USAFSAM-TR-88-3

UNITED STATES AIR FORCE PERSONNEL AND EXPOSURE TO HERBICIDE ORANGE

Richard A. Albanese, M.D.

February 1988

Interim Report for Period March 1984 - February 1988

Approved for public release; distribution is unlimited.

USAF SCHOOL OF AEROSPACE MEDICINE
Human Systems Division (AFSC)
Brooks Air Force Base, TX 78235-5301

NOTICES

This interim report was submitted by personnel of the Radiation Analysis Branch, Radiation Sciences Division, USAF School of Aerospace Medicine, Human Systems Division, AFSC, Brooks Air Force Base, Texas, under job order SUPTXXRH.

When Government drawings, specifications, or other data are used for any purpose other than in connection with a definitely Government-related procurement, the United States Government incurs no responsibility or any obligation whatsoever. The fact that the Government may have formulated or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication, or otherwise in any manner construed, as licensing the holder, or any other person or corporation; or as conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

Richard A. Albanese, M.D.
RICHARD A. ALBANESE, M.D.
Project Scientist

John C Mitchell
JOHN C. MITCHELL, B.S.
Chief, Radiation Sciences Division

JEFFREY G. DAVIS, Colonel, USAF, MC
Commander

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) USAFSAM-TR-88-3	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION USAF School of Aerospace Medicine 6b. OFFICE SYMBOL (If applicable) USAFSAM/RZM	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Human Systems Division (AFSC) Brooks Air Force Base, TX 78235-5301	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION USAF School of Aerospace Medicine 8b. OFFICE SYMBOL (If applicable) USAFSAM/RZM	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Human Systems Division (AFSC) Brooks Air Force Base, TX 78235-5301	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 65306F; PROJECT NO. SUPT; TASK NO. XX; WORK UNIT ACCESSION NO. RH
11. TITLE (Include Security Classification) United States Air Force Personnel and Exposure to Herbicide Orange	
12. PERSONAL AUTHOR(S) Albanese, Richard A.	
13a. TYPE OF REPORT Interim 13b. TIME COVERED FROM 3/84 TO 2/88	14. DATE OF REPORT (Year, Month, Day) 1988, February 15. PAGE COUNT 39
16. SUPPLEMENTARY NOTATION	

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	05	
24	05	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Epidemiologic Investigation; Dioxin; Phenoxy Herbicides; Ranch Hand; Herbicide Orange; Air Force Health Study

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The United States Air Force is conducting an epidemiological study, called the Air Force Health Study (AFHS), to determine whether or not military personnel associated with herbicide spraying during the Vietnam War have experienced any adverse health effects. This report reviews salient findings of the AFHS first morbidity report published in 1984, and presents new work by examining relationships between AFHS findings and the results of laboratory toxicological studies and other epidemiological studies addressing dioxin. Eleven clinical areas have been emphasized based on a toxicological profile developed from the literature and availability of data in the AFHS: weight loss, neoplasia, birth defects, neurological changes, psychological changes, hepatotoxicity, chloracne, cardiovascular changes, immunological deficits, endocrine changes, and mortality. In six of these eleven clinical areas, statistically significant group differences occurred, and in five of these six instances the group differences were in the direction of expected dioxin

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Richard A. Albanese, M.D.	22b. TELEPHONE (Include Area Code) (512)536-3884 22c. OFFICE SYMBOL USAFSAM/RZM

DD Form 1473, JUN 86 — Previous editions are obsolete.

19. ABSTRACT (Continued)

effects. Dioxin cannot be confidently identified as the causative agent of these findings at this time because of several reasons, including the absence of correlations with an exposure index and the incomplete clinical picture. However, dioxin is not exonerated as a causative agent because of the directionality of the observed group differences and the preliminary nature of the exposure index used in the AFHS first morbidity report.

UNITED STATES AIR FORCE PERSONNEL AND EXPOSURE TO HERBICIDE ORANGE

INTRODUCTION

The United States Air Force is conducting an epidemiological study to determine whether or not military personnel associated with herbicide spraying during the Vietnam War have experienced any adverse health effects. The Air Force program responsible for the herbicide spraying was code named Operation Ranch Hand, and the personnel involved in the spray missions are termed Ranch Hands. The current epidemiological study is called the Air Force Health Study (AFHS).

This report reviews salient findings of the AFHS first morbidity report.[1] Building on prior reports, this article presents new work by examining relationships between AFHS findings and the results of laboratory toxicological studies and other epidemiological studies addressing dioxin. This report attempts to assess the extent to which these initial AFHS findings are compatible with toxic effects of 2,3,7,8-tetrachlorodibenzo para dioxin (TCDD) as known from animal experiments and, to a lesser extent, from observation in humans. In the preparation of this report, more than 400 dioxin-related published articles were studied, and a biomedical portrait of dioxin effects emerged. This portrait will change as research proceeds; nevertheless, the current literature is sufficiently mature to permit comparison with AFHS findings.

METHODS

The Protocol

Investigators at the USAF School of Aerospace Medicine developed a comprehensive study protocol to govern the AFHS.[2] The protocol underwent extensive peer review by military and civilian agencies, including: The University of Texas School of Public Health, an Air Force Scientific Advisory Board, the Armed Forces Epidemiological Board, the National Academy of Sciences, and the White House appointed Agent Orange Working Group. This last organization continues to act in an advisory role. Recommendations by these groups were incorporated into the protocol.

Study Design

The study design is a matched cohort design in a nonconcurrent prospective setting. The study addresses mortality and morbidity and includes long-term follow-up activities. A detailed population ascertainment process identified 1,278 Ranch Hand personnel who served in Vietnam during the period 1962 through 1971, when the spraying operation was active. A comparison group was formed by identifying all individuals assigned to Air Force organizational units with a mission of flying cargo to, from, and in Vietnam during the same period. A computerized nearest neighbor selection process was used to match up to 10 comparison individuals to each Ranch Hand. This matching was done by job category, race, and age. The initial comparison group erroneously contained some individuals who did not in fact have any Southeast Asia experience. These individuals (18%) were removed from the comparison population after detailed hand record review, leaving an average of approximately eight comparison individuals matched to each Ranch Hand.

The comparison individuals matched to each Ranch Hand were listed in random order within each set. The first five comparisons were included in the mortality analyses giving these studies a 1:5 design. For each living Ranch Hand, the first living member of his randomized comparison set was selected for participation in a morbidity study consisting of an in-home interview and a comprehensive physical examination. If the matched comparison subject declined to participate or subsequently withdrew from the morbidity study, that individual was replaced by the next living comparison subject from the randomized set who was willing to participate.

Follow-up studies are an important aspect of the AFHS. The follow-up studies consist of mortality and morbidity components. Each Ranch Hand and his set of comparisons will be the subject of mortality evaluations for the next 20 years. In addition, follow-up questionnaires and physical examinations are being offered to participants in 1985, 1987, 1992, 1997, and 2002 in order to bracket the latency periods associated with possible attributable disease.

Inference Concerning Herbicide Causation

In an experiment, members from a single population are randomly assigned to either a treatment (exposed) or control group. In the setting of such completely randomized designs, statistically significant differences between the treated and control group can often be reliably ascribed to the effect of the exposure. The AFHS is the study of an unplanned environmental exposure and thus does not follow the above experimental design. The study has a non-randomized design and is an observational study. In such studies, while the comparison group is chosen to be similar to the exposed group with respect to as many qualities as possible, except exposure status, in the absence of randomization, group differences or the lack thereof cannot be interpreted solely in terms of exposure. For example, in the AFHS the exposed group was stationed in the Republic of Vietnam itself, while most of the comparison

group was quartered in surrounding areas such as Okinawa and Japan. Comparison aircrew members periodically flew into Vietnam while comparison ground support personnel, predominantly enlisted, remained in non-combat areas. Ranch Hand Vietnam tour length was approximately 1 year while comparison tour length was 3 years. These differences and others may or may not influence health and longevity. Thus, group differences or the lack thereof cannot unambiguously be ascribed to herbicide exposure. This emphasizes the importance of relating study results to other studies to see whether common patterns of effect emerge.

Another approach to inference is the use of an exposure measure or index. If one knew exactly how much herbicide each Ranch Hand was exposed to, highly exposed individuals could be contrasted with less exposed individuals within the Ranch Hand group and an estimate of herbicide effect could be constructed. However, once again, since randomization was not employed in the dose assignment, estimates of herbicide effect must be viewed with great care due to the possibility of confounding factors. For example, it could happen that higher exposures occurred for a variety of reasons in the lower socio-economic strata (lower ranks) of the Ranch Hand cohort. Industrial hygiene data concerning herbicide exposure were not collected during the Vietnam era. In any case, however, the use of an exposure index provides another view of exposure effects which can augment interpretation of group differences.

The exposure index used in this report relates to the TCDD-containing herbicides: Herbicide Orange, Herbicide Purple, Herbicide Pink, and Herbicide Green. Archived samples of Herbicide Purple had a mean TCDD concentration of approximately 33 ppm, and archived samples of Herbicide Orange had a mean concentration of 2 ppm. Herbicides Pink and Green contained twice the 2,4,5-T of Herbicide Purple and, therefore, have been estimated to contain TCDD at a concentration of approximately 66 ppm.

Using mission records, it was possible to determine the amount of each herbicide sprayed each month in Vietnam as well as the number of Ranch Hands in each job category who were involved in spraying that month for the period 1962 through 1971. Tour data also allowed determination of the months each individual was involved in the Ranch Hand operation. Using these data, an exposure index was developed for each Ranch Hand. The exposure index is directly proportional to the number of gallons of herbicide sprayed in Vietnam during the individual's tour, where potential exposure to the higher TCDD-containing herbicides (Purple, Pink, Green) has been properly scaled according to dioxin concentration to place them on the same basis as Herbicide Orange. Also, the exposure index is inversely proportional to the number of airmen assigned to the specific subject's job category during his tour.

From the description just given, it should be clear that the current Ranch Hand exposure index is an estimate only, as it applies theater-wide spraying to a single individual, and, since it assumes that all individuals in a job category were equally exposed. Also, the degree to which this calculated index is associated with actual body burden of TCDD is unknown. In short, the absence of a positive association between the index and health outcomes cannot be taken as confirming a lack of herbicide effect, nor can the presence of an association be interpreted as an unambiguous herbicide effect without consideration of possible confounding factors.

Job category matching in the AFHS used five categories: (a) officer-pilot, (b) officer-navigator, (c) officer-other, (d) enlisted-flying, and (e) enlisted-ground. Exposure index analyses used three occupational categories: all officers were combined into one category called "officer" due to the fact that navigators and pilots, while having different jobs, were believed to be exposed in the same manner. For each exposure occupational group (officer, enlisted-flying, enlisted-ground), the calculated exposure index was trichotomized into three levels: low exposure, medium exposure, and high exposure. Since the mode of exposure was judged to be different in each occupational group, statistical analyses with the exposure index were occupational group specific.

Questionnaire and Physical Examination

The AFHS uses a broad medical history and physical examination. The medical history was collected by an extensive in-home questionnaire.[3]

The purpose of the extensive questionnaire was to collect data that could be analyzed for the subjective presence of adverse health effects that might be related to herbicide exposure. In addition to the study participants, the questionnaire contractor was also required to interview the participants' current and former wives, as well as the first-order next-of-kin of deceased individuals to obtain morbidity data as completely as possible.

Physical examinations were performed at a single location by a contractor. All examiners evaluated the participants without knowledge of their exposure status. The number of examiners and the turnover of staff members were kept to a minimum to limit between-examiner variability. All laboratory tests were subjected to rigid standards of quality control, and laboratory and physical examination data were measured on a continuous scale whenever possible to improve statistical power in the analysis.

A general summary of the major components of the examination is presented in Table 1, and the laboratory procedures conducted on each subject are listed in Table 2.

TABLE 1. AFHS PHYSICAL EXAMINATION

General Physical Examination

Neurological Examination

Dermatological Examination

Electrocardiogram

Pulmonary Function Study

Chest X-ray

Nerve Conduction Velocities

Psychological Evaluation:

- Minnesota Multiphasic Personality Inventory (MMPI)
- Cornell
- Wechsler Memory Scale I
- Wechsler Adult Intelligence Scale (WAIS)
- Wide Range Achievement Test (WRAT)
- Halstead-Reitan Neuropsychological Battery

TABLE 2. LABORATORY PROCEDURES

Chemistry Panel:

- Blood Urea Nitrogen (BUN)
- Creatinine
- Cholesterol
- High-Density Lipoprotein
- Triglyceride
- Total Bilirubin
- Direct Bilirubin
- Alkaline Phosphosphatase
- Glucose (Fasting and 2 hour)
- Cortisol (Fasting and 2 hour)
- Serum Glutamic-Oxaloacetic Transaminase (SGOT)
- Serum Glutamic-Pyruvic Transaminase (SGPT)
- Gamma Glutamyl Transpeptidase (GGTP)
- Lactic Acid Dehydrogenase (LDH)
- Creatine Phosphokinase (CPK)
- Blood Alcohol

Hormone Assay:

- Luteinizing Hormone (LH)
- Follicle Stimulating Hormone (FSH)
- Testosterone
- Triiodothyronine (T3) Uptake
- Tetraiodothyronine (T4)
- Free Thyroxine Index (FTI)

Hematology Panel:

- Erythrocyte Sedimentation Rate
- Prothrombin Time
- Serological Test for Syphilis (RPR)
- White Blood Cell Count (with 10,000 cell differential)
- Red Blood Cell Count
- Hemoglobin
- Hematocrit
- Red Cell Indices
- Platelet Count

Urinalysis:

24-Hour Urine
- Volume
- Delta Amino Levulinic Acid
- Coproporphyrins
- Uroporphyrins
- Porphobilinogen
- Creatinine

Semen Analysis:

- Volume
- Count
- Abnormal Forms

Hepatitis B Testing:

- Surface Antigen
- Antibody to Surface Antigen
- Core Antibody

RESULTS

Morbidity

The results of the analysis of the baseline morbidity data were released in February 1984.[1] Of all Ranch Hand and comparison individuals who were selected for the questionnaire and physical examination phases of this study, 99.5% were contacted, eliminating the major concern of selection bias. One thousand one hundred and seventy-four (97%) of the Ranch Hand group and 956 (93%) of the originally selected comparison group participated in the questionnaire portion of the Morbidity Study. An additional 576 comparison subjects were interviewed as substitute subjects, bringing the total number of comparison participants to 1,532. Substitute comparisons were selected to replace comparisons selected erroneously and to replace noncompliant comparisons. Two thousand seven hundred eight current and former wives of the study participants were also interviewed. One thousand and forty-five (87%) of the Ranch Hand group participated in the physical examination, and 773 (76%) of the originally selected comparison subjects participated in the examination process, giving a total of 2,269 participants.

The analyses presented in the baseline morbidity report were largely performed using all available Ranch Hand data (1,045 participants) and data from originally selected comparison subjects (773 participants) yielding a total of 1,818 subjects. Data from the substitute comparison subjects were not used for inference. Due to logistic difficulties, the substitute comparisons were invited to participate in the physical examination later in the study period; therefore the substitute comparisons had a narrower time window within which to travel to the examination site. The substitute comparison subjects, entered early into the study to replace ineligible subjects, were found to be statistically comparable to the original comparisons when evaluated on clinical endpoints. Some principal investigators were concerned that the group substituting later for noncompliant comparisons may have self selected differently due to the reduced scheduling opportunity. Since opinions differed, a management decision was made to use only the originally invited comparisons for inference.

The baseline morbidity report had 13 primary clinical chapters addressing: general health, neoplasia, reproduction, neurological status, psychological status, hepatic function, dermatological findings, cardiovascular conditions, immunological competence, hematopoietic status, and renal, pulmonary, and endocrine functions. More than 190 clinical variables were tabulated. In this report only a subset of these variables will be discussed. The variables selected for emphasis in this report were chosen because of the availability of corresponding or related evaluations in laboratory or other epidemiological studies related to dioxin effects. Thus, this report reflects on the degree to which AFHS findings are compatible with TCDD toxic effects as currently suggested by experiments with animals and observations in humans. Sample sizes may vary in adjusted analyses due to missing covariate or endpoint data. In all analyses, the phrase "statistically significant" refers to a p value of less than or equal to 0.05.

The fixed sample sizes in this study impose limits on its ability to detect small relative risks for rare diseases. This ability is expressed in probabilistic terms using the statistical quantity called power, which is defined as the probability of detecting a group difference of interest. In the case of dichotomous response, such as presence or absence of disease, groups are generally compared with relative risk, defined as the ratio of the probability of disease in the exposed group to the probability of disease in the comparison group. A relative risk of two, for example, would indicate a doubling of the disease rate in the exposed group relative to the comparisons. If the disease incidence in the control group is 1/1000, as is typical for some specific cancers, such as bladder cancer, the AFHS would require 22,840 exposed and an equal number of comparisons to attain a power of 80% to detect a relative risk of two, assuming two-sided testing with a 5% significance level. In fact, the AFHS is unable to detect relative risks less than eight in diseases with a comparison incidence of less than or equal to 1/1000. There is a 0.351 chance of observing no cases at all of a rare disease of incidence 1/1000 in a group of 1,045 subjects. With even rarer diseases of incidence, 1/10,000, there is a 90% probability of observing no cases at all in a group of 1,045 subjects.

This study does have good power to detect small relative risks for diseases having an incidence of 5/100 or greater. For example, the power for detecting a relative risk of 2, when the disease rate in the comparison population is 5/100, is 0.85, based on only 450 pairs in a matched pair analysis. In the case of continuously distributed response variables, such as blood pressure or cholesterol, this study has good power to detect small mean shifts. For example, the probability of detecting a mean shift of 5% in a matched pair analysis utilizing only 450 pairs is at least 0.90, assuming equal variances, two-sided testing and an 0.05 significance level.

The power of the mortality component of this study is similarly constrained. The mortality study design consists of all 1,247 Ranch Hands and up to 5 matched controls per Ranch Hand. The mortality study has a power of 0.85 to detect a relative risk of two for causes of death, such as heart disease, having incidence 1/100 in the comparison population. The corresponding power is less than 0.25 for causes having an incidence of 1/1000 in the control population.

This study, in summary, has good power for detecting relative risks on the order of two or three for common diseases and causes of death and has virtually no power for detecting relative risks of the same order of magnitude for rare diseases. It does have good power for detecting small mean shifts in continuously distributed response variables. Bearing these study power constraints in mind, the following ten areas of clinical morbidity are discussed.

General Health

Weight loss has been frequently reported as a consequence of subacute and chronic administration of TCDD to animals. McNulty[4] noted marked weight loss in two male rhesus monkeys fed diets containing 2 or 20 ppb TCDD. Horses ingesting TCDD-contaminated waste oil sprayed on arenas in Missouri showed significant weight loss.[5,6] Chapman and Schiller[7] report that decreased feed consumption did not account for weight loss in C57 mice given dioxin in their

feed. Seefeld and colleagues[8] conclude that TCDD affects the weight regulation set-point in rats. Weight loss has not been prominently commented on in studies of human exposure. However, Oliver[9] indicates weight loss or loss of appetite in two of three reported cases.

The toxicological literature mentioned here suggests that weight changes might be anticipated in a dioxin-exposed group. In the AFHS, body fat percent was calculated by a formula that uses height and weight as independent variables.[10] No statistically significant differences in the distribution of estimated body fat were detected between the Ranch Hand and comparison group. The basic data are shown in Table 3.

TABLE 3. DISTRIBUTION OF BODY FAT PERCENT

	Lean (<10%)		Normal (10-25%)		Obese (>25%)		
	Number	Percent	Number	Percent	Number	Percent	Total
Ranch Hand	13	(1)	824	(79)	207	(20)	1,044
Comparison	7	(1)	607	(79)	157	(20)	771

The sample sizes in Table 3 (1,044 and 771) are reduced due to missing data for three individuals. A chi-square statistical test using these data indicated no statistically significant difference between the distributions for the groups (p=0.89). Detailed analyses of percent body fat, adjusting for age, race, and occupational category, are described in the baseline report, and these analyses also indicated the absence of a group difference. Also, within the Ranch Hand group no relationship between body fat and the exposure index was found.

Neoplasia

The animal toxicology literature portrays dioxin as a carcinogen, a cocarcinogen, and as having anti-carcinogenic properties. Jackson[11] showed impairment in the functioning of the mitotic apparatus at 0.2 µg/l in dividing endosperm cells of the African blood lily. Kociba et al[12] fed male and female Sprague-Dawley rats on diets supplying 0.1, 0.01, or 0.001 µg of TCDD/kg/day for 2 years. Exposed male rats displayed more stratified squamous cell carcinomas of the hard palate and tongue. However, fewer adenomas of the pancreas and pheochromocytomas of the adrenal were found. Kouri and colleagues[13] conclude that TCDD is a cocarcinogen. They propose that this effect is mediated through aryl hydrocarbon hydroxylase induction. On the other hand, DiGiovanni and colleagues[14] found that TCDD reduced cutaneous papilloma formation by various hydrocarbons, indicating an anti-carcinogenic effect. With respect to carcinogenesis in man, Coggon and Acheson[15] reviewed the available

epidemiological studies and concluded ". . . there is suggestive evidence of a biological association between phenoxy herbicides (or their contaminants) and soft-tissue sarcomas. The evidence relating these products to the occurrence of lymphomas is weaker."

Table 4 summarizes the cancer events that have occurred in the Ranch Hand and comparison groups since these individuals completed their Southeast Asia military tours. All shown cancer cases have been verified by personal medical or pathological records. One comparison individual has had both a skin and systemic cancer. In the table below he is shown as having only a systemic cancer for purposes of the statistical analysis.

TABLE 4. CANCER VERIFIED BY INDIVIDUAL MEDICAL RECORDS OR PATHOLOGY REPORTS

Group	No. with Skin Cancer (%)	No. with Systemic Cancer (%)	No. with No Cancer (%)	Total
Ranch Hand	35 (3.3)	13 (1.2)	997 (95.4)	1,045
Comparison	10 (1.3)	8 (1.0)	755 (97.7)	773

Of 1,045 Ranch Hands, 4.59% have a skin or systemic cancer. Of the 773 comparison individuals, 2.33% have a skin or systemic cancer. Thus, the relative risk for any type of cancer is 1.97 and this relative risk has a probability of less than 0.01 of occurring by chance under the hypothesis of no difference. This statistical test for overall cancer rate difference was the hypothesis test formulated prior to examination of the cancer data set. After this statistical test was performed, detailed review of the cancer data file suggested that the file be partitioned into skin and systemic events based upon the observation that skin cancer comprised a large fraction of the cancer set. The relative risk for skin cancer is 2.59, and this risk has a p value of less than 0.01. The relative risk for systemic cancer is 1.20, and this risk has a probability of 0.67 of occurring by chance.

In the first morbidity report, the authors felt the neoplastic process was confined to skin. This inference cannot be affirmed because the separate skin and systemic hypothesis tests followed rather than preceded review of the cancer data file; thus the critical levels for these tests are unknown. Furthermore, important increments in relative risk for systemic cancer could be missed by chance mechanisms because of the small sample sizes in the AFHS. Neither skin cancer nor systemic cancer rates were correlated with herbicide exposure level; however, these statistical analyses involved a very small number of cases in most of the nine occupation-exposure categories, thus decreasing the precision of rate determinations in these categories.

Reproduction

The literature suggests that dioxin has mutagenic and teratogenic capacity. Some bacterial tests have been positive for TCDD mutagenicity.[16] The baby hamster kidney cell transformation assay was positive for TCDD mutagenicity.[16] Chromosome aberrations have been seen in bone marrow cells of male rats exposed to TCDD.[17] Van Miller and Allen[18] observed reduced spermatogenesis in rats experiencing chronic exposure to TCDD. Seiler[19] observed reduced DNA synthesis in mouse testicle. Courtney and Moore[20] observed kidney abnormalities in fetuses of female rats given 0.5 µg/kg/day of TCDD subcutaneously on days 6-15 of gestation. Cleft palate and renal abnormalities have been produced in the mouse after oral or subcutaneous administration of TCDD to females.[21] Lamb and colleagues[22] showed that exposure of male mice to toxic levels of TCDD with subsequent mating did not affect sperm, mating frequency, or quality of offspring.

This sampling of the literature should be sufficient to indicate the possibility of reproductive changes in exposed human populations. Hanify and colleagues[23] have reported an association of aerial spraying of 2,4,5-T and an excess of talipes in New Zealand. Townsend et al.,[24] in a study of Dow chemical workers' wives, found no statistically significant differences in fetal wastage or birth defects. The Australian government's study of birth defects in Vietnam veterans showed no statistically significant differences in rates between veterans who served in Vietnam and those who did not.[25] A Centers for Disease Control study (CDC) also found Vietnam veterans to have the same overall risk for fathering abnormal offspring as nonveterans. In the CDC study some specific defects were associated with higher exposures, but interpretation of this finding was uncertain.[26]

Male exposure could theoretically lead to unfavorable reproductive outcomes by means of several mechanisms: (a) mutated DNA in sperm, (b) abnormal sperm or testicular function due to biochemical effects, (c) transmission of TCDD to the female by spermatic fluid, and (d) transmission of TCDD to the female by contact with clothes or other objects.

Semen specimens from study participants without vasectomies or orchiectomies evidenced no statistically significant group differences in sperm count or percent abnormal sperm. The data are displayed in Table 5. Sample sizes reflect the number of compliant subjects not previously vasectomized.

TABLE 5. DESCRIPTIVE STATISTICS OF SPERM VARIABLES BY GROUP

Sperm Count (millions/ml)

	$\bar{x}$	σ
Ranch Hand (N=572)	111.5	102.8
Comparison (N=421)	111.9	108.8

Percent Abnormal Sperm

	$\bar{x}$	σ
Ranch Hand (N=560)	9.7	5.5
Comparison (N=409)	9.6	5.2

Conceptions reported by study participants and their spouses were categorized as miscarriages, stillbirths, induced abortions, and live births. Numbers in each category are shown in Table 6 with indication of whether the conception occurred before or following the participant's Southeast Asia tour.

TABLE 6. CONCEPTION OUTCOMES BY GROUP MEMBERSHIP AND TIME

	Pre-SEA			Post-SEA			
	Yes	(%)	No	Yes	(%)	No	P Value
Miscarriages							
Ranch Hand	239	(13.7)	1,505	156	(15.0)	883	0.76
Comparison	172	(11.9)	1,276	104	(12.5)	726	
Stillbirths							
Ranch Hand	9	(0.5)	1,735	12	(1.2)	1,027	1.00
Comparison	8	(0.6)	1,440	8	(1.0)	822	
Induced Abortions							
Ranch Hand	8	(0.5)	1,736	37	(3.6)	1,002	0.89
Comparison	7	(0.5)	1,441	33	(4.0)	797	
Live Births							
Ranch Hand	1,487	(85.3)	25	833	(80.2)	206	0.94
Comparison	1,258	(86.9)	190	682	(82.2)	148	

The data were analyzed using log-linear models[27], adjusting for the factors of maternal age (<35, ≥35), maternal smoking (yes/no), maternal alcohol use (yes/no), and paternal age (<35, ≥35). The four statistical tests all had p values greater than or equal to 0.76. Exposure analyses showed no consistent pattern with exposure level.

Ranch Hand and comparison live births were further analyzed to determine the occurrence of learning di bilities, physical handicaps, infant death, neonatal death, and birth defects. Data on live birth outcomes by group membership and time are shown in Table 7.

TABLE 7. LIVE BIRTH OUTCOMES BY GROUP MEMBERSHIP AND TIME

	Pre-SEA			Post-SEA			
	Yes	(%)	No	Yes	(%)	No	P Value
Learning Disability							
Ranch Hand	57	(3.8)	1,430	75	(9.0)	758	0.19
Comparison	57	(4.5)	1,201	47	(6.9)	635	
Physical Handicap							
Ranch Hand	134	(9.0)	1,353	126	(15.1)	707	0.45
Comparison	103	(8.2)	1,155	77	(11.3)	605	
Infant Death							
Ranch Hand	7	(0.5)	1,480	3	(0.4)	830	0.81
Comparison	2	(0.2)	1,256	1	(0.1)	681	
Birth Defects							
Ranch Hand	78	(5.2)	1,409	76	(9.1)	757	0.04
Comparison	80	(6.4)	1,178	44	(6.5)	638	
Neonatal Death							
Ranch Hand	20	(1.3)	1,467	14	(1.7)	819	0.20
Comparison	17	(1.4)	1,241	3	(0.4)	679	

Analyses of these data, adjusting for maternal age, maternal smoking, maternal alcohol use, and paternal age, reveals a statistically significant increase in reported birth defects in the Ranch Hand group. Subsequent to this observation the birth defects were categorized as severe (life threatening or interfering with normal overall health or socio-economic progress), moderate (not life threatening and, with health care, non-interfering with overall health or socio-economic progress), and limited (non-life threatening, non-interfering, and needing no care). These data are shown in Table 8.

TABLE 8. SEVERITY OF REPORTED BIRTH DEFECTS BY GROUP MEMBERSHIP AND TIME

	Severe	Moderate	Limited	No reported Defects	Total
			Pre-SEA		
	N (%)	N (%)	N (%)	N (%)	
Ranch Hand	51 (3.0)	32 (1.9)	7 (0.4)	1,633 (95)	1,723
Comparison	50 (3.5)	27 (1.9)	10 (0.7)	1,348 (94)	1,435
			Post-SEA		
	N (%)	N (%)	N (%)	N (%)	
Ranch Hand	32 (3.5)	22 (2.4)	26 (2.8)	837 (91)	917
Comparison	18 (2.4)	20 (2.7)	10 (1.3)	696 (94)	744

The above data set is larger than the prior data set since this set contains all reported live births while the prior set consisted of all live births on whom the covariates (maternal age, maternal smoking, maternal alcohol, and paternal age) were available. The larger data set was used since categorizing birth defects as severe, moderate, or limited reduced cell counts. Full covariate adjustment was not possible. The morbidity report can be consulted for details.[1]

Once again, in this larger data set, an increase in reported defects is noted. Specifically, the Ranch Hand to comparison birth defect odds ratio is 0.85 for children born prior to Vietnam, while the post-Vietnam ratio is 1.39. A statistical analysis of the complete data set suggests that the birth defect severity pattern by group relationship changes with time ($p=0.07$). Visual inspection of these data suggests that this nearly significant change may be due to a relatively large number (26) of post-Vietnam Ranch Hand children reported as having limited birth defects; the Ranch Hand to comparison odds ratio in this category is 2.16. However, an excess of severe defects is also seen by visual inspection. A separate analysis on data for defective children only (with the reported non-defective children removed) suggests that the group by severity relationship does not change with time ($p=0.15$) and that the severity pattern does not change with group ($p=0.78$). The statistical analysis of the complete data set is more powerful than these last two analyses since the latter analyses use only the 305 reportedly defective children, which constitutes only 6.3% of the total data set.

In the AFHS first morbidity report, it was asserted that minor skin lesions accounted for the reported birth defects excess. That analysis was incomplete, and we are no longer confident in that inference. Also in the AFHS first morbidity report, it was properly suggested that differential reporting of birth defects could be responsible for the apparent excess. A

preliminary analysis of medical records of children reported abnormal, has indicated that overreporting of defects may not account for the excess; however, intensive work is in progress addressing both differential over- and underreporting.

Exposure index analysis of the birth defect data yields inconsistent findings that are not interpretable as a herbicide effect.

The finding of increased birth defects as reported by study participants, their wives, and partners is under further investigation by review of birth certificates and medical records of all 5,663 children to verify both positive and negative responses.

Neurological Findings

A variety of neurological symptoms have been described following industrial accidents involving TCDD including headaches, asthenia, sleep disturbances, irritability, and confusion. Peripheral polyneuropathy is a specific neurological condition that has also been linked to acute dioxin exposure, and is a condition that is amenable to direct clinical measurement.[28] Elovaara and colleagues[29] found that acid proteinase activity was increased in the brains of Wistar rats after TCDD treatment. Acid proteinase is a lysosomal enzyme responsible for protein destruction in the mammalian brain, and may play a role in degenerative diseases and intoxications.

In the AFHS, neurological examination of the twelve cranial nerves revealed no statistically significant group differences. Assessment of peripheral nerve status included sensitivity to touch, vibration, and test of the patellar, achilles, and biceps reflexes. Again, no statistically significant group differentials were observed.

As shown in Table 9, the groups were not statistically different with respect to nerve conduction velocities. Of interest is the observation (data not shown) that the conduction velocities decreased as expected with increasing self-reported alcohol use (drink-years) and postprandial glucose levels (dichotomized as less than, or equal to or greater than 120 mg/dl). These effects appear to be consistent in both groups. All exposure index analyses were unremarkable.

TABLE 9. NERVE CONDUCTION VELOCITY (M/SEC)

Nerve	Group (N)	Unadjusted Mean	P Value
Ulnar (above the elbow)	Ranch Hand (1,035)	55.9	0.30
	Comparison (769)	56.2	
Ulnar (below the elbow)	Ranch Hand (1042)	60.5	0.39
	Comparison (771)	60.7	
Peroneal	Ranch Hand (1041)	48.2	0.74
	Comparison (769)	48.1	

Psychological Assessment

Working with rats, Creso et al.[30] have noted that TCDD provokes irritability, aggressiveness, and restlessness. They found by in vitro studies that TCDD directly stimulates the striatal and hypothalamic adenylate cyclase of rat. Oliver[9] reports that two of three TCDD-exposed individuals studied expressed the symptom of excessive fatigue, and one communicated loss of ability to concentrate. Bauer et al.[31] studied nine workers with chloracne and noted fatigue and apathy alternating with anger and irritability. Rorshach tests showed a weakened emotional reaction, slowed thought processes, and perseveration. Poland and Smith[32] observed increased values on the MMPI mania scale in the group of workers with chloracne when compared to two groups with less severe acne.

The AFHS disclosed several group differences in psychological testing. Indices developed from the questionnaire relating to fatigue, anger, mental erosion, anxiety, isolation, and depression all showed Ranch Hands to be statistically significantly less well than comparisons. The Cornell index results paralleled the questionnaire indices; however, no increase in Ranch Hand depression was seen. Education strongly related to results of all psychological testing with group differences tending to be most prominent in high-school-only educated individuals rather than college educated. For example, the MMPI among high-school-only educated individuals showed statistically significantly higher hypochondria, mania, and social introversion scores among Ranch Hands. The MMPI among college-educated participants showed only higher social introversion among Ranch Hands. In interpreting these data it must be remembered that there is a very high association between being college educated and having officer status. None of these data had been adjusted for the potentially confounding variable of combat stress. This area was pursued during the 1985 follow-up examination. Tests aiming at neuromuscular and intellectual functioning (WAIS and Halsted-Reitan) showed no group differences, and no consistent patterns emerged from any analyses using the exposure index.

Thirty-six of 1,045 Ranch Handers (3.4%) and 16 of 773 comparisons (2.1%) reported psychological illness (psychosis, alcohol dependence, anxiety, or other neurosis). This group difference is not statistically significant.

Hepatic Examination

Dioxin has been associated with the occurrence of hepatotoxicity. Proliferation of the smooth endoplasmic reticulum, distortion of liver architecture, and increase in liver weight relative to body weight have been seen in rats and mice. Changes in serum enzymes have been observed, but not with consistency. Porphyria has been observed after chronic dosing. A sampling of an extensive literature is given next. Weber et al.[33] observed rats over a 32-week period following a single intraperitoneal dose (20 µg/kg) of TCDD. Centrolobular necrosis, mitochondrial lesions, smooth endoplasmic reticulum increase, and hepatic regeneration were seen. The abnormalities began to regress 16 weeks after exposure. Similar findings in rats have also been reported by King and Roesler.[34] Gupta and colleagues[35] orally administered single, daily, and weekly doses of TCDD to rats, guinea pigs, and mice. Severe liver lesions were only seen in the rat indicating species variation. Kociba et al.[12] noted elevated liver enzyme levels in rats fed diets with TCDD for two years. Porphyria cutanea tarda has occurred in workers exposed to TCDD,[36] and porphyria has been observed in rats.[12]

Sweeney and colleagues[37] have performed work that shows a synergism between the effects of TCDD and systemic iron. Iron deficiency was seen to prevent TCDD-induced porphyria in mice. In iron-deficient animals, TCDD was not able to decrease uridine decarboxylase levels. Iron deficiency protected mice against skin damage and the disruption of hepatic architecture seen with TCDD. Mixed function oxygenase activity was induced by TCDD in iron-deficient animals to a lesser degree than in non-deficient animals, but this difference was not significant. Sinclair and Granick[38] had earlier seen that iron was needed for uroporphyrin formation induced by chlorinated hydrocarbons. Smith et al.[39] noted an increase in hepatic iron content 3 weeks after a 75 µg/kg oral dose of TCDD to C57BL/10 and DBA/2 mice. Increased intestinal uptake of iron has been observed in mice and rats after TCDD exposure.[40]

In the AFHS, nine biochemical determinations of liver function were made: SGOT, SGPT, GGTP, alkaline phosphatase (Alk.Phos.), total bilirubin (T.Bili), direct bilirubin (D.Bili), lactic acid dehydrogenase (LDH), cholesterol (Chol), and triglycerides (Trig). In the analyses of these nine variables, statistical adjustments were made for four covariates: current alcohol ingestion (self-reported in drinks per day), self-reported days of exposure to non-herbicide industrial chemicals, self-reported days of exposure to degreasing chemicals, and presence or absence of antibody to hepatitis B surface antigen (anti-HBsAg).

Table 10 provides unadjusted and adjusted means and percent abnormality by group for the nine hepatic-related variables. The standard age-adjusted criteria for abnormal laboratory values were used throughout. No obvious group differences are apparent in these data. However, the statistical modeling of the dependent variables with the covariables showed three group differences. The Ranch Hand SGOT-alcohol regression slope is 0.0178 base-10 logarithmic units per drink per day while the comparison slope is 0.0113.

These slopes mean that among study participants who report one drink per day, Ranch Hand SGOT levels are 1.5% higher than comparison levels. Among study participants who had four drinks per day, Ranch Hands have SGOT levels 6.8% higher than comparisons.

TABLE 10. UNADJUSTED MEANS, ADJUSTED MEANS, AND PERCENT ABNORMALITY FOR NINE LIVER-RELATED VARIABLES

Variable	Group	Unadjusted Means	Adjusted Means	PCT Outside of Normal Range
SGOT	RH*	33.0	33.0	13.9
	Com**	33.1	33.1	14.8
SGPT	RH	20.3	20.3	7.8
	Com	20.5	20.5	8.6
GGPT	RH	40.2	40.1	10.8
	Com	39.3	39.3	10.3
Alk.Phos.	RH	7.68	7.69	17.3
	Com	7.53	7.52	16.9
T. Bili	RH	0.57	0.57	1.8
	Com	0.58	0.58	2.0
D. Bili	RH	0.23	0.23	29.0
	Com	0.24	0.24	29.7
LDH	RH	142.1	142.1	1.7
	Com	141.7	141.7	2.1
Chol	RH	212.2	212.2	26.0
	Com	216.6	216.6	27.7
Trig	RH	121.8	121.9	34.7
	Com	124.3	124.1	36.1

*RH denotes Ranch Hand
**Com denotes original fully compliant comparisons.

LDH-alcohol slopes were 0.0041 logarithmic units per drink per day in the Ranch Hand cohort and -0.0008 in the comparison group (p=0.011). The LDH-degreasing chemical slopes were 0.000005 and -0.0000008 logarithmic units per day degreasing chemical exposure in the Ranch Hand and comparison groups respectively (p=0.037).

Twenty-four-hour urine collections were obtained for 620 Ranch Hands and 439 comparisons; uroporphyrins, coproporphyrins, and d-aminolevulinic acid were determined. Unadjusted group means are shown in Table 11.

TABLE 11. UNADJUSTED GROUP MEANS FOR THREE COMPOUNDS RELATED TO PORPHYRIN METABOLISM

Uroporphyrin	RH	30.5
	Com	30.8
Coproporphyrin	RH	31.2
	Com	30.8
d-aminolevulinic acid	RH	2328.9
	Com	2383.2

No statistically significant differences are obvious in these data. Detailed statistical analyses were done, simultaneously adjusting for six covariates: current alcohol use, blood urinary nitrogen, creatinine clearance, days of exposure to industrial chemicals, days of exposure to degreasing chemicals, and presence/absence of antibody to hepatitis B surface antigen. A generalized linear model analysis was done for each of the three compounds with all six covariates examined simultaneously. The coproporphyrin-alcohol slope was +0.013 logarithmic units per drink per day in the Ranch Hand group and -0.008 logarithmic units per drink per day in the comparison group ($p=0.045$). No other group differences were statistically significant. The clinical relevance of these differences in slope is unclear.

Sixteen of 1,027 Ranch Handers (1.56%) were diagnosed as having hepatomegaly at physical examination while six of 769 comparisons (0.78%) had that finding ($p=0.138$). Thirteen of 1,032 Ranch Handers had a verified medical history of liver disorder other than hepatitis, jaundice, or cirrhosis verified by medical record while two of 773 comparisons had the same ($p=0.004$).

Throughout the hepatic analyses no variable showed a meaningful relationship with the herbicide exposure index. In all the above analyses no adjustments for iron metabolism were made.

Dermatological Finding

TCDD is known to cause chloracne. Chloracne is an acneiform lesion which tends to predominate in the areas of the face around the eyes, temples, and ears. Chloracne has been frequently seen in humans who have contacted TCDD in the context of industrial accidents. In one study the chloracne resolved within 1 year for the most part with no scarring.[41] Chloracne has been noted

by many investigators years after exposure and is generally recognized as a persistent effect of dioxin exposure. Bovey and Young[42] conclude that "the presence of active chloracne months to years after exposure does not necessarily mean continuing exposure."

In the AFHS, no active chloracne was found in either the exposed or comparison group by examination or review of medical records. Also, as indicated in Table 12, there were no statistically significant group differences with respect to chloracne-related lesions. No statistically significant regression trends were noted with the exposure index.

TABLE 12. PREVALENCE OF DERMATOLOGIC DIAGNOSES IN PERCENT

Diagnoses	Ranch Hand N = 1045	Comparison N = 773	P Value	Relative Risk	95% Conf Int
Comedones	21.7	20.7	0.60	1.05	(.87,1.26)
Acneiform lesions	18.3	17.5	0.66	1.05	(.85,1.29)
Acneiform scars	11.2	10.4	0.57	1.08	(.82,1.43)
Cysts	11.6	10.5	0.46	1.10	(.84,1.46)
Hyperpigmentation	8.3	7.1	0.35	1.17	(.84,1.65)
Other abnormalities	12.6	16.3	0.03	.77	(.81, .98)
Any abnormalities	45.0	44.9	0.97	1.00	(.90,1.11)

Cardiovascular System

TCDD causes a rapid, dose-dependent elevation of lipofuscin in the hearts of female Fischer 344 rats. The authors of the research suggest that TCDD toxicity may be associated with radical-induced lipid peroxidation.[43] Kociba and colleagues[12] saw an increased incidence of arteritis in rats. In 1958, Schmittle and colleagues[44] reported hydropericardium in poultry following ingestion of feeds contaminated with industrial chemicals. In 1969 a dioxin was shown to be the hydropericardium-producing factor in poultry.[45] Jirasek and colleagues[46] report that a 57-year-old male with chloracne develcped unusually severe atherosclerosis and subsequently died. Moses and colleagues[47] found that 17 of 116 workers with chloracne reported myocardial infarction (14.7%) while 7 of 85 (8.2%) without chloracne reported the same ($p > 0.10$). Zack and Suskind[48] observed no excess in circulatory system deaths comparing events in Monsanto Company workers to standard US population rates.

In the AFHS no statistically significant group differences were observed with respect to measurements of systolic and diastolic blood pressures. Also, the groups were not statistically significantly different with respect to numbers of abnormal electrocardiograms. Abnormal funduscopic findings were not associated with group membership nor was the occurrence of carotid bruits. During the physical examination, 10 peripheral pulses were examined: the radial, femoral, popliteal, dorsalis pedis, and posterior tibial pulses. One

or more pulses in this set of pulses were found to be abnormal in 12.8% (106/829) of the non-black Ranch Hands, while 9.4% (56/596) were found abnormal in the comparison group (p=0.05). The group difference was not statistically significant in the data set on Black study participants, but this may only reflect smaller numbers. Peripheral pulse abnormalities tended to aggregate in older individuals (≥ 40 years) who smoked (> 10 pack years). The participants were allowed to smoke prior to the examination of the pulses, and more Ranch Hands smoked at the time of the examination than did comparisons (45.7% versus 40.5%, p=0.03).

Data on the numbers of individuals in the Ranch Hand and the comparison group who had experienced some form of heart disease (ICD-9th edition, CM) or who had experienced a myocardial infarction are shown in Table 13. The numbers shown are supported by medical record verification of participants' self-reporting. These data do not suggest a difference in ischemic heart disease in the two groups.

TABLE 13. HEART DISEASE AND HEART ATTACK IN THE AFHS

	Ranch Hand		Comparison		
	Yes	No	Yes	No	P Value
Verified Heart Disease	147	898	109	664	0.982
Verified Heart Attack	7	1,038	3	770	0.432

Immunological Effects

Clark and colleagues[49] describe thymic atrophy as a consistent observation in all animal species following TCDD exposure. They also observed reduced delayed hypersensitivity reactions assessed by ear swelling following oxazalone sensitization in 6- to 8-week-old mice. Cytotoxic T cell lymphocyte generation was impaired by low doses (0.004 µg/kg) of TCDD. Following a variety of experiments, the authors suggest that TCDD may acutely decrease cytotoxic T lymphocyte generation by promoting the generation of suppressor T cells. In parallel results, Montovani and colleages[50] observed decreased numbers of peritoneal macrophages and splenocytes in TCDD-treated 6- to 8-week-old mice, while cytotoxic capability per unit number of cells was not affected. Van Logten and colleagues[51] conclude that the atrophy of the thymus observed following TCDD administration in rats is not mediated by the adrenal or pituitary glands. Clark and colleagues[52] state that the immunotoxic effects of TCDD in C57B/6 and DBA/2 mice occur at dose levels below those needed to induce hepatic mixed function-oxidase enzymes.

In the AFHS, immunological status was assessed in 592 participants by (1) the enumeration of T-lymphocytes, T-lymphocyte subsets, and B-lymphocytes using monoclonal surface marker analysis, and by (2) the assessment of lymphocyte ability to respond to selected antigen or mitogen stimuli.

The data were analyzed for statistically significant group distributional differences using the Kolmogorov-Smirnov two-sample test. The analysis of the immunological cell count data showed no statistically significant differences. These cell count data are shown in Table 14.

TABLE 14. SELECTED PERCENTILES AND P VALUE FOR KOLMOGOROV-SMIRNOV TESTING OF NUMBERS OF SURFACE MARKER POSITIVE CELLS (THOUSANDS/MM^3)

Variable	Group	N	10%	50%	90%	P Value (comparing distributions)
T_{11}	RH	235	0.70	1.25	1.96	0.74
	Com	144	0.77	1.23	2.02	
T_3	RH	233	0.70	1.27	1.96	0.39
	Com	144	0.73	1.28	2.13	
T_4	RH	231	0.40	0.79	1.25	0.81
	Com	147	0.48	0.78	1.42	
T_8	RH	235	0.30	0.57	0.99	0.34
	Com	147	0.28	0.60	1.17	
B_1	RH	235	0.023	0.071	0.188	0.097
	Com	147	0.022	0.071	0.247	
TLC*	RH	290	1.34	1.92	2.54	0.63
	Com	177	1.35	1.91	2.74	

*Total lymphocyte count

Statistical testing of the four stimulation and two control measurements assessing lymphocyte functional ability is shown in Table 15.

TABLE 15. KOLMOGOROV-SMIRNOV TESTING OF T AND B CELL FUNCTION DATA: THYMIDINE INCORPORATION MEASURED AS COUNTS/MIN

Variable	Group	N	Percentiles 10%	50%	90%	P Value
Control #1	RH	279	140	374	1,320	0.20
	Com	168	138	448	1,483	
After ConA	RH	279	17,741	54,190	91,724	0.38
	Com	168	13,596	58,394	99,104	
After PHA	RH	279	33,027	79,342	130,064	0.51
	Com	168	30,143	84,339	135,684	
Control #2	RH	274	132	388	917	0.85
	Com	168	142	404	1,079	
After PW	RH	274	12,700	29,623	58,288	0.64
	Com	168	12,232	27,916	53,662	
After TT	RH	274	866	3,726	13,979	0.81
	Com	168	1,001	3,719	16,058	

ConA = concanavallin A
PHA = phytohemagglutin
PW = pokeweed mitogen
TT = tetenus toxoid.

No statistically significant group differences are noted in these T and B cell function data. High laboratory variability and small sample sizes led to the decision to not use exposure index analyses with the immunological data. The control #1 and control #2 variables represent the unstimulated activity of the T cells.

Endocrinological Effects

Working with liver homogenates taken from adult male Wistar rats, Nienstedt and colleagues[53] showed that TCDD reduced the catabolism of testosterone. Hook and colleagues[54] also reported reduced metabolism of testosterone. These reports would suggest that elevated testosterone levels could be observed in a recently dioxin-exposed population.

Bastomsky[55] observed serum T_4 levels to be one-half of normal in TCDD-treated animals, but serum T_3 was elevated by 50%. Sephadex uptake of T_3 was statistically significantly decreased. Potter and colleagues[56] observed serum levels of T_4 to fall to 46% of pair-fed controls in TCDD-dosed rats. However, no statistically significant change in serum T_3 occurred. These authors also noted hypoglycemia after a single intraperitoneal dose of TCDD. Rozman and colleagues[57] emphasize the potentially important role of thyroid hormones in certain expressions of TCDD toxicity. Specifically, athyroid rats showed a markedly decreased mortality rate and less weight loss than nonthyroidectomized or thyroidectomized but euthyroid controls.

In the endocrinological portion of the AFHS, five clinical variables were studied: T_3 uptake, serum T_4, free thyroxine index (FTI), 2-hr postprandial glucose, and serum testosterone. One statistical analysis of these variables examined the number of participants below, in, or above the variables' normal ranges. This analysis is summarized in Table 16. Statistically significant difference is seen in these data for the T_3 uptake comparison. The Ranch Hand group was also contrasted with the comparison group in terms of the five endocrinological variables using analysis of covariance, adjusting for age and percent body fat. These analyses are summarized in Table 17. Three group differences are noted in these analyses. In both the Ranch Hand and comparison groups, a decrease in T_3 uptake is observed with advancing age, but the slope is -0.0068% per year in the comparison group and -0.0495% per year in the Ranch Hand group, and this group difference was statistically significant ($p=0.026$). Two-hour postprandial glucose levels increase with age in both the Ranch Hand and comparison groups, but the rate of increase is 1.53 mg/dl per year in the Ranch Hand group and 0.77 mg/dl per year in the comparison group ($p=0.006$). Lastly, Ranch Hands show a higher (but not statistically significant) testosterone level than do comparisons. Both increasing age and body fat were found to be associated with decreasing testosterone levels to the same extent in both groups.

TABLE 16. UNADJUSTED PERCENTAGES FOR FIVE ENDOCRINOLOGICAL VARIABLES BY VARIABLE LEVEL AND GROUP

Variable	Group	N	Variable Level Low	Normal	High	P Value For Group Difference
T_3 Uptake (%)	RH	1,032	5.72%	93.41%	0.87%	0.020
	Com	767	8.47%	91.26%	0.26%	
T_4 (µg/dl)	RH	1,033	0.10%	99.13%	0.77%	0.250
	Com	767	0.39%	99.22%	0.39%	
FTI	RH	1,033	0.00%	99.71%	0.29%	0.085
	Com	767	0.26%	99.74%	0.00%	
2-hr Glucose (mg/dl)	RH	1,040	NA	84.81%	15.19%	0.234
	Com	770	NA	82.73%	17.27%	
Testosterone (ng/dl)	RH	1,034	4.93%	94.58%	0.48%	0.414
	Com	769	6.37%	93.11%	0.52%	

TABLE 17. RANCH HAND COMPARISON GROUP MEANS OF ENDOCRINE VARIABLES

Variable	Group	N	Unadj. Mean	P Value For Unadj. Means	Adjusted Mean	P Value For Adjusted Means	Remarks About Adjusting Covariates
T_3 Uptake (%)	RH	1,037	30.28				Group-by-age interaction (p=0.026)
	Com	770	30.14	0.21	*	*	
T_4 (µg/dl)	RH	1,038	8.46		8.45		
	Com	770	8.39	0.31	8.39	0.38	
FTI	RH	1,038	2.54		2.54		
	Com	770	2.51	0.07	2.51	0.13	
2-hr Glucose (mg/dl)	RH	1,045	104				Group-by-age interaction (p=0.006)
	Com	773	102	0.37	*	*	
Testos-terone (ng/dl)	RH	1,039	654		652		
	Com	772	634	0.02	637	0.06	

*Signifies interaction present rendering group means noninformative.

Mortality

Administration of single doses of dioxin to animals can result in lethality with marked species differences being observed.[42] Repeated daily doses can also lead to death. No studies with large numbers of animals (for instance, with 100 control and 100 exposed animals) are being conducted where the possibility of life shortening by very low doses of dioxin can be evaluated.

The Australian government provides a very complex report addressing mortality among Vietnam veterans.[58] Infantry exhibited a relative mortality rate of 0.96 with 95% confidence interval from 0.7 to 1.3. Engineers exhibited a relative mortality rate of 2.5 (confidence interval 1.4 to 4.0); armour and artillery, 1.06 (confidence interval 0.7 to 1.7); veterans with minor field presence, 1.5 (confidence interval 0.9 to 2.6); and non-field corps, 1.01 (confidence interval 0.7 to 1.5). Thus, only the engineers exhibited a statistically significant difference (p=0.001), and that difference involved increased Vietnam veteran mortality.

In the AFHS, cumulative mortality as of 31 December 1984 displays no statistically significant overall Ranch Hand-comparison differences. Summary counts of death and age standardized mortality ratios (SMR) by rank and occupation are given in Table 18.

TABLE 18. SUMMARY COUNTS, SMRs AND P VALUES FOR DEATH BY RANK AND OCCUPATION

	Ranch Hand			Comparison				
Rank	At Risk	Dead	Rate	At Risk	Dead	Rate	SMR	P Value
Officers	466	16	.034	2278	98	.043	.791	.37
Enlisted	791	39	.049	3893	187	.048	1.03	.89
Occupation								
Flying	646	24	.037	3163	161	.051	.726	.13
Ground	611	31	.051	3008	124	.041	1.23	.33

Further study of these mortality data discloses complex patterns with date of birth (which is related to date of service in Vietnam) and date of death.

CONCLUSION

In this report, eleven clinical areas have been emphasized based on a clinical toxicological profile developed from the literature concerning animal and human responses to dioxin and availability of data in the AFHS. Table 19 lists the general toxicological effects anticipated on the basis of the literature and also summarizes Ranch Hand findings. The toxicological profile we have developed from the literature certainly has an element of subjectivity as articles were selected and interpreted from a very large literature. Similarly, each observation in the AFHS can be challenged, and some specific caveats have already been mentioned.

TABLE 19. SUMMARY OF FINDINGS*

Toxicological Effect Suggested by Animal and Human Literature on Dioxin	Observation In AFHS
Weight Loss	o
Increased Neoplasia	+
Increased Birth Defects	+
Neurological Changes	o
Psychological Changes	+
Hepatotoxicity	+
Chloracne	o
Cardiovascular Changes	+
Immunological Deficits	o
Endocrine Changes	+/-
Increased Mortality	o

* "o" indicates no group differences observed
"+" indicates group difference observed in expected direction
"-" indicates group difference observed but in opposite direction

At this time one cannot ascribe the observed group differences to an effect of dioxin. One cannot implicate dioxin for at least four reasons: (a) the exposure index completely failed to demonstrate any association between increased exposure and increasing adverse outcome; (b) the full clinical profile for dioxin was not realized with the absence of chloracne being particularly noteworthy; (c) uninvestigated confounding variables remain for several of the target clinical endpoints, and resolution of these issues may alter the observed group differences; and (d) the effect of multiple statistical testing is not well defined.

However, the AFHS does not exonerate dioxin as a causative agent of these group differences. This conclusion is supported by three reasons: (a) in six of eleven clinical variables, statistically significant group differences occurred, and in five of these six instances the group differences were in the direction of expected dioxin effects; (b) uninvestigated confounding variables remain for several of the targeted clinical endpoints and resolution of these issues could alter group differences; and (c) the currently available exposure index is only an indication of exposure with unknown precision.

The overall probability of obtaining the AFHS results under the hypothesis of no group difference is not known. The summary in Table 19 cannot be used for statistical inference at this time because the table summarizes the results of hundreds of potentially correlated tests of significance. Further clarification of the role of dioxin in human health must await the results of the follow-up phases of the AFHS and other ongoing epidemiologic studies of dioxin-exposed groups.

ACKNOWLEDGMENT

I thank Ms Edith E. Wood for her professional help in retrieving and organizing the extensive literature review summarized in this report.

REFERENCES

1. Lathrop GD, Wolfe WH, Albanese RA, et al: An Epidemiological Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides: Baseline Morbidity Study Results. USAF School of Aerospace Medicine Report, Available from NTIS, Springfield, VA, 1984.

2. Lathrop GD, Wolfe WH, Albanese RA, et al: Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides: Study Protocol. USAF School of Aerospace Medicine Technical Report 82-44, Available from NTIS, Springfield, VA, 1982.

3. Lathrop GD, Moynahan PM, Albanese RA, et al: Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides: Baseline Questionnaires. USAF School of Aerospace Medicine Technical Report 82-42, Available from NTIS, Springfield, VA, 1982.

4. McNulty WP: Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin for Rhesus Monkeys: Brief Report. Bull Environ Contam Toxicol 1977;18,1:108-109.

5. Case AA, Coffman JR: Waste Oil: Toxic for Horses. Vet Clin North Am 1973;3:273-277.

6. Carter CD, Kimbrough RD, Liddle JA, et al: Tetrachlorodibenzodioxin: An Accidental Poisoning Episode in Horse Arenas. Science 1975;188:738-740.

7. Chapman DE, Schiller CM: Dose-Related Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in C57BL/6J and DBA/2J Mice. Toxicol Appl Pharmacol 1985;78,1:147-157.

8. Seefeld MD, Peterson RE: Starvation-Like Syndrome and 2,3,7,8-Tetrachlorodibenzo-P-Dioxin: New Ideas on the Mode of Action at the Whole Animal Level. in Proc Conf Environ Toxicol 1982:237-249.

9. Oliver RM: Toxic effects of 2,3,7,8 tetrachlorodibenzo 1,4 dioxin in laboratory workers. Br J Ind Med 1975;32:49-53.

10. Hc don JA, Marcinik EJ. Unpublished Data. Department of the Navy, Naval Health Research Center, San Diego, 1983.

11. Jackson WT: Regulation of Mitosis, III. Cytological Effects of 2,4,5-Trichlorophenoxyacetic Acid and of Dioxin Contaminants in 2,4,5-T Formulations. J Cell Sci 1972;10:15-25.

12. Kociba RJ, Keyes DG, Beyer JE, et al: Results of a Two-Year Chronic Toxicity and Oncogenicity Study of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin in Rats. Toxicol Appl Pharmacol 1978;46:279-303.

13. Kouri RE, Rude TH, Joglekar R, et al: 2,3,7,8-Tetrachlorodibenzo-p-dioxin as Cocarcinogen Causing 3-Methylcholanthrene-initiated Subcutaneous Tumors in Mice Genetically "Nonresponsive" at Ah Locus. Cancer Res 1978;38: 2777-2783.

14. DiGiovanni J, Berry DL, Gleason GL, et al: Time-dependent Inhibition by 2,3,7,8-Tetrachlorodibenzo-p-dioxin of Skin Tumorigenesis with Polycyclic Hydrocarbons. Cancer Res 1980;40:1580-1587.

15. Coggon D, Acheson ED: Do Phenoxy Herbicides Cause Cancer in Man? The Lancet 1982;1:1057-1059.

16. Hay A: The Chemical Scythe. Lessons of 2,4,5-T and Dioxin. Plenum Press, NY, 1982.

17. Green S, Moreland F, Sheu C: Cytogenetic Effect of 2,3,7,8-Tetrachlorodibenzo-p-dioxin on Rat Bone Marrow Cells. FDA By-Lines 1977;7,6:292-294.

18. Van Miller JP, Allen JR: Chronic Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin in Rats. Fed Proc Fed Am Soc Exp Biol 1977;36,1:396.

19. Seiler JP: Inhibition of Testicular DNA Synthesis by Chemical Mutagens and Carcinogens. Preliminary Results in the Validation of a Novel Short Term Test. Mutat Res 1977;46:305-310.

20. Courtney KD, Moore JA: Teratology Studies with 2,4,5-Trichlorophenoxyacetic Acid and 2,3,7,8-Tetrachlorodibenzo-P-dioxin. Toxicol Appl Pharmacol 1971;20:396-403.

21. Smith FA, Schwetz BA, Nitschke KD: Teratogenicity of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin in CF-1 Mice. Toxicol Appl Pharmacol 1976;38:517-523.

22. Lamb JC, Moore JA, Marks TA: Evaluation of 2,4-Dichlorophenoxyacetic Acid (2,4-D), 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T), and 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Toxicity in C57BL/6 Mice: Reproduction and Fertility in Treated Male Mice and Evaluation of Congenital Malformations in Their Offspring. National Toxicology Program Report-80-44, 1980.

23. Hanify JA, Metcalf P, Nobbs CL, et al: Aerial Spraying of 2,4,5-T and Human Birth Malformations: An Epidemiological Investigation. Science 1981;212: 349-351.

24. Townsend JC, Bodner KM, Van Peenan PFD, et al: Survey of Reproductive Events of Wives of Employees Exposed to Chlorinated Dioxins. Am J Epidemiol 1982;115,5:695-713.

25. Donovan JW, Adena MA, Rose G, et al: Case-Control Study of Congenital Anomalies and Vietnam Service (Birth Defects Study). Australian Government Publishing Service, Canberra, 1983.

26. Erickson JD, Mulinare J, McClain PW, et al: Vietnam Veterans' Risks for Fathering Babies with Birth Defects. JAMA 1984;252,7:903-912.

27. Bishop YMM, Feinberg SE, Holland PW: Discrete Multivariate Analysis: Theory and Practice. The MIT Press, Cambridge MA, 1975.

28. Pocchiari F, Silano V, Zampieri A: Human Health Effects From Accidental Release of Tetrachlorodibenzo-p-Dioxin (TCDD) at Seveso, Italy. Ann NY Acad Sci 1979;320:311-320.

29. Elovaara E, Savolainen H, Parkki MG, et al: Neurochemical Effects of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin in Wistar and Gunn Rats. Res Commun Chem Pathol and Pharmacol 1977;18,3:487-494.

30. Creso E, DeMarino V, Donatelli L, et al: Neuropsychopharmacological effects of TCDD. Boll Soc Intal Biol Sper 1978;54,17:1592-1596.

31. Bauer H, Schulz KH, Spiegelberg U: Berufliche Vergiftungen bei der Herstellung von Chlorphenol-Verbindungen [Occupational Intoxications in Manufacturing Chlorphenol Compounds]. Archiv Gewerbepath 1961;18:538-555.

32. Poland AP, Smith D: A Health Survey of Workers in a 2,4-D and 2,4,5-T Plant. Arch Environ Health 1971;22:316-327.

33. Weber G, Luzi P, Resi L, et al: Natural History of TCDD-Induced Liver Lesions in Rats as Observed by Transmission Electron Microscopy During a 32-Week Period after a Single Intraperitoneal Injection. J Toxicol Environ Health 1983;12:533-540.

34. King ME, Roesler AR: Subacute Intubation Study on Rats with the Compound 2,3,7,8-Tetrachlorodioxin. IIT Res Inst Rep to EPA No IITRI-L6073-12.

35. Gupta BN, Vos JG, Moore JA, et al: Pathologic Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Laboratory Animals. Environ Health Perspect 1973;5:125-140.

36. Bleiberg J, Wallen M, Brodkin R, et al: Industrially Acquired Porphyria. Arch Dermatol 1964;89:793-797.

37. Sweeney GD, Jones KG, Cole FM, et al: Iron Deficiency Prevents Liver Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin. Science 1979;204:332-335.

38. Sinclair PR, Granick S: Uroporphyrin Formation Induced by Chlorinated Hydrocarbons (Lindane, Polychlorinated Biphenyls, Tetrachlorodibenzo-p-Dioxin). Requirements for Endogenous Iron, Protein Synthesis and Drug-Metabolizing Activity. Biochem Biophys Res Commun 1974;61,1:124-133.

39. Smith AG, Francis JE, Kay SJE, et al: Hepatic Toxicity and Uroporphyrinogen Decarboxylase Activity Following a Single Dose of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin to Mice. Biochem Pharmacol 1981;30,20:2825-2830.

40. Manis J, Kim G: Stimulation of iron absorption by polychlorinated aromatic hydrocarbons. Am J Physiol 1979;236:E763-E768.

41. May G: Chloracne from the accidental production of tetrachlorodibenzo-dioxin. Br J Ind Med 1973;30:276-283.

42. Bovey RW, Young AL: The Science of 2,4,5-T and Associated Phenoxy Herbicides. John Wiley and Sons, NY, 1980.

43. Albro PW, Corbett JT, Harris M, et al: Effects of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin on Lipid Profiles in Tissue of the Fischer Rat. Chem Biol Interact 1978;23:315-330.

44. Schmittle SC, Edwards HM, Morris D: A Disorder of Chickens Probably Due to a Toxic Feed-Preliminary Report. J. Amer Vet Med Assoc 1958;132:216-219.

45. Cantrell JS, Webb NC, Mabis AJ: The Identification and Crystal Structure of a Hydropericardium-Producing Factor: 1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin. Acta Crystallogr 1969;B25:150-156.

46. Jirasek L, Kalensky J, Kubec K, et al: Chlorakne, Porphyria cutanea tarda und andere Intoxikationen durch Herbizide. [Chloracne, porphyria cutane tarda and other herbicide induced intoxications.] Hautarzt 1976;27,7:328-333.

47. Moses M, Lilis R, Crow KD, et al: Health Status of Workers with Past Exposure to 2,3,7,8-Tetrachlorodibenzo-p-dioxin in the Manufacture of 2,4,5-Trichlorophenoxyacetic Acid: Comparison of Findings With and Without Chloracne. Am J Ind Med 1984;5:161-182.

48. Zack JA, Suskind RR: The Mortality Experience of Workers Exposed to Tetrachlorodibenzodioxin in a Trichlorophenol Process Accident. J Occup Med 1980;22,1:11-14.

49. Clark DA, Gauldie J, Szewczuk MR, et al: Enhanced Suppressor Cell Activity as a Mechanism of Immunosuppression by 2,3,7,8-Tetrachlorodibenzo-p-dioxin. Proc Soc Exp Biol Med 1981;168:290-299.

50. Montovani A, Vecchi A, Luini W, et al: Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on macrophage and natural killer cell-mediated cytotoxicity in mice. Biomedicine 1980;32,4:200-204.

51. Van Logten MJ, Gupta BN, McConnell EE, et al: Role of the Endocrine System in the Action of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) on the Thymus. Toxicology 1980;15:135-144.

52. Clark DA, Sweeney G, Safe S, et al: Cellular and genetic basis for suppression of cytotoxic T cell generation by haloaromatic hydrocarbons. Immunopharmacology 1983;6,2:143-153.

53. Nienstedt W, Parkki M, Uotila P, et al: Effect of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin on the Hepatic Metabolism of Testosterone in the Rat. Toxicology 1979;13:233-236.

54. Hook GER, Orton TC, Moore JA, et al: 2,3,7,8-Tetrachlorodibenzo-p-Dioxin-Induced Changes in the Hydroxylation of Biphenyl by Rat Liver Microsomes. Biochem Pharmacol 1975;24:335-340.

55. Bastomsky CH: Enhanced Thyroxine Metabolism and High Uptake Goiters in Rats After a Single Dose of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin. Endocrinology 1977;101:292-296.

56. Potter CL, Sipes IG, Russel DH: Hypothyroxinemia and Hypothermia in Rats in Response to 2,3,7,8-Tetrachlorodibenzo-p-dioxin Administration. Toxicol Appl Pharmacol 1983;69:89-95.

57. Rozman K, Rozman T, Greim H: Effect of Thyroidectomy and Thyroxine on 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Induced Toxicity. Toxicol Appl Pharmacol 1984;72:372-376.

58. Fett MJ, Dunn M, Adena MA, et al: Australian Veterans Health Studies (The Mortality Report), Part I. Australian Government Publishing Service, Canberra, 1984.