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PROTOCOL

PROJECT RANCH HAND II

EPIDEMIOLOGIC INVESTIGATION OF HEALTH EFFECTS IN AIR FORCE PERSONNEL FOLLOWING EXPOSURE TO HERBICIDE ORANGE

MATCHED PAIR COHORT DESIGN

MASTER COPY FOR CORRECTIONS

PREPARED BY: EPIDEMIOLOGY DIVISION
DATA SCIENCES DIVISION
CLINICAL SCIENCES DIVISION

USAF SCHOOL OF AEROSPACE MEDICINE (USAFSAM)

BROOKS AFB, TX

PREPARED FOR: PEER REVIEW AGENCIES

6 August 1979

AIR FORCE WORKING PAPER

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PROJECT RANCH HAND II

EPIDEMIOLOGIC INVESTIGATION OF HEALTH EFFECTS IN AIR FORCE PERSONNEL FOLLOWING EXPOSURE TO HERBICIDE ORANGE

MATCHED PAIR COHORT DESIGN

I. Purpose of the Investigation

The purpose of this investigation is to determine, by epidemiologic techniques, whether long-term health effects exist and can be attributed to occupational exposure to Herbicide Orange.

II. Synopsis of Background

A. Current

News media presentations have recently focused medical, political and lay attention on possible adverse health effects in military personnel, allegedly due to Herbicide Orange [a mixture of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)] which was used as a defoliant during the Vietnam Conflict. This defoliant was later found to have been contaminated with the toxin 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Approximately 500 claims for compensation have been filed against the Veterans Administration (VA), largely by former US Army members. In response to Congress, the General Accounting Office (GAO) investigated the issue and subsequently recommended that the Department of Defense (DOD) conduct a long-term epidemiologic study of the problem. The Department of the Air Force has made a formal commitment to the Congress and the White House to conduct such a study.

B. Use of Herbicides

Research and development on phenoxy herbicides began in the early 1940s. Most of the initial phytotoxic screening programs and development of application technologies were sponsored by the DOD. The military concept for use of these compounds was directed to two purposes: (1) defoliation of vegetation to decrease risk of ambush by improving visibility, and (2) destruction of enemy crops. The first sustained DOD operational use of herbicides was initiated during the Vietman Conflict (Operation RANCH HAND). Data have indicated that the various 2,4,5-T containing-herbicides (code-named

Pink, Purple, and Green) used between 1962 and 1965 contained relatively higher concentrations of TCDD than the herbicide (code-named Orange) used from 1965 through 1970. Concurrent with the change in herbicides, the scope of aerial use shifted from four rotating aircrews to 30 permanent aircrews assigned in Vietnam. Between 1962 and 1970 approximately 10.9 million gallons of phenoxy herbicide, containing approximately 368 pounds of TCDD, were dispersed. Following the announcement in October 1969 that the administration of 2,4,5-T to pregnant rodents caused an increase in the rate of congenital abnormalities, the DOD confined Herbicide Orange spray operations to non-populated areas. In April 1970, all uses of the herbicide were halted. In February 1971, all Herbicide Orange stocks were removed from South Vietnam and transported to Johnston Island, Pacific Ocean, for open storage (Project PACER IVY). The remainder of the military phenoxy herbicide stock was incinerated at sea in 1977 (Project PACER HO). In 1979, the Environmental Protection Agency (EPA) suspended the use of herbicides containing 2,4,5-T because an epidemiologic study in the United States attributed abortogenic effects to its use.

III. Goals of the Investigation

From the above background, three interdependent study goals emerge:

A. Health

(1) To identify veteran and active duty individuals with adverse health effects (physical and psychological) if any, and which are attributed to herbicide exposure, and

(2) To identify other individuals at risk of developing future adverse health effects, if any.

B. Political

To satisfy the social concern for proper investigation voiced both by lay and scientific communities, national and international.

To ✓

C. Legal

GOAL STATEMENT

With regard to the goal of legal clarification, it is apparent that data and conclusions arising from this investigation, positive, negative, or indeterminant, will probably be used in determining compensation ~~to better assess~~ the issue of long-term health effects and resultant compensation.

Pink, Purple, and Green) used between 1962 and 1965 contained relatively higher concentrations of TCDD than the herbicide (code-named Orange) used from 1965 through 1970. Concurrent with the change in herbicides, the scope of aerial use shifted from four rotating aircrews to 30 permanent aircrews assigned in Vietnam. Between 1962 and 1970 approximately 10.9 million gallons of phenoxy herbicide, containing approximately 368 pounds of TCDD, were dispersed. Following the announcement in October 1969 that the administration of 2,4,5-T to pregnant rodents caused an increase in the rate of congenital abnormalities, the DOD confined Herbicide Orange spray operations to non-populated areas. In April 1970, all uses of the herbicide were halted. In February 1971, all Herbicide Orange stocks were removed from South Vietnam and transported to Johnston Island, Pacific Ocean, for open storage (Project PACER IVY). ~~The remainder~~ All of the military phenoxy herbicide stock was were incinerated at sea in 1977 (Project PACER HO). In 1979, the Environmental Protection Agency (EPA) suspended the use of herbicides containing 2,4,5-T because an epidemiologic study in the United States attributed abortogenic effects to its use.

plus additional support personnel

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B. Political

To satisfy the social concern for proper investigation voiced both by lay and scientific communities, national and international.

C. Legal

To clarify the question of compensation award to the 500 claimants.

With regard to the goal of legal clarification, it is apparent that data and conclusions arising from this investigation, positive, negative, or indeterminant, will probably be used to better assess the issue of long-term health effects and resultant

compensation. The operational assumption of this study, therefore, is: Air Force Operation RANCH HAND personnel probably received a greater average occupational exposure to 2,4,5-T and TCDD than US Army ground personnel, implying that RANCH HAND personnel should develop greater numbers of acute and chronic clinical signs/symptoms from the exposure, and should manifest them sooner than US Army personnel, if indeed there are any adverse long-term health effects at all. This dose-response notion suggests that although the Air Force population is not the best one to study, it is probably better than the Army population.

The overall scientific thrust of this investigation is to define the natural history of disease, if any, and its spectrum of illness, by direct and indirect methodology.

IV. Synopsis and Discussion of Literature

A. Overview

More than 20,000 scientific articles relating to the phenoxy herbicides have been published since the 1940's. Many of the articles cite herbicide-caused health effects in a variety of animal species. Most early studies used a myriad of herbicide formulations and unknowingly dealt with physically and chemically impure compounds. The assay technology was far short of today's state-of-the-art. Many human studies have ascribed cause and effect relationships but have suffered from problems of clinical empiricism or questionable methodology. The only consistent and repetitive clinical finding associated with acute exposure to 2,4,5-T herbicide has been chloracne, recognized by most workers as the herald sign of acute overexposure to the herbicide. Sequaelae from chloracne, localized or systemic, appear to be unusual according to the preponderance of the literature. It is appropriate to note that sustained worldwide usage of herbicides for 30 years has not evoked a readily identifiable disease state. It is clear from the literature and the usage history of herbicides that if there are significant attributable long-term health effects, they are either reasonably rare, or of such nonspecific commonality that they blend unnoticeably into the symptoms, syndromes, or diseases associated with increasing age or other factors.

B. Pharmacokinetics of 2,4-D, 2,4,5-T and TCDD

(1) 2,4-D

The pharmocokinetics of 2,4-D have been well studied in

animals. 2,4-D is readily absorbed on oral administration. Initially, it is distributed in high concentrations to the central nervous system and liver. Eventually, all tissues are involved, with the kidneys accumulating twenty times the concentration of the other tissues. The plasma half-life is approximately 3-12 hours, with 2,4-D primarily eliminated from the body by the kidney, the rate of elimination being dose-dependent. Generally, high doses or repeated lower doses result in tissue accumulation. The majority of 2,4-D is eliminated unmetabolized; however, esters of 2,4-D have been shown to undergo hydrolysis prior to excretion. Muscle and fat show the lowest accumulation of 2,4-D on repeated exposure, whereas the kidneys and liver show the highest accumulations. Within 24 hours of single dose administration of 2,4-D, 16.8% was present in the uterus, placenta, fetus and amniotic fluid in rats. In addition, 2,4-D was found in the milk of lactating rats for up to six days following single-dose exposure.

(2) 2,4,5-T

The pharmacokinetics of 2,4,5-T have been well studied in animals. In all animals, 2,4,5-T has been shown to be readily absorbed upon oral administration. However, beyond this point, 2,4,5-T has shown marked variations in its pharmacokinetics in the various animals. These differences are supposedly due to variations in species, age, dose levels, route of administration and chemical formulation used in the various studies. The distribution is generally ubiquitous throughout the body with the exception of hamsters, which show no placental passage, and mice, which show placental passage but only in late gestation. Clearance from plasma and the body varies greatly among animals with rats showing faster clearance than dogs, mice and man. In addition, this clearance appears to be generally dose-dependent. The biological half-life of 2,4,5-T in rats, as estimated by tissue analyses and urinary clearance at administered dosages of 5 mg/kg, is 4.7 hours. However, at 200 mg/kg, the half-life in rats is prolonged to 25 hours. Excretion of 2,4,5-T is primarily via the kidneys. The elimination of 2,4,5-T at low doses is essentially achieved in its unmetabolized form. However, at higher doses or more chronic doses, elimination entails a more active role by the liver (i.e., conjugation). Higher doses and repeated lower doses appear to result in accumulation in animal tissues.

(3) TCDD

The information on the absorption, distribution and excretion of TCDD has been mostly derived from animal models. The

Subsequent to the accidental release of TCDD in Seveso, Italy in July 1976.

only reported human study dealing with pharmacokinetics of TCDD dealt with the analysis of TCDD in tissues at necropsy of one case of confirmed ~~TCDD~~ exposure. Studies in rats, mice and guinea pigs generally show that intestinal absorption of TCDD is relatively complete, with a large proportion of TCDD remaining unmetabolized in the liver. The majority of this TCDD is assumed to be localized in the liver microsomes (centrifugation techniques). Initially, adipose tissue accumulates TCDD, followed later by accumulation in the liver, adrenals, kidneys and lungs. The level of TCDD in the liver and adipose tissue is about ten-fold greater than in other body tissues; however, significant species variability has been observed. The biological half-life of TCDD varies by species, but is reported to range from 12 to 50 days. The major route of excretion is via the feces with urinary excretion occurring at a much reduced rate.

(4) Phenoxy Herbicdes in Humans

Relatively few studies have dealt with the pharmacokinetics of 2,4-D and 2,4,5-T in humans. Numerous reports of occupational exposures in industry and in commercial and private herbicide applications have supported percutaneous entry. Rapid absorption has been observed after oral administration of 2,4-D or 2,4,5-T. The main mode of excretion of the phenoxy herbicides is via the urine with 74% of 2,4-D and 63%-72% of 2,4,5-T being cleared from the body within the first 96 hours. The majority of phenoxy herbicides are unmetabolized prior to excretion. The biological half-life of 2,4-D and 2,4,5-T in humans (as estimated by tissue analyses and urinary excretion) is 33 hours and 18 hours, respectively. Tissue analysis has revealed a ubiquitous distribution of the herbicides after absorption. Limited studies on the accumulation of the phenoxy herbicides following repeated doses suggest that such accumulation in humans is unlikely. This is in contrast to numerous animal studies on 2,4-D and 2,4,5-T which show that such accumulation does occur.

No specific data are available on the odor threshold of Herbicide Orange. Data are availabe however, on the odor threshold of a butyl ester formulation of 2,4,5-T. The odor threshold was found to be about 0.3 ppb (the taste threshold was 1.3 ppb). A Threshold Limit Value (TLV) of 10,000 $\mu g/m^3$ (10ppm) for either 2,4-D or 2,4,5-T has been adopted by the American Conference of Governmental Industrial Hygienists. The TLV is a time-weighted average concentration for a normal 8-hour workday or 40-hour workweek to which workers may be repeatedly exposed, day after day, without,

adverse effect. Analysis of ambient air samples collected adjacent to and downwind from actual dedrumming operations involving Herbicide Orange were at least two orders of magnitude below the TLVs.

C. Proposed Cellular Mechanism of Action for TCDD

TCDD has, in general, three proposed mechanisms of action by which its variety of effects, both documented and suspected, can be understood. All currently available information in this area is derived from animal, plant, and bacterial models. The few human studies dealing with mechanisms are limited to the clinical manifestations of chloracne.

(1) Microsomal Enzyme Induction

TCDD's ability to induce a variety of microsomal enzymes is well documented. The induction of aryl hydrocarbon hydroxylase, delta-aminolevulinic acid synthetase and cytochrome P448/450 ~~P448/450~~ associated enzymes are implicated in the development of cutaneous porphyria. The induction of aryl hydrocarbon hydroxylase and other mixed-function oxygenases/oxidases have been associated with carcinogenesis and tumorogenesis. In addition, TCDD has been shown to be a possible promoter or cocarcinogen of known carcinogens. In some nonhuman studies, TCDD produced a protective effect against endocrine tumors (e.g., pituitary, uterine, pancreatic, adrenal and mammary tumors). TCDD's induction of UDP-glucuronyl transferase, an important enzyme in steroid metabolism, may explain this peculiar effect of TCDD. The induction of DT-diaphorase and lysosomal acid proteinases has been implicated in TCDD's neuropathic effects. These and other biochemical alterations may account for TCDD's clinical manifestation of chloracne resulting from an over production of keratin in the sebaceous ducts.

(2) DNA/TCDD Interaction.

Alterations in the structure and fidelity of transcription of DNA due to TCDD have been indirectly demonstrated. In a similar fashion to the acridine family of compounds, TCDD, because of its planar ring structure, is felt to "intercalate" with DNA resulting in "frame-shift" mutations. A few laboratory studies with bacterial systems, e.g., Escherichia coli and Salmonella typhimurium, or in one plant system, e.g., the African Blood Lily, have identified TCDD as being able to produce chromosome aberrations and perhaps a weak dominant lethal effect. This hypothesized DNA/TCDD interaction could explain the development of chloracne, as well as the suggested mutagenic and carcinogenic effects, if similar mechanisms occur in mammalian species.

(3) Toxicity.

The effect of some nonspecific activity or as of yet unspecified toxicity continues to serve as a reasonable mechanism for TCDD's hepatic and thymus toxicity. TCDD has been described by some as "one of the most potent, low molecular weight toxins known", with extremely low concentrations producing severe liver damage and death in various animal studies. The immune suppression effect of TCDD has been shown to result specifically from its T-cell (thymus) toxicity. In addition, TCDD's concentration in the adipose tissue suggests the possibility that under situations of weight loss (e.g., life style, medical indications, or disease), TCDD may be released into the circulation. Such a hypothesized reemergence of the agent could result in low doses being either detectable and/or toxic at some later point in time. If TCDD's primary toxicity results from low doses (e.g., mutagenic/carcinogenic effect) rather than high doses (e.g.,cellular poisoning and cell death), then the deposition of TCDD in the adipose tissue may have greater significance with respect to delayed effects on the long-term health of the exposed individual. This possibility raises a theoretical dose-response paradox which might "explain" the prevailing preponderance of symptoms in populations which may have been exposed to relatively low doses of TCDD.

D. Animal Studies

A comparison of animal toxicity studies is difficult due to variations in experimental designs which include differences in (1) the species, age, and sex of animals used; (2) the level, route, and length of exposure to chemicals; (3) the purity of the chemicals used; (4) the criteria measured and the time sequence of data collection. Animals have shown a wide range of toxic effects. This range may serve as a guide to anticipate the potential toxic effects in humans following exposure to Herbicide Orange.

A summarization of the literature is presented in the Appendix, Table A-1. It is apparent that the toxic effects of 2,4-D and 2,4,5-T are markedly different from TCDD. TCDD is approximately 1000 times more toxic in acute studies. In addition, the slower clearance time of TCDD may account for the significantly lower daily doses required to elicit chronic toxicity. A consistent finding in TCDD toxicity is depletion of the lymphoid tissues throughout the host. This is readily characterized by involution of the thymus in all species studied. In relationship to the chronic maternal toxic dose, the embryotoxic dose is markedly lower for TCDD than for 2,4-D and 2,4,5-T. Both 2,4,5-T and 2,4-D appear to be very weak teratogens and/or carcinogens at best, but these evaluations are complicated by varying levels of contamination by various dibenzo-p-dioxins. TCDD appears to have significant teratogenic and carcinogenic potential which appears to be species specific.

The most striking observation noted in the literature is a marked variation in response among species. Examples of these variations are in the areas of acute toxicity (TCDD's LD_{50} in the guinea pig is 1 μg/kg compared to 1000 μg/kg in dog), excretion (2,4,5-T plasma half-life in rats is 4.7 hrs compared to 77 hrs in dog), and oncogenicity (TCDD is oncogenic in mice but not shown to be oncogenic in ~~mice~~ rats under similar conditions). Even among strains of the same species (mice) variations in oncogenicity were noted following 2,4,5-T exposures. As noted earlier, this high variability between species is an important consideration in designing human studies.

A second area of interest noted in the literature is a possible dose-response paradox in nonhuman primates (rhesus monkey) following exposure to TCDD. Animals receiving subtoxic doses in single-dose acute toxicity studies (LD_{50} determinations) have not been followed over long periods of time. Animals on chronic exposure studies fed a low level of TCDD in feed ~~(e.g. 50-500 ppm)~~ have shown signs of disease only after several months when the accumulated dose was approximately 1 μg/kg body weight. Therefore, it remains unclear whether the toxicity demonstrated in chronic exposure studies is dependent upon a low level daily exposure accumulated to 1 μg/kg or would also be demonstrated following a single dose of 1 μg/kg.

E. Veteran Complaints

The Veterans Administration Compensation and Pension Service, Washington, DC, provided 361 claims ~~as of the 30th of April~~ 30 April 1979, submitted by veterans alleging an altered health status due to exposure to Herbicide Orange. A review of these claims revealed that less than half of the veterans received detailed physical examinations to evaluate the claims. Numerous media presentations emphasizing both military and civilian herbicide exposures have published a remarkably wide range of symptoms. The present review substantiates the wide spectrum of alleged health effects being claimed. Based on current guidelines established by the Veterans Administration (Program Guide 21-1, Section 0-18 and Title 38 USC), none of the symptoms elicited in these claims were shown to be secondary to exposure to Herbicide Orange. The vast majority of the exposure claims remained unsubstantiated, based on review of military personnel and medical records. The guidelines state that the only chronic residual of defoliant exposure ever incriminated by clinical history has been chloracne. Furthermore, chloracne was associated with prolonged intensive exposure and all other toxic effects of the herbicide were viewed to be rapid in onset and to run

a brief course followed by recovery without residual disease. In fact, the vast majority of the claims alleging exposure to Herbicide Orange were not for chloracne and, as a result, did not satisfy the criteria set forth for compensation. Of the three claims dealing specifically with chloracne, none were confirmed by physical examination.

Table 1 summarizes the descriptive characteristics of the 361 claimants.

Table 1

SUMARY OF DESCRIPTIVE CHARACTERISTICS OF HERBICIDE RELATED CLAIMS SUBMITTED TO THE VERTERANS ADMINISTRATION AS OF 30 APRIL 1979*

Total Number of Claims: 361

Sex: 100% Male (gender identified in 64% of claims)

Mean Age: 34 years (age identified in 96.1% of claims)

Mean Number of Alleged Symptoms per Veteran: 2.3

Branch of Service: (Service history identified in 66.8% of claims)

US Army	66.4%
US Marine Corp	17.4%
US Air Force	11.2%
US Navy	5.0%

*Exact racial distribution unknown; anecdotal information suggests the majority of claimants are non-Caucasian.

The summary shows that the veteran complaining of an altered health status secondary to Herbicide Orange exposure is generally a young

male who served with the Army in Vietnam, however, the Navy and Marine Corp veterans accounted for a significant component (22.4%) of the alleged exposures.

Table 2 summarizes the number of herbicide related complaints by alleged symptom category.

Symptom	Percent
Dermatologic	48.9
Psychiatric	27.6
Ear, Nose & Throat	14.4
Cancer	13.8
Peripheral Neuropathy	12.1
Asthenia	11.2
Gastrointestinal	10.9
Reproductive	10.1
Pulmonary	9.2
Ophthalmologic	8.9
Musculoskeletal	8.1
Cardiovascular	7.5
Genitourinary	4.0
Central Nervous System	3.7
Hepatic	3.5
Pancreatic	2.3
Hematologic	1.4
Collagen-vascular	1.2
Allergic	0.9
Fever	0.9
Thyroid	0.3
Amyloidosis	0.3
Periodontitis	0.3
Poisoning (lateritic soils)	0.3

Note: 13 claims alleged exposure only (without symptoms) as basis for compensation

Study implications that can be drawn from this material are limited due to the lack of knowledge concerning denominator data. Overall, the group of claimants exhibited a uniquely high frequency of readily identifiable disorders (e.g., psychiatric illness and cancer). Further evaluation of the claims revealed that of the total number of claimants, 16.3%, had previous diagnoses of psychiatric disorders (20% of those diagnosed with schizophrenia).

Table 3 summarizes information on the USAF veterans as to general characteristics and alleged symptom category.

Table 3

HERBICIDE RELATED CLAIMS SUBMITTED BY USAF VETERANS BY SYMPTOM CATEGORY AS OF 30 APRIL 1979

Number of USAF Veterans: 28 (Mean age = 35.4 years)

Symptom	Percent
Psychiatric	50
Dermatologic	39
Reproductive	25
Peripheral Neuropathy	14
Cancer	7
Miscellaneous	7

In those identified as USAF veterans, the distribution of symptom categories (especially psychiatric, dermatologic and reproductive) was not representative of the proportion of symptoms exhibited by the veterans in general. No implications can be drawn from this observation due to lack of information on branch of service in 33.2% of the veterans, as well as the weakness of the overall denominator data.

Table A-2, in the Appendix, summarizes the veterans symptoms into general symptom categories and gives the relative frequency of each category. Comparison of Table 2 (percent of veterans having that symptom) and Table A-2 (dealing with the relative frequencies of the symptoms) reveals the relative magnitude of effort, in manpower, history-taking and physical examinations, that will be needed to evaluate each specific symptom category. For example, although dermatologic manifestations were alleged in 48.9% of all veterans

(Table 2), ~~it made up only 22.2% of all symptoms identified by the group of veterans as a whole.~~ Such symptoms comprised The demonstrated lack of an easily identifiable symptom complex on review of the veteran claims clearly requires evaluation of individual symptoms. Therefore, a comprehensive questionnaire and physical examination is required.

F. Case Reports

Much of the medical literature on 2,4-D, 2,4,5-T and TCDD exposures in humans is based on individual case reports. Most of the patients discussed in these reports were exposed to multiple chemical agents and, therefore, it is difficult to determine which agents were responsible for specific symptoms. Nevertheless, the general areas of dermatologic and neuropsychiatric disease have been of primary interest to most investigations. Since the neuropsychiatric symptoms of herbicide exposure are numerous and largely subjective in nature, they have been extremely difficult to assess from a clinical standpoint. Hepatic dysfunction, renal, gastrointestinal and cardiac disturbances are "linked" to exposures to these chlorophenolic compounds.

(1) 2,4-D

A multitude of symptoms have been attributed to 2,4-D, and the ones reported most consistently are listed in the Appendix, Table A-3. Components of some of these selected symptoms/signs are described in Table A-4 of the Appendix. The asthenic syndrome, peripheral neuropathy and hepatic dysfunction are of particular interest. Other symptoms of systemic toxicity occur, but usually resolve within 4-6 weeks. The peripheral neuropathy associated with 2,4-D exposure has been extensively described. It has an early onset, causes prolonged disability of variable degree, and recovery has been incomplete in many cases. Electromyography in some patients has demonstrated denervation, and some studies have detected decreases in nerve conduction times. One autopsy study demonstrated a demyelination process within the brain of a 76-year-old male who committed suicide by ingestion of 2,4-D in kerosene.

(2) 2,4,5-T/TCDD

The human effects of 2,4,5-T are difficult to evaluate since the chemical is contaminated with TCDD in the manufacturing process. The effects of TCDD have been determined from studies of trichlorophenol workers, and from laboratory workers using TCDD. Symptom/sign complexes attributable to exposure to

2,4,5-T and TCDD are listed in Tables A-3 and A-4 of the Appendix. Chloracne usually begins in the zygomatic/temporal region and is often found on and behind the pinna of the ear. This is an oily acne-like skin condition characterized by comedones and inclusion cysts which may result in extensive scarring. In severe cases spread of lesions to the throat, back and inguinal areas has been noted. This skin condition is frequently preceded by erythema and blepharoconjunctivitis. Active lesions usually disappear within two years, but have been found 30 years after exposure. Porphyria cutanea tarda and hypothyroidism have also been linked to 2,4,5-T/TCDD exposure. Other symptoms such as asthenia, liver and renal dysfunction, neuropathy, and gastrointestinal and cardiac disturbances are probably due to mechanisms similar or identical to those of 2,4-D.

Numerous instances of alleged disease due to 2,4-D/2,4,5-T exposure have been the subject of heavy media attention, particularly an episode of alleged 2,4,5-T exposure in Globe, Arizona, in 1969. Despite extensive scientific review and analysis with negative findings, the Globe incident continues to appear in current news media productions. A similar incident in Missouri in 1971 is often cited. Six children and two adults experienced chloracne after accidental exposure to TCDD, but all were healthy after five years of followup study. A final prospective assessment of fertility, teratogenesis and carcinogenesis will probably be made in the future.

G. Epidemiologic Studies

Epidemiologic studies of occupational groups have validated links between exposure to TCDD and the development of chloracne. Associations between TCDD and psychological abnormalities have also been suggested. A 1978 study by Hardell and Sandstrom in Sweden evaluated occupational exposure to chlorophenolic compounds in soft tissue cancer patients by a case-control design. They found an association between cancer and exposure, but methodologic problems have raised questions concerning the value of these findings.

Tung (1973) reported an abnormal increase in the occurrence of primary carcinoma of the liver in Vietnam (26 cases per year during 1955-1961 versus 144 cases per year during 1962-1968). He attributed the increase to a suspected carcinogenic effect of TCDD. His published study, however, has been criticized for failure to contain sufficient data and method descriptions to verify his conclusions. The role of aflatoxin as an alternative cause of liver cancer was not addressed. His study was largely an empiric clinical study. A study sponsored by the US Environmental Protection Agency

in 1979 in Alsea, Oregon, found a statistically significant increase in spontaneous abortion in areas where 2,4,5-T herbicide was routinely used in reforestation programs. EPA concluded, however, that "for all its complexity, this analysis is a correlation analysis, and correlation does not necessarily mean causation." This report is currently the subject of intense scientific criticism. Differences in the availability of specialty obstetrical care and in the patterns of health care delivery existed between the exposed and control areas; these differences were not taken into consideration by the researchers. Variations in the ascertainment of spontaneous abortions in each of the areas severely limited the validity of the data, and of the conclusions derived from them. A recent study conducted in Australia (1978) was unable to find an association between birth defects (neural tube abnormalities) and the use of 2,4,5-T Herbicide.

Epidemiologic studies are continuing in Seveso, Italy. A population of 220,000 was potentially exposed to TCDD following an industrial accident in July 1976. These studies have involved investigations of more than 30,000 children and detailed clinical examinations of 1,024 persons, including the most severely exposed children and adults. Recent data (Homberger, et al., 1979) indicated that most cases of chloracne from this incident (134) cleared rapidly. No evidence of significant hepatotoxicity, deranged porphyrin metabolism, or abnormal neurologic findings have been observed thus far. Growth and development of newborn infants and children, immunological response, chromosome aberrations, the reaction to the challenges of infectious diseases, and the morbidity and mortality patterns of the study population have not been significantly altered by TCDD exposure to date. Thirty-eight cases of birth defects were reported in early 1977, approximately 6-8 months after the industrial accident. However, the authors ascribe this increase to an artifact of surveillance. The social pressures operating in the Seveso population prior to the accident fostered underreporting of birth defects, while the atmosphere after the accident made the occurrence of a birth defect more socially acceptable. The post accident malformation rate is not significantly different than the rate in similar areas of Central Europe. Similarly, ascertainment and surveillance of spontaneous abortions after July 1976 is hampered by the lack of valid baselines for the pre-accident period. Chloracne appears to be the only significant adverse effect in the exposed population noted to date.

A 2,4,5-T Dispute Resolution Conference was held in Arlington, Virginia, from 3 to 7 June 1979. Fifty-six recognized experts from the United States and seven foreign nations were actively involved in the deliberations of the conference. Human Exposure, Carcinogenicity/Mutagenicity, and Teratogenicity Working Groups independently reached conclusions that there was no valid scientific evidence linking fetotoxicity, teratogenicity or carcinogenicity to 2,4,5-T/TCDD exposures in humans. The Human Exposure Working Group also concluded that there were no epidemiologic data associating TCDD with any long-term health effect in humans other than persistent chloracne. While they did not find evidence of serious long-term health effects, neither could they find strong evidence for lack of effect. Most previous epidemiologic studies have not had sufficient statistical power to detect increased risks of low incidence/prevalence conditions in the observed populations, and the period of observation in many prospective studies has been less than ideal.

Several potentially valuable epidemiologic studies are currently in progress. Two independent and comprehensive studies of workers exposed to TCDD at a Monsanto manufacturing plant in Nitro, West Virginia, are currently being conducted (Mt. Sinai Medical Center, New York, and the Kettering Laboratory, University of Cincinnati, Ohio). These chemical industry workers were exposed over long periods of time and were previously evaluated in 1953 and 1956, following an industrial accident which occurred in 1949. The Dow Chemical Company is currently analyzing data from a reproductive survey of the spouses of 2,4,5-T/TCDD exposed workers. A Czechoslovakian study involving a 10 year followup of TCDD exposed workers, and a US National Cancer Institute (NCI) mortality study of 4,400 structural pest control workers are also underway.

These new studies, and the continuing evaluations of the Seveso, Italy, population, should provide valuable data. The large study groups involved in the Seveso and NCI studies should provide good statistical power, and the Nitro, West Virginia, and Czechoslovakian efforts will evaluate the effects of exposure after prolonged periods of time (10-30 years). The results of these studies should fill major gaps in the knowledge of 2,4,5-T/TCDD epidemiology, and should prove to be useful in evaluating the long-term effects of these compounds on health and reproductive outcomes.

V. Epidemiologic Study Design: Matched Pair - Cohort

A. Design Consideration

The proposed goals for this study clearly mandate a broad comprehensive epidemiologic approach, incorporating retrospective, cross-sectional, and prospective elements. The primary issue is time. Exposure to herbicides during the 1962-1970 time period may have initiated long-term health effects that may or may not be progressive. If such effects are detectable by retrospective techniques, and are verified, there will be direct links to compensation. Current health status, as mirrored by the large number of recent VA claims, becomes of strong interest, because it might be confirmable by comprehensive physical examination. In the event both retrospective and cross-sectional methodologies yield indeterminant or weakly suggestive findings, it may be that sufficient time has not yet passed for substantial emergence of long-term health effects. This dictates a requirement for a prospective element to the study.

The Air Force staff does not propose to any Peer Review group agency that the following stratified matched pair cohort design is the best technique for any given study phase. Many methodological shortcomings are inherent in each phase of this comprehensive study. To some extent, the classical deficiencies of each particular epidemiologic approach are compensated by the concurrent use of the other methods. For example, the low chance of identifying a relatively uncommon disease solely by the use of a cohort study is offset by the inclusion of the retrospective element. The relatively quick feedback that can be attained from the retrospective and cross-sectional studies will serve to better define the prospective phase and will help to alleviate problems that arise in cohort studies as a result of changes in diagnostic criteria and methods with time. Nevertheless, there will remain many problems that will affect ascertainment of disease in all phases of the study. The problem with patient recall of antecedent events, the distortion of information by knowledge of the disease, as well as participant or observer knowledge of their exposure status can only be corrected to a limited extent by review of records for symptom validation and "blind" assessment protocols. In addition, fundamental problems dealing with adequate selection of a control group and limiting loss to study can jeopardize even the most comprehensive epidemiologic investigation. These and other pitfalls in study design will be discussed in more detail in Section VIII.

The matched pair - cohort design was recommended as the best technique to be employed in this investigation.

~~The staff does note that~~ Since the study has three phases and confronts a health issue with undefined endpoints, including strong bias and political pressure with severe time constraints, the following design may represent the best overall framework for achieving validity. The design process is complex and in itself time dependent. All epidemiologic techniques used are time-compressed. Unique record searching systems within the Air Force, and computer and clinical capabilities, as well as bias and loss-to-study correctors, will work toward making this effort achievable.

B. Ascertainment of Exposed and Control Group Populations

(1) Exposed Group

Operation RANCH HAND personnel primarily flew C-123 aircraft in Vietnam during 1962-1970. Data from hand-compiled lists obtained through the RANCH HAND Association (a reunion organization), Air Force personnel computer entries, historical records and actual C-123 flight orders, place the estimated study population at 1000 (range 800-1200). Of those now confirmed in the computer system, 25% are still on active duty, with the remainder being composed of retired or separated persons. An indepth search is being conducted of all organizational records stored at the Military Records Division, National Personnel Records Center, St. Louis, Missouri, to identify all RANCH HAND participants. Detailed advertisements in active/retired military trade journals, VA publications, and local newspapers will be pursued in the near future to insure total ascertainment/indentification of the exposed group. Introductory letters will be sent to the last known address of all identified persons; and nonresponse will be pursued by cross-locator systems available within the government (e.g., Social Security Administration, VA, Internal Revenue Service). Significant effort will be made to account for at least 99% of the total population (see Table A-5, Appendix). Because of the limited number of estimated RANCH HAND personnel (1000), no subsampling is planned in any phase of the study. All members will be invited to participate in all phases of the investigation.

INSERT #D

(a) Known or Predicted Characteristics of the Exposed Group

All exposed aircrew personnel are males currently ranging in age from approximately 38-58 years. As the normal C-123 crew composition was one pilot and one copilot-navigator (both officers) and one spray equipment console operator in the rear of the aircraft (enlisted), the overall officer-enlisted ratio will be approximately 2:1. While almost all officers were Caucasian, approximately 10-14% of the enlisted men were Black. Because of the

significant combat hazard associated with low, slow flying missions, all RANCH HAND personnel were elite volunteers (see Risk-Taker Bias, Section VIII). In fact, RANCH HAND crew members comprised one of the most highly decorated units during the Vietnam Conflict. Anecdotal stories reveal that most crew members were, on occasion, heavily exposed to Herbicide Orange due to normal or combat induced equipment malfunctions within the aircraft. Many former RANCH HAND personnel are expected to be currently employed in the aerospace industry as commercial airline pilots, airline managers, and flight mechanics. RANCH HAND personnel still on active duty are expected to be found in senior management positions.

(2) Ancillary Study Groups (Non-RANCH HAND personnel)

Air Force handlers of herbicide drums in Vietnam were exposed to herbicides because of drum leakage. Advertisements similar to those proposed for the RANCH HAND personnel will be issued in attempts to define this population. As the drum handlers were ad lib participants, no personnel designator was assigned to these individuals, thus prohibiting computer tracking and identification. The population is unknown, but expected to be low (less than 200) as the majority of drum handlers were known to be Vietnamese. Additional study groups such as flightline mechanics servicing the C-123 aircraft, US Army personnel (officer and enlisted) who flew as observers, may be injected into the study proper. Specific epidemiologic/clinical studies for these groups will be planned by a separate protocol following ascertainment; control group selection will be difficult or moot. It is intended that all data derived from the ancillary study groups will be subsetted for separate analysis; these data will be treated as anecdotal to the primary study.

(a) Known or Predicted Characteristics of the Ancillary Study Groups

All members of these groups are expected to be males, ranging in age from 28-68 years. The officer-enlisted ratio is estimated at 1:10. Approximately 10-18% of these populations are expected to be Black. Low numbers of respondents are expected (and with significant discordance to the above estimated characteristics due to socio-economic-race bias). Population at risk ascertainments are not possible.

(3) Control Group (Not exposed to Herbicide Orange)

The ideal control group, the non-RANCH HAND C-123 population, is known to be too small (approximately 3000) to provide adequate sampling flexibility and replacement under the proposed best match variable concept (see below and Section VI, A). Many of the RANCH HAND aircraft were reconfigured for transport and insecticide missions. Thus, non-RANCH HAND crews responsible for these other missions, may have been exposed to Orange Herbicide residues in these aircraft. This group may not be truly unexposed to herbicides and therefore may not be an appropriate control population. The C-7 crewmembers have also been considered as a potential control group. This latter group, however, was comprised of only 1000 to 1200 individuals. Accordingly, aircrew members who flew C-130 aircraft in Vietnam during 1962-1970 will be selected as controls to the C-123 RANCH HAND population. Total ascertainment of this C-130 population is being conducted by computer selection for specific mililtary flying organizations, foreign country service, etc. Over 2.3 million personnel records have already been scanned and the approximate C-130 sample size is 25,000 aircrew members. The proportions in active duty, and non-active duty status are expected to parallel the patterns in the exposed group.

The absolute "best fit" matched C-130 controls will be used for the retrospective mortality analysis (see below) versus all ascertained RANCH HAND members. In the event of mortality or nonresponse from a best fit control, the next best fit C-130 crew member will be selected for replacement for the cross-sectional and prospective study elements of the study.

(a) Known or Predicted Characteristics of the Control Group

All C-130 aircrew members will be tightly matched on a 1:1 basis to the exposed group with respect to age, sex, race, and other factors listed below. The normal crew composition of a C-130 is three officers and two enlisted personnel. The control group will be "pure" from the standpoint of nonoccupational exposure to herbicide. The entire control group will be considered "nonvolunteer" with respect to abnormally high combat risk. While in general they will probably possess similar lifestyle characteristics and socio-economic backgrounds, their overall combat morbidity/mortality and resultant stress influences upon general health may be slightly less than the exposed group. For those separated and retired C-130 controls, similar proportions to the exposed group are expected to be employed in the aerospace industry.

(4) Matched Pair Procedures and Rationale

Each member of the exposed group will be computer matched for four variables to a set of at least 10 control C-130 crew members. Since the two groups are highly selected and inherently similar with respect to many variables, very close

matches are feasible. This epidemiologic design incorporates a matched pair concept because: (1) a matched pair with stratification will provide maximum test power throughout retrospective, cross-sectional, and prospective phases, (2) statistical intergroup comparisons may be made without normalization by four key variables known to effect symptom frequencies of interest, thus providing greater power for complex multivariate testing, and (3) extremely close matching is feasible and necessary for some of the anticipated analyses of the physical examination findings. Matches will not necessarily be rigidly maintained throughout the data analysis phase, depending upon the particular analysis. It is apparent that following the match, both exposed and control populations will be very nearly identical with respect to the four influencing variables, and that in the event of frequent match breaks, stratification of a controlling variable can be made with enough precision to ensure proper adjustment.

Matching will be conducted for (1) age, by year of birth, and closest month possible, (2) ~~crew position (officer versus enlisted)~~ Air Force Specialty Code as an absolute match, (3) length of time spent in Vietnam, to the closest month, and (4) race (Caucasian versus non-Caucasian) as an absolute match. These variables are listed in priority order of the match sequence. Specific rationale for these varibles is as follows: (1) many clinical symptoms and signs allegedly attributed to herbicide exposure (see literature review) can also be attributed to an aging effect, or to collateral diseases more commonly associated with advancing age, (2) crew position (rank) (as well as crew member and non-crewmember) controls specifically for officer-enlisted status, a variable strongly linked to educational background, current socio-economic status, and moderately linked to age (5 year median difference) and socio-economic background, (3) length of tour in Vietnam (measured in months, or actual flying hours, if feasible) controls for the generalized probability of combat morbidity, mortality, and for combat induced neuro-psychiatric disorders [additionally, length of tour may reflect effects related to intensity of alcohol consumption, drug consumption (chemoprophylactic or illicit), and degree of disease acquisition] and (4) race controls for difficulty in diagnosis of dermatitis, socio-economic background, etc. (note possible racial discordance for VA claimants).

An intragroup comparison in the exposed group of health effects to an index of exposure to herbicides will be made. By using the length of time in Vietnam, as measured by exact flying hours coupled to the exact time of the tour when the concentration of TCDD contamination changed, a crude exposure index can be constructed, normalized, and tested.

C. Retrospective Phase

(1) Introduction

The term "retrospective" has been used generically in this discussion of the design protocol to address the mechanics of selection of the exposed and nonexposed groups, as well as the various means of tracing these groups to the present. In reality, the retrospective and prospective phases are components of a "nonconcurrent" prospective study used in the observation, starting from some date in the past, of a specially exposed group or industrial population. The availability of employment, medical or other types of records is an obvious requisite for such a study. The classical "case-control", retrospective study is not operative in this protocol because of the open-ended nature of the alleged effects and lack of a definable disease syndrome.

(2) Data Collection Methods

All exposed members and their matched controls will be given a comprehensive personal and family health questionnaire via telephone. The questionnaire (see Section XIII) will emphasize identification data, Vietnam tour history, dermatologic conditions, neuropsychiatric conditions, fertility aberrations, genetic defects in offspring, sensory defects, and personality factors, including assessments of Risk-Taking Behavior. The questionnaire will be limited to a 30-45 minute telephonic interaction with participants and it will take 10 to 12 months to complete all initial questionnaires on both groups. The questionnaire will be "Field-Tested" on a group of 25 to 30 former Air Force pilots with Vietnam combat experience. Specific questions on the questionnaire will be directed to verifiable information, wherever possible. Inclusion of specific verifying questions and bias indicator questions (nonsense symptoms) have as yet not been included, since they are still under development. They will be added and appropriately sequenced immediately prior to the start of the study. Questionnaire data will be linked and integrated with medical record information and physical examination findings. Each participant will be asked to sign release forms so that all civilian health records, including those of dependents, can be reviewed as necessary. Federal health records on all family members on file in the St. Louis Repository will be retrieved. All retreived medical records will be reviewed, scored, compared to questionnaire data for reliabililty, and then be entered into a respository system. Identified participants who are non-responsive to questionnaire will be pursued to determine status, disinterest, moribund state or death, etc. These individuals will be cross-referenced to other federal accounting systems in an attempt to achieve total ascertainment.

Death certificates will be retrieved for all identified deaths exposed and control members. Appropriate birth/death certificates will be sought for all appropriate offspring.

D. Cross-Sectional Phase

(1) Selection/Entry Criteria

A voluntary comprehensive physical examination will be offered to all individuals in both the exposed and control populations. The condition for study entry will be completion of the baseline questionnaire. In the event that the "best fit" control does not complete both the questionnaire and the physical examination, the next best fit will be selected, and so on, until a willing control is obtained. Statistical testing will be conducted by a variety of techniques on both questionnaire and examination findings (see VI, Statistical Methodology below). At the time of physical examination, an extensive indepth interview will be conducted. A standardized protocol will be used to insure comparability of inteview data. This will provide cross-reference data to the initial questionnaire and to medical record data, if retrievable. Specific verifier and bias indicator questions will be included during the interview as well.

(2) Physical Examination Parameters

A comprehensive physical examination will be conducted on all willing participants. The examination will be structured as outlined below and in Section XIV.

General Physical Examination

FBS, 2 Hr Post Prandial	CBC	CPK
Urinalysis	RBC Indices	ECG
BUN/Creatinine	Sedimentation Rate	Chest X-Ray
Cholesterol/HDL Cholesterol	Cortisol (Matching)	VDRL/FTA
Triglycerides	Thyroid Profile (RIA)	

Dermatologic Examination

Urine Porphyrins
Urine ~~Porphobilinogen~~ Porphobilinogen
DEHA-aminolevulenic Acid

Neuro-Psychiatric

Nerve Conduction Velocities
Psychological Battery
MMPI
WAIS
Halstead-Reitan
Wechsler Memory Scale Subtests
Cornell Index

Reproductive Examination

LH, FSH, Testosterone

Neoplastic/Hepatic Examination

SGOT
SGPT
Alkaline Phosphatase
LDH (Isoenzymes if ~~evaluated~~ elevated)

Additional Studies (Individuals with abnormal history or examination)

SEMEN Analysis
Karyotyping
Additional consultations as required
Hepatitis Antigens (A and B)
Mono Spot Test
Anti-nuclear Antibody

Examinations will be performed in regional federal medical facilities in the United States and overseas having dermatologic, neurologic and electromyogram/nerve conduction capabilities (Section XII, ~~Appendix~~, Figure A-1). This will generate better participation than if all examinations were conducted at a single location. Special Air Force authorization will be obtained to conduct such examinations on individuals separated from the service and informed consent forms will be obtained for nerve conduction tests. Physicians and technicians will handle all participants without a knowledge of exposed or control status, and will conduct the examinations by standardized protocols to minimize variability. Clinical specimens will be forwarded to USAFSAM where the laboratory procedures will be conducted. All laboratory tests ~~would~~ will thus be subject to the same technology and rigid quality control.

Special contingencies will be made for unusal laboratory testing. Karyotyping of the individual and his family members will be performed if clinical history or physical examination findings are suggestive of this need. Most well conducted

Semen analysis will also be requested from participants if infertility is suspected after completion of the initial questionnaire.

studies have shown that, when present, chromosomal abnormalities due to TCDD are transient. If on detailed analysis of the baseline examination and questionnaire, reproductive areas are heavily affected, routine karyotyping may be included in the test battery for the prospectus. TCDD analysis on blood and urine will be considered in the future provided that (1) strong cause and effect relationships can be ascribed to Herbicide Orange (~~positive Vietnam exposure index versus negative crude geographical exposure index~~), and (2) high resolution mass spectrometry technology achieves 10 femtogram sensitivity with high specificity. Appropriate specimens will be obtained from all participants, aliquoted, and preserved at -70°C for possible future analysis.

Physical examination and laboratory data will be placed in the member's master file for detailed cross-analysis to questionnaire data. Information identifiable to the subject will not be released without his consent in accordance with the Privacy Act. (Exceptions: In accordance with Air Force regulations, all active duty flying personnel and air traffic controllers found to have disqualifying defects will be temporarily grounded pending resolution; in accordance with federal regulations, all commercial airline pilots and air traffic controllers found to have disqualifying defects will be reported to the Federal Aviation Administration.)

E. Prospective Phase

(1) Study Adaptations

Following complete data analysis of the retrospective and cross-sectional phases, an adaptive or restrictive health survey will be developed and annually administered for five years. Similarly, a condensed physical exam profile that will achieve adequate sensitivity and specificity for prospective diagnosis will be developed. This adaptive physical examination by protocol will be applied to all prospective phase participants, and will be conducted at regional federal medical facilities every two years at government expense.

(2) Entry Criteria

All exposed or control individuals completing the baseline questionnaire and physical examination will be entered into the prospectus; further continuation will depend upon the member's willingness/ability to participate in additional health surveys and condensed examinations.

(3) Loss to Study

At the initiation of the prospective phase loss of an exposed member will not be cause to cease surveillance of his matched control. In the event of a control loss, the next best fit match control will be brought to study, the comprehensive questionnaire will be administered, and the adaptive examination offered. In all cases of loss-to-study, specific reasons will be sought. Medical record reviews of new entrants and death certificate reviews will continue throughout the prospective phase. Mortality analysis based upon time and cause of death within the ascertained groups will be conducted through the study.

(4) Study Length

The prospective phase is initially planned for five consecutive years. Results of the entire effort will be presented to a neutral scientific body. Their recommendation for continuance/discontinuance will be forwarded to the Air Force Surgeon General for final decision.

F. Determination of "Disease"

(1) Introduction

Since this study is dealing with an unknown clinical endpoint, determination of disease state by statistical methodology is a prime scientific thrust of the investigation. From the literature, chloracne is the only recognized disease associated with high exposure to dioxin. The questions to be answered are: (1) Does a history of chloracne invariably lead to future disease? and (2) In the absence of chloracne, is there emergence of other attributable diseases? Under a broad concept of "spectrum of illness", it is probably correct that either of these two conditions are possible, and the clarification of their respective contributions to the natural history of subsequent "disease" is extremely difficult.

(2) Discussion

Inferences about a disease state from this study can be derived from several logical approaches. These approaches can be categorized into two directions: (1) those that deal with symptoms in order to construct a symptom complex that may represent disease, and (2) those that deal with physical signs which in themselves

represent disease. In the former, one can form a subset of individuals that have symptoms (e.g. infertility) and study them during the "retrospective" and prospective phase. Focusing on the overall patterns of alleged symptoms and categorizing them into a symptom complex may identify those individuals with a disease syndrome, or those at higher risk of developing disease (e.g. genetic disorders, cancer). In the latter approach, data on abnormal physical signs (e.g., genetic defects in offspring) and laboratory results can be compared between exposed and non-exposed groups in an attempt to again establish the presence or absence of disease. By putting this array of data into a logical decision-making scheme, specific relative risks can be calculated in the prospective phase.

By the use of combinational and correlational analyses, statements about the probability of a disease state, a subclinical state, and over-reporting bias can be attempted. If the development of symptoms in the exposed group is positively correlated with physical findings, and this correlation is absent in the control group, a statement concerning the existence of a possible disease state can be made. By taking these possible combinations of observations and viewing them in the context of associated positive verifiers, negative bias indicators, and positive exposure index, the probability of over-reporting bias acting in these circumstances can be substantially reduced and, as a result, any statement concerning the existence of disease is strengthened. Similarly, if symptoms in the exposed group do not correlate with the development of findings, but are associated with positive laboratory results, a statement concerning the existence of a subclinical disease state can be made. On the other hand, if comparisons within the RANCH HAND group reveal a negative correlation between reported symptoms and the presence of abnormal physical signs, then an over-reporting bias and/or subclinical disease state is suggested.

Another approach to the determination of a disease state is by taking a normalized exposure index and applying regression techniques to the resulting curve. If there is a positive correlation between increased exposure and the presence of various abnormal physical signs and/or verifiable symptoms, then a symptom complex or disease syndrome is suggested. Factors suspected of altering the classical dose-response curve include cellular repair mechanisms and the release of TCDD from adipose tissue following weight loss. The addition of multivariate techniques to the regression analyses will strengthen statements about the presence of disease. Beyond

these pair-wise and group comparisons, newer techniques of pattern recognition, such as Factor Analysis and Cluster Theory, are being considered in order to achieve a more automatic and objective analysis.

The strength of any inferences made from these analyses is dependent upon the statistical power inherent in the study. In addition, due to the possibility of latency being a factor in this study, a negative analysis at any time within the study does not categorically imply lack of disease, since sufficient time for emergence may not have passed.

C. Exposure Estimates

The exposure to Herbicide Orange and/or other herbicides by RANCH HAND personnel was frequent (almost daily), extensive (anecdotal information suggests that many had direct skin contact) and repetitive over a long period of time (one-year tour for most individuals). Anecdotal information also suggests that most RANCH HAND personnel felt that the herbicides employed in the operations were nontoxic to animals and man and hence, did not exercise the caution in handling that is recommended today. The available records on Operation RANCH HAND indicated that aircrews assigned to the project seldom had a "routine" work schedule or environment. Thus, numerous factors influenced the level of herbicide exposure that RANCH HAND personnel may have received. Such factors included the length of tour, number of tours, crew position, number of herbicide dissemination missions, herbicides employed (Orange, Blue or White), time to and from mission locations, and multiple routes of exposure (inhalation, ingestion and/or percutaneous absorption).

Although industrial hygiene data are not available from defoliation operations during the Vietnam War, the Air Force did conduct extensive industrial hygiene monitoring programs during the dedrumming and incineration of Herbicide Orange during Project PACER HO (see Young, et. al., 1978). These monitoring data (e.g., breathing zone data) and recently unpublished data on percutaneous absorption of 2,4,5-T in humans during actual spray operations (DOW Chemical U.S.A., Midland MI, 1979), when combined with data on characteristics of the C-123 aircraft, number of missions and crew position may permit calculations of exposure estimates.

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SECTION.

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VI. Statistical Methodology

A. Introduction

As described in previous sections on study design, data from all RANCH HAND personnel and their matched controls will be available for statistical analysis. The design of the study is presented in schematic form in Figure 1. $\overline{R}$ refers to the RANCH HAND personnel and $\overline{C}'$ refers to the collection of C-130 crewmembers. As defined, $\overline{R}$ and $\overline{C}'$ will contain individuals who are deceased. Since it appears that there are approximately 25 members of $\overline{C}'$ for every member of $\overline{R}$, significant matching is likely to be possible between the groups (Carpenter, 1977; McKinlay, 1977). The groups will be matched in a 1 to 1 manner as closely as is possible using: age, Vietnam tour lengths, crew position and race. This matching will be accomplished without regard to whether the individual is currently living or dead so that an assessment of mortality can be accomplished. Statistical aspects of this mortality analysis will be described in more detail below.

Referring again to Figure 1, R and C indicate living RANCH HAND members and matched controls. If m_R is the proportion of $\overline{R}$ found to be deceased, then,

$$R = (1 - m_R)\,\overline{R}$$

If the best match for an exposed individual is in fact deceased, the next best control will be selected to complete the study pair for subsequent study phases so that R will equal C and 1 to 1 matching will be preserved.

The questionnaire will provide data concerning specific symptoms and other findings in the R and C groups. Thus various questionnaire finding rates in R, s_R, will be calculated and compared with the corresponding rates in C, s_C.

The questionnaire will allow allocation of RANCH HAND personnel into those with symptoms on questionnaire, indicated by RS, and those without, $R\overline{S}$. Similarly, the control individuals will be placed into symptomatic, indicated CS, and asymptomatic, $C\overline{S}$ groups.

The physical examination performed on individuals from R and C will allow estimation and comparison of rates of physical findings in these groups. Rates of abnormal physical findings can be symbolically indicated as f_R and f_C for RANCH HAND and control groups respectively. Comparison of these rates is very important and details will be discussed below.

Let f_{RS} be the rate of physical findings among RANCH HAND personnel with findings by questionnaire and let $f_{R\bar{S}}$ be the rate of physical findings among RANCH HAND people with no findings of note on their questionnaire. For most disease processes it would be expected that f_{RS} should be a larger rate than $f_{R\bar{S}}$. If f_{RS} is observed to be equal to or less than $f_{R\bar{S}}$, an interpretation of over-reporting may be warranted, although the possibility of sub-clinical disease is recognized. Rates f_{CS} and $f_{C\bar{S}}$ will also be estimated, and comparisons between f_{RS}, f_{CS}, $f_{R\bar{S}}$ and $f_{C\bar{S}}$ will be accomplished.

B. General Concerns

Before proceeding to statistical details regarding this design, two general concerns about the overall design will be discussed.

(1) Adequacy of the Control Group

The RANCH HAND personnel may be characterized as greater risk takers than their controls. It is possible that risk taking behavior could be correlated with differing patterns of disease; e.g., type A personality could be more common among RANCH HAND personnel, thus increasing the incidence of cardiovascular disease. Further, in relation to herbicide exposure, an increased accidental death rate among RANCH HAND personnel could well be a sign of herbicide induced peripheral neuropathy. However, higher accidental death rates in general are expected among risk takers.

(2) Adequacy of Sample Sizes

While this subject will be treated in greater detail below, some general observations are in order. The size of $\bar{R}$ is approximately 1000 individuals. Without formal statistical analysis it should be quite clear that a lethal effect of herbicide which occurs in only 1 out of 2000 controls will be quite difficult to detect unless the herbicide effect is very strong. For example, at a rate of 1 in 2000, 0.5 effected controls are expected. If the basic rate is doubled by herbicide to 2 per 2000, one effected RANCH HAND individual would be expected. At a rate of 1 per 2000 for controls and a rate of 2 per 2000 for RANCH HAND personnel, the probability of observing no affected individuals in both groups is

$$(1 - 1/2000)^{1000} (1 - 2/2000)^{1000} = .22$$

or, in other words, "there is a 22% chance" that no affected individuals will be found in this study. In a population of 100,000 exposed individuals, 100 cases would be expected, 50 of which would be due to herbicide. In short, this study has little statistical power to define the relationship of herbicide to the rarer diseases.

C. Analysis of Mortality Data

Considering the basic cohorts $\overline{R}$ and $\overline{C}$, individuals will be classified into three categories: alive, dead, unaccounted. If a large number of individuals of each group are unaccounted for, the study can obviously be severely biased. Thus, significant effort must be expended to reduce the unaccounted category as far as possible. An initial assessment suggests that at most 1 to 3 percent of both groups can remain unaccounted, with a 1% rate being preferred. This is seen estimating that the mortality rate in $\overline{C}$ may be approximately 0.15 and an unaccountability rate of 0.01 is 6.6% of this basal mortality rate. Whatever the unaccountability rates, the pattern of unaccountability must also be compared between groups $\overline{R}$ and $\overline{C}$. For example, the possibility of age differences or Vietnam tour length differences must be examined, particularly if the unaccountability rates are high. The following will discuss analysis of mortality per se under the assumption that low unaccountability rates have rendered the mortality analysis meaningful.

The mortality data will be analyzed using several different approaches. Crude age-specific death rates will first be calculated and tabulated. Age will be divided into k strata, and person-years will be observed for each stratum as will be the number of deaths in each stratum. In this manner a tabular display will be developed as shown in table 4.

Table 4

STRATIFIED FORMAT OF AGE-SPECIFIC DEATH RATES

	Ranch Hand			Controls		
Age Group	Person Years	Deaths	Death Rate	Person Years	Deaths	Death Rate
1	P_{11}	m_{11}	r_{11}	P_{21}	m_{21}	r_{21}
2	P_{12}	m_{12}	r_{12}	P_{22}	m_{22}	r_{22}
3	P_{13}	m_{13}	r_{13}	P_{23}	m_{23}	r_{23}
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
k	P_{1k}	m_{1k}	r_{1k}	P_{2k}	m_{2k}	r_{2k}

Since the death rates r_{1j} and r_{2j} are Poisson variables, they can be contrasted directly. If the relationship r_{1j} to r_{2j} is found to be consistent between age strata (with statistical variability), a summary mortality index may be calculated. One summary index that will be calculated is the Standarized Mortality Ratio (SMR) which is (Armitage, 1971):

$$SMR = M \times 100$$

$$M = \frac{\sum m_{1j}}{\sum p_{1j} r_{2j}}$$

The term $\sum m_{1j}$ is the total number of deaths observed in the RANCH HAND group while $\sum p_{1j} r_{2j}$ is the number of deaths that would be expected were the age-specific RANCH HAND death rates the same as the age-specific control death rates. Thus the concern is for an SMR greater than 100%. If a crude rate for controls, r_c, is calculated as

$$r_c = \frac{\sum p_{2j} r_{2j}}{\sum p_{2j}}$$

then the indirectly standardized crude rate for the RANCH HAND group r_{RH} is

$$r_{RH} = Mr_c.$$

An approximate statistical test would regard r_{RH} as a Poisson random variable with mean r_c. An alternative approach to the provision of a standarized mortality ratio is that of Breslow and Day (1975). In this treatment, a multiplicative model is employed, for example:

$$\lambda_{ijk} = \theta_i \varphi_j \psi_k$$

where λ_{ijk} is mortality rate, θ_i is the contribution due to population differences (RANCH HAND versus Control), φ_j is the contribution due to age group, and ψ_k is the contribution due to tour length, etc. The statistical approach here is via maximum likelihood.

Logistic models (Walker and Duncan, 1967) have been extensively studied at USAFSAM for application in cardiovascular disease. These models, in the herbicide context would have this form

$$P = \frac{e^{\alpha + \beta_1 A + \beta_2 T + \beta_3 R + \beta_4 E + \beta_5 AE \cdots}}{1 + e^{\alpha + \beta_1 A + \beta_2 T + \beta_3 R + \beta_4 E + \beta_5 AE \cdots}}$$

where

P = probability of death
A = age in years
T = Vietnam tour length
R = indicator variable for race
E = exposure variable

and where α, β_j $(j=1,\ldots)$ are coefficients to be estimated from the data. Testing for a herbicide exposure effect can be accomplished by estimating β_4 and interaction coefficients such as β_5. If all interaction coefficients involving the exposure variable E are zero and E is treated as a 0/1 variable, Cox (1958a, 1958b) has shown that the most powerful test for non-zero β_4 is McNemar's test. This latter test makes full use of the paired design of the study. For McNemar's test, the data are cast into a 2 x 2 table as shown in Table 5. In this table, "a" is the number of pairs in which both members have died, "b" is the number of pairs in which only the RANCH HAND person has died, etc. Using McNemar's test, the test statistic

$$\chi^2 = \frac{|b-c|^2}{b+c}$$

is calculated and referred to the chi-squared distribution with one degree of freedom. Of course the above analyses will be accomplished considering all deaths, and deaths by specific cause.

Table 5

FORMAT OF McNEMAR'S TEST

CONTROLS

RANCH HAND PERSONNEL	DEAD	ALIVE	TOTAL
Dead	a	b	a+b
Alive	c	d	c+d
Total	a+c	b+d	n

D. Analysis of Questionnaire and Physical Examination Data

The Questionnnaire and Physical Examination will produce data of three types: (1) dichotomous, (2) polytomous and (3) continuous.

Dichotomous (present-absent) rates will be evaluated using the tools described above for mortality analysis. For example, the questionnaire will provide data concerning the first occurrence of disease states by age, and standardized rates and relative risks may be calculated. The occurrence of such findings can be related to age, tour length exposure and other variables using logistic models followed by McNemar's test where appropriate. These tests will examine the presence or absence of herbicide effect and allow assessment of the statistical significance on non-unity relative risks. Returning to Figure 1, the eight rates m_R, m_C, s_R, s_C, f_{RS}, $f_{R\bar{S}}$, f_{CS}, $f_{C\bar{S}}$ fully characterize this study in a sense. In this figure, "Vertical comparisons," that is, m_R/m_C, s_R/s_C, f_{RS}/f_{CS}, $f_{R\bar{S}}/f_{C\bar{S}}$ are relative risks of central importance in defining herbicide effects. "Horizontal comparisons," that is f_{RS}/s_R, $f_{R\bar{S}}/(1-s_R)$, f_{CS}/s_C, and $f_{C\bar{S}}/(1-s_C)$ will enable interpretation of over-reporting and subclinical disease.

Polytomous or categorical findings will occur in both questionnaire and physical examination responses. As an example consider retinal findings categorized into four grades, and studied as a function of age and exposure group as represented in Table 6. In this table the x_{ijk}'s are counts of occurrence. In analyzing tables such as these, techniques as described by Bishop, Fienberg and Holland (1975) will be used. Specifically, if m_{ijk} is the expected value of x_{ijk}, general log-linear models of the form

$$\ln m_{ijk} = u + u_1(i) + u_2(j) + u_3(k) + u_{12}(ij) + u_{13}(ik) + u_{23}(jk) + u_{123}(ijk)$$

will be used, where $u_1(i)$ is the effect of RANCH HAND membership alone on cell frequency, $u_{12}(ij)$ is the effect of an interaction on RANCH HAND membership with retinal grade, etc. Under appropriate conditions on expected values of entries in Table 6, the pairing in the study design can be used with the data being organized as shown in Table 7. In Table 7, N_{ij} is the number of pairs such that the exposed person has retinal grade i, and the control person has retinal grade j. Appropriate tests for this setting are indicated by Fleiss (1973).

Table 6

FORMAT CATEGORICAL REPRESENTATION OF RETINAL CHANGES

Retinal Category \ Age Category	RANCH HAND PERSONNEL				CONTROLS			
	1	2	3	4	1	2	3	4
1	X_{111}	X_{112}	X_{113}	X_{114}	X_{211}	X_{212}	X_{213}	X_{214}
2	X_{121}	X_{122}	X_{123}	X_{124}	X_{221}	X_{222}	X_{223}	X_{224}
3	X_{131}	X_{132}	X_{133}	X_{134}	X_{231}	X_{232}	X_{233}	X_{234}
4	X_{141}	X_{142}	X_{143}	X_{144}	X_{241}	X_{242}	X_{243}	X_{244}

Table 7

FORMAT OF PAIRING FOR LOG-LINEAR MODELS OF GRADES OF RETINAL FINDINGS.

RANCH HAND Grade \ Control Grade	1	2	3	4
1	N_{11}	N_{12}	N_{13}	N_{14}
2	N_{21}	N_{22}	N_{23}	N_{24}
3	N_{31}	N_{32}	N_{33}	N_{34}
4	N_{41}	N_{42}	N_{43}	N_{44}

E. Statistical Power

The power $(1 - \beta)$ of a study design is the probability that a specified difference between populations will be detected if it in fact exists. In general, power is a direct function of sample size; that is, for a particular study design, the more subjects measured the larger the study power. Essentially all animal and

human studies concerning herbicide suffer from a lack of adequate consideration of study power. While the present study is not a powerful one against less common disease states as already discussed, it is obviously important nonetheless to exactly specify just what the study can and cannot accomplish. The following presents a preliminary analysis of study power for the case of continuous and dichotomous variables expected from the study.

(1) Power in Continuous Variable Case

Assume that blood cholesterol levels are being compared between RANCH HAND and control groups, and that the coefficient of variation for cholesterol in the control group is 0.1, where the coefficient of variation is the ratio σ_C / μ_C. Assume σ_{RH} equals σ_C. The symbol α is the probability that the study will indicate an effect where none exists, and $1-\beta$ is power as defined before. Consider that the RANCH HAND mean cholesterol μ_{RH} is shifted from the control mean μ_C by the fraction γ. A natural question is to inquire about the study power as a function of available pairs (n) and mean shift γ.

Table 8

POWER CALCULATIONS

ASSUMPTIONS: $\alpha=0.05$, $\sigma_C/\mu_C=0.1$, $\gamma = \mu_{RH}/\mu_C$

		Power = $1-\beta$	
R	γ	n=180	n=450
.20	1.01	.20	.38
.20	1.02	.55	.88
.20	1.05	>.995	>.995
.70	1.01	.86	>.995
.70	1.02	>.995	>.995
.70	1.05	>.995	>.995

Power calculations are displayed in Table 8. Study power in the case of a matched pair design is strongly dependent on the degree of positive correlation produced between the involved groups by the matching procedure. Of course, the degree of correlation can be expressed by the correlation coefficient R which can take values between -1 (negative correlation) and + 1 (positive correlation), and two values of R have been employed in Table 8. From this

table it is seen that if only 450 pairs are studied a 1% shift in mean ($\gamma = 1.01$) will not be reliably detected, but a 2% shift will be detected with a probability of 0.88 if R = 0.2 at least. From this calculation one can infer the need to examine at least 450 pairs to obtain the 2% shift, and to strive for more if possible.

(2) Power in the Dichotomous Variable Case. There is significant discussion in the mathematical statistics literature concerning the efficacy of paired designs in the setting of dichotomous responses (Billewicz, 1964; Ury, 1975; Miettinen, 1970; and several others). Table 9 shows a set of power calculations which are applicable to the present study.

Table 9
POWER CALCULATIONS FOR DICHOTOMOUS VARIABLE CASE AS A FUNCTION OF EFFICACY OF PAIRED DESIGNS

				POWER = $1-\beta$					
P_1	P_2	Rel. Risk	R	n= 160	n= 200	n= 250	n= 300	n= 350	
.05	.01	5	0	.71	.78	.84	.89	.92	*
.04	.01	4	0	.56	.64	.72	.79	.84	
.03	.01	3	0	.40	.45	.51	.57	.61	
.10	.05	2	0	.54	.61	.69	.76	.81	
.20	.10	2	0	.80	.86	.92	.95	>.95	
.05	.01	5	.1	.65/.02	.82/.033	.89/.029	.94/.038	.96/.032	**
.04	.01	4	.1	-	.54/.020	.72/.033	.79/.029	.87/.038	
.03	.01	3	.1	-	-	.38/.020	.55/.033	.68/.046	
.10	.05	2	.1	.60/.058	.67/.054	.76/.055	.77/.036	.85/.048	
.20	.10	2	.1	.81/.036	.92/.056	.94/.043	>.96/.038	>.98/.046	

* $\alpha = .050$
** α as indicated

In this figure, R is again the correlation coefficient indicating the degree of correlation induced between the involved groups by the matching procedure. The probability of the disease among RANCH HAND personnel is symbolized as p_1, while p_2 is the probability of the disease among the controls. Relative risk is the ratio p_1/p_2. With R = 0.1, sign test power tables were used as an exact version of McNemar's test, and therefore different α levels are shown under each power number. Table 9 shows the positive influence of effective pairing in the higher power levels noted. Also, it appears that for $p_2 = 0.01$ and $p_1 = 0.03$, physical examination of 350 pairs (700 examinations) will disclose the three-fold relative risk with probability less than .80. In other words, there is a greater than "20% chance" that a three-fold relative risk on a 1/100 disease state will go undetected in this study if only 350 pairs are examined and if low correlations occur. Once again the need to examine increased numbers of pairs in the study is seen.

To present these dichotomous power calculations more clearly, calculations in the context of actual disease states have been accomplished. The diseases considered are cardiovascular disease and cancer, corresponding to high and low rate illnesses for the age groups presently under investigation.

Cardiovascular Disease. A logistic risk function was fitted to data from 17,455 autopsies gathered in a WHO collaborative study in Czechoslovakia, Sweden and the USSR. The function fitted has the form

$$p = \frac{e^{\alpha + \beta(x-.5) + \gamma(y-.5)}}{1 + e^{\alpha + \beta(x-.5) + \gamma(y-.5)}}$$

where

p = the probability of a complicated coronary lesion

x = age scaled linearly so that x = 0 is equivalent to 38 years, and x = 1 is equivalent to 58 years (the age span of the current study)

y = 1 or 0 if the subject is exposed or not

and α and β were obtained from the data. The function represents a fairly high rate disease in that at 40 years of age 7% of the group had the lesion and at 60 years of age 20% had the lesion. The coefficient γ, represents the exposure effect. Power calculations for $\gamma = \beta$ and $\gamma = .8\beta$ are shown in Table 10. This figure suggests that

if, as a cell toxin, herbicide exposure accelerates cardiovascular disease, this study has a good chance of detecting that acceleration if the herbicide effect is comparable to the age effect. Once again, beneficial effect of pairing is seen.

Cancer. A logistic risk function was fitted to breast cancer data presented by Breslow and Day (1975). The function fitted represents a low rate disease in that at 35 years of age only .000336 of the group had the lesion while at 70 years of age .00676 of the group will have the lesion. Using pairing to achieve a power of 0.80 in this setting, 1312 pairs would be needed, when the exposure effect is equal to the age effect. This exceeds the size of our RANCH HAND cohort, and reinforces the fact that herbicide exposure effects on rarer diseases will not have a high likelihood of being detected by this study, and again supports an attempt to examine as many pairs as possible.

Table 10

POWER CALCULATIONS AS OF A FUNCTION OF HERBICIDE EFFECT

ASSUMPTION: $\alpha = 0.05$

	$\gamma = \beta$		$\gamma = .8\beta$	
Number of Pairs	Power Neglecting Pairing	Power With Pairing	Power Neglecting Pairing	Power With Pairing
100	.69	.93	.81	.82
160	.89	.98	.86	.87
200	> .95	> .995	.93	.95

F. Multivariate Analysis

Some questionnaire and physical examination data naturally fall into groups; for example, fertility/reproduction data, liver function tests, cardiovascular examination tests. In these cases, multivariate analysis may be in order. When the response variables are continuous, they will be analyzed by the well-known multivariate extensions of the generalized linear model.

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VI. Statistical Methodology

A. Introduction

As described in previous sections on study design, data from all Ranch Hand personnel and matched controls will be available for statistical analysis. The design of the study is presented in schematic form in Figure 1. $\mathcal{R}$ refers to the Ranch Hand personnel, and $\mathcal{C}'$ refers to the collection of all possible control crewmembers. As defined, $\mathcal{R}$ and $\mathcal{C}'$ will contain individuals who are deceased. Since $\mathcal{C}'$ may be 10 to 25 times larger than $\mathcal{R}$, a subsample $\mathcal{C}$ of $\mathcal{C}'$ may be constructed matched to $\mathcal{R}$ in a pairwise or 1 to 1 manner (Carpenter, 1977; McKinlay, 1977). The control group $\mathcal{C}$ will be matched to $\mathcal{R}$ as closely as possible using: age, Vietnam tour length, crew position and race. Of course, $\mathcal{C}$ will be constructed without regard to whether the individual is currently living or dead so that an assessment of mortality can be accomplished. Statistical aspects of this mortality analysis will be described in more detail below. If $\mathcal{C}$ cannot be constructed from $\mathcal{C}'$ using pairwise or 1 to 1 matching, it will be constructed using stratified random sampling.

Referring again to Figure 1, R and C indicate living RANCH HAND members and matched controls. If m_R is the proportion of $\mathcal{R}$ found to be deceased, then,

$$R = (1 - m_R)\,\mathcal{R}$$

If the best match for an exposed individual is in fact deceased, the next best control will be selected to complete the study pair for subsequent study phases so that R will equal C and 1 to 1 matching will be preserved.

The questionnaire will provide data concerning specific symptoms and other findings in the R and C groups. Thus various questionnaire finding rates in R, s_R, will be calculated and compared with the corresponding rates in C, s_C.

The questionnaire will allow allocation of RANCH HAND personnel into those with symptoms on questionnaire, indicated by RS, and those without, $R\bar{S}$. Similarly, the control individuals will be placed into symptomatic, indicated CS, and asymptomatic, $C\bar{S}$ groups.

The physical examination performed on individuals from R and C will allow estimation and comparison of rates of physical findings in these groups. Rates of abnormal physical findings can be symbolically indicated as f_R and f_C for RANCH HAND and control groups respectively. Comparison of these rates is very important and details will be discussed below.

Let f_{RS} be the rate of physical findings among RANCH HAND personnel with findings by questionnaire and let $f_{R\bar{S}}$ be the rate of physical findings among RANCH HAND people with no findings of note on their questionnaire. For most disease processes it would be expected that f_{RS} should be a larger rate than $f_{R\bar{S}}$. If f_{RS} is observed to be equal to or less than $f_{R\bar{S}}$, an interpretation of over-reporting may be warranted, although the possibility of sub-clinical disease is recognized. Rates f_{CS} and $f_{C\bar{S}}$ will also be estimated, and comparisons between f_{RS}, f_{CS}, $f_{R\bar{S}}$ and $f_{C\bar{S}}$ will be accomplished.

B. General Concerns

Before proceeding to statistical details regarding this design, three general concerns about the overall design will be discussed.

(1) Adequacy of the Control Group.

Candidate groups comprising C', the set of all possible control crewmembers, ~~include: C-130 crewmembers, C-7 crewmembers, and~~ non-Ranch Hand ~~C-123 crewmembers.~~ Known and estimated factors relevant to these potential control groups and Ranch Hand personnel are listed and evaluated in Table below. Considering the estimated factors, a subjective estimate from 1+ to 4+ is provided. At the present time, no data have been found to suggest that aircraft fuels ~~of the~~ type involved in these aircraft here have the capability to exert a health impact considering exposures and exposure routes experienced. On the other hand, exposure to insecticide may be a significant confounding factor. Further, Ranch Hand personnel did have more combat exposure than the other groups thus far considered.

and this stressful exposure could exert a long term effect on morbidity and mortality rates in this group. ~~For example~~ also, the fact that many Ranch Hand personnel volunteered for their hazardous duty suggests that this group may contain individuals who are ~~significant~~ risk takers. This factor could inject ~~a~~ significant bias into the study. For example, it is ~~possible~~ that risk taking behavior could be correlated with differing patterns of disease; for instance, type A personality type could be more common among the intense, risk taking Ranch Hand personnel, thus increasing the incidence of cardiovascular disease. ~~Additionally,~~ In relation to herbicide exposure, an increased accidental death rate among Ranch Hand personnel could well be a sign of an indicator herbicide induced peripheral neuropathy. However, higher accidental death rates in general are expected among risk takers. Actual time spent in Vietnam could also be an important factor to control on. First, the total time spent in Vietnam indicates a ~~total~~ magnitude of stress imposed on the individual. Further, recognizing that herbicide effects include hepatic hyperplasia, hepatoma and other injury, it seems appropriate to control on exposure to hepatic parasites and aflotoxin, which would be related to exposure

COMPARISON OF THE STUDY GROUP TO POSSIBLE CONTROL GROUPS BY

KNOWN AND ESTIMATED FACTORS

KNOWN FACTORS	STUDY GROUP	POSSIBLE CONTROL GROUPS		
	Ranch Hand C123	Non-Ranch Hand C123	C-7	C-130
POPULATION RANGE	800-1200	4000-6000	4000-6000	20,000-25,000
OFFICER/ENLISTED CREW RATIO	2:1	2:1	2:1	3:2
AIRCRAFT FUEL (AV-GAS)	YES/NO*	YES	YES	NO (JP-4)
OCCUPATIONAL HERBICIDE EXPOSURE	YES	NO/YES**	NO	NO
ESTIMATED FACTORS				
OCCUPATIONAL INSECTICIDE EXPOSURE	2+	4+	0	0
COMBAT HAZARD	4+	3+	3+	1+
RVN-IN COUNTRY ASSIGNMENT	4+	4+	4+	1+

* - KC-123 (modified from BC-123 in 196X) has booster jets employed JP-4

** Contaminated Aircraft re-configured for transport in any hand resulted in herbicide exposure by non RANCH HAND

total time in Vietnam.

As indicated in Table no available control group is fully ideal. satisfactory. Differences in risk taking, occupational insecticide exposure and aircraft fuel exposure appear most important. Risk taking differences can be approached using a risk taking scale and this will be discussed under mortality analysis and under analysis of questionnaire and physical examination data. Aircraft fuel differences may well be dismissed as an unlikely or nonexistent effect with further literature search and further literature search is also required as regards the possibility of an insecticide effect.

(2) Adequacy of Sample Sizes

While this subject will be treated in greater detail below, some general observations are in order. The size of R is approximately 1000 individuals. Without formal statistical analysis it should be quite clear that a lethal effect of herbicide which occurs in only 1 out of 2000 controls will be quite difficult to detect unless the herbicide effect is very strong. For example, at a rate of 1 in 2000, 0.5 effected controls are expected. If the basic rate is doubled by herbicide to 2 per 2000, one effected RANCH HAND individual would be expected. At a rate of 1 per 2000 for controls and a rate of 2 per 2000 for RANCH HAND personnel, the probability of observing no affected individuals in both groups is

$$(1 - 1/2000)^{1000} (1 - 2/2000)^{1000} = .22$$

or, in other words, "there is a 22% chance" that no affected individuals will be found in this study. In a population of 100,000 exposed individuals, 100 cases would be expected, 50 of which would be due to herbicide. In short, this study has little statistical power to define the relationship of herbicide to the rarer diseases.

(3) Additional Study Biases

Section VI B (1) has already addressed potential elements of bias in this study concerned with specific differences between Ranch Hand personnel and the study control group. Some additional bias sources are discussed in this section. Not all of these biases can be corrected, indeed many will have to be simply acknowledged as weaknesses of the study.

The telephone interview. Here there will be at least two types of epidemiological bias: lying and nonresponse. Military subjects close to their retirement date may tend to ~~lie~~ exaggerate about their health- exaggerating their symptoms so that they may become eligible for retirement benefits. Those subjects who are pilots or who have other careers requiring a clean physical record, may lie by suppressing their symptoms. There will be some subjects who simply will not talk with any government related interviewer. Patterns of lying and nonresponse may be quite different between the two involved groups (Ranch Hand and control).

To understand the effect of lying on the estimation of relative risk and the odds ratio, let S stand for the presence of a certain symptom, and $\bar{S}$ denote its abscence. Misclassification due to lying may be represented as:

REPORTED STATUS	ACTUAL STATUS S	ACTUAL STATUS $\bar{S}$	TOTAL
S	A	B	A + B
$\bar{S}$	C	D	C + D
	A + C	B + D	

The proportion of correctly classified positives is defined by A/(A+C) and is called the sensitivity of the classification scheme; the proportion of correctly classified negatives D/(B+C) is called the specificity.

When there is non-differential misclassification, that is, when the sensitivity is the same among the exposed and nonexposed, and the specificity is the same among the exposed and nonexposed, the bias induced in the estimate of relative risk will be toward the null value. The situation may be summarized by the following presentation:

REPORTED STATUS	ACTUAL STATUS: EXPOSED S	EXPOSED $\bar{S}$	EXPOSED TOTAL	NONEXPOSED S	NONEXPOSED $\bar{S}$	NONEXPOSED TOTAL
S	a	b	a+b	e	f	e+f
$\bar{S}$	c	d	c+d	g	h	g+h
	a+c	b+d	n	e+g	f+h	n

Using this representation, the true relative risk is $(a+c)/n \div (e+g)/n$, and the apparent relative risk is $(a+b)/n \div (e+f)/n$. Figure provides a graphic representation of how apparent relative risk varies as a function of sensitivity and specificity. For these curves the true relative risk is 2 with the exposed population having a symptom incidence of 0.1 and the nonexposed population having a symptom incidence of 0.05 (Copeland et al., 1977). The effect of non-differential misclassificati on the odds ratio is as severe as that shown in Figure for relative risk. A technique does exist for correcting the estimate of relative risk to account for misclassification, but the technique requires knowledge of the sensitivity and specificity of the classification scheme, knowledge that may not exist in this study at least initially. (~~It~~ may be possible to estimate the sensitivity and specificity after the physical examination data are available.) It should be noted that since the above remarks are concerned with relative risk, the number n of subjects in each group is irrelevant, as the results shown are independent of n.

If the misclassification is differential, an estimate of relative risk that is biased away from the null value can result. This will occur in situations in which the Ranch Hand personnel and controls do not lie in the same way about their symptoms. (Copeland, et al., 1977).

The effect of questionnaire nonresponse will be twofold: (a) it will lower the sample size, and (b) since nonresponse will ~~no doubt~~ may occur for different reasons in the two groups, it will lead to bias in the apparent relative risk in a manner similar to that described above.

The prospective phase. Loss to follow up may occur for different reasons in the Ranch Hand group than in the Control group. In addition, this censoring may be related to the death experience, especially in the Ranch Hand group. For example, the disappearance of a Ranch Hand individual may be due to the effect of exposure, making loss to follow up dependent on the death process. It should be noted that all currently available survival analysis techniques are founded on the assumption that censoring is independent of the death process, and all comparative tests require that the censoring mechanism operate in the same way in both groups considered. The effect of departures from the assumptions is not well understood.

The physical examination. Non compliance will be a general problem in both the exposed and nonexposed groups. If the reasons for noncompliance are systematically different in the two groups, significant bias can result. For example, a number of Ranch Hand personnel may refuse to comply acting under advice from legal counsel. Noncompliant controls would not likely have this cause but be more motivated by reasons such as inability to take time from work. Ranch Hand individuals motivated by legal advice or

other similar reasons, may in fact be symptomatic, thus decreasing the number of positive findings in this study.

In concluding this section on study concerns, it seems appropriate to remind the reader that no single observational study such as the present one is likely to resolve all questions. Rather, any single study must be evaluated in the light of other available data and other similar efforts (McKinlay, 1975).

C. Analysis of Mortality Data

Considering the basic cohorts $\mathcal{R}$ and $\mathcal{C}$, individuals will be classified into three categories: alive, dead, unaccounted. If a large number of individuals of each group are unaccounted for, the study can obviously be severely biased. Thus, significant effort must be expended to reduce the unaccounted category as far as possible. An initial assessment suggests that at most 1 to 3 percent of both groups can remain unaccounted, with a 1% rate being preferred. This is seen estimating that the mortality rate in $\mathcal{C}$ may be approximately 0.15 and an unaccountability rate of 0.01 is 6.6% of this basal mortality rate. Whatever the unaccountability rates, the pattern of unaccountability must also be compared between groups $\mathcal{R}$ and $\mathcal{C}$. For example, the possibility of age differences or Vietnam tour length differences must be examined, particularly if the unaccountability rates are high. The following will discuss analysis of mortality per se under the assumption that low unaccountability rates have rendered the mortality analysis meaningful.

The mortality data will be analyzed using several different approaches. Crude age-specific death rates will first be calculated and tabulated. Age will be divided into k strata, and person-years will be observed for each strata as will be the number of deaths in each strata. In this manner a tabular display will be developed as shown in table 4.

Table 4

STRATIFIED FORMAT OF AGE-SPECIFIC DEATH RATES

	Ranch Hand			Controls		
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1	P_{11}	m_{11}	r_{11}	P_{21}	m_{21}	r_{21}
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3	P_{13}	m_{13}	r_{13}	P_{23}	m_{23}	r_{23}
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
k	P_{1k}	m_{1k}	r_{1k}	P_{2k}	m_{2k}	r_{2k}

Since the death rates r_{1j} and r_{2j} are Poisson variables, they can be contrasted directly. If the relationship r_{1j} to r_{2j} is found to be consistent between age strata (within statistical variability), a summary mortality index may be calculated. One summary index that will be calculated is the Standarized Mortality Ratio (SMR) which is (Armitage, 1971):

$$SMR = M \times 100$$

$$M = \frac{\sum m_{1j}}{\sum p_{1j} r_{2j}}$$

The term $\sum m_{1j}$ is the total number of deaths observed in the RANCH HAND group while $\sum p_{1j} r_{2j}$ is the number of deaths that would be expected were the age-specific RANCH HAND death rates the same as the age-specific control deaths rates. Thus the concern is for an SMR greater than 100%. If a crude rate for controls, r_c, is calculated as

$$r_c = \frac{\sum p_{2j} r_{2j}}{\sum p_{2j}}$$

then the indirectly standardized crude rate for the RANCH HAND group r_{RH} is

$$r_{RH} = M r_c.$$

An approximate statistical test would regard r_{RH} as a Poisson random variable with mean r_c. An alternative approach to the provision of a standarized mortality ratio is that of Breslow and Day (1975). In this treatment, a multiplicative model is employed, for example:

$$\lambda_{ijk} = \theta_i \varphi_j \psi_k$$

where λ_{ijk} is mortality rate, θ_i is the contribution due to population differences (RANCH HAND versus Control), φ_j is the contribution due to age group, and ψ_k is the contribution due to tour length, etc. The statistical approach here is via maximum likelihood.

Logistic models (Walker and Duncan, 1967) have been extensively studied at USAFSAM for application in cardiovascular disease. These models, in the herbicide context would have the form

$$P = \frac{e^{\alpha + \beta_1 A + \beta_2 T + \beta_3 R + \beta_4 E + \beta_5 AE + \cdots}}{1 + e^{\alpha + \beta_1 A + \beta_2 T + \beta_3 R + \beta_4 E + \beta_5 AE + \cdots}}$$

where

P = probability of death
A = age in years
T = Vietnam tour length
R = indicator variable for race
E = exposure variable

and where α, β_j $(j=1,\ldots)$ are coefficients to be estimated from the data. Testing for a herbicide exposure effect can be accomplished by estimating β_4 and interaction coefficients such as β_5.
If all interaction coefficients involving the exposure variable E are zero and E is treated as a 0/1 varialble, Cox (1958a, 1958b) has shown that the most powerful test for non-zero β_4 is McNemar's test. This latter test makes full use of the paired design of the study. For McNemar's test, the data are cast into a 2 x 2 table as shown in Table 5. In this table, "a" is the number of pairs in which both members have died, "b" is the number of pairs in which only the RANCH HAND person has died, etc. Using McNemar's test, the test statistic

$$\chi^2 = \frac{|b-c|^2}{b+c}$$

is calculated and referred to the chi-squared distribution with one degree of freedom. Of course the above analyses will be accomplished considering all deaths, and deaths by specific cause.

Table 5

FORMAT OF McNEMAR'S TEST

CONTROLS

RANCH HAND PERSONNEL	DEAD	ALIVE	TOTAL
Dead	a	b	a+b
Alive	c	d	c+d
Total	a+c	b+d	n

As discussed in section VI.B.(1) above, it is postulated that Ranch Hand personnel may be properly characterized as risk takers. This risk taking behavior may be associated with increased mortality from a variety of causes. Let us first consider accidental death. If herbicide exposure has caused neuropathy in the Ranch Hand personnel, one should anticipate that this disability would increase the probability of accidental death. However, accidental death rates among Ranch Hand personnel must surely be corrected for ~~their possible~~ risk taking tendency. A method of accomplishing this correction for risk taking would be to employ a psychological instrument such as the Life Experience Inventory (Torrance, 1954) of the Sensation Seeking Scale (Zuckerman, 1972). Both control and Ranch Hand mortality could be corrected using these measures, with the resultant rates being perhaps less biased and therefore a better indicator of herbicide effect. The same argument may apply to death rates from cancer under the hypothesis that risk taking behavior whould tend to increase the probability that a Ranch Hand individual would ~~more likely encounter an environment that was not well controlled, and was therefore~~ experienced increased carcinogen exposure. The situation concerning mortality and morbidity from cardiovascular disease is very interesting. Ranch Hand personnel in many instances volunteered for their particular duty. This step had the well known effect of improving ~~their~~ officer evaluation score. This volunteerism may be part of a Type A behavior syndrome which has been correlated with enhanced atherosclerosis. Instruments for determining Type A behavior have been developed and these scores may be profitably used to correct cardiovascular mortality and morbidity rates.

D. Analysis of Questionnaire and Physical Examination Data

The Questionnnaire and Physical Examination will produce data of three types: (1) dichotomous, (2) polytomous and (3) continuous.

Dichotomous (present-absent) rates will be evaluated using the tools described above for mortality analysis. For example, the questionnaire will provide data concerning the first occurrence of disease states by age, and standardized rates and relative risks may be calculated. The occurrence of such findings can be related to age, tour length exposure and other variables using logistic models followed by McNemar's test where appropriate. These tests will examine the presence or absence of herbicide effect and allow assessment of the statistical significance on non-unity relative risks. Returning to Figure 1, the eight rates m_R, m_C, s_R, s_C, f_{RS}, $f_{R\bar{S}}$, f_{CS}, $f_{C\bar{S}}$ fully characterize this study in a sense. In this figure, "Vertical comparisons," that is, m_R/m_C, s_R/s_C, f_{RS}/f_{CS}, $f_{R\bar{S}}/f_{C\bar{S}}$ are relative risks of central importance in defining herbicide effects. "Horizontal comparisons," that is f_{RS}/s_R, $f_{R\bar{S}}/(1-s_R)$, f_{CS}/s_C, and $f_{C\bar{S}}/(1-s_C)$ will enable interpretation of over-reporting and subclinical disease.

Polytomous or categorical findings will occur in both questionnaire and physical examination responses. As an example consider retinal findings categorized into four grades, and studied as a function of age and exposure group as represented in Table 6. In this table the x_{ijk}'s are counts of occurrence. In analyzing tables such as these, techniques as described by Bishop, Fienberg and Holland (1975) will be used. Specifically, if m_{ijk} is the expected value of x_{ijk}, general log-linear models of the form

$$\ln m_{ijk} = u + u_1(i) + u_2(j) + u_3(k) + u_{12}(ij) + u_{13}(ik) + u_{23}(jk) + u_{123}(ijk)$$

will be used, where $u_1(i)$ is the effect of RANCH HAND membership alone on cell frequency, $u_{12}(ij)$ is the effect of an interaction on RANCH HAND membership with retinal grade, etc. Under appropriate conditions on expected values of entries in Table 6, the pairing in the study design can be used with the data being organized as shown in Table 7. In Table 7, N_{ij} is the number of pairs such that the exposed person has retinal grade i, and the control person has retinal grade j. Appropriate tests for this setting are indicated by Fleiss (1973).

Table 6

FORMAT CATEGORICAL REPRESENTATION OF RETINAL CHANGES

Retinal Category \ Age Category	RANCH HAND PERSONNEL				CONTROLS			
	1	2	3	4	1	2	3	4
1	X_{111}	X_{112}	X_{113}	X_{114}	X_{211}	X_{212}	X_{213}	X_{214}
2	X_{121}	X_{122}	X_{123}	X_{124}	X_{221}	X_{222}	X_{223}	X_{224}
3	X_{131}	X_{132}	X_{133}	X_{134}	X_{231}	X_{232}	X_{233}	X_{234}
4	X_{141}	X_{142}	X_{143}	X_{144}	X_{241}	X_{242}	X_{243}	X_{244}

Table 7

FORMAT OF PAIRING FOR LOG-LINEAR MODELS OF GRADES OF RETINAL FINDINGS.

RANCH HAND Grade \ Control Grade	1	2	3	4
1	N_{11}	N_{12}	N_{13}	N_{14}
2	N_{21}	N_{22}	N_{23}	N_{24}
3	N_{31}	N_{32}	N_{33}	N_{34}
4	N_{41}	N_{42}	N_{43}	N_{44}

As indicated in section VI.E concerning analysis of mortality data, risk taking behavior among Ranch Hand personnel could be correlated with changed mortality and morbidity patterns. Morbidity from cancer could be examined against a risk taking scale, and morbidity from cardiovascular disease could be corrected for personality type effect.

Analysis of Fertility/Reproduction Data. The herbicides under consideration in this study have been alleged to effect fertility and/or reproductive functioning. Decreased libido, increased number of miscarriages and increased number of abnormal offspring have been alleged effects. We will attempt to address these allegations in this study by analyzing three primary variables, at least total number of conceptions since exposure in Vietnam, number of miscarriages in spouses since exposure in Vietnam, and number of abnormal offspring since exposure in Vietnam. The study questionnaire will provide [redacted] the number of miscarriages, abnormal offspring and total number of live births. The sum of the number of miscarriages and the number of live births will provide an estimate of the total number of conceptions. There has been some indication that the divorce rate may be higher in the Ranch Hand group than in the controls and this may render the average number of years of marriage and the distribution of the years of marriage different in the two groups. This will be investigated and adjusted for if need be, either by analyzing total number of conceptions divided by (or normalized by) the number of years of marriage, or by using a more detailed covariance analysis. Further, the ratio of the number of miscarriages to adjusted total conceptions will be calculated and compared as will be the ratio of the number of abnormal births and adjusted total conceptions.

E. Survival Analysis

This section is written to extend and complement sections C and D. The defining common attribute of the techniques discussed in this section is that they deal with events which (a) correspond to categorical changes in health status, and (b) occur at definite and observable times. These methods may therefore be applied to studies of mortality (as the name"survival analysis" implies) as well as to studies in morbidity.

This section consists of two parts. Methods of comparing survival distributions which neglect possibly confonding effects of covariables are first presented. Then, methods for dealing with covariates are briefly reviewed. The approaches disussed here are just a small fraction of what is available.

Survival analysis without covariates. The first step in the statistical analysis of survival data is descriptive,i.e., construction of summary measures which provide a basis for comparing different exposure groups without any allowance for the effects of possibly confounding variables (e.g. age) except perhaps for some limited stratification. Since one must expect many "losses to follow-up", only methods which take full cognizance of this complication will be considered. It should be pointed out that all the methods described below assume independence between censoring (e.g. loss to follow-up) and death or morbid event, although some techniques permit different patterns of censoring in different exposure groups.

The life table method can be adapted to obtain a step-function approximation to survival distributions in the presence of censoring (Chiang, 1968, Gross and Clark, 1975). However, the product-limit estimator due to Kaplan and Meier (1958) may be preferred due to its intrinsic properties and its relationship to more refined methods.

The failure time distribution is the function $F^0(t)$ which provides the probability of death at or before time t in the study. The Kaplan-Meier estimator of $F^0(t)$ is $\hat{F}^0(t)$ where

$$\hat{F}^0(t) = 1 - \prod_{i \in \mathcal{D}(t)} \left[1 - 1/R(T_i)\right]$$

In this equation, $\mathcal{D}(t)$ is the "death set " at time t, i.e., the set of all indices i of individuals who were observed to fail before time t. $R(T_i)$ is the number of indivudals who were at risk just before time T_i, the time of death (or morbid event) of the i^{th} study individual, $i \in \mathcal{D}(t)$. This product-limit estimator due to Kaplan and Meier is maximum likelihood in the class of all possible failure time distribution functions. This estimator can be computed and graphed using a component of the BMDP survival analysis computer package.

Assuming that failure time distributions have been calculated for Ranch Hand individuals and controls, the next question concerns testing the null hypothesis of equality between the distributions. When only two such distributions are being

compared, one may use the nonparametric procedures generalizing Wilcoxon's statistic proposed by E. Gehan (1965a,b) and discussed by N. Mantel (1967). When more than two such distributions are being compared one may use the nonparametric procedure generaliz ing the Kruskal-Wallis statistic proposed by N. Breslow (1970). The Breslow statistic is calculated as part of the BMDP survival analysis package.

Survival analysis with covariates. These methods allow adjustment of mortality rates or morbidity rates using covariates such as age, race, Vietnam tour length , crew position, risk taking score etc. For the purposes of this discussion it will be assumed that the covariables are categorical, that there are only two such covariables and that the covariables do not interact in affecting the hazard of death or morbidity. These assumptions can all be relaxed using av ailable methods.

The hazard function for the ith individual in the study is the function denoted $h_i(t)$ which provides the probability of death or morbid event in the time interval $(t, t+dt)$. The function $H_i(t)$ where

$$H_i(t) = \int_0^t h_i(\tau)d\tau$$

is called the cummulative hazard for the ith individual. It is readily seen that the failure distribution time $F_i^0(t)$ is given by:

$$F_i^0(t) = 1 - \exp(-H_i(t))$$

From this last equation it follows that h_i and F_i^o are transforms of each other, whence the dependence of F_i^o on covariables may be modeled via h_i. This may be accomplished as follows: Let $X_i(t)$ and $Y_i(t)$ denote discrete valued stochastic processes pertaining to the ith individual and describing two covariates of interest (e.g. one may be an exposure variable and the other may be a covariate such as age or crew position). The basic model for hazard is:

$$h_i(t) = \exp\left[\xi X_i(t) + \eta Y_i(t) \right]$$

where ξ and η are "log-relative risks". A thorough study of statistical inference on these relative risks is provided in Frank (1977).

F. Statistical Power

The power $(1 - \beta)$ of a study design is the probability that a specified difference between populations will be detected if it in fact exists. In general, power is a direct function of sample size; that is, for a particular study design, the more subjects measured the larger the study power. Essentially all animal and

human studies concerning herbicide suffer from a lack of adequate consideration of study power. While the present study is not a powerful one against less common disease states as already discussed, it is obviously important nonetheless to exactly specify just what the study can and cannot accomplish. The following presents a preliminary analysis of study power for the case of continuous and dichotomous variables expected from the study.

(1) Power in Continuous Variable Case

Assume that blood cholesterol levels are being compared between RANCH HAND and control groups, and that the coefficient of variation for cholesterol in the control group is 0.1, where the coefficient of variation is the ratio σ_c / μ_c . Assume σ_{RH} equals σ_C. The symbol α is the probability that the study will indicate an effect where none exists, and $1-\beta$ is power as defined before. Consider that the RANCH HAND mean cholesterol μ_{RH} is shifted from the control mean μ_c by the fraction γ . A natural question is to inquire about the study power as a function of available pairs (n) and mean shift γ.

Table 8

POWER CALCULATIONS

ASSUMPTIONS: $\alpha=0.05$, $\sigma_c/\mu_c=0.1$, $\gamma = \mu_{RH}/\mu_c$

		Power = $1-\beta$	
R	γ	n=180	n=450
.20	1.01	.20	.38
.20	1.02	.55	.88
.20	1.05	>.995	>.995
.70	1.01	.86	>.995
.70	1.02	>.995	>.995
.70	1.05	>.995	>.995

Power calculations are displayed in Table 8. Study power in the case of a matched pair design is strongly dependent on the degree of positive correlation produced between the involved groups by the matching procedure. Of course, the degree of correlation can be expressed by the correlation coefficient R which can take values between −1 (negative correlation) and + 1 (positive correlation), and two values of R have been employed in Table 8. From this

table it is seen that if only 450 pairs are studied a 1% shift in mean ($Y = 1.01$) will not be reliably detected, but a 2% shift will be detected with a probability of 0.88 if $R = 0.2$ at least. From this calculation one can infer the need to examine at least 450 pairs to obtain the 2% shift, and to strive for more if possible.

(2) Power in the Dichotomous Variable Case. There is significant discussion in the mathematical statistics literature concerning the efficacy of paired designs in the setting of dichotomous responses (Billewicz, 1964; Ury, 1975; Miettinen, 1970; and several others). Table 9 shows a set of power calculations which are applicable to the present study.

Table 9
POWER CALCULATIONS FOR DICHOTOMOUS VARIABLE CASE AS A FUNCTION OF EFFICACY OF PAIRED DESIGNS

				POWER = $1-\beta$					
P_1	P_2	Rel. Risk	R	n= 160	n= 200	n= 250	n= 300	n= 350	
.05	.01	5	0	.71	.78	.84	.89	.92	*
.04	.01	4	0	.56	.64	.72	.79	.84	
.03	.01	3	0	.40	.45	.51	.57	.61	
.10	.05	2	0	.54	.61	.69	.76	.81	
.20	.10	2	0	.80	.86	.92	.95	>.95	
.05	.01	5	.1	.65/.02	.82/.033	.89/.029	.94/.038	.96/.032	**
.04	.01	4	.1	-	.54/.020	.72/.033	.79/.029	.87/.038	
.03	.01	3	.1	-	-	.38/.020	.55/.033	.68/.046	
.10	.05	2	.1	.60/.058	.67/.054	.76/.055	.77/.036	.85/.048	
.20	.10	2	.1	.81/.036	.92/.056	.94/.043	>.96/.038	>.98/.046	

* $\alpha = .050$
** α as indicated

In this figure, R is again the correlation coefficient indicating the degree of correlation induced between the involved groups by the matching procedure. The probability of the disease among RANCH HAND personnel is symbolized as p_1, while p_2 is the probability of the disease among the controls. Relative risk is the ratio p_1/p_2. With R = 0.1, sign test power tables were used as an exact version of McNemar's test, and therefore different α levels are shown under each power number. Table 9 shows the positive influence of effective pairing in the higher power levels noted. Also, it appears that for $p_2 = 0.01$ and $p_1 = 0.03$, physical examination of 350 pairs (700 examinations) will disclose the three-fold relative risk with probability less than .80. In other words, there is a greater than "20% chance" that a three-fold relative risk on a 1/100 disease state will go undetected in this study if only 350 pairs are examined and if low correlations occur. Once again the need to examine increased numbers of pairs in the study is seen.

To present these dichotomous power calculations more clearly, calculations in the context of actual disease states have been accomplished. The diseases considered are cardiovascular disease and cancer, corresponding to high and low rate illnesses for the age groups presently under investigation.

Cardiovascular Disease. A logistic risk function was fitted to data from 17,455 autopsies gathered in a WHO collaborative study in Czechoslovakia, Sweden and the USSR. The function fitted has the form

$$p = \frac{e^{\alpha + \beta(x-.5) + \gamma(y-.5)}}{1 + e^{\alpha + \beta(x-.5) - \gamma(y-.5)}}$$

where

p = the probability of a complicated coronary lesion

x = age scaled linearly so that x = 0 is equivalent to 38 years, and x = 1 is equivalent to 58 years (the age span of the current study)

y = 1 or 0 if the subject is exposed or not

and α and β were obtained from the data. The function represents a fairly high rate disease in that at 40 years of age 7% of the group had the lesion and at 60 years of age 20% had the lesion. The coefficient γ, represents the exposure effect. Power calculations for $\gamma = \beta$ and $\gamma = .8\beta$ are shown in Table 10. This figure suggests that

if, as a cell toxin, herbicide exposure accelerates cardiovascular disease, this study has a good chance of detecting that acceleration if the herbicide effect is comparable to the age effect. Once again, beneficial effect of pairing is seen.

Cancer. A logistic risk function was fitted to breast cancer data presented by Breslow and Day (1975). The function fitted represents a low rate disease in that at 35 years of age only .000336 of the group had the lesion while at 70 years of age .00676 of the group will have the lesion. Using pairing to achieve a power of 0.80 in this setting, 1312 pairs would be needed, when the exposure effect is equal to the age effect. This exceeds the size of our RANCH HAND cohort, and reinforces the fact that herbicide exposure effects on rarer diseases will not have a high likelihood of being detected by this study, and again supports an attempt to examine as many pairs as possible.

Table 10

POWER CALCULATIONS AS OF A FUNCTION OF HERBICIDE EFFECT

ASSUMPTION: $\alpha = 0.05$

	$\gamma = \beta$		$\gamma = .8\beta$	
Number of Pairs	Power Neglecting Pairing	Power With Pairing	Power Neglecting Pairing	Power With Pairing
100	.69	.93	.81	.82
160	.89	.98	.86	.87
200	> .95	> .995	.93	.95

6. Multivariate Analysis

Some questionnaire and physical examination data naturally fall into groups; for example, fertility/reproduction data, liver function tests, cardiovascular examination tests. In these cases, multivariate analysis may be in order. When the response variables are continuous, they will be analyzed by the well-known multivariate extensions of the generalized linear model.

The general approach to multivariate analysis of polytomous data considers all classification factors and all variables as "factors" in a multi-way contingency table. Log-linear models as described above for polytomous data will be employed where appropriate.

N. Indices of Exposure and Response.

Indices can be useful to sharpen a statistical analysis and can be helpful in summarizing responses. In this discussion, ~~three~~ two types of indices will be considered: indices related to Vietnam herbicide exposure, and indices related to ~~CONUS~~ herbicide exposure independent of Vietnam experience, ~~and indices related to fertility and reproductive outcome in study personnel.~~

Vietnam herbicide exposure. In the above discussion of statistical methodologies, exposure variables appeared. In the logistic formula on page 33, the variable E was shown which could be either dicotomous, polytomous or continuous. In the use of logistic functions to discuss study power, the exposure variable was taken as dicotomous. If a polytomous or continuous exposure variable E can be constructed, significant sharpening of the study analysis would be accomplished. For example, biases in this study could lead one to suspect that differences between Ranch Hand personnel and controls were in fact due to factors other than a herbicide effect. If however, in addition to differences between Ranch Hand personnel and controls, one were able to show a regression of mortality and/or morbidity on an exposure index E, the case for a bone fide herbicide effect would be firmer. ~~How then could one construct~~ The exposure index E? Assuming that Ranch Hand personnel were exposed to herbicide only during actual spraying missions, total flying time spent on such missions by an individual, becomes a meaningful measure of his exposure.

Presently, records are being sought which would provide this measure of exposure (total flying time spent on spraying missions) by individual. Literature review has indicated that herbicidal agents used differ in their toxicity, so that, if possible, flying times spent spraying should be broken out by formulation sprayed.

CONUS Herbicide exposure. Individuals in both the Ranch Hand and control groups will have had varying exposure to herbicide in the United States. Particularly, individuals from specific farming and/or foresting areas, may have had a significant background exposure. Data on place of residence in the CONUS, and information concerning home practices (gardening etc.) could be used to build a background exposure index E_b . In lieu of consturcting this index, one can hope that randomization would even out background exposure between Ranch Hand and control groups, however, good statistical practice would seem to require that this randomization be in fact tested for.

Exposure weights

I. Next Steps

This statistical protocol is evolving as more is learned about the cohorts to be studied. The next weeks and months will be the occasion for the examination of several issues not addressed above or only touched upon. There must be further consideration of RANCH HAND personnel as risk takers. Also, there must be further analysis of bias due to selective participation in the study. In the analysis of mortality, questionnaire and physical examination data, more attention must be paid to the construction of meaningful exposure and disease indices. More details concerning survival analysis methods will be developed in the future with examination of the details of application to this study and power.

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VII. Data Repository

Throughout the 6-year period of this investigation data collection methods will be integrated by use of computer systems. A data repository will be established at the USAFSAM. A master file will be formed for each exposed member and his matched control. The individual master files will be keyed to one or more identifiers.

Individual data bits and their sources are as follows:

(1)	Questionnaire	a. Initial (telephone) b. Indepth interview (personal and telephone) c. Prospective (telephone)
(2)	Psychological Battery	a. Initial b. Prospective
(3)	Physical Examination	a. Initial b. Prospective
(4)	Medical Records	a. Active duty b. VA c. Civilian d. Dependent
(5)	Historical	a. Military personnel files b. Flight records c. Military unit
(6)	Death Certificates	a. Study members b. Dependents
(7)	Birth Certificates	a. Dependents

The computer software for the data analysis phase will be prepared to assure proper data conversion, quality control and standardization of test measurements. Quality control areas will include verification of identification data, range checks, and identification/correction of ambiguous or conflicting data. The repository capability of this investigation will allow complete computer files on the exposed/control populations with potential momentary recall.

VIII. Recognized Study Difficulties and Corrective Measures

A. Medical Precedence

(1) Problem

A departure from the usual methodological approach characterizes this particular epidemiological investigation. Clearly there is no historical "roadmap of methodology" to conduct this study, complete with all of its constraints. Most occupational exposure studies utilize the presentation of an unusual disease to justify the initiation of a comprehensive study. A rare disease or a common disease in an uncommon site, or one with an unusual presentation appearing in space-time clusters, often in an unusual population or age group, generates the requirement for a new study. In the case of Herbicide Orange, the evidence for long-term human effects has remained extremely tenuous and controversial. Despite the unique problems that this study possesses, such as the lack of clinically defined endpoints, there are many problems that it shares with other occupationally related exposure studies. For example, the question of a latent period in the development of symptoms/signs, the lack of accurate dose-response relationships, and the possibility of a synergistic effect with other toxins/carcinogens are all operating in this study. Although most cohort studies of occupational mortality use the general population as a standard for deriving the expected number of deaths, preemployment selection ("healthy worker" bias) affects the comparative experience. Age-standardized mortality ratios (SMR's) in general are 60-90 percent of the standard in the working population. Similarly, conflicting results can occur utilizing even the matched-pair cohort method proposed in this study design. Statistical verification of the validity of utilizing such a control for a summary mortality index (e.g., SMR) has been infrequently attempted in the past. Inability to verify the validity of the more classical methods of comparing mortality will necessitate the use of multiplicative and/or logistic models to obtain a valid standardized mortality ratio.

(2) Corrective Measures

Unprecedented study designs forced by unprecedented presentations of occupationally related medical complaints force novel approaches, reorientation and standardization of thinking; all of which require an effective Peer Review system, prior to Study Initiation. Beyond even the immediacy of the current study is the growing problem of a myriad of occupationally-related exposures, both in the military and civilian sector, which will require similar epidemiological studies in the future in order to make some judgment as to whether or not an association is of causal significance.

Duplicate with different headings & composition

B. Group Accountability Bias

(1) Problem

The numerous media presentations on Herbicide Orange issues have focused attention on the RANCH HAND group, and several attempts have been made to construct lists of former members this study, complete with all of its constraints. Most occupational exposure studies utilize the presentation of an unusual disease to justify the initiation of a comprehensive study. A rare disease or a common disease in an uncommon site, or one with an unusual presentation appearing in space-time clusters, often in an unusual population or age group, generates the requirement for a new study. In the case of Herbicide Orange, the evidence for long-term human effects has remained extremely tenuous and controversial. Despite the unique problems that this study possesses, such as the lack of clinically defined endpoints, there are many problems that it shares with other occupationally related exposure studies. For example, the question of a latent period in the development of symptoms/signs, the lack of accurate dose-response relationships, and the possibility of a synergistic effect with other toxins/carcinogens are all operating in this study. Although most cohort studies of occupational mortality use the general population as a standard for deriving the expected number of deaths, preemployment selection ("healthy worker" bias) affects the comparative experience. Age-standardized mortality ratios (SMR's) in general are 60-90 percent of the standard in the working population. Similarly conflicting results can occur utilizing even the matched-pair cohort method proposed in this study design. Statistical verification of the validity of utilizing such a control for a summary mortality index (e.g., SMR) has been infrequently attempted in the past. Inability to verify the validity of the more classical methods of comparing mortality will necessitate the use of multiplicative and/or logistic models to obtain a valid standardized mortality ratio.

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B. Group Accountability Bias

(1) Problem

The numerous media presentations on Herbicide Orange issues have focused attention on the RANCH HAND group, and several attempts have been made to construct lists of former members of this group. The RANCH HAND population should be easier to locate and contact than the control population. This difference will be particularly evident with respect to reported mortality experience. The incentives for cooperation and study participation are likely to be greater in the exposed group than in the controls. Also, the close knit reunion association of former RANCH HAND personnel will lead to a more precise reporting of morbidity and mortality in that group. Such group identity tends to decrease the degree of unaccountability in the exposed group while its absence in the controls may lead to under ascertainment of mortality. This could then lead to the attribution of excess mortality in the exposed population.

(2) Corrective Measures

Unaccountability bias will be minimized by continuing attempting to keep the percentages of unaccounted for study subjects below 1% in both exposed and control groups. The morbidity and mortality status of all individuals selected for the study will be strongly pursued utilizing a variety of techniques previously described.

C. "Risk Taking" Behavior Bias

(1) Problem

The early aircrew RANCH HAND population was an exclusively volunteer group; the C-130 control population, while volunteers in the Air Force, were not volunteers for special hazardous missions. RANCH HAND mission conditions were considered to be more dangerous than those encountered in the normal combat environment. This suggests that some differences may exist in the psychological profiles of the two groups. A sensation seeking or risk taking psychological orientation may have altered the accident mortality or morbidity patterns of the exposed group. In addition, an accident rate affected by peripheral neuropathy could be masked by undetected risk taking behavior bias.

(2) Corrective Measures

In an attempt to correct for the unique psychological factors that affect the choice of an aeronautical career, and to adjust for the effects of combat stress, transport aircrew

members were matched with crewmembers of similar transport aircraft. However, the volunteer nature of the RANCH HAND operation casts doubt on the adequacy of this basic matching as an attempt to control for the psychological effects of combat stress. The factors of volunteerism and risk-taking behavior must be considered from both the individual and group perspectives. The assessment of individual risk-taking behavior has been quantified by psychological instruments such as the Sensation Seeking Scale (SSS) of Zuckerman, et al. and the Life Experience Inventory (Torrance). The SSS has been demonstrated to have considerable validity in measuring a variety of phenomena including volunteerism and participation in risky activities and has been applied to Naval Aviation Trainees (Waters). These models will be adapted for use throughout all phases of the study. The classical model of field dependence/independence will be used to assess the group effect in this area.

D. Response Bias

(1) Problem

False positive response is anticipated as the primary bias operating in this study. Compensation issues arising from individual claims to the VA or from class action suits, heightened health concern generated by extensive publicity, disenchantment with military service, and the simple desire to please the interviewer may introduce positive responses that exceed the study's ability to correct or adjust. False negative response will also operate, and such bias is even more difficult to assess than the spurious response in a positive direction. Significant factors in this direction include: issues of patriotism and loyalty, personal conviction as to the propriety of the defoliation program and their participation in it, the strong virility orientation of the pilot/aircrew population (particularly with reference to questions of libido and fertility), personal inconvenience caused by study participation, errors of memory, and fear of the adverse effects on career goals that abnormal physical examination results could produce (a significant problem for active civilian and military pilots).

(2) Corrective Measures

The primary correction technique for questionnaire response bias will be a carefully constructed and standardized physical examination. Multiple verification and bias indicator questions will be designed and included in the initial questionnaire. Memory verification will be conducted by cross-referencing responses to medical and personnel records. Detailed statistical correlations between the questionnaire reponses and the physical examination results will be conducted. All telephone interviews and physical examinations will be conducted on a "blind" basis to the maximum

extent possible. Self-administered and group-administered questionnaires will not be conducted. Log linear models of anticipated biases and their estimated impact on the study will be attempted prior to the final analysis of any phase in order to justify the analytic methods used. Conclusions drawn from this study will be predicated and coupled to a bias estimate.

E. Interview Bias

(1) Problem

Voice inflection, speed of interview, intonation and ethnicity etc. are recognized factors which can affect positive or negative interview response. These factors will definitely operate in this study.

(2) Corrective Measures

An extensive interviewer training program will be conducted in order to limit the effects of interview bias. The Survey Research Center of the University of Illinois and the Center for Disease Control, Venereal Disease Training Branch, Atlanta, Georgia, will assist in this effort. The training will concentrate on techniques to elicit sensitive personal and medical information in an accurate manner, while minimizing discomfort to the subject and the interviewer. Quality assurance methodology and information verification techniques will also be included in the training. Interviews will be randomly monitored by the supervisor in an unannounced and undetectable manner. For particularly sensitive questions (e.g. illicit drug usage), randomized response techniques (coin flip method) will be used, recognizing that responses will be valid on a group basis only.

F. Political Implications

(1) Problem

The question of adverse health effects due to Herbicide Orange exposure in Vietnam has evoked many strong emotions. The actions of consumer groups, environmentalists, and other special interest groups have generated defensive responses on the part of some governmental agencies, and reactive decisions by others. Frequently, these responses have been based on unsubstantiated claims and/or scientific evidence of questionable validity. As a result of these governmental actions, the political impact on the planning of this study has been substantial. Suggestions to increase the scope of the effort to include other "exposed" individuals or poorly defined groups continue to surface. However, monumental problems of group ascertainment, exposure validation, control

Duplicate

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group selection, and control of additional bias make the inclusion of such individuals undesirable from a sound scientific perspective. If such decisions are made without regard for their scientific impact, compromise of study validity is assured.

(2) Corrective Measures

The dilution of the scientific credibility of this effort by politically motivated decisions will be diplomatically resisted. While all suggested improvements will be considered, any alterations or corrections to the study protocol will be based on sound scientific assessments of the proposed changes. Such issues will be clearly presented to appropriate peer review agencies for comment.

G. Loss to Study

(1) Problem

Loss to study in the RANCH HAND group poses a major problem to the validity of the inferences that can be made from any subsequent comparisons between or within groups. The avenues of loss will conceivably arise from individual apathy (volunteer bias), lack of appropriate financial reimbursement for lost worktime, the presence or absence of illness (perception of health), and the lack of a desire for "treatment." Losses in the matched controls at any phase of the study, though predictably greater than in the exposed group can be managed by resampling from the best-of-fit matches from the C-130 population. Consequently, additional decrements in statistical power will not result from losses in this group. However, significant losses in the exposed group will have irreparable adverse effects on the power of the statistical analyses. The estimated allocation of participants in this study appears in Table A-5, Section IV, of the Appendix. It is estimated that the response rate of the accessible, identified exposed group will be 70% for both the initial questionnaire and the physical examination phases of the study. This is expected to occur despite great efforts to keep the questionnaire at an acceptable length, to coordinate questionnaire administration with the subject's personal schedule, and to make the questions as innocuous as possible. It is also estimated that only 80% of those who respond positively to the opportunity for physical examinations will actually present themselves for examination.

(2) Corrective Measures

Loss to study problems in the study participants will be avoided as much as possible by detailed and exhaustive efforts to contact and followup each identified participant. Nonparticipants will be encouraged to reconsider their initial decisions. Design considerations have been made to minimize loss to study in both the exposed and control populations. Federal regional hospitals in the United States and overseas will be used to facilitate the ease of obtaining physical examinations, and thus participation in the cross-sectional and prospective study phases will be increased. It is felt that physical examination variances due to slight differences in technique between hospitals and physicians will be less damaging to the validity of the study than the effects resulting from attempts to conduct all of the examinations at a single facility, i.e., examination at a single facility would reduce participation rates, and therefore would severely compromise the overall statistical power of the study.

H. Statistical Power Limitations

(1) Problem

As discussed above, statistical power considerations are heavily dependent on loss to study rates. Since the design of the study is limited by the small exposed population, statistical power for identifying the relative risk of an uncommon disease or symptom-complex (1/100) is very low (.50). This study will, to a greater extent, be able to detect increased risks only in common diseases or symptom-complexes (1/100).

(2) Discussion

The "herald sign" of TCDD exposure, chloracne, is expected to have the greatest likelihood of achieving adequate statistical power in this study. Recent findings from Seveso, Italy, support the importance of chloracne as the primary marker symptom. The incidence of chloracne has been reported by Reggiani (personal communication) and Homberger, et al., to be 14.9 cases per 1000 residents in the region of highest contamination of Seveso (Zone A) and 6-12 cases per 1000 in the Seveso community as a whole. These rates vary by age group, with children being at highest risk. Only 1-5 cases per 1000 were seen in other regions of Northern Italy (Milan, Como, and Lecco). The incidence of adolescent acne in all of these populations varies between 21 and 30%. These incidence

rates probably place chloracne at the lower limit of adequate statistical power within the constraints imposed on this study. In the Nitro, West Virginia studies, residuals of chloracne, as well as exacerbations of previously active disease, continue to be seen 10 years after the most recent exposures, and 30 years after the industrial accident. Thus, it is likely that any chloracne in the exposed population may be detected, despite the intervening years since RANCH HAND exposures.

I. Variablility of Procedures

(1) Problem

The variance of physical examination findings from technique differences and the random errors inherent in laboratory testing are items of concern, particularly if attributable health effects are subtle or of low magnitude. Nonstandardized procedures and techniques are major contributors to this variance.

(2) Corrective Measures

Variability in examination procedures will be minimized by the use of standardized procedures, examination protocols, and training. Most laboratory procedures will be conducted centrally at the USAF School of Aerospace Medicine, and quality control will be stressed at all times.

J. Confounding Exposure Factors

(1) Problem

While virtually all of the media attention has been directed toward the 2,4,5-T containing herbicide formulations, other herbicides were applied concurrently by the C-123 aircrews in Vietnam. Herbicide Blue (Cacodylic acid with 15.4% pentavalent arsenic) and Herbicide White (2,4-D and Picloram) were used throughout the 1962-1970 time period. Any long-term health effects from these additional compounds may confound the results of the study. Peripheral neuritis, tremors, skin and lung cancer, loss of hair and nails, skin rashes, and gastric symptoms have been alleged after exposure to arsenical pesticides. The organophosphate insecticide, Malathion, was also sprayed by many of these same aircrewmembers when RANCH HAND duties permitted their temporary assignment to mosquito/malaria control units. Many of these individuals were involved in the aerial spray application of these and other pesticides both before, during, and after their Vietnam service. Long-term

effects from these chemicals would confound the study results. The small size of the RANCH HAND population will allow very little opportunity for analytic stratification for these confounding variables. Differing patterns of exposure to aircraft fuels in the study populations have been suggested as confounding factors. The C-130 aircraft were powered by turbo-prop engines which used jet fuel (JP-4), while the C-123 and C-7 aircraft were powered by standard reciprocating engines which used leaded aviation fuel (AV-GAS). After June 1968, many C-123s were modified by the addition of auxilliary jet engine boosters for added power on take-offs and emergencies.

(2) Corrective Measures

While the extent of confounding caused by exposure to these other pesticides is undetermined at this time, assessment of its magnitude must rely on responses of the subjects to that portion of the questionnaire dealing with other occupational exposures. Variations in fuel between C-130 and C-123 aircraft would be significant factors if individuals in the study were heavily and repetitively exposed. However, the normal duties of the study participants did not involve aircraft refueling or other fuel handling ~~refueling~~ activities. Thus, fuel exposures can be rejected as confounding factors.

IX. Reporting Procedures

Interim synoptic progress reports will be provided to the Surgeon General through Quarterly Management Reviews conducted each January, April, July and October. Key data analyses will be displayed, but inferences and conclusions will await full data analysis at the conclusion of each phase. A formal report for each of the three phases will be completed with forecasted submission dates of : Retrospective Study, July 1981; Cross-Sectional Study, October 1981; and Prospective Study, April 1986. Findings and conclusions of each phase will be published in a journal of stature. Total study design, findings, and conclusions will be published in the USAFSAM Aeromedical Reviews or Technical Reports.

PRINCIPAL:

George D. Lathrop, MD, MPH, PhD
Colonel, USAF, MC
Chief, Epidemiology Division
USAF School of Aerospace Medicine
Brooks AFB, TX 78235

William H. Wolfe, MD, MPH
Lt Colonel, USAF, MC
Chief, Disease Surveillance Branch
Epidemiology Division
USAF School of Aerospace Medicine
Brooks AFB, TX 78235

Clarence F. Watson, Jr., MD, MPH
Colonel, USAF, MC
Chief, Clinical Sciences Divsion
USAF School of Aerospace Medicine
Brooks AFB, TX 78235

Richard A. Albanese, MD, GS-15
Chief, Biomathematical Modeling Branch
Data Sciences Division
USAF School of Aerospace Medicine
Brooks AFB, TX 78235

Patricia M. Moynahan, BSN, MS,
Lt Colonel, USAF, NC
Chief, Epidemiologic Investigative Section
Disease Surveillance Branch
Epidemiology Division
USAF School of Aerospace Medicine
Brooks AFB, TX 78235

CO-INVESTIGATORS:

Alvin L. Young, BS, MS, PhD
Major, USAF
Environmental Sciences Consultant
Epidemiology Division
USAF School of Aerospace Medicine
Brooks, AFB, TX 78235

CO-INVESTIGATORS (Cont'd)

Michael A. Sauri, MD, MPH
Captain, USAF, MC
Staff Epidemiologist
Epidemiology Division
USAF School of Aerospace Medicine
Brooks AFB, TX 78235

Richie S. Dryden, MD, MPH
Lt Colonel, USAF, MC
Chief, Flight Medicine Branch
Clinical Sciences Division
USAF School of Aerospace Medicine
Brooks AFB, TX 78235

Phelps P. Crump, PhD, GS-15
Chief, Consultation & Training Branch
Data Sciences Division
USAF School of Aerospace Medicine
Brooks AFB, TX 78235

Jeffrey E. Kantor, PhD, GS-13
Acting Chief
Demographic & Attitudinal Research Branch
AF Human Resources Laboratory
Brooks, AFB, TX 78235

Thomas V. Murphy, MBA, GS-9
Statistician
Disease Surveillance Branch
Epidemiology Division
USAF School of Aerospace Medicine
Brooks AFB, TX 78235

Richard C. McNee, MS, GS-14
Chief, Advanced Analysis Branch
Data Sciences Division
USAF School of Aerospace Medicine
Brooks AFB, TX 78235

Robert A. Bottenberg, PhD, GS-15
Chief, Computational Sciences Division
AF Human Resources Laboratory
Brooks AFB, TX 78235

CO-INVESTIGATORS (Cont'd)

William E. Allen PhD, GS-13
Supervisory Research Psychologist
Occupation and Manpower Research Division
AF Human Resources Laboratory
Brooks AFB, TX 78235

William J. Phalen
Research Psychologist
Occupation and Manpower Research Division
AF Human Resources Laboratory
Brooks AFB, TX 78235

Jimmy D. Souter, BA, GS-14
Chief, Analysis & Programming Branch
Computational Sciences Division
AF Human Resources Laboratory
Brooks AFB, Tx 78235

Alton J. Rahe, MS, GS-13
Mathematical Statistician
Data Sciences Division
USAF School of Aerospace Medicine
Brooks AFB, TX 78235

Joel E. Michalek, PhD. GS-13
Mathematical Statistician
Data Sciences Division
USAF School of Aerospace Medicine
Brooks AFB, TX 78235

Calvin C. Fresney, GS-12
Chief, Data Base Management Section
Computational Sciences Division
AF Human Resources Laboratory
Brooks AFB, TX 78235

Henry W. Clark, BA, GS-12
Supervisory Computer Systems Analysist
Computational Sciences Division
AF Human Resources Laboratory
Brooks, AFB, TX 78235

TABLE A-3 "SYMPTOM COMPLEX" DERIVED FROM LITERATURE REVIEW OF CASE STUDIES EXPOSED TO 2,4-D; 2,4,5-T AND/OR TCDD

2,4-D	2,4,5-T (+ TCDD)	TCDD
	CHLORACNE	CHLORACNE
	PORPHYRIA	PORPHYRIA
	HYPERPIGMENTATION	HYPERPIGMENTATION
ASTHENIA	ASTHENIA	ASTHENIA
PERIPHERAL NEUROPATHY	PERIPHERAL NEUROPATHY	PERIPHERAL NEUROPATHY
SWEATING/FEVER		
CARDIAC DISTURBANCE	CARDIAC DISTURBANCE	CARDIAC DISTURBANCE
RENAL DYSFUNCTION		RENAL DYSFUNCTION
LIVER DYSFUNCTION	LIVER DYSFUNCTION	LIVER DYSFUNCTION
GI DISTURBANCE	GI DISTURBANCE	GI DISTURBANCE
HEADACHE		
PNEUMONITIS		
CSF PROTEIN ALTERATIONS		HYPOTHYROIDISM
CONVULSIONS		HEARING/SMELL DISTURBANCES

TABLE A-4 DETAILED LISTING OF SYMPTOMS/SIGNS BY MAJOR CATEGORY FROM LITERATURE REVIEW OF CASE STUDIES EXPOSED TO 2,4-D; 2,4,5-T AND/OR TCDD

NEURO-PSYCHIATRIC ABNORMALITIES

AESTHENIA	PERIPHERAL NEUROPATHY
ANXIETY	HYPOREFLEXIA
DEPRESSION	WEAKNESS
FATIGUE	PARESTHESIAS
APATHY	EXTREMITY NUMBNESS
LOSS OF DRIVE	MYALGIA
LIBIDO	GAIT DISTURBANCE
IMPOTENCY	"MILD" PARESIS
SLEEPLESSNESS	
EMOTIONAL INSTABILITY	
ANOREXIA	
DIZZINESS	
DECREASED LEARNING ABILITY	

TABLE A-4 (CONTINUED) DETAILED LISTING OF SYMPTOMS/SIGNS BY MAJOR CATEGORY FROM LITERATURE REVIEW OF CASE STUDIES EXPOSED TO 2,4-D, 2,4,5-T AND/OR TCDD

DERMATOLOGIC DISEASE

CHLORACNE

PORPHYRIA CUTANEA TARDA

HYPERPIGMENTATION

HIRSUTISM (BODY)

ALOPECIA OF THE SCALP

OTHER DISORDERS

HEPATIC DYSFUNCTION

Increased CHOLESTEROL & Triglyceride

~~SGOT, SGPT, LDH~~ Increased Liver Function Test

RENAL DYSFUNCTION

PROTEINURIA

DECREASED OUTPUT

TUBULAR DEGENERATION

GLOMERULAR DEGENERATION

RENAL GLUCOSURIA

GI DISTURBANCE

NAUSEA

VOMITING

DIARRHEA

GASTRITIS

ABDOMINAL PAIN

CARDIAC DISTURBANCE

BRADYCARDIA

TACHYCARDIA

ATRIAL FIBRILLATION

USAFSAM CODE _____

RANCH HAND II - DRAFT QUESTIONNAIRE

INTERVIEWER _____

DATE _____

TIME STARTED _____

PERSONAL IDENTIFICATION:

1. YOUR NAME: _____ (Last First M.I.) 2. DATE OF BIRTH: YR ☐☐ MO ☐☐ DAY ☐☐

3. CURRENT ADDRESS: _____ (Number Street) _____ (City) _____ (State) _____ (Zip)

4. SSAN: ☐☐☐ ☐☐ ☐☐☐☐ 5. PREVIOUS SERVICE NUMBERS: ☐☐☐☐☐☐ ☐☐☐☐☐☐☐ ☐☐☐☐☐☐☐

6. PHONE NUMBER (WORK): AC ☐☐☐ ☐☐☐ ☐☐☐☐ (HOME): AC ☐☐☐ ☐☐☐ ☐☐☐☐

7. RACE/ETHNIC GROUP: CAUCASIAN ☐ BLACK ☐ MEXICAN AMERICAN ☐ ORIENTAL ☐ OTHER ☐ (Specify) _____

8. MILITARY STATUS: ACTIVE DUTY ☐ RETIRED (YR) ☐ SEPARATED (YR) ☐

9. WHICH OF THE FOLLOWING WORDS BEST DESCRIBES YOUR PRESENT STATE OF HEALTH:

a. VERY POOR ☐ b. POOR ☐ c. FAIR ☐ d. GOOD ☐ e. EXCELLENT ☐

10. Where are your Air Force medical records now? _____

11. Where are your dependents' Air Force medical records now? _____

12. MEDICAL PROBLEMS

	NO	YES: PRIOR TO VIETNAM DUTY	YES: DURING VIETNAM DUTY	YES: AFTER RETURN TO US	YES: NOW	DID YOU SEEK MEDICAL ADVICE? YES	NO	DIAGNOSIS	ICDA CODE NR.
a. HEADACHES	☐	☐	☐	☐	☐	☐	☐	_____	___
b. HOARSENESS	☐	☐	☐	☐	☐	☐	☐	_____	___
c. SKIN RASH									
(1) EARS	☐	☐	☐	☐	☐	☐	☐	_____	___
(2) EYES	☐	☐	☐	☐	☐	☐	☐	_____	___
(3) TEMPLES	☐	☐	☐	☐	☐	☐	☐	_____	___

	NO	YES: PRIOR TO VIETNAM DUTY	YES: DURING VIETNAM DUTY	YES: AFTER RETURN TO US	YES: NOW	DID YOU SEEK MEDICAL ADVICE? YES	NO	DIAGNOSIS	ICDA CODE NR.
(4) CHEEKS	☐	☐	☐	☐	☐	☐	☐		
(5) NECK	☐	☐	☐	☐	☐	☐	☐		
(6) ARMS	☐	☐	☐	☐	☐	☐	☐		
(7) LEGS	☐	☐	☐	☐	☐	☐	☐		
(8) CHEST	☐	☐	☐	☐	☐	☐	☐		
(9) BACK	☐	☐	☐	☐	☐	☐	☐		
(10) GROIN	☐	☐	☐	☐	☐	☐	☐		
d. DEPRESSION	☐	☐	☐	☐	☐	☐	☐		
e. ANXIETY	☐	☐	☐	☐	☐	☐	☐		
f. APATHY	☐	☐	☐	☐	☐	☐	☐		
g. LOSS OF DRIVE	☐	☐	☐	☐	☐	☐	☐		
h. EMOTIONAL INSTBL	☐	☐	☐	☐	☐	☐	☐		
i. MUSCULAR WKNSS	☐	☐	☐	☐	☐	☐	☐		
j. HYPOREFLEXIA	☐	☐	☐	☐	☐	☐	☐		
k. PARESTHESIAS	☐	☐	☐	☐	☐	☐	☐		
l. MYALGIA	☐	☐	☐	☐	☐	☐	☐		
m. STYLE OF WALKING	☐	☐	☐	☐	☐	☐	☐		
n. MILD PARESIS	☐	☐	☐	☐	☐	☐	☐		
o. FATIGUE	☐	☐	☐	☐	☐	☐	☐		
p. DECREASE IN LRNG ABILITY	☐	☐	☐	☐	☐	☐	☐		
q. DECREASED MEMORY	☐	☐	☐	☐	☐	☐	☐		
r. UNDSRD WT LOSS (10 lbs)	☐	☐	☐	☐	☐	☐	☐		
s. ANOREXIA	☐	☐	☐	☐	☐	☐	☐		

	NO	YES: PRIOR TO VIETNAM DUTY	YES: DURING VIETNAM DUTY	YES: AFTER RETURN TO US	YES: NOW	DID YOU SEEK MEDICAL ADVICE? YES	NO	DIAGNOSIS	ICDA CODE NR.
rr. LIBIDO	☐	☐	☐	☐	☐	☐	☐		
ss. IMPOTENCE	☐	☐	☐	☐	☐	☐	☐		
tt. STERILITY	☐	☐	☐	☐	☐	☐	☐		
uu. RESP INFECTNS	☐	☐	☐	☐	☐	☐	☐		
vv. NOSEBLEEDS	☐	☐	☐	☐	☐	☐	☐		
ww. ABDOMINAL PAIN	☐	☐	☐	☐	☐	☐	☐		

13. SMOKING HISTORY

a. DID YOU EVER SMOKE MARIJUANA ☐ ☐ ☐ ☐ ☐ ☐ ☐

b. DID YOU EVER SMOKE CIGARETTES ☐ ☐ ☐ ☐ ☐

c. IF YES, HOW MUCH DID YOU SMOKE

CODES
1 Light 1 pk/day
2 Moderate 1-2 pks/day
3 Heavy 2 pks/day
4 None

14. a. DID YOU EVER ☐ ☐ ☐ ☐ DRINK ALCOHOLIC BEVERAGES

b. IF YES, WOULD YOU CONSIDER YOURSELF

CODES
1 Light
2 Moderate
3 Heavy
4 None

15. HAVE YOU EVER TAKEN PRESCRIPTION MEDICATION ON A LONG-TERM BASIS (60 CONTINUOUS DAYS OR LONGER) ☐ ☐ ☐ ☐ ☐

NAME OF DRUG
_____
_____
_____
_____

FAMILY HISTORY:

16. ARE YOU OR WERE YOU EVER MARRIED: YES ☐ NO ☐

a. IF YES, HOW MANY TIMES: ☐☐

17. DATES OF YOUR MARRIAGES:

	YR	MO	REASON FOR SEPARATION		
			DIVORCE/LEGAL SEPARATION	DEATH	N/A
a.	☐☐	☐☐	☐	☐	☐
b.	☐☐	☐☐	☐	☐	☐
c.	☐☐	☐☐	☐	☐	☐
d.	☐☐	☐☐	☐	☐	☐

18. HAVE YOU EVER FATHERED CHILDREN: YES ☐ NO ☐

19. OF CHILDREN FATHERED, PLEASE SUPPLY THE FOLLOWING INFORMATION (TO THE BEST OF YOUR KNOWLEDGE.

BIRTH YR MO	WAS THIS CHILD PREMATURE			DID THIS CHILD HAVE ANY BIRTH DEFECTS			WHAT IS THIS CHILD'S CURRENT HEALTH STATUS					
	YES	NO	UNK	YES	NO	UNK	VERY POOR	POOR	FAIR	GOOD	EXCELLENT	UNK
______	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
______	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
______	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
______	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
______	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
______	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
______	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

20. HAS YOUR WIFE (WIVES) EVER HAD A MISCARRIAGE: YES ☐ NO ☐ UNK ☐

IF SO, APPROXIMATELY HOW MAY AND WHEN: NUMBER ☐ WHEN (YR) ☐☐

21. HAS YOUR WIFE (WIVES) EVER HAD A STILL BIRTH: YES ☐ NO ☐ UNK ☐

IF SO, HOW MANY AND WHEN: NUMBER ☐ WHEN (YR) ☐☐

22. HAS DOCTOR EVER ADVISED YOUR WIFE(S) NOT TO GET PREGNANT: YES ☐ NO ☐ UNK ☐

IF YES, WHY ______________________________ (ICDA CODE NR) __________

	MOTHER	FATHER	GRAND- MOTHER	GRAND- FATHER	BROTHER	SISTER	CODE NR.

23. IS THERE A HISTORY OF CANCER IN YOUR IMMEDIATE FAMILY:

YES ☐ NO ☐ UNK ☐

TYPE _____ IN	☐	☐	☐	☐	☐	☐	_____
TYPE _____ IN	☐	☐	☐	☐	☐	☐	_____
TYPE _____ IN	☐	☐	☐	☐	☐	☐	_____
TYPE _____ IN	☐	☐	☐	☐	☐	☐	_____

24. IS THERE A HISTORY OF HEART DISEASE IN YOUR IMMEDIATE FAMILY:

YES ☐ NO ☐ UNK ☐

TYPE _____ IN	☐	☐	☐	☐	☐	☐	_____
TYPE _____ IN	☐	☐	☐	☐	☐	☐	_____
TYPE _____ IN	☐	☐	☐	☐	☐	☐	_____
TYPE _____ IN	☐	☐	☐	☐	☐	☐	_____

25. DO YOU KNOW OF ANY SPECIFIC INHERITED DISEASE/CONDITION ON EITHER SIDE OF YOUR OR YOUR WIFE'S FAMILY:

YES ☐ NO ☐ UNK ☐

TYPE _____ IN	☐	☐	☐	☐	☐	☐	_____
TYPE _____ IN	☐	☐	☐	☐	☐	☐	_____
TYPE _____ IN	☐	☐	☐	☐	☐	☐	_____
TYPE _____ IN	☐	☐	☐	☐	☐	☐	_____

26. DO ANY OF YOUR BROTHERS OR SISTERS HAVE CHILDREN WITH CONGENITAL ABNORMALITIES:

YES ☐ NO ☐ UNK ☐

IF YES, SPECIFY TYPE _____ _____

_____ _____

_____ _____

27. DO ANY OF YOUR WIFE'S BROTHERS OR SISTERS HAVE CHILDREN WITH CONGENITAL ABNORMALITIES:

IF YES, SPECIFY TYPE _____ _____

_____ _____

_____ _____

_____ _____

EMPLOYMENT/HOBBIES:

28. WHAT IS YOUR CURRENT JOB: _____ DO YOU REGULARLY COME IN CONTACT WITH

- ☐ ASBESTOS
- ☐ RADIATION
- ☐ HERBICIDES
- ☐ INDUSTRIAL CHEMICALS
- ☐ PESTICIDE

29. IN OTHER JOBS SINCE YOUR VIETNAM TOUR, DID YOU REGULARLY COME IN CONTACT WITH

- ☐ ASBESTOS
- ☐ RADIATION
- ☐ HERBICIDES
- ☐ INDUSTRIAL CHEMICALS
- ☐ PESTICIDE

30. IN YOUR HOBBIES, DO YOU REGULARLY COME IN CONTACT WITH

- ☐ ASBESTOS
- ☐ RADIATION
- ☐ HERBICIDES
- ☐ INDUSTRIAL CHEMICALS
- ☐ PESTICIDE

SOUTHEAST ASIA TOUR INFORMATION

31. VIETNAM SERVICE (SOURCE: COMPUTER/INTERVIEW)

TOUR	DATES yr	mo		yr	mo	UNIT OF ASSIGNMENT	AFSC	FLYING TIME
1ST	☐☐	☐☐	to	☐☐	☐☐	_____	____	_____
2ND	☐☐	☐☐	to	☐☐	☐☐	_____	____	_____
3RD	☐☐	☐☐	to	☐☐	☐☐	_____	____	_____
4TH	☐☐	☐☐	to	☐☐	☐☐	_____	____	_____
5TH	☐☐	☐☐	to	☐☐	☐☐	_____	____	_____
6TH	☐☐	☐☐	to	☐☐	☐☐	_____	____	_____
7TH	☐☐	☐☐	to	☐☐	☐☐	_____	____	_____

32. WERE YOU EVER FORCE DOWN IN COMBAT: YES ☐ NO ☐

33. WERE YOU EVER INVOLVED IN A MAJOR AIRCRAFT ACCIDENT: YES ☐ NO ☐

34. WERE YOU EVER WOUNDED IN COMBAT: YES ☐ NO ☐

35. a. IN VIETNAM, WERE YOU EXPOSED TO: b. IF YES, WAS YOUR EXPOSURE:

	YES	NO	DAILY	MONTHLY	YEARLY
INSECTICIDES	☐	☐	☐	☐	☐
DEFOLIANTS/HERBICIDES	☐	☐	☐	☐	☐
DEGREASING CHEMICALS	☐	☐	☐	☐	☐

36. IF YES TO HERBICIDES, WHICH OF THE FOLLOWING STATEMENTS WOULD BEST DESCRIBE YOUR TYPICAL EXPOSURE:

☐ MY FLIGHT SUIT WAS SATURATED

☐ SEVERAL SPOTS OF THE SUBSTANCE ON MY FLIGHT SUIT

☐ NONE ON MY FLIGHT SUIT, BUT I COULD SMELL IT

☐ NONE ON MY FLIGHT SUIT, I COULD NOT SMELL IT, BUT I COULD SEE THE SPRAY MIST

☐ WAS NOT AWARE OF IT

37. WHAT STATES HAVE YOU LIVED IN (CONTINUOUSLY FOR 1 YEAR OR MORE) SINCE YOUR BIRTH, AND FOR HOW LONG: (CHRONOLOGICAL ORDER)

a. STATE	b. LENGTH	c. URBAN/RURAL
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

38. WOULD YOU BE WILLING TO COME TO BROOKS AFB FOR AN AIR FORCE FUNDED PHYSICAL EXAMINATION: YES NO

IF NOT, WHY?

a. ☐ EXCESSIVE COST

b. ☐ CANNOT GET THE TIME OFF WORK

c. ☐ NOT INTERESTED

d. ☐ WASTE OF TIME

e. ☐ OTHER _____

39. WOULD YOU BE WILLING TO UNDERGO A COMPLETE PHYSICAL EXAM AT THE FEDERAL HOSPITAL WITHIN 250 MILES OF YOUR HOME IF THE AIR FORCE PAID YOUR TRAVEL COSTS?

YES ☐ NO ☐

40. "SENSATION SEEKING SCALE" QUESTIONS

TIME COMPLETED _____

[Corrected version of protocol]

PROTOCOL

PROJECT RANCH HAND II

EPIDEMIOLOGIC INVESTIGATION OF HEALTH EFFECTS IN AIR FORCE PERSONNEL FOLLOWING EXPOSURE TO HERBICIDE ORANGE

MATCHED PAIR COHORT DESIGN

PREPARED BY: EPIDEMIOLOGY DIVISION
DATA SCIENCES DIVISION
CLINICAL SCIENCES DIVISION

USAF SCHOOL OF AEROSPACE MEDICINE (USAFSAM)

BROOKS AFB, TX

PREPARED FOR: PEER REVIEW AGENCIES

AIR FORCE WORKING PAPER

PROJECT RANCH HAND II

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PROJECT RANCH HAND II

EPIDEMIOLOGIC INVESTIGATION OF HEALTH EFFECTS
IN AIR FORCE PERSONNEL FOLLOWING EXPOSURE TO HERBICIDE ORANGE

MATCHED PAIR COHORT DESIGN

I. Purpose of the Investigation

The purpose of this investigation is to determine, by epidemiologic techniques, whether long-term health effects exist and can be attributed to occupational exposure to Herbicide Orange.

II. Synopsis of Background

A. Current

News media presentations have recently focused medical, political and lay attention on possible adverse health effects in military personnel, allegedly due to Herbicide Orange [a mixture of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)] which was used as a defoliant during the Vietnam Conflict. This defoliant was later found to have been contaminated with the toxin 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Approximately 500 claims for compensation have been filed against the Veterans Administration (VA), largely by former US Army members. In response to Congress, the General Accounting Office (GAO) investigated the issue and subsequently recommended that the Department of Defense (DOD) conduct a long-term epidemiologic study of the problem. The Department of the Air Force has made a formal commitment to the Congress and the White House to conduct such a study.

B. Use of Herbicides

Research and development on phenoxy herbicides began in the early 1940s. Most of the initial phytotoxic screening programs and development of application technologies were sponsored by the DOD. The military concept for use of these compounds was directed to two purposes: (1) defoliation of vegetation to decrease risk of ambush by improving visibility, and (2) destruction of enemy crops. The first sustained DOD operational use of herbicides was initiated during the Vietman Conflict (Operation RANCH HAND). Data have indicated that the various 2,4,5-T containing-herbicides (code-named

Pink, Purple, and Green) used between 1962 and 1965 contained relatively higher concentrations of TCDD than the herbicide (code-named Orange) used from 1965 through 1970. Concurrent with the change in herbicides, the scope of aerial use shifted from four rotating aircrews to 30 permanently assigned aircrews and additional support personnel. Between 1962 and 1970 approximately 10.9 million gallons of phenoxy herbicide, containing approximately 368 pounds of TCDD, were dispersed. Following the announcement in October 1969 that the administration of 2,4,5-T to pregnant rodents caused an increase in the rate of congenital abnormalities, the DOD confined Herbicide Orange spray operations to non-populated areas. In April 1970, all uses of the herbicide were halted. In February 1971, all Herbicide Orange stocks were removed from South Vietnam and transported to Johnston Island, Pacific Ocean, for open storage (Project PACER IVY). All of the military phenoxy herbicide stock were incinerated at sea in 1977 (Project PACER HO). In 1979, the Environmental Protection Agency (EPA) suspended the use of herbicides containing 2,4,5-T because an epidemiologic study in the United States attributed abortogenic effects to its use.

III. Goals of the Investigation

From the above background, three interdependent study goals emerge:

A. Health

(1) To identify veteran and active duty individuals with adverse health effects (physical and psychological) if any, and which are attributed to herbicide exposure, and

(2) To identify other individuals at risk of developing future adverse health effects, if any.

B. Political

To satisfy the social concern for proper investigation voiced both by lay and scientific communities, national and international.

C. Legal

To clarify the question of compensation award to the 500 claimants.

With regard to the goal of legal clarification, it is apparent that data and conclusions arising from this investigation, positive, negative, or indeterminant, will probably be used to better assess the issue of long-term health effects and resultant

compensation. The operational assumption of this study, therefore, is: Air Force Operation RANCH HAND personnel probably received a greater average occupational exposure to 2,4,5-T and TCDD than US Army ground personnel, implying that RANCH HAND personnel should develop greater numbers of acute and chronic clinical signs/symptoms from the exposure, and should manifest them sooner than US Army personnel, if indeed there are any adverse long-term health effects at all. This dose-response notion suggests that although the Air Force population is not the best one to study, it is probably better than the Army population.

The overall scientific thrust of this investigation is to define the natural history of disease, if any, and its spectrum of illness, by direct and indirect methodology.

IV. Synopsis and Discussion of Literature

A. Overview

More than 20,000 scientific articles relating to the phenoxy herbicides have been published since the 1940's. Many of the articles cite herbicide-caused health effects in a variety of animal species. Most early studies used a myriad of herbicide formulations and unknowingly dealt with physically and chemically impure compounds. The assay technology was far short of today's state-of-the-art. Many human studies have ascribed cause and effect relationships but have suffered from problems of clinical empiricism or questionable methodology. The only consistent and repetitive clinical finding associated with acute exposure to 2,4,5-T herbicide has been chloracne, recognized by most workers as the herald sign of acute overexposure to the herbicide. It is now recognized that the chloracne was caused by the presence of TCDD Rather than the 2,4,5-T. Sequaelae from chloracne, localized or systemic, appear to be unusual according to the preponderance of the literature. It is appropriate to note that sustained worldwide usage of herbicides for 30 years has not evoked a readily identifiable disease state. It is clear from the literature and the usage history of herbicides that if there are significant attributable long-term health effects, they are either reasonably rare, or of such nonspecific commonality that they blend unnoticeably into the symptoms, syndromes, or diseases associated with increasing age or other factors.

B. Pharmacokinetics of 2,4-D, 2,4,5-T and TCDD

(1) 2,4-D

The pharmocokinetics of 2,4-D have been well studied in animals. 2,4-D is readily absorbed on oral administration. Initially, it is distributed in high concentrations to the central

nervous system and liver. Eventually, all tissues are involved, with the kidneys accumulating twenty times the concentration of the other tissues. The plasma half-life is approximately 3-12 hours, with 2,4-D primarily eliminated from the body by the kidney, the rate of elimination being dose-dependent. Generally, high doses or repeated lower doses result in tissue accumulation. The majority of 2,4-D is eliminated unmetabolized; however, esters of 2,4-D have been shown to undergo hydrolysis prior to excretion. Muscle and fat show the lowest accumulation of 2,4-D on repeated exposure, whereas the kidneys and liver show the highest accumulations. Within 24 hours of single dose administration of 2,4-D, 16.8% was present in the uterus, placenta, fetus and amniotic fluid in gravid rats. In addition, 2,4-D was found in the milk of lactating rats for up to six days following single-dose exposure.

(2) 2,4,5-T

The pharmacokinetics of 2,4,5-T have been well studied in animals. In all animals, 2,4,5-T has been shown to be readily absorbed upon oral administration. However, beyond this point, 2,4,5-T has shown marked variations in its pharmacokinetics in the various animals. These differences are supposedly due to variations in species, age, dose levels, route of administration and chemical formulation used in the various studies. The distribution is generally ubiquitous throughout the body with the exception of hamsters, which show no placental passage, and mice, which show placental passage but only in late gestation. Clearance from plasma and the body varies greatly among animals with rats showing faster clearance than dogs, mice and man. In addition, this clearance appears to be generally dose-dependent. The biological half-life of 2,4,5-T in rats, as estimated by tissue analyses and urinary clearance at administered dosages of 5 mg/kg, is 4.7 hours. However, at 200 mg/kg, the half-life in rats is prolonged to 25 hours. Excretion of 2,4,5-T is primarily via the kidneys. The elimination of 2,4,5-T at low doses is essentially achieved in its unmetabolized form. However, at higher doses or more chronic doses, elimination entails a more active role by the liver (i.e.,conjugation). Higher doses and repeated lower doses appear to result in accumulation in animal tissues.

(3) TCDD

The information on the absorption, distribution and excretion of TCDD has been mostly derived from animal models. The

only reported human study dealing with pharmacokinetics of TCDD dealt with the analysis of TCDD in tissues at necropsy of one case of confirmed TCDD exposure subsequent to the acidental release of TCDD in Seveso, Italy in July 1976. Studies in rats, mice and guinea pigs generally show that intestinal absorption of TCDD is relatively complete, with a large proportion of TCDD remaining unmetabolized inthe liver. The majority of this TCDD is assumed to be localized in the liver microsomes (centrifugation techniques). Initially, adipose tissue accumulates TCDD, followed later by accumulation in the liver, adrenals, kidneys and lungs. The level of TCDD in the liver and adipose tissue is about ten-fold greater than in other body tissues; however, significant species variability has been observed. The biological half-life of TCDD varies by species, but is reported to range from 12 to 50 days. The major route of excretion is via the feces with urinary excretion occurring at a much reduced rate.

(4) Phenoxy Herbicides in Humans

Relatively few studies have dealt with the pharmacokinetics of 2,4-D and 2,4,5-T in humans. Numerous reports of occupational exposures in industry and in commercial and private herbicide applications have supported percutaneous entry. Rapid absorption has been observed after oral administration of 2,4-D or 2,4,5-T. The main mode of excretion of the phenoxy herbicides is via the urine with 74% of 2,4-D and 63%-72% of 2,4,5-T being cleared from the body within the first 96 hours. The majority of phenoxy herbicides are unmetabolized prior to excretion. The biological half-life of 2,4-D and 2,4,5-T in humans (as estimated by tissue analyses and urinary excretion) is 33 hours and 18 hours, respectively. Tissue analysis has revealed a ubiquitous distribution of the herbicides after absorption. Limited studies on the accumulation of the phenoxy herbicides following repeated doses suggest that such accumulation in humans is unlikely. This is in contrast to numerous animal studies on 2,4-D and 2,4,5-T which show that such accumulation does occur.

No specific data are available on the odor threshold of Herbicide Orange. Data are availabe, however, on the odor threshold of a butyl ester formulation of 2,4,5-T. The odor threshold was found to be about 0.3 ppb (the taste threshold was 1.3 ppb). A Threshold Limit Value (TLV) of 10,000 g/m^3 (10ppm) for either 2,4-D or 2,4,5-T has been adopted by the American Conference of Governmental Industrial Hygienists. The TLV is a time-weighted average concentration for a normal 8-hour workday or 40-hour workweek to which workers may be repeatedly exposed, day after day, without,

adverse effect. Analysis of ambient air samples collected adjacent to and downwind from actual dedrumming operations involving Herbicide Orange were at least two orders of magnitude below the TLVs.

C. Proposed Cellular Mechanism of Action for TCDD

TCDD has, in general, three proposed mechanisms of action by which its variety of effects, both documented and suspected, can be understood. All currently available information in this area is derived from animal, plant, and bacterial models. The few human studies dealing with mechanisms are limited to the clinical manifestations of chloracne.

(1) Microsomal Enzyme Induction

TCDD's ability to induce a variety of microsomal enzymes is well documented. The induction of aryl hydrocarbon hydroxylase, delta-aminolevulinic acid synthetase and cytochrome P-448/P-450 associated enzymes are implicated in the development of cutaneous porphyria. The induction of aryl hydrocarbon hydroxylase and other mixed-function oxygenases/oxidases have been associated with carcinogenesis and tumorogenesis. In addition, TCDD has been shown to be a possible promoter or cocarcinogen of known carcinogens. In some nonhuman studies, TCDD produced a protective effect against endocrine tumors (e.g., pituitary, uterine, pancreatic, adrenal and mammary tumors). TCDD's induction of UDP-glucuronyl transferase, an important enzyme in steroid metabolism, may explain this peculiar effect of TCDD. The induction of DT-diaphorase and lysosomal acid proteinases has been implicated in TCDD's neuropathic effects. These and other biochemical alterations may account for TCDD's clinical manifestation of chloracne resulting from an over production of keratin in the sebaceous ducts.

(2) DNA/TCDD Interaction.

Alterations in the structure and fidelity of transcription of DNA due to TCDD have been indirectly demonstrated. In a similar fashion to the acridine family of compounds, TCDD, because of its planar ring structure, is felt to "intercalate" with DNA resulting in "frame-shift" mutations. A few laboratory studies with bacterial systems, e.g., Escherichia coli and Salmonella typhimurium, or in one plant system, e.g., the African Blood Lily, have identified TCDD as being able to produce chromosome aberrations and perhaps a weak dominant lethal effect. This hypothesized DNA/TCDD interaction could explain the development of chloracne, as well as the suggested mutagenic and carcinogenic effects, if similar mechanisms occur in mammalian species.

(3) Toxicity.

The effect of some nonspecific activity or as of yet unspecified toxicity continues to serve as a reasonable mechanism for TCDD's hepatic and thymus toxicity. TCDD has been described by some as "one of the most potent, low molecular weight toxins known", with extremely low concentrations producing severe liver damage and death in various animal studies. The immune suppression effect of TCDD has been shown to result specifically from its T-cell (thymus) toxicity. In addition, TCDD's concentration in the adipose tissue suggests the possibility that under situations of weight loss (e.g., life style, medical indications, or disease), TCDD may be released into the circulation. Such a hypothesized reemergence of the agent could result in low doses being either detectable and/or toxic at some later point in time. If TCDD's primary toxicity results from low doses (e.g., mutagenic/carcinogenic effect) rather than high doses (e.g., cellular poisoning and cell death), then the deposition of TCDD in the adipose tissue may have greater significance with respect to delayed effects on the longterm health of the exposed individual. This possibility raises a theoretical dose-response paradox which might "explain" the prevailing preponderance of symptoms in populations which may have been exposed to relatively low doses of TCDD.

delete

D. Animal Studies

A comparison of animal toxicity studies is difficult due to variations in experimental designs which include differences in (1) the species, age, and sex of animals used; (2) the level, route, and length of exposure to chemicals; (3) the purity of the chemicals used; and (4) the criteria measured and the time sequence of data collection. Animals have shown a wide range of toxic effects. This range may serve as a guide to anticipate the potential toxic effects in humans following exposure to Herbicide Orange.

A summarization of the literature is presented in the Appendix, Table A-1. It is apparent that the toxic effects of 2,4-D and 2,4,5-T are markedly different from TCDD. TCDD is approximately 1000 times more toxic in acute studies. In addition, the slower clearance time of TCDD may account for the significantly lower daily doses required to elicit chronic toxicity. A consistent finding in TCDD toxicity is depletion of the lymphoid tissues throughout the host. This is readily characterized by involution of the thymus in all species studied. In relationship to the chronic maternal toxic dose, the embryotoxic dose is markedly lower for TCDD than for 2,4-D and 2,4,5-T. Both 2,4,5-T and 2,4-D appear to be very weak teratogens and/or carcinogens at best, but these evaluations are complicated by varying levels of contamination by various dibenzo-p-dioxins. TCDD appears to have significant teratogenic and carcinogenic potential which appears to be species specific.

The most striking observation noted in the literature is a marked variation in response among species. Examples of these variations are in the areas of acute toxicity (TCDD's LD_{50} in the guinea pig is 1 g/kg compared to 1000 g/kg in dog), excretion (2,4,5-T plasma half-life in rats is 4.7 hrs compared to 77 hrs in dog), and oncogenicity (TCDD is oncogenic in rats but not shown to beoncogenic in mice under similar conditions). Even among strains of the same species (mice) variations in oncogenicity were noted following 2,4,5-T exposures. As noted earlier, this high variability between species is an important consideration in designing human studies.

A second area of interest noted in the literature is a possible dose-response paradox in nonhuman primates (rhesus monkey) following exposure to TCDD. Animals receiving subtoxic doses in single-dose acute toxicity studies (LD_{50} determinations) have not been followed over long periods of time. Animals on chronic exposure studies fed a low level of TCDD in feed [e.g., 50-500 parts per trillion (ppt)] have shown signs of disease only after several months when the accumulated dose was approximately 1 g/kg body weight. Therefore, it remains unclear whether the toxicity demonstrated in chronic exposure studies is dependent upon a low level daily exposure accumulated to 1 g/kg or would also be demonstrated following a single dose of 1 g/kg.

E. Case Reports

Much of the medical literature on 2,4-D, 2,4,5-T and TCDD exposures in humans is based on individual case reports. Most of the patients discussed in these reports were exposed to multiple chemical agents and, therefore, it is difficult to determine which agents were responsible for specific symptoms. Nevertheless, the general areas of dermatologic and neuropsychiatric disease have been of primary interest to most investigations. Since the neuropsychiatric symptoms of herbicide exposure are numerous and largely subjective in nature, they have been extremely difficult to assess from a clinical standpoint. Hepatic dysfunction, renal, gastrointestinal and cardiac disturbances are "linked" to exposures to these chlorophenolic compounds.

(1) 2,4-D

A multitude of symptoms have been attributed to 2,4-D, and the ones reported most consistently are listed in the Appendix, Table A-3. Components of some of these selected symptoms/signs are described in Table A-4 of the Appendix. The asthenic syndrome, peripheral neuropathy and hepatic dysfunction are of particular interest. Other symptoms of systemic toxicity occur, but usually resolve within 4-6 weeks. The peripheral neuropathy associated with 2,4-D exposure has been extensively described. It has an early onset, causes prolonged disability of variable degree,

and recovery has been incomplete in many cases. Electromyography in some patients has demonstrated denervation, and some studies have detected decreases in nerve conduction times. One autopsy study demonstrated a demyelination process within the brain of a 76-year-old male who committed suicide by ingestion of 2,4-D in kerosene.

(2) 2,4,5-T/TCDD

The human effects of 2,4,5-T are difficult to evaluate since the chemical is contaminated with TCDD in the manufacturing process. The effects of TCDD have been determined from studies of trichlorophenol workers, and from laboratory workers using TCDD. Symptom/sign complexes attributable to exposure to 2,4,5-T and TCDD are listed in Tables A-3 and A-4 of the Appendix. Chloracne usually begins in the zygomatic/temporal region and is often found on and behind the pinna of the ear. This is an oily acne-like skin condition characterized by comedones and inclusion cysts which may result in extensive scarring. In severe cases spread of lesions to the throat, back and inguinal areas has been noted. This skin condition is frequently preceded by erythema and blepharoconjunctivitis. Active lesions usually disappear within two years, but have been found 30 years after exposure. Porphyria cutanea tarda and hypothyroidism have also been linked to 2,4,5-T/TCDD exposure. Other symptoms such as asthenia, liver and renal dysfunction, neuropathy, and gastrointestinal and cardiac disturbances are probably due to mechanisms similar or identical to those of 2,4-D.

Numerous instances of alleged disease due to 2,4-D/2,4,5-T exposure have been the subject of heavy media attention, particularly an episode of alleged 2,4,5-T exposure in Globe, Arizona, in 1969. Despite extensive scientific review and analysis with negative findings, the Globe incident continues to appear in current news media productions. A similar incident in Missouri in 1971 is often cited. Six children and two adults experienced chloracne after accidental exposure to TCDD, but all were healthy after five years of followup study. A final prospective assessment of fertility, teratogenesis and carcinogenesis will probably be made in the future.

F. Veteran Complaints

The Veterans Administration Compensation and Pension Service, Washington, DC, provided data on 361 claims filed as of 30 April 1979, submitted by veterans alleging an altered health status due to exposure to Herbicide Orange. A review of these claims revealed that less than half of the veterans received detailed physical examinations to evaluate the claims. Numerous media presentations emphasizing both military and civilian herbicide exposures have alleged a remarkably wide spectrum of health effects

being claimed. Based on current guidelines established by the Veterans Administration (Program Guide 21-1, Section 0-18 and Title 38 USC), none of the symptoms elicited in these claims were shown to be secondary to exposure to Herbicide Orange. The vast majority of the exposure claims remained unsubstantiated, based on review of military personnel and medical records. The guidelines state that the only chronic residual of defoliant exposure ever incriminated by clinical history has been chloracne. Furthermore, chloracne was associated with prolonged intensive exposure and all other toxic effects of the herbicide were viewed to be rapid in onset and to run a brief course followed by recovery without residual disease. In fact, the vast majority of the claims alleging exposure to Herbicide Orange were not for chloracne and, as a result, did not satisfy the criteria set forth for compensation. Of the three claims dealing specifically with chloracne, none were confirmed by physical examination.

Table 1 summarizes the descriptive characteristics of the 361 claimants, while Table 2 summarizes the percent of symptom category complaints.

Table 1

SUMARY OF DESCRIPTIVE CHARACTERISTICS OF HERBICIDE RELATED CLAIMS SUBMITTED TO THE VERTERANS ADMINISTRATION AS OF 30 APRIL 1979*

Total Number of Claims:	361
Sex:	100% Male
Mean Age:	34 years
Mean Number of Alleged Symptoms per Veteran:	2.3
Branch of Service: (Service history identified in 66.8% of claims)	
US Army	66.4%
US Marine Corp	17.4%
US Air Force	11.2%
US Navy	5.0%

*Exact racial distribution unknown; anecdotal information suggests the majority of claimants are non-Caucasian.

Table 2

HERBICIDE RELATED CLAIMS SUBMITTED TO THE VERTERANS ADMINISTRATION BY SYMPTON CATEGORY AS OF 30 APRIL 1979

Total Number of Claims: 361-13 = 348*

		PERCENT
DERMATOLOGIC	(hairloss; chloracne; tinea, eczema, contact dermatititis, keloid, vitiligo, tumors, porphyria)	48.9
PSYCHIATRIC	(personality disorders, anxiety neurosis, depression, psychoses, pedophilia, alcoholism, adjustment reactions)	27.6
EAR, NOSE, & THROAT	(hearing loss, tinnitus, voice loss, sinusitis)	14.4
CANCER	(lung, bone, pancreas, brain, thyroid, larynx, colon, skin, soft palate, leukemia, lymphomas, Hodgkins Disease)	13.8
PERIPHERAL NEUROPATHY	(numbness, paresthesia, weakness, tingling, Guillan-Barre Syndrome, Multiple Sclerosis, Amylotrophic Lateral Sclerosis)	12.1
ASTHENIA	(headache, weight loss/gain, dizziness, fainting/blackouts, fatigue, lethargy)	11.2
GASTRO-INTESTINAL	(pain, ulcers, diarrhea, bleeding, hemorrhoids, colitis, achalasia, regional enteritis)	10.9
REPRODUCTIVE	(decreaed sex drive, impotence, decreased fertility, miscarriages, sterility; 15 genetic defects in offspring)	10.1
PULMONARY	(asthma, shortness of breath, infiltrates, chest pain, bronchitis, pulmonary hypertension, lung disease)	9.2
OPHTHALMOLOGIC	(conjunctivitis, visual loss, pterygium, blurred vision, light sensitivity, optic atrophy)	8.9
MUSCULO-SKELETAL	(arthritis, gout, fractures, stiffness, spasm, hernia, bone disease, strains)	8.1
CARDIO-VASCULAR	(hypertension, arrhythmias, myocardial infarction, peripheral vascular disease, heart problems)	7.5
GENITO-URINARY	(urethritis, stones, renal disease, prostatitis, epididymitis, testicular mass)	4.0
CENTRAL NERVOUS SYSTEM	(strokes, loss of memory, seizures, tremors, meningoencephalitis,speech impairment)	3.7
HEPATIC	(hepatitis, liver disease, gall-bladder disease, jaundice)	3.5

TABLE 2 (CONTINUED)

		PERCENT
PANCREATIC	(Diabetes Mellitus, pancreatitis, reactive hypoglycemia, increased amylase levels)	2.3
HERMTOLOGIC	(Pernicious Anemia, blood disorders, lymphnode disease, spleen disease, Polycythemia Vera)	1.4
COLLAGEN-VASCULAR	(Systemic Lupus Erythematosis, Rheumatoid Arthritis, Polymyosistis, Sarcoidosis)	1.2
ALLERGIC	(allergic reactions)	0.9
FEVER	(low-grade fever, fever of unknown origin)	0.9
POISONING (LATERITIC SOILS)		0.3
PERIODONTITIS		0.3
AMYLOIDOSIS		0.3
HYPERTHYROIDISM		0.3

*NOTE: 13 CLAIMS ALLEGED EXPOSURE ONLY (WITHOUT SYMPTOMS) AS BASIS FOR COMPENSATION

Study design implications that can be drawn from these tables are limited due to the lack of knowledge concerning denominator data. Overall, the group of claimants exhibited a high frequency of readily identifiable disorders (e.g., dematologic, psychiatric, and cancer, etc.). Further evaluation of the claims revealed that of the total number of claimants, 16.3%, had previous diagnoses of psychiatric disorders (20% of these diagnosed with schizophrenia).

Table 3 summarizes information on the USAF veterans as to general characteristics and alleged symptom category.

Table 3

HERBICIDE RELATED CLAIMS SUBMITTED BY USAF VETERANS BY SYMPTOM CATEGORY AS OF 30 APRIL 1979

Number of USAF Veterans: 28 (Mean age = 35.4 years)

Symptom	Percent
Psychiatric	50
Dermatologic	39
Reproductive	25
Peripheral Neuropathy	14
Cancer	7
Miscellaneous	7

The demonstrated lack of an easily identifiable symptom complex on review of the veteran claims clearly requires evaluation of individual symptoms. Therefore, a comprehensive questionnaire and physical examination is required.

G. Epidemiologic Studies

Epidemiologic studies of occupational groups have validated links between exposure to TCDD and the development of chloracne. Associations between TCDD and psychological abnormalities have also been suggested. A 1978 study by Hardell and Sandstrom in Sweden evaluated occupational exposure to chlorophenolic compounds in soft tissue cancer patients by a case-control design. They found an association between cancer and exposure, but methodologic problems have raised questions concerning the value of these findings.

Tung (1973) reported an abnormal increase in the occurrence of primary carcinoma of the liver in Vietnam (26 cases per year during 1955-1961 versus 144 cases per year during 1962-1968). He attributed the increase to a suspected carcinogenic effect of TCDD. His published study, however, has been criticized for failure to contain sufficient data and method descriptions to verify his conclusions. The role of aflatoxin as an alternative cause of liver cancer was not addressed. His study was largely an empiric clinical study. A study sponsored by the ~~US Environmental Protection Agency~~ EPA in 1979 in Alsea, Oregon, found a statistically significant increase in spontaneous abortion in areas where 2,4,5-T herbicide was routinely used in reforestation programs. EPA concluded, however, that "for all its complexity, this analysis is a correlation analysis, and correlation does not necessarily mean causation." This report is currently the subject of intense scientific criticism. Differences in the availability of specialty obstetrical care and in the patterns of health care delivery existed between the exposed and control areas; these differences were not taken into consideration by the researchers. Variations in the ascertainment of spontaneous abortions in each of the areas severely limited the validity of the data, and of the conclusions derived from them. A recent study conducted in Australia (1978) was unable to find an association between birth defects (neural tube abnormalities) and the use of 2,4,5-T Herbicide.

Epidemiologic studies are continuing in Seveso, Italy. A population of 220,000 was potentially exposed to TCDD following an industrial accident in July 1976. These studies have involved investigations of more than 30,000 children and detailed clinical examinations of 1,024 persons, including the most severely exposed children and adults. Recent data (Homberger, et al., 1979) indicated that most cases of chloracne from this incident (134) cleared rapidly. No evidence of significant hepatotoxicity, deranged porphyrin metabolism, or abnormal neurologic findings have been observed thus far. Growth and development of newborn infants

and children, immunological response, chromosome aberrations, the reaction to the challenges of infectious diseases, and the morbidity and mortality patterns of the study population have not been significantly altered by TCDD exposure to date. Thirty-eight cases of birth defects were reported in early 1977, approximately 6-8 months after the industrial accident. However, the authors ascribe this increase to an artifact of surveillance. The social pressures operating in the Seveso population prior to the accident fostered underreporting of birth defects, while the atmosphere after the accident made the occurrence of a birth defect more socially acceptable. The post accident malformation rate is not significantly different than the rate in similar areas of Central Europe. Similarly, ascertainment and surveillance of spontaneous abortions after July 1976 is hampered by the lack of valid baselines for the pre-accident period. Chloracne appears to be the only significant adverse effect in the exposed population noted to date.

A 2,4,5-T Dispute Resolution Conference was held in Arlington, Virginia, from 3 to 7 June 1979. Fifty-six recognized experts from the United States and seven foreign nations were actively involved in the deliberations of the conference. Human Exposure, Carcinogenicity/Mutagenicity, and Teratogenicity Working Groups independently reached conclusions that there was no valid scientific evidence linking fetotoxicity, teratogenicity or carcinogenicity to 2,4,5-T/TCDD exposures in humans. The Human Exposure Working Group also concluded that there were no epidemiologic data associating TCDD with any long-term health effect in humans other than persistent chloracne. While they did not find evidence of serious long-term health effects, neither could they find strong evidence for lack of effect. Most previous epidemiologic studies have not had sufficient statistical power to detect increased risks of low incidence/prevalence conditions in the observed populations, and the period of observation in many prospective studies has been less than ideal.

Several potentially valuable epidemiologic studies are currently in progress. Two independent and comprehensive studies of workers exposed to TCDD at a Monsanto manufacturing plant in Nitro, West Virginia, are currently being conducted (Mt. Sinai Medical Center, New York, and the Kettering Laboratory, University of Cincinnati, Ohio). These chemical industry workers were exposed over long periods of time and were previously evaluated in 1953 and 1956, following an industrial accident which occurred in 1949. The Dow Chemical Company is currently analyzing data from a reproductive survey of the spouses of 2,4,5-T/TCDD exposed workers. A Czechoslovakian study involving a 10 year followup of TCDD exposed workers, and a US National Cancer Institute (NCI) mortality study of 4,400 structural pest control workers are also underway.

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These new studies, and the continuing evaluations of the Seveso, Italy, population, should provide valuable data. The large study groups involved in the Seveso and NCI studies should provide good statistical power, and the Nitro, West Virginia, and Czechoslovakian efforts will evaluate the effects of exposure after prolonged periods of time (10-30 years). The results of these studies should fill major gaps in the knowledge of 2,4,5-T/TCDD epidemiology, and should prove to be useful in evaluating the long-term effects of these compounds on health and reproductive outcomes.

V. Epidemiologic Study Design: Matched Pair - Cohort

A. Design Consideration

The proposed goals for this study clearly mandate a broad comprehensive epidemiologic approach, incorporating retrospective, cross-sectional, and prospective elements. The primary issue is time. Exposure to herbicides during the 1962-1970 time period may have initiated long-term health effects that may or may not be progressive. If such effects are detectable by retrospective techniques, and are verified, there will be direct links to compensation. Current health status, as mirrored by the large number of recent VA claims, becomes of strong interest, because it might be confirmable by comprehensive physical examination. In the event both retrospective and cross-sectional methodologies yield indeterminant or weakly suggestive findings, it may be that sufficient time has not yet passed for substantial emergence of long-term health effects. This dictates a requirement for a prospective element to the study.

Many methodological shortcomings are inherent in each phase of this comprehensive study. To some extent, the classical deficiencies of each particular epidemiologic approach are compensated by the concurrent use of the other methods. For example, the low chance of identifying a relatively uncommon disease solely by the use of a cohort study is offset by the inclusion of the retrospective element. The relatively quick feedback that can be attained from the retrospective and cross-sectional studies will serve to better define the prospective phase and will help to alleviate problems that arise in cohort studies as a result of changes in diagnostic criteria and methods with time. Nevertheless, there will remain many problems that will affect ascertainment of disease in all phases of the study. The problem with patient recall of antecedent events, the distortion of information by knowledge of the disease, as well as participant or observer knowledge of their exposure status can only be corrected to a limited extent by review of records for symptom validation and "blind" assessment protocols. In addition, fundamental problems dealing with adequate selection of a control group and limiting loss to study can jeopardize even

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the most comprehensive epidemiologic investigation. These and other pitfalls in study design will be discussed in more detail in Section VIII.

Since the study has three phases and confronts a health issue with undefined endpoints, including strong bias and political pressure with severe time constraints, the following design may represent the best overall framework for achieving validity. The design process is complex and in itself time dependent. All epidemiologic techniques used are time-compressed. Unique record searching systems within the Air Force, and computer and clinical capabilities, as well as bias and loss-to-study correctors, will work toward making this effort achievable.

B. Ascertainment of Exposed and Control Group Populations

(1) Exposed Group

Operation RANCH HAND personnel primarily flew C-123 aircraft in Vietnam during 1962-1970. Data from hand-compile lists obtained through the RANCH HAND Association (a reunion organization), Air Force personnel computer entries, historical records and actual C-123 flight orders, place the estimated study population at 1000 (range 800-1200). Of those crewmembers now confirmed in the computer system, 25% are still on active duty, with the remainder being composed of retired or separated persons. An indepth search is being conducted of all organizational records stored at the Military Records Division, National Personnel Records Center, St. Louis, Missouri, to identify all RANCH HAND participants. The flight line maintenance personnel assigned directly to RANCH HAND can also be expected to range in age from 28-58. This group was almost exclusively enlisted with approximately 10-14% being Black. Detailed advertisements in active/retired military trade journals, VA publications, and local newspapers will be pursued in the near future to insure total ascertainment/indentification of the exposed group. Introductory letters will be sent to the last known address of all identified persons, and nonresponse will be pursued by cross-locator systems available within the government (e.g., Social Security Administration, VA, Internal Revenue Service). Significant effort will be made to account for at least 99% of the total population (see Table A-5, Section XII). Because of the limited number of estimated RANCH HAND personnel (1000), no subsampling is planned in any phase of the study. All members will be invited to participate in all phases of the investigation.

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(a) Known or Predicted Characteristics of the Exposed Group

All exposed aircrew personnel are males currently ranging in age from approximately 28-58 years. As the normal C-123 crew composition was one pilot and one copilot/

navigator (both officers) and one spray equipment console operator in the rear of the aircraft (enlisted), the overall officer-enlisted ratio will be approximately 2:1. While almost all officers were Caucasian, approximately 10-14% of the enlisted men were Black. Attempts will be made to identify all maintenance personnel assigned to the RANCH HAND units. Maintenance of the RANCH HAND aircraft was performed within a stepwise organizational structure. Routine daily maintenance was conducted by flight line personnel who were often dedicated exclusively to RANCH HAND operations. More extensive maintenance (secondary) was carried out by consolidated units at the base level, wqhich were also responsible for nonRANCH HAND C-123s as well. Major aircraft overhauls and modification were conducted by maintenance units at Clark Air Base, Philippines. The maintenance personnel in these centralized units were not directly assigned to RANCH HAND, and their exposures to RANCH HAND C-123 aircraft and herbicide cannot be validated. From 1962 through 1964, the primary flight line maintenance teams were dedicated to RANCH HAND aircraft and these individuals can be identified by the mechanisms described above to identify the flight crew personnel. In 1965, flight line maintenance was performed by personnel of the centralized maintenance organization (secondary) and the individual working on the RANCH HAND C-123s cannot be specifically identified from available records. After 1966, the RANCH HAND organization transferred their base of operations to a new location, and primary maintenance was once again performed by personnel assigned specifically to RANCH HAND. These individuals can again be readily identified. Thus, maintenance personnel directly assigned to RANCH HAND will be included in the study, but data from this group will be analyzed separately from the aircrew data. These complexities are summarized in Table 4.

Table 4

FEASIBILITY FOR IDENTIFYING AIRCRAFT MAINTENANCE PERSONNEL (TOTAL POPULATION) EXPOSED TO HERBICIDE ORANGE

Time	Primary Maint Personnel[1]	Secondary Maint Personnel[2]
Jan 1962-Jul 1964	Yes	No
Aug 1964-Dec 1966	Yes/No	No
Jan 1967-Apr 1970	Yes	No

[1]individual assigned to RH; denominator known
[2]individual not assigned specifically to RH, although may have served the aircraft; denominator not ascertainable

Because of the significant combat hazard associated with low, slow flying missions, all early RANCH HAND crewmembers were elite volunteers (see Risk-Taker Bias, Section VIII). In fact, RANCH HAND crew members comprised one of the most highly decorated units during the Vietnam Conflict. Anecdotal stories reveal that most crew members were, on occasion, heavily exposed to Herbicide Orange due to normal or combat induced equipment malfunctions within the aircraft. Many former RANCH HAND personnel are expected to be currently employed in the aerospace industry as commercial airline pilots, airline managers, and flight mechanics. RANCH HAND personnel still on active duty are expected to be found in senior management positions.

(2) Ancillary Study Groups (Non-RANCH HAND personnel)

Air Force handlers of herbicide drums in Vietnam were exposed to herbicides because of drum leakage. Advertisements similar to those proposed for the RANCH HAND personnel will be issued in attempts to define this population. As the drum handlers were ad lib participants, no personnel designator was assigned to these individuals, thus prohibiting computer tracking and identification. The population is unknown, but expected to be low (less than 200) as the majority of drum handlers were known to be Vietnamese. Additional study groups such as US Army personnel (officer and enlisted) who flew as observers, US Army helicopter crews, as well as experimental fighter-bomber spray personnel, may be injected into the study proper. Specific epidemiologic/clinical studies for these groups will be planned by a separate protocol following ascertainment; control group selection will be difficult or moot. It is intended that all data derived from the ancillary study groups will be subsetted for separate analysis; these data will be treated as anecdotal to the primary study.

(a) Known or Predicted Characteristics of the Ancillary Study Groups

All members of these groups are expected to be males, ranging in age from 28-68 years. The officer-enlisted ratio is estimated at 1:10. Approximately 10-18% of these populations are expected to be Black. Low numbers of respondents are expected (and with significant discordance to the above estimated characteristics due to socio-economic-race bias). Population at risk ascertainments are not possible.

(3) Control Group (Not exposed to Herbicide Orange)

The ideal control group, the non-RANCH HAND C-123 population, is known to be too small (approximately 3000) to provide adequate sampling flexibility and replacement under the proposed best match variable concept (see below and Section VI, A). Many of the RANCH HAND aircraft were reconfigured for transport and

COMPARISON OF THE STUDY GROUP TO POSSIBLE CONTROL GROUPS BY

KNOWN AND ESTIMATED FACTORS

KNOWN FACTORS	STUDY GROUP	POSSIBLE CONTROL GROUPS		
	RANCH HAND C-123	Non-RANCH HAND C-123	C-7	C-130
POPULATION RANGE	800-1200	3000	1200	20,000-25,000
OFFICER/ENLISTED CREW RATIO	2:1	2:1	2:1	3:2
AIRCRAFT FUEL (AV-GAS)	YES (+JP-4)*	YES (+JP-4)*	YES	NO (JP-4)
OCCUPATIONAL HERBICIDE EXPOSURE	YES	YES/NO**	NO	NO
ESTIMATED FACTORS				
OCCUPATIONAL INSECTICIDE EXPOSURE	2+	1+ to 4+	0	0
COMBAT HAZARD	4+	3+	3+	2+
RVN-IN COUNTRY ASSIGNMENT	4+	4+	4+	2+

*In 1968, aircraft were modified with a JP-4 booster.
**Contaminated aircraft reconfigured for transport may have resulted in exposure to non-RANCH HAND personnel.

insecticide missions. Thus, non-RANCH HAND crews responsible for these other missions, may have been exposed to Orange Herbicide residues in these aircraft. This group may not be truly unexposed to herbicides and therefore may not be an appropriate control population. The C-7 crewmembers have also been considered as a potential control group. This latter group, however, was comprised of only 1000 to 1200 individuals. Accordingly, aircrew members who flew C-130 aircraft in Vietnam during 1962-1970 will be selected as controls to the RANCH HAND aircrew population. Total ascertainment of this C-130 population is being conducted by computer selection for specific mililtary flying organizations, foreign country service, etc. Over 2.3 million personnel records have already been scanned and the approximate C-130 sample size is 25,000 aircrew members. The C-130 flight line maintenance population will be ascertained from personnel records by similar mechanisms, and will serve as the specific control population for the RANCH HAND maintenance personnel. The proportions in active duty, and non-active duty status are expected to parallel the patterns in the exposed group.

The absolute "best fit" matched C-130 controls will be used for the retrospective mortality analysis (see below) versus all ascertained RANCH HAND members. In the event of mortality or nonresponse from a best fit control, the next best fit C-130 control will be selected for replacement for the cross-sectional and prospective study elements of the study.

(a) Known or Predicted Characteristics of the Control Group

All C-130 aircrew members and appropriate maintenance personnel will be tightly matched on a 1:1 basis to the exposed group with respect to age, sex, race, and other factors listed below. The normal crew composition of a C-130 is three officers and two enlisted personnel. The control group will be "pure" from the standpoint of nonoccupational exposure to herbicide. The entire control group will be considered "nonvolunteer" with respect to abnormally high combat risk. While in general they will probably possess similar lifestyle characteristics and socio-economic backgrounds, their overall combat morbidity/ mortality and resultant stress influences upon general health may be slightly less than the exposed group. For those separated and retired C-130 controls, similar proportions to the exposed group are expected to be employed in the aerospace industry. Known and factors of the control and exposed populations are summarized in Table 5.

(4) Matched Pair Procedures and Rationale

Each member of the exposed group will be computer matched for four variables to a set of at least 10 control C-130

crewmembers. Since the two groups are highly selected and inherently similar with respect to many variables, very close matches are feasible. This epidemiologic design incorporates a matched pair concept because: (1) a matched pair with stratification will provide maximum test power throughout retrospective, cross-sectional, and prospective phases, (2) statistical intergroup comparisons may be made without normalization by four key variables known to effect symptom frequencies of interest, thus providing greater power for complex multivariate testing, and (3) extremely close matching is feasible and necessary for some of the anticipated analyses of the physical examination findings. Matches will not necessarily be rigidly maintained throughout the data analysis phase, depending upon the particular analysis. It is apparent that following the match, both exposed and control populations will be very nearly identical with respect to the four influencing variables, and that in the event of frequent match breaks, stratification of a controlling variable can be made with enough precision to ensure proper adjustment.

Matching will be conducted for (1) age, by year of birth, and closest month possible, (2) Air Force Speciality Code (AFSC) as an absolute match, (3) length of time spent in Vietnam, to the closest six month period, and (4) race (Caucasian versus non-Caucasian) as an absolute match. These variables are listed in priority order of the match sequence. Specific rationale for these variables is as follows: (1) many clinical symptoms and signs allegedly attributed to herbicide exposure (see literature review) can also be attributed to an aging effect, or to collateral diseases more commonly associated with advancing age, (2) AFSC controls specifically for officer-enlisted status (as well as crewmember or noncrewmember status), a variable strongly linked to educational background, current socio-economic status, and moderately linked to age (5 year median difference) and socio-economic background, (3) length of tour in Vietnam (measured in six month intervals, or actual flying hours, if feasible) controls for the generalized probability of combat morbidity, mortality, and for combat induced neuro-psychiatric disorders [additionally, length of tour may reflect effects related to intensity of alcohol consumption, drug consumption (chemoprophylactic or illicit), and degree of disease acquisition] and (4) race controls for difficulty in diagnosis of dermatitis, socio-economic background, etc. (note possible racial discordance for VA claimants).

An intragroup comparison in the exposed group of health effects to an index of exposure to herbicides will be made. By using the length of time in Vietnam, as measured by exact flying hours coupled to the exact time of the tour when the concentration of TCDD contamination changed, a crude exposure index can be constructed, normalized, and tested.

C. Retrospective Phase

(1) Introduction

The term "retrospective" has been used generically in this discussion of the design protocol to address the mechanics of selection of the exposed and nonexposed groups, as well as the various means of tracing these groups to the present. In reality, the retrospective and prospective phases are components of a "non-concurrent" prospective study used in the observation, starting from some date in the past, of a specially exposed group or industrial population. The availability of employment, medical or other types of records is an obvious requisite for such a study. The classical "case-control", retrospective study is not operative in this protocol because of the open-ended nature of the alleged effects and lack of a definable disease syndrome.

(2) Data Collection Methods

All exposed members and their matched controls will be given a comprehensive personal and family health questionnaire via telephone. The questionnaire (see Section XIII) will emphasize identification data, Vietnam tour history, dermatologic conditions, neuropsychiatric conditions, fertility aberrations, genetic defects in offspring, sensory defects, and personality factors, including assessments of risk-taking behavior. The questionnaire will be limited to a 30-45 minute telephonic interaction with participants, and it will take 10 to 12 months to complete all initial questionnaires on both groups. The questionnaire will be "field-tested" on a group of 25 to 30 former Air Force pilots with Vietnam combat experience. Specific questions on the questionnaire will be directed to verifiable information, wherever possible. Inclusion of specific verifying questions and bias indicator questions (nonsense symptoms) have as yet not been included, since they are still under development. They will be added and appropriately sequenced immediately prior to the start of the study. Questionnaire data will be cross-linked and integrated with medical record information and physical examination findings. Each participant will be asked to sign release forms so that all civilian health records, including those of dependents, can be reviewed as necessary. Federal health records on all family members on file in the St. Louis Repository will be retrieved. For retired members, and separated members with VA priviledge, all available medical records will be obtained. All retrieved medical records will be reviewed, scored, compared to questionnaire data for reliabilility, and then be entered into a repository system. Identified participants who are non-responsive to questionnaire will be pursued to determine status, disinterest, moribund state or death, etc. These individuals will be cross-referenced to other federal accounting systems in an attempt to achieve total ascertainment.

Death certificates will be retrieved on all dead exposed and control subjects. Birth/death certificates will be sought for all offspring, born subsequent to the study subject's Vietnam duty.

D. Cross-Sectional Phase

(1) Selection/Entry Criteria

A voluntary comprehensive physical examination will be offered to all individuals in both the exposed and control study groups. The condition for entry into this phase will be completion of the baseline questionnaire. In the event that the "best fit" control does not complete both the questionnaire and the physical examination, the next "best fit" will be selected, and so on, until a willing control is obtained. Statistical testing will be conducted by a variety of techniques on both questionnaire and examination findings (see VI, Statistical Methodology below). At the time of physical examination, an extensive indepth interview will be conducted. A standardized protocol will be used to insure comparability of inteview data. This will provide cross-reference data to the initial questionnaire and to medical record data, if retrievable. Specific response verification and bias indicator questions will be included during the interview as well.

(2) Physical Examination Parameters

A comprehensive physical examination will be conducted on all willing participants. The examination will be structured as outlined below and in Section XIV.

General Physical Examination

FBS, 2 Hr Post Prandial	CBC →	CPK →
Urinalysis	RBC Indices	ECG
BUN/Creatinine	Sedimentation Rate	Chest X-Ray
Cholesterol/HDL Cholesterol	Cortisol (Matching)	VDRL/FTA
Triglycerides	Thyroid Profile (RIA)	

Dermatologic Examination

Urine Porphyrins
Urine Porphobilinogen
DELTA-aminolevulenic Acid

Neuro-Psychiatric

Nerve Conduction Velocities
Psychological Battery
MMPI
WAIS

Halstead-Reitan
Wechsler Memory Scale Subtests
Cornell Index

Reproductive Examination

LH, FSH, Testosterone
SEMEN Analysis

Neoplastic/Hepatic Examination

SGOT	Alkaline Phosphatase
SGPT	LDH (Isoenzymes if elevated)

Additional Studies (Individuals with abnormal history or examination)

Karyotyping	Hepatitis Antigens (A and B)
Additional Consultations as Required	Mono Spot Test
	Anti-Nuclear Antibody

Examinations will be performed in regional federal medical facilities having dermatologic, neurologic and electromyogram/nerve conduction capabilities in the United States and overseas (Section XII, Figure A-1). This will generate better participation than if all examinations were conducted at a single location. Special Air Force authorization will be obtained to conduct such examinations on individuals separated from the service and informed consent forms will be obtained for nerve conduction tests. Physicians and technicians will handle all participants without a knowledge of exposed or control status, and will conduct the examinations by standardized protocols to minimize variability. Clinical specimens will be forwarded to USAFSAM where the laboratory procedures will be conducted. All laboratory tests will thus be subject to the same technology and rigid quality control.

qualified and specifically trained?

Special contingencies will be made for unusal laboratory testing. Karyotyping of the individual and his family members will be performed if clinical history or physical examination findings are suggestive of this need. Most well conducted studies have shown that, when present, chromosomal abnormalities due to TCDD are transient. If on detailed analysis of the baseline examination and questionnaire, reproductive areas are heavily affected, routine karyotyping may be included in the test battery for the prospectus. TCDD analysis on blood and urine will be considered in the future provided that (1) strong cause and effect relationships can be ascribed to Herbicide Orange and (2) high resolution mass spectrometry technology achieves 10 femtogram sensitivity with high specificity. Appropriate specimens will be obtained from all participants, aliquoted, and preserved at -70°C for possible future analysis in the future.

Physical examination and laboratory data will be placed in the member's master file for detailed cross-analysis to questionnaire data. Information identifiable to the subject will not be released without his consent in accordance with the Privacy Act. (Exceptions: In accordance with Air Force regulations, all active duty flying personnel and air traffic controllers found to have disqualifying defects will be temporarily grounded pending resolution; in accordance with federal regulations, all commercial airline pilots and air traffic controllers found to have disqualifying defects will be reported to the Federal Aviation Administration.)

E. Prospective Phase

(1) Study Adaptations

Following complete data analysis of the retrospective and cross-sectional phases, an adaptive or restrictive health survey will be developed and annually administered for five years. Similarly, a condensed physical examination profile that will achieve adequate sensitivity and specificity for prospective diagnosis will be developed. This adaptive physical examination by protocol will be applied to all prospective phase participants, and will be conducted at regional federal medical facilities every two years at government expense.

(2) Entry Criteria

All exposed or control individuals completing the baseline questionnaire and physical examination will be entered into the prospectus; further continuation will depend upon the member's willingness/ability to participate in additional health surveys and condensed examinations.

(3) Loss to Study

At the initiation of the prospective phase loss of an exposed member will not be cause to cease surveillance of his matched control. In the event of a control loss, the next "best fit" match control will be brought to study, the comprehensive questionnaire will be administered, and the adaptive examination offered. In all cases of loss-to-study, specific reasons will be sought. Medical record reviews of new entrants and death certificate reviews will continue throughout the prospective phase. Mortality analysis based upon time and cause of death within the ascertained groups will be conducted throughout the study.

(4) Study Length

The prospective phase is initially planned for five consecutive years. Results of the entire effort will be presented

to a neutral scientific body. Their recommendation for continuance/discontinuance will be forwarded to the Air Force Surgeon General for final decision.

F. Determination of "Disease"

(1) Introduction

Since this study is dealing with an unknown clinical endpoint, determination of disease state by statistical methodology is a prime scientific thrust of the investigation. From the literature, chloracne is the only recognized disease associated with high exposure to dioxin. The questions of primary interest are: (1) Does a history of chloracne invariably lead to future disease? and (2) In the absence of chloracne, is there emergence of other attributable diseases? Under a broad concept of "spectrum of illness", either or both of these conditions are possible. The clarification of their respective contributions to the natural history of subsequent "disease" is extremely difficult.

(2) Discussion

Inferences about a disease state from this study can be derived from several logical approaches. These approaches can be grouped into two categories: (1) those dealing with symptoms which can be used to construct a symptom complex that may represent disease, and (2) those dealing with physical signs which in themselves represent disease. In the former, one can form a subset of individuals that have symptoms (e.g., infertility) and study them during the "retrospective" and prospective phase. Focusing on the overall patterns of alleged symptoms and categorizing them into a symptom complex may identify those individuals with a disease syndrome, or those at higher risk of developing disease (e.g., genetic disorders, cancer). In the latter approach, data on abnormal physical signs (e.g., genetic defects in offspring) and laboratory results can be compared between exposed and non-exposed groups in an attempt to again establish the presence or absence of disease. By putting this array of data into a logical decision-making scheme, specific relative risks can be calculated in the prospective phase.

By the use of combinational and correlational analyses, statements about the probability of a disease state, a subclinical state, and over-reporting bias can be attempted. If the development of symptoms in the exposed group is positively correlated with physical findings, and this correlation is absent in the control group, a statement concerning the existence of a possible disease state can be made. By taking these possible combinations of observations and viewing them in the context of associated positive

verifiers, negative bias indicators, and positive exposure index, the probability of over-reporting bias acting in these circumstances can be substantially reduced and, as a result, any statement concerning the existence of disease is strengthened. Similarly, if symptoms in the exposed group do not correlate with the development of findings, but are associated with positive laboratory results, a statement concerning the existence of a subclinical disease state can be made. On the other hand, if comparisons within the RANCH HAND group reveal a negative correlation between reported symptoms and the presence of abnormal physical signs, then an over-reporting bias and/or subclinical disease state is suggested.

Another approach to the determination of a disease state is by taking a normalized exposure index and applying regression techniques to the resulting curve. If there is a positive correlation between increased exposure and the presence of various abnormal physical signs and/or verifiable symptoms, then a symptom complex or disease syndrome is suggested. Factors suspected of altering the classical dose-response curve include cellular repair mechanisms and the release of TCDD from adipose tissue following weight loss. The addition of multivariate techniques to the regression analyses will strengthen statements about the presence of disease. Beyond these pair-wise and group comparisons, newer techniques of pattern recognition, such as Factor Analysis and Cluster Theory, are being considered in order to achieve a more automatic and objective analysis.

The strength of any inferences made from these analyses is dependent upon the statistical power inherent in the study. In addition, due to the possibility of latency being a factor in this study, a negative analysis at any time within the study does not categorically imply lack of disease, since sufficient time for emergence may not have passed.

G. Exposure Estimates

The exposure to Herbicide Orange and/or other herbicides by RANCH HAND personnel was frequent (almost daily), extensive (anecdotal information suggests that many had direct skin contact) and repetitive over a long period of time (one-year tour for most individuals). Anecdotal information also suggests that most RANCH HAND personnel felt that the herbicides employed in the operations were nontoxic to animals and man and hence, did not exercise the caution in handling that is recommended today. The available records on Operation RANCH HAND indicate that aircrews assigned to the project seldom had a "routine" work schedule or environment. Thus, numerous factors influenced the level of herbicide exposure that RANCH HAND personnel may have received. Such factors included the length of tour, number of tours, crew position, number of herbicide dissemination missions, herbicides

employed (Orange, Blue or White), time to and from mission locations, and multiple routes of exposure (inhalation, ingestion and/or percutaneous absorption).

Although industrial hygiene data are not available from defoliation operations during the Vietnam War, the Air Force did conduct extensive industrial hygiene monitoring programs during the dedrumming and incineration of Herbicide Orange during Project PACER HO (see Young, et. al., 1978). These monitoring data (e.g., breathing zone data) and recently unpublished data on percutaneous absorption of 2,4,5-T in humans during actual spray operations (DOW Chemical U.S.A., Midland MI, 1979), when combined with data on characteristics of the C-123 aircraft, number of missions and crew position may permit crude calculations of exposure estimates.

VI. Statistical Methodology

A. Introduction

The design of the study is presented in schematic form in Figure 1. R' refers to the RANCH HAND personnel and C" refers to the collection of all possible control crewmembers. As defined, R' and C" will contain individuals who are deceased. Since C" may be 10 to 25 times larger than R', a subsample C' of C" may be constructed matched to R' in a pairwise or 1 to 1 manner (Carpenter, 1977; McKinlay, 1977). The control group C' will be matched to R' as closely as possible using: age, AFSC, Vietnam tour length, and race. C' will be constructed without regard to whether the individual is currently living or dead so that an assessment of mortality can be accomplished. Statistical aspects of this mortality analysis will be described in more detail below. If C'cannot be constructed from C" using pairwise or 1 to 1 matching, it will be constructed using stratified random sampling.

Referring again to Figure 1, R and C indicate living RANCH HAND members and matched controls. If $m_{R'}$ is the proportion of R' found to be deceased, then,

$$R = (1 - mR')R'$$

If the best match for an exposed individual is in fact deceased, the next best control will be selected to complete the study pair for subsequent study phases so that the number of individuals in R will equal the number of individuals in C and 1 to 1 matching will be preserved.

The questionnaire will provide data concerning specific symptoms and other findings in the R and C groups. Thus various questionnaire finding rates in R, s_R, will be calculated and compared with the corresponding rates in C, s_C.

The questionnaire will allow allocation of RANCH HAND personnel into those with symptoms on questionnaire, indicated by RS, and those without, RS. Similarly, the control individuals will be placed into symptomatic, indicated CS, and asymptomatic, CS groups.

The physical examination performed on individuals from R and C will allow estimation and comparison of rates of physical findings in these groups. Rates of abnormal physical findings can be symbolically indicated as f_R and f_C for RANCH HAND and control groups respectively. Comparison of these rates is very important and details will be discussed below.

Let f_{RS} be the rate of physical findings among RANCH HAND personnel with findings by questionnaire and let $f_{\overline{RS}}$ be the rate of physical findings among RANCH HAND people with no findings on their questionnaire. For most disease processes it would be expected that f_{RS} should be a larger rate than $f_{\overline{RS}}$. If f_{RS} is observed to be equal to or less than $f_{\overline{RS}}$, an interpretation of over-reporting may be warranted, although the possibility of subclinical disease is recognized. Rates $f_{\overline{CS}}$ and f_{CS} will also be estimated, and comparisons between f_{RS}, $f_{\overline{CS}}$, $f_{\overline{RS}}$ and f_{CS} will be accomplished.

B. General Concerns

Before proceeding to statistical details regarding this design, three general concerns about the overall design will be discussed.

(1) Adequacy of the Control Group

Candidate groups comprising C", the set of all possible control crewmembers, include: C-130, C-7, and non-Ranch Hand C-123 crewmembers. Known and estimated factors relevant to these potential control groups and RANCH HAND personnel are listed and evaluated in Table 5 below. Considering the estimated factors, a subjective estimate from 0 to 4+ is provided. At the present time, no data have been found to suggest that the fuels involved in these aircraft have the capability to adversely effect health when the levels of exposure and exposure routes are considered. On the other hand, exposure to insecticide may be a significant confounding factor. Further, RANCH HAND personnel did have more combat involvement than the other groups thus far considered, and this stressful experience could exert a long term effect on morbidity and mortality rates in this group. Also, the fact that many RANCH HAND personnel volunteered for their hazardous duty suggests that this group may include individuals who are risk takers. This factor could inject significant bias into the study. For example, it is possible that risk taking behavior could be correlated with different patterns of disease; for instance, type A personality type could be more common among the intense, risk taking RANCH HAND personnel, thus increasing the incidence of cardiovascular disease. In relation to herbicide exposure, an increased accidental death rate among RANCH HAND personnel could well be an indication of herbicide induced peripheral neuropathy. However, higher accidental death rates in general are expected among risk takers. Actual time spent in Vietnam could also be an important factor to control. The total time spent in Vietnam indicates a magnitude of stress imposed on the individual.

As indicated in Table 5 no available control group is ideal. Variations in risk taking behavior, occupational insecticide exposure and aircraft fuel exposure appear most important. Risk taking differences can be approached using a risk taking scale and this will be discussed under mortality analysis and under analysis of questionnaire and physical examination data. Aircraft fuel differences may well be dismissed as an unlikely or nonexistent effect with further literature search, and further literature search is also required as regards the possibility of an insecticide effect.

(2) Adequacy of Sample Sizes

While this subject will be treated in greater detail below, some general observations are in order. The size of R' is approximately 1000 individuals. Without formal statistical analysis it should be quite clear that a lethal effect of herbicide which occurs in only 1 out of 2000 controls will be quite difficult to detect unless the herbicide effect is very strong. For example, at a rate of 1 in 2000, 0.5 effected controls are expected. If the basic rate is doubled by herbicide to 2 per 2000, one effected RANCH HAND individual would be expected. At a rate of 1 per 2000 for controls and a rate of 2 per 2000 for RANCH HAND personnel, the probability of observing no affected individuals in both groups is

$$(1 - 1/2000)^{1000} (1 - 2/2000)^{1000} = .22$$

or, in other words, "there is a 22% chance" that no affected individuals will be found in this study. In a population of 100,000 exposed individuals, 100 cases would be expected, 50 of which would be due to herbicide. In short, this study has little statistical power to define the relationship of herbicide to the rarer diseases.

(3) Misclassification

To understand the effect of misrepresentation on the estimof relative risk and the odds ratio, let S stand for presence of a cesymptom, and S denote its absence. This misclassification may be represented as in Figure 2.

delete space

FIGURE 2 MISCLASSIFICATION

REPORTED STATUS	ACTUAL STATUS S	ACTUAL STATUS S	TOTAL
S	A	B	A + B
S	C	D	C + D
	A + C	B + D	

The proportion of correctly classified positives is defined by A/(A+C) and is called the sensitivity of the classification scheme; the proportion of correctly classified negatives D/(B+D) is called the specificity.

When there is non-differential misclassification, that is, when the sensitivity is the same among the exposed and nonexposed, and the specificity is the same among the exposed and nonexposed, the bias induced in the estimate of relative risk will be toward the null value. The situation may be summarized by Figure 3.

FIGURE 3

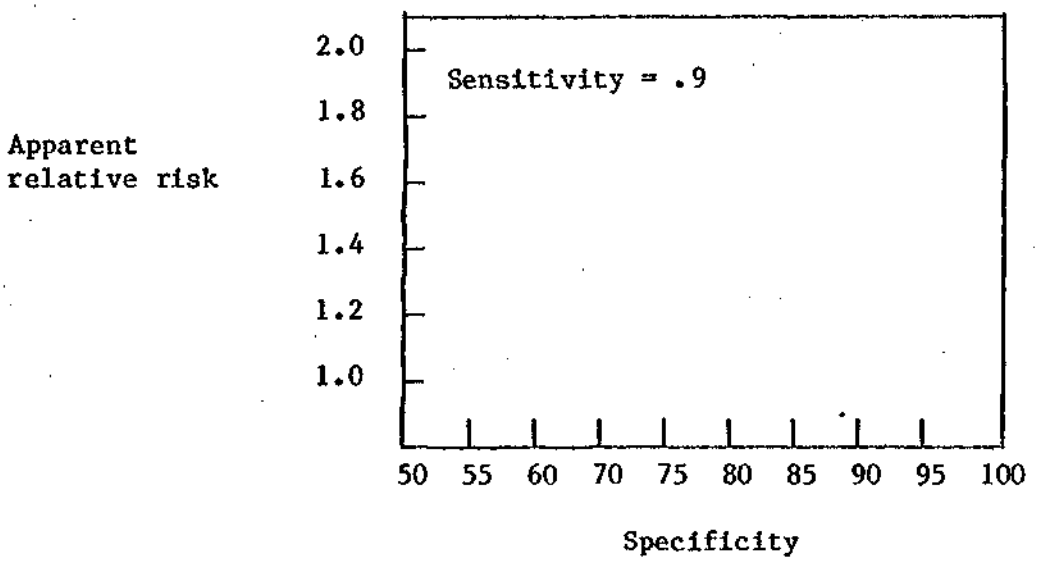
MISCLASSIFICATION IN RANCH HAND II

		ACTUAL STATUS EXPOSED S	EXPOSED S	EXPOSED TOTAL	NONEXPOSED S	NONEXPOSED S	NONEXPOSED TOTAL
REPORTED STATUS	S	a	b	a + b	e	f	e +f
	S	c	d	c + d	g	h	g + h
		a + c	b + d	n	e + g	f + h	n

Using this representation, the true relative risk is (a+c)/n - (e+g)/n , and the apparent relative risk is (a+b)/n - (e+f)/n. Figure 4 provides a graphic representation of how apparent relative risk varies as a function of sensitivity and specificity. For these curves, the true relative risk is 2 with the exposed population having a symptom incidence of 0.1 and the nonexposed population having a symptom incidence of 0.05 (Copeland et. al. 1977). The

effect of non-differential misclassification on the odds ratio is nearly as severe as that shown in Figure 4 for relative risk. A technique does exist for correcting the estimate of relative risk to account for misclassification, but the technique requires knowledge of the sensitivity and specificity of the classification scheme, knowledge that may not exist in this study. It should be noted that since the above remarks are concerned with relative risk, the number n of subjects in each group is irrelevant, as the results shown are independent of n.

FIGURE 4
APPARENT RELATIVE RISK VERSUS
SPECIFICITY



If the misclassification is differential, an estimate of relative risk that is biased away from the null value can result. This will occur in situations in which the RANCH HAND personnel and controls do not misrepresent their symptoms in the same manner. (Copeland et. al. 1977).

(4) Nonresponse and Noncompliance

The prospective phase. Loss to follow up may occur for different reasons in the RANCH HAND group than in the Control group. In addition, this censoring may be related to the death experience, especially in the RANCH HAND group. For example, the disappearance of a RANCH HAND individual may be due to the effect of exposure, making loss to follow up dependent on the death process. It should be noted that all currently available survival analysis techniques are founded on the assumption that censoring is independent of the death process, and all comparative tests require that the censoring mechanism operate in the same way in both groups considered. The effect of departure from the assumptions is not well understood.

The physical examination. Noncompliance will be a general problem in both the exposed and nonexposed groups. If the reasons for noncompliance are systematically different in the two groups, significant bias can result. For example, a number of RANCH HAND personnel may refuse to comply acting under advice from legal counsel. Noncomplaint controls would not likely have this cause but be more motivated by reasons such as inability to take time from work. RANCH HAND individuals motivated by legal advice or other similar reasons, may in fact be symptomatic, thus decreasing the number of positive findings in this study.

C. Analysis of Mortality Data

Considering the basic cohorts R' and C', individuals will be classified into three categories: alive, dead, unaccounted. If a large number of individuals of each group are unaccounted for, the study can obviously be severely biased. Thus, significant effort must be expended to reduce the unaccounted category as far as possible. At most 1 to 3 percent of both groups can be allowed to remain unaccounted, with a 1% rate being preferred. If for example, the mortality rate in C' is 0.15, then an unaccountability rate of 0.01 is 6.6% of this mortality. Whatever the unaccountability rates, the pattern of unaccountability must also be compared between groups R' and C'. For example, the possibility of age differences or Vietnam tour length differences must be examined, particularly if the unaccountability rates are high. The following will discuss analysis of mortality under the assumption that low unaccountability rates have rendered the mortality analysis meaningful.

The mortality data will be analyzed using several different approaches. Crude age-specific death rates will first be calculated and tabulated. Age will be divided into k strata, and person-years will be observed for each strata as will be the number of deaths in each strata. In this manner a tabular display will be developed as shown in table 6.

SPACE FOR ADJUSTMENT

Table 6

STRATIFIED FORMAT OF AGE-SPECIFIC DEATH RATES

	Ranch Hand			Controls		
Age Group	Person Years	Deaths	Death Rate	Person Years	Deaths	Death Rate
1	P_{11}	m_{11}	r_{11}	P_{21}	m_{21}	r_{21}
2	P_{12}	m_{12}	r_{12}	P_{22}	m_{22}	r_{22}
3	P_{13}	m_{13}	r_{13}	P_{23}	m_{23}	r_{23}
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
k	P_{1k}	m_{1k}	r_{1k}	P_{2k}	m_{2k}	r_{2k}

Since the death rates r_{1j} and r_{2j} are Poisson variables, they can be contrasted directly. If the relationship of r_{1j} to r_{2j} is found to be consistent between age strata (with statistical variability), a summary mortality index may be calculated. One summary index that will be calculated is Proportionate Mortality Ratio (PMR) which is:

Classical standardized mortality ratios will not be calculated for RANCH HAND II due to the effects of the Health Worker Phenomenon. The term is the total number of deaths observed in the RANCH HAND group while is the number of deaths that would be expected were the age-specific RANCH HAND death rates the same as

the age-specific control deaths rates. Thus the concern is for an PMR greater than 100%. If a crude rate for controls, d_C, is calculated as

then the proportionate crude rate for the RANCH HAND group d_{RH} is

$$d_{RH} = Md_C.$$

An approximate statistical test would regard r_{RH} as a Poisson random variable with mean d_C.

An alternative approach to the provision of a proportionate mortality ratio is that of Breslow and Day (1975). In this treatment, a multiplicative model is employed, for example:

where is mortality rate, is the contribution due to population differences (RANCH HAND versus Control), is the contribution due to age group, and is the contribution due to tour length, etc. The statistical approach here is via maximum likelihood.

Logistic models (Walker and Duncan, 1967) have been extensively studied at USAFSAM for application in cardiovascular disease. These models, in the herbicide context would have the form

where

P = probability of death
A = age in years
T = Vietnam tour length
R = indicator variable for race
E = exposure variable

and where are coefficients to be estimated from the data. Testing for a group difference can be accomplished by estimating and interaction coefficients such as.

If all interaction coefficients involving the exposure variable E are zero and E is treated as a 0/1 variable, Cox (1958a, 1958b) has shown that the most powerful test for non-zero is McNemar's test. This latter test makes full use of the paired design of the study. For McNemar's test, the data are cast into a 2 x 2 table as

shown in Table 7. In this table, "a" is the number of pairs in which both members have died, "b" is the number of pairs in which only the RANCH HAND person has died, etc. Using McNemar's test, the test statistic

is calculated and referred to the chi-squared distribution with one degree of freedom. Of course the above analyses will be accomplished considering all deaths, and deaths by specific cause.

Table 7

FORMAT OF McNEMAR'S TEST

CONTROLS

RANCH HAND PERSONNEL	DEAD	ALIVE	TOTAL
Dead	a	b	a+b
Alive	c	d	c+d
Total	a+c	b+d	n

As discussed in section VI.B(1) above, it is postulated that RANCH HAND personnel may be properly characterized as risk takers. This risk taking behavior may be associated with increased mortality from a variety of causes. Let us first consider accidental death. If herbicide exposure has caused neuropathy in the RANCH HAND personnel, one should anticipate that this disability could increase the probability of accidental death. However, accidental death rates among RANCH HAND participants must surely be corrected for risk taking tendency. A method of accomplishing this correction for risk taking would be to employ a psychological instrument such as the Life Experience Inventory (Torrance, 1954) or the Sensation Seeking Scale (Zuckerman, 1972). Both control and RANCH HAND mortality could be corrected using these measures, with the resultant rates being perhaps less biased and, therefore, a better indicator of exposed versus control effect. The same argument may apply to death rates from cancer under the hypothesis that risk taking behavior would tend to increase the likelihood that a RANCH HAND individual would experience increased carcinogen exposure. The situation concerning mortality and morbidity from cardiovascular disease is noteworthy. RANCH HAND personnel in many instances volunteered for their particular duty. This step had the well known effect of improving officer evaluation score. This volunteerism may be part of a Type A behavior syndrome which has been correlated with enhanced atherosclerosis. Instruments for determining Type A behavior have been developed and these scores may be profitably used to correct cardiovascular mortality and morbidity rates.

D. Analysis of Questionnaire and Physical Examination Data

The Questionnnaire and Physical Examination will produce data of three types: (1) dichotomous, (2) polytomous and (3) continuous.

Dichotomous (present-absent) rates will be evaluated using the tools described above for mortality analysis. For example, the questionnaire will provide data concerning the first occurrence of disease states by age, and standardized rates and relative risks may be calculated. The occurrence of such findings can be related to age, tour length exposure and other variables using logistic models followed by McNemar's test where appropriate. These tests will examine the presence or absence of group effect and allow assessment of the statistical significance on non-unity relative risks. Returning to Figure 1, the eight rates m_R', m_C', s_R, s_C, f_{RS}, f_{RS}, f_{CS}, f_{CS} fully characterize this study in a sense. In this figure, "Vertical comparisons," that is, m_R/m_C, s_R/s_C, f_{RS}/f_{CS}, f_{RS}/f_{CS} are relative risks of central importance in defining herbicide effects. "Horizontal comparisons," that is f_{RS}/s_R, $f_{RS}/(1-s_R)$, f_{CS}/s_C, and $f_{CS}/(1-s_C)$ will enable interpretation of over-reporting and subclinical disease.

Polytomous findings will occur in both questionnaire and physical examination responses. As an example consider retinal findings categorized into four grades, and studied as a function of age and exposure group as represented in Table 8. In this table the are counts of occurrence. In analyzing tables such as these, techniques as described by Bishop, Fienberg and Holland (1975) will be used. Specifically, if is the expected value of , general log-linear models of the form

will be used, where is the effect of RANCH HAND membership alone on cell frequency, is the effect of an interaction on RANCH HAND membership with retinal grade, etc. The reader will note that of course this model can work with dichotomous as well as polytomous data. Under appropriate conditions on expected values of entries in Table 8, the pairing in the study design can be used with the data being organized as shown in Table 9. In Table 9, N_{ij} is the number of pairs such that the exposed person has retinal grade i, and the control person has retinal grade j. Appropriate tests for this setting are indicated by Fleiss (1973).

Table 8

FORMAT CATEGORICAL REPRESENTATION OF RETINAL CHANGES

	RANCH HAND PERSONNEL				CONTROLS			
Age Category								
Retinal Category	1	2	3	4	1	2	3	4
1	X_{111}	X_{112}	X_{113}	X_{114}	X_{211}	X_{212}	X_{213}	X_{214}
2	X_{121}	X_{122}	X_{123}	X_{124}	X_{221}	X_{222}	X_{223}	X_{224}
3	X_{131}	X_{132}	X_{133}	X_{134}	X_{231}	X_{232}	X_{233}	X_{234}
4	X_{141}	X_{142}	X_{143}	X_{144}	X_{241}	X_{242}	X_{243}	X_{244}

Table 9

FORMAT OF PAIRING FOR LOG-LINEAR MODELS OF GRADES OF RETINAL FINDINGS.

	Control Grade			
RANCH HAND Grade	1	2	3	4
1	N_{11}	N_{12}	N_{13}	N_{14}
2	N_{21}	N_{22}	N_{23}	N_{24}
3	N_{31}	N_{32}	N_{33}	N_{34}
4	N_{41}	N_{42}	N_{43}	N_{44}

As indicated in section VI.E concerning the analysis of mortality data, risk taking behavior among RANCH HAND personnel could be correlated with changed mortality and morbidity patterns. Morbidity from cancer could be examined against a risk taking scale, and morbidity from cardiovascular disease could be corrected for personality type effect.

Analysis of Fertility/Reproduction Data. The herbicides under consideration in this study have been alleged to effect fertility and/or reproductive functioning. An attempt will be made to address these allegations by analyzing at least three primary

variables: the total number of conceptions since exposure in Vietnam, the number of miscarriages in spouses since exposure in Vietnam, the number of abnormal offspring since exposure in Vietnam. The study questionnaire will provide the number of miscarriages, abnormal offspring and total number of live births. The sum of the number of miscarriages and the number of live births will provide an estimate of the total number of conceptions. If differing divorce rates are found in the RANCH HAND and control groups, this may render the average number of years of marriage and the distribution of the years of marriage different in the two groups. This will be investigated and adjusted for if need be, either by analyzing total number of conceptions divided by (or normalized by) the number of years of marriage, or by using a more detailed covariance analysis. Further, the ratio of the number of miscarriages to adjusted total conceptions will be calculated and compared as will be the ratio of the number of abnormal births and adjusted total conceptions.

E. Survival Analysis

This section is written to extend and complement sections C and D. The defining common attribute of the techniques discussed in this section is that they deal with events which (a) correspond to categorical changes in health status, and (b) occur at definite and observable times. These methods may, therefore, be applied to studies of mortality (as the name "survival analysis" implies) as well as to studies in morbidity.

Survival analysis without covariates. The first step in the statistical analysis of survival data is descriptive, i.e., construction of summary measures which provide a basis for comparing different exposure groups without any allowance for the effects of possibly confounding variables (e.g., age) except perhaps for some limited stratification. Since one must expect many "losses to follow-up", only methods which take full cognizance of this complication will be considered. It should be pointed out that all the methods described below assume independence between censoring (e.g., loss to follow-up) and death or morbid event, although some techniques permit different patterns of censoring in different exposure groups.

The life table method can be adapted to obtain a step-function approximation to survival distributions in the presence of censoring (Chiang, 1968, Gross and Clark, 1975). However, the product-limit estimator of Kaplan and Meier (1958) may be preferred due to its intrinsic properties and its relationship to more refined methods.

The failure time distribution is the function $F^o(t)$ which provides the probability of death at or before time t in the study. The Kaplan-Meier estimator of $F^o(t)$ is $F^o(t)$ where

$$F^o(t) = 1 - \quad 1 \quad = \quad 1/R(T_i)$$

In this equation, is the "death set" at time t, i.e., the set of all indices i of individuals who were observed to fail before time t. $R(T_i)$ is the number of individuals who were at risk just before time T_i, the time of death (or morbid event) of the i^{th} study individual in . This product-limit estimator of Kaplan and Meier is maximum likelihood in the class of all possible failure time distribution functions.

Assuming that failure time distributions have been calculated for RANCH HAND individuals and controls, the next question concerns testing the null hypothesis of equality between the distributions. When only two such distributions are being compared, one may use the nonparametric procedures generalizing Wilcoxon's statistic proposed by E. Gehan (1965a, b) and discussed by N. Mantel (1967). When more than two such distributions are being compared, one may use the nonparametric procedure generalizing the Kruskal-Wallis statistic proposed by N. Breslow (1970).

Survival analysis with covariates. These methods allow adjustment of mortality rates or morbidity rates using covariates such as age, race, Vietnam tour length, AFSC, risk taking score etc. For the purposes of this discussion it will be assumed that the covariables are categorical, that there are only two such covariable and that the covariables do not interact in affecting the hazard of death or morbidity. These assumptions can all be relaxed using available methods.

The hazard function $h_i(t)$ for the ith individual in the study is the function which provides the conditional probability of death or morbid event in the time interval (t, t+dt) given his survival up to time t. The function $H_i(t)$ where

$$H_i(t) = \quad h_i(\ )d$$

is called the cumulative hazard for the ith individual. It is readily shown that the failure time distribution $F_i(t)$ is given by:

$$F_i^o(t) = 1 - \exp(-H_i(t))$$

From this last equation it follows that h_i and F_i^{φ} are transforms of each other, whence the dependence of F_i^{φ} on covariables may be modeled via h_i. This may be accomplished as follows. Let $X_i(t)$ and $Y_i(t)$ denote discrete valued stochastic processes pertaining to the ith individual and describing two covariates of interest (e.g., one may be an exposure variable and the other may be covariate such as age or crew position). The basic model for hazard is:

$$h_i(t) = \exp$$

where and are "log-relative risks". It is shown in J. Frank (1977) that this model may be extended to allow for any number of possibly interacting factors. Inference about log-relative risks may be drawn using either an approach derived from D. R. Cox (1972) by E. Peritz and R. Ray (1978) or using an approach described by Frank (1977).

E. Statistical Power

The power (1 -) of a study design is the probability that a specified difference between populations will be detected if it in fact exists. In general, power is a direct function of sample size; that is, for a particular study design, the more subjects measured the larger the study power. Essentially all animal and human studies concerning herbicide suffer from a lack of adequate consideration of study power. While the present study is not a powerful one against less common disease states as already discussed, it is obviously important nonetheless to exactly specify just what the study can and cannot accomplish. The following presents a preliminary analysis of study power for the case of continuous and dichotomous variables expected from the study.

(1) Power in Continuous Variable Case

Assume that blood cholesterol levels are being compared between RANCH HAND and control groups, and that the coefficient of variation for cholesterol in the control group is 0.1, where the coefficient of variation is the ratio . Assume . The symbol is the probability that the study will indicate an effect where none exists, and 1- is power as defined before. Consider that the RANCH HAND mean cholesterol is shifted from the control mean . A natural question is to inquire about the study power as a function of available pairs (n) and mean ratio .

Table 10

POWER CALCULATIONS

ASSUMPTIONS: =0.05, / =0.1,

R		Power = 1-	
		n=180	n=450
.20	1.01	.20	.38
.20	1.02	.55	.88
.20	1.05	.995	.995
.70	1.01	.86	.995
.70	1.02	.995	.995
.70	1.05	.995	.995

Power calculations are displayed in Table 10. Study power in the case of a matched pair design is strongly dependent on the degree of positive correlation produced between the involved groups by the matching procedure. Of course, the degree of correlation can be expressed by the correlation coefficient r which can take values between -1 (negative correlation) and + 1 (positive correlation), and two values of r have been employed in Table 10. From this table it is seen that if only 450 pairs are studied a 1% shift in mean (= 1.01) will not be reliably detected, but a 2% shift will be detected with a probability of 0.88 if r = 0.2 at least. From this calculation one can infer the need to examine at least 450 pairs to obtain the 2% shift, and to strive for more if possible.

(2) Power in the Dichotomous Variable Case. There is significant discussion in the mathematical statistics literature concerning the efficacy of paired designs in the setting of dichotomous responses (Billewicz, 1974; Ury, 1975; Miettinen, 1970; and several others). Table 11 shows a set of calculations which are applicable to the present study.

Table 11

POWER CALCULATIONS FOR DICHOTOMOUS VARIABLE CASE AS A FUNCTION OF EFFICACY OF PAIRED DESIGNS

				POWER = 1-					
P_1	P_2	Rel. Risk	R	n= 160	n= 200	n= 250	n= 300	n= 350	
.05	.01	5	0	.71	.78	.84	.89	.92	*
.04	.01	4	0	.56	.64	.72	.79	.84	
.03	.01	3	0	.40	.45	.51	.57	.61	
.10	.05	2	0	.54	.61	.69	.76	.81	
.20	.10	2	0	.80	.86	.92	.95	.95	
.05	.01	5	.1	.65/.02	.82/.033	.89/.029	.94/.038	.96/.032	
.04	.01	4	.1	-	.54/.020	.72/.033	.79/.029	.87/.038	**
.03	.01	3	.1	-	-	.38/.020	.55/.033	.68/.046	
.10	.05	2	.1	.60/.058	.67/.054	.76/.055	.77/.036	.85/.048	
.20	.10	2	.1	.81/.036	.92/.056	.94/.043	.96/.038	.98/.046	

* = .050
** as indicated

In this figure, r is again the correlation coefficient indicating the degree of correlation induced between the involved groups by the matching procedure. The probability of the disease among RANCH HAND personnel is symbolized as p_1, while p_2 is the probability of the disease among the controls. Relative risk is the ratio p_1/p_2. With r = 0.1, sign test power tables were used as an exact version of McNemar's test, and therefore different levels are shown under each power number. Table 11 shows the positive influence of effective pairing in the higher power levels noted. Also, it appears that for p_2 = 0.01 and p_1 = 0.03, physical examination of 350 pairs (700 examinations) will disclose the three-fold relative risk with probability less than the minimum target .80. In other words, there is a greater than "20% chance" that a three-fold relative risk on a 1/100 disease state will go undetected in this study if only 350 pairs are examined and if low correlations occur. Once again the need to examine increased numbers of pairs in the study is seen.

To present these dichotomous power calculations more clearly, calculations in the context of actual disease states have been accomplished. The diseases considered are cardiovascular disease and cancer, corresponding to high and low rate illnesses for the age groups presently under investigation.

Cardiovascular Disease. A logistic risk function was fitted to data from 17,455 autopsies gathered in a WHO collaborative study in Czechoslovakia, Sweden and the USSR. The function fitted has the form

where

p = the probability of a complicated coronary lesion

x = age scaled linearly so that x = 0 is equivalent to 3 years, and x = 1 is equivalent to 58 years (the age span of the current study)

y = 1 or 0 if the subject is exposed or not

and and were obtained from the data. The function represents a fairly high rate disease in that at 40 years of age 7% of the group had the lesion and at 60 years of age 20% had the lesion. The coefficient , represents the exposure effect. Power calculations for and are shown in Table 12. This figure suggests that if, as a cell toxin, herbicide exposure accelerates cardiovascular disease, this study has a good chance of detecting that acceleration if the herbicide effect is comparable to the age effect. Once again, beneficial effect of pairing is seen.

Cancer. A logistic risk function was fitted to breast cancer data presented by Breslow and Day (1975). The function fitted represents a low rate disease in that at 35 years of age only .000336 of the group had the lesion while at 70 years of age .00676 of the group will have the lesion. Using pairing to achieve a power of 0.80 in this setting, 1312 pairs would be needed, when the exposure effect is equal to the age effect. This exceeds the size of our RANCH HAND cohort, and reinforces the fact that herbicide exposure effects on rarer diseases will not have a high likelihood of being detected by this study, and again supports an attempt to examine as many pairs as possible.

Table 12

POWER CALCULATIONS AS OF A FUNCTION OF HERBICIDE EFFECT

ASSUMPTION: = 0.05

Number of Pairs	= Power Neglecting Pairing	= Power With Pairing	=.8 Power Neglecting Pairing	=.8 Power With Pairing
100	.69	.93	.81	.82
160	.89	.98	.86	.87
200	.95	.995	.93	.95

G. Multivariate Analysis

Some questionnaire and physical examination data naturally fall into groups; for example, fertility/reproduction data, liver function tests, cardiovascular examination tests. In these cases, multivariate analysis may be in order. When the response variables are continuous, they will be analyzed by the wellknown multivariate extensions of the generalized linear model.

The general approach to multivariate analysis of polytomous data considers all classification factors and all variables as "factors" in a multi-way contingency table. Log-linear models as described above for polytomous data will be employed where appropriate.

H. Indices and Estimates and Exposure

Exposure estimates can be used to sharpen a statistical analysis and can be helpful in summarizing responses. In this discussion, two estimates will be considered: one related to Vietnam herbicide exposure, and another related to domestic (US) herbicide exposure independent of Vietnam experience.

Vietnam herbicide exposure. In the above discussion of statistical methodologies, exposure variables appeared. In the logistic formula on page , the variable E was shown which could be either dichotomous, polytomous or continuous. In the use of logistic functions to discuss study power, the exposure variable was taken as dichotomous. If a polytomous or continuous exposure variable E is constructed, significant sharpening of the study analysis would be accomplished. For example, biases in this study could lead

one to suspect that differences between RANCH HAND personnel and controls were in fact due to factors other than a herbicide effect. If however, in addition to differences between RANCH HAND and personnel and controls, one was able to show a regression of mortality and/or morbidity on an exposure index E, the case for a bona fide herbicide effect would be firmer. How then could one construct an exposure index E? The reconstruction of exposures is discussed in Section V(c).

<u>Domestic herbicide exposure.</u> Individuals in both the RANCH HAND and control groups will have had varying exposure to herbicide in the United States. Particularly, individuals from specific farming and/or foresting areas, may have had and continue to have a significant background exposure. Data on place of residence and information concerning home practices (gardening etc.) could be used to build a background exposure index E_b. In lieu of constructing this index, one can hope that randomization would even out background exposure between RANCH HAND and control groups, however, good statistical practice would seem to require that such randomization be in fact tested.

I. <u>Next Steps</u>

This statistical protocol is evolving as more is learned about the cohorts to be studied. The next weeks and months will be the occasion for the examination of several issues not addressed above or only touched upon. There must be further consideration of RANCH HAND personnel as risk takers. Also, there must be further analysis of bias due to selective participation in the study. In the analysis of mortality, questionnaire and physical examination data, more attention will be paid to the construction of meaningful exposure and disease indices.

VII. <u>Data Repository</u>

Throughout the 6-year period of this investigation, data collection methods will be integrated by use of computer systems. A data repository will be established at the USAFSAM. A master file will be formed on each exposed member and for his matched control. The individual master files will be keyed to one or more identifiers.

Individual data bits and their sources are as follows:

(1)	Questionnaire	a. Initial (telephone) b. Indepth interview (personal and telephone) c. Prospective (telephone)
(2)	Psychological Battery	a. Initial b. Prospective

(3)	Physical Examination	a. Initial b. Prospective
(4)	Medical Records	a. Active duty b. VA c. Civilian d. Dependent
(5)	Historical Data	a. Military personnel files b. Flight records c. Military unit
(6)	Death Certificates	a. Study members b. Dependents
(7)	Birth Certificates	a. Dependents

The computer software for the data analysis phase will be prepared to assure proper data conversion, quality control and standardization of test measurements. Quality control areas will include verification of identification data, range checks, and identification/correction of ambiguous or conflicting data. The repository capability of this investigation will allow complete computer files on the exposed/control populations with potential momentary recall. IN ADDITION, THIS CAPABILITY WILL PERMIT INTEGRATION OF DATA COLLECTION METHODS UTILIZED THROUGHOUT THE SIX YEAR STUDY PERIOD, THEREBY SERVING AS A EFFICIENT CROSS LINK OF RECORDS.

VIII. Recognized Study Difficulties and Corrective Measures

A. Medical Precedence

(1) Problem

A departure from the usual methodological approach characterizes this particular epidemiological investigation. Clearly there is no historical "roadmap of methodology" to conduct this study. Most occupational exposure studies use the presentation of an unusual disease to justify the initiation of a comprehensive study. A rare disease or a common disease in an uncommon site, or one with an unusual presentation appearing in space-time clusters, often in an unusual population or age group, generates the requirement for a new study. In the case of Herbicide Orange, the evidence for long-term human effects has remained extremely tenuous and controversial. Despite the unique problems that this study possesses, such as the lack of clinically defined endpoints, there are many problems that it shares with other occupationally related exposure studies. For example, the question of a latent period in the development of symptoms/signs, the lack of accurate doseresponse relationships, and the possibility of a synergistic effect with other toxins/carcinogens are all operating in this study. Since most cohort studies of occupational mortality use the general population as a standard for deriving the expected number of deaths, preemployment selection ("healthy worker" bias) affects the

comparative experience. Age-standardized mortality ratios (SMR's) in general are 60-90 percent of the standard in the working population. Similarly, conflicting results can occur using the matched-pair cohort method proposed in this study design. Statistical verification of the validity of utilizing such a control for a summary mortality index (e.g.,SMR) has been infrequently attempted in the past. Inability to verify the validity of the more classical methods of comparing mortality will necessitate the use of multiplicative and/or logistic models to obtain a valid standardized mortality ratio.

(2) Corrective Measures

Unprecedented study designs forced by unprecedented occurences of occupationally related medical complaints require novel approaches, and reorientation and standardization of thinking; all of which require on an effective Peer Review system. Beyond even the immediacy of the current study is the growing problem of a myriad of occupationally-related exposures, both in the military and civilian sector, which will require similar epidemiological studies in the future in order to make some judgment as to whether or not an association is of causal significance.

B. Group Accountability Bias

(1) Problem

The numerous media presentations on Herbicide Orange issues have focused attention on the RANCH HAND group, and several attempts have been made to construct lists of former members of this group. The RANCH HAND population should be easier to locate and contact than the control population. This difference will be particularly evident with respect to reported mortality experience. The incentives for cooperation and study participation are likely to be greater in the exposed group than in the controls. Also, the close knit reunion association of former RANCH HAND personnel will lead to a more precise reporting of morbidity and mortality in that group. Such group identity tends to decrease the degree of unaccountability in the exposed group while its absence in the controls may lead to under ascertainment of mortality. This could then lead to the attribution of excess mortality in the exposed population.

(2) Corrective Measures

Unaccountability bias will be minimized by attempting to keep the percentages of unaccounted for study subjects below 1% in both exposed and control groups. The morbidity and mortality status of all individuals selected for the study will be strongly pursued utilizing a variety of techniques previously described.

C. "Risk Taking" Behavior Bias

(1) Problem

The early RANCH HAND aircrew population was an exclusively volunteer group; the C-130 control population, while volunteers in the Air Force, were not volunteers for special hazardous missions. RANCH HAND mission conditions were considered to be more dangerous than those encountered in the normal combat environment. This suggests that some differences may exist in the psychological profiles of the two groups. A sensation seeking or risk taking psychological orientation may have altered the accident mortality or morbidity patterns of the exposed group. In addition, an accident rate affected by peripheral neuropathy could be masked by undetected risk taking behavior bias.

(2) Corrective Measures

In an attempt to correct for the unique psychological factors that affect the choice of an aeronautical career, and to adjust for the effects of combat stress, transport aircrew members were matched with crewmembers of similar transport aircraft. However, the volunteer nature of the early RANCH HAND operation casts doubt on the adequacy of this basic matching as an attempt to control for the psychological effects of combat stress. The factors of volunteerism and risk-taking behavior must be considered from both the individual and group perspectives. The assessment of individual risk-taking behavior has been quantified by psychological instruments such as the Sensation Seeking Scale (SSS) of Zuckerman, et al. and the Life Experience Inventory (Torrance). The SSS has been demonstrated to have considerable validity in measuring a variety of phenomena including volunteerism and participation in risky activities and has been applied to naval aviation trainees (Waters). These models will be adapted for use throughout all phases of the study. The classical model of field dependence/independence will be used to assess the group effect in this area.

D. Response Bias

(1) Problem

False positive response is anticipated as the primary bias operating in this study. Compensation issues arising from individual claims to the VA or from class action suits, heightened health concern generated by extensive publicity, disenchantment with military service, and the simple desire to please the interviewer may introduce positive responses that exceed the study's ability to correct or adjust. False negative response will also operate, and such bias is even more difficult to assess than the spurious response in a positive direction. Significant factors in this direction include: issues of patriotism and loyalty, personal conviction

as to the propriety of the defoliation program and their participation in it, the strong virility orientation of the pilot/aircrew population (particularly with reference to questions of libido and fertility), personal inconvenience caused by study participation, errors of memory, and fear of the adverse effects on career goals that abnormal physical examination results could produce (a significant problem for active civilian and military pilots).

(2) Pending Retirement Bias:

The military retirement system also creates a potential source of positive bias. A "pending-retirement phenomenon" personnel who are approaching end of their careers exaggerate their systems so that they may become eligible for disability benefits.

(3) Corrective Measures

The primary correction technique for questionnaire response bias will be a carefully constructed and standardized physical examination. Multiple verification and bias indicator questions will be designed and included in the initial questionnaire. Memory verification will be conducted by cross-referencing responses to medical and personnel records. Detailed statistical correlations between the questionnaire reponses and the physical examination results will be conducted. All telephone interviews and physical examinations will be conducted on a "blind" basis to the maximum extent possible. Self-administered and group-administered questionnaires will not be conducted. Log linear models of anticipated biases and their estimated impact on the study will be attempted prior to the final analysis of any phase in order to justify the analytic methods used. Conclusions drawn from this study will be predicated and coupled to a bias estimate.

E. Interview Bias

(1) Problem

Voice inflection, speed of interview, intonation and ethnicity are recognized factors which can affect positive or negative interview response. These factors will definitely operate in this study.

(2) Corrective Measures

An extensive interviewer training program will be conducted in order to limit the effects of interview bias. The Survey Research Center of the University of Illinois and the Center for Disease Control, Venereal Disease Training Branch, Atlanta, Georgia, will assist in this effort. The training will concentrate on techniques to elicit sensitive personal and medical information in an accurate manner, while minimizing discomfort to the subject

and the interviewer. Quality assurance methodology and information verification techniques will also be included in the training. Interviews will be randomly monitored by the supervisor in an unannounced and undetectable manner. For particularly sensitive questions (e.g. illicit drug usage), randomized response techniques (coin flip method) will be used, recognizing that responses will be valid on a group basis only.

F. Political Implications

(1) Problem

The question of adverse health effects due to Herbicide Orange exposure in Vietnam has evoked many strong emotions. The actions of consumer groups, environmentalists, and other special interest groups have generated defensive responses on the part of some governmental agencies, and reactive decisions by others. Frequently, these responses have been based on unsubstantiated claims and/or scientific evidence of questionable validity. As a result of these governmental actions, the political impact on the planning of this study has been substantial. Suggestions to increase the scope of the effort to include other "exposed" individuals or poorly defined groups continue to surface. However, monumental problems of group ascertainment, exposure validation, control group selection, and control of additional bias make the inclusion of such individuals undesirable from a sound scientific perspective. If such decisions are made without regard for their scientific impact, compromise of study validity is assured.

(2) Corrective Measures

The dilution of the scientific credibility of this effort by politically motivated decisions will be diplomatically resisted. While all suggested improvements will be considered, any alterations or corrections to the study protocol will be based on sound scientific assessments of the proposed changes. Such issues will be clearly presented to appropriate peer review agencies for comment.

G. Loss to Study

(1) Problem

Loss to study in the RANCH HAND group poses a major problem to the validity of the inferences that can be made from any subsequent comparisons between or within groups. The avenues of loss will conceivably arise from individual apathy (volunteer bias), lack of appropriate financial reimbursement for lost worktime, the presence or absence of illness (perception of health), and the lack of a desire for "treatment." Losses in the matched controls at any phase of the study, though predictably greater than in the exposed group can be managed by resampling from the best-of-fit matches from

the C-130 population. Consequently, additional decrements in statistical power will not result from losses in this group. However, significant losses in the exposed group will have irreparable adverse effects on the power of the statistical analyses. The estimated allocation of participants in this study is shown in Section XII, Table A-5. It is estimated that the response rate of the accessible, identified exposed group will be 70% for both the initial questionnaire and the physical examination phases of the study. This is expected to occur despite great efforts to keep the questionnaire at an acceptable length, to coordinate questionnaire administration with the subject's personal schedule, and to make the questions as innocuous as possible. It is also estimated that only 80% of those who respond positively to the opportunity for physical examinations will actually present themselves for examination.

(2) Corrective Measures

Loss to study problems in the study participants will be avoided as much as possible by detailed and exhaustive efforts to contact and followup each identified participant. Non-participants will be encouraged to reconsider their initial decisions. Design considerations have been made to minimize loss to study in both the exposed and control populations. Federal regional hospitals in the United States and overseas will be used to facilitate the ease of obtaining physical examinations, and thus participation in the cross-sectional and prospective study phases will be increased. It is felt that physical examination variances due to slight differences in technique between hospitals and physicians will be less damaging to the validity of the study than the effects resulting from attempts to conduct all of the examinations at a single facility, i.e., examination at a single facility would reduce participation rates, and therefore would severely compromise the overall statistical power of the study.

H. Statistical Power Limitations

(1) Problem

As discussed above, statistical power considerations are heavily dependent on loss to study rates. Since the design of the study is limited by the small exposed population, statistical power for identifying the relative risk of an uncommon disease or symptom-complex (1/100) is very low (.50). This study will, to a greater extent, be able to detect increased risks only in common diseases or symptom-complexes (1/100).

(2) Discussion

The "herald sign" of TCDD exposure, chloracne, is expected to have the greatest likelihood of achieving adequate statistical power in this study. Recent findings from Seveso, Italy, support the importance of chloracne as the primary marker symptom.

The incidence of chloracne has been reported by Reggiani (personal communication) and Homberger, et al., to be 14.9 cases per 1000 residents in the region of highest contamination of Seveso (Zone A) and 6-12 cases per 1000 in the Seveso community as a whole. These rates vary by age group, with children being at highest risk. Only 1-5 cases per 1000 were seen in other regions of Northern Italy (Milan, Como, and Lecco). The incidence of adolescent acne in all of these populations varies between 21 and 30%. These incidence rates probably place chloracne at the lower limit of adequate statistical power within the constraints imposed on this study. In the Nitro, West Virginia studies, residuals of chloracne, as well as exacerbations of previously active disease, continue to be seen 10 years after the most recent exposures, and 30 years after the industrial accident. Thus, it is likely that any chloracne in the exposed population may be detected, despite the intervening years since RANCH HAND exposures.

SINCE CONTINUOUS DATA WILL NOT SUFFER FROM STATISTICAL POWER LIMITATIONS AS DOES DICHOTOMOUS DATA, EMPHASIS WILL BE ON STRUCTURING THE PHYSICAL EXAMINATION TO DEVELOP CONTINUOUS DATA AS MUCH AS POSSIBLE

I. Variability of Procedures

(1) Problem

The variance of physical examination findings from technique differences and the random errors inherent in laboratory testing are items of concern, particularly if attributable health effects are subtle or of low magnitude. Nonstandardized procedures and techniques are major contributors to this variance.

(2) Corrective Measures

Variability in examination procedures will be minimized by the use of standardized procedures, examination protocols, and training. Most laboratory procedures will be conducted centrally at the USAFSAM, and quality control will be stressed at all times.

J. Confounding Exposure Factors

(1) Problem

While virtually all of the media attention has been directed toward the 2,4,5-T containing herbicide formulations, other herbicides were applied concurrently by the C-123 aircrews in Vietnam. Herbicide Blue (Cacodylic acid with 15.4% pentavalent arsenic) and Herbicide White (2,4-D and Picloram) were used throughout the 1962-1970 time period. Any long-term health effects from these additional compounds may confound the results of the study. Peripheral neuritis, tremors, skin and lung cancer, loss of hair and nails, skin rashes, and gastric symptoms have been alleged after exposure to arsenical pesticides. The organophosphate insecticide, Malathion, was also sprayed by many of these same aircrewmembers

when RANCH HAND duties permitted their temporary assignment to mosquito/malaria control units. Many of these individuals were involved in the aerial spray application of these and other pesticides both before, during, and after their Vietnam service. Long-term effects from these chemicals would confound the study results. The small size of the RANCH HAND population will allow very little opportunity for analytic stratification for these confounding variables. Differing patterns of exposure to aircraft fuels in the study populations have been suggested as confounding factors. The C-130 aircraft were powered by turbo-prop engines which used jet fuel (JP-4), while the C-123 and C-7 aircraft were powered by standard reciprocating engines which used leaded aviation fuel (AV-GAS). After June 1968, many C-123s were modified by the addition of auxilliary jet engine boosters for added power on take-offs and emergencies.

(2) Corrective Measures

While the extent of confounding caused by exposure to these other pesticides is undetermined at this time, assessment of its magnitude must rely on responses of the subjects to that portion of the questionnaire dealing with other occupational exposures. Variations in fuel between C-130 and C-123 aircraft would be significant factors if individuals in the study were heavily and repetitively exposed. However, the normal duties of the study participants did not involve aircraft refueling or other fuel handling activities. Thus, fuel exposures can be rejected as confounding factors.

IX. Reporting Procedures

Interim synoptic progress reports will be provided to the Surgeon General through Quarterly Management Reviews conducted each January, April, July and October. Key data analyses will be displayed, but inferences and conclusions will await full data analysis at the conclusion of each phase. A formal report for each of the three phases will be completed with forecasted submission dates of : Retrospective Study, July 1981; Cross-Sectional Study, October 1981; and Prospective Study, April 1986. Findings and conclusions of each phase will be published in a journal of stature. Total study design, findings, and conclusions will be published in the USAFSAM Aeromedical Reviews or Technical Reports.