Air Force Health Study

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

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SERUM DIOXIN ANALYSIS OF 1987 EXAMINATION RESULTS

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

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AIR FORCE HEALTH STUDY

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

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VOLUME I

SERUM DIOXIN ANALYSIS OF 1987 EXAMINATION RESULTS

EPIDEMIOLOGY RESEARCH DIVISION
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NOTICE

This report presents the results of analyses comparing the serum dioxin assays with physical examination data collected in 1987. This serum dioxin report is an addendum to the Ranch Hand versus Comparison group contrasts contained in the 1987 examination report published in February 1990. That report was the third in a series of epidemiologic studies to investigate the health effects in Air Force personnel following exposure to herbicides. The results of preceding studies (the 1982 Baseline and 1985 examinations) were presented in the Baseline Morbidity Study Results (February 1984) and the Air Force Health Study First Followup Examination Results (October 1987). Given the relationship of the serum dioxin analyses to the previous studies, portions of these earlier documents have been reproduced or paraphrased in this report. The purpose of this notice is to acknowledge the authors of these documents and to refer the reader to the 1987 examination report for additional background details regarding this study. No further references are made.

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EXECUTIVE SUMMARY

SERUM DIOXIN ANALYSIS OF THE 1987 AIR FORCE HEALTH STUDY EXAMINATIONS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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CHAPTER 1

INTRODUCTION

AIR FORCE HEALTH STUDY

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

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

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

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

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

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

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

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

Questionnaire Methodology

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

Physical Examination Methodology

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

- Review-of-systems questionnaire
- Psychological testing

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

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

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

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

Quality Control

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

Administrative Quality Control

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

Questionnaire, Physical, and Psychological Quality Control

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

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

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

Clinical Laboratory and Immunology Laboratory Quality Control

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

Data Management Quality Control

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

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

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

Statistical Analysis Quality Control

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

Statistical Models

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

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

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

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

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

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

Organization of the Report

This report is organized as follows:

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

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

INTERPRETIVE CONSIDERATIONS

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

Bias

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

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

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

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

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

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

Adjustments for Covariates and Interactions

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

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

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

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

Consistency

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

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

Multiple Testing

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