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PROTOCOL

Validation Study Comparing Military Records-based Estimates of Likelihood of Exposure to Agent Orange with Plasma Levels of 2,3,7,8-TCDD

September 1986

Agent Orange Projects
Division of Chronic Disease Control
Center for Environmental Health
Centers for Disease Control

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SUMMARY

The purpose of the proposed study is to determine if military records can be used to derive valid estimates of possible exposure to Agent Orange. The study is made feasible by the recent development of a reliable laboratory method to measure 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD) levels in plasma (see Appendix A), so that exposure estimates from military records can be compared with plasma TCDD levels, which are, presumably, a more valid marker of previous exposure to Agent Orange.

Because of uncertainties about the ecologic distribution, absorption, and pharmacotoxicology of Agent Orange or TCDD and inherent limitations with available military records, it is not possible to propose a definitive method for using military record information to estimate possible Agent Orange exposure. Based on studies to date and a preliminary evaluation of the troop location and spray data, the initial method we propose to evaluate is based on a "hits" exposure model. Only those occasions on which a veteran was within close proximity to a known Agent Orange application within a few days of the application are counted as potential exposures or "hits". We propose using 2 kilometers and 6 days as the time and distance cut-off criteria for a "hit". For each veteran in the study, we will calculate a hits score by summing all of his "hits" during his tour in Vietnam. The hits score will form the basis of participant selection. A "high" likelihood of exposure group will consist of veterans who have hits scores of 10 or more. A "low" likelihood of exposure group will consist of veterans with hits scores of zero for Agent Orange applications, no more than one "hit" for "unknown" agent applications, and no more than 30 days with missing location information.

In addition to the "hits" method, we also will be able to study some different exposure scoring methods which make different assumptions about possible sources of exposure to TCDD or which may be less dependent on the completeness and accuracy of available military records.

From the potential participants, we expect to obtain plasma samples from 222 veterans in the "high" group and 185 veterans in the "low" group. These sample sizes allow detection of a 71% difference in mean plasma TCDD levels between the two groups with 95% power. The procedures for locating, interviewing, and examining participants will be the same as those used in the Vietnam Experience Study. Plasma samples will be collected at Lovelace Medical Foundation in Albuquerque, New Mexico, the current medical examination contractor. Laboratory assays for plasma TCDD will be performed by the Division of Environmental Health Laboratory Sciences of CDC using High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS). Statistical analyses will focus on measures of association between the military records-based exposure scores and plasma TCDD levels.

The results of this validation study would be used to guide any decision on the advisability of proceeding with a full-scale Agent Orange Study. The validation study is not intended to determine whether identified differences, if any, in TCDD levels are "biologically meaningful." Such a determination would have to rest with a full-scale study, if it appears one is warranted.

I. Objective

The objective of the proposed study is to validate indirect estimates of exposure to Agent Orange based on military records by comparing such measures with plasma 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) levels, presumably a more precise and valid marker of prior exposure to Agent Orange.

II. Background

A. Use of military records to estimate possible Agent Orange exposure

The Agent Orange Study was originally developed to assess the possible health effects of the type of exposures to Agent Orange typical of Vietnam veterans who served in heavily sprayed areas of Vietnam. The ideal epidemiologic study of the influence of exposure to Agent Orange on the health of Vietnam veterans would be based on a valid and precise estimate of exposure of each veteran to Agent Orange. It was recognized when the Agent Orange Study was first designed that military records do not permit determination of individual exposure to Agent Orange or TCDD in any quantitative sense. At best, available data permit only a probabilistic assessment of opportunity for exposure. Reasons for the inability to calculate an accurate individual exposure to Agent Orange include: 1) limited knowledge about the dispersion, biodegradation, and ecologic disposition of Agent Orange in Vietnam; 2) uncertainties about the absorption of Agent Orange or TCDD in humans, including

the influence of different routes of exposure; and 3) inherent limitations in available data which prevent the determination of the precise location of individual soldiers in relation to Agent Orange applications. Because of these uncertainties, the November 1983 protocol for the Agent Orange Study indicated that further pilot testing would be needed before a decision could be made to proceed with the full scale study. Working with the U.S. Army and Joint Services Environmental Support Group (ESG), CDC prepared and submitted to the Office of Technology Assessment (OTA) two reports on exposure assessment in February and November 1985. These reports did not provide enough evidence that military records could be used to locate individual men with enough precision to allow an acceptable estimate of possible exposure to Agent Orange applications. In addition, some reviewers were concerned that too few men were identified who had "meaningfully high" exposure opportunity scores to warrant proceeding with a full scale study.

As a result of the identified problems, it was decided not to proceed with the full-scale Agent Orange Study at that time. The Agent Orange Working Group (AOWG) convened a subpanel of its science panel to review the pertinent information and records related to exposure assessment. As part of this assessment, ESG was asked to perform an independent pilot study of selected battalions with presumed high exposure to Agent Orange. After reviewing the pertinent information and the ESG pilot study results, the AOWG Science Panel recommended that "...the potential for misclassification of exposure status of ground troops... will

preclude scientifically valid results from any epidemiological study based on military records alone" (see Appendix B).

Two issues were specifically confirmed by the pilot study as influencing the degree of misclassification:

- Unit dispersion - On a substantial number of days, personnel in combat units eligible for the study were not located together as a unit; rather they were dispersed geographically up to 20 kilometers that same day.

- Incomplete spray records - Expert opinion suggested that an unknown but apparently large proportion of fire-base perimeter spray operations were never recorded and the degree to which these unrecorded operations may have influenced exposure is unknown. The record of aerial sprays, i.e., the Ranch Hand missions on the so-called HERBS Tapes, are thought to be complete.

This protocol has been developed in response to the above concerns. In the proposed study, plasma levels of TCDD would be used to verify indirect estimates of possible exposure to Agent Orange based on military record information.

B. Tissue dioxin levels as a marker of Agent Orange exposure

Prior to recent studies, estimation of TCDD half-lives in humans had been based on extrapolation from animal models which suggested

that the half-life in humans should be about one year. Such a short half-life would make determinations of current TCDD levels of little use in estimating exposure to Agent Orange 15 to 20 years ago during the Vietnam conflict. Recent studies, however, indicate that the half-life in humans may be much longer--on the order of five years or more (1-3). If the half-life of TCDD in human adipose tissue is five years or more, it should be possible to detect appreciable elevations in TCDD levels in Vietnam veterans 15 to 20 years after exposure (i.e., after 3 or 4 half-lives). At least two studies suggest that this may be the case.

A study in 1984 found a correlation between degree of exposure to Agent Orange in Vietnam and tissue levels of TCDD (4). Adipose tissue samples were obtained from 23 self-selected Vietnam veterans who believed they had been exposed to Agent Orange. All samples were taken eight or more years after presumed exposure. Adipose samples were also obtained during elective surgery from ten veterans who had not been in Vietnam. Classification of likelihood of exposure to Agent Orange was initially done by the Veterans Administration (VA) based on the veterans' reported information. An additional classification was performed by the Army Agent Orange Task Force (AAOTF) based on the veterans' military records. Two of the three veterans classified as "heavily exposed" by the VA had the highest TCDD levels--99 and 35 parts per trillion (ppt). The third "heavily exposed" veteran did not have TCDD detected, but the results of this particular veteran's assay are questionable because only 20% of the internal standard was recovered. An assay of

another sample of the same veteran's adipose tissue performed by another laboratory detected TCDD at a level of 20 ppt. These three veterans classified as "heavily exposed" by the VA were also in the highest exposure category as determined by the AAOTF. The less heavily exposed veterans by either classification did not differ appreciably in TCDD levels from unexposed controls; mean TCDD levels in these groups were 5-6 ppt.

Another study found that 14 years after the last known application of Agent Orange, levels of TCDD in adipose tissue of Vietnamese living in the South of Vietnam were, on average, higher than in persons living in the North (5). Adipose tissue specimens were collected in 1984 in hospitals in Hanoi and Ho Chi Minh City from patients undergoing surgery or from autopsy cases. The authors stated that most of the specimens from the South came from persons who lived in outlying provinces away from Ho Chi Minh City. Twelve of fifteen specimens obtained from the South had detectable TCDD levels with an average concentration of 28 ppt (range: 3-103 ppt). Samples in the North were taken from people who had never been in the southern part of Vietnam and had no known exposure to Agent Orange. None of the nine samples from the North had detectable levels of TCDD (detection limit 2-3 ppt). The authors concluded that at least part of the TCDD in the south Vietnamese samples was due to Agent Orange exposure.

The above studies suggest that TCDD persists in human tissues for several years and that some Vietnam veterans may have been exposed

to high enough levels of TCDD to have detectably elevated levels 15 to 20 years after exposure. These results raise the possibility of using current tissue levels of TCDD in Vietnam veterans as an objective marker of previous Agent Orange exposure.

To date, as was the case in the above studies, measurement of tissue levels of TCDD has relied on levels in adipose tissue. To obtain the adipose sample requires a surgical procedure, which has made the assay impractical to use in any large-scale study. During the past year, the Division of Environmental Health Laboratory Sciences (DEHLS) of the Center for Environmental Health (CEH), CDC, has developed a reliable method to measure TCDD levels in plasma instead of fat (see Appendix A). The ability to make TCDD measurements on plasma makes a validation study of indirect estimates of possible Agent Orange exposure feasible.

While TCDD assays hold promise of providing an objective assessment of Agent Orange exposure, current TCDD levels will bear an imperfect correlation with exposure to Agent Orange because of interpersonal variability in both the metabolic half-life of dioxin and post-service exposure to dioxin-containing compounds. Since these characteristics are unlikely to be associated with the opportunity for exposure to Agent Orange in Vietnam, they should not prevent the use of TCDD levels as a validation of approaches for assessing exposure opportunity based on previous proximity of veterans to Agent Orange applications. Furthermore, extensive information on post-service exposures to dioxin-containing

compounds would be obtained from study participants and permit controlling for these exposures in the analyses.

We stress that the proposed study will measure TCDD levels as a marker of prior exposure to Agent Orange. The study is not intended to detect "biologically meaningful" differences in plasma TCDD levels because:

1. Levels of TCDD in man at which adverse health effects become manifest are not known.

2. Even if "toxic levels" of TCDD were known, the tissue half-life of TCDD in man is not precisely known, so that it would be difficult to extrapolate current levels in veterans back 20 years to the time when exposure may have occurred.

3. The component of Agent Orange that may have been responsible for adverse health effects is not known. Although attention has focused on the TCDD contaminant, the two active ingredients, 2,4-D and 2,4,5-T, also may cause adverse health effects (6-7). In theory, different components of Agent Orange may have been responsible for different manifestations (8).

III. Comparability of Plasma and Adipose Levels of TCDD

The DEHLS laboratory recently completed an evaluation of its methodology for measuring TCDD levels in plasma. A detailed description of the

study is provided in Appendix A. In brief, 50 paired specimens of adipose tissue and plasma were obtained from Missouri residents who participated in a previous study of dioxin and health effects. The participants included persons with known residential, occupational or recreational exposure to dioxin as well as persons with no known exposure to dioxin. About 20 grams of adipose tissue and 15 to 200 ml of plasma were obtained from each participant. The TCDD levels in both types of samples were measured using High Resolution Gas Chromatography/High Resolution Mass Spectrometry.

The study found a correlation of 0.97 and 0.98 between adipose tissue and serum 2,3,7,8-TCDD levels, on a whole weight and lipid weight basis, respectively. The high correlation indicates that serum levels provide a valid measure of 2,3,7,8-TCDD body burden concentration.

For the same reason, wherever it is feasible, as in the validation study proposed here, a sufficient volume of blood must be obtained to analyze each veteran separately.

IV. Indirect Methods for Estimating Exposure Opportunity

This section will review relevant background information on exposure assessment, discuss several alternative approaches to estimating a veteran's opportunity of exposure, and outline methods for the proposed validation study.

A. Background

1. Ecologic and Toxicologic Data

Disappearance of TCDD from the environment depends on several processes including photodegradation, volatilization, and transport. Many factors, including the amount of sunlight and other climatic conditions, area of the environment in which the dioxin is deposited, and the vehicle in which the dioxin is applied influence the rate of disappearance of TCDD. Table I shows that published half-lives of TCDD in the environment may be divided into three groups or compartments: a "fast decay compartment" with a half-life of a few hours (e.g., TCDD found on foliage), an "intermediate decay compartment" with a half-life of several days (e.g., TCDD found near the ground or on the soil surface), and a "slow decay compartment" with a half-life of several years (e.g., TCDD adsorbed to the soil) (9-14). While the relevance of the half-life estimates to the conditions in Vietnam can be questioned, the data do support the existence of at least three environmental compartments with half-lives differing by one or more orders of magnitude.

Dispersion of the herbicide following aerial spraying has been evaluated by flying test missions over a sampling grid under controlled conditions (15-16). While many experimental conditions such as the spray system and air craft were similar to those used in Operation Ranch Hand, other experimental

Table I

Estimated TCDD Half-Lives, by Deposition in Environment

Half-Life	Deposition	Report
Fast Compartment		
2 Hours	leaves, foliage	Crosby and Wong (1977)
Intermediate Compartment		
2 days	soil surface	Crosby and Wong (1977)
4-7 days	grass (in silvex)	Nash and Beal (1980)
6 days	grass (in herbicide)	Jensen (1983)
Slow Compartment		
0.5 yrs	soil, initially	DiDomenico (1980)
10 yrs	soil	Wipf and Schmid (1983)
5 yrs	pond, predicted	EPA (1985)

conditions differed from those in Vietnam so that results can only be used as rough estimates of herbicide dispersion. Nevertheless, these data indicate a rapid decrease in spray concentration with increasing distance from the spray path. Based on an exponential decay, at a distance of one-half kilometer from the flight path, the amount deposited would be about 2% of that found near the center of the flight path, and at one kilometer from the flight path the amount would be 0.03 percent. We know of no similar, quantitative studies of the dispersion of Agent Orange when applied from jeep-mounted tanks, back pack sprayers, or jerry-rigged helicopter devices such as were used in perimeter sprayings.

Experimental data document TCDD absorption from the gastrointestinal tract and percutaneously in animals (14). TCDD appears to be absorbed even if administered with soil, although the percentage absorbed may be lower. No information is available concerning absorption through the respiratory tract. The half-life of TCDD in humans is not well established although recent evidence suggests that it may be on the order of 5 to 8 years rather than approximately 1 year as believed previously (see Section II.B.).

2. Data on Study Subjects and Spray Applications

For each subject the primary information available with which to assess exposure opportunity are daily troop location records and computerized data files of dates and locations of herbicide applications. Briefly, location data for entire battalions and individual companies or parts of companies are recorded in various battalion and brigade level records, generally to the nearest tenth of a kilometer. Companies are the smallest troop unit for which records exist to allow placing locations of individual men on particular days.

Spray application information is available on Operation Ranch Hand missions in the "Herbs" tape, compiled by the Military Assistance Command in 1971, and on perimeter sprayings and miscellaneous applications in the "Services Herbs" tape, compiled and updated by ESG from extant records from chemical

units and certain other units operating in Vietnam. These computer tapes contain data on spray location, herbicide type, and, on the Herbs tape, number of airplanes involved in the mission and gallons of herbicide applied.

The completeness of the Herbs tape has been evaluated by the National Academy of Sciences and by Australian scientists conducting the recently published study of the health of Australian veterans who served in Vietnam (17). These studies conclude that, although not definitive, the data appear to be consistent with the information available for validation. Evaluation of data quality and completeness of the Services Herbs tape has not been published. However, the AOWG Subpanel felt that considerable undocumented spraying is likely (see Appendix B).

3. ESG Pilot Study Findings

The ESG pilot study results provide helpful guidance on the factors and uncertainties that need to be considered when using available military records to estimate possible exposure to Agent Orange. The pilot study found that although the majority of Agent Orange used in Vietnam was delivered via "Ranch Hand" fixed-wing aircraft, most documentable exposure of troops is from helicopter and ground spraying, including perimeter sprays of fire bases and similar encampments. In addition, the ESG pilot study showed that, at least in the seven pilot study

battalions, there was substantially more potential exposure from helicopter and ground spraying with unknown agents (type not specified in the military records) than from documented Agent Orange sprayings.

B. "Hits" Method for Estimating Exposure Opportunity

Because of the uncertainties about the ecologic distribution and absorption of Agent Orange or TCDD and the inherent limitations of available records, it is not possible to propose a clearly superior method for estimating possible exposure to Agent Orange. The initial method we plan to evaluate as an estimator of exposure, and the method which will form the basis of participant selection, will focus on exposures which may have occurred within a few days of a documented Agent Orange application. This method will assume that available troop location and herbicide application records are sufficiently complete and accurate to allow reliable estimation of individual veterans' proximity to Agent Orange applications. This method will make the additional assumption that the predominant exposure to Agent Orange or TCDD was from Agent Orange which was on foliage or near the ground (i.e., potential exposures to TCDD adsorbed in soil will be ignored).

The relative likelihoods of exposure for individual veterans will be estimated using a "hits" model in which a "hit" will be defined as any occasion on which a veteran's company had a recorded location within two kilometers of an area that had been sprayed

with Agent Orange in the previous six days. For each veteran, a hits score will be computed by summing all of his "hits" during his tour in Vietnam. The two kilometer distance criterion is based primarily on troop movement and dispersion of individuals within a company. The amount of TCDD at distances greater than a kilometer from a spray path is probably quite small (15-16). Nevertheless, troops in a company may have been spread out over several kilometers around recorded locations so that a man whose company location according to written records was several kilometers from a spray path still had some likelihood of being in or moving into the sprayed area. If more than one location is reported for a company on a particular day, the distance will be taken from the location point nearest to the spray line. The six day cut-off criterion is based on the half-life of TCDD on leaves and near the soil surface.

The "hits" method has practical advantages. Because scores would be easy to calculate, determination of exposure opportunity category could be done early in the subject selection process in a full-scale Agent Orange Study (if one were to be done) and would tend to minimize record abstraction for subjects who would ultimately not be included in the study (i.e., because they fall into an intermediate exposure opportunity category). Furthermore, the scores obtained by the "hits" model are highly correlated ($r > .90$) with scores obtained from more complex models which include decay factors for both the time and distance parameters.

C. Alternative Methods for Estimating Exposure Opportunity

As stated above, the "hits" model will form the basis of participant selection and will be the initial method we will evaluate via blood TCDD correlation. This selection scheme, however, should not preclude us from being able to study other scoring methods which reflect different assumptions about the relative importance of the various environmental compartments for dioxin exposure as well as the completeness and accuracy of available records. The other methods we will study will include:

E3 Score

Area Score

"Unknown" agents

1. E3 Score based on "slow decay"

One of the alternative approaches we will evaluate for estimating possible exposure will reflect the possibility that dioxin which may have been adsorbed to the soil also could have been a significant source of exposure. This method attempts to address the concern of some veterans that there is a risk to health from being in a defoliated area <u>any time</u> after the spraying of Agent Orange. The location and spray data currently available indicate that a substantial number of veterans were frequently in close proximity to areas where Agent Orange had been applied at some time in the past. The

recently published findings of abnormalities in certain liver function and immunologic indices among residents of a Missouri trailer park, where dioxin contaminated oil had been sprayed in 1971 for dust control, suggest that long term exposure to dioxin in soil may be important (18).

Dioxin adsorbed in soil has a half-life of several years and thus this method would count as a potential exposure any occasion on which a veteran's company was in close proximity to a known Agent Orange application, even after several years from the date of the application. The score for the "slow decay compartment" (E3 score) will be calculated assuming an exponential decrease with a half-life of five years and will be calculated according to the following formula:

$$E3 = \sum_i [D_i \exp(-.139 \times t_i)]$$

where $D_i = 1$ if the distance from the spray application is less than 2 kilometers

0 otherwise

t_i is the time since spray application in years

Summation is over each day and each spraying

The term -.139 reflects a half-life of five years

Like the hits score, the E3 score is dependent upon the completeness and accuracy of troop location and herbicide application records.

2. Area Score

Another alternative score which we will investigate, the "area score," is less dependent on completeness or accuracy of extant records of troop locations or spray applications. The background and rationale for this method is provided in Appendix C. Briefly, the score is calculated as follows:

First, five large regions or zones are defined in which spray applications were relatively common during the war and other zones in which the potential for exposure was relatively less likely because of less frequent or better controlled spray applications (such as base camps). Four of the "high" zones correspond to previously defined regions: War Zone C, War Zone D, Rung Sat Special Zone, and the Iron Triangle (see Figure 1). These four zones include approximately 75% of known applications in III Corps from October 1966 to December 1968. A fifth zone (east of Rung Sat) has been tentatively outlined based on mappings of spray applications so that the great majority of spray applications are included.

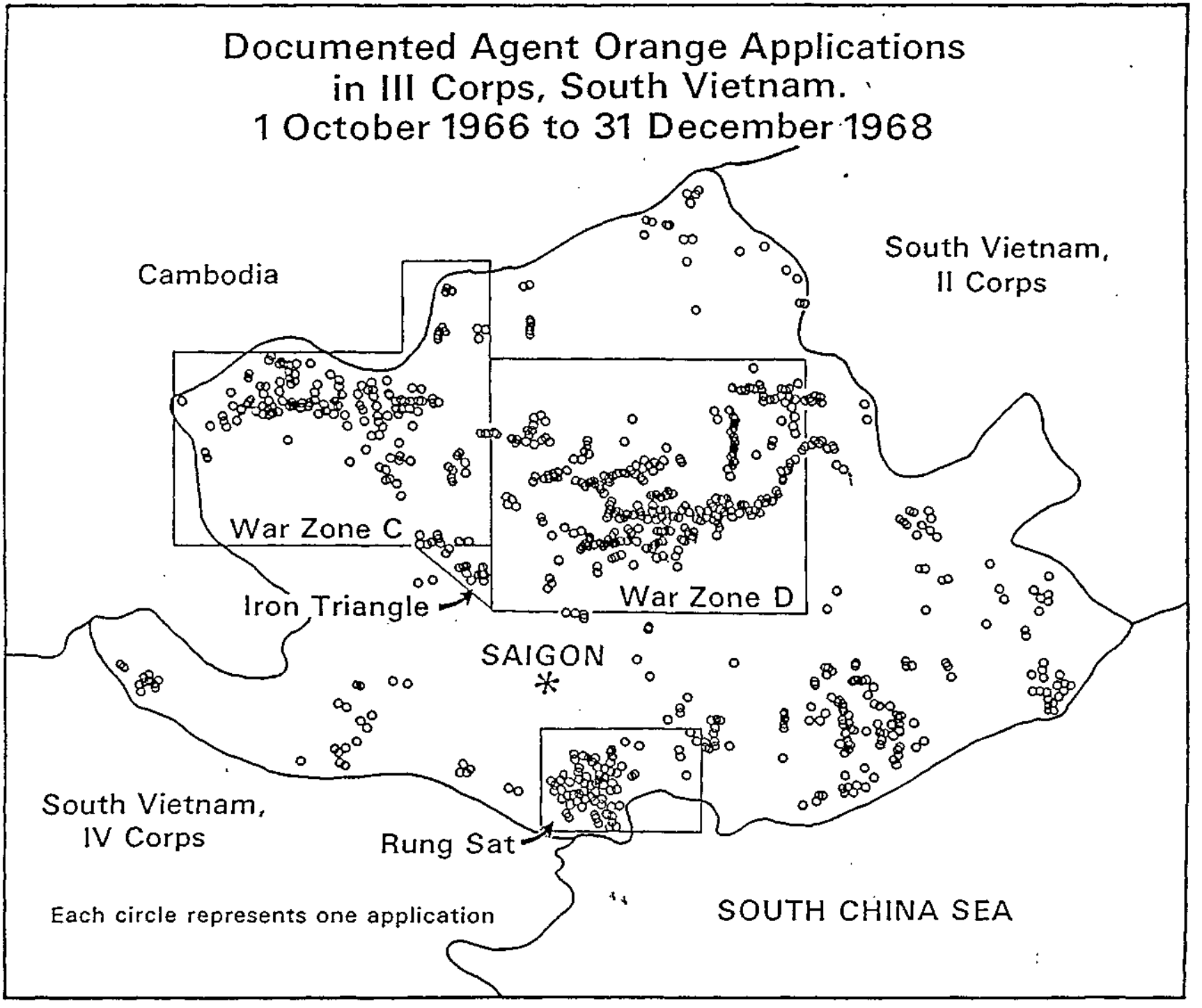
The next step is to determine the days each battalion was in one area or the other (a "high" or a "low" spray area). This information requires less detailed knowledge than does location of companies within a half kilometer or so (as is necessary for the hits and E3 scores). Each veteran would be assigned a score proportional to the number of days his battalion(s) spent in one of the "high" areas.

While the proposed score is admittedly arbitrary, it does allow for separation of areas with heavy spraying from those with lighter spraying. Furthermore, if spraying such as perimeter spraying was undocumented and if such spraying occurred more frequently in areas where known spraying occurred (see Appendix C), then the score would reflect that undocumented exposure. A major drawback, though, is that the score does not reflect the information which is available such as time (or even year) of spraying and therefore incompletely uses available data.

3. "Unknown" agents

For a large number of herbicide spray applications documented in available records (particularly the Services Herbs tape) the type of herbicide applied is listed as "unknown." Both the encounters score and E3 score as described above would be based on documented Agent Orange spraying and would exclude "unknown sprays" from possible exposure estimates. Since an unknown proportion of "unknown" sprays were undoubtedly of Agent Orange, we will perform additional analyses in which hits and E3 scores based on "unknown" sprays will be calculated and compared with TCDD levels.

FIGURE 1


Documented Agent Orange Applications
in III Corps, South Vietnam.
1 October 1966 to 31 December 1968
Cambodia
South Vietnam,
II Corps
War Zone C
War Zone D
Iron Triangle
SAIGON
South Vietnam,
IV Corps
Rung Sat
Each circle represents one application
SOUTH CHINA SEA

D. Comparison with TCDD levels of non-Vietnam Veterans

Each of the proposed methods for assessing exposure opportunity will compare TCDD levels among Vietnam veterans who have different levels of possible exposure opportunity. It is possible that Vietnam veterans were exposed to substantial levels of Agent Orange but that none of our methods of indirect assessment are adequate to distinguish clearly between exposed and unexposed veterans. This would result in our finding no association between any of the exposure scores and TCDD levels, but we would not be able to determine whether this reflected extreme misclassification or rather that few Vietnam veterans had been exposed to sufficient TCDD to still have distinguishably elevated residual levels nearly twenty years later. To address this question, in the event that we should find no association between any of the indirect exposure assessment methods and plasma TCDD levels, we plan to compare the validation study TCDD levels against several serum pools from non-Vietnam veterans who participated in the Vietnam Experience Study. Pooling sera from several non-Vietnam veterans will be necessary because 50 to 75 ml of serum are currently required to perform the TCDD assay, and we have only a few ml's of frozen serum available from each of the participants in the Vietnam Experience Study. The serum pools from the non-Vietnam veterans should give us a good idea of what the level of serum dioxin should be in comparable Vietnam-era veterans who almost certainly were not exposed to Agent Orange while in the Army. If the levels in the Vietnam veterans who participate in the validation study are

similar to the mean levels found in the non-Vietnam veteran pools, it would suggest that few if any Vietnam veterans were exposed to sufficient Agent Orange to have detectably elevated levels of TCDD after almost twenty years. If, on the other hand, the levels in the Vietnam veterans are higher than the levels found in the pooled serum of non-Vietnam veterans, it would suggest that sufficient exposure to TCDD, through Agent Orange, did occur among some veterans in Vietnam, but military and spray records are inadequate for distinguishing the exposed and unexposed veterans.

Participant Selection

A. Source of Potential Participants

The participants for the validation study will be selected from veterans for whom we currently have some tracking information available. As part of the initial work in preparing for the Agent Orange Study, ESG identified 65 combat battalions which served 18 months or longer in III Corps during 1967 and 1968. The 65 battalions were selected from 122 battalions which spent any time in III Corps during the study period. For individual companies in the 65 battalions, ESG abstracted recorded daily locations from military records. On average, however, company-level locations were not recorded for 50% of the days. As part of its pilot study, ESG filled in most of the missing locations for all companies in the seven battalions between October 1, 1966, through March 31, 1969 (see Appendix B). The updated tracking information on these

companies has been computerized and a tape containing the information has been provided to CDC.

As of December 1985, CDC had received names and personnel file abstracts on about 10,000 veterans who had location information available for at least some of the days during their tour in Vietnam. Participants for the validation study will be chosen from these 10,000 veterans, including veterans who served in the seven ESG pilot study battalions.

B. Selection of Study Groups Based on Hits Scores

The goal of the study will be to compare TCDD levels in two groups of veterans: one with presumed low opportunity for exposure to Agent Orange and one with presumed high opportunity for exposure. The two groups will be selected based on hits scores as follows:

"High" group

We will select those veterans with 10 or more "hits". Table 2 shows the distribution of hits scores among available veterans and indicates that a score of 10 or more "hits" will yield 461 potential participants for the "high" group. These scores are based on incomplete location information and thus represent minimun hits scores (mean number of days with missing location = 57). Most (85%) of the veterans in the "high" group served in the seven ESG pilot study battalions.

"Low" group

This group will be made up of 385 veterans with zero "hits" for known Agent Orange applications, no more than one "hit" for "unknown" agent applications, and no more than 30 days with missing location information.

Our rationale for selecting participants for the validation study from the extreme ends of the "hits" score distribution is that we wish to maximize the possibility of detecting a difference in TCDD levels. From a comparison of 2 groups with "hits" scores as maximally different as we can reasonably select, a finding of no association between exposure opportunity scores and TCDD levels would be most convincing that a study based on military records is not feasible. We are attempting to detect differences in TCDD levels several half-lives after exposure has occurred. If we were to select participants on less extreme differences in exposure scores, or a random sample of exposure scores, a finding of no association could always be questioned as being due to not having evaluated enough veterans with high scores or to a small separation of exposures. By evaluating the two extremes, a finding of no association would provide the most convincing evidence that military record information is not adequate for distinguishing groups of veterans with different exposures to Agent Orange during the Vietnam era.

Table 2 Distribution of "Hits"

	Number of "Hits"*									
	0	1–3	4–6	7–9	10–14	15–19	20–29	30–39	40–49	≥50
Number of Veterans	7944	1423	392	99	177	9	150	2	82	41

* Any occasion on which a veteran's company was within 2 kilometers of a documented Agent Orange application within 6 days of the application.

We realize we may be sacrificing some generalizability of the validation study findings to the entire population of potentially exposed veterans and that this could make designing a full-scale Agent Orange Study difficult (particularly finding enough veterans for the "high" group). Also, since we are limited to selecting men for whom we currently have some tracking information, the issue of generalizability would still be a concern no matter what criteria are used to select individual veterans for this study. Nonetheless, we feel we will be able to use the validation study results to guide the design of a full-scale Agent Orange Study, if it appears that one is warranted. For example, a strong association between hits scores and TCDD levels, might suggest that we could relax the criteria for the "high" group in a full-scale study so that a larger number of veterans could be included. Also, we might be able to adjust the sampling ratios of the two groups to less than one-to-one, without an appreciable loss in study power. Even then, it would be necessary for ESG to determine if enough men could be found from the military records to carry out a full-scale Agent Orange Study.

VI. DATA ANALYSIS

In this section we describe the major techniques that will be used to analyze the data. Separate analyses are described for assessing each of the three different exposure opportunity scoring methods' association, if any, with plasma TCDD levels.

A. Association of hits scores with serum TCDD levels

Analyses will reflect the study design in which two groups of men are selected, one group with "low" likelihood of exposure and the second group with "high" likelihood of exposure. The major measures that will be used to assess the association between TCDD levels and hits scores are summarized in Table 3. First, the distribution of TCDD levels in the "high" exposure group will be summarized and compared with that in the "low" exposure group. Techniques will include bivariate plotting and calculation of geometric means for the "high" and for the "low" exposure groups. The difference between the mean TCDD level of the "high" group and that of the "low" group will be tested for statistical significance using t-tests.

The proportion of participants with high TCDD levels also will be compared. The ninetieth or the ninety-fifth percentile for TCDD levels in the "low" group will be determined and compared with the proportion of men in the "high" group whose TCDD level is higher than that level. The statistical significance of the difference in these proportions will be tested using standard Chi-square tests.

Further analyses will reflect the interval structure of the hits score. Standard least squares linear regression techniques will be applied to estimate and to test the association between hits scores and TCDD levels. A variable indicating membership in the high group will be included in the model, if necessary (e.g., a simple

TABLE 3

MEASURES FOR ASSESSING THE ASSOCIATION BETWEEN "HITS" SCORES AND TCDD

MEASURE	COMMENT
geometric mean TCDD, "high" group	"average" TCDD in high group
geometric mean TCDD, "low" group	"average" TCDD in low group
difference in above means	"average" TCDD difference between groups
regression coefficient, "b"	estimates increase in TCDD per unit increase in "hits" score
% with elevated TCDD by group	used to compare proportions of abnormals

model to be evaluated: $Y = a + b_1 I + b_2 S*I + E$, where a, b_1, and b_2 are the regression parameters to be estimated, Y is the serum TCDD level or an appropriate transform thereof, S is the "hits" score, I is 1 if the "hits" score is 8 or more and 0 otherwise, and E is the error). In additional analyses we will use linear regression to adjust for potentially confounding covariates, such as potential post-service herbicide exposures.

For all statistical analyses, appropriate transformations of dependent and/or independent variables will be used as necessary (data from the Missouri study suggest that the logarithms of serum TCDD levels have an approximate normal distribution).

B. Association of E3 scores or area scores with TCDD levels

Assessment of the association of E3 scores or area scores with TCDD levels will include bivariate plots and linear regression.

Additional analyses will address possible confounding by covariates, such as post-service occupation, using linear regression.

Although the study groups will be selected based on hits scores, the distributions of E3 scores and area scores in the two study groups reasonably reflect the range of E3 and area scores in the entire available population (see Appendix D). Therefore, we can still study the association between E3 or area scores and TCDD levels using linear regression techniques. In any event, the strength of the association will tend to be underestimated because the location information is incomplete, resulting in underestimation of actual scores.

If more than one type of score is found to be associated with TCDD levels, then additional analyses based on linear regression using all scores as independent variables simultaneously will be done to determine which score or combination of scores is most closely associated with TCDD level. (The correlation between the hits score and E3, the hits score and the area score, and E3 and the area score are .45, .15, and .35, respectively so that multicollinearity should not be a problem in such analyses).

C. Analyses of "Unknown" sprays

Finally, we will assess the association of TCDD levels with hits scores and E3 scores based on sprays for which the type of

herbicide was unknown. The analyses will use linear regression in a similar manner as described above.

D. Comparison of TCDD levels of Vietnam Veterans with those of non-Vietnam veterans

As indicated in section V.D., if none of the indirect measures of exposure opportunity are associated with plasma TCDD levels, we plan to perform additional comparisons using serum collected in the Vietnam Experience Study (VES). In these analyses, the TCDD levels of the veterans in the validation study (all of whom would be Vietnam veterans) will be compared with those of non-Vietnam veterans from the VES. The TCDD level in each of twenty non-Vietnam pooled samples will be determined, each sample consisting of pooled serum from several non-Vietnam veterans who were examined in the VES study. For these analyses, the mean TCDD level of the twenty pooled samples from non-Vietnam veterans will be calculated and compared with the mean TCDD level of the validation study Vietnam veterans. It is anticipated that the distribution of TCDD levels in the non-Vietnam veteran serum pools will differ from the distribution of individual values in the Vietnam group. Those differences will be investigated and statistical analyses will be modified appropriately to reflect differences in distribution, including differences in variances.

VII. Sample Size, Power and Participation Rates

A critical decision in determining the study's power, given the available sample size, is the difference in TCDD levels to be detected. It is difficult to propose a minimum difference the study should be able to detect considering that we are attempting to detect differences nearly 20 years after potential exposure, during which time most of the TCDD from exposures in Vietnam will have been eliminated from tissue stores. In this case, it may be preferrable to indicate the minimium difference which can be detected for a given level of power. With the available sample size, we expect to have 95% power to detect a 71% difference between the geometric mean TCDD concentrations of the groups.

The minimum difference to be detected was calculated according to the formula:

$$\Delta = (Z_\alpha - Z_\beta)\ (V^{1/2})\ (1/n_1 + 1/n_2)^{1/2}, \text{ where}$$

logarithm of TCDD is normally distributed

Δ = minimum detectable difference in means, log scale

n_1 = Number of samples in "low" group = 185

n_2 = Number of samples in "high" group = 222

V = Variance (log scale) = 2.7

Alpha-error (1-sided) = .05 (Z_α = 1.645)

Beta-error = .05 (Z_β = -1.645)

The estimated variance is the sample variance of the logarithm of serum TCDD levels of Missouri residents who participated in the laboratory study of serum/adipose tissue correlations described in Appendix A.

The expected number of plasma specimens in each group is based upon the following expected losses during the process from initial selection to actually obtaining the serum sample: We expect that of the originally selected participants, 5% will have died. Based on the results of the VES and an anticipated 4-month interval for tracing and interviewing, we expect that RTI will be able to interview 78% of the remaining potential participants. Of those interviewed we anticipate that 65% will agree to undergo the medical examination and provide the blood sample for TCDD measurement. (Since the interview contractor and examination contractor each are expected to have only 4 months for their respective activities, the expected participation rates have been adjusted downward from levels achieved in the VES due to the reduced time window.) Applying the preceeding percentages to the 461 potential "high" group participants and 385 potential "low" group participants, results in an expected number of 222 and 185 plasma samples in each group, respectively.

Although we expect to obtain specimens on only about half of the potential participants, we do not think this relatively low participation rate will result in substantial selection bias. The two measures of primary interest in this study, exposure scores and plasma TCDD levels, will not be known to the potential participants. It is difficult to conceive that either measure could have a substantial influence on participation. Differential participation according to exposure scores would not bias the results unless it was also accompanied by differential participation according to plasma TCDD levels. Theoretically, differential participation according to TCDD

levels alone could bias the findings and would tend to obscure the association. It is possible that such a situation could arise if elevated TCDD levels are associated with poor health and an inability to participate. We will have vital status information on all potential participants, detailed medical histories on all participants in the telephone interview, and reason for not participating on all located non-participants so that we will be able to evaluate whether ill health (including death) materially affected participation rates.

VIII. Study Procedures

The pool of potential study participants which we currently have available at CDC was selected according to the following process. From the companies which had been tracked as of December 1985, the U.S. Army Reserve Components Personnel and Administration Center (RCPAC) reviewed company morning reports for the individual companies to list the individuals who served in these companies during the study period, October 1, 1966, through March 31, 1969. The lists of names were sent to ESG where the data were processed and edited to eliminate duplicate names, match military service numbers and social security numbers, and identify the accession number used in filing each individual's personnel file (201 File) at the National Personnel Records Center (NPRC) in St. Louis. These data were forwarded to NPRC who pulled the personnel files and sent the files through RCPAC to ESG for review in qualification of veterans (according to the basic eligibility criteria specified in the original protocol for the Agent Orange Study in November 1983). ESG completed a data abstraction form on each qualified individual and transmitted these forms to CDC.

For the proposed validation study, the procedures for tracing, contacting, interviewing, and examining participants will be similar to procedures used in the Vietnam Experience Study (VES). For the approximately 850 potential study participants, CDC will prepare data tapes containing names, social security numbers, addresses and other locating information. These tapes will be submitted to the Internal Revenue Service (IRS), the Social Security Administration (SSA), the Veterans Administration (VA), and the National Center for Health Statistics (NCHS), to obtain more recent locating information (e.g., addresses) and to identify veterans who have died since discharge from the Army. At the same time these various agencies are searching their data files, ESG will carry out quality control activities to verify that the men in the "high" group were actually with their companies on the days when a "hit" occurred by reviewing morning reports and other records. Once the locating and mortality data are obtained from the various agencies, CDC will prepare and forward data tapes containing names, addresses, and other locating information to Research Triangle Institute (RTI), the contractor for telephone interviews. RTI will be responsible for tracing and contacting potential study participants. Briefly, the process consists of telephone tracing (using directory assistance), credit bureau searches, drivers license records, and various local sources such as town directories, and contacts with relatives and former neighbors and employers. We plan to administer the same questionnaire as was used in the VES to all validation study participants.

All participants in the telephone interview will be invited to take part in a comprehensive medical and psychological evaluation at Lovelace Medical Foundation (LMF) in Albuquerque, New Mexico, the current examination contractor. As part of the medical examinations, the necessary 150 ml of plasma for TCDD measurements will be collected. The medical evaluation will be similar to the evaluation conducted in the VES except for the collection of additional blood for TCDD measurement and the addition to the medical history of more detailed questions regarding potential civilian exposures to dioxin as well as questions of the veteran's self-assessed exposure to herbicides while in Vietnam.

We feel strongly, in terms of fairness and quality of service provided to the participants, that the veterans who participate in the validation study deserve the same complete health evaluation as has been provided to veterans who have participated in the VES and which may be provided to future participants in a full-scale Agent Orange Study. In addition, using the same procedures and contractors in the validation study as have been used in the VES has advantages. The procedures are well established, well tested and have proved themselves successful. We know from the VES experience what the anticipated participation rates would be, which is important for planning purposes. All of the mechanisms for performing the study are already in place so that the validation study could be implemented quickly upon approval. The blood collection would be done in a controlled and safe clinical environment. The specimen collection would be performed by a staff which has developed considerable experience and expertise in

conducting research studies, resulting in high levels of standardization and quality control in the collection, processing, and shipping of specimens. Furthermore, by performing the same health evaluation as would be performed in a full-scale Agent Orange Study, it may be possible to use the data from the participants in the validation study in the full-scale study analysis. Finally, providing a comprehensive clinical evaluation will help ensure maximal participation rates.

As was stated above, the major modification to current VES procedures would be that additional blood would be drawn on study participants for measurement of TCDD levels. The current laboratory TCDD measurement method requires 75 ml of plasma. We plan to collect 150 ml of plasma so that we will have a back-up specimen to cover possible losses during processing. Necessary approvals for the additional blood collection will be obtained from the internal review boards of CDC and Lovelace Medical Foundation.

The additional questions on potential exposures to dioxin will be added to the medical history that is obtained at LMF (see Appendix E). The purpose of the additional questions is to get more detailed information on possible civilian exposures to dioxin, which will allow adjusting for any differences in such exposures between the two groups in the analysis. Also, we will obtain more detailed information on veterans' self-assessed exposures to herbicides while in Vietnam. There also will be a short series of questions on units of assignment while in Vietnam, types of units, locations served, and primary duties. These

questions are not related to the validation study. They are questions which come from the questionnaire being used in the Selected Cancers Study (SCS) and we would like to include them in this study to compare veterans' recall with information recorded in military records. These questions may form the basis for an exposure index in the SCS and the proposed validation study provides a valuable opportunity to evaluate the reliability of this critical questionnaire information.

The serum which is collected at the examination clinic will be frozen and shipped to the laboratory of the Division of Environmental Health Laboratory Sciences (DEHLS) of the Center for Environmental Health (CEH) of CDC. The serum TCDD levels will be measured using High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS). The laboratory methods are described in Appendix A.

IX. Interpretation of Results

The different possible results from the validation study each would have different implications for a full-scale Agent Orange Study:

1) There is a strong association between at least one of the indirect measures of exposure opportunity and plasma TCDD levels.

   This result would mean that we would be able to distinguish veterans' likelihoods of exposure using indirect assessment methods based on military records alone and there would be a sound scientific basis for proceeding with a full-scale Agent Orange

Study whose purpose would be to determine whether the different exposure levels are associated with any differences in adverse health outcomes.

2) There is little or no association between all the indirect measures of exposure opportunity and plasma TCDD levels.

If none of the indirect measures of exposure opportunity show an association with plasma TCDD levels, we will compare the TCDD levels of the validation study participants with levels found in several serum pools of non-Vietnam veterans who participated in the Vietnam Experience Study examinations. If the Vietnam veterans as a group appear to have TCDD levels appreciably above the levels found in the non-Vietnam veterans, it would indicate that there are Vietnam veterans who were exposed to substantial levels of dioxin in Vietnam (assuming other exposures have been ruled out), but that our indirect exposure assessment methods are inadequate for predicting who these men might be, either because of misclassification or other inadequacies of available data. If such a result is found, consideration would have to be given to conducting a full-scale Agent Orange Study in which plasma levels of TCDD would be measured on all participants.

If the plasma TCDD levels in the Vietnam veterans are found to be similar to the levels in the non-Vietnam veteran serum pools, it would indicate that the levels of dioxin to which study participants were exposed were not high enough to result in

elevated TCDD levels 15 to 20 years after exposure or, perhaps, that the TCDD half-life is less than 5 years. Such a result might provide reassurance to most Vietnam veterans that the levels of dioxin to which they may have been exposed in Vietnam were either quite small or at least have been eliminated from the body in the intervening years. If such a result is found, a decision would have to be made at that time concerning the advisability of proceeding with a full-scale study of Agent Orange exposure and health outcomes.

X. Timetable

The timetable outlined below is tentative and depends on the timeliness of the review cycles.

DATE	MAJOR MILESTONE
9/86	- Validation Study protocol submitted to AOWG/OTA
10/86	- Validation Study protocol submitted to IRB/OMB
10/86	- AOWG/OMB/OTA protocol approval
11/86	- Validation Study interviews begin
12/86	- Medical Examinations begin
3/87	- Complete interviews
4/87	- Complete medical examinations
5/87	- Complete Laboratory Analyses
6/87	- Report Validation Study Findings

REFERENCES

1. Poiger H, Schlatter C. Pharmacokinetics of 2,3,7,8-TCDD in Man. Fifth International Symposium on Chlorinated Dioxins and Related Compounds. Bayrueth (FRG), September 16-19, 1985.

2. Rappe C, Nyguen M, et al. Identification of 2,3,7,8-Substituted Polychlorinated Dioxins (TCDDs) and Dibenzofurans (PCDFs) in Environmental and Human Samples. Presented at American Chemistry Society, Miami, 1985.

3. Patterson DG, Jr., Hoffman RE, Needham LL, et al. Levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin in adipose tissue of exposed and control persons in Missouri: An interim reported. Submitted for publication.

4. Gross ML, Jackson O, et al. 2,3,7,8-tetrachloro-dibenzo-p-dioxin levels in adipose tissue of Vietnam veterans. Environmental Research 1985; 33:261-8.

5. Schecter E, Ryan J, et al. Agent Orange in Vietnam: Follow-up Studies of Dioxin Levels in Adipose Tissue and Milk Samples from Hanoi and Ho Chi Minh City Hospitals, 1984. Annual meeting of the American Public Health Association. Washington, D.C., November 17-21, 1985.

6. Eriksson M., Hardell L., Berg NO, et al. Soft-tissue sarcomas and exposure to chemical substances: A case-referent study. Brit. J. Ind. Med. 38:27-33, 1981.

7. Hoar SK, Blair A, Holmes FF, et al. Agricultureal herbicide use and risk of lymphoma and soft-tissue sarcoma. JAMA 1986; 256:1141-7.

8. Hatch MC, Stein ZA. Agent Orange and risks to reproduction: The limits of epidemiology. Teratogenesis, Carcinogenesis, and Mutagenesis 1986; 6:185-202.

9. Crosby DG, and Wong AS. Environmental Degradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Science 195:1337-1338, 1977.

10. Nash RG, Beall ML. Distribution of Silvex, 2,4-D, and TCDD applied to turf in chambers and field plots. J. Agric. Food Chem. 28:614-623, 1980.

11. Jensen DJ, Getzendaner ME, et al. Residue studies for (2,4,5-trichlorophenoxy) acetic acid and 2,3,7,8 Tetrachlorodibenzo-p-Dioxin in grass and rice. J. Agric. Food Chem. 31:118-122, 1983.

12. DiDomenico AG, Viviano G, Zapponi. Environmental Persistence of 2,3,7,8-TCDD at Seveso. In: Chlorinated Dioxins and Related Compounds: Impact on the Environment. O. Hutzinger et al. Pergamon Press, NY, 105-114, 1982.

13. Wipf HK, Schmid J. Seveso - An environmental assessment. In: Human and Environmental Risks of chlorinated dioxins and related compounds. R.E. Tucker et al. Plenum Publishing Corp., NY, p. 255-274, 1983.

14. Environmnetal Protection Agency. Health Assessment Document for Polychlorinated Dibenzo-p-dioxins. Final report, EPA/600/8-84/014f, Cincinnati, OH, 1985.

15. Harrigan ET Technical report ADTC-TR-70-36, Calibration Test of the UC-123K/A/A45Y-1 Spray System, Armament Development and Test Center, Air Force Systems Command, Eglin Air Force Base, Florida. February 1970.

16. Joint Munitions Effectiveness Manual (JMEM). Government document TH 61A1-1-4; FM 101-50-1; NAVAIR 00-130-AS-1-3, 1971.

17. Forcier L, Hudson HM, and Fett MJ. Australian Veterans Health Studies: The Mortality Report, Part III: The Relationship between Aspects of Vietnam Service and Subsequent Mortality among Australian National Servicemen of the Vietnam Conflict Era. Australian Government Publishing Service, Canberra, Australia, 1984.

18. Hoffman RE, Stehr-Green PA, Webb KB, et al. Health effects of long-term exposure to 2,3,7,8-tetrachloro-dibenzo-p-dioxin. JAMA 1986; 255:2031-8.