipose tissue and 20 ml blood) are taken from each case for future analyses. Blood samples also are collected from all controls, as are adipose tissue samples. The biologic samples are stored frozen to –20° C. Direct measurements of exposure will be possible when stored biologic samples from the recruited subjects are analyzed at the end of the recruitment period. The direct exposure measures will be used as an additional source of information on exposure and may be used for reciprocal validation of several exposure measures collected within the study.

Discussion

Carrying out these studies in Vietnam has numerous advantages. Large amounts of Agent Orange have been dispersed in South Vietnam. Fifteen years after spraying ended, several studies showed that dioxin levels were higher in adipose tissues of subjects from South Vietnam than in those from industrialized countries (*46*). For areas in North Vietnam that were not sprayed, no detectable dioxin was found in adipose tissues of people who lived there (*46*). The large variation in degree of contamination of the different treated regions in South Vietnam allows study of the dose–response relationship. A large proportion of the population was exposed either directly (living in sprayed areas) or indirectly (consuming contaminated food and water).

Exposure Assessment

Most populations in developed countries are exposed to environmental TCDD, its main source being food (*47,48*). The background levels of serum TCDD are of the order of nanogram per kilogram (parts per trillion) on a lipid basis. Epidemiologic studies assessing the possible association between TCDD and cancer occurrence have therefore focused on groups of people who were subjected to higher than background exposure levels. These include workers exposed during the manufacture or use of herbicides (*18–36,38,39,49*); populations exposed to industrial accidents [Seveso, Italy, in 1976 (*50*), a German chemical plant in 1953 (*37*)]; and populations exposed to Agent Orange [the Vietnamese population living in the sprayed areas and American veterans who served in Vietnam (*44–46,51,52*)].

Based on animal data, however, the calculated risk from relatively large levels of dioxin exposures like those in Seveso and in the participants of the U.S. NIOSH [National Institute of Occupational Safety and Health] Dioxin Registry is still moderate compared with high background exposures present in most industrialized countries (*16*). If TCDD caused an increase in total cancers or of a common cancer (lung), the expected increase in risk was below the limit of detectability in epidemiologic studies. If, on the other hand, dioxin caused a tumor with incidence comparable to that of soft-tissue sarcomas, the calculated expected increases should be detectable in dioxin-exposed chemical workers and in the Seveso population. The failure of epidemiologic studies to produce convincing evidence of any chronic human effects from dioxin probably results from the relatively low exposures experienced by humans. There is no reason to allude to differential sensitivities between test animals and humans (*53*).

Although it is of primary importance, the exposure assessment is the weakest aspect of epidemiologic studies to evaluate the relationship between exposure to herbicides and occurrence of cancer. Precise information on the intensity and duration of individual exposure is usually missing. Rather, surrogate measures are used as an approximation of exposure. Several methods of exposure assessment are used but no method is entirely satisfactory in its own right.

Self-Reports. Self-reports from questionnaires may be affected by misclassification due to difficulties of accurate recall of past exposures and consequently to loss of statistical power. Other dangers include possible confusion of herbicides with other products such as malathion (and reporting false-positive exposure), denial of exposure because the subject was exposed unknowingly (false-negative reports), or recall bias (*44,45*).

Exposure Proxy Indices. A combination of records and individual recollections was most useful in historic exposure reconstruction. Summation of individual exposure histories results in a parameter or index that can be used in epidemiologic models to assess exposure–risk association. Such an index is then sometimes compared with exposure measures obtained in a different way, such as serum level of a significant biomarker. The exposure effect thus obtained is usually diluted—some subjects who were not exposed may be classified in the exposed group and vice versa—but the result nonetheless proves useful in comparisons on the level of exposure groups.

A number of indices were developed to assess exposure (*34,54–56*).

Like other exposure estimation strategies for retrospective studies, all of these strategies may be valid; however, they vary in precision and in the degree to which they contribute to assessing a particular exposure–disease association.

The Stellman and Stellman Exposure Index

The method of Stellman and Stellman seemed the most feasible for setting our two case–control studies. The necessary information is the residence history and information recorded on the HERBS tape. Including the report of the location rather than a recall of exposure per se is likely to reduce the recall bias. In addition, this index was tested on 478 U.S. military veterans (*44*). No direct validation of the estimates was possible, but the indices appeared consistent with other measures or proxies of exposure. A modification of the Stellman and Stellman index was also used in the study of evaluation of the risk of gestational trophoblastic disease (*57*).

The Stellman and Stellman indices have been developed expressly for the purpose of assigning probabilities of exposure to herbicides in epidemiologic studies of large numbers of veterans and are not claimed to represent actual exposures perfectly (*44*). As with all indirect exposure measures, some deficiencies must be considered for correct interpretation of potential associations.

First, the information used for calculation of the indices is not perfect; the potential misclassification and other fallacies of

self-reports are transferred into the index. Approximate coordinates were used when a village could not be located on the map. The starting and ending dates of residence in a particular location are given with precision only to a year. The distance from the spraying mission is calculated with respect to the start or end rather than the center of a leg. Different authors' estimations of the swath width of the aerial spray vary from 80 m to 20 km per aircraft (*1,58*). On the HERBS tape the assumed swath width was 80 m. The coordinates for each leg represented the middle of the mission, which usually consisted of three aircraft, but as many as 12 to 16 were used for some missions.

Similarly, meteorologic conditions are important for the spray dispersion, but they are not controlled for in calculation of the index. Although most of the missions were flown early in the morning to avoid spray drift, this drift could not be avoided entirely. Another situation is also not considered —a plane flying against a hill would produce a different spread of herbicides than one flying parallel with a hill or yet another flying over flat areas.

Further, no account is taken of actual movements of the settlers around their place of residence. In rural areas, Vietnamese villages are often divided into several hamlets between which the distance may vary from a few hundred meters to a few kilometers; village inhabitants and farmers usually move about within a radius of a few kilometers of their villages. Short-term temporary places of residence also are not recorded.

A constant Agent Orange concentration was assumed for all missions, which might be so in the majority of cases because the ratio of gallon to area was nearly constant; however, it might have produced slightly different estimates in almost 20% of cases (*44*).

In the literature, the half-life of dioxin in the environment was estimated to range from 9 years (95% CI 6–17) (*59*) to 10 to 12 years (*60*). In the Stellman and Stellman index (*44*) it was assumed to be 1 year, based on several environmental studies (*61–63*). The function describing dioxin persistence in soil was an exponential function (*44*). However, a better agreement with present knowledge about dioxin distribution among the different ecologic compartments and about its degradation processes may be obtained by a double exponential function (*61*), which takes into account an initial fast disappearance phase [the dioxin half-life estimated in Seveso was 23 days (*65*)] and a slow disappearance phase of several years' half-life.

No adjustment is made for residual exposure via ingestion of polluted food or water (*44*). A possibility of other sources of dioxin contamination (fires, chlorophenols used by the French and U.S. armies) is also not considered.

Unfortunately the HERBS tape itself is incomplete. Three percent of Agent Orange was sprayed before year 1965, and this amount is not recorded. The sprays of Agents Pink and Purple were also not recorded (those chemicals had the active agent 2,4,5-T) (*1*), nor were helicopter and ground spraying.

Despite the limitations, the method described here for measuring exposure to Agent Orange seems to be the best available method at present. Exposure levels of relatively large comparison groups should reflect differences in exposure of the groups even if individually calculated indices may be inaccurate. There is a potential for improving the complexity of the index, notably by inclusion of both environmental and biologic decay of TCDD in the assumptions used in index calculation.

Determination of Dioxin Body Burden. Serum and fat biopsy samples from individuals with unusually high past exposures indicate that TCDD may remain in the body for many years after exposure (*66*). Current tissue TCDD levels may therefore be useful in distinguishing the groups of individuals exposed to high levels of TCDD 15 to 20 years ago (*67*). In 1984 Schecter and colleagues reanalyzed archived specimens of human milk from the 1970s (preserved at Harvard University [Cambridge, MA] in a freezer at –70°C) using improved techniques. TCDD levels measured in aliquots of these archived samples proved almost identical to the values reported originally from the same samples (*46*).

However, there are also disadvantages to using tissue measurements. Dioxin analysis of body tissues is exceedingly expensive. In addition, withdrawal of adipose tissue and collection of blood sometimes raise ethical issues because such procedures are fairly invasive. Apart from these restrictions, there are more essential limiting factors for using the TCDD tissue measurements as a gold standard for exposure evaluation.

Dioxin measurement usually suffers from a lack of accuracy when levels are < 100 ng/kg (ppt). Errors range from 20 to 50% (*68,69*). Dioxin measurements probably do not perfectly reflect the amounts actually absorbed because human dioxin metabolism is subject to individual variations and changes over time (*14*).

Adjusted for the background TCDD levels, the median half-life of TCDD in humans was estimated to be 7.1 years (95% CI 5.8–9.6 years) based on measurements of 36 veterans (*70*). Other authors estimated biologic half-life of dioxin at 6 years (*71,72*). An extended study of 337 Operation Ranch Hand veterans estimated a median half-life of 8.7 years (95% CI 8.0–9.7) (*54*). In a study of 27 persons exposed in Seveso in 1976 and followed for 16 years, a half-life of 8.2 years was found (*73*). Among 48 German workers, half-life was estimated at 7.2 years and an increase in half-life with increasing age and amount of body fat was noted (*74*). In the index calculated in Hanoi, TCDD half-life was assumed to be 6 years.

These investigations show that the half-life is not stable and that it depends on individual metabolism, time since exposure, age, race, body mass index, and region of residence (*14*). Kang and colleagues (*52*) showed that the year sampling was done accounts for much of the variability in dioxin levels. Half-life increased with increasing amount of body fat (35% body fat = 20 years; 15% body fat = 7 years) (*54*).

Variations in TCDD half-life estimates are likely to result from changes in weight and percent body fat that typically occur with aging. When body fat content increases with age, it is possible that a small or moderate intake of dioxin may no longer be detectable by using current serum TCDD level as a biomarker (*75*). It has also been suggested that disease may affect serum TCDD levels (reverse causality) (*76,77*). Also, background levels appear to be lognormally distributed and to increase with age (*78*).

Although serum TCDD measures are useful in epidemiologic studies, they should not be taken as a gold standard of exposure level for individuals (in particular because of poorly understood variations in TCDD metabolism among individuals). However, group differences in serum TCDD levels probably indicate a difference in TCDD exposure between groups, although it is unknown how fast this difference may disappear with the passage of time between exposure and measurement.

According to the protocol of our two studies, a validation of exposure indices is envisaged against the measurements of tissue TCDD levels. In a pilot study, 27 Vietnamese subjects were examined to determine if there was an association

between the environmental exposure assessed from the personal history of residence and the HERBS tape and dioxin levels measured in the subjects' adipose tissue (*45*). The geometric mean dioxin level found was 7.8 ng/kg (ppt) on a lipid basis; dioxin and furan congener profiles were similar to those already reported in industrialized countries. Levels in three samples were below the detection limit. The Pearson correlation coefficient between the dioxin levels and the exposure index of 0.36 was not statistically significant ($p = 0.07$), but when the correlation analysis was restricted to 22 subjects who had a positive exposure index, the correlation coefficient rose to 0.50 ($p = 0.02$). This correlation was observed exclusively for TCDD isomer (*45*). Some of the described imperfections of the Stellman and Stellman index may explain why dioxin was detected in biologic material of the subjects who had a 0 exposure index; notably, intake of contaminated food by subjects in unsprayed areas.

In the final phase of these two case–control studies, special attention will be devoted to certain issues. First, careful verification of calculation of the exposure index will continue and the algorithm of calculation of the exposure index may be further developed. The relationship between the exposure index and the self-report will be clarified. Second, the entire database will be analyzed and the risk of exposure to Agent Orange for soft-tissue sarcomas and non-Hodgkin lymphoma will be quantified with respect to other covariates. Third, the exposure index will be validated by direct measurement of dioxin contamination in the human tissues collected from the recruited subjects and the association with the disease outcome will be evaluated.

Conclusion

Although quantitative measures of exposure are highly desirable, it may still be valid to use approximate measures of exposure or even surrogate data such as the amount of time spent in a military occupation or in a location in which the toxin was likely to have been used. Thus a biomarker, especially one gathered years after the exposure, is not necessarily better than measures based on spraying records. Group differences in serum TCDD levels can be useful in confirming that exposure measures reflect true differences in exposure.

On the other hand, quantitative assessment of exposure was not attempted in the majority of studies conducted to date on the topic. A combination of several sources of exposure assessment in these two case–control studies may ultimately provide quantitative risk determination of TCDD exposure.

REFERENCES AND NOTES

1. Westing AH. Herbicides in War: The Long-Term Ecological and Human Consequences. London:Taylor & Francis, 1984.
2. Young AL, Reggiani GM, eds. Agent Orange and Its Associated Dioxin: Assessment of a Controversy. Amsterdam:Elsevier, 1988.
3. National Academy of Sciences. The Effects of Herbicides in South Vietnam. Washington:National Academy Press, 1974.
4. Rappe C, Buser HR, Boshardt HP. Dioxins, dibenzofurans and other polyhalogenated aromatics: production, use, formation, and destruction. Ann NY Acad Sci 320:1–18 (1979).
5. Trost C. Elements of Risk. New York:New York Times Books, 1984.
6. U.S. EPA. Health Assessment Document for Polychlorinated Dibenzo-*p*-dioxins. Cincinnati, OH:U.S. Environmental Protection Agency, 1984.
7. WHO. 2,4-Dichlorophenoxyacetic Acid (2,4-D). Environmental Health Criteria Doc 29. Geneva:World Health Organization, 1984.
8. Young AL, Calcagani JA, Thalken CE, Tremblay JW. The Toxicology, Environmental Fate and Human Risk of Herbicide Orange and Its Associated Dioxin. USAF OEHL-TR-78-92. Brooks Air Force Base, TX:Medical Division, 1978.
9. Huff JE, Salmon AG, Hooper NK, Zeise L. Long-term carcinogenesis studies on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and hexachlorodibenzo-*p*-dioxins. Cell Biol Toxicol 7:67–94 (1991).
10. Ahlborg UG, Brouwer A, Fingerhut MA, Jacobson JL, Jacobson SW, Kennedy SW, Kettrup AAF, Koeman JH, Poiger H, Rappe C et al. Impact of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls on human and environmental health, with special emphasis on application of the toxic equivalency factor concept. Eur J Pharmacol Environ Toxicol Pharmacol Sect 228:179–199 (1992).
11. Ryan JJ, Norstrom RJ. Occurrence of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in humans and major exposure routes. In: Environmental Carcinogens: Methods of Analysis and Exposure Measurement. Vol 11: Polychlorinated Dioxins and Dibenzofurans (Rappe C, Buser HR, Dodet B, O'Neill IK, eds). IARC Sci Publ No 108. Lyon:International Agency for Research on Cancer, 1991;5–29.
12. Lilienfeld DE, Gallo MA. 2,4-D, 2,4,5-T and 2,3,7,8-TCDD: an overview. Epidemiol Rev 11:28–58 (1989).
13. Kahn PC, Gochfeld M, Nygren M, Hansson M, Rappe C, Velez H, Ghent-Guenther T, Wilson WP. Dioxins and dibenzofurans in blood and adipose tissue of Agent Orange-exposed Vietnam veterans and matched controls. J Am Med Assoc 259:1661–1667 (1988).
14. Devine OJ, Karon JM, Flanders WD, Needham LL, Patterson DG. Relationships between concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin serum and personal characteristics in U.S. Army Vietnam veterans. Chemosphere 20:681–691 (1990).
15. IARC. Chlorinated dibenzodioxins. In: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol 15: Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals. Lyon:International Agency for Research on Cancer, 1977; 41–102.
16. Tollefson L. Use of epidemiology data to assess the cancer risk of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Regul Toxicol Pharmacol 13:150–169 (1991).
17. IARC. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol 69: Polychlorinated Dibenzo-*para*-dioxins and Polychlorinated Dibenzofurans. Lyon:International Agency for Research on Cancer, 1997.
18. Hardell L, Sändstrom A. Case-control study: soft-tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. Br J Cancer 39:711–717 (1979).
19. Eriksson M, Hardell L, Berg NO, Moller T, Axelson O. Soft-tissue sarcomas and exposure to chemical substances: a case-referent study. Br J Ind Med 38:27–33 (1981).
20. Hardell L, Eriksson M. The association between soft-tissue sarcoma and exposure to phenoxyacetic acids. A new case-referent study. Cancer 62:652–656 (1988).
21. Eriksson M, Hardell L, Adami HO. Exposure to dioxins as a risk factor for soft tissue sarcoma: a population-based case-control study. J Natl Cancer Inst 82:486–490 (1990).
22. Hardell L, Eriksson M, Axelson O, Fredriksson M. Letter to the editor: Dioxin and mortality from cancer. New Engl J Med 324:1810–1811 (1991).

23. Hardell l, Eriksson M, Lenner P, Lundgren E. Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: a case-control study. Br J Cancer 43:169–176 (1981).
24. Smith AH, Pearce NE, Fisher DO, Giles HJ, Teague CA, Howard JK. Soft tissue sarcoma and exposure to phenoxy herbicides and chlorophenols in New Zealand. J Natl Cancer Inst 73:1111–1117 (1984).
25. Smith AH, Pearce NE. Update on soft tissue sarcoma and phenoxy herbicides in New Zealand. Chemosphere 15:1795–1799 (1986).
26. Pearce NE, Sheppard RA, Smith AH, Teague CA. Non-Hodgkin's lymphoma and farming: an expanded case-control study. Intl J Cancer 39:155–161 (1987).
27. Vineis P, Terracini B, Ciccone G, Cignetti A, Colombo E, Donna A, Maffi L, Pisa R, Ricci P, Zanini E et al. Phenoxy herbicides and soft tissue sarcomas in female rice weeders: a population-based case-referent study. Scand J Work Environ Health 13:9–17 (1986).
28. Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R, Fraumeni JF. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. J Am Med Assoc 256:1114–1147 (1986).
29. Woods JS, Polissar L, Severson RK, Heuser LS, Kulander BG. Soft tissue sarcoma and non-Hodgkin's lymphoma in relation to phenoxy herbicide and chlorinated phenol exposure in western Washington. J Natl Cancer Inst 78:899–910 (1987).
30. Woods JS, Polissar L. Non-Hodgkin's lymphoma among phenoxy herbicide-exposed farm workers in western Washington State. Chemosphere 18:401–406 (1989).
31. Cantor KP, Blair A, Everett G, Gibson R, Burmeister LF, Brown LM, Schuman L, Dick FR. Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Res 52:2447–2455 (1992).
32. Smith JG, Christophers AJ. Phenoxy herbicides and chlorophenols: a case-control study on soft tissue sarcoma and malignant lymphoma. Br J Cancer 65:442–448 (1992).
33. Olsson H, Brandt L. Risk of non-Hodgkin's lymphoma among men occupationally exposed to organic solvents. Scand J Work Environ Health 14:246–251 (1988).
34. Fingerhut M, Halperin W, Marlow D, Piacitelli L, Honchar P, Sweeney M, Gerife A, Dill P, Steenland K, Suruda A. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. New Engl J Med 324:212–218 (1991).
35. Becher H, Flesch-Janys D, Kauppinen T, Kogevinas M, Steindorf K, Manz A, Wahrendorf J. Cancer mortality in German male workers exposed to phenoxy herbicides and dioxins. Cancer Causes Control 7:312–321 (1996).
36. Hooiveld M, Heederik D, Bueno de Mesquita HB. Preliminary results of the second follow-up of a Dutch cohort of workers occupationally exposed to phenoxy herbicides, chlorophenols and contaminants. Organohalogen Compounds 30:185–189 (1996).
37. Ott MG, Zober A. Cause specific mortality and cancer incidence among employees exposed to 2,3,7,8-TCDD after a 1953 reactor accident. Occup Environ Med 53:606–612 (1996).
38. Kogevinas M, Becher H, Benn T, Bertazzi PA, Boffetta P, Bueno de Mesquita HB, Coggon D, Colin D, Flesch-Janys D, Fingerhut M et al. Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols and dioxins: an expanded and updated international cohort study. Am J Epidemiol 12:1061–1075 (1997).
39. Kogevinas M, Kauppinen T, Winkelman R, Becher H, Bertazzi PA, Bueno de Mesquita HB, Coggon D, Green L, Johnson E, Littorin M et al. Soft tissue sarcoma and non-Hodgkin's lymphoma in workers exposed to phenoxy herbicides, chlorophenols, and dioxins: two nested case-control studies. Epidemiology 6:396–402 (1995).
40. Linet MS, Brookmeyer R. Use of cancer controls in case-control cancer studies. Am J Epidemiol 125:1–11 (1987).
41. Smith AH, Pearce NE, Callas PW. Case-control studies using other cancers as controls. Int J Epidemiol 17:298–306 (1988).
42. Miettienen OS. The "case-control" study: valid selection of subjects. J Chron Dis 7:543–548 (1985).
43. Pearce N, Checkoway H. Case-control studies using other diseases. Am J Epidemiol 127:851–856 (1988).
44. Stellman SD, Stellman JM. Estimation of exposure to Agent Orange and other defoliants among American troops in Vietnam: a methodological approach. Am J Ind Med 9:305–321 (1986).
45. Verger P, Cordier S, Thuy LT, Bard D, Dai LC, Phiet PH, Gonnord MF, Abenhaim L. Correlation between dioxin levels in adipose tissue and estimated exposure to Agent Orange in South Vietnamese residents. Environ Res 65:226–242 (1994).
46. Schecter A, Dai LC, Thuy LC, Quynh HT, Minh DQ, Cau HD, Phiet PH, Phuong NT, Constable JD, Baughman R et al. Agent Orange and the Vietnamese: the persistence of elevated dioxin levels in human tissues. Am J Public Health 85:516–522 (1995).
47. Geyer H, Scheunert I, Korte F. Bioconcentration potential of organic environmental chemicals in humans. Regul Toxicol Pharmacol 6:313–347 (1986).
48. Byard JL. The toxicological significance of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related compounds in human adipose tissue. J Toxicol Environ Health 22:381–403 (1987).
49. Saracci R, Kogevinas M, Bertazzi PA, Bueno de Mesquita HB, Coggon D, Green LM, Kauppinen T, L'Appé KA, Littorin M, Lynge E et al. Cancer mortality in workers exposed to chlorophenoxy herbicides and chlorophenols. Lancet 338:1027–1032 (1991).
50. Bertazzi PA, Pesatori AC, Consonni D, Tironi A, Landi MT, Zocchetti C. Cancer incidence in a population accidentally exposed to 2,3,7,8-tetrachlorodibenzo-*para*-dioxin. Epidemiology 4:398–406 (1993).
51. Nygren M, Rappe C, Lindström G, Hansson M, Bergqvist PA, Marklund S, Domellöf L, Hardell L, Olsson M. Identification of 2,3,7,8–substituted polychlorinated dioxins and dibenzofurans in environmental and human samples. In: Chlorinated Dioxins and Dibenzofurans in Perspective (Rappe C, Choudhary G, Keith LH, eds). Chelsea, MI:Lewis Publishers, 1986;17–34.
52. Kang HK, Watanabe KK, Breen J, Remmers J, Conomos MG, Stanley J, Flicker M. Dioxins and dibenzofurans in adipose tissue of U.S. Vietnam veterans and controls. Am J Public Health 81:344–349 (1991).
53. Boroush M, Gough M. Can cohort studies detect any human cancer excess that may result from exposure to dioxin? Maybe. Regul Toxicol Pharmacol 20:198–210 (1994).
54. Michalek JE, Pirkle JL, Caudill SP, Tripathi RC, Patterson G, Needham LL. Pharmacokinetics of TCDD in veterans of Operation Ranch Hand: 10-year follow-up. J Toxicol Environ Health 47:209–220 (1995).
55. Erickson JD, Mulinare J, McClain PW. Vietnam veterans' risk for fathering babies with birth defects. J Am Med Assoc 252:903–912 (1984).
56. Erickson JD, Mulinare J, McClain PW, Fitch TG, James LM, McClearn AB, Adams MJ. Vietnam Veterans' Risk for Fathering Babies with Birth Defects. Atlanta:U.S. Department of Health and Human Services, Centers for Disease Control, 1984.
57. Cordier S, Ha MC, Bard D, Le TB, Hoang AH, Hoang TQ, Le CD, Abenhaim L, Nguyen TN. Agent Orange and the risk of gestational trophoblastic disease in Vietnam. Arch Environ Health 51:368–374 (1996).
58. Smith C, Watkins D, eds. The Vietnam Map Book: A Self-Guide to Herbicide Exposure. Berkeley, CA:Winter Soldier Archives, 1981.
59. Cerlesi S, Domenico A, Ratti S. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) persistence in the Seveso (Milan, Italy) soil. Ecotoxicol Environ Saf 18:149–164 (1989).
60. Young AL. Long-term studies on the persistence and movement of TCDD in a natural ecosystem. In: Human and

Environmental Risks of Chlorinated Dioxins and Related Compounds (Tucker RE, Young AL, Gray P, eds). New York:Plenum Press, 1983;173–190.
61. DiDomenico A, Silano V, Vivano G, Zapponi G. Accidental release of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at Seveso, Italy. V: Environmental persistence of TCDD in soil. Ecotoxicol Environ Safety 4:339–345 (1980).
62. Young AL, Thalken CE, Arnold EL, Cupello JM, Cockerham LG. Fate of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) in the Environment: Summary and Decontamination Recommendations. USAFA-TR-76-18. U.S. Air Force Academy, CO: Department of Chemistry and Biological Sciences, 1976.
63. Kearny PC, Woolson EA, Ellington CP. Persistence and metabolism of chlorodioxins in soils. Environ Sci Technol 6:1017–1019 (1972).
64. Mill T. Prediction of the environmental fate of tetrachlorodibenzodioxin. In: Dioxins in the Environment (Kamrin MA, Rodgers PW, eds). Washington:Hemisphere, 1985;173–193.
65. Di Domencio A, Cerlesi, S, Ratti S. A two exponential model to describe the vanishing trend of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the soil at Seveso, northern Italy. Chemosphere 20:1559–1566 (1990).
66. Gross ML, Lay JO, Lyon PA, Lippstreu D, Kangas N, Harless RL, Taylor SE, Dupuy AE. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin levels in adipose tissue of Vietnam veterans. Environ Res 33:261–268 (1984).
67. Centers for Disease Control. Health Status of Vietnam Veterans. Vietnam Experience Study. Vols I–V Supplements A–C. Atlanta, GA:U.S. Department of Health and Human Services, 1989.
68. Huteau B. Identification et dosage de traces d'organohalogénés par des méthodes séparatives et spectrométrie de masse. Application aux matrices biologiques. Thèse de Doctorat de l'Université de Paris XI, Spécialité Chimie Analytique, 1991.
69. Fachetti S. Distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the tissues of persons exposed to the toxic cloud at Seveso. Adv Mass Spectrom 88:1405–1414 (1980).
70. Pirkle JL, Wolfe WH, Patterson DG, Needham LL, Michalek JE, Miner JC, Peterson MR, Phillips DL. Estimates of the half-life of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in Vietnam veterans of Operation Ranch Hand. J Toxicol Environ Health 27:165–171 (1989)
71. Patterson DG, Holler JS, Smith SJ, Liddle JA, Sampson EJ, Needham LL. Human adipose data for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in certain U.S. samples. Chemosphere 15:2055–2060 (1986).
72. Poiger H, Schlatter C, Pharmacokinetics of 2,3,7,8-TCDD in man. Chemosphere 15:1489–1494 (1986).
73. Needham LL, Gerthoux PM, Patterson DG, Brambilla P, Pirkle JL, Tramacere PI, Turner WE, Beretta C, Sampson EJ, Mocarelli P. Half-life of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in serum of Seveso adults: interim report. Organohalogen Compounds 21:81–85 (1994).
74. Flesch-Janys D, Gurn P, Jung D, Konietzko J, Päpke O. First results of an investigation of the elimination of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F) in occupationally exposed persons. Organohalogen Compounds 21:93–99 (1994).
75. Schlatter C. Data on kinetics of PCDDs and PCDFs as a prerequisite for human risk assessment. In: Biological Basis for Risk Assessment of Dioxins and Related Compounds (Gallo MA, Scheuplein RJ, van der Heijden KA, eds). Banbury Rpt 35. Plainview, NY:Cold Spring Harbor Laboratory Press, 1991;215–227.
76. Michalek JE, Tripathi RC. Predicting Checkmark Patterns in the Air Force Health Study. Brooks Air Force Base, TX:Armstrong Laboratory, 1992.
77. Flanders WD, Lin L, Pirkle JL, Caudill SP. Assessing the direction of causality in cross-sectional studies. Am J Epidemiol 135:926–935 (1992).
78. Sielken RL. Statistical evaluations reflecting the skewness in the distribution of TCDD levels in human adipose tissue. Chemosphere 16:2135–2140 (1987).