Air Force Health Study

An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides

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Introduction, Background and Conclusions
(Chapters 1-5, 18, 19)

SERUM DIOXIN ANALYSIS OF
1987 EXAMINATION RESULTS

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) This report presents the results of the serum dioxin analysis of the Air Force Health Study 1987 examination cycle. The purpose of the study is to determine whether long-term health effects are associated with serum dioxin levels for the participants in the study. For each health effect variable, analyses were performed to evaluate the relationships with initial serum dioxin (extrapolated from a first-order pharmacokinetics model); current serum dioxin and time since tour; and categorized current dioxin levels (providing contrasts of Ranch Hands with specified current dioxin levels versus Comparisons with background levels). Significant associations between serum dioxin and several lipid-related health indices were found in these analyses. Specifically, significant associations with dioxin were found for diabetes, percent body fat, cholesterol, HDL, and cholesterol-HDL ratio. Other variables, such as the spirometric indices in the pulmonary assessment and benign systemic neoplasms (over 70% were lipomas) in the malignancy assessment, showed significant associations that may be related to the body fat results. These findings and their possible relationship to dioxin elimination will be explored in future examination cycles. Other health variables revealed no patterns within or across clinical assessments that were indicative of a health detriment due to dioxin.

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AIR FORCE HEALTH STUDY

An Epidemiologic Investigation of
Health Effects in Air Force Personnel
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SERUM DIOXIN ANALYSIS OF
1987 EXAMINATION RESULTS

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NOTICE

This report presents excerpts of analyses comparing the serum dioxin assays with physical examination data collected in 1987. It is intended to serve as an extended summary of the study's background, the serum dioxin assay, the findings and conclusions. If additional detail is required, the reader may refer to specific discussions in each clinical area (Chapters 6 - 17) found in the complete report.

EXECUTIVE SUMMARY

SERUM DIOXIN ANALYSIS OF THE 1987 AIR FORCE HEALTH STUDY EXAMINATIONS

This publication is the fourth morbidity report resulting from the Air Force Health Study (AFHS), an epidemiologic investigation of the possible association between occupational exposure to Herbicide Orange (and its dioxin contaminant) and adverse health experienced by Air Force personnel who served in Operation Ranch Hand units in Vietnam from 1962 to 1971. A Comparison group was formed from Air Force veterans who flew or maintained C-130 aircraft in Southeast Asia during the same time period. The 1982 Baseline examination, summarized in the first report, was followed by additional studies in 1985 and 1987. Additional evaluations are planned for 1992, 1997, and 2002.

The 19 chapters of this report present conclusions drawn from statistical analyses of approximately 300 health-related endpoints in 12 clinical areas: general health, malignancy, neurology, psychology, gastrointestinal, dermatology, cardiovascular, hematology, renal, endocrine, immunology, and pulmonary. The analyses focused on dioxin measurements in serum collected from 1,670 participants as part of the 1987 examination.

This report summarizes the first large-scale study of dose-response effects based on an accurate measurement of current dioxin levels. This investigation is an important enhancement of the AFHS and supplements previous AFHS reports, which focused on group contrasts between exposed (Ranch Hand) and unexposed (Comparison) cohorts.

Three statistical models were used to evaluate associations between the health of study participants and their serum dioxin levels:

- Model 1: Estimated initial dioxin levels, using Ranch Hand participants only
- Model 2: Current serum dioxin levels and time since military service in Vietnam, using Ranch Hand participants only
- Model 3: Categories of current dioxin levels, using both Ranch Hand and Comparison participants.

Analyses based on model 1 depend directly on first-order kinetics and a constant dioxin decay rate, while those based on model 2 assume nothing about dioxin elimination other than that Ranch Hands were exposed in Vietnam and that their body burdens have decreased in an unspecified manner over time. All health data were analyzed using both of these models to reduce the likelihood that an effect would be missed because of incorrect assumptions regarding dioxin elimination. Models 1 and 2 were implemented under two assumptions—minimal and maximal. The minimal assumption included only Ranch Hands with current dioxin levels above 10 parts per trillion (ppt) (n=521); the maximal assumption expanded the analysis to include all Ranch Hands with current dioxin levels above 5 ppt (n=742).

In addition, model 3, using both Ranch Hands and Comparisons, assessed the health consequences of current dioxin levels above background. This assessment required no assumptions about when or how increased dioxin body burdens were attained.

Statistical analyses were often applied to clinical endpoints in continuous (i.e., original measurement) and discrete (i.e., measurements grouped into categories based on abnormal levels) forms. Analyses were also performed to account for the effects that demographic and personal characteristics may have on the clinical measurements. Such analyses are termed "adjusted analyses."

The general health assessment found that higher levels of body fat and the erythrocyte sedimentation rate were significantly related to both the initial and current serum levels of dioxin. The findings for body fat are consistent with the association between dioxin and diabetes mellitus in the endocrine assessment and lipids in the gastrointestinal assessment. The sedimentation rate findings raise the possibility that a subtle, chronic inflammatory response may be related to higher levels of dioxin exposure.

The malignancy assessment determined that serum dioxin levels were not significantly associated with the incidence of skin neoplasms, except for an increase of basal cell carcinoma on sites other than the ear, face, head, or neck in Ranch Hand enlisted flyers. However, these results may be the result of a multiple-testing artifact, because they were not noted for the enlisted groundcrew who, as a group, had higher levels of serum dioxin than the enlisted flyers. Previous AFHS reports showed that the Ranch Hand group had a significantly increased risk of basal cell carcinoma relative to the Comparison group; however, the skin neoplasm findings in this report did not support a positive dose-response relationship. The serum dioxin analyses detected significantly increased risks of benign, but not malignant, systemic neoplasms (approximately 75% of the benign neoplasms in Ranch Hands and 70% in Comparisons were lipomas). There was one verified case of non-Hodgkin's lymphoma in a Ranch Hand at the 1987 examination.

The neurological analyses revealed no consistent evidence to indicate that dioxin was associated with neurological disease. The adjusted analyses for the verified neurological disorders were not significant. Dioxin was found to be significantly associated with coordination and a central nervous system index, but cranial nerve function and peripheral nerve status were not associated with dioxin.

Higher serum dioxin levels were unrelated to verified psychological and reported sleep disorders. Results of the two clinical psychological tests (the Symptom Check List-90-Revised [SCL-90-R] and the Millon Clinical Multiaxial Inventory [MCMI]) were inconsistent. Most of the adjusted results for the SCL-90-R variables were not significant. Many of the adjusted MCMI results were significant, but substantial overlap and correlation between test scales of the MCMI limit the clinical importance of these statistical differences.

The serum dioxin levels showed no association with verified liver diseases. However, the laboratory results showed a consistent pattern suggestive of a subclinical effect on lipid metabolism, possibly related to the positive association between dioxin and body fat observed in the general health assessment.

Dermatologic endpoints were not consistently associated with dioxin concentrations. For Ranch Hands with a later tour of duty in Vietnam (time since tour≤18.6 years), there were significant or marginally significant positive associations between current levels of dioxin and post-Southeast Asia acne and several of the other acne-related physical examination variables. However, the corresponding adjusted relative risks for Ranch Hands with an early tour (time since tour>18.6 years) were not significant or were significantly less than 1.

The cardiovascular findings offered no consistent evidence of an adverse dioxin effect among nondiabetics. There was a significantly increased risk of essential hypertension for Ranch Hands in the high current dioxin category (>33.3 ppt) relative to Comparisons in the background category (≤10 ppt) when the effect of body fat was not considered. By contrast, the analyses of verified heart disease (excluding essential hypertension) found that the adjusted relative risk was significantly less than 1 for Ranch Hands in the high current dioxin category. The analyses of systolic blood pressure and diastolic blood pressure in their continuous forms found that the adjusted mean level for both variables was significantly higher for Ranch Hands in the high current dioxin category relative to Comparisons in the background category when the effect of body fat was not considered. However, the corresponding analyses of the percentage of participants with abnormally high systolic or diastolic blood pressures did not show an association with dioxin. The assessment of peripheral vascular function found significant associations between dioxin and decreases in the peripheral pulses.

The hematologic results revealed no evidence that overt hematopoietic toxicity was related to dioxin exposure. The white blood cell count revealed statistically significant associations consistent with a positive dose-response effect in all three models; consistently significant results were not found for the other variables. A significant increased risk of an elevated platelet count was found for Ranch Hands in the high current dioxin category relative to the Comparisons in the background category. These findings suggest the presence of a low-level, chronic inflammatory response related to higher levels of dioxin exposure.

The analyses did not indicate any relationship between renal health and dioxin. Under the maximal assumption (but not the minimal), the initial dioxin analyses found a significantly increased risk of urinary occult blood cells, but results were not significant for the other models. Statistically significant results were not noted for the other variables.

The endocrine assessment established a strong positive association between glucose intolerance and dioxin, but concluding that dioxin directly causes diabetes would be premature. The initial and current levels of serum dioxin both were associated significantly with an increased incidence of diabetes. Significant positive associations also were noted for the analyses of fasting glucose and 2-hour postprandial glucose. These findings may be related to the association between dioxin and body fat observed in the general health assessment. The basis of these relationships will be investigated during subsequent phases of this study.

Assessment of testicular size as evaluated at the physical examination revealed significant positive associations in all three models between serum dioxin and decreased size. The serum dioxin analyses did not reveal a significant association with abnormally low

levels of serum testosterone, but the analyses found a significant negative correlation with testosterone when the effect of body fat was not considered. The clinical meaning of these findings is unclear. The results for thyroid stimulating hormone and T_3 % uptake treated as continuous variables were consistent with subclinical decreases in thyroid function related to dioxin exposure. However, the corresponding analyses on the percentage of participants with abnormally high levels for these variables did not show an association with dioxin.

The immunologic assessment did not find any clinically significant alterations related to the current or initial levels of serum dioxin. An evaluation of immunoglobulins found a significant association between initial dioxin level and increased IgA levels, consistent with a subtle inflammatory response. The analyses of the other immunoglobulins (IgG and IgM) did not indicate the presence of any dioxin-related effects. Analyses for the other laboratory variables revealed several statistically significant findings, but they either were internally inconsistent or were not in a direction expected in an impaired immune system. Serum dioxin was not significantly associated with delayed hypersensitivity skin-test response. The previous report of the 1987 examination data had showed that significantly more Ranch Hands had possibly abnormal skin-test reactions than Comparisons. These new analyses suggest that the previously noted group difference may not be related to dioxin.

Analyses of the pulmonary disease history found no evidence of a dioxin relationship for the five respiratory illnesses studied. However, based on physical examination results, the risk of thorax and lung abnormalities for Ranch Hands in the high current dioxin category was significantly increased relative to Comparisons in the background category. Abnormal spirometric measurements were often significantly associated with dioxin levels, but the differences in the mean levels between high- and low-exposed participants were not clinically important. These findings may be related to the association between dioxin and body fat noted in the general health assessment because obesity is known to cause a reduction in vital capacity. These relationships will be investigated during subsequent phases of the study.

Extrapolation of the serum dioxin results to the general population of ground troops who served in Vietnam is difficult because Ranch Hand and ground troop exposure situations were quite different. Based on serum dioxin testing results done by others, nearly all ground troops tested currently have levels of dioxin similar to background levels. Even the ground troops who served in herbicide-sprayed areas of Vietnam had current levels indistinguishable from those of men who never left the United States. The AFHS subgroup most like the ground troops in terms of current dioxin levels is those Ranch Hands who currently have background levels of dioxin (designated as the "unknown" category in the model 3 analyses). Therefore, if the results of the AFHS are applied to the general population of Vietnam veterans, the focus should be on the unknown Ranch Hand versus background Comparison contrasts. However, extrapolating the results of these analyses to Vietnam veterans should still be made cautiously. In general, the adjusted model 3 analyses found that Ranch Hands in the unknown category did not show a significant health detriment relative to Comparisons in the background category.

Small but significant mean differences in a continuously measured health variable when there are no corresponding differences in the percentage of abnormal tests are difficult to assess in any study. For example, in the discrete analysis of serum testosterone, abnormally

low levels were not significantly associated with dioxin. However, the adjusted continuous analysis found a significant negative association between dioxin and testosterone when the effect of body fat was not considered. The continuous and discrete analyses of systolic and diastolic blood pressure also exhibited conflicting results. Observations such as these could represent an early subclinical effect, or they could be the result of a multiple testing artifact. Significant trends in the mean with increasing levels of dioxin are interpreted as a dioxin-related effect if a corresponding trend is seen in the proportion above or below the normal range. These observations emphasize the importance of continued evaluation of a broad spectrum of health endpoints in the subsequent physical examination phases of the AFHS.

The serum dioxin analyses in this report detected significant associations with lipid-related health indices. In particular, diabetes and body fat were associated positively with dioxin. Cholesterol, high-density lipoprotein (HDL), cholesterol-HDL ratio, and 2-hour postprandial glucose also were associated significantly with dioxin. Erythrocyte sedimentation rate, white blood cell count, platelet count, and IgA were positively associated with dioxin, suggesting the presence of a chronic dose-related inflammatory response. Other variables, such as the spirometric indices in the pulmonary assessment and benign systemic neoplasms in the malignancy assessment showed significant associations with dioxin that may be related to body fat (approximately 75% of the benign neoplasms in Ranch Hands and 70% in Comparisons were lipomas). These findings and their possible relationship to dioxin elimination will be explored in future examination cycles. The serum dioxin analyses also revealed a significant positive association between dioxin and decreased testicular size, but the importance of this finding is unclear (fertility and other reproductive outcomes will be assessed in a separate report). Results for other variables revealed no consistent pattern, within or across clinical areas, indicative of a health detriment due to dioxin exposure.

In summary, many of the findings in this report reveal a consistent relationship between dioxin and body fat. Two hypotheses may explain the observed relationships. In one, dioxin could cause an increase in body fat, or the level of body fat could influence the dioxin decay rate, which in turn alters physiologic outcomes, such as blood pressure, serum lipid alterations, and blood sugar levels. An alternative hypothesis involves dioxin as a direct cause of two or more of the observed endpoints, including body fat. Whether dioxin causes these observed effects directly or is a step in an extended causal pathway cannot be determined from these data. Additional analyses following the physical examination scheduled for 1992 may help resolve this question.

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TABLE OF CONTENTS

INTRODUCTION, BACKGROUND AND CONCLUSIONS
(Chapters 1-5, 18, 19)

PAGE

EXECUTIVE SUMMARY v

ACKNOWLEDGMENTS xi

TABLE OF CONTENTS xv

1. INTRODUCTION 1-1

AIR FORCE HEALTH STUDY 1-1

Questionnaire Methodology 1-2
Physical Examination Methodology 1-2
Quality Control 1-3
Statistical Models 1-5
Organization of the Report 1-6

INTERPRETIVE CONSIDERATIONS 1-7

Bias 1-7
Adjustments for Covariates and Interactions 1-8
Consistency 1-9
Multiple Testing 1-9
Trends 1-10
Power Limitations 1-10
Strength of Association 1-10
Biological Credibility 1-10
Interpretation of Negative Results 1-11
Interpretation of the Coefficient of Determination 1-11
Clinic Interpretation of Discrete versus Continuous Data 1-11
Minimal versus Maximal Results 1-11
Graphics 1-12
The Checkmark Pattern 1-12
Extrapolation to Army Ground Troops 1-12
Summary of Results 1-13

CONCLUSION 1-13

REFERENCES 1-14

TABLE OF CONTENTS (Continued)

PAGE

2. DIOXIN ASSAY 2-1

SAMPLE ACQUISITION 2-1
ANALYTICAL METHOD 2-1
QUALITY CONTROL 2-1
DATA DELIVERED TO THE AIR FORCE BY THE CENTERS
FOR DISEASE CONTROL 2-2

REFERENCES 2-6

3. THE RELATIONSHIP BETWEEN THE EXPOSURE INDEX AND
DIOXIN BODY BURDENS IN RANCH HANDS 3-1

INTRODUCTION 3-1

Exposure Index Definition 3-1
The Dioxin Assay 3-3
The Exposure Index versus the Dioxin Assay 3-3

SUMMARY 3-15
REFERENCES 3-18

4. STATISTICAL METHODS 4-1

MODELS AND ASSUMPTIONS 4-1

Prior Knowledge Regarding Dioxin 4-1
Fundamental Limitations of the Serum Dioxin Data 4-2
Health versus Dioxin in Ranch Hands 4-2
Health versus Dioxin in Ranch Hands and Comparisons 4-6
Data Error 4-9
Bias Calculations 4-9
The Correlation Between Initial Dioxin and Current Dioxin 4-12

FACTORS DETERMINING ANALYTICAL METHOD 4-12
ANALYSIS METHODOLOGIES 4-14
MODELING STRATEGY 4-15
POWER 4-21
EXPLANATION OF TABLES 4-25

Continuous Variables 4-25
Discrete Variables 4-29

TABLE OF CONTENTS (Continued)

PAGE

GRAPHICS 4-33

Data Plots/Histograms 4-33
Interaction Plots/Histograms 4-36
Statistical Analysis Protocol 4-36

REFERENCES 4-37

5. COVARIATE ASSOCIATIONS 5-1

INTRODUCTION 5-1
MATCHING VARIABLES (AGE, RACE, AND OCCUPATION) 5-1
DRINKING HABITS 5-14
SMOKING HABITS 5-16
SUN EXPOSURE CHARACTERISTICS 5-16
EXPOSURE TO CARCINOGENS 5-18
PERSONAL AND FAMILY HEALTH 5-21
OTHER CHARACTERISTICS 5-23
SUMMARY 5-24
CONCLUSION 5-25

REFERENCES 5-26

18. CONCLUSIONS 18-1

INTRODUCTION 18-1

Statistical Models 18-1

RESULTS 18-2

General Health Assessment 18-2
Malignancy Assessment 18-2
Neurological Assessment 18-4
Psychological Assessment 18-5
Gastrointestinal Assessment 18-6
Dermatologic Assessment 18-6
Cardiovascular Assessment 18-7
Hematologic Assessment 18-8
Renal Assessment 18-9
Endocrine Assessment 18-9
Immunologic Assessment 18-10
Pulmonary Assessment 18-10
Extrapolation of Results 18-11

SUMMARY 18-12

19. FUTURE DIRECTIONS 19-1

TABLE OF CONTENTS - REPORT

VOLUME I

EXECUTIVE SUMMARY
ACKNOWLEDGMENTS
CHAPTER 1 - Introduction
CHAPTER 2 - Dioxin Assay
CHAPTER 3 - The Relationship Between the Exposure Index and Dioxin Body Burdens in Ranch Hands
CHAPTER 4 - Statistical Methods Models and Assumptions
CHAPTER 5 - Covariate Associations
CHAPTER 6 - General Health Assessment

VOLUME II

CHAPTER 7 - Malignancy Assessment

VOLUME III

CHAPTER 8 - Neurological Assessment
CHAPTER 9 - Psychological Assessment

VOLUME IV

CHAPTER 10 - Gastrointestinal Assessment
CHAPTER 11 - Dermatologic Assessment

VOLUME V

CHAPTER 12 - Cardiovascular Assessment
CHAPTER 13 - Hematologic Assessment

VOLUME VI

CHAPTER 14 - Renal Assessment
CHAPTER 15 - Endocrine Assessment
CHAPTER 16 - Immunologic Assessment

VOLUME VII

CHAPTER 17 - Pulmonary Assessment
CHAPTER 18 - Conclusions
CHAPTER 19 - Future Directions

VOLUME VIII

APPENDIX A through J

VOLUME IX

APPENDIX K through R

CHAPTER 1

INTRODUCTION

AIR FORCE HEALTH STUDY

The Air Force Health Study (AFHS) is an epidemiologic investigation to determine whether occupational exposure to Herbicide Orange in a group of U.S. Air Force personnel is associated with adverse health effects. During the Vietnam conflict, Herbicide Orange was the primary herbicide used in a military operation, code-named Operation Ranch Hand, which disseminated the herbicide through aerial spraying for purposes of defoliation and crop destruction.

As documented in prespecified analytical plans and predecessor reports, the AFHS is based on a cohort design in a nonconcurrent prospective setting. The study design consisted of a baseline morbidity assessment that is to be complemented by five followup morbidity evaluations over a 20-year period. The baseline morbidity evaluation, conducted in 1982, was performed by the Air Force. Followup evaluations were conducted in 1985 and 1987. The 1985 and 1987 evaluations (also known as the third- and fifth-year studies, respectively) were performed, under contract to the Air Force, by Science Applications International Corporation (SAIC), in conjunction with Scripps Clinic and Research Foundation (SCRF) and the National Opinion Research Center (NORC). Future evaluations are planned for 1992, 1997, and 2002 (i.e., the 10-year, 15-year, and 20-year followup studies, respectively).

For the Baseline and the 1985 and 1987 studies, the major focus of the analyses was to compare the health status of the Ranch Hands (i.e., the exposed cohort) with that of the Comparisons (i.e., the unexposed cohort). An ancillary analysis used an approximate estimate of exposure (low, medium, and high) that was constructed for each Ranch Hand using historical military record information with herbicide procurement and usage records. For the most part, the constructed exposure index failed to display consistent and/or meaningful dose-response relationships.

During the conduct of the 1987 physical examination, the Air Force initiated a collaborative study with the Centers for Disease Control (CDC) to measure dioxin levels in the serum of Ranch Hands and Comparisons. The purpose of this report is to perform a thorough statistical evaluation to assess dose-response relationships between various measures of dioxin and approximately 300 health-related endpoints in 12 clinical areas. The statistical analyses associated with the serum data will evaluate the association between a specified health endpoint and dioxin among the Ranch Hands, as well as contrast the health of various categories of Ranch Hands having differing serum dioxin levels with the health of Comparisons having background levels of dioxin in their blood. The analysis of dose-response relationships based on serum assays provides an important enhancement over the previous AFHS investigations. This research is the first large-scale study of dose-response effects based on an accurate measurement of current dioxin. The results of this study supplement the findings of previous AFHS reports, which have focused on group contrasts between exposed and unexposed cohorts, rather than on the dose-response relationships in this report.

Of the 995 Ranch Hands who were fully compliant to the 1987 physical examination, 932 had serum specimens analyzed by CDC; 64 of these 932 specimens were reported by CDC as not quantifiable by the analytical method. Two of the 932 participants provided blood but were not part of the 1987 examination. The Ranch Hand participants used for the statistical analyses of the serum data excluded the 66 Ranch Hands specified above. Thus, the serum levels of the remaining 866 Ranch Hands were candidates for evaluating the association between health status and level of dioxin. Current dioxin levels exceeded 5 ppt for 742 of the Ranch Hands, and exceeded 10 ppt for 521 Ranch Hands. These two Ranch Hand groups are the maximal and minimal cohorts, described later in this chapter.

Of the 1,299 Comparisons who completed the 1987 physical examination, 1,198 had serum specimens analyzed by CDC. Dioxin assay information on a randomly selected subset of 888 Comparisons was received from CDC by January 1990, at which time statistical analyses involving Comparison data began. Eighty-three of the 887 Comparisons who completed the physical examination had a current dioxin level reported by CDC as not quantifiable. Therefore, 804 Comparisons were candidates for use in the statistical analyses.

An additional 314 Comparison dioxin assay results were subsequently received. Of these results, 311 were based on Comparisons who had completed the physical examination, and 3 were reanalyses of specimens of 3 Comparisons who completed the examination but whose dioxin result was indeterminant.

Chapter 2, Dioxin Assay, contains a more complete discussion of the dioxin assay, the 888 and the subsequently received 314 Comparison assay results.

Questionnaire Methodology

One source of information used in the statistical analyses for the AFHS was the participant questionnaire. For the 1982 Baseline study, the questionnaire was administered at the participant's home. The questionnaires of the 1985 and 1987 followup cycles were administered at the physical examination site. New participants or participants who refused to take part in the 1982 and 1985 examinations had the option of responding to the Baseline questionnaire either at their residence or at the physical examination site. The instruments provided baseline or updated information on such items as: demographic characteristics, education, occupation, medical history, study compliance, toxic exposures, reproductive experience, personality type, sleep disorders, and risk factors for skin cancer. For a detailed discussion of the development, expansion, and implementation of the questionnaire (i.e., interviewer training, scheduling of participants, data collection, and data processing), the reader is referred to Chapter 3, Questionnaire Methodology, AFHS 1987 examination (1).

Physical Examination Methodology

Another major source of information for the analyses in the AFHS resulted from the various health evaluations performed at SCRF in 1987. The evaluations consisted of the following major elements:

- Review-of-systems questionnaire
- Psychological testing

- Physical examination
- Laboratory testing
- Specialized testing (e.g., phlebotomy for measurement of serum dioxin)
- Psychological and medical outbriefings.

The logistical efforts involved in contacting, transporting, and examining the study participants for the 1987 phase of the AFHS are described in Chapter 4, Physical Examination Methodology, of the AFHS 1987 examination report (1).

During the clinical examinations, data were collected in the laboratory and by a general and two subspecialty (dermatological and neurological) examinations. In the clinical laboratory, cutpoints between normal and abnormal measurements are in most cases well defined. In the physical examinations that were conducted by multiple examiners, however, some subjective variation in data collection would be anticipated. By adhering to a strict examination protocol and by blinding the examiners to the exposure status of all participants, a group bias was avoided.

The format of the physical examination was designed to address the wide range of body organ systems suggested by the scientific literature on both human and animal studies, the spectrum of health problems reported by Vietnam Veterans listed in the Agent Orange Repository of the Department of Veterans Affairs, and concerns expressed in the press. The examiners were kept strictly unaware of the exposure status of each participant and were required to conduct their examinations in a standardized and consistent manner. Each participant was provided with all of his examination results by a specialist in internal medicine and a clinical psychologist. Whenever a condition requiring prompt medical followup or further evaluation was identified by one of these debriefers, arrangements and appointments were made with a referral physician before the participant departed from the clinic. In this manner, continuing treatment of important medical conditions was not overlooked.

Quality Control

Throughout the 1987 examination, a number of steps were taken to maintain stringent quality control (QC) and quality review standards. In general, quality assurance (QA) activities were defined and implemented in the areas of administrative QA; questionnaire, physical, and psychological examination QC; laboratory QC measures; data management QC; and statistical QC. Chapter 6, Quality Control, of the AFHS report on the 1987 examination contains detailed descriptions of these quality control efforts (1).

Administrative Quality Control

For the 1985 and 1987 examinations, and the associated serum dioxin analyses presented in this report, an internal Quality Review Committee (QRC) was convened by the prime contractor. QRC members provided independent reviews and comments on draft report materials submitted to the Air Force. The QRC also provided advice on issues that might affect study quality.

Questionnaire, Physical, and Psychological Quality Control

For administration of the 1987 questionnaires, interviewers were provided specific training and detailed instructions by NORC on conducting the interviews. In addition, schedulers were trained to perform initial contacts with individuals to invite them to participate in the 1987 examination cycle. Conversion specialists were used to contact refusals or to identify replacements for unwilling Comparisons. Site supervisors monitored a sample of interviews from each interviewer. If necessary, immediate onsite retraining was provided for interviewers to ensure proper administration of the questionnaire. A rigorous review process for monitoring the completeness and quality of responses to the questionnaire items was followed.

After the questionnaires were reviewed for completeness and data validity, the questionnaire and physical examination records were provided to the Air Force for medical coding of the reported information. Once the medical coding was completed, the questionnaire information was provided to NORC for data processing. Various edit and data verification procedures were performed and discrepancies were resolved on a case-by-case basis. All corrections were documented and entered into the data base. QA reports were generated monthly and the review process was continued until no errors or discrepancies were found.

The physical examination provided most of the health status information used for clinical and statistical evaluation. Hence, a number of steps were taken to guarantee the quality and completeness of the information generated during the physical examination. The steps included a stringent selection process for all personnel directly involved with the study participants; a complete pretest of the physical examination, interview, psychological test, and laboratory test procedures before the start of the study; refresher training for diagnostic procedures (e.g., to diagnose chloracne); weekly review of participant critique forms; timely review, and revision if necessary, of items reported on the physical examination forms; and daily monitoring of clinical examination activities by the onsite Air Force monitor and the SCRF Medical Project Director.

Clinical Laboratory and Immunology Laboratory Quality Control

Multiple actions were implemented in the area of QC for the clinical laboratory. An integrated medical laboratory management information system was used to provide direct device to data base interfaces for automated testing equipment; stringent calibration standards were maintained for all automated equipment; control samples were used to monitor test quality; formal analysis and review of QC data was performed on a weekly basis; and CUSUM and FIR CUSUM techniques were used to detect calibration problems. A stringent QC procedure was also implemented in the cellular immunology component of the AFHS to address problems in assay performance, reagent validity, data analysis, and results reporting. Chapter 6 of the 1987 examination report provides an indepth discussion of the clinical and immunologic QC procedures (1).

Data Management Quality Control

The QC program for the data management activity consisted of multiple checks at all steps of the examination, data collection, and data processing cycle. Data QC procedures for data collection, conversion, and integration were developed before the clinical examinations

began. Pretesting of forms, procedures, and logistical arrangements was conducted 3 weeks before the examinations actually began.

Five interwoven layers of QC were instituted to ensure data integrity: data processing system design; design and administration of all exams or questionnaires; data completeness checks; data validation techniques; and quality control medical records coding.

Statistical Analysis Quality Control

QC was exercised in the following areas addressing the statistical analysis: construction of data bases for the statistical analysis of each clinical chapter, the statistical analysis, and the preparation of the clinical chapters containing the results of the statistical analyses. Each clinical area data base was examined for extreme and improbable values. Discrepancies were resolved through contact with the organization responsible for the data item of interest (e.g., SCRF or NORC). Technical issues related to statistical analysis were discussed, and resolved through frequent telephone and/or written communications between the SAIC statisticians and the Air Force principal investigators. The content of the report was verified for accuracy and validity among the reported text and tables, and for consistency with the output results generated by the statistical software.

Statistical Models

The serum dioxin measurements were used in three different ways to assess the relationships between current health status and dioxin. Within a specified clinical area, the results of three analyses performed for each dependent variable were described under sections titled:

- Model 1: Ranch Hands - Log_2 (Initial Dioxin)
- Model 2: Ranch Hands - Log_2 (Current Dioxin) and Time
- Model 3: Ranch Hands and Comparisons by Current Dioxin Category.

Models 1 and 2 used serum dioxin values for only the Ranch Hands. For model 1, the dependent variable for each Ranch Hand was regressed on an initial dioxin level. The initial dioxin value was estimated retrospectively from a first-order pharmacokinetic half-life model using the measured current dioxin, the estimated half-life of 7.1 years (2) and time since the end of each Ranch Hand's tour of duty in Vietnam. For model 2, regression relationships were developed between the dependent variable for each Ranch Hand and the measured current dioxin level and time since the end of the tour in Vietnam. The latter model was implemented as an alternative to model 1 which was based on assuming a particular half-life model. Both of these models were implemented with and without adjustment for covariate information. While the overall analysis in model 2 specifically assesses the effect of differences between time strata, a current dioxin effect can be seen in the time stratified portions of the analyses as well.

Models 1 and 2 were also applied under two assumptions concerning exposure: the minimal assumption and the maximal assumption. Under the minimal assumption, the analyses are based on those Ranch Hands with current dioxin levels above 10 ppt. The basis

for the minimal assumption is that Ranch Hands currently having dioxin levels at or below 10 ppt are assumed not to have been exposed to dioxin during their Ranch Hand tour. Under the maximal assumption, the analyses are based on Ranch Hands with current dioxin levels above 5 ppt. The maximal assumption presumes that Ranch Hands with levels between 5 ppt and 10 ppt were only exposed to such an extent that their body burden of dioxin has just recently decayed to levels equivalent to normal background. Ranch Hands with current dioxin levels at or below 5 ppt were excluded from the analyses because of concerns raised by the CDC regarding the validity of the half-life model to extrapolate initial dioxin levels using such low dioxin levels. The minimal assumption is an attempt to focus the analyses on Ranch Hands who are more likely to have been exposed during their tour. The maximal assumption focuses on those participants known to be part of Operation Ranch Hand but the analyses may include some participants who possibly may not have been exposed to dioxin during their tours. Each assumption defines the size of the Ranch Hand groups being analyzed. The use of the terms "minimal" and "maximal" should not be interpreted as identifying those participants with a particular level or magnitude of dioxin exposure.

The analyses identified under model 3 compare the health of Ranch Hands with current dioxin values categorized as unknown (current dioxin at or below 10 ppt), low (current dioxin above 15 ppt but not above 33.3 ppt), and high (current dioxin above 33.3 ppt) with Comparisons having background levels (current dioxin at or below 10 ppt). "Unknown" is used as a description for Ranch Hands with current serum dioxin levels at background. Ranch Hands with current dioxin levels at or below 10 ppt were placed in a separate category (i.e., unknown) because the exposure resulting from their Vietnam tour could not be differentiated from background levels. Separating the unknown and low exposure categories by 5 ppt reduces concerns about the assignment of a Ranch Hand to either of the categories when the current level is very near a defined cutpoint. To remove any doubt about possible exposure in the Comparison group, any Comparisons having a current dioxin level above 10 ppt were excluded. Eighteen Comparisons had a current dioxin level above 10 ppt. Chapter 3 graphically displays distributions of serum levels for Ranch Hands and Comparisons.

Organization of the Report

This report is organized as follows:

- Chapter 1 (Introduction) provides summary background information on AFHS and the serum dioxin analysis; and discusses specific technical items/issues that may affect the results of the different clinical area assessments.
- Chapter 2 (Dioxin Assay) describes the blood draw procedure used to determine the serum dioxin measurements; the analytical method used to determine the dioxin level from the serum; and QC procedures associated with the serum dioxin data.
- Chapter 3 (Relationship of Estimates of Dioxin and Exposure Index) provides a comparison of the constructed exposure index used in previous reports to the estimates of dioxin body burden used in this report.
- Chapter 4 (Statistical Methods) documents the statistical methods used in the individual clinical area assessments; and the statistical procedures and results of the half-life analyses performed by the Air Force.

- Chapter 5 (Covariate Associations) examines the associations between dioxin and the individual covariates used in the different clinical assessments.
- Chapters 6 through 17 present the results and medical discussion for each clinical area from the statistical analyses of the dependent variables using the three models described earlier in this chapter. Each chapter contains a brief overview of pertinent scientific literature. More detailed summaries can be found in the report of the 1987 examination (1).
- Chapter 18 (Conclusions) summarizes the findings and medical discussion of the statistical analyses performed for each of the 12 clinical areas.
- Chapter 19 (Future Directions) summarizes the anticipated future activities, and possible modifications to the existing instruments and methodologies used to investigate the association between health status and dioxin exposure.

INTERPRETIVE CONSIDERATIONS

When interpreting the data presented in this report, careful consideration must be given to bias, interactions, consistency, multiple testing, dose-response patterns, trends, power limitations, strength of association, and biological credibility. Problems in evaluating negative results, extrapolating to other populations, and summarizing results also should be considered.

Bias

With the introduction of the dioxin assay as the measure of exposure, important sources of bias are reduced to violations of the underlying assumptions of the three models upon which all analyses in this report are based. Closely associated with violation of assumptions is the possibility that an important covariate may have been overlooked.

Biased results will be produced if the assumptions underlying any of the three statistical models are violated. Of the three models, model 1 (see Chapter 4, Statistical Methods) is the most vulnerable to this kind of bias, since it depends directly on two unvalidated assumptions: (a) that dioxin elimination is by first-order pharmacokinetics and (b) that all Ranch Hands have the same dioxin half-life (7.1 years). If dioxin elimination is first-order, but some Ranch Hands have a shorter half-life than others (as suggested by unpublished analysis of paired dioxin measurements on 36 Ranch Hands, see Chapter 4, pages 4-9 through 4-12), then there would have been misclassification of initial dioxin exposure. If the clinical endpoint is not associated with a factor (e.g., relative weight change) that affects the elimination rate, then estimates of the odds ratio for common diseases associated with low and high levels of initial dioxin will, in general, be biased toward unity. However, if the clinical endpoint is associated with a factor that affects the elimination rate, then the odds ratio will be biased away from unity.

The validity of the constant half-life assumption cannot be assessed until the half-life study is expanded to all 500 Ranch Hands with current levels above background (above 10 ppt). Paired dioxin measurements on each of these 500 Ranch Hands, one derived from frozen serum samples collected in 1982 and the other from serum collected in 1987, will permit investigation of half-life variability with changes in weight, percent body fat, and disease since exposure. Assessment of the first-order elimination assumption will be based

on up to five dioxin measurements collected serially on each of 20 males who were exposed during a factory explosion near Seveso, Italy (3). The additional Air Force and Seveso data will be available in 1991.

Estimates of health effects derived from model 2 also could be biased if, for example, some Ranch Hands were fast dioxin eliminators (have a short dioxin half-life) and some were slow eliminators (have a long half-life). If this phenomenon was associated with a covariate (e.g., relative weight change between 1982 and 1987), lack of adjustment for this covariate would bias estimates of the slope or relative risk toward the null values (slope=0 and relative risk=1). Further investigation of this possibility will occur during the expanded half-life study, which is scheduled to begin in early 1991. A similar concern arises regarding estimates of effect derived from model 3. If, for example, a health effect was expressed many years after exposure, such an effect would probably be apparent in contrasts in disease rates between the background group and Ranch Hands in the high current dioxin category with the earliest tours of duty. The categorized current dioxin analyses were not adjusted for time since tour, however. Hence, it might not be possible to detect such an effect with that model because time since tour was not used for adjustment. This shortcoming is partially overcome by analyses based on model 2, which are adjusted for time since tour and the interaction between current dioxin and time.

Information bias, represented by overreporting disease symptoms, was precluded by verifying all diseases and conditions with medical records. It is possible that Ranch Hand conditions may be more verifiable because they may have been seen by physicians more often than Comparisons; this would be revealed by group differences in the quantity and content of medical records. Because currently there is no way to quantify these aspects, this potential source of bias remains unexplored. This source, however, if it exists, would affect only estimates of health effects derived from model 3 because Comparison data were not used in the model 1 and model 2 analyses. Information bias due to errors in the data introduced through data entry or machine error is negligible. All laboratory results were subject to strict quality control procedures. Medical coding data were verified completely by medical record review.

Adjustments for Covariates and Interactions

In previous reports, the focus was on overall group contrasts between all Ranch Hands and all Comparisons, which took advantage of the matched design. In those analyses, the matching variables age, race, and occupation were eliminated effectively as confounders. The present dioxin analyses within Ranch Hands and the categorized current dioxin analyses within Ranch Hands and Comparisons are not benefited by the matched design. Military occupation is a strong confounder because it is highly correlated with current dioxin levels in Ranch Hands and is related to some health variables through socioeconomic differences between officers and enlisted personnel. Education is highly associated with military occupation and certain psychometric results.

In addition, some covariates (e.g., percent body fat) may themselves be associated with current dioxin level and, perhaps, through their relationship with dioxin, may be related to the dependent health variable. In this situation, analyses of covariance adjusted for such a covariate are not valid, since the assumed independence of the "treatment" (current or initial dioxin) and the covariate is not met (4). There is no recourse but to analyze the data with

and without adjustment for the covariate; both analyses potentially are biased. Thus, unadjusted analyses must be viewed with caution and circumspection. Because some covariates may act in an intervening manner relating the "treatment" to the dependent variable, some adjusted analyses of covariance are themselves subject to bias. Bias introduced by intervening covariates is unavoidable in an observational study.

The adjusted models assessed the statistical significance of interactions between dioxin and the covariates to determine whether the relationship between dioxin and the dependent variable (health-related endpoint) differed across levels of the covariate. In many instances the clinical importance of a statistically significant dioxin-by-covariate interaction is unknown or uncertain. The clinical relevance of a statistically significant interaction would be strengthened if the same interaction persisted among related endpoints. It is recognized that due to the large number of dioxin-by-covariate interactions that were examined for approximately 300 variables, some of the dioxin-by-covariate interactions judged significant at the 0.05 level might be spurious (i.e., chance occurrences not of biological or clinical relevance). This should be considered when significant dioxin-by-covariate interactions are interpreted. It is important that the size of the p-value associated with each dioxin-by-covariate interaction be weighed carefully. For this reason models without the dioxin-by-covariate interaction were implemented to address the possibility that some interactions may arise from multiple testing (see Chapter 4).

Consistency

Ideally, an adverse health effect in Ranch Hands attributable to herbicide or dioxin would be revealed by internally and externally consistent findings. An internally consistent finding does not contradict prior information, other findings, or medical knowledge. An externally consistent finding has been established either previously in theory or empirically as related to exposure.

The findings of positive trends of increasing abnormalities with increasing levels of current dioxin with regard to lipids, percent body fat, and diabetes are internally consistent. The observed associations between dioxin and Millon Clinical Multiaxial Inventory scale scores appear inconsistent and isolated. They are not consistent between themselves or with known patterns of psychological disorder.

Multiple Testing

Numerous dependent variables were considered because of the lack of a predefined medical endpoint. Each dependent variable was analyzed in many different ways to accommodate covariate information and different statistical models. In the hypothetical case when Ranch Hand physical health is not related to dioxin, about 5 percent of the many statistical tests of hypotheses (dioxin effects and dioxin-by-covariate interactions) shown in this report should be expected to detect an association between dioxin and health in Ranch Hands (p-values<0.05). Observing significant results due to multiple testing, even when there is no relationship between dioxin and health, is known as the multiple-testing artifact and is common in large studies. Unfortunately, there is no statistical procedure available to distinguish between those statistically significant results that arise due to the multiple testing artifact and those that may be due to a bona fide dioxin effect. Instead, in order to weigh and interpret the findings, the authors have considered the strength of the association, consistency, dose-response patterns, and biologic credibility.

Trends

Assessing consistent and meaningful trends is essential when interpreting any large study with multiple endpoints, clinical areas, and covariates. However, caution must be used when assessing trends. Increased numbers of abnormalities or means with increased dioxin levels across medically related variables within a clinical area might indicate a dioxin effect. In this case, it is important to note that there is a moderate-to-strong correlation between some endpoints. Hence, the strength of the trends also must be considered when assessing the suspected association.

Power Limitations

The fixed size of the Ranch Hand cohort limits the ability of this study to detect a dioxin association. This limitation is most obvious concerning specific types of cancer, such as soft tissue sarcoma and non-Hodgkin's lymphoma, which are so uncommon that fewer than two cases are expected in this study, indicating that this study has virtually no statistical power to detect low-to-moderate associations (relative risks less than 5) with dioxin. On the other hand, these sample sizes are sufficient to detect very small mean shifts in the continuously distributed variables (see Chapter 4). For example, with regard to IgG, this study has approximately 90 percent power to detect a mean shift of 1 percent. The detection of significant mean shifts without a corresponding indication of increased Ranch Hand abnormalities or disease is considered to be of little importance or it may be an artifact of multiple testing. This study has good power to detect relative risks of 2.0 or more with respect to diseases, such as heart disease and basal cell carcinoma, occurring at prevalences of at least 5 percent in unexposed populations.

In an attempt to overcome the lack of power to detect group differences for specific types of systemic cancer, all types of systemic cancer were combined into a single variable. It is still possible, however, that an increased risk could exist for a particularly rare type of cancer, allowing that increased risk to be missed in this study.

Strength of Association

Ideally, an adverse effect, if it exists, would be revealed by a strong association between categorized current dioxin and a disease condition; that is, by a statistically significant relative risk greater than 2.0 for Ranch Hands in the high current dioxin category relative to the unexposed Comparisons (5). Statistically significant relative risks less than 2.0 are considered to be less important than larger risks because the relative risks less than 2.0 can easily arise due to unperceived bias or confounding. Relative risks greater than 5.0 are less subject to this concern. The numbers 2 and 5 are rules of thumb regarding analyses of association between a dichotomous endpoint (disease, no disease) and dichotomized exposure (exposed, unexposed). No such rules have been published regarding the analysis of continuously distributed endpoints (such as cholesterol) versus continuously distributed exposure (such as initial or current dioxin in models 1 and 2).

Biological Credibility

The assessment of biological credibility requires consideration of the following question. In biological terms, can it be understood how the exposure under study could produce the effect of interest? While a lack of biological credibility or even a contradiction of biological knowledge can lead to the dismissal of a significant result, the failure to perceive a

mechanism may reflect only ignorance of the state of nature. On the other hand, it is easy to ascribe biological mechanisms that relate almost any exposure to almost any cancer. Thus, while pertinent, the response to this question is not always convincing.

Interpretation of Negative Results

A 1985 study (6) presents minimal sample-size criteria for proof of safety and hazard in studies of environmental and occupational exposures. The study was directed at rectifying widespread misconceptions about proof of safety in the medical and scientific establishments and in other groups involved in public health and safety. Thus, a lack of significant results relating dioxin to a particular disease only means that this study is unable to detect a relationship between dioxin and health. This does not imply that a relationship does not exist, but that, if it does exist, it was not detected. A lack of significant results does not mean that dioxin is safe or that there is no relationship between dioxin and health, because this study is not designed, nor was it intended, to establish safety. This study was designed to determine whether a hazard existed for the exposed personnel and not whether dioxin was "safe."

Interpretation of the Coefficient of Determination

The coefficient of determination, R^2, measures the proportionate reduction of the total variation in a continuously distributed health variable y associated with the set of independent variables in a linear regression. A large value of R^2 does not necessarily imply that the fitted model is a useful one. Large values of R^2 would occur, for example, if y is regressed on an independent variable with only two observed values. On the other hand, very small values of R^2 are generally seen in observational studies because little or no control has been applied in the assignment of the values of the "treatment" (initial or current dioxin) or the conditions under which the "treatment" has been applied. In this study, the dioxin measurements were taken many years after exposure and are themselves subject to measurement error. Thus, in most analyses, the values of R^2 in this study are small.

Clinical Interpretation of Discrete versus Continuous Data

Small but significant mean differences in a continuously measured health variable (e.g., systolic blood pressure) between exposed and unexposed groups when there are no corresponding differences in the percentage of abnormal tests are difficult to assess in any study. In this study, significant mean differences are sometimes observed without a corresponding group difference in the proportion outside the normal range. Such contrasting situations may be interpreted as spurious outcomes of no clinical consequence, or as a subclinical dioxin effect. Significant trends in the mean with increasing levels of dioxin are interpreted as a dioxin-related effect if a corresponding trend is seen in the proportion above or below the normal range.

Minimal versus Maximal Results

The minimal and maximal assumptions for Ranch Hands having background dioxin levels (≤10 ppt) were imposed to address the unknown exposure history of this subgroup. There were 345 Ranch Hands in this "unknown" category. In the minimal analyses, all of these were excluded from the data set. In the maximal analyses, only those with less than or equal to 5 ppt (n=124) were excluded. The intent of these two analyses was to "trap" the true dioxin versus health relationship between them. The results of the maximal analyses

appear to be statistically significant more often than those of the minimal analyses. This could be due to the larger sample size of the maximal cohort or it could be due to the uncertainty of true exposure in Ranch Hands between 5 ppt and 10 ppt. There are no additional data available at this time with which to resolve these two interpretations.

Graphics

The histograms, scatter plots, and graphical descriptions of interactions were included as aids to interpretation. The graphics alone are not sufficient to assess the relationship between dioxin and health. For example, a trend may be seen in a plot, but it could be statistically nonsignificant because the number of abnormalities is small. On the other hand, a statistically significant result can be clarified by the graphics, especially if the result depends on a few data points that appear far from the main cluster. Such points are termed "outliers" by statisticians. Outside of the initial quality control review activities, no additional effort was made to identify statistically significant outliers in this report.

The Checkmark Pattern

In many model 3 analyses, the "unknown" Ranch Hand group has the lowest percentage of abnormalities; this phenomenon is termed "the checkmark pattern." These patterns are interesting but are without explanation at this time. Some reanalyses were accomplished with adjustment for military rank (officers, enlisted personnel), but the checkmark pattern remained after adjustment. This effect will be a subject of continued focus in future reports.

Extrapolation to Army Ground Troops

Extrapolation of the serum dioxin results to the general population of ground troops who served in Vietnam is difficult because Ranch Hand and ground troop exposure situations were quite different. Based on serum dioxin testing results done by CDC (7) and others (8), nearly all ground troops tested have current levels of dioxin similar to background levels. Even ground troops who served in herbicide-sprayed areas of Vietnam had current levels indistinguishable from levels in men who never left the United States (with means of 4.2 ppt and 4.1 ppt, respectively). The AFHS subgroup most like the ground troops in terms of current dioxin levels are Ranch Hands who currently have background levels of dioxin (10 ppt or less—designated as the "unknown" current dioxin category in the model 3 analyses). Therefore, if the results of the AFHS are applied to the general population of Vietnam veterans, the focus should be on the unknown Ranch Hand versus background Comparison contrast in the model 3 analyses. However, extrapolating the results of these analyses to Vietnam veterans should still be made cautiously. There may be demographic distinctions between the unknown group of Ranch Hands and other Vietnam veterans that may be related to health. Also, if Ranch Hands in the unknown current dioxin category showed a significant health detriment relative to Comparisons in the background category, but there was no significant detriment for Ranch Hands in the high current dioxin category, the biological plausibility of such an effect would be questionable because this would not indicate a dose-response effect. In general, the adjusted model 3 analyses found that Ranch Hands in the unknown current dioxin category did not show a significant health detriment relative to Comparisons in the background current dioxin category. This was particularly true for the variables that exhibited a significant high versus background contrast.

Summary of Results

Many readers of this report will attempt to tally statistically significant results across clinical areas and study cycles. A study of this scope with a multitude of endpoints and no prescribed strength of association to declare an effect demands, and at the same time defies, meaningful summary tabulation. Such summaries can be misleading because they ignore correlations between the endpoints, correlations between study-cycle results, and the nonquantifiable medical importance of each endpoint. In fact, many endpoints are redundant (e.g., psychological scales and indices developed from combining multiple variables) so as not to miss a dioxin effect and some (such as those arising from measures of pulmonary function) were not suspected beforehand to be related to dioxin exposure.

In addition, such tabulations combine endpoints that medically are not comparable. For example, a diminished sense of smell is of less medical importance than the presence of malignant neoplasm. Statisticians have attempted to summarize multidimensional repeated measures data with growth curve analyses. Such methods were not used in this study because they apply to continuously distributed data only, do not account for medical importance, and reduce the data too much.

Nevertheless, given the lack of adequate summary statistics, the tally of significant results will occur. Such summaries can be misleading and must be interpreted carefully.

CONCLUSION

The interpretation of the AFHS requires careful consideration of potential biases, interactions, consistency of results, the multiple-testing artifact, dose-response patterns, trends, power limitations, strength of association, and biological credibility.

CHAPTER 1

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CHAPTER 2

DIOXIN ASSAY

SAMPLE ACQUISITION

Blood for the serum dioxin assay was drawn on the morning of the second day of the physical examination in 1987. Participants who volunteered to give blood for the dioxin assay fasted after midnight (water was allowed). Blood was drawn from the participants with a 15-gauge needle into a blood pack unit without anticoagulant. The blood pack units had been tested previously by the Centers for Disease Control (CDC) and were found to be free of dioxin contamination. Participants selected for the immunology studies had 250 ml of blood drawn; all others had 350 ml of blood drawn. After drawing, the bags were clamped, labeled, placed upright at room temperature, and allowed to clot for 7 hours. Appendix B-1 contains the Scripps Clinic and Research Foundation's (SCRF) procedure for the dioxin blood collection and processing.

The unit bags were centrifuged for 15 minutes at 4500 RPM at a temperature of 4°C to 10°C. The serum was then transferred to transfer packs (also dioxin-free) from the spun unit bag by a plasma extractor. The transfer packs were spun for 15 minutes at 4500 RPM. The serum was then placed into four Wheaton bottles: two 4-ounce bottles for the serum dioxin analysis, a 5 ml bottle for the lipid profile, and a 10 ml bottle for reserve serum. Samples were logged and stored at -20°C or less until shipment. Frozen samples, packed in dry ice in styrofoam boxes, were shipped twice weekly from SCRF, La Jolla, California, to Brooks Air Force Base, Texas. At Brooks Air Force Base, inventory was taken and the specimens were stored at -70°C until shipment to the CDC. All samples were coded so that the CDC was blinded to the group status (Ranch Hand, Comparison) of each specimen.

ANALYTICAL METHOD

The serum samples were analyzed for dioxin in analytical runs that consisted of a method blank, three unknown samples, and a quality control pool sample (1, 2). Cholesterol esters, triglycerides, and high-density lipoprotein cholesterol were determined in duplicate by standard methods. Total phospholipids were determined in duplicate by modifying (3) the Folch et al. procedure (4). Fresh cholesterol was determined in duplicate by an enzymatic method (5). For each analysis, the results of the duplicate analyses were averaged and the mean was used. These results were used to calculate the concentrations of (a) total lipids using the summation method (6), (b) low-density lipoprotein cholesterol, and (c) very low-density lipoprotein cholesterol (7).

QUALITY CONTROL

Quality assurance was maintained with matrix-based materials that are well characterized for dioxin concentration and isotope ratios to ensure that the analytical system was in control. Quality control (QC) charts were maintained for each of these materials (five serum pools). The concentration in the QC sample from each analytical run must be within 99 percent confidence limits established for the QC material (8, 9). The unlabeled and carbon-13 labeled internal standard isotope ratios must be within 95 percent confidence limits. All analytical runs for the dioxin and lipid measurements were in control. No dioxin was detected

TABLE 2-1.

Report Field Definition

Report Field Value	Definition
G	Good result
GML	Good result, missing lipids
GND	Good result, below limit of detection
GNQ	Good result, below limit of quantitation
NR	No result

in the blanks (on-column injection of 100 femtograms from a standard solution produces detectable signals that are greater than three times the background noise).

DATA DELIVERED TO THE AIR FORCE BY THE CENTERS FOR DISEASE CONTROL

The dioxin data used in this report were derived from a data base of results on 932 Ranch Hands and 888 Comparisons delivered by the CDC in January 1990. The CDC sent data on whole-weight and lipid-weight dioxin concentrations to the Air Force together with the total sample weight, weights of lipid fractions, total lipid weight, the detection limit, quantitation limit, and all associated QC information, including results from blank samples. Table 2-1 defines a "report" field in the data base.

Some participants (150 Ranch Hands and 50 Comparisons) participated in a pilot dioxin study in April 1987 (8). Four of these (three Ranch Hands and one Comparison) had a missing dioxin result (report=NR), the rest had good results (report=G). The remaining 147 Ranch Hands and 49 Comparisons were included in the dioxin data base from which the analysis data set for this report was derived. Of these, 145 Ranch Hands and 48 Comparisons were also fully compliant to the 1987 physical examination. Forty-seven of the pilot study participants (43 Ranch Hands and 4 Comparisons) also had blood drawn for the dioxin assay at the 1987 physical examination (May 1987 through March 1988). If a participant was assayed during the pilot study but not at the 1987 physical examination, or if he was assayed at the pilot study and at the 1987 physical examination, then his pilot study assay was used.

Table 2-2 shows counts of study participants by group, report, and compliance to the 1987 physical examination.

TABLE 2-2.

Sample Sizes by Group, Report, and Compliance to the 1987 Physical Examination

	Ranch Hand		Comparison	
Report	Fully Compliant	Noncompliant	Fully Compliant	Noncompliant
G	858	2	761	1
GML	0	0	1	0
GND	8	0	43	0
GNQ	20	0	51	0
NR	44	0	31	0
Total	930	2	887	1

Missing dioxin results (report=NR or GML) and nonquantitatable dioxin results (report=GNQ) were excluded from analysis in this report. The resulting effective sample sizes (866 Ranch Hands and 804 Comparisons) were determined by the condition that the participants were fully compliant to the 1987 physical examination. Table 2-3 summarizes this sample size reduction.

TABLE 2-3.

Sample Sizes Used in This Report

		Ranch Hand	Comparison
Fully compliant to 1987 physical examination and assayed for dioxin		930	887
	Report		
Less	GNQ	(20)	(51)
	NR	(44)	(31)
	GML	(0)	(1)
Total		866	804

TABLE 2-4.

Dioxin Result Summary of 866 Ranch Hands and 804 Comparisons

	Ranch Hands			Comparisons		
Stratum	n	Median	Range	n	Median	Range
Officer	319	7.8	0-42.6	291	4.7	0-18.5
Enlisted Flyer	148	18.1	0-195.5	127	4.0	0-12.8
Enlisted Groundcrew	399	24.0	0-617.8	386	4.0	0-54.8
Total	866	12.8	0-617.8	804	4.2	0-54.8

Table 2-4 summarizes, by military occupation and group, the dioxin results among the 866 Ranch Hands and 804 Comparisons whose results were used in analyses of dioxin versus health in this report.

The 95th, 98th, and 99th percentiles of the Ranch Hand dioxin distribution were 110.8, 168.0, and 211.0 ppt; the corresponding Comparison percentiles were 8.3, 10.2, and 14.2 ppt.

CDC subsequently provided 314 Comparison dioxin results after January 1990 (the beginning date for statistical analyses involving Comparison data). Of these 314 dioxin results, 253 had a report field value of G or GND, 24 had a report field value of GNQ, and 37 had a report field value of NR (no result). Of the 253 Comparisons, the median current dioxin result was 4.1 ppt, the range of levels was between 0 ppt and 13.6 ppt, and the first and third quartiles were 2.9 ppt and 5.8 ppt. The percentages of the 253 Comparisons and of the 804 Comparisons analyzed in this report, having levels less than 10 ppt, were 97.8 and 97.6, respectively. A statistical contrast of the dioxin distributions of these 253 and the 804 Comparisons included in this report revealed no significant difference ($p=0.15$), as expected.

The phrase "serum dioxin" is used throughout this report and is defined as the serum lipid-weight concentration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Its relationship with dioxin concentrations in other compartments, such as adipose tissue, is a subject of continuing research. The lipid-weight dioxin measurement, also called "current dioxin body burden" in this report, is a derived quantity calculated from the formula $ppt = ppq \cdot 102.6/W$, where ppt is the lipid-weight concentration, ppq is the actual weight of dioxin in the sample in femtograms, 102.6 corrects for the average density of serum, and W is the total lipid weight of the sample (9). The correlation between the serum lipid-weight concentration and adipose tissue lipid-weight concentration of TCDD has been observed to be 0.98 in 50 persons from Missouri (10). Using the same data, Patterson et al. calculated the partitioning ratio of dioxin between adipose tissue and serum on a lipid-weight basis as 1.09 (95% C.I.: [0.97,1.21]). On the basis of these data, a one-to-one partitioning ratio of dioxin between lipids in adipose tissue and the lipids in serum cannot be excluded. Measurements of dioxin in adipose tissue generally have been accepted as representing the body burden concentration of dioxin. The

high correlation between serum dioxin levels and adipose tissue dioxin levels in their study suggests that serum dioxin is also a valid measurement of dioxin body burden.

CHAPTER 2

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CHAPTER 3

THE RELATIONSHIP BETWEEN THE EXPOSURE INDEX AND DIOXIN BODY BURDENS IN RANCH HANDS

INTRODUCTION

An increased prevalence of adverse health effects at higher levels of exposure represents the classic dose-response relationship sought in any study of environmental or occupational exposure to potentially toxic substances. In previous Air Force Health Study (AFHS) reports, the potential relationship between clinical endpoints and herbicide exposure in Ranch Hands was assessed using a calculated estimate of TCDD exposure, hereafter called the exposure index.

The exposure index was constructed solely from available historical data to measure the potential exposure of a Ranch Hand to any of four 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-containing herbicides: Herbicides Orange, Purple, Pink, and Green (1). The index was only an estimate of exposure, because the actual concentration of TCDD in the herbicides varied with type and lot as well as with individual work habits and duties. The calculation of the index was necessary because actual measures of dioxin exposure on individuals during or just after their Southeast Asia tours were not feasible at that time.

Exposure Index Definition

The exposure index for a Ranch Hand was defined as the product of a TCDD weighting factor and the gallons of TCDD herbicides sprayed during his tour divided by the number of Ranch Hands sharing his duties during his tour. The TCDD weighting factor reflected the estimated relative concentration of TCDD in the herbicides sprayed; these were 2 ppm in Herbicide Orange, 33 ppm in Herbicide Purple, 66 ppm in Herbicide Pink, and 66 ppm in Herbicide Green, as determined from archived samples (1). Based on procurement records and historical spray records, a combination of Herbicides Green, Pink, and Purple was sprayed between January 1962 and June 1965. The estimated mean concentration of TCDD in this combination during that period was 48 ppm. The "Herbs" tape and other data sources (1) indicate that only Herbicide Orange was sprayed by Operation Ranch Hand after 1 July 1965. Normalizing to Herbicide Orange, the weighting factor was defined as 24 for a Ranch Hand with a tour of duty before 1 July 1965 and as 1 for a Ranch Hand with a tour of duty after 1 July 1965.

A table showing gallons of TCDD-containing herbicide sprayed for each month of the Ranch Hand operation was constructed using data derived from the Herbs tape, Contemporary Historical Evaluation and Combat Reports, and quarterly operations reports. Gallons of Herbicides Purple, Pink, and Green were converted to Herbicide Orange equivalents based on the TCDD weighting factor. Appendix B-2 contains this table.

The tour dates and military occupation of each Ranch Hand were verified by review of military records. The study design reduced the many occupational categories (specified by an Air Force Specialty Code) to five: (1) officer-pilot, (2) officer-navigator, (3) officer-nonflying, (4) enlisted flyer, and (5) enlisted groundcrew. After computing the index for each Ranch Hand, he was placed in one of three exposure categories ("low," "medium," and "high")

TABLE 3-1.

Exposure Index Categorization of 866 Fully Compliant Ranch Hands With TCDD Results

Occupation	Exposure Index Category	Effective Herbicide Orange Gallons Corresponding to Exposure Index Category	Number of Ranch Hand Participants in Exposure Index Category
Officer	Low	<35,000	109
	Medium	35,000-70,000	104
	High	>70,000	106
Enlisted Flyer	Low	<50,000	43
	Medium	50,000-85,000	57
	High	>85,000	48
Enlisted Groundcrew	Low	<20,000	127
	Medium	20,000-27,000	139
	High	>27,000	133
Total			866

according to the tertiles of the index in three occupational categories: officer, enlisted flyer, and enlisted groundcrew. The officer category consisted of officers who were pilots, navigators, or nonflyers. Table 3-1 shows counts of the 866 Ranch Hands who subsequently had serum levels determined and who were fully compliant to the 1987 examination according to their assigned exposure index category. Nonflying officers were assigned an exposure index value of zero and were placed in the "low" category of exposure.

The index was not useful for assessing the exposure of any specific individual because it did not account for variation in exposures due to work habits and duties. For example, it was known that some Ranch Hand enlisted ground personnel primarily were occupied with administrative duties and probably had little actual contact with herbicides. Other enlisted Ranch Hands periodically greased an emergency dump valve inside the spray tank. To do this, the Ranch Hand had to enter the spray tank and apply the grease to a valve at the bottom of the tank which contained at least 2 inches of herbicide.

In past reports, every clinical endpoint was evaluated for a dose-response effect versus the calculated exposure index. Few significant trends were found. Those that were found were not consistent with other findings or were medically implausible or both.

The Dioxin Assay

The dioxin assay provides a direct measurement of current dioxin burden which, together with assumptions regarding the decay process, provides an approximate measure of TCDD exposure in Ranch Hands and Comparisons. The assay is preferred over the calculated exposure index, because it is a direct rather than indirect measure of TCDD exposure. Confidence in the assay as a measure of TCDD exposure is heightened by the following: (a) Ranch Hand results are generally greater than those of the Comparisons, and (b) Ranch Hand results are logically placed relative to those of industrially exposed individuals and people exposed to TCDD in Seveso, Italy (2). Additionally, differences in TCDD body burdens between the three occupational groups within the Ranch Hand group are in accordance with recent information regarding the relative exposure of the occupational cohorts gleaned from interviews of two Ranch Hand crew chiefs, administered before any Ranch Hands were assayed for TCDD. Based on those interviews, it appears that Ranch Hand groundcrew had more opportunity for cutaneous exposure than enlisted flyers or officers and that enlisted flyers had more opportunity than officers for cutaneous exposure and inhalation of herbicide spray. These aspects will be investigated during an analysis of a questionnaire administered to all assayed Ranch Hand enlisted ground personnel before they received their serum dioxin assay results. These men were asked whether they entered the spray tank to service the dump valve and if so, how often. Other questions addressed daily exposures reported by crew chiefs during in-person interviews at Brooks Air Force Base, Texas, in 1988.

The relative position of the Ranch Hand results in contrast to other study cohorts lends credence to the assay as a measure of TCDD exposure. The Ranch Hand serum dioxin results are less than those observed in people exposed in Seveso, Italy, and are greater than those observed in U.S. Army ground troops and the Air Force Comparison cohort. Ranch Hand dioxin results are also generally less than those observed in a National Institute for Occupational Safety and Health study of workers who produced trichlorophenol and its derivatives (3).

The Exposure Index versus the Dioxin Assay

The relationship between the assay results and the exposure index provides an indication of the extent to which Ranch Hands have been misclassified by the exposure index. Figure 3-1 shows a scatter plot of the extrapolated initial dioxin concentrations of the 742 Ranch Hands in the maximal cohort (having current dioxin greater than 5 ppt; see Chapter 4, Statistical Methods) versus the continuously distributed exposure index. The extrapolated initial dioxin concentration (I) was computed from the current dioxin level (C) and the time in years between the end of the Vietnam tour and the dioxin blood draw (T) with the formula $I = C \cdot 2^P$, where $P = T/7.1$.

Both distributions are highly skewed, hence the concentration of observations near the origin. Figure 3-2 shows the bivariate scatter plot of the logarithms of these quantities. The logarithms are taken to the base 2 and 1 was added to the exposure index prior to taking the logarithm.

The corresponding scatter plots of current dioxin versus the exposure index and the logarithms of these quantities in all 866 Ranch Hands fully compliant to the 1987 examination

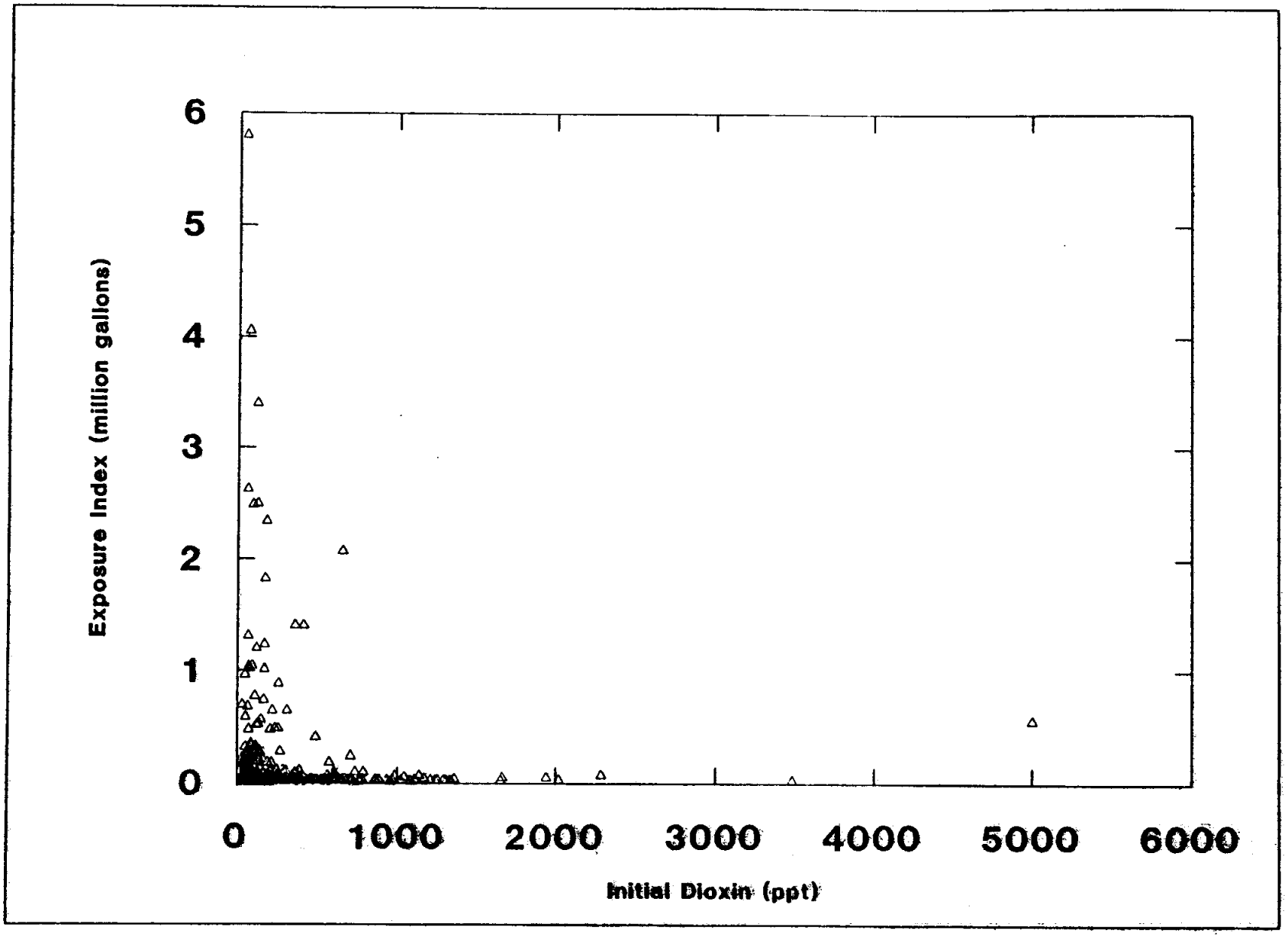

FIGURE 3-1. Initial Dioxin versus the Exposure Index in Ranch Hands With Current Dioxin Greater Than 5 ppt (N=742)

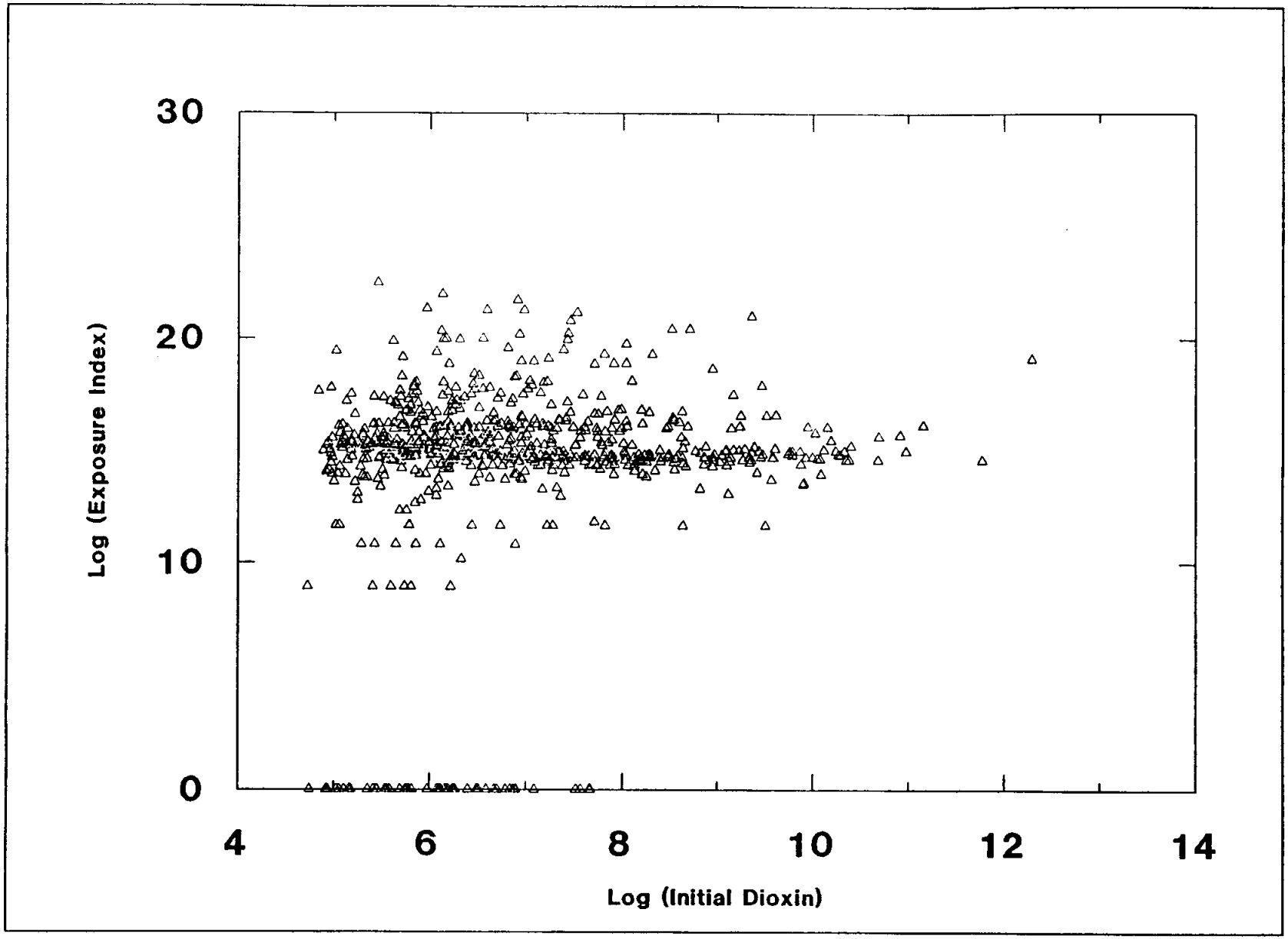

FIGURE 3-2. Logarithm of Initial Dioxin versus Logarithm of the Exposure Index in Ranch Hands With Current Dioxin Greater Than 5 ppt (N=742)

having a dioxin result are shown in Figures 3-3 and 3-4. Figures 3-5 through 3-7 show the logarithmic scatter plots within each of the three occupational strata (officer, enlisted flyer, enlisted groundcrew). One ppt was added to each current dioxin concentration value before taking the logarithm.

The relationship between the assay result and the exposure index is weak in view of these scatter plots; the same situation holds within each of the three occupational categories, as evident from the plots. Using only nonzero dioxin and exposure index values, Table 3-2 presents correlations between the logarithm of the dioxin results and the logarithm of the exposure index.

Because the categorized exposure index, rather than the continuously distributed index shown in the plots, was used in the assessment of exposure trends in prior reports, the relationship between this categorized index and categories of current dioxin is also of interest. Table 3-3 shows a cross-tabulation of Ranch Hands using the prior exposure index versus current dioxin levels. The cutpoints for the low, medium, and high current dioxin levels

TABLE 3-2.

Correlations Between Log (Current Dioxin) and Log (Exposure Index) in Ranch Hands With Current Dioxin and Exposure Greater Than Zero

Stratum	N	Correlation	p-Value
Officer	295	0.10	0.082
Enlisted Flyer	143	0.33	<0.001
Enlisted Groundcrew	347	0.12	0.024
All	785	-0.10	0.003

TABLE 3-3.

Categorized Exposure Index versus Current Dioxin Levels in Ranch Hands

Current Dioxin Level	Exposure Index				
	Zero	Low	Medium	High	Total
0-5 ppt	7	52	28	37	124
Low	6	76	52	51	185
Medium	6	109	134	121	370
High	0	23	86	78	187
Total	19	260	300	287	866

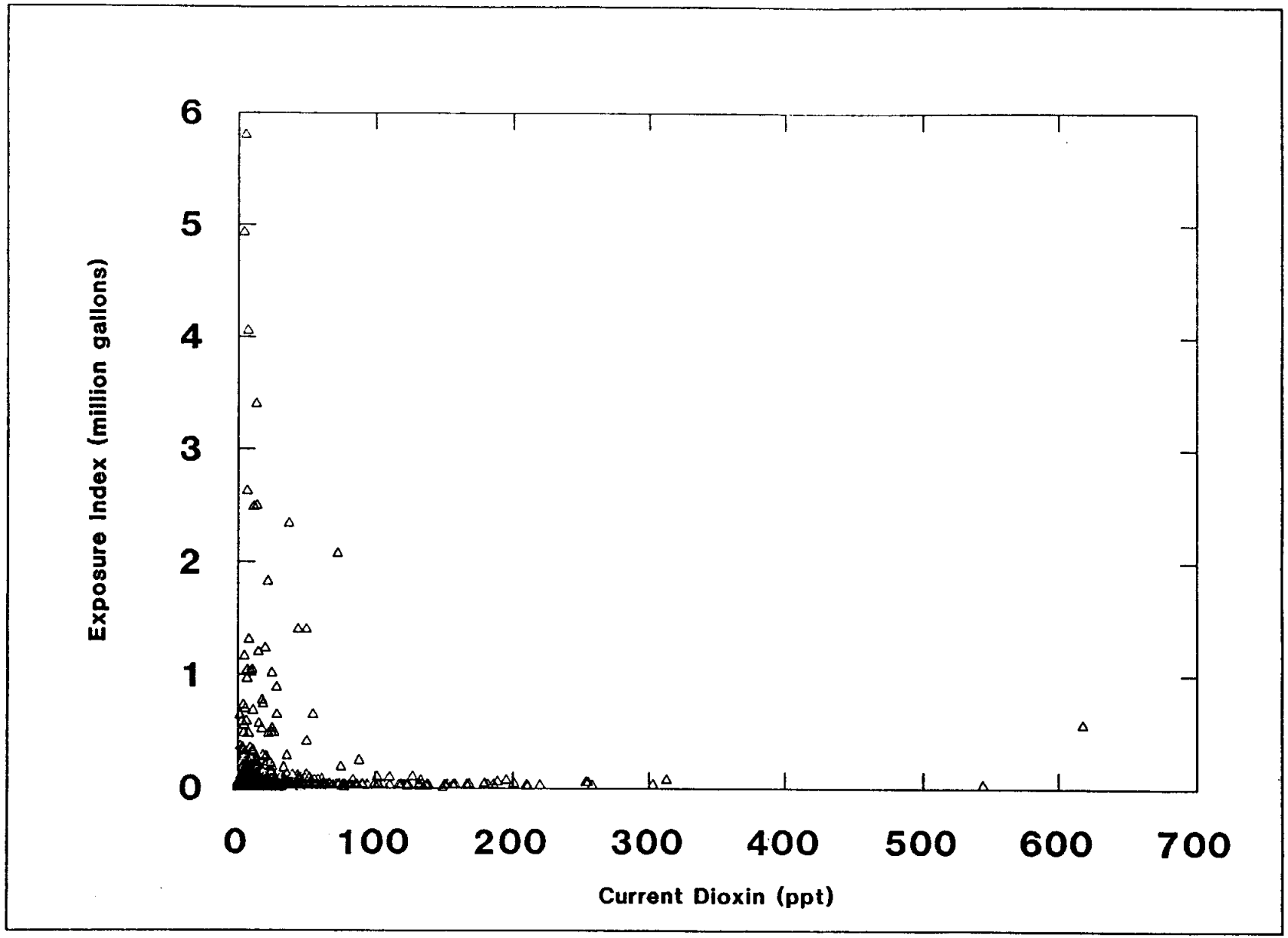

FIGURE 3-3. Current Dioxin versus the Exposure Index in Ranch Hands (N=866)

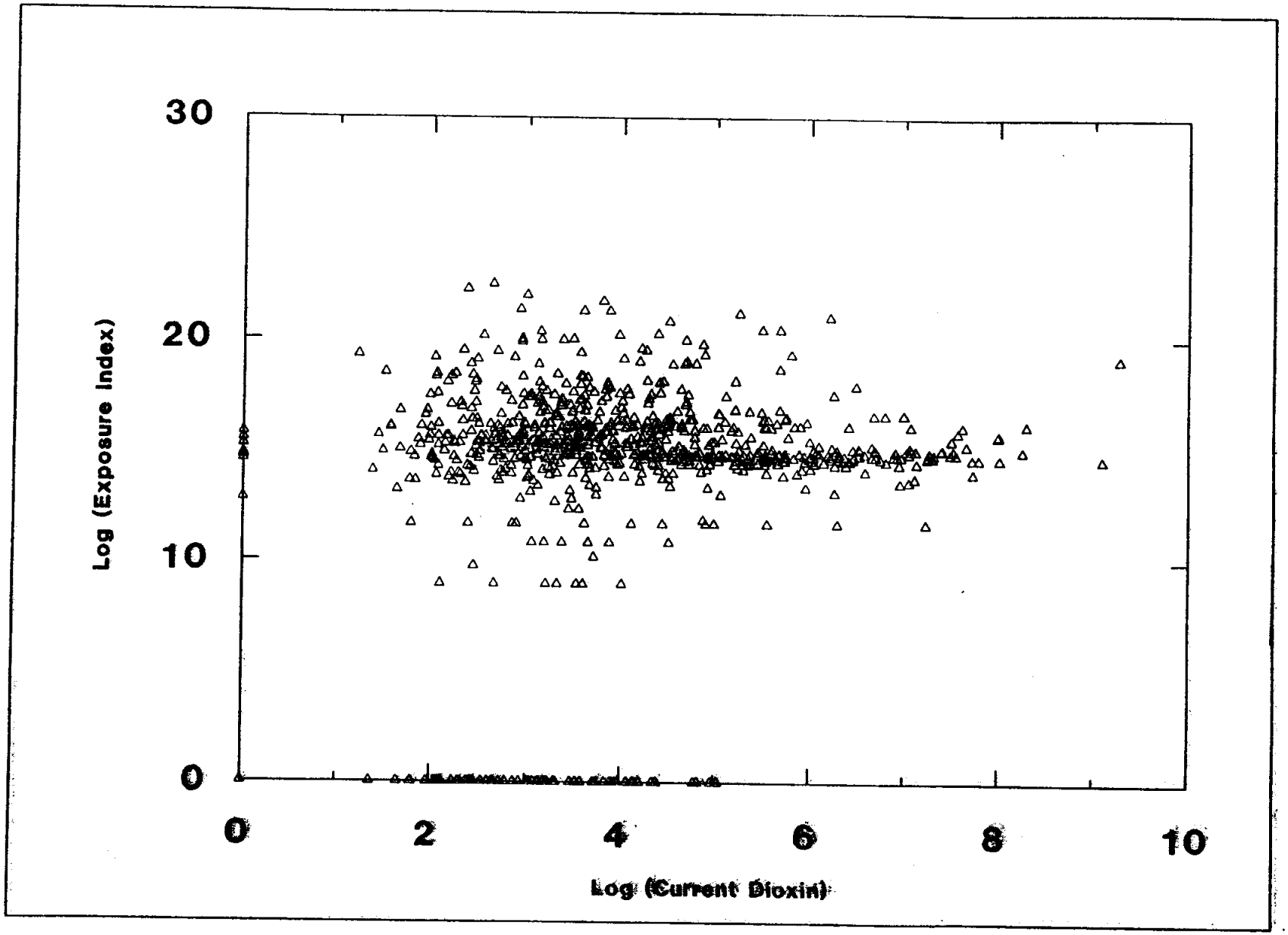

FIGURE 3-4. Logarithm of Current Dioxin versus Logarithm of the Exposure Index in Ranch Hands (N=866)

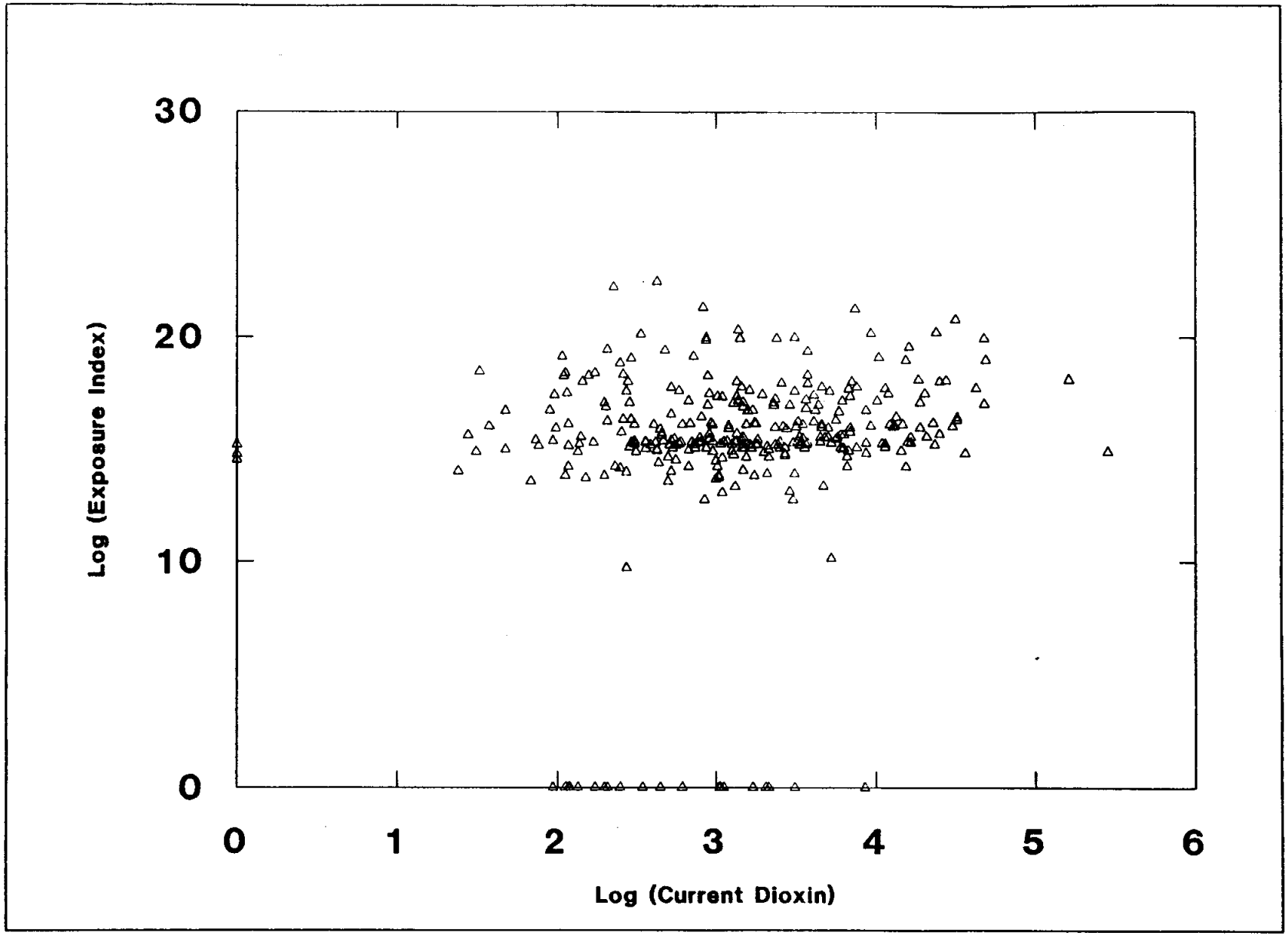

FIGURE 3-5. Logarithm of Current Dioxin versus Logarithm of the Exposure Index in Ranch Hand Officers (N=319)

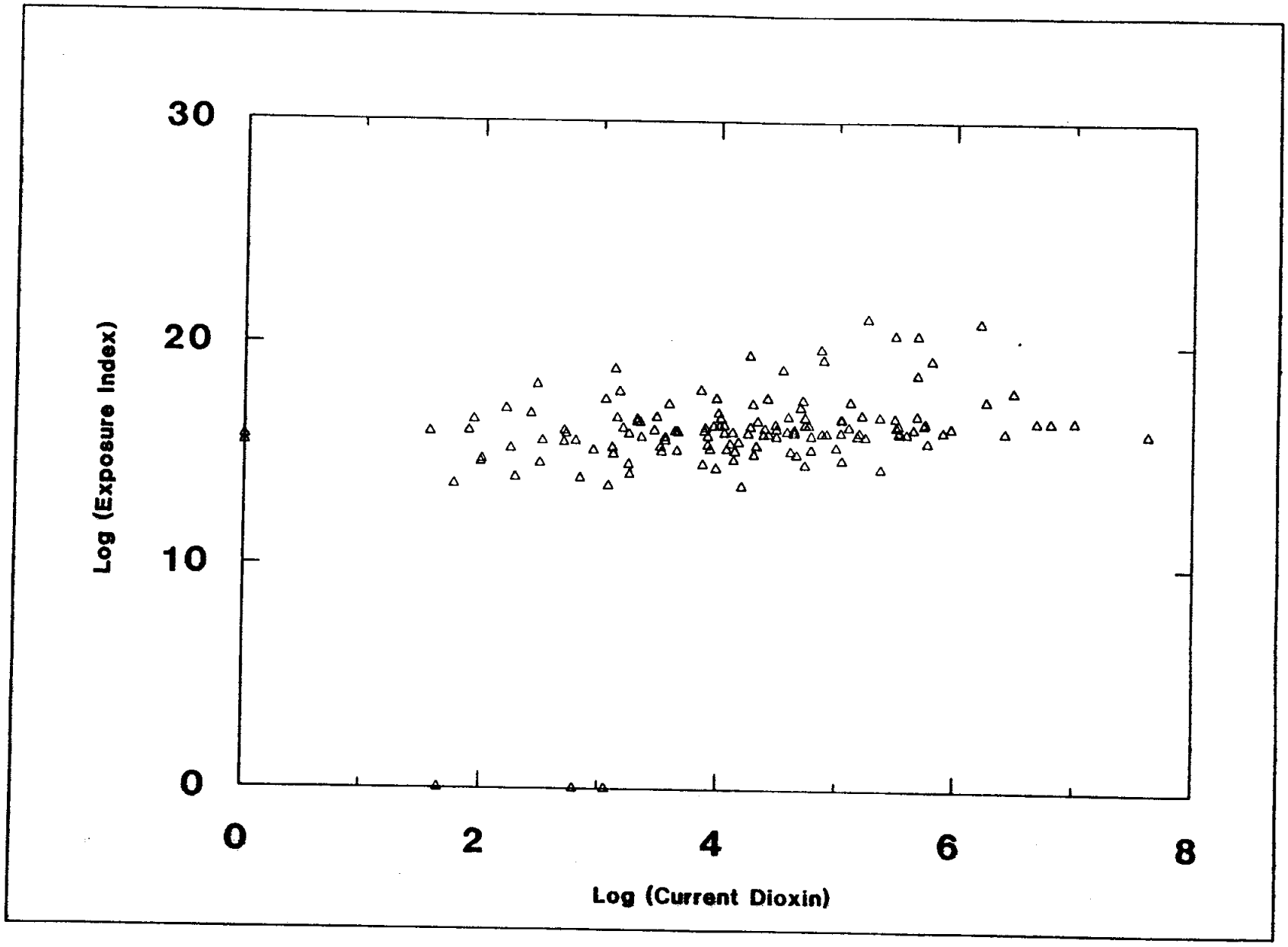

FIGURE 3-6. Logarithm of Current Dioxin versus Logarithm of the Exposure Index in Ranch Hand Enlisted Flyers (N=148)

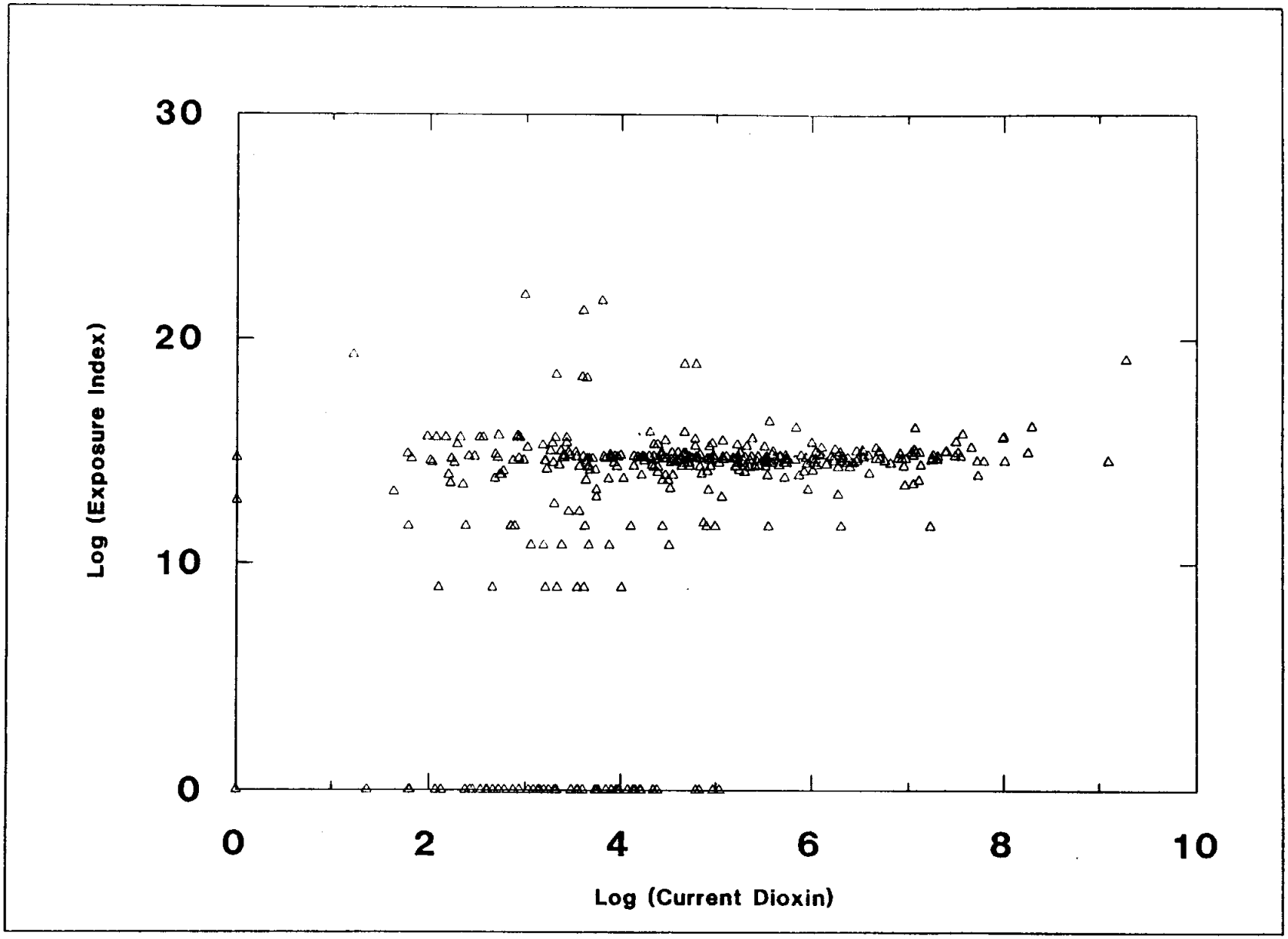

FIGURE 3-7. Logarithm of Current Dioxin versus Logarithm of the Exposure Index in Ranch Hand Enlisted Groundcrew (N=399)

are those used in tabular displays for the maximal assumption (see Explanation of Tables section in Chapter 4). The 0-5 ppt level was, of course, excluded under the maximal assumption.

Table 3-4 presents a breakdown within each of the three occupational strata.

Tables 3-5 and 3-6 show the relationship between initial dioxin body burden levels and the categorized exposure index. Ranch Hands with current dioxin less than or equal to 5 ppt were assigned a "missing" initial dioxin level. The cutpoints for the low, medium, and high initial dioxin levels are those used in tabular displays for the maximal assumption (see Explanation of Tables section in Chapter 4).

The logarithm of the current dioxin concentration is approximately lognormally distributed. Figure 3-8 shows the distribution of the logarithm of one plus the current dioxin concentration among the 804 Comparisons fully compliant to the 1987 examination and having

TABLE 3-4.

Categorized Exposure Index versus Current Dioxin Levels in Ranch Hands by Occupation

Occupation	Current Dioxin Level	Exposure Index				
		Zero	Low	Medium	High	Total
Officer	0-5 ppt	7	25	19	22	73
	Low	6	38	41	33	118
	Medium	6	26	44	50	126
	High	0	1	0	1	2
	Total	19	90	104	106	319
Enlisted Flyer	0-5 ppt	0	9	3	4	16
	Low	0	11	4	6	21
	Medium	0	21	35	20	76
	High	0	2	15	18	35
	Total	0	43	57	48	148
Enlisted Groundcrew	0-5 ppt	0	18	6	11	35
	Low	0	27	7	12	46
	Medium	0	62	55	51	168
	High	0	20	71	59	150
	Total	0	127	139	133	399

TABLE 3-5.

Categorized Exposure Index versus Initial Dioxin Level in Ranch Hands

Initial Dioxin Level	Exposure Index				
	Zero	Low	Medium	High	Total
Missing	7	52	28	37	124
Low	5	87	53	40	185
Medium	7	99	138	127	371
High	0	22	81	83	186
Total	19	260	300	287	866

TABLE 3-6.

Categorized Exposure Index versus Initial Dioxin Level in Ranch Hands by Occupation

Occupation	Initial Dioxin Level	Exposure Index				
		Zero	Low	Medium	High	Total
Officer	Missing	7	25	19	22	73
	Low	5	44	39	30	118
	Medium	7	20	46	53	126
	High	0	1	0	1	2
	Total	19	90	104	106	319
Enlisted Flyer	Missing	0	9	3	4	16
	Low	0	11	6	3	20
	Medium	0	21	34	21	76
	High	0	2	14	20	36
	Total	0	43	57	48	148
Enlisted Groundcrew	Missing	0	18	6	11	35
	Low	0	32	8	7	47
	Medium	0	58	58	53	169
	High	0	19	67	62	148
	Total	0	127	139	133	399

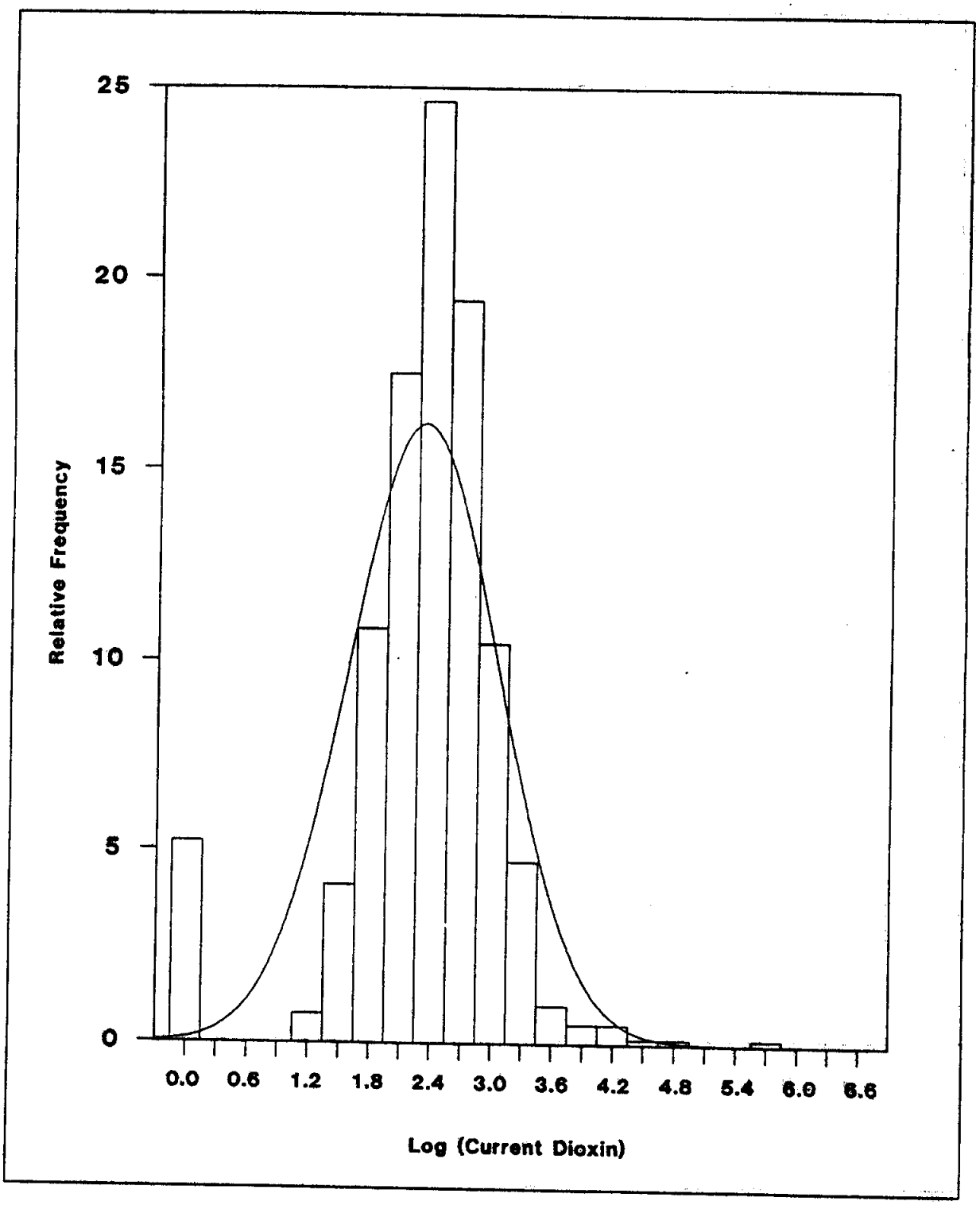

FIGURE 3-8. Relative Frequency Distribution of the Logarithm of Current Dioxin in Comparisons (N=804)

a dioxin assay result. A normal distribution was fit to these data and a multiple of the probability density function is plotted on the same graph. The fit is improved when the histogram is restricted to those Comparisons (n=762) having positive concentrations, as shown in Figure 3-9. The histogram of the logarithm of one plus current dioxin body burden in Ranch Hands is shown in Figure 3-10 with a multiple of the probability density function of the fitted normal distribution shown on the same plot.

SUMMARY

The indirectly calculated exposure index derived solely from personnel records and historical information has wide precedent in epidemiology. These data suggest that the work history-based exposure index methodology should be reconsidered in studies with exposures of short duration and low relative risks. The correlation between the AFHS exposure index and the dioxin body burden (current or initial levels) is weak although statistically significant. Cross tabulations of dioxin body burden levels versus the categorized exposure index, shown in Tables 3-2 through 3-6, indicate considerable misclassification if the dioxin measure (initial or current dioxin) is taken as the standard.

The dioxin measure is the preferred index of exposure because (a) it is a direct, rather than indirect measure of exposure, (b) the Ranch Hand levels appear logically placed relative to other cohorts, and (c) the within-occupation stratum levels appear to agree with exposure patterns described in Ranch Hand crew chief interviews conducted before the assay became available to participants in the AFHS.

Estimates of initial dioxin exposure will be improved with increased knowledge regarding its elimination in humans. New data in the Ranch Hand cohort and in people exposed to dioxin in Seveso, Italy, will be collected. The Seveso data will be used to evaluate the first-order elimination assumption. Variation in half-life with disease and changes in weight and body fat will be assessed with Ranch Hand data if the first-order elimination assumption (see Chapter 4) is supported by the Seveso data.

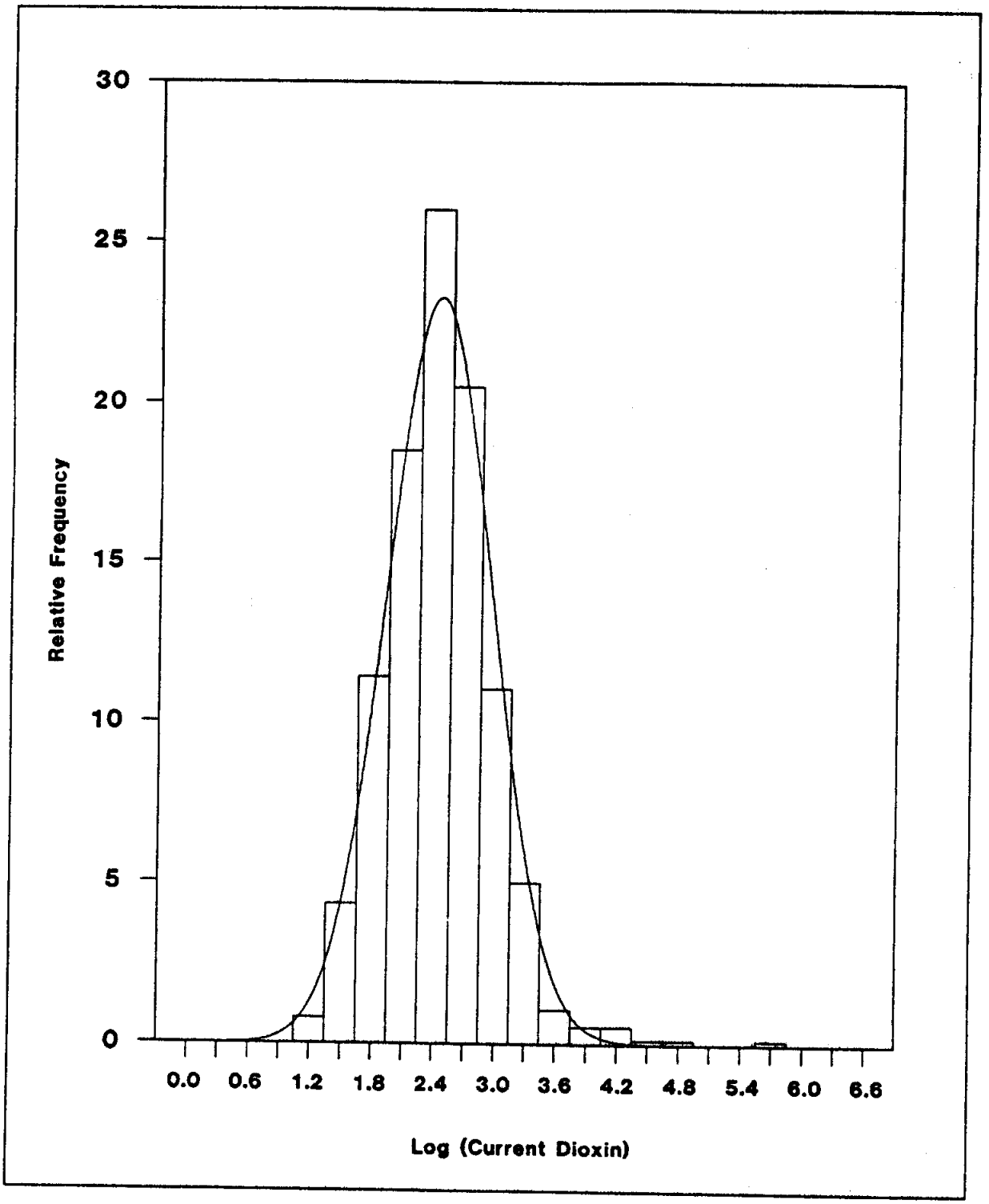

FIGURE 3-9. Relative Frequency Distribution of the Logarithm of Current Dioxin in Comparisons With Current Dioxin Greater Than Zero (N=762)

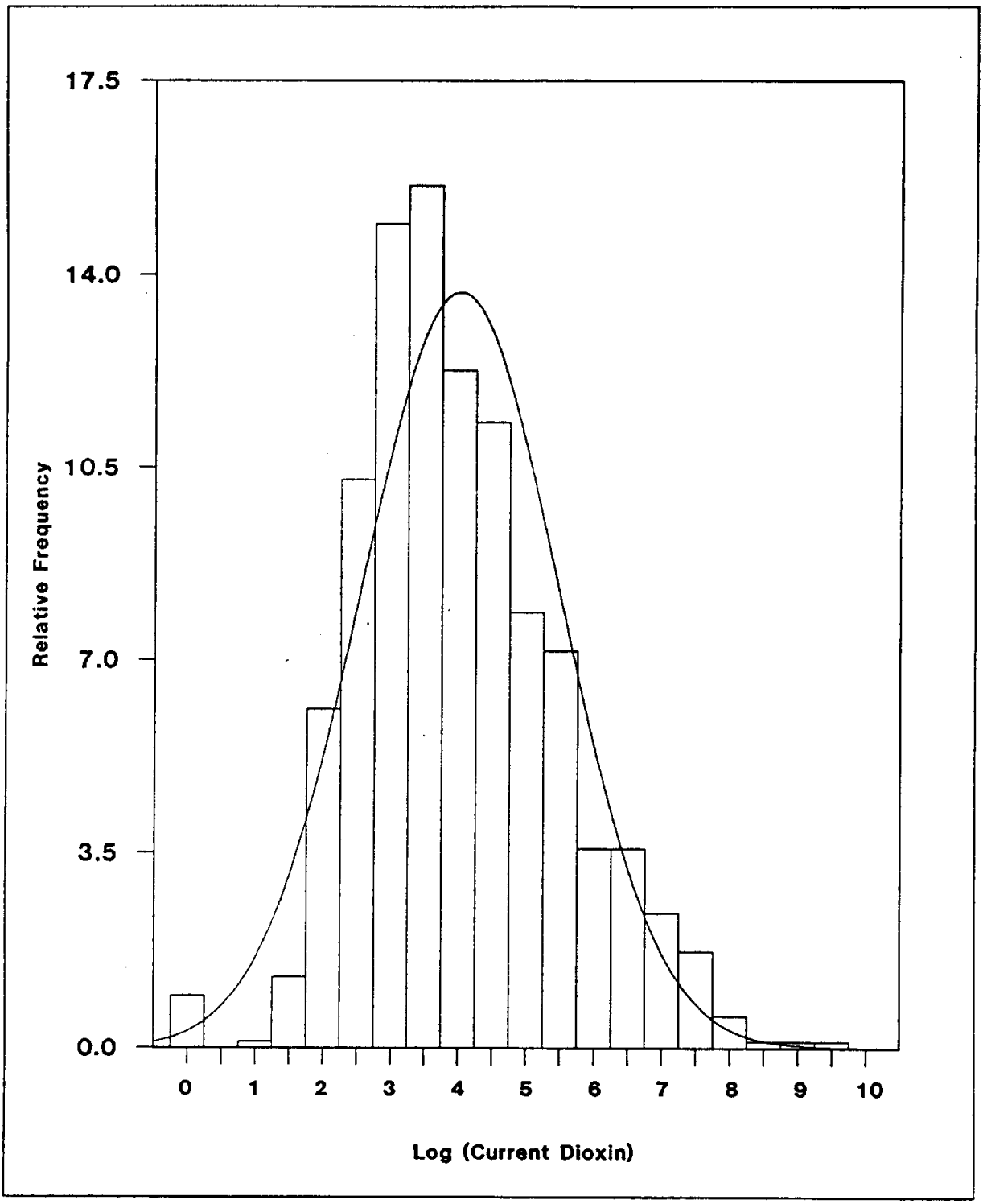

FIGURE 3-10. Relative Frequency Distribution of the Logarithm of Current Dioxin in Ranch Hands (N=866)

CHAPTER 3

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CHAPTER 4

STATISTICAL METHODS

This chapter summarizes statistical methods that were used for investigating relationships between serum dioxin measurements and health status of Ranch Hands and Comparisons. Current body burden dioxin levels were determined by the Centers for Disease Control (CDC) from serum samples taken from Ranch Hands and Comparisons. A variety of statistical procedures were applied to evaluate the relationships between specific health endpoints and dioxin, as measured from these serum samples.

MODELS AND ASSUMPTIONS

Prior Knowledge Regarding Dioxin

This study presents statistical analyses based on assumptions and models that were conceived in 1988 after the publication of the Ranch Hand dioxin pilot study and half-life substudy. At that time, available data regarding the elimination of dioxin in humans suggested that

- Measurements following the ingestion of dioxin by an individual showed that dioxin elimination appeared to be by first-order mechanisms (1).
- Air Force data on 36 Ranch Hand veterans with dioxin body burdens measured in blood drawn in 1982 and in 1987 produced a median half-life estimate of 7.1 years (2). The lack of correlation between individual half-lives and current dioxin levels supported the first-order elimination assumption.
- Assay results on 932 Ranch Hands and 888 Comparisons showed that the concentrations were lognormally distributed with the Ranch Hand distribution significantly shifted to the right of the Comparison distribution. The Comparison median was 4.2 ppt; the 98th percentile of the Comparison distribution was 10.17 ppt. The Ranch Hand median was 12.8 ppt and the 98th percentile was 168 ppt. Based on these data, levels at or below 10 ppt were considered background.

The term "elimination" denotes the overall removal of dioxin from the body. Some analyses in this report assume that the amount of dioxin in the body (C) decays exponentially with time according to the model $C = I \cdot \exp(-rT)$, where I is the initial level, $r = \log 2/H$, H is the half-life, and T is the time between the end of the Vietnam tour and the dioxin blood draw at the 1987 physical examination; this exponential decay law is termed first-order elimination in this report.

The first-order elimination assumption is not equivalent to assuming a one compartment model for dioxin distribution within the body. While a multicompartment model incorporating body composition and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) binding to tissue receptors would provide a detailed description of dioxin concentrations in different compartments, published multicompartment models for TCDD distribution within the body predict first-order elimination of TCDD, overwhelmingly due to fecal excretion (3). Direct

assessment of the first-order assumption with serial dioxin results taken over many years on a number of exposed individuals has not been, as yet, carried out.

The term "body burden" refers to the serum lipid-weight concentration of TCDD, expressed in parts per trillion (4, 5). The lipid-weight dioxin measurement, also called current dioxin body burden in this report, is a derived quantity calculated from the formula ppt = ppq•102.6/W, where ppt is the lipid-weight concentration, ppq is the actual weight of dioxin in the sample in femtograms, 102.6 corrects for the average density of serum, and W is the total lipid weight of the sample (4).

The relationship between the serum lipid-weight concentration of dioxin and lipid-weight concentrations in adipose tissue is a subject of continuing research. The correlation between the serum lipid-weight concentration and adipose tissue lipid-weight concentration of dioxin has been observed by Patterson et al. to be 0.98 in 50 persons from Missouri (6). Using the same data, Patterson et al. calculated the partitioning ratio of dioxin between adipose tissue and serum on a lipid-weight basis as 1.09 (95% C.I.: [0.97,1.21]). On the basis of these data, a one-to-one partitioning ratio of dioxin between lipids in adipose tissue and the lipids in serum cannot be excluded. Measurements of dioxin in adipose tissue generally have been accepted as representing the body burden concentration of dioxin. The high correlation between serum dioxin levels and adipose tissue dioxin levels in the Patterson et al. study suggests that serum dioxin is also a valid measurement of dioxin body burden.

Fundamental Limitations of the Serum Dioxin Data

There are two evident limitations to the available data:

1) While Ranch Hand and ingestion data do not appear to violate a first-order elimination assumption, no serially repeated dioxin assay results taken over many years are available yet with which to evaluate directly the adequacy of the first-order elimination model in humans.

2) At this time, it has not been determined whether Ranch Hands with dioxin burdens at or below 10 ppt were exposed and their body burdens had decayed to background levels since their duty in Vietnam or whether they were not exposed at all during their tour in Vietnam.

Health versus Dioxin in Ranch Hands

Because first-order elimination is suggested, but not validated directly in humans, the dioxin versus health relationship was assessed within Ranch Hands using two models. The first model directly depends upon the first-order elimination assumption; the second does not. In combination, these two models circumvent the first fundamental limitation by assessing the dioxin versus health relationship with and without first-order elimination. Table 4-1 shows these two models, their assumptions, advantages, and disadvantages for a continuously distributed health variable y.

In Table 4-1, the phrase "single dioxin dose" is a simplification of the process by which Ranch Hands accumulated dioxin during their tour of duty in Vietnam. This process, which undoubtedly varied from individual to individual, is unknown. However, the Ranch Hand tours generally were short (1 to 3 years) relative to the time elapsed since their tours. Hence, additional knowledge regarding the accumulation of dioxin during an individual Ranch Hand's tour, were it to become available, likely would not change conclusions drawn from any of the statistical analyses presented in this report.

Analyses based on model 1 are dependent directly on the first-order elimination assumption, while those based on model 2 are not. With model 1 one assumes that elimination is first-order and that the half-life is 7.1 years for all Ranch Hands. With model 2 one assumes nothing about the kinetics of dioxin elimination other than Ranch Hands received a dose in Vietnam and that their body burdens have decreased in an unspecified manner with time. Thus, with model 1 one assumes "everything" is known about dioxin elimination in Ranch Hands; with model 2 one assumes "nothing" about dioxin elimination in Ranch Hands. All health data were analyzed with both models to reduce the likelihood that an effect would be missed due to incorrect assumptions regarding dioxin elimination.

The introduction of the time-by-current dioxin interaction term ($b_3 T \log_2 [C]$) in model 2 allows investigation of the dioxin health relationship with respect to time. For example, such an effect would be detected by model 2 if there was no relationship between health and dioxin in the first few years after exposure and a strong positive relationship many years after exposure. In this case, if the effect were strong enough, it would be detected by the interaction coefficient (b_3) being significantly different from zero. Following that, analyses within time strata would find the coefficient (b_1) of $\log_2$ (C) significantly different from zero and positive for large values of time (T); no significant difference between b_1 and 0 for small values of T would be found. It is important to note that a significant effect of this kind could be due to the passage of time or to a higher initial dioxin level received by Ranch Hands in the later time stratum or both of these.

Analyses based on models 1 and 2 were carried out both adjusted and unadjusted for covariates.

No additional data or other information exist to determine whether any of the Ranch Hands with background levels (≤10 ppt) of current dioxin (n=345) received a dose above background levels in Vietnam. To accommodate this lack of knowledge, all analyses based on models 1 and 2 were carried out with these Ranch Hands excluded. Additionally, since 10 ppt may be considered arbitrary or too conservative, all analyses based on models 1 and 2 were carried out with Ranch Hands having less than or equal to 5 ppt (n=124) excluded. With the second approach, it is assumed that Ranch Hands currently having more than 5 ppt (the approximate Comparison median) were exposed in Vietnam and those with less than 5 ppt were not. These two assumptions are termed "minimal" (Ranch Hands with more than 10 ppt were exposed in Vietnam) and "maximal" (Ranch Hands with more than 5 ppt were exposed in Vietnam).

TABLE 4-1.

Models 1 and 2 for Assessing Health versus Dioxin in Ranch Hands Only: Assumptions, Advantages, and Disadvantages

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Model 1: $y = \beta_0 + \beta_1 \log_2(I) + e$

where

- y = health variable
- I = extrapolated initial dose, assuming first-order elimination, $I = C \cdot \exp(\log 2 \cdot T/H)$
- T = time between the end of the Vietnam Ranch Hand tour of duty and the 1987 physical examination
- C = current dioxin body burden, determined in 1987
- H = dioxin half-life in Ranch Hands assuming first-order elimination (7.1 years)
- e = zero mean normal error

Assumptions:	Ranch Hands received a single dioxin dose in Vietnam and background exposure thereafter.
	Ranch Hands experienced first-order dioxin elimination with a constant known half-life of 7.1 years.
	The error variance does not change with health status (y) or initial dioxin dose (I).
Advantages:	Easily interpretable.
	Most efficient if first-order elimination and constant half-life are valid assumptions and y is linearly related to $\log_2(I)$
Disadvantages:	Will be biased if first-order elimination or constant half-life assumption is not valid.
	Does not address time-related effects.

TABLE 4-1. (Continued)

Models 1 and 2 for Assessing Health versus Dioxin in Ranch Hands Only: Assumptions, Advantages, and Disadvantages

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Model 2: $y = \beta_0 + \beta_1 \log_2(C) + \beta_2 T + \beta_3 T \log_2(C) + e$

where

y = health variable
T = time between the end of the Vietnam Ranch Hand tour of duty and the 1987 physical examination
C = current dioxin body burden, determined in 1987
e = zero mean normal error

Assumptions:	Ranch Hands received a single dioxin dose in Vietnam and background exposure thereafter.
	Ranch Hand dioxin body burdens changed with time (T) in the same way for all individuals.
	The dioxin versus health relationship may change with time (T).
	The error variance does not change with values of the health variable (y), the current dioxin body burden (C), time (T), or the product of time and the logarithm of the current dioxin body burden ($T \log_2[C]$).
Advantages:	Does not depend on any particular elimination law or half-life assumptions.
	Assesses time-related effects.
Disadvantages:	Less easily interpreted than model 1.
	Less efficient than model 1 if first-order elimination and constant half-life are valid assumptions and y is linearly related to $\log_2(I)$.
	Biased if any of the assumptions are violated.

In summary, to address the second fundamental limitation, two assumptions about Ranch Hands with current dioxin body burdens less than 10 ppt were made. These minimal and maximal assumptions are

- *Minimal assumption: Ranch Hands with less than or equal to 10 ppt were not exposed to dioxin in Vietnam*
- *Maximal assumption: Ranch Hands with less than or equal to 5 ppt were not exposed to dioxin in Vietnam.*

The terms minimal and maximal were given because fewer Ranch Hands were exposed under the minimal than under the maximal assumption. The numbers 5 and 10 correspond to the approximate median and 98th percentile of the Comparison current dioxin distribution. Based on this Comparison dioxin distribution, current dioxin levels less than 10 ppt are called background levels.

To assess the dioxin versus health relationship while addressing the second fundamental limitation, all analyses based on models 1 and 2 were carried out under the minimal and again under the maximal assumptions. Under the minimal assumption, Ranch Hands with less than or equal to 10 ppt were excluded from the analyses. Under the maximal assumption, Ranch Hands with less than or equal to 5 ppt were excluded from the analyses.

Table 4-2 shows counts of exposed Ranch Hands under the minimal and maximal assumptions with initial and current dioxin trichotomized for tabular presentation. Ranch Hands under the maximal assumption are termed the "maximal cohort"; those under the minimal assumption are termed the "minimal cohort." The time between the end of tour and the 1987 physical examination is dichotomized at 18.6 years (corresponding approximately to the year 1969), the approximate median of the maximal cohort. The cutpoints for stratifying dioxin levels (I and C) were the approximate 25th and 75th percentiles and were specific to a particular cohort.

Health versus Dioxin in Ranch Hands and Comparisons

Finally, an assessment of the health consequences of current dioxin body burdens above background was carried out with a third model (model 3) that required no assumptions about when or how increased dioxin body burdens were attained and was applied to both Ranch Hand and Comparison data. This model assessed health versus categorized current dioxin body burden (D) with four levels, found in Table 4-3.

The cutpoint between the low and high categories, 33.3 ppt, is the approximate median dioxin level of Ranch Hands having more than 15 ppt. Ranch Hands having between 10 ppt and 15 ppt were excluded from these categorized dioxin analyses in an attempt to avoid misclassification of Ranch Hands to the unknown and low categories due to various sources of variation in the dioxin measurement.

Table 4-4 shows counts of participants within each level of categorized current dioxin. The relationship between current health and categorized dioxin body burden was based on the model shown in Table 4-5.

TABLE 4-2.

Ranch Hand Sample Sizes Under the Minimal and Maximal Assumptions

Assumption	Stratum Name	Initial Dioxin (I) Stratum	Initial Dioxin (I) Count	Current Dioxin (C) Stratum	Current Dioxin (C) T≤18.6 Count	Current Dioxin (C) T>18.6 Count
Minimal	Low	52<I≤93	130	10<C≤14.65	72	58
	Medium	93<I≤292	260	14.65<C≤45.75	128	132
	High	292<I	131	45.75<C	54	77
		Total	521		254	267
Maximal	Low	25<I≤56.9	185	5<C≤9.01	106	79
	Medium	56.9<I≤218	371	9.01<C≤33.3	191	179
	High	218<I	186	33.3<C	83	104
		Total	742		380	362

TABLE 4-3.

Current Dioxin Body Burden (D) Categorized in Ranch Hands and Comparisons for Model 3

Value	Definition
Background	Comparisons with up to 10 ppt
Unknown	Ranch Hands with up to 10 ppt
Low	Ranch Hands with more than 15 and up to 33.3 ppt
High	Ranch Hands with more than 33.3 ppt

TABLE 4-4.

Counts of Participants by Level of Categorized Current Dioxin (D)

Level	Count
Background	786
Unknown	345
Low	196
High	187
Total	1,514

TABLE 4-5.

Model 3 for Assessing Health versus Categorized Current Dioxin Body Burden in Ranch Hands and Comparisons

Model 3: $y = \beta_0 + \beta_1 D + e$

where

y = health variable
D = categorized current dioxin
e = zero mean normal error

Assumptions: Dioxin body burden has accumulated with time.
The error variance does not change with categorized current dioxin body burden (D).

Advantage: Requires no assumption regarding the time course of dioxin accumulation or elimination.

Disadvantages: Makes no use of prior belief that Ranch Hands received an unusually large dioxin dose in Vietnam.
Does not address time-related effects.

In addition to assessing the overall mean change in the health variable (y) with levels of categorized current dioxin (D), the mean values of y within the unknown, low, and high categories were contrasted with the mean values of y within the background category.

Figure 4-1 summarizes the current dioxin levels used in models 1, 2, and 3.

Data Error

After the serum dioxin analyses were well underway, an error was discovered with respect to the race of one Comparison. The participant (subject 36410) was listed in the data base as a non-Black when in fact he was a Black. The Comparison was a 49-year-old at the Baseline examination and he was a member of the enlisted groundcrew cohort. His current serum dioxin value was 3.97 ppt as determined from the assay performed on the 1987 examination serum sample. The following abnormal medical conditions were noted for this individual: hepatomegaly, reported and verified hypertension, hyperpigmentation, and acne. The data error was corrected for the cardiovascular, malignancy, and dermatology assessments. Because the individual was a Comparison only the model 3 analyses of the other clinical area assessments were affected.

Bias Calculations

In any epidemiologic study, investigators must be concerned with avoiding spurious conclusions that are attributable to limitations in study design or analysis. The introduction of the dioxin assay as the measure of exposure in this study has provided the best available information regarding dioxin exposure in Ranch Hands and Comparisons. Uncertainties remain, however, regarding the choice of statistical models with which to assess the relationship between dioxin and health.

Biased results will be produced if the assumptions underlying any of the three statistical models are violated. Of the three models, model 1 is the most vulnerable to this kind of bias, since it depends directly on two unvalidated assumptions: (a) that dioxin elimination is first-order and (b) all Ranch Hands eliminate dioxin at the same rate (all Ranch Hands have the same dioxin half-life of 7.1 years). Air Force investigators currently are gathering additional data to evaluate both assumptions. The original half-life study on 36 Ranch Hands is being expanded to approximately 500 Ranch Hands. Assuming that dioxin elimination is first-order, this larger study will allow an assessment of half-life variability with weight changes, percent body fat changes, and disease since exposure. Additionally, the Air Force is collaborating with the CDC and Italian health authorities to assay serum collected periodically from people exposed in the Seveso accident. These data will consist of five dioxin measurements taken over a period of 10 years on 20 males who were adults at the time of the accident and will allow, for the first time, a direct assessment of the first-order elimination assumption in humans.

Until the Ranch Hand half-life study is expanded, the only available information regarding half-life variation in Ranch Hands is that derived from the smaller cohort of 36 subjects. Unpublished analyses of half-life heterogeneity among those 36 Ranch Hands suggest that half-life varies with relative weight changes between 1982 and 1987. With relative weight changes dichotomized at the median (2.7%), the 18 Ranch Hands below the median have an estimated half-life of 9.7 years (95% C.I.: [6.8,17.3]) and the 18 Ranch

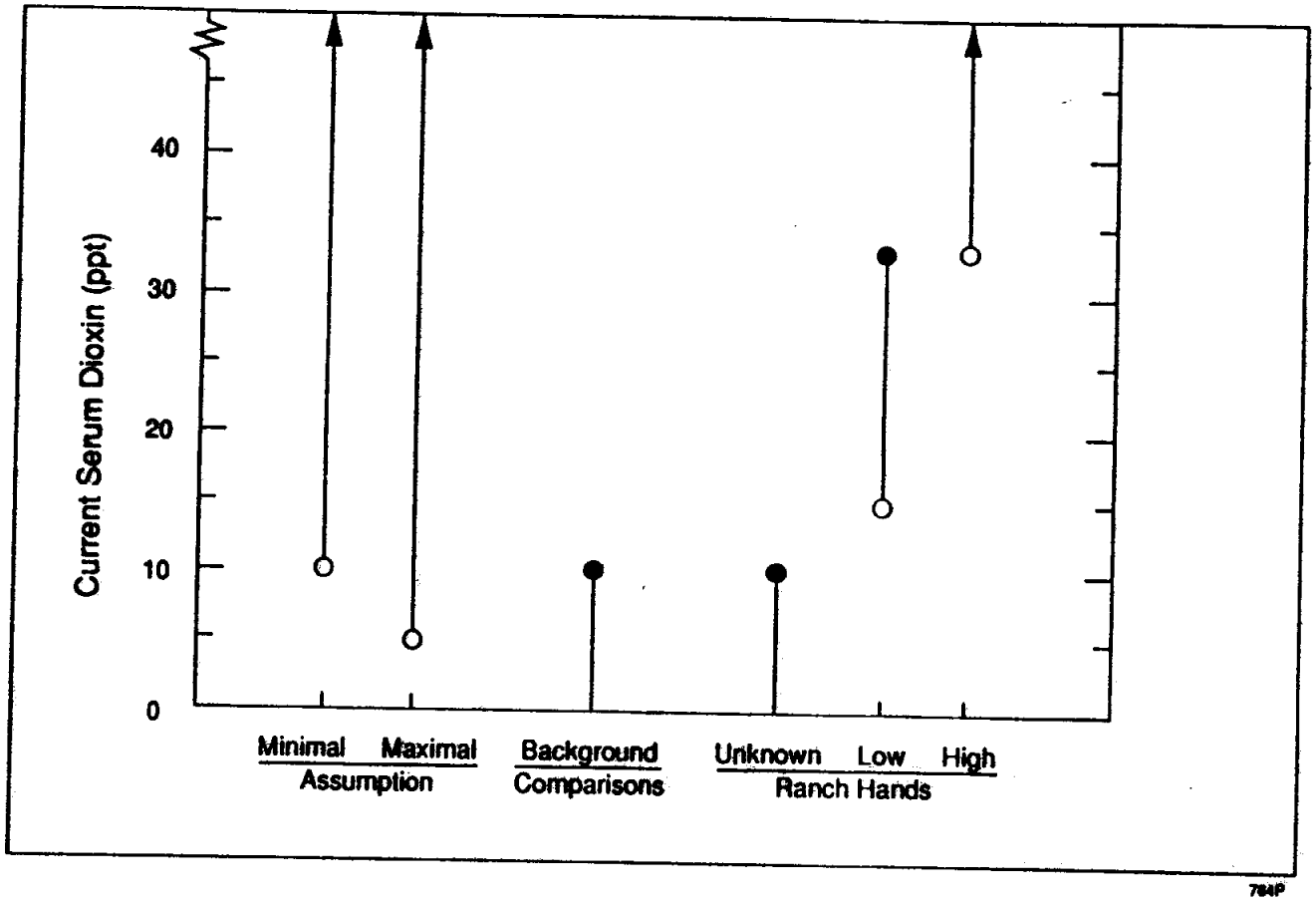

FIGURE 4-1. Ranges of Current Serum Dioxin Levels Used in Different Analysis Models

Hands above the median have an estimated half-life of 6.2 years (95% C.I.: [5.0,8.0]). The analysis showed a significant difference between these two half-lives (p=0.02). The two confidence intervals overlap because they are not derivable from the test for equality of half-lives. "Apparent" half-life decreases may be due to weight gain because of dilution of the body burden when it is redistributed to the new adipose tissue. Conversely, when there has been weight loss, the body burden may be redistributed in less adipose tissue and the serum concentration increases.

If these results are generalized to all Ranch Hands, statistical inference based on model 1 will be biased. For example, if the first-order elimination assumption is valid, but the constant half-life assumption is not, and there is no misclassification with regard to health status, odds ratios expressing the relationship between health and dioxin based on model 1 will be biased toward unity. That is, a misspecification of a constant half-life when, in fact, half-life changes with weight changes, will lead to misclassification with regard to dioxin level and therefore reduce our ability to detect an association between health and dioxin. To evaluate this possibility, the bias induced in the odds ratio under the maximal assumption and the computation of initial dioxin body burden assuming a constant half-life of 7.1 years (when in fact 50 percent of Ranch Hands have a dioxin half-life of 6 years and the other 50 percent have a dioxin half-life of 10 years) was calculated (7). In carrying out this calculation, it was assumed that initial dioxin had been dichotomized to high and low, with Ranch Hands assigned to the high category if their calculated initial dioxin level was greater than 218 ppt and assigned to the low category if their level was less than 218 ppt. The sample sizes of the real maximal cohort were used in the calculation; 186 Ranch Hands had a high initial dose and 556 had a low initial dose. With these assumptions, 76.3 percent of Ranch Hands assigned to the high category and 6.1 percent assigned to the low category truly had an initial dose above 218 ppt. The resultant bias in the odds ratio due to this misclassification depends on the true value of the odds ratio and the disease prevalence in the low category. For example, if the true odds ratio is 2.0 and the disease prevalence in the low initial dioxin category is 5 percent, this misclassification will produce an odds ratio of 1.7. Table 4-6 shows other values of the biased odds ratio produced by this misclassification for true odds ratios from 1 to 3 and the disease prevalence in the low initial dioxin category held fixed at 5 percent. There is no bias under assumptions if there is no association between initial dioxin and disease (true odds ratio equal to 1.0).

Model 2 also may be biased if, as suggested by the weight change analysis on the 36 Ranch Hands in the half-life study, 50 percent of Ranch Hands are fast dioxin eliminators (having a short half-life) and 50 percent of Ranch Hands are slow eliminators (with a longer half-life). If this attribute is not taken into account in the analysis (such as through adjustment for relative weight change), then the odds ratio relating disease to dioxin exposure will be biased toward unity. Again, disease status is assumed to be determined without error. For example, if slow eliminators experience an effect that does not become expressed until 20 years after exposure, if fast eliminators do not experience the effect, and if the analysis is not adjusted for relative weight change, then the ability of the model to detect the effect will be attenuated by the lack of adjustment. The extent of this bias toward the null depends on the nature of the four-factor interaction between health, current dioxin, time, and relative weight change, as well as upon the disease prevalence among Ranch Hands with low dioxin levels at each combination of categories of time and relative weight change. Bias calculations for this scenario, therefore, are more complicated and speculative than those presented for model 1 and were not pursued further.

TABLE 4-6.

Biased Odds Ratios Produced by a Misspecification of the Half-Life in the Calculation of the Initial Dioxin Body Burden in Model 1, Assuming a Disease Prevalence of 5 Percent in Ranch Hands Having a Low Calculated Initial Dose

True Odds Ratio	Biased Odds Ratio
1.0	1.0
1.5	1.3
2.0	1.7
2.5	2.0
3.0	2.2

Model 3 requires fewer assumptions than models 1 or 2, but is susceptible to bias due to misclassification or incorrect modeling. Biased results most likely are to occur with model 3 due to the failure to adjust for an important covariate. Every attempt, however, has been made in this report to adjust for all known important covariates.

The Correlation Between Initial Dioxin and Current Dioxin

The extrapolated initial dioxin dose is correlated highly with current dioxin level (correlation coefficient >0.98 for both the minimal and maximal cohorts). The same high correlation is, of course, seen between the logarithms of these quantities. The reason for the high correlation is that the initial dioxin dose is the current dioxin body burden multiplied by 2 raised to the power T/7.1. This high correlation is simply an expression of the fact that if the first-order model is valid and if dioxin half-life is constant, then models 1 and 2 nearly are redundant because the variation of time (T) is relatively small (see Figure 4-2).

FACTORS DETERMINING ANALYTICAL METHOD

For a specified questionnaire-based or clinical measurement determined from the physical or laboratory examination, the selection of an analytical method was dependent on each of the following:

- Dependent Variable Form — Continuous or discrete
- Serum Dioxin Estimate — Initial dioxin, current dioxin and time since tour, or categorized current dioxin incorporating group membership
- Analysis Type — Unadjusted, adjusted, or longitudinal

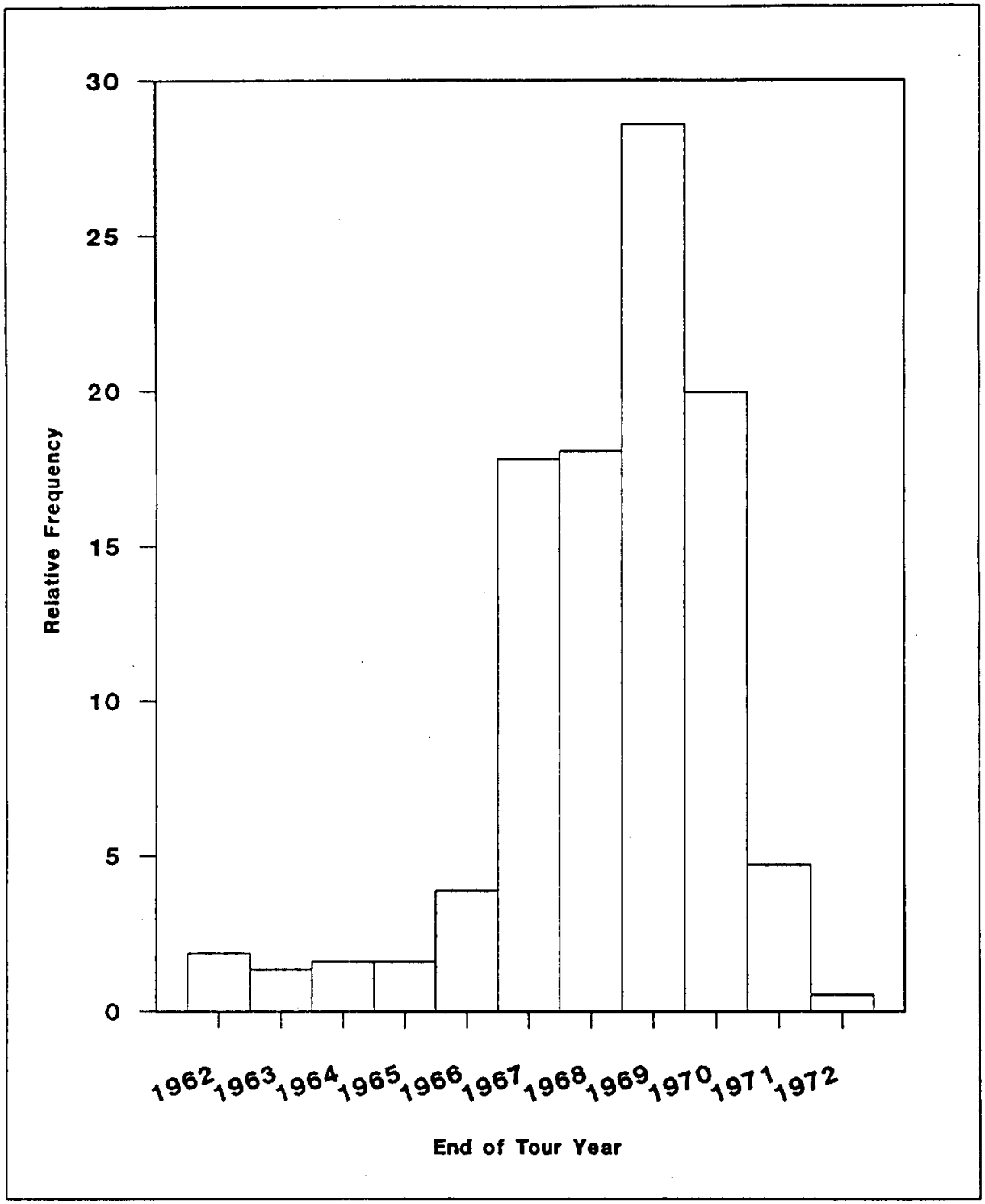

FIGURE 4-2. Relative Frequency Distribution of End of Tour Year in Ranch Hands Under the Maximal Assumption (N=742)

- Analysis Cohort(s) — Ranch Hands: minimal assumption, Ranch Hands: maximal assumption, and defined subsets of Ranch Hands and Comparisons for the categorized current dioxin variable.

Appendix Table C-1 specifies 30 separate analysis situations based on dependent variable form, serum dioxin estimate, analysis type, and analysis cohort. For each of the 30 situations, the statistical method is specified.

ANALYSIS METHODOLOGIES

As in previous Air Force Health Study reports, current health dependent variables can be either continuous or discrete. For the former case, the general linear model approach is the basis for applying such techniques as simple and multiple linear regression, analysis of variance, analysis of covariance, and repeated measures analysis. This approach permits model fitting of the dependent variable as a function of dioxin, relevant covariates, dioxin-by-covariate interactions, and interactions between covariates. As part of the previous analyses of 1987 data, the health variables were examined to ensure that assumptions underlying statistical methods were met. Transformations used to enhance normality for specific continuous health variables in the previous analyses of 1987 data also were used for the serum dioxin analysis. For these continuous analyses, SAS® GLM (8) was used. When a "best" model was fitted, tests of significance for a dioxin effect were made. Associations with a p-value less than or equal to 0.05 are described as significant, and associations with a p-value greater than 0.05 but less than or equal to 0.10 are termed marginally significant or borderline significant. If there was a significant interaction between the dioxin variable and any covariate, the dioxin effect was assessed using stratification by different levels of the covariate(s) involved in the interaction.

Discrete dependent variables were analyzed by methods parallel to those used for continuous variables. For dichotomous variables, logistic regression was performed using BMDP®-LR (9). For polychotomous dependent variables, log-linear modeling was performed using BMDP®-4F (9) by incorporating the full k-factor interaction term involving the k covariates used in the model. For the log-linear modeling approach, covariate information must be categorized. Because of this required categorization of the covariate(s), the marginals were fixed in the log-linear model (10), effectively converting the log-linear model into a logit model. For the log-linear model, the significance of the relative risk for a particular categorized dioxin variable (i.e., categorized initial dioxin, categorized current dioxin and categorized time, or categorized current dioxin for specified subsets of Ranch Hands and Comparisons) was determined by examination of the appropriate model, as determined by the model that includes all statistically significant effects and a dioxin measure, or by examination of the significant interactions. Adjusted relative risks were derived from the coefficients of the appropriate model.

Selected longitudinal analyses were performed investigating changes in health status between 1982 and 1987, for each of the three dioxin analysis models. The variables selected for longitudinal study were chosen prior to all 1987 examination data analyses. In the longitudinal analysis of discrete variables, only those participants whose health was classified as normal in 1982 were included in the analysis of the participants' health at the

1987 examination. Analysis was performed in this manner to investigate any temporal effects of dioxin in the subgroup at risk (i.e., those participants who could become abnormal over the time span). The rate of abnormalities under this restriction approximates an incidence rate between 1982 and 1987. The dependent variable in this type of analysis was the health of participants at the 1987 examination whose health was normal in 1982. The independent variable(s) were the appropriate dioxin measures.

For some variables, measurements in 1985 were substituted for 1982 measurements because the variable was not analyzed at the 1982 examination or inherently was different from the 1987 variable. For example, to enhance comparability, the longitudinal analyses for the neurological assessment were based on changes between 1985 and 1987 because SCRF conducted both of these examinations.

Both the general linear model and the logistic regression model approaches were applied using covariate information in either the discrete or the continuous form. Table 4-7 provides a summary of the basic statistical methods for the serum dioxin analyses.

MODELING STRATEGY

In each clinical category, many covariates were considered for inclusion in the statistical models relating specific health endpoints and dioxin. The large number of covariates, consequent interaction terms, and resulting difficulties of interpretation obligated the adoption of a strategy for identifying a moderately simple model using a stepwise strategy, as defined below. Interpretation of possible dioxin relationships was then made in the context of this simpler model.

In general, based on one of the adjusted analysis models described in Appendix Table C-1, an initial model was constructed containing any requisite two or three-factor interaction terms. As a first step, screening was performed at the 0.15 significance level to eliminate unnecessary two- and three-factor interactions. A hierarchical stepwise deletion strategy was applied at the 0.15 significance level on the set of main effect covariates (to address possible confounding effects between the covariates and dioxin) and at the 0.05 significance level for interactions. In general, the only effects not subject to the deletion strategy were the serum dioxin variables of interest (i.e., initial dioxin; current dioxin, time since tour, and current dioxin-by-time interaction; categorized current dioxin). With the objective of producing the simplest model, other lower-order effects were retained in the model only if involved in significant higher-order interactions. Significant interactions between covariates were retained as terms in the model.

The modeling strategy was refined slightly for adjusted statistical analyses of discrete dependent variables for particular clinical areas where a large number of covariates and/or sparse number of abnormalities were encountered. In these situations, the starting model included all main effects and excluded all interactions. Main effects were stepped out of the model if the associated p-value was greater than 0.15 and interactions were entered into the model if the associated p-value was less than or equal to 0.05. The alternative strategy was used to avoid overspecification of the model and minimize collinearity among terms that can lead to imprecise parameter and standard error estimates.

TABLE 4-7.

Summary of Statistical Procedures

Chi-square Contingency Table Test

The chi-square test of independence (11) is calculated for a contingency table by the following formula:

$$\chi^2 = \Sigma(f_o - f_e)^2/f_e$$

where the sum is taken over all cells of the contingency table and

f_o = observed frequency in a cell

f_e = expected frequency under the hypothesis of independence.

Large values indicate deviations from the null hypothesis and are tested for significance by comparing the calculated χ^2 to the tables of the chi-square distribution.

Fisher's Exact Test

Fisher's exact test (11) is a randomization test of the hypothesis of independence for a 2 x 2 contingency table. This technique was used for small samples and sparse cells. This is a permutation test based on the exact probability of observing the particular set of frequencies, or of one more extreme.

Correlation Coefficient (Pearson's Product-Moment)

The population correlation coefficient (12), ρ, measures the strength of the linear relationship between two random variables X and Y. A commonly used sample-based estimate of this correlation coefficient is

$$r = \frac{\Sigma(x_i - \bar{x})(y_i - \bar{y})}{\left[\Sigma(x_i - \bar{x})^2 \Sigma(y_i - \bar{y})^2\right]^{1/2}}$$

where the sum is taken over all (x,y) pairs in the sample. A Student's t-test based on this estimator is used to test for a significant correlation between the two random variables of interest. For the sample size of 521 (the size of the Ranch Hand cohort under the minimal assumption), a sample correlation coefficient of ±0.086 is sufficient to attain a statistically significant correlation at a 5 percent level for a two-sided hypothesis test. Assuming normality of X and Y for the sample size of 742 under the maximal assumption, a sample coefficient of ±0.072 is sufficient.

TABLE 4-7. (Continued)

Summary of Statistical Procedures

General Linear Models Analysis

The form of the general linear model (13) for two independent variables is

$$Y = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \varepsilon$$

where

Y	=	dependent variable (continuous)
α	=	level of Y at $X_1 = 0$ and $X_2 = 0$, i.e., the intercept
X_1, X_2	=	measured value of the first and second independent variables, respectively, which may be continuous or discrete
β_1, β_2	=	coefficient indicating linear association between Y and X_1, Y and X_2, respectively; each coefficient reflects the effect on the model of the corresponding independent variable adjusted for the effect of the other independent variable.
β_{12}	=	coefficient reflecting the linear interaction of X_1 and X_2, adjusted for linear main effects
ε	=	error term.

This model assumes that the error terms are independent and normally distributed with a mean of 0 and a constant variance. Extension to more than two independent variables and interaction terms is immediate.

Simple linear regression, multiple linear regression, analysis of variance, analysis of covariance, and repeated measures analysis of variance are all examples of general linear models analysis.

Logistic Regression Analysis

The logistic regression model (11, 14) enables a dichotomous dependent variable to be modeled in a regression framework with continuous and/or discrete independent variables. For two risk factors, such as dioxin and age, the logistic regression model would be

$$\text{logit } P = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \varepsilon$$

TABLE 4-7. (Continued)

Summary of Statistical Procedures

where

P	=	probability of disease for an individual with risk factors X_1 and X_2
logit P	=	ln (P/1-P), i.e., the log odds for disease
X_1	=	first risk factor, e.g., dioxin
X_2	=	second risk factor, e.g., age.

The parameters are interpreted as follows:

α	=	log odds for the disease when $X_1 = 0$ and $X_2 = 0$
β_1	=	coefficient indicating the dioxin effect adjusted for age
β_2	=	coefficient indicating the age effect adjusted for dioxin
β_{12}	=	coefficient indicating the interaction between dioxin and age, adjusted for linear main effects
ε	=	error term.

In the absence of an interaction ($\beta_{12} = 0$) for a dichotomous risk factor (e.g., Comparisons, Ranch Hands), $\exp(\beta_1)$ reflects the adjusted odds ratio for individuals in group 1 ($X_1 = 1$) relative to group 0 ($X_1 = 0$). If the probability of disease is small, the odds ratio will be approximately equal to the relative risk. In the absence of an interaction for a continuous risk factor (e.g., initial dioxin in its continuous form), $\exp(\beta_1)$ reflects the adjusted odds ratio for a unit increase in the risk factor. If the risk factor is expressed in logarithmic (base 2) form, $\exp(\beta_1)$ reflects the adjusted odds ratio for a twofold increase in the risk factor.

Throughout this report, the adjusted odds ratios will be referred to as adjusted relative risks. Correspondingly, in the absence of covariates (i.e., unadjusted analysis), the odds ratios will be referred to as estimated relative risks.

This technique will also be used for longitudinal analyses of dichotomous dependent variables to examine changes in health status between 1982 (or 1985) and 1987 in relation to the dioxin measures.

TABLE 4-7. (Continued)

Summary of Statistical Procedures

Log-linear Analysis

Log-linear analysis (11) is a statistical technique for analyzing cross-classified data or contingency tables. A saturated log-linear model for a three-way table is

$$\ln (Z_{ijk}) = U_o + U_{1(i)} + U_{2(j)} + U_{3(k)} + U_{12(ij)} + U_{23(jk)} + U_{13(ik)} + U_{123(ijk)}$$

where

Z_{ijk} = expected cell count

$U_{1(i)}$ = specific one-factor effect

$U_{12(ij)}$ = specific two-factor effect or interaction

$U_{123(ijk)}$ = three-factor effect or interaction.

The simplest models are obtained by including only the significant U-terms. Adjusted relative risks are derived from the estimated U-terms from an adequately fitting model.

In the analysis of a particular health variable, when no dioxin-by-covariate interactions were significant at the 0.05 level, adjusted means (15) or relative risks were presented. If a dioxin-by-covariate interaction was significant at the 0.05 level, the behavior of the dioxin variable was explored for different levels (categories) of the covariate to identify subpopulations for which a dioxin relationship might exist. Further, for illustrative purposes, if any dioxin-by-covariate interaction was significant at a level between 0.01 and 0.05, the adjusted means or relative risks also were presented, after dropping the interaction terms from the model.

In some instances a followup model also was performed that excluded a highly significant interaction ($p<0.01$). This optional model was run at the discretion of the analyst in an attempt to simplify the interpretation that may be complicated by an interaction difficult to explain from a clinical perspective.

For all models that included a dioxin-by-covariate interaction, the stratified results presented in the appendices display adjusted relative risks, confidence intervals, and associated p-values determined from a model that included the interaction term. However, in the model 2 analyses the p-values for the stratified current dioxin-by-time since tour interaction terms were determined from separate models for each covariate stratum; similarly in the model 3 analyses, the overall p-values were determined from separate models.

The adjusted models assessed the statistical significance of interactions between dioxin and the covariates to determine whether the relationship between dioxin and the dependent variable (health-related endpoint) differed across levels of the covariate. In many instances the clinical importance of a statistically significant dioxin-by-covariate interaction is unknown or uncertain. The clinical relevanee of a statistically significant interaction would be strengthened if the same interaction persisted among related endpoints. It is recognized that due to the large number of dioxin-by-covariate interactions that were examined for approximately 300 variables, some of the dioxin-by-covariate interactions judged significant at the 0.05 level might be spurious; i.e., chance occurrences not of biological/clinical relevance. This should be considered when significant dioxin-by-covariate interactions are interpreted. It is important that the size of the p-value associated with each dioxin-by-covariate interaction be weighed carefully; for this reason, if the p-value for a dioxin-by-covariate interaction was between 0.01 and 0.05, the adjusted means or relative risks (omitting the interaction) were reported.

For the neurology, cardiovascular, renal, and endocrine clinical assessments, additional analyses were performed when certain covariates were retained in the final model. These covariates were variables that may have been affected by dioxin exposure and included diabetic class (neurology and renal), percent body fat (cardiovascular and endocrine), and cholesterol (cardiovascular). Due to the association between these covariates and dioxin, both the statistical and clinical interpretation of other health variables can be affected. Analyses were consequently performed with these covariates in the final model, and with the covariates removed from the model. Tabular results with these covariates in the model are given in the body of the clinical chapter; results with these covariates removed are given in the associated chapter appendix.

POWER

Conducting a statistical test using a type I error, also called alpha level, of 0.05 means that, on the average in 5 cases out of 100, a false conclusion would be made that an association (dioxin effect) exists when, in reality, there is no association. The other possible inference error (called a type II error) is the failure to detect an association when one actually exists. The probability of a type II error for a statistical test is 1 minus the power of the test. The power of the test is the probability that the test will reject the hypothesis of no dioxin effect when an effect does in fact exist. The power of a test depends on the distribution of the dioxin data, the sample size, the disease prevalence rate, and the true dioxin effect measured in terms of the relative risk.

Table 4-8 contains the approximate power for detecting specified relative risks for a given prevalence rate (discrete dependent variable), using initial dioxin in its continuous form and an alpha level of 0.05 for a two-sided test under the minimal assumption (n=521). The corresponding power under the maximal assumption is slightly higher. Figure 4-3 presents a graphical display of the power at different prevalence rates, where the different curves represent relative risks of 1.1, 1.2, 1.3, 1.4, and 1.5. Power calculations were performed using the logarithm (base 2) of initial dioxin, and consequently the relative risk is for a twofold increase in initial dioxin. These calculations also assume approximate prevalences at the mean $\log_2$ (initial dioxin) value of 7.49, corresponding to an initial dioxin level of 180 ppt.

TABLE 4-8.

Power to Detect an Initial Dioxin Effect Based on the Minimal Assumption at a 5 Percent Significance Level (Discrete Dependent Variable)

Prevalence Rate of Disease	Relative Risk						
	1.10	1.20	1.30	1.40	1.50	1.75	2.00
0.005	0.05	0.07	0.09	0.12	0.17	0.33	0.54
0.01	0.06	0.09	0.13	0.20	0.29	0.56	0.80
0.02	0.07	0.12	0.21	0.34	0.49	0.82	0.96
0.03	0.08	0.16	0.29	0.46	0.64	0.93	0.99
0.04	0.08	0.19	0.36	0.57	0.75	0.97	1.00
0.05	0.09	0.22	0.43	0.65	0.83	0.99	1.00
0.10	0.13	0.36	0.66	0.88	0.97	1.00	1.00
0.15	0.16	0.47	0.79	0.95	0.99	1.00	1.00
0.20	0.18	0.55	0.86	0.97	1.00	1.00	1.00

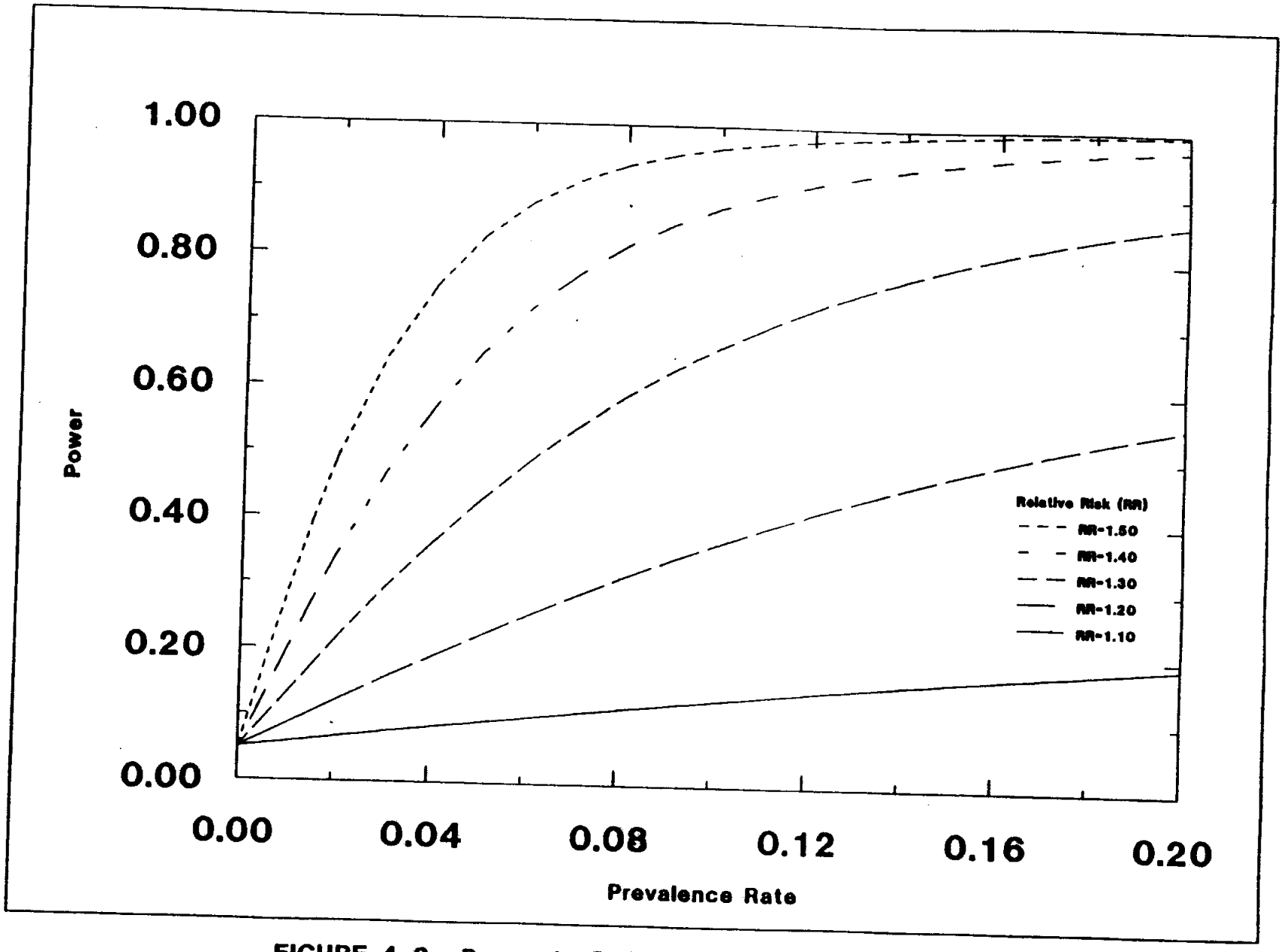

FIGURE 4-3. Power to Detect an Initial Dioxin Effect (Discrete Dependent Variable)

As an example, using age-adjusted incidence rates for all U.S. males (based on data from the Surveillance Epidemiology and End Results program of the National Cancer Institute), prevalence rates for all cancers, non-Hodgkin's lymphoma (NHL), and soft tissue sarcoma (STS) were estimated as 0.07, 0.002, and 0.001, respectively. Thus, Table 4-8 shows at least a power of 0.80 to detect a relative risk of 1.5 or greater given an estimated prevalence of 0.07 for all cancers. For the estimated prevalences of NHL and STS, the power to detect a relative risk of 2.0 would be less than 0.50.

Table 4-9 provides the same information for continuous variables in terms of coefficients of variation (100 times the standard deviation of the dependent variable divided by the mean of the dependent variable) and the proportion mean change. The proportion mean change in this table is defined as the change in the expected value (mean) of the dependent variable for a twofold increase in initial dioxin relative to the dependent variable mean. These mean changes are evaluated at the mean $\log_2$ (initial dioxin) value of 7.49, corresponding to an initial dioxin level of 180 ppt. The proportion mean change corresponds mathematically to the slope of the initial dioxin variable divided by the dependent variable mean, assuming no transformation of the dependent variable. An analogous quantity can be derived based on transformed statistics. Figure 4-4 shows a graphical display of the power at a given proportion mean change, where the different curves represent coefficients of variation of 5, 10, 25, 50, and 75. In this study, continuously distributed laboratory results were subject to a laboratory-error coefficient of variation of less than 3 percent.

TABLE 4-9.

Power to Detect an Initial Dioxin Effect Based on the Minimal Assumption at a 5 Percent Significance Level (Continuous Dependent Variable)

	Coefficient of Variation (σ/μ)				
Mean Change	5	10	25	50	75
0.005	0.78	0.28	0.09	0.06	0.05
0.01	1.00	0.78	0.20	0.09	0.07
0.02	1.00	1.00	0.59	0.20	0.11
0.03	1.00	1.00	0.91	0.38	0.20
0.04	1.00	1.00	0.99	0.59	0.31
0.05	1.00	1.00	1.00	0.78	0.45
0.10	1.00	1.00	1.00	1.00	0.96

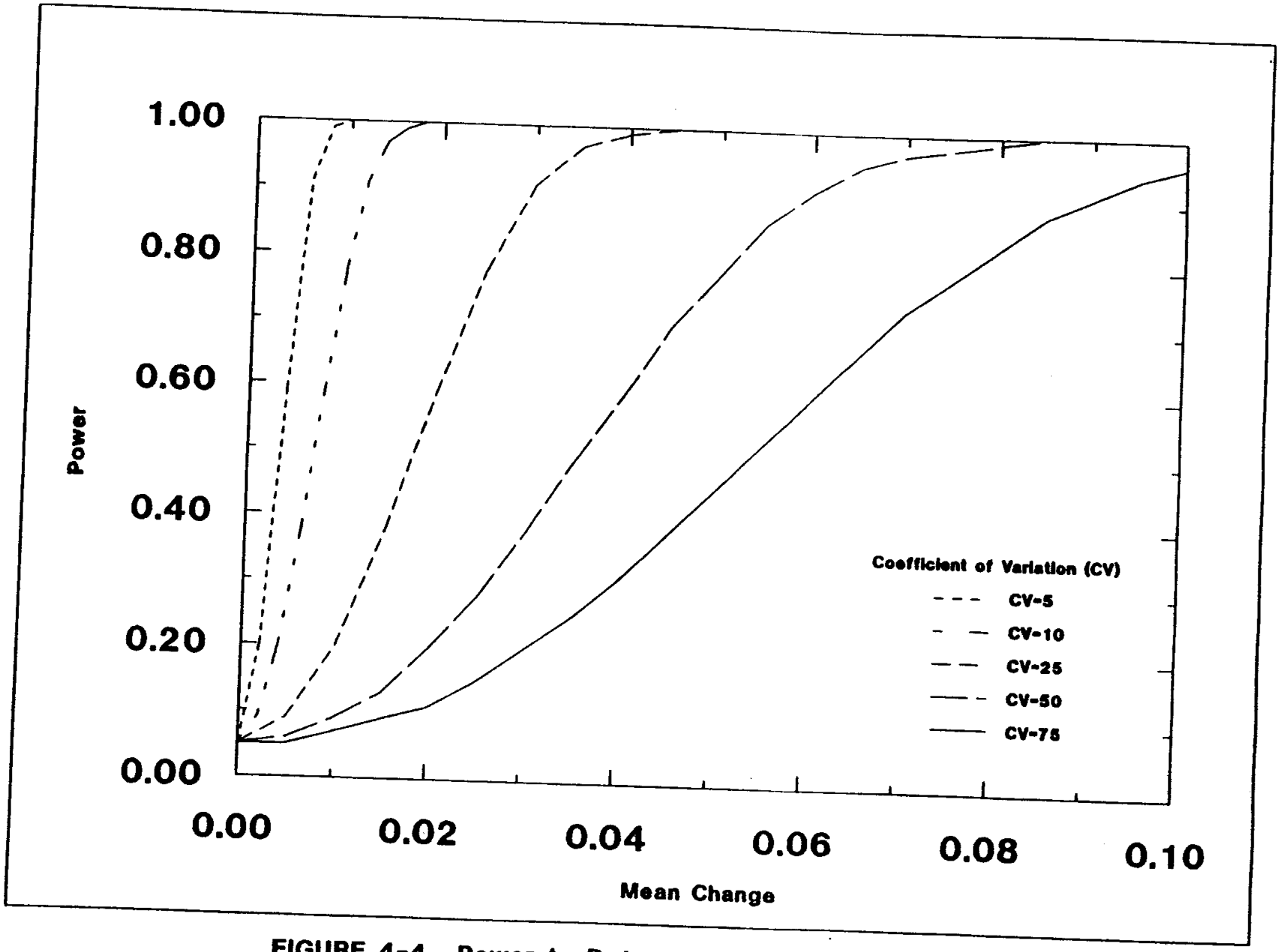

FIGURE 4-4. Power to Detect an Initial Dioxin Effect (Continuous Dependent Variable)

TABLE 4-10.

Location of Table Results from Different Analysis Models

Subpanel in Table	Dioxin Estimate	Type of Analysis	Assumption
a	initial[a]	unadjusted	minimal
b	initial[a]	unadjusted	maximal
c	initial[a]	adjusted	minimal
d	initial[a]	adjusted	maximal
e	current, time[a]	unadjusted	minimal
f	current, time[a]	unadjusted	maximal
g	current, time[a]	adjusted	minimal
h	current, time[a]	adjusted	maximal
i	current[b]	unadjusted	- -
j	current[b]	adjusted	- -

[a]Ranch Hands only.
[b]Categorized current dioxin, Ranch Hands and Comparisons.

EXPLANATION OF TABLES

This section introduces the reader to the contents of the tables that are used to report the results of the analyses for continuous and discrete dependent variables (two levels and more than two levels). Selected results from the statistical analysis methods applied in the hematology assessment (see Chapter 13, Hematologic Assessment) will be referenced throughout this discussion. The contents of each summary table depend on the form of the health status endpoint (i.e., whether the dependent variable under analysis is a continuous or discrete variable). Generally, the results of the various analyses will be summarized in subpanels within each table as specified in Table 4-10. The subpanel specifications may be slightly different when adjusted analyses are not performed. This section also provides an explanation of the information contained in these tables.

Continuous Variables

Table 13-3 presents an example of the results of analysis when the dependent variable is continuous. Subpanels (a) and (b) report summary statistics (for the minimal and maximal assumptions, respectively) assessing the association between the dependent variable and initial dioxin without adjusting for covariate information. Immediately below the specified assumption, the aggregate sample size (n) and the coefficient of determination (R^2) associated with the simple linear regression of the continuous dependent variable on $\log_2$ (initial dioxin) are presented. Sample sizes also are presented for low, medium, and high categories of initial dioxin. The numerical values defining these categories are specified in a table footnote. The low, medium, and high categories are based on the lower 25th percent, the 25th to 75th percent, and the upper 25th percent of the initial dioxin estimates for the cohort corresponding to the specified assumption. Means of the dependent variable (transformed to the original units, if necessary) are calculated from the data and are

presented for the low, medium, and high initial dioxin categories. Based on the simple linear regression analysis, the estimated slope and its associated standard error are reported for each assumption. If the dependent variable was transformed for the regression analysis, the means, slope, and standard error are footnoted and the transformation is identified in the footnote. The p-value associated with testing whether the estimated slope is equal to zero also is presented under both assumptions.

Based on analyses that incorporate covariate and interaction information, subpanels (c) and (d) report summary statistics (for the minimal and maximal assumptions, respectively) assessing the association between the dependent variable and initial dioxin. Immediately below the specified assumption, the aggregate sample size (n) and the multiple coefficient of determination (R^2) are presented for a multiple linear regression of the continuous dependent variable on $\log_2$ (initial dioxin) including covariate and interaction effect terms in the adjusted model. Similar to the unadjusted analyses, sample sizes are also presented for low, medium, and high categories of initial dioxin. The numerical values defining these categories are specified in a table footnote. Sample sizes for corresponding panels of unadjusted and adjusted analyses may differ because of missing covariate information. Adjusted means of the dependent variable (transformed to the original units, if necessary) also are presented. The adjusted means are presented for the low, medium, and high initial dioxin categories. Based on the multiple linear regression analysis, the adjusted slope for the $\log_2$ (initial dioxin) term and its associated standard error are reported for each assumption. If the dependent variable was transformed for the regression analysis, the adjusted means, adjusted slope, and standard error are footnoted and the transformation is identified in the footnote. The p-value for testing whether the adjusted slope is equal to zero also is presented under both assumptions.

Covariates with p-values less than or equal to 0.15 and interactions with p-values or equal to 0.05 retained in the multiple regression model after implementing the modeling strategy are presented under covariate remarks, along with the associated p-values. If the multiple regression model contains a significant initial dioxin-by-covariate interaction with an associated p-value less than or equal to 0.01, then the adjusted means, adjusted slope, standard error, and p-value generally are not reported. The entries for these statistics are reported as four asterisks (****) and are identified by a table footnote. Covariates and interactions retained in the model are, however, reported under covariate remarks. For some clinical assessments, an analyst may exercise discretion and report the adjusted means, adjusted slope, standard error, and a p-value from a model that excludes the interaction having a p-value less than 0.01. When these discretionary followup analyses are performed, the results are reported along with three asterisks (***) and are explained by a table footnote. If the multiple regression model contains a significant initial dioxin-by-covariate interaction with an associated p-value between 0.01 and 0.05, then the adjusted means, adjusted slope, standard error, and p-value are reported from a model that excludes that interaction. The entries for these statistics are reported along with two asterisks (**) accompanied by a table footnote. In either case (i.e., $p \leq 0.01$ or $0.01 < p \leq 0.05$), stratified analyses are undertaken and the results are reported in an associated appendix for each individual clinical area.

Subpanels (e) and (f) of Table 13-3, for example, report summary statistics (for the minimal and maximal assumptions, respectively) assessing the association of the dependent variable with current dioxin and time since tour without adjusting for covariate information.

Multiple regression techniques are used to generate the statistics provided in both panels. In the multiple regression model, current dioxin is included as a continuous variable and time since tour as a discrete variable. The interaction of current dioxin and time since tour also is included. For these models, time since tour is dichotomized and separate statistics are presented on the association between the dependent variable and current dioxin within each time stratum. For each subpanel, the aggregate sample size (n) and the coefficient of determination (R^2) are presented, under each specified assumption, for the multiple linear regression model. For presentation purposes, current dioxin and time since tour both are categorized. The numerical values defining the current dioxin categories are specified in a table footnote. The low, medium, and high categories are based on the lower 25th percent, the 25th to 75th percent, and the upper 25th percent of the current dioxin estimates for the cohort corresponding to the specified assumption. The value of 18.6 years for time since tour corresponds to approximately the median value of time since tour in the Ranch Hand cohort. The means of the dependent variable (transformed to the original units, if necessary) are calculated from the data and are presented, along with sample size, for the combinations of trichotomized current dioxin and dichotomized time since tour. The first p-value within each subpanel evaluates the interaction term of the multiple regression using current dioxin in continuous form and time since tour in discrete form. The p-value for the interaction term provides a test of the equality of the slopes for the two time strata. For each time stratum, a simple linear regression model of the dependent variable on current dioxin ($\log_2$ scale) provides an estimated slope, associated standard error, and p-value for testing the significance of the slope. If the dependent variable was transformed for regression analysis, the means, slope, and standard error are footnoted and the transformation identified in the footnote.

Incorporating covariate and current dioxin-by-time-by-covariate interaction information into the analysis, subpanels (g) and (h) report summary statistics (for the minimal and maximal assumptions, respectively) assessing the association of the dependent variable with current dioxin, time since tour, and the current dioxin-by-time interaction. Multiple linear regression techniques are used to generate the statistics provided. In the overall multiple regression model, current dioxin is included as a continuous variable and time since tour as a discrete variable. The interaction of current dioxin and time since tour also is included. The test of the interaction of current dioxin and time since tour (i.e., the first p-value in each subpanel) determines whether the adjusted slopes of the two time strata differ significantly.

Immediately below the specified assumption, the aggregate sample size (n) and the multiple coefficient of determination (R^2) are presented for the multiple linear regression of the continuous dependent variable on current dioxin ($\log_2$ scale), time since tour, the current dioxin-by-time interaction, covariates, and other interactions retained in the model. For each time stratum ($\leq$18.6 years or >18.6 years), separate statistics relating the dependent variable to current dioxin ($\log_2$ scale) are presented. In particular, based on the multiple linear regression analysis, the adjusted slope for the current dioxin term ($\log_2$ scale), its associated standard error, and a p-value for testing the significance of the slope are reported.

Sample sizes also are presented for combinations of low, medium, and high categories of current dioxin and dichotomized time since tour. The numerical values defining these categories are specified in a table footnote. Sample sizes for corresponding panels of unadjusted and adjusted analyses may differ because of missing covariate information.

Adjusted means of the dependent variable (transformed to the original units, if necessary) are presented. The adjusted means are presented for the combinations of trichotomized current dioxin and dichotomized time since tour. If the dependent variable was transformed for the regression analysis, the adjusted means, adjusted slope, and standard error are footnoted and the transformation is identified in the footnote.

Covariates (p-values less than or equal to 0.15) and interactions (p-values less than or equal to 0.05) retained in the multiple regression model after implementing the modeling strategy are presented under covariate remarks, along with the associated p-values. If the multiple regression model contains a significant current dioxin-by-time-by-covariate interaction term with an associated p-value less than or equal to 0.01, then the adjusted means, adjusted slope, standard error, and p-value generally are not reported. The entries for these statistics are reported as four asterisks (****) and are identified by a table footnote. Covariates and interactions retained in the model are, however, reported under covariate remarks. For some clinical assessments, an analyst may exercise discretion and report adjusted means, adjusted slope, standard error, and a p-value from a model that excludes the interaction having a p-value less than 0.01. When these discretionary followup analyses are performed, the results are reported along with three asterisks (***) and are explained by a table footnote. If the multiple regression model contains a significant current dioxin-by-time-by-covariate interaction with an associated p-value between 0.01 and 0.05, then the adjusted means, adjusted slope, standard error, and p-value are reported from a model that excludes that interaction. The entries for these statistics are reported along with two asterisks (**) accompanied by a table footnote. In either case, interactions are investigated within strata of the covariate and reported in an associated appendix for each clinical area.

Subpanels (i) and (j) of Table 13-3, for example, show the results of unadjusted and adjusted analyses that compare the means of a continuous dependent variable for Ranch Hands with high, low, and unknown current dioxin levels and for Comparisons having background current dioxin levels. The note at the bottom of the table defines the four current dioxin categories. Sample sizes for each category and across the four categories are reported. The coefficient of determination (R^2) also is presented.

For the unadjusted analysis, dependent variable means are presented for each category. If the dependent variable was transformed for the analysis, the means of the transformed values are converted to the original scale and the column heading is footnoted. A test of the simultaneous equality of the four category means is evaluated by the first p-value cited. If the analysis was performed on a transformed scale, the p-value column is footnoted to indicate that the p-value is based on the difference of means on a transformed scale. For the individual contrasts of the three Ranch Hand categories versus Comparison background category, differences in means are reported on the original scale. If the analyses were performed on a transformed scale, 95 percent confidence intervals on the differences of means are not presented and the column is footnoted. A p-value also is reported to determine whether a difference in means for a specified contrast is significantly different from zero.

For an adjusted analysis, the table is modified to include adjusted means, differences in adjusted means (reported on the original scale), 95 percent confidence intervals on the differences in adjusted means (if the analysis was performed on the original scale), and any

covariates and interactions retained in the adjusted model along with their associated p-values.

Discrete Variables

Discrete Variable With Two Categories

Table 13-4 presents an example of the results of analysis when the dependent variable is discrete and dichotomous in form. Subpanels (a) and (b) report summary statistics (for the minimal and maximal assumptions, respectively) assessing the association between the dependent variable and initial dioxin without adjusting for covariate information. Immediately below the specified assumption, the aggregate sample size (n) associated with the simple logistic regression of the continuous dependent variable on $\log_2$ (initial dioxin) is presented. Sample sizes also are presented for low, medium, and high categories of initial dioxin. The numerical values defining these categories are specified in a table footnote. The low, medium, and high categories are based on the lower 25th percent, the 25th to 75th percent, and the upper 25th percent of the initial dioxin estimates for the cohort corresponding to the specified assumption. The percentage of Ranch Hands with the specified dichotomous characteristic (as cited in the column heading) is calculated from the data and presented for the low, medium, and high initial dioxin categories. Based on the simple logistic regression model, an estimated relative risk and its associated 95 percent confidence interval are reported for each assumption. The p-value associated with testing whether the relative risk is equal to one also is presented for both assumptions. The relative risk, confidence interval, and p-value are based on $\log_2$ (initial dioxin) in its continuous form.

Results may exhibit a significant ($p \leq 0.05$) p-value associated with testing whether the relative risk is equal to 1.00, while the corresponding 95 percent confidence interval on the relative risk contains the number 1.00. These results occur because the BMDP®-LR procedure uses a normal distribution in calculating an approximate 95 percent confidence interval and a chi-square distribution based on a likelihood radio statistic (9) in the determination of a p-value. Similarly, the results may exhibit a 95 percent confidence interval of a relative risk that does not contain the number 1.00, while the corresponding p-value is not significant ($p > 0.05$) for the reasons stated above.

Incorporating covariate and interaction information, subpanels (c) and (d) report summary statistics (for the minimal and maximal assumptions, respectively) assessing the association between the discrete dependent variable and initial dioxin. Immediately below the specified assumption, the aggregate sample size (n) is presented for a multiple logistic regression of the discrete dependent variable on $\log_2$ (initial dioxin) including covariate and interactions in the adjusted model. Based on the multiple logistic regression model, the adjusted relative risk for the $\log_2$ (initial dioxin) term and its associated 95 percent confidence interval are reported for each assumption. The p-value for testing whether the adjusted relative risk is equal to 1 also is presented under both assumptions. Covariates (p-values less than or equal to 0.15) and interactions (p-values less than or equal to 0.05) retained in the multiple regression model after implementing the modeling strategy are presented under covariate remarks, along with the associated p-values. If the multiple logistic regression model contains a significant initial dioxin-by-covariate interaction with an associated p-value less than or equal to 0.01, then the adjusted relative risk, 95 percent confidence interval, and associated p-value generally are not reported. The entries for these statistics are reported

as four asterisks (****) and are identified by a table footnote. Covariates and interactions retained in the model are, however, reported under covariate remarks. For some clinical assessments, an analyst may exercise discretion and report an adjusted relative risk, 95 percent confidence interval, and an associated p-value from a model that excludes the interaction having a p-value less than 0.01. When these discretionary followup analyses are performed, the results are reported along with three asterisks (***) and are explained by a table footnote. If the multiple logistic regression model contains a significant initial dioxin-by-covariate interaction with a p-value between 0.01 and 0.05, then the adjusted relative risk, 95 percent confidence interval, and associated p-value are reported from a model that excludes that interaction. The entries for these statistics are reported along with two asterisks (**) accompanied by a table footnote. In either case (i.e., $p \leq 0.01$ or $0.01 < p \leq 0.05$), stratified analyses are undertaken and the results are reported in an appropriate appendix.

Subpanels (e) and (f) of Table 13-4, for example, report summary statistics (for the minimal and maximal assumptions, respectively) assessing the association of the discrete dependent variable with current dioxin and time since tour without adjusting for covariate information. Multiple logistic regression techniques are used to generate the statistics provided in both panels. In the multiple logistic regression model, current dioxin is treated as a continuous variable and time since tour as a discrete variable. The interaction of current dioxin and time since tour also is included in the model. For the logistic regression model, time since tour is dichotomized and separate statistics are presented for the association between the dependent variable and current dioxin within each time stratum. For each subpanel, the aggregate sample size (n) is presented under each specified assumption for the multiple logistic regression model. For presentation purposes, current dioxin and time since tour both are categorized. The numerical values defining the current dioxin categories are specified in a table footnote. The low, medium, and high categories are based on the lower 25th percent, the 25th to 75th percent, and the upper 25th percent of the measured current dioxin for the cohort corresponding to the specified assumption. The value of 18.6 years for time since tour corresponds to approximately the median value in the Ranch Hand cohort. The percentage of Ranch Hands with the specified dichotomous characteristic (as cited in the column heading) is calculated from the data and presented, along with sample size, for the combinations of trichotomized current dioxin and dichotomized time since tour. Each panel also contains a p-value (i.e., the first p-value in each subpanel) for the interaction of the multiple logistic regression using current dioxin in continuous form and time since tour in discrete form. The p-value for the interaction term provides a test of the equality of the relative risks for the two time strata. For each time stratum, the logistic regression on current dioxin ($\log_2$ scale) provides an estimated relative risk, associated 95 percent confidence interval, and p-value for testing the significance of the relative risk.

Incorporating covariate and interaction information into the analysis, subpanels (g) and (h) report summary statistics (for the minimal and maximal assumptions, respectively) assessing the association of the discrete dependent variable with current dioxin, time since tour, and the current dioxin-by-time interaction. Multiple logistic regression techniques are used to generate the statistics provided. In the multiple logistic regression model, current dioxin is included as a continuous variable and time since tour as a discrete variable. The interaction of current dioxin and time since tour also is included. The test of the interaction of current dioxin and time since tour (i.e., the first p-value in each subpanel) determines whether the adjusted relative risks of the two time strata differ significantly.

Immediately below the specified assumption, the aggregate sample size (n) is presented for the multiple logistic regression of the continuous dependent variable on $\log_2$ (current dioxin), time since tour, the current dioxin-by-time interaction, covariates, and other interactions retained in the model. For each time stratum (≤18.6 years or >18.6 years), separate statistics relating the dependent variable to current dioxin ($\log_2$ scale) are presented. Based on the multiple logistic regression analysis, the adjusted relative risk for the $\log_2$ (current dioxin) term, its associated 95 percent confidence interval, and a p-value for testing the significance of the adjusted relative risk are reported.

Covariates (p-values less than or equal to 0.15) and interactions (p-values less than or equal to 0.05) retained in the multiple logistic regression model after implementing the modeling strategy are presented under covariate remarks, along with the associated p-values. If the multiple logistic regression model contains a significant current dioxin-by-time-by-covariate interaction term such that the associated p-value is less than or equal to 0.01, then the adjusted relative risk, associated 95 percent confidence interval, and p-value generally are not reported. The entries for these statistics are reported as four asterisks (****) and are identified by a table footnote. Covariates and interactions retained in the model, however, are reported under covariate remarks. For some clinical assessments, an analyst may exercise discretion and report an adjusted relative risk, 95 percent confidence interval, and an associated p-value from a model that excludes the interaction having a p-value less than 0.01. When these discretionary followup analyses are performed, the results will be reported along with three asterisks (***) and are explained by a table footnote. If the multiple logistic regression model contains a significant current dioxin-by-time-by-covariate interaction such that the interaction lies between 0.01 and 0.05, then the adjusted relative risk, 95 percent confidence interval, and p-value are reported from a model that excludes that interaction. The entries for these statistics are reported along with two asterisks (**) accompanied by a table footnote. In either case ($p \leq 0.01$ or $0.01 < p \leq 0.05$), stratified analyses are undertaken and reported in the appropriate appendix.

Subpanels (i) and (j) of Table 13-4, for example, show the results of unadjusted and adjusted analyses that compare Ranch Hands with high, low, and unknown current dioxin levels and Comparisons having background current dioxin levels on the relative frequency for a specified discrete dependent variable (e.g., percent of participants in a current dioxin category with an abnormal condition). The note at the bottom of the table defines the four categories. Sample sizes for each category and across the four categories are reported.

For the unadjusted analysis, a relative frequency is presented for each current dioxin category. The simultaneous equality of the four category relative frequencies is evaluated by the first p-value cited. For the individual contrasts of the three Ranch Hand categories versus Comparison background category, relative risks, associated 95 percent confidence intervals for the relative risks, and p-values to evaluate if the risks differ significantly from 1 are presented.

Results may exhibit a significant ($p \leq 0.05$) p-value associated with testing whether the relative risk is equal to 1.00, while the corresponding 95 percent confidence interval on the relative risk contains the number 1.00. Similarly, the results may exhibit a 95 percent confidence interval of a relative risk that does not contain the number 1.00, while the

corresponding p-value is not significant ($p>0.05$). These patterns are due to the use of the normal distribution in calculating an approximate 95 percent confidence interval and the use of Fisher's exact test for unadjusted analyses in the determination of the corresponding p-values in the event of sparse data.

For an adjusted analysis, the table presents adjusted relative risks, 95 percent confidence intervals on the adjusted relative risks, and covariates and interactions retained in the adjusted model along with their associated p-values.

Discrete Variable With More Than Two Categories

Log-linear analysis techniques were used to analyze discrete dependent variables having more than two levels (e.g., low, normal, high—see Table 13-6). For the unadjusted and adjusted analyses relating such discrete dependent variables to initial dioxin, summary tables present sample sizes, relative frequencies, relative risks, 95 percent confidence intervals for the relative risks, and associated p-values. For the adjusted analyses, any covariates and interactions retained in the model along with their associated p-values also are presented. One difference between the table presentations for dichotomous dependent variables and discrete dependent variables with more than two levels is that relative frequencies of Ranch Hands belonging to each of the dependent variable categories are summarized with respect to each initial dioxin category (i.e., low, medium, and high initial dioxin). Therefore, for each initial dioxin level, the relative frequencies sum to 100 percent across the dependent variable categories. Also, for specified pairs of dependent variable levels (e.g., low and normal or high and normal for the discrete dependent variable), contrasts for high initial dioxin versus low initial dioxin, and medium initial dioxin versus low initial dioxin, are constructed with relative risks, 95 percent confidence intervals, and associated contrast p-values. Contrasts are based on a categorized form (i.e., low, medium, and high) of initial dioxin rather than $\log_2$ (initial dioxin). A p-value for an overall test of independence between the dependent variable and initial dioxin also is reported.

Similar to the log-linear analysis using initial dioxin, unadjusted and adjusted analyses of discrete dependent variables with more than two categories were performed using current dioxin and time since tour. For the unadjusted analysis, sample sizes, relative frequencies (within each current dioxin level), current dioxin contrasts for specified pairs of dependent variable levels with relative risks, 95 percent confidence intervals on the relative risks, and associated contrast p-values were reported for each time since tour stratum. For these analyses a categorized form of current dioxin (i.e., low, medium, and high), rather than the continuous form of $\log_2$ (current dioxin), is used. For the adjusted analysis, contrast-specific adjusted relative risks with 95 percent confidence intervals, associated contrast p-values, and covariates and interactions retained in the model along with associated p-values are presented. For both the unadjusted and the adjusted analyses, a p-value is provided that tests the significance of the interaction between current dioxin and time since tour and, for each time stratum, another p-value is reported as an overall test of independence between the discrete dependent variable and current dioxin.

For log-linear analyses of initial dioxin, and those concerning current dioxin and time since tour, the cutpoints between the three dioxin categories (i.e., between low and medium dioxin, and between medium and high dioxin) are the same under both the minimal and

maximal assumptions. The actual cutpoints are relevant for log-linear analyses, and this standardization was done to permit a more valid comparison of category contrasts between the minimal and maximal assumptions.

Unadjusted and adjusted analyses comparing relative frequencies for discrete dependent variables of more than two categories also were performed to compare the four current dioxin categories. For the unadjusted analysis, sample sizes, relative frequencies (within each of the four categories), Ranch Hand versus Comparison contrasts for specified pairs of dependent variable levels with relative risks, 95 percent confidence intervals on the relative risks, and associated contrast p-values were reported. For the adjusted analysis, sample sizes, contrast-specific adjusted relative risks with 95 confidence intervals, associated contrast p-values, and covariates and interactions retained in the model along with associated p-values are presented. For both the unadjusted and the adjusted analyses, an all categories p-value is provided that tests the independence of the categories and the discrete dependent variable.

GRAPHICS

The analytic activities for the serum dioxin analyses were supplemented by two sets of graphic displays: data plots/histograms and interaction plots/histograms. These graphics were produced using the SYSTAT® graphics procedure (16).

Data Plots/Histograms

As part of the serum dioxin analyses, graphic displays were produced describing the relationship between each dependent variable and serum dioxin level, as well as relevant covariates and serum dioxin level. Evaluations of the relationships between dioxin and the covariates were carefully made because such relationships particularly are important in the interpretation of dioxin effects for this study (see Chapter 5, Covariate Associations). Initial and current dioxin levels were used in continuous form. Transformations used in statistical analyses also were incorporated into the graphic presentations.

For initial dioxin, dependent variable and covariate relationships were displayed separately for Ranch Hands under the minimal and maximal assumptions. In addition, graphic relationships between dependent health variables and current dioxin level, as well as relevant covariates and current dioxin level, were presented separately for all Comparisons and Ranch Hands.

For continuous dependent variables, bivariate scatterplots were produced. For binary or categorical dependent variables, bar charts with percentages of participants classified as abnormal for common interval groupings of dioxin were generated for each of the clinical areas. For the covariate associations section, relative frequency histograms were produced for each level of the covariate.

Figure 4-5 presents an illustration of the bar charts seen in the appendix for each clinical area. Figures 4-5(a), (b), and (c) display a positive relationship, no relationship, and a negative relationship between the percentage of participants classified as abnormal and dioxin. These displays were generated assuming equal sample sizes for each bar; inference based on unequal sample sizes is not straightforward. Figures 4-6(a), (b), and (c) illustrate

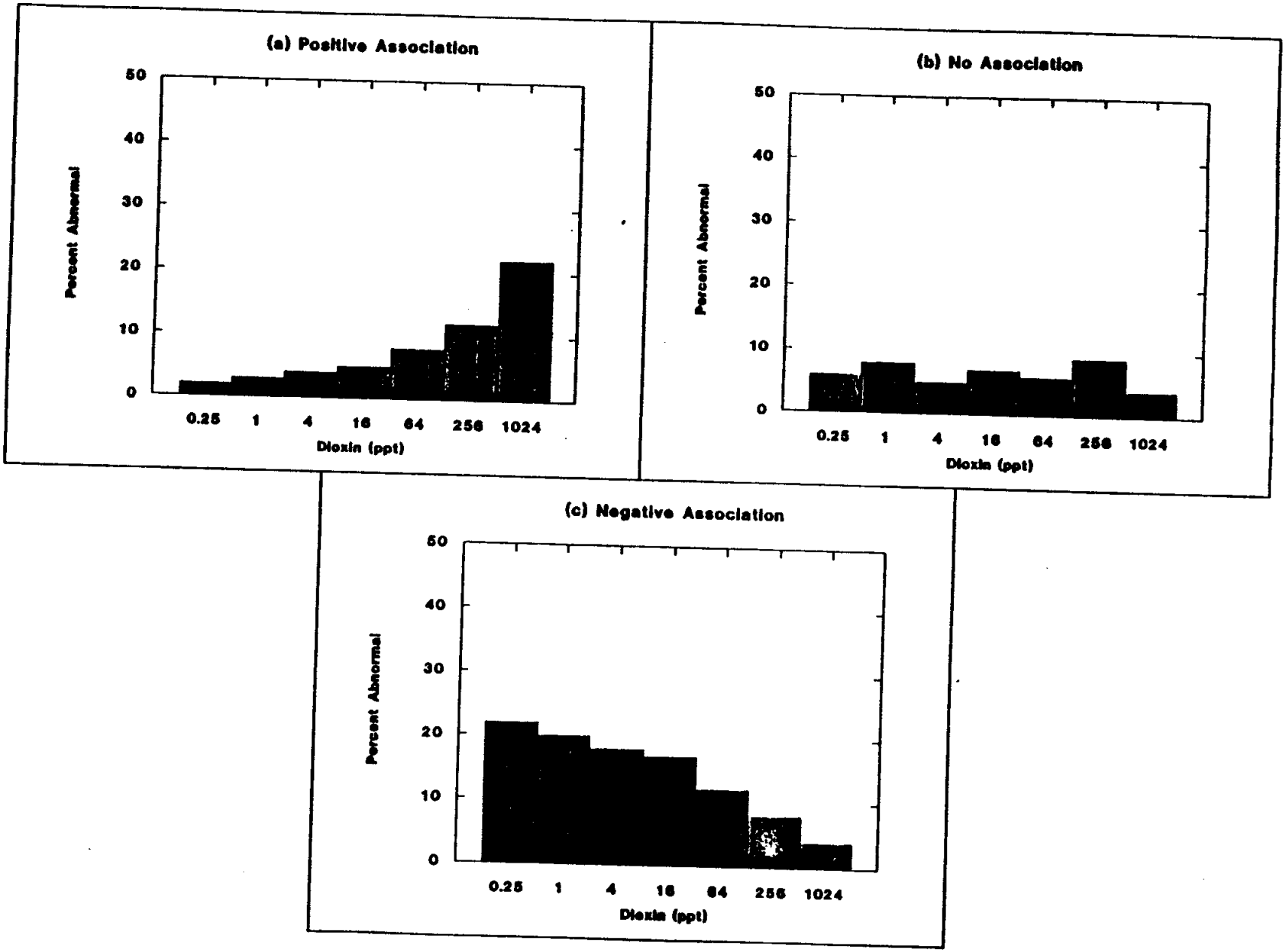

FIGURE 4-5. Hypothetical Data (Discrete Dependent Variable) versus Dioxin

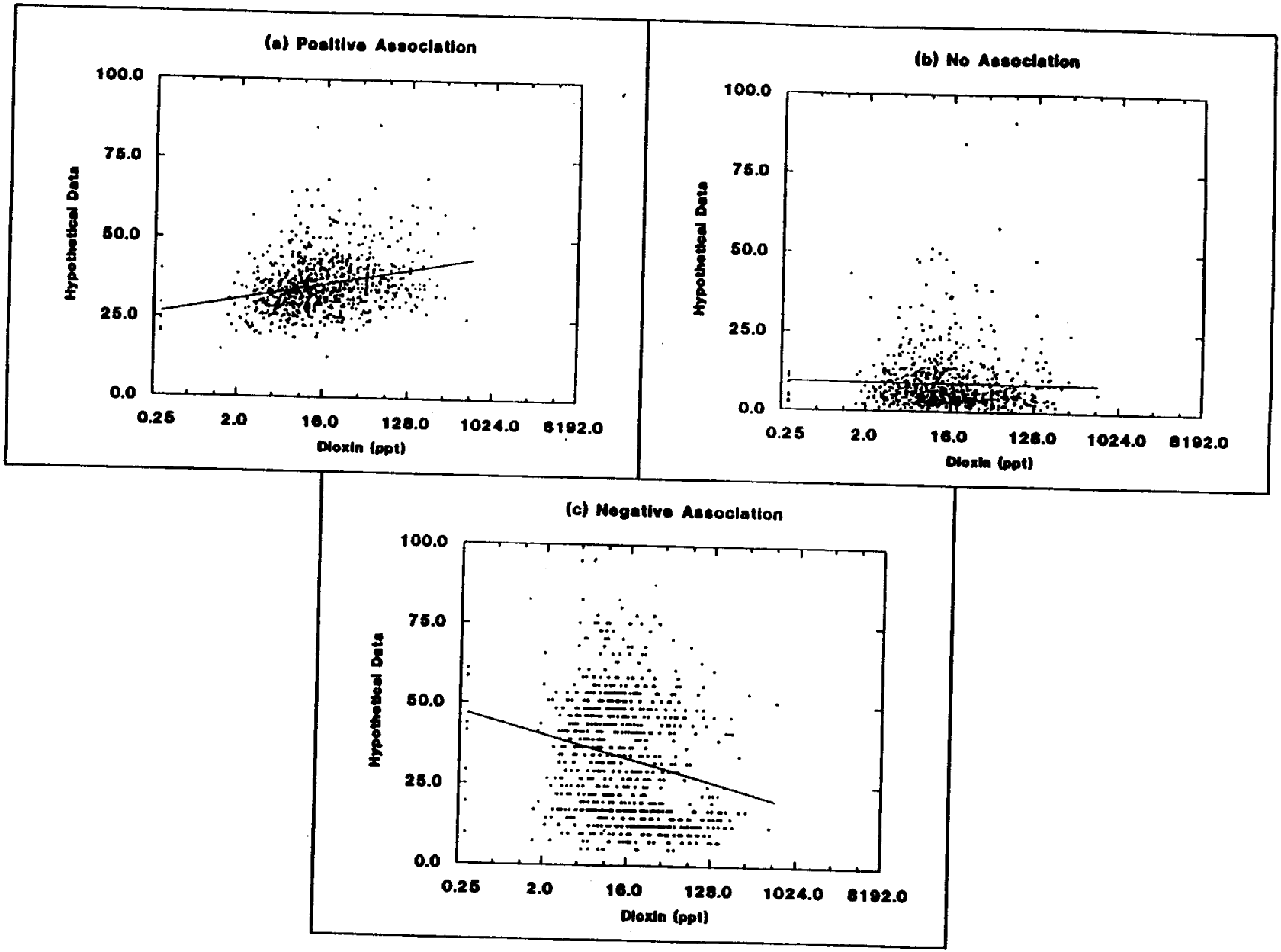

FIGURE 4-6. Hypothetical Data (Continuous Dependent Variable) versus Dioxin

examples of a positive relationship, no relationship, and a negative relationship between a dependent health variable and dioxin.

Interaction Plots/Histograms

Dioxin-by-covariate interactions also were investigated through appropriate graphic displays. Analogous to the data plots/histograms, transformations were used in the presentations when appropriate. If the dependent variable was continuous (e.g., blood urea nitrogen), a significant interaction between dioxin level (e.g., initial dioxin) and a covariate (e.g., age) was presented as a set of bivariate scatterplots (dependent variable versus initial dioxin) for each level of a categorized covariate. For a discrete dependent variable (e.g., kidney disease: yes versus no), a significant interaction between initial dioxin and a covariate was displayed using bar charts at each level of a categorized covariate. The bar charts contrasted percentages of participants classified as abnormal for common interval groupings of initial dioxin.

Statistical Analysis Protocol

Except for changes suggested by the Advisory Committee (deletioning conditional analyses and moving fasting glucose from Chapter 10, Gastrointestinal Assessment to Chapter 15, Endocrine Assessment), all statistical analyses summarized in this report were carried out as specified in an analytical plan (17) written in July 1989 and the contract Statement of Work; the analyses began in October 1989 and concluded in November 1990. The analytical plan specified statistical methods, dependent variables, covariates, and exclusions. These analyses did not deviate from those specified in the plan. In certain cases, clarification analyses were carried out, however. Strict adherence to the plan was maintained to avoid the possibility that some analyses might be conducted based on the observation of significant results. Such analyses are called "post hoc" and are known to be biased (18).

CHAPTER 4

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CHAPTER 5

COVARIATE ASSOCIATIONS

INTRODUCTION

This chapter evaluates the covariates used in adjusted statistical analyses for significant associations with initial dioxin levels for the Ranch Hand participants and current dioxin levels for the Ranch Hands and the Comparisons. The evaluation, with respect to initial dioxin levels for the Ranch Hand participants, was performed under both the minimal and the maximal assumptions (i.e., Ranch Hands with current dioxin above 10 ppt and above 5 ppt, respectively; see Chapter 4, Statistical Methods, for a further discussion of these assumptions). Associations between the covariates and the health status variables are documented in the previous Air Force Health Study report of the 1987 examination data (1).

Table 5-1 presents geometric mean dioxin levels (transformed from the logarithm base 2 scale) and sample sizes by covariate category under both assumptions for initial dioxin and under both group classifications (i.e., Ranch Hands and Comparisons) for current dioxin. Mean dioxin levels, expressed in parts per trillion (ppt), were evaluated for statistical significance across the defined categories of a particular covariate (e.g., under both assumptions, initial dioxin means of Black and non-Black Ranch Hand participants were compared for a statistically significant difference). The aggregate sample size and the significance probability associated with comparing dioxin means across covariate levels are included in the table. Aggregate sample sizes may differ from covariate to covariate because of missing covariate information. The significance probability was determined from statistics calculated on the logarithm base 2 scale of the serum dioxin concentration. For covariates on a continuous scale, the correlation coefficient and the associated significance probability are presented in the table. The correlation coefficient is based on the association between the covariate and the logarithm base 2 of the serum dioxin concentration. Dioxin levels equal to zero were assigned a value of 0.1 ppt due to the logarithmic transformation used in the analyses of all Ranch Hands and all Comparisons.

MATCHING VARIABLES (AGE, RACE, AND OCCUPATION)

The variables age, race, and military occupation were used in the design of the Air Force Health Study to match Ranch Hand participants with Comparisons and thus reduce the association between these variables and group status. It was not possible to eliminate the association of these variables with serum dioxin through the study design, however.

In general, age at Baseline (1982) exhibited a significant negative correlation with initial dioxin ($p<0.001$ under both the minimal and maximal assumptions). For Ranch Hands born in or after 1942, and for those born before 1942, initial dioxin means were 226.6 ppt and 148.5 ppt under the minimal assumption. Corresponding means of initial dioxin under the maximal assumption were 149.9 and 101.6 ppt, respectively. For all Ranch Hand participants a significant negative correlation between age and current dioxin was exhibited ($p<0.001$). The current dioxin means were 19.3 ppt and 11.7 ppt for Ranch Hands born in or after 1942 and Ranch Hands born before 1942. For the Comparisons the correlation between age and current dioxin was also significant, but positive ($p<0.001$). The current dioxin means were 3.0 ppt for Comparisons born in or after 1942 and 4.0 ppt for Comparisons born before 1942.

TABLE 5-1.

Relationship of Covariates to Initial and Current Dioxin

Variable	Statistic	Initial Dioxin (Ranch Hands) Assumption: Minimal	Initial Dioxin (Ranch Hands) Assumption: Maximal	Current Dioxin: Ranch Hand	Current Dioxin: Comparison
Matching Variables					
Age (continuous)	n	521	742	866	804
	Correlation	-0.240	-0.200	-0.205	0.155
	p-Value	<0.001	<0.001	<0.001	<0.001
Age (year of birth) (discrete)	n	521	742	866	804
	Mean (n)				
	Born≥1942	226.6 (237)	149.9 (314)	19.3 (355)	3.0 (330)
	Born<1942	148.5 (284)	101.6 (428)	11.7 (511)	4.0 (474)
	p-Value	<0.001	<0.001	<0.001	<0.001
Race	n	521	742	866	804
	Mean (n)				
	Black	134.5 (32)	114.7 (38)	14.6 (44)	2.9 (49)
	Non-Black	183.5 (489)	120.0 (704)	14.4 (822)	3.6 (755)
	p-Value	0.011	0.701	0.904	0.288
Occupation	n	521	742	866	804
	Mean (n)				
	Officer	91.7 (108)	61.4 (246)	7.7 (319)	4.0 (291)
	Enlisted Flyer	172.3 (108)	134.7 (132)	16.3 (148)	3.7 (127)
	Enlisted Groundcrew	232.1 (305)	180.2 (364)	23.2 (399)	3.2 (386)
	p-Value	<0.001	<0.001	<0.001	0.007
Alcohol Variables					
Current Alcohol Use (continuous)	n	518	737	861	804
	Correlation	0.043	0.014	0.039	0.023
	p-Value	0.326	0.703	0.255	0.523
Current Alcohol Use (drinks/day) (discrete)	n	518	737	861	804
	Mean (n)				
	0-1	181.8 (420)	121.4 (594)	14.3 (696)	3.6 (630)
	>1-4	158.4 (83)	105.5 (124)	13.6 (143)	3.2 (143)
	>4	276.6 (15)	182.2 (19)	22.3 (22)	4.5 (31)
	p-Value	0.051	0.049	0.171	0.100

TABLE 5-1. (Continued)

Relationship of Covariates to Initial and Current Dioxin

		Initial Dioxin (Ranch Hands)			
		Assumption		Current Dioxin	
Variable	Statistic	Minimal	Maximal	Ranch Hand	Comparison
Lifetime Alcohol History (continuous)	n	515	733	857	802
	Correlation	0.044	0.057	0.012	0.005
	p-Value	0.318	0.125	0.728	0.894
Lifetime Alcohol History (drink-years) (discrete)	n	515	733	857	802
	Mean (n)				
	0	233.7 (57)	163.7 (73)	18.7 (85)	3.8 (61)
	>0-40	167.5 (345)	110.1 (507)	13.4 (599)	3.5 (547)
	>40	192.8 (113)	134.3 (153)	15.8 (173)	3.6 (194)
	p-Value	0.012	0.001	0.021	0.810
Current Wine Use (continuous)	n	517	737	861	803
	Correlation	-0.111	-0.110	-0.054	-0.007
	p-Value	0.011	0.003	0.110	0.853
Current Wine Use (drinks/day) (discrete)	n	517	737	861	803
	Mean (n)				
	0	197.2 (349)	139.9 (459)	16.7 (526)	3.6 (458)
	>0	148.5 (168)	92.1 (278)	11.3 (335)	3.5 (345)
	p-Value	<0.001	<0.001	<0.001	0.656
Lifetime Wine History (continuous)	n	517	736	860	802
	Correlation	-0.160	-0.107	-0.059	0.018
	p-Value	<0.001	0.004	0.086	0.603
Lifetime Wine History (drink-years) (discrete)	n	517	736	860	802
	Mean (n)				
	0	207.4 (301)	144.2 (398)	16.9 (458)	3.6 (403)
	>0-10	151.9 (191)	97.1 (302)	11.8 (363)	3.5 (367)
	>10	117.9 (25)	87.5 (36)	12.9 (39)	4.3 (32)
	p-Value	<0.001	<0.001	<0.001	0.482

TABLE 5-1. (Continued)

Relationship of Covariates to Initial and Current Dioxin

		Initial Dioxin (Ranch Hands)			
		Assumption		Current Dioxin	
Variable	Statistic	Minimal	Maximal	Ranch Hand	Comparison
Smoking Variables					
Current Cigarette Smoking (continuous)	n	521	742	866	804
	Correlation	0.013	0.034	-0.067	-0.074
	p-Value	0.758	0.355	0.049	0.035
Current Cigarette Smoking (cigarettes/ day) (discrete)	n	521	742	866	804
	Mean (n)				
	0-Never	189.0 (135)	114.1 (207)	15.2 (236)	4.3 (223)
	0-Former	169.1 (196)	113.6 (282)	14.5 (323)	3.5 (336)
	>0-20	187.9 (101)	137.4 (131)	14.5 (159)	2.9 (128)
	>20	182.7 (89)	126.6 (122)	12.9 (148)	3.1 (117)
	p-Value	0.603	0.208	0.587	<0.001
Lifetime Cigarette Smoking History (continuous)	n	521	742	866	804
	Correlation	-0.064	-0.010	-0.094	-0.013
	p-Value	0.147	0.783	0.006	0.719
Lifetime Cigarette Smoking History (pack-years) (discrete)	n	521	742	866	804
	Mean (n)				
	0	187.7 (136)	113.8 (208)	15.1 (237)	4.3 (223)
	>0-10	180.6 (152)	124.5 (206)	15.3 (237)	2.9 (218)
	>10	175.3 (233)	120.7 (328)	13.5 (392)	3.6 (363)
	p-Value	0.749	0.621	0.297	<0.001
Sun Exposure-Related Variables					
Average Lifetime Residential Latitude[a]	n	489	704	821	750
	Mean (n)				
	Latitude <37°	196.5 (205)	126.1 (295)	14.8 (344)	3.7 (385)
	Latitude ≥37°	174.6 (284)	115.8 (409)	14.2 (477)	3.6 (365)
	p-Value	0.128	0.247	0.596	0.786

TABLE 5-1. (Continued)

Relationship of Covariates to Initial and Current Dioxin

Variable	Statistic	Initial Dioxin (Ranch Hands) Assumption: Minimal	Initial Dioxin (Ranch Hands) Assumption: Maximal	Current Dioxin: Ranch Hand	Current Dioxin: Comparison
Ethnic Background[a,b]	n	476	687	801	738
	Mean (n)				
	AB	179.8 (447)	116.5 (654)	14.0 (767)	3.7 (701)
	CDE	260.4 (29)	214.8 (33)	29.1 (34)	2.9 (37)
	p-Value	0.022	<0.001	<0.001	0.115
Skin Color[a]	n	489	703	821	755
	Mean (n)				
	Peach	183.3 (395)	122.5 (559)	14.7 (651)	3.6 (615)
	Non-Peach	184.3 (94)	111.5 (144)	13.4 (170)	3.5 (140)
	p-Value	0.952	0.293	0.354	0.582
Hair Color[a]	n	489	704	822	754
	Mean (n)				
	Black/Dark Brown	196.7 (332)	129.0 (467)	15.7 (541)	3.6 (524)
	Other	158.4 (157)	104.2 (237)	12.2 (281)	3.7 (230)
	p-Value	0.008	0.005	0.004	0.486
Eye Color[a]	n	488	703	821	753
	Mean (n)				
	Brown	206.2 (150)	135.4 (211)	16.4 (242)	3.4 (227)
	Hazel/Green	167.8 (144)	113.5 (205)	13.3 (241)	3.4 (188)
	Grey/Blue	179.6 (194)	114.4 (287)	13.8 (338)	3.9 (338)
	p-Value	0.101	0.097	0.103	0.072
Reaction of Skin to Sun After at Least 2 Hours, After First Exposure[a]	n	489	704	822	755
	Mean (n)				
	Burned Painfully	182.6 (35)	123.3 (48)	14.8 (56)	5.0 (48)
	Burned	170.1 (63)	117.6 (87)	14.9 (102)	3.7 (90)
	Became Red	192.8 (195)	120.1 (292)	14.2 (345)	3.5 (326)
	No Reaction	179.1 (196)	120.1 (277)	14.3 (319)	3.5 (291)
	p-Value	0.720	0.995	0.997	0.062

TABLE 5-1. (Continued)

Relationship of Covariates to Initial and Current Dioxin

Variable	Statistic	Initial Dioxin (Ranch Hands) Assumption: Minimal	Initial Dioxin (Ranch Hands) Assumption: Maximal	Current Dioxin: Ranch Hand	Current Dioxin: Comparison
Reaction of Skin to Sun After Repeated Exposure[a]	n	489	704	822	754
	Mean (n)				
	Freckled-No Tan	202.4 (11)	138.1 (15)	15.9 (18)	5.6 (18)
	Tanned Mildly	207.2 (74)	149.4 (95)	16.1 (119)	3.4 (109)
	Tanned Moderately	178.3 (246)	113.8 (366)	14.5 (417)	3.8 (393)
	Tanned Deep Brown	179.9 (158)	118.2 (228)	13.4 (268)	3.4 (234)
	p-Value	0.565	0.094	0.507	0.088
Composite Sun Reaction Index[a,c]	n	489	704	822	754
	Mean (n)				
	Low	180.7 (358)	116.5 (526)	14.0 (609)	3.5 (557)
	Medium	194.3 (90)	134.5 (121)	15.8 (147)	3.4 (139)
	High	184.9 (41)	124.4 (57)	15.1 (66)	5.1 (58)
	p-Value	0.764	0.319	0.496	0.008
Carcinogen Exposure Variables					
Asbestos Exposure	n	521	742	866	804
	Mean (n)				
	Yes	183.6 (129)	121.3 (185)	14.6 (212)	3.7 (195)
	No	178.8 (392)	119.3 (557)	14.3 (654)	3.5 (609)
	p-Value	0.754	0.832	0.802	0.580
Ionizing Radiation Exposure	n	521	742	866	804
	Mean (n)				
	Yes	160.6 (105)	115.7 (143)	12.3 (175)	3.5 (212)
	No	185.2 (416)	120.8 (599)	15.0 (691)	3.6 (592)
	p-Value	0.118	0.626	0.070	0.833
Industrial Chemical Exposure	n	521	742	866	804
	Mean (n)				
	Yes	196.8 (311)	138.8 (408)	16.6 (470)	3.4 (443)
	No	157.8 (210)	100.0 (334)	12.1 (396)	3.8 (361)
	p-Value	0.003	<0.001	<0.001	0.043

TABLE 5-1. (Continued)

Relationship of Covariates to Initial and Current Dioxin

		Initial Dioxin (Ranch Hands)			
		Assumption		Current Dioxin	
Variable	Statistic	Minimal	Maximal	Ranch Hand	Comparison
Herbicide Exposure	n	521	742	866	804
	Mean (n)				
	Yes	180.5 (493)	119.7 (703)	14.6 (816)	3.8 (263)
	No	170.6 (28)	121.3 (39)	11.9 (50)	3.5 (541)
	p-Value	0.728	0.933	0.227	0.151
Insecticide Exposure	n	521	742	866	804
	Mean (n)				
	Yes	173.0 (381)	118.0 (537)	14.1 (626)	3.7 (454)
	No	200.5 (140)	124.6 (205)	15.2 (240)	3.5 (350)
	p-Value	0.074	0.484	0.391	0.430
Degreasing Chemical Exposure	n	521	742	866	804
	Mean (n)				
	Yes	196.0 (353)	137.3 (471)	17.1 (529)	3.6 (496)
	No	150.5 (168)	94.5 (271)	10.9 (337)	3.6 (308)
	p-Value	0.001	<0.001	<0.001	0.926
Anthracene Exposure	n	521	742	866	803
	Mean (n)				
	Yes	83.4 (1)	83.4 (1)	15.0 (1)	4.0 (3)
	No	180.3 (520)	119.8 (741)	14.4 (865)	3.6 (800)
	p-Value	0.357	0.704	0.971	0.832
Arsenic Exposure	n	521	741	865	803
	Mean (n)				
	Yes	156.0 (11)	100.5 (18)	12.9 (21)	3.1 (13)
	No	180.6 (510)	120.4 (723)	14.4 (844)	3.6 (790)
	p-Value	0.567	0.426	0.669	0.557
Benzene Exposure	n	521	742	866	804
	Mean (n)				
	Yes	226.2 (21)	162.6 (27)	16.9 (33)	3.7 (21)
	No	178.3 (500)	118.4 (715)	14.3 (833)	3.6 (783)
	p-Value	0.201	0.089	0.522	0.893

TABLE 5-1. (Continued)

Relationship of Covariates to Initial and Current Dioxin

Variable	Statistic	Initial Dioxin (Ranch Hands) Assumption: Minimal	Initial Dioxin (Ranch Hands) Assumption: Maximal	Current Dioxin: Ranch Hand	Current Dioxin: Comparison
Benzidine Exposure	n	521	742	866	802
	Mean (n)				
	Yes	127.5 (5)	93.8 (7)	7.5 (9)	3.7 (9)
	No	180.6 (516)	120.0 (735)	14.5 (857)	3.6 (793)
	p-Value	0.355	0.495	0.313	0.929
Chromate Exposure	n	519	739	863	804
	Mean (n)				
	Yes	232.5 (36)	159.2 (47)	17.8 (55)	3.3 (39)
	No	176.6 (483)	117.5 (692)	14.2 (808)	3.6 (765)
	p-Value	0.057	0.034	0.160	0.593
Coal Tar Exposure	n	521	742	866	804
	Mean (n)				
	Yes	137.0 (18)	121.7 (20)	9.7 (27)	4.1 (27)
	No	181.8 (503)	119.7 (722)	14.6 (839)	3.6 (777)
	p-Value	0.158	0.940	0.207	0.459
Creosote Exposure	n	521	742	866	804
	Mean (n)				
	Yes	175.7 (47)	125.6 (62)	13.8 (76)	3.2 (63)
	No	180.4 (474)	119.2 (680)	14.4 (790)	3.6 (741)
	p-Value	0.837	0.683	0.752	0.381
Aminodiphenyl Exposure	n	521	742	866	802
	Mean (n)				
	Yes	83.2 (2)	83.2 (2)	14.4 (2)	4.4 (4)
	No	180.5 (519)	119.9 (740)	14.4 (864)	3.6 (798)
	p-Value	<0.001	<0.001	0.998	0.649
Chloromethyl Ether Exposure	n	520	740	864	804
	Mean (n)				
	Yes	144.3 (3)	65.4 (8)	6.0 (10)	4.2 (11)
	No	180.1 (517)	120.5 (732)	14.5 (854)	3.6 (793)
	p-Value	0.648	0.070	0.015	0.267

TABLE 5-1. (Continued)

Relationship of Covariates to Initial and Current Dioxin

Variable	Statistic	Initial Dioxin (Ranch Hands) Assumption: Minimal	Initial Dioxin (Ranch Hands) Assumption: Maximal	Current Dioxin: Ranch Hand	Current Dioxin: Comparison
Mustard Gas Exposure	n	521	742	866	804
	Mean (n)				
	Yes	126.3 (3)	126.3 (3)	10.2 (4)	3.8 (4)
	No	180.4 (518)	119.7 (739)	14.4 (862)	3.6 (800)
	p-Value	0.461	0.923	0.553	0.633
Naphthylamine Exposure	n	521	741	865	803
	Mean (n)				
	Yes	219.1 (23)	179.5 (26)	19.9 (30)	3.3 (20)
	No	178.4 (498)	118.2 (715)	14.2 (835)	3.6 (783)
	p-Value	0.249	0.028	0.217	0.759
Cutting Oils Exposure	n	521	742	866	804
	Mean (n)				
	Yes	174.1 (76)	118.8 (107)	13.9 (124)	3.0 (102)
	No	181.0 (445)	119.9 (635)	14.5 (742)	3.7 (702)
	p-Value	0.706	0.924	0.693	0.076
Trichloro-ethylene Exposure	n	518	738	862	804
	Mean (n)				
	Yes	207.5 (57)	142.4 (76)	15.5 (91)	3.3 (71)
	No	176.7 (461)	117.3 (662)	14.2 (771)	3.6 (733)
	p-Value	0.170	0.092	0.547	0.386
Ultraviolet Light (Not Sun) Exposure	n	521	742	866	803
	Mean (n)				
	Yes	142.7 (13)	101.1 (18)	13.8 (20)	4.2 (17)
	No	181.1 (508)	120.3 (724)	14.4 (846)	3.6 (786)
	p-Value	0.311	0.445	0.808	0.232
Vinyl Chloride Exposure	n	520	741	865	803
	Mean (n)				
	Yes	209.1 (10)	144.1 (13)	17.0 (15)	4.1 (11)
	No	179.5 (510)	119.3 (728)	14.3 (850)	3.6 (792)
	p-Value	0.568	0.478	0.564	0.363

TABLE 5-1. (Continued)

Relationship of Covariates to Initial and Current Dioxin

Variable	Statistic	Initial Dioxin (Ranch Hands) Assumption: Minimal	Initial Dioxin (Ranch Hands) Assumption: Maximal	Current Dioxin: Ranch Hand	Current Dioxin: Comparison
Composite Carcinogen Exposure	n	515	731	855	796
	Mean (n)				
	Yes	192.9 (155)	134.2 (208)	16.4 (236)	3.3 (179)
	No	174.3 (360)	114.7 (523)	13.6 (619)	3.6 (617)
	p-Value	0.209	0.045	0.038	0.157
Personal and Family Health Variables					
Cholesterol (continuous)	n	521	742	866	804
	Correlation	0.054	0.046	0.051	0.046
	p-Value	0.217	0.215	0.137	0.196
Cholesterol (mg/dl) (discrete)	n	521	742	866	804
	Mean (n)				
	≤200	168.4 (163)	112.0 (238)	13.0 (287)	3.4 (281)
	>200-230	175.8 (177)	120.7 (244)	15.2 (275)	3.4 (244)
	>230	195.6 (181)	126.4 (260)	15.1 (304)	3.9 (279)
	p-Value	0.227	0.362	0.175	0.139
HDL (continuous)	n	521	742	866	804
	Correlation	-0.074	-0.142	-0.136	-0.099
	p-Value	0.090	<0.001	<0.001	0.005
HDL (µg/dl) (discrete)	n	521	742	866	804
	Mean (n)				
	≤40	182.7 (206)	138.6 (261)	17.5 (289)	3.9 (264)
	>40-50	188.6 (173)	121.7 (251)	14.5 (294)	3.7 (294)
	>50	166.5 (142)	99.6 (230)	11.6 (283)	3.1 (246)
	p-Value	0.400	<0.001	<0.001	0.008
Cholesterol-HDL Ratio (continuous)	n	521	742	866	804
	Correlation	0.078	0.146	0.148	0.109
	p-Value	0.076	<0.001	<0.001	0.002

TABLE 5-1. (Continued)

Relationship of Covariates to Initial and Current Dioxin

		Initial Dioxin (Ranch Hands)			
		Assumption		Current Dioxin	
Variable	Statistic	Minimal	Maximal	Ranch Hand	Comparison
Cholesterol-HDL Ratio (discrete)	n	521	742	866	804
	Mean (n)				
	≤4.2	158.1 (138)	97.0 (222)	11.3 (274)	3.0 (264)
	>4.2-5.5	187.9 (199)	124.5 (283)	15.2 (322)	3.9 (286)
	>5.5	189.3 (184)	139.3 (237)	17.2 (270)	3.9 (254)
	p-Value	0.104	<0.001	<0.001	0.001
Diabetic Class[d]	n	519	740	863	802
	Mean (n)				
	Normal	174.4 (371)	112.8 (548)	13.5 (648)	3.4 (620)
	Impaired	176.2 (82)	123.7 (110)	14.8 (130)	4.0 (115)
	Diabetic	221.9 (66)	169.9 (82)	21.9 (85)	4.5 (67)
	p-Value	0.095	0.001	0.001	0.028
Differential Cortisol Response (continuous)	n	509	721	839	770
	Correlation	-0.024	-0.059	-0.076	-0.052
	p-Value	0.583	0.112	0.027	0.152
Differential Cortisol Response (mg/dl) (discrete)	n	509	721	839	770
	Mean (n)				
	≤0.6	191.7 (185)	132.0 (251)	15.7 (288)	3.6 (275)
	>0.6-4.0	189.0 (192)	127.5 (265)	16.4 (299)	3.8 (262)
	>4.0	155.5 (132)	101.4 (205)	11.5 (252)	3.3 (233)
	p-Value	0.056	0.007	<0.001	0.315
Percent Body Fat (continuous)	n	521	742	866	804
	Correlation	0.139	0.210	0.300	0.154
	p-Value	0.001	<0.001	<0.001	<0.001
Percent Body Fat (discrete)	n	521	742	866	804
	Mean (n)				
	Lean/Normal: ≤25%	170.4 (389)	110.2 (579)	12.9 (693)	3.3 (608)
	Obese: >25%	211.4 (132)	161.1 (163)	22.4 (173)	4.4 (196)
	p-Value	0.018	<0.001	<0.001	<0.001

TABLE 5-1. (Continued)

Relationship of Covariates to Initial and Current Dioxin

Variable	Statistic	Initial Dioxin (Ranch Hands) Assumption: Minimal	Initial Dioxin (Ranch Hands) Assumption: Maximal	Current Dioxin: Ranch Hand	Current Dioxin: Comparison
Family History of Heart Disease	n	521	742	866	804
	Mean (n)				
	Yes	176.9 (125)	118.5 (178)	13.9 (208)	3.5 (177)
	No	181.0 (396)	120.2 (564)	14.6 (658)	3.6 (627)
	p-Value	0.793	0.867	0.591	0.765
Family History of Heart Disease Before Age 50	n	521	742	866	804
	Mean (n)				
	Yes	179.0 (17)	106.5 (27)	14.5 (30)	2.3 (26)
	No	180.0 (504)	120.3 (715)	14.4 (836)	3.6 (778)
	p-Value	0.979	0.515	0.970	0.134
Other Variables					
Education	n	517	737	860	799
	Mean (n)				
	High School	198.0 (322)	153.1 (395)	18.2 (448)	3.5 (400)
	College	153.4 (195)	89.8 (342)	11.1 (412)	3.7 (399)
	p-Value	0.001	<0.001	<0.001	0.378
Blood Type	n	519	738	861	802
	Mean (n)				
	A	182.4 (224)	125.0 (307)	15.0 (351)	3.6 (311)
	AB	171.9 (18)	111.8 (27)	14.6 (31)	4.3 (24)
	B	184.5 (54)	128.5 (72)	14.9 (87)	3.8 (98)
	O	177.3 (223)	114.4 (332)	13.8 (392)	3.4 (369)
	p-Value	0.973	0.593	0.773	0.469
Presence of Pre-SEA Acne	n	521	742	866	804
	Mean (n)				
	Yes	193.0 (53)	133.6 (71)	15.1 (88)	2.8 (88)
	No	178.6 (468)	118.4 (671)	14.3 (778)	3.4 (716)
	p-Value	0.523	0.309	0.819	0.246

TABLE 5-1. (Continued)

Relationship of Covariates to Initial and Current Dioxin

		Initial Dioxin (Ranch Hands)			
		Assumption		Current Dioxin	
Variable	Statistic	Minimal	Maximal	Ranch Hand	Comparison
Personality Type	n	506	717	834	769
	Mean (n)				
	Type A	173.9 (222)	112.3 (331)	13.6 (381)	3.5 (325)
	Type B	185.2 (284)	128.3 (386)	15.3 (453)	3.6 (444)
	p-Value	0.401	0.061	0.148	0.685

[a]Blacks excluded.

[b]Ethnic Background – A: English, Welsh, Scottish, or Irish
B: Scandinavian, German, Polish, Russian, Other Slavic, Jewish, or French
C: Spanish, Italian, or Greek
D: Mexican, American Indian, or Asian
E: African
AB: A or B
CDE: C, D, or E.

[c]Composite Sun Reaction Index (from reaction of skin after at least 2 hours after first exposure and reaction of skin after repeated exposure) – High: Burns painfully and/or freckles with no tan
Medium: Burns and/or tans mildly
Low: All other reactions.

[d]Diabetic Class – Normal: <140 mg/dl 2-hour postprandial glucose
Impaired: ≥140-<200 mg/dl 2-hour postprandial glucose
Diabetic: Verified past history of diabetes or ≥200 mg/dl 2-hour postprandial glucose.

Note: All means expressed in parts per trillion and have been transformed from the logarithm (base 2) scale.

Under the minimal assumption, the Black and non-Black Ranch Hand categories had significantly different initial dioxin means (134.5 ppt versus 183.5 ppt, p=0.011). Under the maximal assumption, the initial dioxin means were not significantly different between the race categories (p=0.701). The current dioxin means were also not significantly different between the race categories for all Ranch Hand participants and for all Comparisons (Ranch Hands, p=0.904; Comparisons, p=0.288).

As expected, the initial dioxin means differed significantly, under both assumptions, among the Ranch Hands who served as officers, enlisted flyers, and enlisted groundcrew (minimal, p<0.001; maximal, p<0.001). The initial dioxin means, under the minimal assumption, were 91.7 ppt for the officers, 172.3 ppt for the enlisted flyers, and 232.1 ppt for the enlisted groundcrew. The corresponding means under the maximal assumption were 61.4, 134.7, and 180.2 ppt, respectively. The current dioxin means also differed significantly for all Ranch Hands (p<0.001) and for all Comparisons (p=0.007). However, for the Ranch Hands, the enlisted groundcrew had the highest current dioxin mean (officers: 7.7 ppt; enlisted flyers: 16.3 ppt; enlisted groundcrew: 23.2 ppt), whereas, for the Comparisons, the officers had the highest current dioxin mean (officers: 4.0 ppt; enlisted flyers: 3.7 ppt; enlisted groundcrew: 3.2 ppt). (See Chapter 2, Dioxin Assay, for a further discussion of these results.)

DRINKING HABITS

Drinking habits were analyzed on the basis of current alcohol use, lifetime alcohol history, current wine use, and lifetime wine history.

Under the minimal assumption, the mean initial dioxin levels for Ranch Hands with current alcohol use values categorized as zero to one drink per day, over one but no more than four drinks per day, and over four drinks per day were marginally significant (p=0.051; 0-1 drink per day: 181.8 ppt; >1-4 drinks per day: 158.4 ppt; >4 drinks per day: 276.6 ppt). Under the maximal assumption, the mean initial dioxin levels differed significantly (p=0.049) with corresponding means of 121.4 ppt, 105.5 ppt, and 182.2 ppt for increasing current alcohol use categories. However, when current alcohol use was treated as a continuous variable, the correlation between current alcohol use and initial dioxin was not significant under both assumptions (minimal, p=0.326; maximal, p=0.703).

For all Ranch Hand participants, the mean current dioxin levels did not differ significantly among the current alcohol use categories (p=0.171). The differences were marginally significant for all Comparisons (p=0.100; 0-1 drink per day: 3.6 ppt; >1-4 drinks per day: 3.2 ppt; >4 drinks per day: 4.5 ppt). The correlation between current alcohol use, when treated as a continuous variable, and current dioxin was nonsignificant for both groups (Ranch Hands, p=0.255; Comparisons, p=0.523).

Under both assumptions, mean initial dioxin levels differed significantly among Ranch Hands who had lifetime alcohol history values of 0 drink-years, over 0 but no more than 40 drink-years, and over 40 drink-years (minimal, p=0.012; maximal, p=0.001). (See Chapter 7, Malignancy Assessment, for a definition of drink-years.) For these lifetime alcohol history categories, the mean initial dioxin levels for the minimal cohort were 233.7, 167.5, and 192.8 ppt, respectively. For the maximal cohort, the corresponding mean initial dioxin levels were 163.7, 110.1, and 134.3 ppt, respectively. Under both assumptions, however, the correlation

between lifetime alcohol history and initial dioxin was not significant when lifetime alcohol history was treated as a continuous variable (minimal, p=0.318; maximal, p=0.125).

The mean current dioxin levels were significantly different among the lifetime alcohol categories for all Ranch Hand participants (p=0.021). The current dioxin means for the categories of 0 drink-years, over 0 but no more than 40 drink-years, and over 40 drink-years were 18.7, 13.4, and 15.8 ppt. For all Comparisons, the differences in the mean current dioxin levels were not significant (p=0.810). When lifetime alcohol history was treated as a continuous variable, the correlation between lifetime alcohol history and current dioxin was not significant for both groups (Ranch Hands, p=0.728; Comparisons, p=0.894).

Under both the minimal and maximal assumptions, the mean initial dioxin levels differed significantly between Ranch Hands who reported they did not drink wine and Ranch Hands who reported they drank wine at the time of the 1987 examination (minimal, p<0.001; maximal, p<0.001). The mean initial dioxin levels for the minimal cohort were 197.2 ppt for Ranch Hands with zero drinks per day and 148.5 ppt for Ranch Hands with more than zero drinks per day. For the maximal cohort, the corresponding mean initial dioxin levels were 139.9 ppt and 92.1 ppt. When current wine use was treated as a continuous variable, a significant negative correlation between current wine use and initial dioxin was exhibited under both assumptions (minimal, p=0.011; maximal, p=0.003).

For all Ranch Hand participants, the mean current dioxin level was significantly higher for Ranch Hands who reported they did not drink wine than for Ranch Hands who reported they drank wine at the time of the 1987 examination (p<0.001). The current dioxin means were 16.7 ppt and 11.3 ppt for the two current wine use strata (i.e., 0 drinks per day and >0 drinks per day). However, the correlation between current wine use, when treated as a continuous variable, and current dioxin was nonsignificant for all Ranch Hand participants (p=0.110). For all Comparisons, the current dioxin means did not differ significantly between the two current wine use categories (p=0.656). The correlation between current wine use and current dioxin was also nonsignificant for the Comparisons (p=0.853).

The mean initial dioxin levels differed significantly among the lifetime wine history categories (0 drink-years, >0-10 drink-years, and >10 drink-years) under both assumptions (minimal, p<0.001; maximal, p<0.001). Under the minimal assumption, the mean initial dioxin levels were 207.4, 151.9, and 117.9 ppt for the lifetime wine history categories (0 drink-years, >0-10 drink-years, and >10 drink-years). Under the maximal assumption, the corresponding means were 144.2, 97.1, and 87.5 ppt, respectively. When lifetime wine history was treated as a continuous variable, a significant negative correlation between lifetime wine history and current dioxin was exhibited under both assumptions (minimal, p<0.001; maximal, p=0.004).

There was a significant difference in the mean current dioxin levels for all Ranch Hand participants with lifetime wine history values of 0 drink-years, greater than 0 but no more than 10 drink-years, and greater than 10 drink-years (p<0.001). The mean current dioxin levels were 16.9, 11.8, and 12.9 ppt for the lifetime wine history categories, respectively. For all Ranch Hand participants, there was a marginally significant negative correlation between lifetime wine history, when treated as a continuous variable, and current dioxin (p=0.086). For all Comparisons, the difference in mean current dioxin levels among the lifetime wine

history categories was not significant (p=0.482). In contrast to the Ranch Hands, the correlation between lifetime wine history and current dioxin was positive, but nonsignificant for all Comparisons (p=0.603).

SMOKING HABITS

The covariates used to evaluate smoking habits were current cigarette smoking and lifetime cigarette smoking history.

Under the minimal and maximal assumptions, the mean initial dioxin levels were not significantly different for Ranch Hands with current cigarette smoking habits categorized as follows: never smoked, formerly smoked, smoked no more than 20 cigarettes per day, and smoked over 20 cigarettes per day (minimal, p=0.603; maximal, p=0.208). Similarly, the mean current dioxin levels were not significantly different among the defined current cigarette smoking categories for all Ranch Hand participants (p=0.587). However, for all Comparisons, there was a significant difference in the mean current dioxin levels among the current cigarette smoking categories (p<0.001). The mean current dioxin levels were 4.3 ppt for those who never smoked, 3.5 ppt for those who formerly smoked, 2.9 ppt for those who smoked no more than 20 cigarettes per day, and 3.1 ppt for those who smoked over 20 cigarettes per day.

When current cigarette smoking was treated as a continuous variable, the correlation between initial dioxin and current cigarette smoking was not significant under both assumptions (minimal, p=0.758; maximal, p=0.355). However, for all Ranch Hand participants, the correlation between current dioxin and current cigarette smoking was significantly negative (p=0.049). For all Comparisons, there was also a significant negative association between current dioxin and current cigarette smoking (p=0.035).

Mean initial dioxin levels were compared for Ranch Hands who had categorized lifetime cigarette smoking history values of 0 pack-years, up to 10 pack-years, and over 10 pack-years. (See Chapter 7 for a definition of pack-years.) Under both assumptions, the means were not significantly different (minimal, p=0.749; maximal, p=0.621). In addition, mean current dioxin levels also did not differ significantly among all Ranch Hand participants for the categorized lifetime cigarette smoking history values (p=0.297). However, there was a significant difference in mean current dioxin levels for all Comparisons (p<0.001; 0 pack-years: 4.3 ppt; >0-10 pack-years: 2.9 ppt; >10 pack-years: 3.6 ppt).

The correlation between initial dioxin and lifetime cigarette smoking, when treated as a continuous variable, was not significant under both assumptions (minimal, p=0.147; maximal, p=0.783). Likewise, the correlation between current dioxin and lifetime cigarette smoking was not significant for all Comparisons (p=0.719). However, for all Ranch Hand participants, there was a significant negative correlation between current dioxin and lifetime cigarette smoking (p=0.006).

SUN EXPOSURE CHARACTERISTICS

The following covariates characterize sun exposure and reaction to sun exposure: average lifetime residential latitude, ethnic background, skin color, hair color, eye color, reaction of skin to sun after at least 2 hours of exposure after first exposure, reaction of skin

to sun after repeated exposure, and a composite sun-reaction index. These variables were candidate covariates for the skin neoplasm analyses. Since Blacks were excluded in the analyses of skin neoplasms, they were also excluded in these analyses.

A line connecting San Francisco, California, and Richmond, Virginia, approximates 37 degrees North latitude. Participants were classified into two categories depending on whether their average lifetime residential latitude was above or below 37 degrees North latitude. The determination of each participant's average lifetime residential latitude is discussed in Chapter 7. Under both the minimal and maximal assumptions, the initial dioxin means did not differ significantly between Ranch Hands who resided in the northern latitudes ($\geq 37^{\circ}$ N. latitude) and those who resided in the southern latitudes ($<37^{\circ}$ N. latitude) (minimal, $p=0.128$; maximal, $p=0.247$). The current dioxin means also did not differ significantly between the north and the south for all Ranch Hand participants ($p=0.596$) and for all Comparisons ($p=0.786$).

For this study, ethnic background was divided into five categories (A: English, Welsh, Scottish, or Irish; B: Scandinavian, German, Polish, Russian, Other Slavic, Jewish, or French; C: Spanish, Italian, or Greek; D: Mexican, American Indian, or Asian; E: African). These five categories were combined into two categories for this analysis (A and B in one category; C, D, and E in the other). Under the minimal assumption, there was a significant difference in the mean initial dioxin levels between these two categories ($p=0.022$; AB: 179.8 ppt, CDE: 260.4 ppt). The mean initial dioxin levels also differed significantly under the maximal assumption ($p<0.001$; AB: 116.5 ppt; CDE: 214.8 ppt). For all Ranch Hand participants there was a significant difference in the mean current dioxin levels ($p<0.001$; AB: 14.0 ppt; CDE: 29.1 ppt), but, for all Comparisons, the difference in the current dioxin means was not significant ($p=0.115$). For the Ranch Hands, the current dioxin mean was greater for the CDE category, whereas, for the Comparisons, the AB category had the larger current dioxin mean.

There were no significant differences, under either assumption, in the mean initial dioxin levels between Ranch Hands with skin color categorized as peach and those whose skin color was not peach (minimal, $p=0.952$; maximal, $p=0.293$). The difference in the mean current dioxin levels was nonsignificant for all Ranch Hand participants ($p=0.354$) and for all Comparisons ($p=0.582$).

Under both assumptions, the initial dioxin means were significantly different between Ranch Hands with black or dark brown hair and other Ranch Hands (minimal, $p=0.008$; maximal, $p=0.005$). The means, under the minimal assumption, were 196.7 ppt for black or dark brown hair and 158.4 ppt for other hair colors. Under the maximal assumption, the corresponding means were 129.0 and 104.2 ppt. The difference in the current dioxin means was significant for all Ranch Hand participants ($p=0.004$), but not for all Comparisons ($p=0.486$). For the Ranch Hands, the current dioxin means were 15.7 ppt (black/dark brown) and 12.2 ppt (other); whereas, for the Comparisons, the current dioxin mean was lower for the black/dark brown hair category than for the other category.

No significant association was found between eye color and initial dioxin under the minimal assumption ($p=0.101$). However, under the maximal assumption, there was a

marginally significant difference in the initial dioxin means among the eye color categories of brown, hazel/green, and grey/blue (p=0.097). The initial dioxin means were 135.4, 113.5, and 114.4 ppt, respectively. For all Ranch Hand participants, the association between eye color and current dioxin was nonsignificant (p=0.103). There was, however, a marginally significant association for all Comparisons (p=0.072). The current dioxin means for the Comparisons were 3.4, 3.4, and 3.9 ppt for the brown, hazel/green, and grey/blue categories.

The reaction of one's skin after at least 2 hours of exposure to the sun, after the first exposure, was not significantly associated with initial dioxin under either assumption (minimal, p=0.720; maximal, p=0.995). There was also no significant association with current dioxin for all Ranch Hand participants (p=0.997). For all Comparisons, however, there was a marginally significant difference in the current dioxin means among the skin reaction categories (p=0.062). The means were 3.5 ppt for Comparisons who reported they experienced no reaction, 3.5 ppt for those who became red, 3.7 ppt for those who burned, and 5.0 ppt for those who burned painfully.

The reaction of one's skin, after repeated exposure to the sun, was not significantly associated with initial dioxin under the minimal assumption (p=0.565). However, under the maximal assumption, there was a marginally significant association (p=0.094). The initial dioxin means were 118.2 ppt for those who reported they tanned deep brown, 113.8 ppt for those who tanned moderately, 149.4 ppt for those who tanned mildly, and 138.1 ppt for those who freckled with no tan. For all Ranch Hand participants, there was no significant association between current dioxin and skin reaction to repeated sun exposure (p=0.507). For all Comparisons, however, the differences in the current dioxin means among the skin reaction categories (tanned deep brown, tanned moderately, tanned mildly, and freckled with no tan) were marginally significant (p=0.088). The current dioxin means were 3.4, 3.8, 3.4, and 5.6 ppt, respectively.

A composite sun-reaction index was formed from the two skin reaction measures and categorized as follows: high (burns painfully and/or freckles with no tan), medium (burns and/or tans mildly), and low (all other reactions). The mean initial dioxin levels for these categories did not differ significantly under both the minimal and the maximal assumptions (minimal, p=0.764; maximal, p=0.319). There were also no significant differences in the mean current dioxin levels for all Ranch Hand participants (p=0.496). However, for all Comparisons, the current dioxin means differed significantly (p=0.008) with means of 3.5, 3.4, and 5.1 ppt for the low, medium, and high sun reaction categories.

EXPOSURE TO CARCINOGENS

Information was gathered on each participant's exposure to 21 different carcinogens. (See Chapter 7 for a discussion of these carcinogens.) These carcinogens were divided into two sets. The first set consisted of asbestos, ionizing radiation, industrial chemicals, herbicides, insecticides, and degreasing chemicals. The other set contained anthracene, arsenic, benzene, benzidine, chromate, coal tar, creosote, aminodiphenyl, chloromethyl ether, mustard gas, naphthylamine, cutting oils, trichloroethylene, ultraviolet light, and vinyl chloride. A composite carcinogen exposure variable was created from the second set. The response was coded as "yes" if the individual had been exposed to any of the 15 carcinogens.

The mean initial dioxin levels did not differ between those Ranch Hands who had been exposed to ionizing radiation and those who had not been exposed (minimal, p=0.118; maximal, p=0.626). There was also no significant difference in the current dioxin means for all Comparisons (p=0.833). However, for all Ranch Hands, there was a marginally significant difference in the current dioxin means between those who had been exposed to ionizing radiation and those who had not been exposed (p=0.070; exposed: 12.3 ppt, not exposed: 15.0 ppt).

Under both the minimal and maximal assumptions, Ranch Hands who had been exposed to industrial chemicals had a significantly higher mean initial dioxin level than those who had not been exposed (minimal, p=0.003; maximal, p<0.001). Under the minimal assumption, the mean initial dioxin levels were 196.8 ppt for those who had been exposed and 157.8 ppt for those who had not been exposed. Under the maximal assumption, the means were 138.8 ppt and 100.0 ppt. Ranch Hand participants who had been exposed to industrial chemicals also had a higher mean current dioxin level than those who had not been exposed (p<0.001; exposed: 16.6 ppt; not exposed: 12.1 ppt). There was also a significant difference for all Comparisons (p=0.043), but the exposed category had a lower current dioxin level mean than the nonexposed category (exposed: 3.4 ppt; not exposed: 3.8 ppt).

Under the minimal assumption, there was a marginally significant difference in the mean initial dioxin levels between Ranch Hands who had been exposed to insecticides and those who had not been exposed (173.0 ppt versus 200.5 ppt; p=0.074). Under the maximal assumption, the difference was not significant (p=0.484). For all Ranch Hand participants and for all Comparisons, the mean current dioxin levels did not differ between the two insecticide exposure categories (Ranch Hands, p=0.391; Comparisons, p=0.430).

Under both assumptions, the Ranch Hands who reported being exposed to degreasing chemicals had a higher mean initial dioxin level than those who had not been exposed (minimal, p=0.001; maximal, p<0.001). The means, under the minimal assumption, were 196.0 ppt for those who had been exposed and 150.5 ppt for those who had not been exposed. Under the maximal assumption, the corresponding means were 137.3 ppt and 94.5 ppt, respectively. The mean current dioxin level was also higher for all Ranch Hand participants who reported exposure to degreasing chemicals than for those who reported no exposure (17.1 ppt versus 10.9 ppt; p<0.001). For all Comparisons, the difference was nonsignificant (p=0.926).

For the other two carcinogens in the first set (asbestos and herbicides), no significant differences in the initial dioxin means were found between the exposed category and the nonexposed category, under both assumptions. There were also no significant differences in the current dioxin means for all Ranch Hands and all Comparisons (see Table 5-1 for the associated significance probabilities).

There was no significant difference, under the minimal assumption, between the initial dioxin mean for those who had been exposed to benzene and the initial dioxin mean for those who had not been exposed (p=0.201). However, under the maximal assumption, those who had been exposed to benzene had a marginally higher initial dioxin mean than those who had not been exposed (162.6 ppt versus 118.4 ppt; p=0.089). The current dioxin means did not

differ significantly for all Ranch Hand participants and for all Comparisons (Ranch Hands, p=0.522; Comparisons, p=0.893).

Ranch Hands who had been exposed to chromate had a marginally higher initial dioxin mean, under the minimal assumption, and a significantly higher initial dioxin mean, under the maximal assumption, than those who had not been exposed (minimal, p=0.057; maximal, p=0.034). The means under the minimal assumption were 232.5 ppt for the exposed category and 176.6 ppt for the nonexposed category. Under the maximal assumption, the corresponding means were 159.2 ppt and 117.5 ppt, respectively. For all Ranch Hand participants and for all Comparisons, the current dioxin means did not differ significantly (Ranch Hands, p=0.160; Comparisons, p=0.593).

The mean initial dioxin levels differed significantly between Ranch Hands who had been exposed to aminodiphenyl and those who had not been exposed, under both assumptions (minimal, p<0.001; maximal, p<0.001). Those who had been exposed had a lower mean than those who had not been exposed (minimal, 83.2 ppt versus 180.5 ppt; maximal, 83.2 ppt versus 119.9 ppt). For all Ranch Hand participants and for all Comparisons, the mean current dioxin levels did not differ significantly (Ranch Hands, p=0.998; Comparisons, p=0.649). However, there were only two Ranch Hand participants and four Comparisons who had been exposed to aminodiphenyl.

Under the minimal assumption, there was no significant difference between the initial dioxin mean for Ranch Hands who had been exposed to chloromethyl ether and the mean for those who had not been exposed (p=0.648). Under the maximal assumption, the difference was marginally significant (p=0.070). The means were 65.4 ppt for those who reported being exposed to chloromethyl ether and 120.5 ppt for those who reported no exposure. There were, however, only three Ranch Hands in the minimal cohort and eight in the maximal cohort who had been exposed to chloromethyl ether. The current dioxin means for the two exposure categories did not differ significantly for all Comparisons (p=0.267), but did differ significantly for all Ranch Hand participants (p=0.015; exposed: 6.0 ppt, not exposed: 14.5 ppt).

Under the maximal assumption, the mean initial dioxin level for those Ranch Hands who had been exposed to naphthylamine was significantly higher than for those who had not been exposed (179.5 ppt versus 118.2 ppt; p=0.028). The difference was not significant under the minimal assumption (p=0.249). For all Ranch Hand participants and for all Comparisons, there was no significant difference between the naphthylamine exposure categories (Ranch Hands, p=0.217; Comparisons, p=0.759).

Under both assumptions, there was no significant difference in the initial dioxin means for Ranch Hands who were exposed to cutting oils and those who were not (minimal, p=0.706; maximal, p=0.924). There was also no significant difference in the current dioxin means for all Ranch Hand participants (p=0.693). For all Comparisons, however, the current dioxin mean was marginally lower for those who had been exposed to cutting oils than for those who had not been exposed (3.0 ppt versus 3.7 ppt; p=0.076).

Ranch Hands in the maximal cohort who had been exposed to trichloroethylene had a marginally higher initial dioxin mean than those who had not been exposed (142.4 ppt versus

117.3 ppt; p=0.092). The difference was not significant under the minimal assumption (p=0.170). There was also no significant difference in the current dioxin means for all Ranch Hand participants and for all Comparisons (Ranch Hands, p=0.547; Comparisons, p=0.386).

With respect to the remaining carcinogens in the second set (anthracene, arsenic, benzidine, coal tar, creosote, mustard gas, ultraviolet light, and vinyl chloride), the initial dioxin means did not differ significantly between the exposed and nonexposed categories. Similarly, for all Ranch Hand participants and all Comparisons, the current dioxin means were not significantly different between the exposed and nonexposed categories. Table 5-1 presents the associated significance probabilities.

For the composite carcinogen exposure variable, under the minimal assumption, there was no significant difference between the initial dioxin mean of the exposed category and the initial dioxin mean of the nonexposed category (p=0.209). Under the maximal assumption, those Ranch Hands who had been exposed to any of the carcinogens in the second set had a significantly higher initial dioxin mean than those who had not been exposed (134.2 ppt versus 114.7 ppt; p=0.045). The mean current dioxin level was also significantly higher for all Ranch Hands who had been exposed, as compared to those who had not been exposed (16.4 ppt versus 13.6 ppt; p=0.038). In contrast, for all Comparisons, those who had not been exposed to any of the carcinogens had a higher current dioxin mean (3.6 ppt) than those who had been exposed (3.3 ppt), but the difference was not significant (p=0.157).

PERSONAL AND FAMILY HEALTH

The personal health covariates used in this study were cholesterol, high-density lipoprotein (HDL), cholesterol-HDL ratio, diabetic class, differential cortisol response, and percent body fat. Family health was also taken into account by means of family history of heart disease and family history of heart disease before the age of 50. No participants were excluded from the association analyses for these variables.

The correlation between cholesterol and initial dioxin was not significant under either assumption (minimal, p=0.217; maximal, p=0.215). The differences in the initial dioxin means for the three cholesterol categories (≤200 mg/dl; >200-230 mg/dl; >230 mg/dl) were also nonsignificant under both assumptions (minimal, p=0.227; maximal, p=0.362). For all Ranch Hand participants and for all Comparisons, the correlation between current dioxin and cholesterol was not significant (Ranch Hands, p=0.137; Comparisons, p=0.196). The current dioxin means also did not differ significantly among the cholesterol categories (Ranch Hands, p=0.175; Comparisons, p=0.139).

Under the minimal assumption, there was a marginally significant negative correlation between HDL and initial dioxin (p=0.090). However, the initial dioxin means for the three HDL categories (≤40 mg/dl; >40-50 mg/dl; >50 mg/dl) did not differ significantly (p=0.400). Under the maximal assumption, there was a significant negative correlation between HDL and initial dioxin (p<0.001), and the differences in the initial dioxin means among the HDL categories was also significant (p<0.001; ≤40 mg/dl: 138.6 ppt; >40-50 mg/dl: 121.7 ppt; >50 mg/dl: 99.6 ppt). The correlation between current dioxin and HDL was significant for all Ranch Hand participants (p<0.001) and for all Comparisons (p=0.005). The mean current dioxin levels also differed significantly among the HDL categories for both groups (Ranch

Hands, p<0.001; Comparisons, p=0.008). For all Ranch Hand participants, the means were 17.5, 14.5, and 11.6 ppt for the HDL categories (≤40 mg/dl, >40-50 mg/dl, and >50 mg/dl). For all Comparisons, the corresponding means were 3.9, 3.7, and 3.1 ppt, respectively.

The results for the cholesterol-HDL ratio were similar, but in the opposite direction, to the HDL results. Under the minimal assumption, there was a marginally significant positive correlation between initial dioxin and the cholesterol-HDL ratio (p=0.076), but the initial dioxin means did not differ significantly among the cholesterol-HDL categories (p=0.104). Under the maximal assumption, there was a significant correlation between initial dioxin and the cholesterol-HDL ratio (p<0.001) and there was a significant difference in the initial dioxin means (p<0.001; ≤4.2: 97.0 ppt; >4.2-5.5: 124.5 ppt; >5.5: 139.3 ppt). For all Ranch Hand participants and for all Comparisons, there was a significant positive correlation between current dioxin and the cholesterol-HDL ratio (Ranch Hands, p<0.001; Comparisons, p=0.002). The current dioxin means for the cholesterol-HDL categories also differed significantly for both groups (Ranch Hands, p<0.001; Comparisons, p=0.001). For the cholesterol-HDL ratio categories (≤4.2, >4.2-5.5, and >5.5), the current dioxin means were 11.3, 15.2, and 17.2 ppt for the Ranch Hands and 3.0, 3.9, and 3.9 ppt for the Comparisons.

Under the minimal assumption, there was a marginally significant difference in the mean initial dioxin levels for Ranch Hands classified as normal, impaired, and diabetic (p=0.095). The mean initial dioxin levels were 174.4, 176.2, and 221.9 ppt for the normal, impaired, and diabetic classes. Under the maximal assumption, the mean initial dioxin levels differed significantly among the three diabetic classes (p=0.001; normal: 112.8 ppt; impaired: 123.7 ppt; diabetic: 169.9 ppt).

For all Ranch Hand participants, a significant difference in the mean current dioxin levels was exhibited among the three diabetic classes (p=0.001). The means were 13.5, 14.8, and 21.9 ppt for the normal, impaired, and diabetic classifications. For all Comparisons, there was also a significant difference in the mean current dioxin levels for the three diabetic classes (p=0.028). The means were 3.4, 4.0, and 4.5 ppt, respectively.

The correlation between initial dioxin and differential cortisol response was not significant under either the minimal or maximal assumptions (minimal, p=0.583; maximal, p=0.112). However, the differences in the initial dioxin means among the differential cortisol response categories (≤0.6 μg/dl; >0.6-4.0 μg/dl; >4.0 μg/dl) were marginally significant under the minimal assumption (p=0.056) and significant under the maximal assumption (p=0.007). The initial dioxin means were 191.7, 189.0, and 155.5 ppt under the minimal assumption and 132.0, 127.5, and 101.4 ppt under the maximal assumption. For all Ranch Hand participants, there was a significant negative correlation between current dioxin and differential cortisol response (p=0.027) and a significant difference in the current dioxin means among the differential cortisol response categories (p<0.001; ≤0.6 μg/dl: 15.7 ppt; >0.6-4.0 μg/dl: 16.4 ppt; >4.0 μg/dl: 11.5 ppt). For all Comparisons, neither the correlation between current dioxin and differential cortisol response (p=0.152) nor the difference in the current dioxin means among the differential cortisol response categories (p=0.315) was significant.

Percent body fat and initial dioxin exhibited a significant positive correlation under both assumptions (minimal, p=0.001; maximal, p<0.001). There was also a significant positive

correlation between percent body fat and current dioxin for all Ranch Hand participants and for all Comparisons (Ranch Hands, $p<0.001$; Comparisons, $p<0.001$).

Under both the minimal and maximal assumptions, Ranch Hands who had been classified as obese had a significantly higher mean initial dioxin level than those who had been classified as normal or lean (minimal, $p=0.018$; maximal, $p<0.001$). The means, under the minimal assumption, were 211.4 ppt for the obese category and 170.4 ppt for the normal/lean category. Under the maximal assumption, the corresponding means were 161.1 ppt and 110.2 ppt, respectively. Similarly, for current dioxin levels, all Ranch Hands who had been classified as obese had a higher mean current dioxin level than those who had been classified as normal or lean ($p<0.001$; obese: 22.4 ppt; normal/lean: 12.9 ppt). The mean current dioxin level for all Comparisons who had been classified as obese was also higher than the mean for all Comparisons who had been classified as normal or lean ($p<0.001$; obese: 4.4 ppt; normal/lean: 3.3 ppt).

Under both the minimal and the maximal assumptions, there was no significant association between initial dioxin and either family history of heart disease (minimal, $p=0.793$; maximal, $p=0.867$) or family history of heart disease before the age of 50 (minimal, $p=0.979$; maximal, $p=0.515$). For all Ranch Hand participants and for all Comparisons, the association with current dioxin was also nonsignificant for family history of heart disease (Ranch Hands, $p=0.591$; Comparisons, $p=0.765$) and for family history of heart disease before the age of 50 (Ranch Hands, $p=0.970$; Comparisons, $p=0.134$).

OTHER CHARACTERISTICS

The relationship with initial and current dioxin was also examined for education, blood type, presence of pre-Southeast Asia (SEA) acne, and personality type.

Ranch Hands with only a high school education had a significantly higher mean initial dioxin level than those with a college education, under both assumptions (minimal, $p=0.001$; maximal, $p<0.001$). Under the minimal assumption, the means were 198.0 ppt and 153.4 ppt for the high school and college categories. Under the maximal assumption, the means were 153.1 ppt and 89.8 ppt, respectively. The mean current dioxin level for all Ranch Hand participants with only a high school education was significantly greater than the mean for all Ranch Hand participants with a college education (18.2 ppt versus 11.1 ppt; $p<0.001$). For all Comparisons, the college graduates had a larger current dioxin mean than those with only a high school education, but the difference was not significant ($p=0.378$).

No significant differences in the mean initial dioxin levels were found among the four blood types (A, B, AB, and O) under either the minimal or the maximal assumption (minimal, $p=0.973$; maximal, $p=0.593$). For all Ranch Hand participants and for all Comparisons the differences in the mean current dioxin levels among the four blood types were also nonsignificant (Ranch Hands, $p=0.773$; Comparisons, $p=0.469$).

Under the minimal and maximal assumptions, the initial dioxin mean for the Ranch Hands with acne prior to their first SEA tour was not significantly different from the mean for those without acne before their first SEA tour (minimal, $p=0.523$; maximal, $p=0.309$). The current dioxin means also did not differ significantly between the Ranch Hand participants

with pre-SEA acne and those without (p=0.819) nor between the Comparisons with and without pre-SEA acne (p=0.246).

Under the minimal assumption, the mean initial dioxin levels for individuals classified as either type A or type B (by the Jenkins Activity Survey administered at the 1985 followup examination) were not significantly different (p=0.401). However, under the maximal assumption, the mean initial dioxin levels for Ranch Hands classified as type A (112.3 ppt) and Ranch Hands classified as type B (128.3 ppt) were marginally different (p=0.061). For all Ranch Hand participants, the difference in the mean current dioxin levels between type A and type B individuals was not significant (p=0.148). For all Comparisons, there was also no significant difference in the mean current dioxin levels (p=0.685).

SUMMARY

Among the matching variables, age and occupation exhibited a significant association with dioxin in one direction for Ranch Hands and in the opposite direction for Comparisons. Age had a negative correlation with initial dioxin for Ranch Hands under the minimal and maximal assumptions and a negative correlation with current dioxin for all Ranch Hands; whereas, for all Comparisons, age and current dioxin were positively correlated. In the analysis of occupation, the dioxin means were greatest for Ranch Hands in the enlisted groundcrew, but for Comparisons, the officers had the greater dioxin means, although all Comparison means were below generally accepted background levels (10 ppt).

For most of the alcohol variables, a significant association was exhibited with initial dioxin for the minimal and maximal cohorts, and with current dioxin for all Ranch Hands. However, for all Comparisons, the association with current dioxin was not significant. For Ranch Hands, the correlations between alcohol use and dioxin, when significant, tended to be negative.

For both smoking variables (current cigarette smoking and lifetime cigarette smoking history), the current dioxin means differed significantly among the smoking categories for all Comparisons. In both cases the correlation between smoking and dioxin was negative. In contrast, for the minimal and maximal cohorts and for all Ranch Hands, the dioxin means did not differ significantly.

The only sun exposure-related variables that had a significant association with dioxin were ethnic background and hair color for Ranch Hands and the composite sun reaction index for Comparisons.

In the analyses of the carcinogen exposure variables—degreasing chemicals, chromate, and naphthylamine—the exposed category had a higher dioxin mean than the nonexposed category, when the dioxin means differed significantly. In the analyses of aminodiphenyl and chloromethyl ether, the nonexposed category had a higher mean than the exposed category. Ranch Hands (including those in the minimal and maximal cohorts and all Ranch Hands) who had been exposed to industrial chemicals had higher dioxin means than those who had not been exposed; whereas, Comparisons who had been exposed to industrial chemicals had a lower dioxin mean than those who had not been exposed. For the composite carcinogen

exposure variable, Ranch Hands with an affirmative response had a higher dioxin mean than those who had not been exposed to any of the 15 specific carcinogens.

Among the personal and family health variables, percent body fat and the cholesterol-HDL ratio showed a significant positive correlation with dioxin for Ranch Hands and Comparisons, and HDL showed a significant negative correlation with dioxin. For both Ranch Hands and Comparisons, diabetic class also exhibited a significant association with dioxin, in which the dioxin means were greatest for the diabetic category.

Education was the only other variable to be significantly associated with dioxin. This association, in which college graduates had a lower dioxin mean than high school graduates, was only significant for Ranch Hands.

CONCLUSION

Many of the significant associations between dioxin and the covariates in the Ranch Hand group can be attributed to an indirect effect of occupational rank, which is highly associated with current serum levels of dioxin. For example, the decreasing relationship between age and dioxin occurred because enlisted groundcrew, who have the highest current dioxin levels of the Ranch Hands, were also the youngest occupational category, while officers, who have the lowest levels, were the oldest occupational category. Adjusting for occupation, the association between dioxin and age became nonsignificant under both the minimal ($p=0.138$) and maximal ($p=0.712$) assumptions. By contrast, the reason for the significant positive association with age in the Comparison group is not as apparent, but may be due to accumulation of normal background levels with time.

Significant associations in the Ranch Hand group between dioxin and education, industrial chemical exposure, degreasing chemical exposure, and wine consumption can also be explained by occupational differences (officers were more likely to be college educated, less likely to have been exposed to industrial or degreasing chemicals, and more likely to drink wine than the enlisted personnel). As with age, these associations (except for lifetime wine consumption under the minimal assumption) became nonsignificant after adjusting for occupation.

More difficult to understand are the associations in the Comparison group between current levels of dioxin with several of the covariates. Most of the Comparison group are assumed to have background levels (97.8% are less than 10 ppt) and there is no obvious related factor (such as occupation) that could explain the associations. Of the 51 covariates (discrete and continuous versions counted as one), 9 were significant at or below the 0.05 level. By chance alone, one would expect about two significant associations. The interrelatedness of some of the covariates may have inflated the number of significant results observed. Most of the significant associations were for the health variables (HDL, cholesterol-HDL ratio, diabetes, and percent body fat) that were also associated significantly with dioxin in the Ranch Hand group.

CHAPTER 5

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CHAPTER 18

CONCLUSIONS

INTRODUCTION

This chapter summarizes the conclusions drawn from statistical analyses performed on approximately 300 health-related endpoints in 12 clinical areas. The analyses focused on dioxin measurements obtained from serum samples collected at the 1987 Air Force Health Study (AFHS) examination. This report summarizes the first large-scale study of dose-response effects based on an accurate measurement of current dioxin levels. This investigation is an important enhancement of the AFHS and supplements previous AFHS reports, which focused on group contrasts between exposed (Ranch Hand) and unexposed (Comparison) cohorts.

Appendix Tables Q-1 to Q-18 summarize the results of the statistical analyses. The significant results discussed in this chapter describe associations between clinical endpoints and dioxin; however, independent of outside criteria (e.g., strength and consistency of association, biological plausibility), they do not necessarily imply cause and effect.

Statistical Models

Three statistical models were used to evaluate associations between the health of study participants and their serum dioxin levels:

- Model 1: Estimated initial dioxin levels using Ranch Hand participants only
- Model 2: Current serum dioxin levels and time since military service in Vietnam using Ranch Hand participants only
- Model 3: Four categories of current dioxin levels using both Ranch Hand and Comparison participants.

Analyses based on model 1 depend directly on first-order kinetics and a constant dioxin decay rate, while analyses based on model 2 assume nothing about dioxin elimination other than that Ranch Hands were exposed in Vietnam and that their body burdens have decreased over time in an unspecified manner. Thus, with model 1, one assumes everything is known about dioxin elimination in Ranch Hands; with model 2, one assumes nothing about dioxin elimination in Ranch Hands. All health data were analyzed using both of these models to reduce the likelihood that an effect would be missed due to incorrect assumptions regarding dioxin elimination. Models 1 and 2 were implemented under two assumptions: a minimal assumption and a maximal assumption. The minimal assumption included only Ranch Hands with current dioxin levels above 10 parts per trillion (ppt) (n=521); the maximal assumption expanded the analysis to include all Ranch Hands with current dioxin levels above 5 ppt (n=742). In addition, model 3, using both Ranch Hands and Comparisons, assessed the health consequences of current dioxin body burdens above background. This assessment required no assumptions about when or how increased dioxin body burdens were attained.

Statistical analyses often were applied to clinical endpoints in continuous (i.e., original measurement) and discrete (i.e., measurements grouped into categories based on abnormal levels) forms. Analyses also were performed to account for the effects that demographic and personal characteristics may have had on the clinical measurements. Such analyses are termed "adjusted analyses."

RESULTS

General Health Assessment

The general health assessment examined the associations between serum dioxin levels and the following five variables: self-perception of health, appearance of illness or distress at physical examination, relative age, percent body fat, and erythrocyte sedimentation rate. These variables were thought to be sensitive to the overall state of health rather than to any single organ system. Of these variables, the percent body fat and sedimentation rate consistently showed significant positive associations with the initial and current levels of dioxin.

Percent body fat can serve as an indicator of the presence of subclinical disease. For these analyses, percent body fat was determined from a formula using weight and height. The findings for percent body fat are consistent with the association between dioxin and diabetes mellitus in the endocrine assessment and lipids in the gastrointestinal assessment.

Sedimentation rate can be a sensitive, although nonspecific, index of general health. Consistent with the findings in this report, the Ranch Hand group had a significantly higher mean sedimentation rate than the Comparison group in both the previous report of the 1987 examination and the 1985 examination report. The results from these reports suggest that a subtle, chronic inflammatory response may be related to higher levels of dioxin exposure. The association between diabetes and dioxin noted in the endocrine assessment might be responsible for the sedimentation rate findings. However, a significant positive association between dioxin and sedimentation rate remained when the analysis was restricted to nondiabetics. As expected, diabetics had higher sedimentation rates than nondiabetics.

The longitudinal analyses of self-perception of health demonstrated significant positive associations with initial dioxin and current dioxin. However, the percentage of participants who reported fair or poor health decreased by more than 50 percent from 1982 to 1987. In the longitudinal analyses of sedimentation rate, the percentages of abnormalities in 1987 differed significantly among the current dioxin categories, with Ranch Hands in the low and high current dioxin categories exhibiting significant positive relative risks in relation to the background current dioxin category.

Malignancy Assessment

The relationship between dioxin and malignant and benign neoplasms, carcinomas in situ, and neoplasms of uncertain behavior or unspecified nature was assessed. Neoplasm refers to any new growth that may or may not be malignant; malignant neoplasms are neoplasms capable of invasion and metastasis. The evaluation was based on the incidence of neoplasms after the Southeast Asia (SEA) tours. Skin and systemic neoplasms were studied separately. The malignant skin neoplasm analyses were based on non-Blacks only;

the benign skin neoplasms analyses included Blacks. Risk factors such as age, ability to tan, cumulative sun exposure, and parental ethnicity, as well as eye, skin, and hair color, were considered as candidate covariates in the analyses of skin neoplasms.

The results of the serum dioxin analyses for the skin neoplasm assessment did not support a positive dose-response relationship. In each of the three previous AFHS reports, an increased risk of basal cell carcinomas was noted in the Ranch Hand group relative to the Comparison group. According to the group contrasts for the 1985 and 1987 examinations, Ranch Hands also had a significantly increased risk of sun exposure-related skin neoplasms (predominantly basal cell carcinoma, but also squamous cell carcinoma, melanoma, and malignant epithelial neoplasms not otherwise specified).

In this report, the initial dioxin analyses and the current dioxin and time since tour analyses found that the relative risks for basal cell carcinoma and sun exposure-related skin neoplasms often were less than 1. The relative risk was significantly less than 1 for the adjusted model 1 analyses of basal cell carcinoma on the ear, face, head, and neck under the minimal assumption. There was no increased risk for the development of any skin neoplasm related to dioxin except for occupation-specific analyses.

Analyses were performed within military occupational groups (officer, enlisted flyer, and enlisted groundcrew). Statistically significant increases in the incidence of basal cell carcinoma of sites other than the ear, face, head, or neck were found for the associations with the initial and current serum dioxin levels for Ranch Hand enlisted flyers. However, these results may be the result of a multiple-testing artifact, since they were not noted for the Ranch Hand enlisted groundcrew who, as a group, had higher levels of serum dioxin than the enlisted flyers. The Air Force will continue to monitor basal cell carcinoma in subsequent phases of the study.

The serum dioxin analyses detected significantly increased risks of benign, but not malignant, systemic neoplasms. Under the maximal assumption, Ranch Hand participants with high levels of initial dioxin had a greater incidence of benign systemic neoplasms (9.7%) than did Ranch Hands with medium (5.7%) or low (1.6%) levels (approximately 75% of benign neoplasms in Ranch Hands and 70% in Comparisons were lipomas). Under both assumptions, the adjusted current dioxin and time since tour analyses revealed that current dioxin was associated with significantly increased risks of benign systemic neoplasms for Ranch Hands with earlier tours. In the categorized current dioxin analyses, the adjusted relative risk was 2.13 for Ranch Hands in the high current dioxin category (>33.3 ppt).

By contrast, the adjusted initial dioxin analyses found that the relative risks were less than 1, but not significant, for malignant systemic neoplasms. The model 3 analyses showed that Ranch Hands in the low current dioxin category (>15-33.3 ppt) had a significantly increased risk relative to Comparisons in the background category (10 ppt or less), but none of the Ranch Hands in the high current dioxin category had any malignant systemic neoplasms.

At the 1985 examination, one Ranch Hand and one Comparison had verified soft tissue sarcoma (STS) (fibrous histiocytoma and fibrosarcoma, respectively). The Ranch Hand was

not part of the 1987 study because he died prior to his scheduled examination; the Comparison with the fibrosarcoma participated in the 1987 examination. At the 1985 examination, one Ranch Hand was classified as having a suspected leukemia, Hodgkin's disease (HD), or non-Hodgkin's lymphoma (NHL). He was subsequently diagnosed as having leukemia by the time of the 1987 examination. There was one verified case of NHL in a Ranch Hand at the 1987 examination.

In summary, dioxin was significantly associated with an increased risk of benign, but not malignant, systemic neoplasms. The study provided no evidence of increased risk for the neoplasms most commonly thought to be linked with exposure to chlorophenols (HD, NHL, and STS). However, the number of participants with these specific neoplasms was very small, limiting the statistical power to detect a significant relative risk. The increased incidence of basal cell skin neoplasms in Ranch Hands documented in previous examination cycles was not associated positively with serum dioxin, except among the enlisted flyers with basal cell carcinomas at sites other than the ear, face, head, or neck.

Neurological Assessment

The neurological assessment was based primarily on extensive physical examination data on cranial nerve function (CNF), peripheral nerve status, and central nervous system (CNS) coordination processes. These data were supplemented by verified histories of neurological diseases.

The neurological analyses revealed no consistent evidence to indicate that dioxin was associated with neurological disease. The analyses of hereditary and degenerative diseases found no association with dioxin, in contrast to the finding from the previous report that showed a significant increase in benign essential tremor in Ranch Hands. The adjusted analyses for the other historical variables also were not significant.

There were few statistically significant associations between dioxin and the physical examination variables. The power to detect significant results was limited for many of the CNF and CNS variables because there were few abnormalities. Peripheral neuropathy clearly has been shown to be associated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure in other studies, but no significant findings were noted for the eight peripheral motor and sensory indices.

Most of the significant findings in the neurological assessment were noted for the CNS variables. For the adjusted model 3 analyses, there was a significantly increased risk of coordination abnormalities for Ranch Hands in the high current dioxin category relative to Comparisons in the background current dioxin category. This finding is consistent with the previous report, which found that Ranch Hands on the whole had significantly more coordination abnormalities than Comparisons. The adjusted model 3 results for the CNS index (a composite variable based on coordination, tremor, and gait) displayed similar findings. The adjusted model 1 results were marginally significant for coordination and significant for the CNS index. The longitudinal analyses of the CNS index under the maximal assumption revealed a marginally significant positive association with initial dioxin.

In summary, dioxin was associated significantly with coordination and the CNS index, but CNF and peripheral nerve status variables were not associated with dioxin.

Psychological Assessment

The psychological assessment examined verified psychological disorders, reported sleep disorders, and the results of two clinical psychological tests: the Symptom Check List-90-Revised (SCL-90-R) and the Millon Clinical Multiaxial Inventory (MCMI). The SCL-90-R is a 90-item checklist of physical and mental symptoms that provides a reasonable measure of health-related concerns and associated anxiety, depression, and general emotional discomfort. The MCMI provides backup measures of depression, anxiety, somatization, and hypochondriasis for the SCL-90-R, while also screening for personality patterns, disorders, and major psychiatric syndromes, including psychoses. Both the SCL-90-R and the MCMI have been used extensively in research and in some clinical settings requiring economical assessment of psychiatric disorders, physical disability status, and response to specific therapies. The SCL-90-R variables were discretized as abnormal or normal for analysis, while each MCMI variable was analyzed in its continuous form.

The serum dioxin analyses did not indicate that dioxin was associated significantly with either the verified questionnaire disorders or the reported sleep disorders. The unadjusted analyses for the SCL-90-R variables were often significant, but in most cases became nonsignificant after covariate adjustment. This was due primarily to adjustment for education. The education covariate was associated indirectly with serum dioxin levels because both of these variables were highly associated with military occupation and rank.

By contrast, the adjusted analyses for many of the MCMI variables showed a significant association with dioxin. After adjustment, the initial dioxin analyses found that 9 of the 20 MCMI scale results were significant under either the minimal or maximal assumption (positive: schizoid, avoidant, dependent, schizotypal, somatoform, psychotic thinking, and psychotic depression scores; negative: histrionic and narcissistic scores). Most of these variables also were associated significantly with current dioxin levels in the model 2 analyses based on Ranch Hands with more than 18.6 years since service in SEA. The adjusted model 3 analyses detected fewer significant results. Ranch Hands in the high current dioxin category relative to Comparisons in the background current dioxin category had significantly higher mean schizoid and schizotypal scores and a significantly lower mean histrionic score.

Although the MCMI results suggest the possibility of a relationship between dioxin and personality disturbances and/or psychotic disorders, they are inconsistent with the results for the verified questionnaire data and the SCL-90-R scales. The relatively large number of statistically significant MCMI results may be explained in part by substantial overlap inherent to the construction of test scales. Because there was a lack of consistency across similar variables included in the SCL-90-R, MCMI, and verified disorders, the available evidence does not suggest that the body burden of dioxin is related to psychological or psychophysiological disorders.

Gastrointestinal Assessment

The gastrointestinal assessment examined the history of eight categories of liver disease: viral hepatitis, acute and subacute necrosis of the liver, chronic liver disease and cirrhosis (alcohol-related and nonalcohol-related were analyzed separately), liver abscess and sequelae of chronic liver disease, other disorders of the liver (abnormal liver scans, abnormal enzyme elevations, unspecified hepatitis, and unspecified disorders of the liver), hepatomegaly, and jaundice. Verified histories of ulcers and skin patches, bruises, and sensitivity also were analyzed. Hepatomegaly diagnosed at the physical examination was investigated in addition to 13 laboratory variables (aspartate aminotransferase [AST], alanine aminotransferase [ALT], gamma-glutamyl transpeptidase [GGT], alkaline phosphatase, d-glucaric acid, total bilirubin, direct bilirubin, lactic dehydrogenase, cholesterol, high-density lipoprotein [HDL], the cholesterol-HDL ratio, triglycerides, and creatine kinase).

The serum dioxin analyses of the hepatic enzymes found significant positive associations with ALT and GGT, but the findings were not significant for the other enzymes. The lipid indices were associated significantly and consistently with dioxin. The analyses of the variable triglycerides showed strong positive associations with both the initial levels of dioxin and the current serum levels; this variable is highly sensitive to body fat. Cholesterol, HDL, and the cholesterol-HDL ratio also showed significant associations with dioxin.

Initial dioxin level was associated with a significantly increased risk of an abnormally high level for several of the laboratory variables (AST, ALT, GGT, and the cholesterol-HDL ratio) in Ranch Hands who had reported no exposure to degreasing chemicals. This pattern is puzzling since it contradicts a synergistic effect of degreasing chemicals and dioxin exposure. The relative risk for these variables was not significant for Ranch Hands who had reported exposure to degreasing chemicals.

In summary, the gastrointestinal analyses did not indicate that the historical liver conditions were associated with dioxin at this time. However, laboratory results showed a consistent pattern suggestive of a subclinical effect on lipid metabolism, possibly related to the positive association between dioxin and percent body fat that was observed in the general health assessment.

Dermatologic Assessment

The dermatologic evaluation was based on verified questionnaire data on the occurrence and location of acne (lifetime and relative to SEA tour). These data were supplemented with eight variables derived from the physical examination: comedones, acneiform lesions, acneiform scars, depigmentation, inclusion cysts, hyperpigmentation, other abnormalities, and a dermatology index. The "other abnormalities" variable included jaundice, spider angiomata, palmar erythema, palmar keratoses, actinic keratoses, petechiae, ecchymoses, conjunctival abnormality, oral mucosal abnormality, fingernail abnormality, toenail abnormality, dermatographia, cutis rhomboidalis, nevus, and other abnormalities. The dermatology index was based on the presence of comedones, acneiform lesions, acneiform scars, and inclusion cysts.

The clinical endpoint, chloracne, has been linked conclusively to topical dioxin exposure in other studies. No evidence of active chloracne has been detected at any of the three AFHS examination cycles. This is not surprising since the concentrations of TCDD exposure in Ranch Hands probably were much less than the concentrations needed to produce overt lesions, based on animal and human studies. Recognizing the remote possibility that chloracne may have occurred in acute form and been resolved, the physical examination emphasized chronic cutaneous conditions, such as scarring and pigmentation, which are complications of all forms of acne.

Dermatologic endpoints were not consistently associated with dioxin concentrations. The adjusted current dioxin and time since tour analyses displayed several statistically significant findings, but they were not supported by the adjusted initial dioxin analyses or the adjusted categorized current dioxin results. The adjusted current dioxin and time since tour analyses under the maximal assumption for Ranch Hands with a later tour of duty in Vietnam (time since tour$\leq$18.6 years) showed significant or marginally significant positive associations between current levels of dioxin and the occurrence of acne and several of the other acne-related physical examination variables after service in SEA. The corresponding adjusted relative risks for Ranch Hands with an early tour (time since tour>18.6 years) were either not significant or were significantly less than 1. Under the minimal assumption (but not the maximal), current levels of dioxin were associated with an increased risk of acne on the temples, eyes, and ears for Ranch Hands with an early tour.

The adjusted initial dioxin analyses found a significantly increased risk of hyperpigmentation abnormalities under the maximal assumption, but the adjusted relative risk was not significant under the minimal assumption. The questionnaire variables were not associated significantly with initial dioxin in the adjusted analyses. The adjusted categorized current dioxin results did not find that Ranch Hands in the high current dioxin category differed significantly with Comparisons in the background current dioxin category for any of the variables analyzed.

Cardiovascular Assessment

The cardiovascular assessment was based on reported and verified heart disease (essential hypertension, overall heart disease, and myocardial infarction) and the measurement of central cardiac function and peripheral vascular function. The analyses of central cardiac function examined systolic blood pressure, heart sounds, and several variables from the electrocardiograph (ECG) readings (overall interpretation, right bundle branch block, left bundle branch block, nonspecific ST- and T-wave changes, bradycardia, arrhythmia, and other diagnoses). The evaluation of peripheral vascular function was based on diastolic blood pressure, fundoscopic examination of small vessels, carotid bruits, and manual palpation of the radial, femoral, popliteal, dorsalis pedis, and posterior pulses.

Diabetes is a major risk factor for the development of cardiovascular disease. The cardiovascular assessment excluded diabetics from most analyses so that the serum dioxin findings would be based on cardiovascular endpoints independent of a diabetes-related etiology. Additional analyses based on diabetics only were done for myocardial infarction and leg pulses.

An increased risk of cardiovascular disease was not associated with dioxin levels. There was a significantly increased risk of essential hypertension for Ranch Hands in the high current dioxin category relative to Comparisons in the background current dioxin category, when the effect of body fat was not considered. Because body fat is associated with dioxin levels, adjustment for body fat could mask a dioxin effect. By contrast, the analyses of verified heart disease (excluding essential hypertension) found that the adjusted relative risk was significantly less than 1 for Ranch Hands in the high current dioxin category; the association with initial dioxin also exhibited a significantly decreased risk under the maximal assumption. In the assessment of central cardiac function, the analyses of systolic blood pressure in its continuous form displayed significant associations with dioxin when the effect of body fat was not considered, but the corresponding discrete analyses did not show a significant increase in the prevalence of abnormally high levels of systolic blood pressure (>140 mm Hg). By contrast, the adjusted analyses for "other ECG diagnoses" found significantly decreased risks in the adjusted current dioxin and time since tour analyses and in the adjusted categorized current dioxin analyses. Longitudinal analyses of the overall ECG displayed significant negative associations with dioxin.

The assessment of peripheral vascular function found significant associations between dioxin and decreases in the peripheral pulses. The adjusted categorized current dioxin analyses showed that Ranch Hands in the high current dioxin category had significantly more peripheral pulse abnormalities than Comparisons in the background current dioxin category, although Ranch Hands in the low current dioxin category had the most peripheral pulse abnormalities. Consistent with the systolic blood pressure findings, the adjusted mean diastolic blood pressure was significantly higher for Ranch Hands in the high current dioxin category relative to the background current dioxin category when the effect of body fat was not considered. However, the corresponding discrete analysis did not show a significantly increased risk of an abnormally high level of diastolic blood pressure (>90 mm Hg).

The cardiovascular findings in this report offered no consistent evidence of an adverse dioxin effect. At present, there is no evidence that humans experience cardiovascular sequelae related to chronic low-dose dioxin exposure. However, the blood pressure and pulse observations could represent early subclinical effects and emphasize the need for continued evaluation in subsequent phases of the study.

Hematologic Assessment

The hematologic assessment examined nine laboratory variables: red blood cell count, white blood cell (WBC) count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, and prothrombin time. These variables can be used to indicate hematopoietic disease and, perhaps more often, to alert the clinician to the presence of disease in other organ systems.

The hematologic results revealed no evidence that overt hematopoietic toxicity was related to dioxin exposure. The WBC count revealed statistically significant increases consistent with a dose-response effect in all three models; consistent significant results were not found for the other variables. The adjusted categorized current dioxin analyses for platelet count found a significantly increased risk of an elevated platelet count for Ranch Hands in the high current dioxin category relative to the Comparisons in the background current dioxin category. These findings suggest the presence of a low-level, chronic

inflammatory response that may not be considered clinically significant, but underscores the need for continued surveillance.

Renal Assessment

History of kidney disease and five laboratory variables (urinary protein, urinary occult blood, urinary white blood cell count, blood urea nitrogen, and urine specific gravity) were analyzed in the assessment of renal function. The statistical analyses did not indicate any renal health detriment related to dioxin. Under the maximal assumption of model 1 (but not the minimal), the initial dioxin analyses found a significantly increased risk of urinary occult blood cells, but results were not significant for the model 2 and model 3 analyses. Statistically significant results were not noted for the other variables. These results are consistent with the renal assessments from the previous AFHS reports, which did not find significant differences between the Ranch Hand and Comparison groups.

Endocrine Assessment

The endocrine assessment focused on thyroid, testicular, and pancreatic functions. Seven laboratory variables were analyzed: T_3 % uptake, thyroid stimulating hormone (TSH), follicle stimulating hormone, testosterone, fasting glucose, 2-hour postprandial glucose, and a composite diabetes indicator. Physical examination data for the thyroid gland and the testes also were evaluated.

In the evaluation of thyroid function, the categorized current dioxin analyses found a significant decrease in the mean T_3 % uptake and a significant increase in the mean TSH for Ranch Hands in the high current dioxin category relative to Comparisons in the background current dioxin category. There was a significant negative correlation between initial dioxin and T_3 % uptake. Though these results were consistent with subclinical decreases in thyroid function related to dioxin exposure, the magnitude of the differences between Ranch Hands in the higher and lower dioxin categories were not considered physiologically significant. In addition, the discrete analyses for these variables did not indicate that dioxin was associated with an increase in the prevalence of abnormal levels.

Decreased testicular size was associated significantly with initial and current levels of serum dioxin. The clinical meaning of this finding is unclear at this time. The discrete analyses of testosterone did not find a significant association between dioxin and abnormally low levels of serum testosterone (<260 ng/dl), but the continuous analyses detected a significant negative association with dioxin when the effect of body fat was not considered. Fertility and other reproductive outcomes will be assessed in a separate report.

Initial dioxin and current levels of serum dioxin both were associated highly with an increased risk of diabetes. Significant positive associations were noted for the analyses of fasting glucose and 2-hour postprandial glucose. The results clearly established a strong association between glucose intolerance and dioxin, but concluding that dioxin directly causes diabetes would be premature. Clinically, obesity is recognized as the most common cause of adult-onset diabetes mellitus. The general health assessment revealed a strong positive association between serum dioxin levels and percent body fat, but the diabetes findings remained significant after adjusting for body fat. Whether these findings imply an increase in diabetes or the earlier appearance of clinical diabetes in susceptible men remains an open

question. The basis of these relationships will be investigated during subsequent phases of this study.

Immunologic Assessment

The immunologic assessment analyzed composite skin-reaction test results in addition to various laboratory measurements consisting of cell surface marker studies, three groups of functional stimulation tests, and quantitative immunoglobulins. The evaluated indices of immunologic capability provide a comprehensive reflection of in vivo and in vitro immune function in the study population. Because of the complexity of the assays and the expense of the immunologic tests, a random sample of approximately 40 percent of the participants was chosen to receive these tests. Of the subset of participants chosen for immunologic testing and assayed for serum dioxin levels, 9.9 percent were diabetic. This percentage was not significantly different from the percentage of assayed participants not selected for immunologic testing (8.3%).

Previously reported Ranch Hand and Comparison group contrasts based on the 1987 physical examination found that significantly more Ranch Hands than Comparisons had possibly abnormal reactions on the delayed hypersensitivity skin-test response, but results for the laboratory variables revealed no medically important differences. In this report, the serum dioxin analyses for the composite skin test diagnosis did not support a dioxin-related effect, suggesting that the previously noted group difference may not be related to dioxin.

Evaluation of the immunoglobulins found a significant association between initial dioxin and IgA increases, consistent with a subtle inflammatory response. The analyses of the other immunoglobulins (IgG and IgM) did not indicate the presence of any dioxin-related effects. Analyses for the other laboratory variables revealed several statistically significant findings, but they either were internally inconsistent or were not in a direction expected in an impaired immune system. In conclusion, the immunologic assessment did not exhibit clinically significant trends related to the current serum dioxin level or the initial level.

Pulmonary Assessment

The pulmonary assessment was based on the verified histories for five self-reported illnesses, five physical examination variables, and seven laboratory measurements from the pulmonary function tests.

Analyses of pulmonary disease history found no evidence of a dioxin effect on the five verified respiratory illnesses studied (asthma, bronchitis, pleurisy, pneumonia, and tuberculosis). Consistent with the findings from the previous report of the 1987 examination data, none of these conditions was significantly associated with either the initial level or the current level of serum dioxin.

The five physical examination variables were hyperresonance, dullness, wheezes, rales, and a composite of these variables (denoted as thorax and lung abnormalities). These variables can provide valuable clues to the presence of pulmonary disease, but are of limited use in confirming a diagnosis because of their lack of specificity. Wheezes and hyperresonance, for example, will occur in obstructive airway disease, in asthma, or in chronic obstructive pulmonary disease (COPD or emphysema) secondary to cigarette use.

The dioxin analyses of the physical examination variables detected increased risks of statistical significance or marginal significance for each variable in at least one adjusted analysis. The adjusted model 3 analyses found that the relative risk for each variable (other than dullness, which had only three abnormalities) was significantly or marginally more than 1 for Ranch Hands in the high current dioxin category relative to Comparisons in the background current dioxin category. The previous 1987 examination finding that Ranch Hands had marginally more thorax and lung abnormalities than Comparisons in an adjusted analysis is consistent with these results.

The laboratory variables evaluated for this study were x-ray interpretation, forced vital capacity (FVC), forced expiratory volume at 1 second (FEV_1), forced expiratory flow maximum (FEFmax), the ratio of observed FEV_1 to observed FVC, loss of vital capacity, and obstructive abnormality. The x ray, when normal, is highly reliable for excluding pulmonary parenchymal disease. The spirometric indices are designed to measure lung volume (FVC) and respiratory air flow (FEV), and are used to help diagnose restrictive and obstructive disease. Restrictive disease is characterized by reduced vital capacity as seen in interstitial fibrosis or reduced lung volume after surgical resection. The flow dependent indices (FEV_1 and FEFmax) are abnormally prolonged in obstructive airways disease, usually COPD.

Serum dioxin was not associated significantly with the x-ray interpretation. By contrast, the analyses of the spirometric indices were often significant, but the differences in the mean levels were not clinically important. Initial dioxin was significantly associated with decreases in FVC, FEV_1, and FEFmax in addition to a significant increase in the ratio of observed FEV_1 to observed FVC. Adjusted results for the model 2 and model 3 analyses also displayed significant findings for these variables. In clinical practice, obesity is known to cause a reduction in vital capacity. The results described here may be due in part to the significant positive association between serum dioxin and percent body fat that is detailed in the general health assessment. Accordingly, interpretation of these results must await further evaluation of the dioxin and body fat relationship.

In the longitudinal analysis of the ratio of observed FEV_1 to observed FVC, there was a significant positive association with current dioxin and a significant difference among the current dioxin categories.

Extrapolation of Results

Extrapolation of the serum dioxin results to the general population of ground troops who served in Vietnam is difficult because Ranch Hand and ground-troop exposure situations were quite different. Based on serum dioxin testing results done by other researchers, nearly all ground troops tested have current levels of dioxin similar to background levels. Even ground troops who served in herbicide-sprayed areas of Vietnam had current levels indistinguishable from levels in men who never left the United States. The AFHS subgroup most like the ground troops in terms of current dioxin levels are Ranch Hands who currently have background levels of dioxin (10 ppt or less, designated as the "unknown" current dioxin category in the model 3 analyses). Therefore, if the results of the AFHS are applied to the general population of Vietnam veterans, the focus should be on the unknown Ranch Hand versus background Comparison contrast in the model 3 analyses. However, extrapolating the

results of these analyses to Vietnam veterans should still be made cautiously. There may be demographic distinctions between the unknown group of Ranch Hands and other Vietnam veterans that may be health-related. In general, the adjusted model 3 analyses found that Ranch Hands in the unknown current dioxin category did not show a significant health detriment relative to Comparisons in the background current dioxin category. This was particularly true for the variables that exhibited a significant high versus background contrast.

SUMMARY

The serum dioxin analyses in this report detected significant associations with lipid-related health indices. In particular, diabetes and body fat were associated positively with dioxin. Cholesterol, HDL, the cholesterol-HDL ratio, and 2-hour postprandial glucose also were associated significantly with dioxin. Erythrocyte sedimentation rate, WBC count, IgA, and platelet count were positively associated with dioxin, suggesting the presence of a chronic dose-related inflammatory process. Other variables, such as the spirometric indices in the pulmonary assessment and benign systemic neoplasms in the malignancy assessment, showed significant associations with dioxin that may be related to the body fat results (approximately 75% of the benign systemic neoplasms in Ranch Hands and 70% in Comparisons were lipomas). These findings and their possible relationship to dioxin elimination will be explored in future examination cycles. The serum dioxin analyses also revealed a significant positive association between dioxin and decreased testicular size, but the importance of this finding is unclear (fertility and other reproductive outcomes will be assessed in a separate report). Results for the other variables revealed no consistent pattern (within or across clinical areas) indicative of a health detriment due to dioxin exposure.

Occasionally there was a significant finding in the analysis of a variable in its continuous form, but the corresponding results for the discrete analysis were not significant. Small but significant mean differences in a continuously measured health variable when there are no corresponding differences in the percentage of abnormal tests are difficult to assess in any study. For example, in the discrete analysis of serum testosterone, abnormally low levels were not associated significantly with dioxin. However, the adjusted continuous analysis found a significant negative association between dioxin and testosterone when the effect of body fat was not considered. The continuous and discrete analyses of systolic and diastolic blood pressure also exhibited conflicting results. Observations such as these could represent an early subclinical effect, or they could be the result of a multiple-testing artifact. Significant trends in the mean with increasing levels of dioxin are interpreted as a dioxin-related effect if a corresponding trend is seen in the proportion above or below the normal range. These observations emphasize the importance of continued evaluation of a broad spectrum of health endpoints in the subsequent physical examination phases of the AFHS.

The graphical displays for fasting and 2-hour postprandial glucose, AST, cholesterol, the cholesterol/HDL ratio, and diastolic blood pressure show a remarkable similarity in the pattern of results between the Comparisons and those Ranch Hands with dioxin levels below 15 ppt. Often, a dioxin-related increase is seen in the Comparisons as well as the Ranch Hands, without an obvious threshold. The medical importance of these observations is not clear, but these data suggest that there may not be a threshold for a subtle dioxin effect, even at levels considered to be at or near background.

In summary, many of the findings in this report reveal a consistent relationship between dioxin and body fat. Two hypotheses may explain the observed relationships. In one, dioxin could cause an increase in body fat, or the level of body fat could influence the dioxin decay rate, which, in turn, alters physiologic outcomes, such as blood pressure, serum lipid alterations, and blood sugar levels. An alternative hypothesis involves dioxin as a direct cause of two or more of the observed endpoints, including body fat. Whether dioxin causes these observed effects directly or is a step in an extended causal pathway cannot be determined from these data. Additional analyses following the next physical examination scheduled for 1992 may help resolve this question.

CHAPTER 19

FUTURE DIRECTIONS

The development of a method to determine levels of dioxin in serum has been a significant enhancement to this study. This procedure permitted the study scientists to develop a measure of exposure for each individual that did not require making assumptions of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure based on a surrogate indicator (developed from available historical data on fixed-wing spray missions used to disseminate Herbicide Orange, Herbicide Purple, Herbicide Pink, and Herbicide Green). The method provided the opportunity to move from relatively simple group contrasts of Ranch Hands and Comparisons to detailed analyses of dose response on an individual basis.

While this breakthrough has led to dramatic improvements in the study, it also has highlighted opportunities for further refinements to the study that will be implemented for the next phase of the study scheduled for 1992. These refinements in exposure assessment will include an evaluation of the pattern of dioxin isomers in the serum of a selected group of participants. These participants would include all Comparisons with 1987 TCDD levels above 20 ppt and a random sample of Ranch Hands. Additionally, serum samples will be collected for dioxin assays from all participants who did not provide blood for testing in 1987 or whose assays did not result in a valid determination in the laboratory. The Air Force also plans to obtain serum samples at the 1992 examination on a selected group of Ranch Hands so that a third data point will be available in the determination of dioxin half-life over the 10 years since 1982. These data, coupled with the results of half-life studies in men exposed to TCDD in Seveso, Italy, in 1976, will be used to assess the validity of the first-order pharmacokinetics assumption for dioxin elimination in humans.

Data on weight changes and intervening illness also will be included in half-life determinations for the 1992 examination. If the first-order elimination assumption is supported by the Seveso data, a specific half-life determination for each individual will be determined for use in the statistical analyses rather than the single value used for everyone in this report.

Modifications to the format of the physical examination also are envisioned for 1992. These include the determination of serum insulin levels, Doppler studies of peripheral arterial circulation, replacement of the T_3 % uptake with a refined methodology to measure the thyroid stimulating hormone accurately in both hyperthyroid and hypothyroid conditions, and the collection of data on the presence of claudication and peripheral vascular insufficiency. In addition, the components of the immunological assessment will be evaluated to ensure that the most current measures of immunological function are used.