Air Force Health Study

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

SAIC Team
Russell H. Roegner, Ph.D.
William D. Grubbs, Ph.D.
Michael B. Lustik, M.S.
Amy S. Brockman, M.S.
Scott C. Henderson, M.S.
David E. Williams, M.D., SCRF

Air Force Team
Col William H. Wolfe, M.D., M.P.H.
Joel E. Michalek, Ph.D.
Col Judson C. Miner, D.V.M., M.P.H.

Project Manager: R.H. Roegner
Statistical Task Manager: W.D. Grubbs
SAIC Quality Review Chair: W.F. Thomas
SAIC Editors: Cynthia A. Marut
Elisabeth M. Smeda

Program Manager: R.W. Ogershok

SCIENCE APPLICATIONS INTERNATIONAL CORPORATION
8400 Westpark Drive
McLean, VA 22102

EPIDEMIOLOGY RESEARCH DIVISION
ARMSTRONG LABORATORY
HUMAN SYSTEMS DIVISION (AFSC)
Brooks Air Force Base, TX 78235

In conjunction with

SCRIPPS CLINIC & RESEARCH FOUNDATION,
LA JOLLA, CA

NATIONAL OPINION RESEARCH CENTER,
CHICAGO, IL

March 1991

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

SERUM DIOXIN ANALYSIS OF
1987 EXAMINATION RESULTS

Contract Number: F41689-85-D-0010
SAIC Project Number: 1-813-X4-105/254/437/011/942/943

(Distribution Unlimited)
An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides, Serum Dioxin Analysis of 1987 Examination Results

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.
AIR FORCE HEALTH STUDY

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

March 1991

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

SERUM DIOXIN ANALYSIS OF 1987 EXAMINATION RESULTS

EPIDEMIOLOGY RESEARCH DIVISION
ARMSTRONG LABORATORY
HUMAN SYSTEMS DIVISION (AFSC)
Brooks Air Force Base, Texas 78235
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 [MCMII]) were inconsistent. Most of the adjusted results for the SCL-90-R variables were not significant. Many of the adjusted MCMII results were significant, but substantial overlap and correlation between test scales of the MCMII 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.
ACKNOWLEDGMENTS

The authors of the report gratefully acknowledge the outstanding support of all the contributors to this project. To all the individuals, named and unnamed, whose dedication and hard work over the past 5 years have made this report possible, the authors wish to express their sincere appreciation.

U.S. Air Force Coinvestigators:

Vincent V. Elequin, Medical Record Librarian
Alton Rahe, QuesTech, Inc., Mathematical Statistician
Lt. Col. John Silva, USAF, MC, Consultant, Immunology

Support in conducting the statistical analysis:

Joseph Benvenuto, SAIC
Lewis Pfister, SAIC
Dung B. Phan, SAIC
Vanessa K. Rocconi, SAIC

Data processing and management support:

Mary E. Carpentier, SAIC
Carol A. Carroll, SAIC Consultant
Melody Darby, USAF
Christie L. Dyer, SAIC Task Manager
Linda S. Pinarock, SAIC Consultant
Lydia Sanchez, QuesTech, Inc.
Jane Tsui-Wu, QuesTech, Inc.
John K. Whiteman, M.D., SAIC Consultant
Mary G. Whiteman, SAIC Consultant

Conduct of the medical records coding:

TSgt. Gregorio Faragoza, USAF
Calvin E. Holloman, USAF
Maricela Luna, USAF
Earl A. Metts, USAF
SSgt. Tracey H. Wilkinson, USAF
Edward E. Zimmerman, USAF
Air Force Onsite Monitors*

Maj. Robert W. Carr, USAF, MC
Col. Alan H. Mumm, USAF, MC
Lt. Col. Michael Peterson, USAF, BSC
Lt. Col. Cynthia A. Smith, USAF, BSC

Conduct of the physical examinations and preparation of the clinical interpretations**:

Maung H. Aung, M.D., SCRF
Dianna M. Cooper, R.N., SCRF
Roger C. Cornell, M.D., SCRF
Donald J. Dalessio, M.D., SCRF
William R. Dito, M.D., SCRF
Janet Gervin, R.N., SCRF
Gene T. Izuno, M.D., SCRF
L. Dee Jacobsen, Ph.D., SCRF
Sharon Law, SIRL
Tony P. Lopez, M.D., SCRF
David A. Mathison, M.D., SCRF
Richard A. McPherson, M.D., SCRF
Anthony P. Moore, M.D., SCRF
Robert M. Nakamura, M.D., SCRF
Shirley M. Ouel, M.D., SCRF
J. Mark Roberts, SCRF
Myrna Roberts, Ph.D., SCRF
John S. Romine, M.D., SCRF
Kathleen Rooney, SCRF
Stephen K. Sargeant, M.D., SCRF
Stanley G. Seat, M.D., SCRF
Abbas Sedaghat, M.D., SCRF
Marjorie E. Seybold, M.D., SCRF
Robert B. Sigafoes, M.D., SCRF
Jack C. Sipe, M.D., SCRF
Ernest S. Tucker, III, M.D., SIRL
Tonia Vyenielo, M.D., SCRF
Cindy Wiesner, SCRF


**David E. Williams, M.D., SCRF, also participated in conducting the physical examinations and preparing the clinical interpretations.
Conduct of serum dioxin testing:

Larry L. Needham, Ph.D., CDC
Donald G. Patterson, Ph.D., CDC
James L. Pirkle, M.D., CDC

Questionnaire administration and scheduling:

Ellwood Carter, NORC
Jan Dyson, NORC
Charlene Harris, NORC
Cynthia Peters, NORC
Jacques Van der Ven, NORC
Belinda Willis, NORC

Logistical arrangements:

Joyce A. Douglass, SAIC
Jacqueline P. Kirk, SAIC Task Manager

Editorial support and report production:

Kathleen A. Dunk, SAIC
Rochelle Gary, NORC
Abby A. Johnson, SAIC
Genean Jones, SAIC
Debbie Schlieth, SAIC
Kristy Shank, SAIC
Margaret I. Siriano, SAIC
Frank B. Tennant, SAIC
Kay Torpey, SAIC
Grace Verchek, SAIC
Lenore C. Wagner, SAIC
Sunita White, SAIC

Management and quality review:

Donna L. Bareis, Ph.D., SAIC
Leon B. Ellwein, Ph.D., SAIC Consultant
Charles Fricker, SAIC Consultant
Tricia A. Graves, SAIC
Michael J. Higgins, SAIC Consultant
James A. Lonergan, Ph.D., SAIC
Wanda F. Thomas, SAIC
Peter Wise, SAIC
Richard W. Ogershok, USAF
Contractual and administrative support:

Annette F. Bermea, USAF  
Linda W. Campbell, USAF  
Loretta Chavana, QuesTech, Inc.  
Mark S. Colangelo, SAIC  
Karyn E. Davis, SAIC  
James E. Ellison, USAF  
Manual Franco, USAF  
Elizabeth Faykus, USAF  
Ronald P. Littman, USAF  
Cindy J. Peterson, USAF  
A1C Leslie Walker, USAF

Advisory Committee on Special Studies Relating to the Possible Long-Term Health Effects of Phenoxy Herbicides and Contaminants:

Earl P. Benditt, M.D., University of Washington School of Medicine  
Turner Camp, Ph.D., Veterans of Foreign Wars  
Captain Ronald F. Coene, National Center for Toxicological Research  
Michael Gough, Ph.D., Resources for the Future  
Leonard T. Kurland, M.D., Mayo Clinic and Mayo Foundation  
Peter C. O'Brien, M.D., Mayo Clinic and Mayo Graduate School  
Dolores C. Shockley, Ph.D., Meharry Medical College  
Ellen K. Silbergeld, Ph.D., Environmental Defense Fund  
Paul D. Stolley, M.D., University of Pennsylvania School of Medicine  
M. Donald Wharton, M.D., ENSR Health Services  
John Young, Ph.D., National Center for Toxicological Research

Advisory Committee consultants:

Harland Austin, Ph.D., University of Alabama, Birmingham  
Irene J. Check, M.D., Emory University  
James S. Taylor, M.D., Cleveland Clinic

Support and Encouragement:

Ranch Hand Association Members

And, for making this study possible:

All Study Participants
TABLE OF CONTENTS
INTRODUCTION, BACKGROUND AND CONCLUSIONS
(Chapters 1-5, 18, 19)

<table>
<thead>
<tr>
<th>SECTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXECUTIVE SUMMARY</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>xi</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>xv</td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1-1</td>
</tr>
<tr>
<td>AIR FORCE HEALTH STUDY</td>
<td>1-1</td>
</tr>
<tr>
<td>Questionnaire Methodology</td>
<td>1-2</td>
</tr>
<tr>
<td>Physical Examination Methodology</td>
<td>1-2</td>
</tr>
<tr>
<td>Quality Control</td>
<td>1-3</td>
</tr>
<tr>
<td>Statistical Models</td>
<td>1-5</td>
</tr>
<tr>
<td>Organization of the Report</td>
<td>1-6</td>
</tr>
<tr>
<td>INTERPRETIVE CONSIDERATIONS</td>
<td>1-7</td>
</tr>
<tr>
<td>Bias</td>
<td>1-7</td>
</tr>
<tr>
<td>Adjustments for Covariates and Interactions</td>
<td>1-8</td>
</tr>
<tr>
<td>Consistency</td>
<td>1-9</td>
</tr>
<tr>
<td>Multiple Testing</td>
<td>1-9</td>
</tr>
<tr>
<td>Trends</td>
<td>1-10</td>
</tr>
<tr>
<td>Power Limitations</td>
<td>1-10</td>
</tr>
<tr>
<td>Strength of Association</td>
<td>1-10</td>
</tr>
<tr>
<td>Biological Credibility</td>
<td>1-10</td>
</tr>
<tr>
<td>Interpretation of Negative Results</td>
<td>1-11</td>
</tr>
<tr>
<td>Interpretation of the Coefficient of Determination</td>
<td>1-11</td>
</tr>
<tr>
<td>Clinic Interpretation of Discrete versus Continuous Data</td>
<td>1-11</td>
</tr>
<tr>
<td>Minimal versus Maximal Results</td>
<td>1-11</td>
</tr>
<tr>
<td>Graphics</td>
<td>1-12</td>
</tr>
<tr>
<td>The Checkmark Pattern</td>
<td>1-12</td>
</tr>
<tr>
<td>Extrapolation to Army Ground Troops</td>
<td>1-12</td>
</tr>
<tr>
<td>Summary of Results</td>
<td>1-13</td>
</tr>
<tr>
<td>CONCLUSION</td>
<td>1-13</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>1-14</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS (Continued)

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)

<table>
<thead>
<tr>
<th>GRAPHICS</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data Plots/Histograms</td>
<td>4-33</td>
</tr>
<tr>
<td>Interaction Plots/Histograms</td>
<td>4-36</td>
</tr>
<tr>
<td>Statistical Analysis Protocol</td>
<td>4-36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>REFERENCES</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4-37</td>
</tr>
</tbody>
</table>

5. COVARIATE ASSOCIATIONS ............................................. 5-1

<table>
<thead>
<tr>
<th>INTRODUCTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MATCHING VARIABLES (AGE, RACE, AND OCCUPATION)</td>
<td>5-1</td>
</tr>
<tr>
<td>DRINKING HABITS</td>
<td>5-14</td>
</tr>
<tr>
<td>SMOKING HABITS</td>
<td>5-16</td>
</tr>
<tr>
<td>SUN EXPOSURE CHARACTERISTICS</td>
<td>5-16</td>
</tr>
<tr>
<td>EXPOSURE TO CARCINOGENS</td>
<td>5-18</td>
</tr>
<tr>
<td>PERSONAL AND FAMILY HEALTH</td>
<td>5-21</td>
</tr>
<tr>
<td>OTHER CHARACTERISTICS</td>
<td>5-23</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>5-24</td>
</tr>
<tr>
<td>CONCLUSION</td>
<td>5-25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>REFERENCES</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-26</td>
</tr>
</tbody>
</table>

18. CONCLUSIONS .......................................................... 18-1

<table>
<thead>
<tr>
<th>INTRODUCTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistical Models</td>
<td>18-1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RESULTS</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Health Assessment</td>
<td>18-2</td>
</tr>
<tr>
<td>Malignancy Assessment</td>
<td>18-2</td>
</tr>
<tr>
<td>Neurological Assessment</td>
<td>18-4</td>
</tr>
<tr>
<td>Psychological Assessment</td>
<td>18-5</td>
</tr>
<tr>
<td>Gastrointestinal Assessment</td>
<td>18-6</td>
</tr>
<tr>
<td>Dermatologic Assessment</td>
<td>18-6</td>
</tr>
<tr>
<td>Cardiovascular Assessment</td>
<td>18-7</td>
</tr>
<tr>
<td>Hematologic Assessment</td>
<td>18-8</td>
</tr>
<tr>
<td>Renal Assessment</td>
<td>18-9</td>
</tr>
<tr>
<td>Endocrine Assessment</td>
<td>18-9</td>
</tr>
<tr>
<td>Immunologic Assessment</td>
<td>18-10</td>
</tr>
<tr>
<td>Pulmonary Assessment</td>
<td>18-10</td>
</tr>
<tr>
<td>Extrapolation of Results</td>
<td>18-11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SUMMARY</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18-12</td>
</tr>
</tbody>
</table>

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 - Log2 (Initial Dioxin)
- Model 2: Ranch Hands - Log2 (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 occupa-
tion 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 ($\leq 10$ 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
REFERENCES


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

<table>
<thead>
<tr>
<th>Report Field Value</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>Good result</td>
</tr>
<tr>
<td>GML</td>
<td>Good result, missing lipids</td>
</tr>
<tr>
<td>GND</td>
<td>Good result, below limit of detection</td>
</tr>
<tr>
<td>GNQ</td>
<td>Good result, below limit of quantitation</td>
</tr>
<tr>
<td>NR</td>
<td>No result</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Report</th>
<th>Ranch Hand</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fully Compliant</td>
<td>Noncompliant</td>
</tr>
<tr>
<td>G</td>
<td>858</td>
<td>2</td>
</tr>
<tr>
<td>GML</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GND</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>GNQ</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>NR</td>
<td>44</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>930</td>
<td>2</td>
</tr>
</tbody>
</table>

Missing dioxin results (report=NR or GML) and nonquantifiable 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

<table>
<thead>
<tr>
<th>Report</th>
<th>Ranch Hand</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully compliant to 1987 physical examination and assayed for dioxin</td>
<td>930</td>
<td>887</td>
</tr>
</tbody>
</table>

Report

<table>
<thead>
<tr>
<th>Less</th>
<th>Ranch Hand</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNQ</td>
<td>(20)</td>
<td>(51)</td>
</tr>
<tr>
<td>NR</td>
<td>(44)</td>
<td>(31)</td>
</tr>
<tr>
<td>GML</td>
<td>(0)</td>
<td>(1)</td>
</tr>
<tr>
<td>Total</td>
<td>866</td>
<td>804</td>
</tr>
</tbody>
</table>
TABLE 2-4.
Dioxin Result Summary of 866 Ranch Hands and 804 Comparisons

<table>
<thead>
<tr>
<th>Stratum</th>
<th>Ranch Hands</th>
<th></th>
<th></th>
<th>Comparisons</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Median</td>
<td>Range</td>
<td>n</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>Officer</td>
<td>319</td>
<td>7.8</td>
<td>0-42.6</td>
<td>291</td>
<td>4.7</td>
<td>0-18.5</td>
</tr>
<tr>
<td>Enlisted Flyer</td>
<td>148</td>
<td>18.1</td>
<td>0-195.5</td>
<td>127</td>
<td>4.0</td>
<td>0-12.8</td>
</tr>
<tr>
<td>Enlisted Groundcrew</td>
<td>399</td>
<td>24.0</td>
<td>0-617.8</td>
<td>386</td>
<td>4.0</td>
<td>0-54.8</td>
</tr>
<tr>
<td>Total</td>
<td>866</td>
<td>12.8</td>
<td>0-617.8</td>
<td>804</td>
<td>4.2</td>
<td>0-54.8</td>
</tr>
</tbody>
</table>

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*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

REFERENCES


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

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

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 nonfliers. 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·2^T, 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

3-3
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>
<thead>
<tr>
<th>Stratum</th>
<th>N</th>
<th>Correlation</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Officer</td>
<td>295</td>
<td>0.10</td>
<td>0.082</td>
</tr>
<tr>
<td>Enlisted Flyer</td>
<td>143</td>
<td>0.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Enlisted Groundcrew</td>
<td>347</td>
<td>0.12</td>
<td>0.024</td>
</tr>
<tr>
<td>All</td>
<td>785</td>
<td>-0.10</td>
<td>0.003</td>
</tr>
</tbody>
</table>

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

TABLE 3-2.
Correlations Between Log (Current Dioxin) and Log (Exposure Index) in Ranch Hands With Current Dioxin and Exposure Greater Than Zero

TABLE 3-3.
Categorized Exposure Index versus Current Dioxin Levels in Ranch Hands
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)