

## CHAPTER 21

### CONCLUSIONS

#### INTRODUCTION

This section summarizes the conclusions drawn from the statistical analyses of data from the 1992 followup examination of the Air Force Health Study (AFHS). The 1992 followup was an extension of the Baseline, 1985, and 1987 followup examinations. Health endpoints measured at the 1992 examination were analyzed for associations with dioxin (TCDD) exposure and body burden of serum dioxin, and were examined longitudinally in relation to data from the previous examination cycles.

#### STUDY PERFORMANCE ASPECTS

Participation at the 1992 followup examination remained high. Of the 1,148 eligible Ranch Hands, 952 participated in the 1992 followup examination, while 912 of the 1,191 eligible Comparisons from the Baseline examination participated in the 1992 followup. Of the 571 Comparisons identified as replacements for Original Comparisons, 369 participated in the 1992 followup. Ninety-one percent of living Ranch Hands and 92 percent of living Comparisons who were fully compliant at the Baseline examination returned for the 1992 followup examination. Each of the 952 Ranch Hands and 1,281 Comparisons at the 1992 followup completed the physical examination, but two participants refused to complete the questionnaire. Despite requirements in the Study Protocol, 62 of 279 noncompliant Comparisons were not replaced as they should have been. However, the total number of fully compliant participants would have increased by less than 3 percent and any biasing effect is considered negligible.

#### POPULATION CHARACTERISTICS

Overall, Ranch Hands and Comparisons had similar personal characteristics and lifestyle habits. However, notable exceptions included duration of combat service, reported herbicide exposure, and high-density lipoprotein (HDL). Ranch Hands tended to serve in combat longer than Comparisons, because Ranch Hands were stationed in combat areas for their entire time of duty in Southeast Asia (SEA), whereas Comparisons returned to stations outside of combat areas between missions. A possible explanation for a greater percentage of Ranch Hands than Comparisons reporting herbicide exposure may have been the tendency of Ranch Hands to report their exposure during their time of duty in SEA, although the questionnaire was designed to capture post-SEA exposure only. The relationship between group and HDL is not clear—the group means are not significantly different, but the percentage of Ranch Hands considered abnormal (less than 35 mg/dl) is significantly greater than the percentage of Comparisons. In Ranch Hands, most of the significant associations between dioxin and the covariates can be attributed to, or partially explained by, the effects of occupation, age, or body fat.

## STATISTICAL MODELS

The analysis of the 1992 followup examination results employed six statistical models to evaluate the relationship between the health status of study participants and their dioxin exposure and serum dioxin levels. The first model specifies contrasts between Ranch Hands and Comparisons using group as a proxy for exposure and does not incorporate serum dioxin measurements. The remaining five models all incorporate serum dioxin measurements in either current or initial form. The six models are summarized as follows:

- Model 1: Ranch Hands versus Comparisons
- Model 2: Estimated initial serum dioxin levels using Ranch Hand participants with greater than 10 ppt of current lipid-adjusted dioxin
- Model 3: Ranch Hands categorized according to serum dioxin levels versus Comparisons with 10 ppt of current lipid-adjusted dioxin or less
- Model 4: Current lipid-adjusted serum dioxin using Ranch Hands only
- Model 5: Current whole-weight serum dioxin using Ranch Hands only
- Model 6: Current whole-weight serum dioxin, adjusted for total lipids, using Ranch Hands only.

In Model 1, the use of group and occupation as a surrogate for exposure is not subject to the possible biases based on health conditions that can occur with serum dioxin estimates. However, an implicit underlying assumption is that Ranch Hands were exposed and Comparisons were not exposed. Model 2 is based on initial dioxin levels that were extrapolated from current lipid-adjusted dioxin measurements above background levels (10 ppt), assuming first-order kinetics and a constant dioxin decay rate. Model 3 is less dependent on the accuracy of the initial dioxin estimation algorithm, but all Ranch Hands with high serum dioxin levels are treated alike without emphasizing the unusually large dioxin doses received by some Ranch Hands. Models 4, 5, and 6 are based on current dioxin measurements from the 1987 examination and assume nothing about dioxin elimination other than that Ranch Hands were exposed in Vietnam and their body-burdens have decreased over time in an unspecified manner. However, current dioxin may not be a good surrogate for exposure if elimination rates differ among individuals.

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

## CLINICAL RESULTS

This section provides the conclusions from the analyses of the twelve clinical areas—general health, neoplasia, neurology, psychology, gastrointestinal, dermatology, cardiovascular, hematology, renal, endocrine, immunology, and pulmonary. Appendix Tables Q-1-1 through Q-1-24 of Appendix Q-1 present the results for each of the six models for more than 300 health endpoints analyzed in the 12 clinical chapters. Appendix Q-2 presents graphical displays of 26 selected continuous health measurements versus the logarithm (base 2) of current lipid-adjusted serum dioxin. These graphics represent scatterplots, unadjusted for any covariates, of the data used in Model 4 analyses.

### General Health Assessment

General health was assessed by five measures, selected for sensitivity to the overall state of health rather than specific to any organ system; the five measures were: self-perception of health, appearance of illness or distress as assessed by a physician, relative age as assessed by a physician, percent body fat, and sedimentation rate.

At the 1992 examination, Ranch Hands perceived themselves as less healthy than Comparisons, just as they had at the 1982 and 1985 examinations (though not at the 1987 examination). Enlisted groundcrew, who experienced the highest levels of dioxin exposure, were particularly inclined to view their health negatively. A highly significant association between the current level of serum dioxin and a negative self-perception of health also was found in Ranch Hands. Because participants were aware of their serum dioxin levels, the possibility of bias in these results should be considered. Participants who knew they possessed an elevated dioxin level, or whose occupation implied a greater risk for exposure (i.e., enlisted groundcrew), may have consciously or subconsciously perceived their health as poorer than that of their Comparisons. Indeed, apart from the self-perceived health status, the examining physicians, in their objective observations, recorded no significant group differences as to the appearance of illness or distress and appearance in terms of relative age.

The prevalence of obesity was similar in the Ranch Hand and Comparison cohorts. However, in Ranch Hands, a highly significant positive association between percent body fat and current serum dioxin was found in all of the occupational categories. These results imply a difference in the dioxin pharmacokinetics in obese versus lean participants; but clinically, it is difficult to explain the higher levels of serum dioxin in obese participants relative to any health detriment. It is not clear whether a causal relationship exists between dioxin exposure and increased body fat.

In previous AFHS examinations, sedimentation rate, a sensitive, but nonspecific index of general health usually associated with serious underlying disease, was significantly higher in Ranch Hands than in Comparisons. However, the 1992 examination revealed only a slight clinically insignificant difference in the Ranch Hand enlisted groundcrew and their Comparisons. Analyses showed a statistically significant dose-response effect in the association between sedimentation rate and current serum dioxin in Ranch Hands, but the biological significance is uncertain.

The longitudinal analyses revealed that results from the 1992 examination contrasted with those of previous examinations. Between 1982 and 1987, the percentage of Ranch Hands and Comparisons reporting fair or poor self-perceptions of health was greatly reduced and the difference between the groups had narrowed. However, in the 1992 examination, the change in self-perception of health between 1982 and 1992 was significantly associated with calculated initial serum dioxin levels (of which participants had become aware). The potentially negative effect of known exposure status and serum dioxin level may have affected the more recent results.

In conclusion, the general health of the Ranch Hands and Comparisons appeared comparable by all objective indices; however significant, although possibly biased, group differences were evident in self-perceived health status. Percent body fat and sedimentation rate displayed significant associations with current serum dioxin levels, but the biological significance is uncertain.

### **Neoplasia Assessment**

In the neoplasia assessment, skin and systemic neoplasms were evaluated by behavior, cell type, and location or site. As the anatomic point of contact with industrial toxins and as the only organ system with a clearly defined clinical endpoint (i.e., chloracne) for TCDD exposure, the skin deserves the special emphasis it has received in this study. Although there is no evidence that TCDD exposure causes—or that chloracne is associated with—basal cell carcinomas, the Ranch Hand cohort was found to be at increased risk for the occurrence of these skin cancers in each of the three prior examination cycles.

In the analyses of the 1992 examination, Ranch Hands continued to have a slightly higher prevalence of benign and malignant skin neoplasms than did Comparisons, including basal cell skin cancers at all sites. However, these group differences are no longer statistically significant. Consistent with results from the 1987 examinations, many analyses revealed a significant inverse dose-response with current serum dioxin levels.

Consistent with all previous examinations, none of the analyses revealed any significant group differences in the prevalence of systemic malignancies in the Ranch Hand and Comparison cohorts; neither did the analyses disclose an increased risk of any systemic malignancy in association with either the current or extrapolated initial levels of serum dioxin in Ranch Hands. Longitudinal analyses discovered no significant group differences in the incidence of benign or malignant neoplasms including those thought by some to be related to herbicide exposure (i.e., Hodgkin's disease, non-Hodgkin's lymphoma, and soft tissue sarcoma [STS]).

In summary, at the end of a decade of surveillance, Ranch Hands and Comparisons appear to be at equal risk for the development of all forms of neoplastic disease and there is no evidence to suggest a positive dose-response relationship between body burden of dioxin and neoplastic disease.

## **Neurological Assessment**

The neurological assessment examined historical neurological disorders in addition to central nervous system (CNS), cranial, and peripheral nerve indices, all of which can provide specific clues to the anatomical site of neurological lesions and clarify the need for additional diagnostic studies. The neurological examination is highly sensitive in detecting the presence of peripheral neuropathy, a suspect clinical condition related to TCDD exposure.

The prevalence of historical neurological disorders was similar in the Ranch Hand and Comparison cohorts. In contrast, but of doubtful clinical significance, an inverse dose-response was noted in the analyses relating current serum dioxin to the history of hereditary and degenerative disorders.

In the analyses of the physical examination variables, Ranch Hand enlisted groundcrew, the occupation category with the highest current levels of dioxin, had significantly more cranial nerve index abnormalities than Comparison enlisted groundcrew, but there was no evidence of a dose-response relationship in the serum dioxin analyses. In relation to the extrapolated initial level of serum dioxin, no significant associations were noted for any of the directly measured physical examination variables. The analyses employing current serum dioxin yielded inconsistent results. A positive association was noted in relation to the cranial nerve motor variable smile and the peripheral nerve variables pin prick and patellar reflex, while inverse dose-response patterns were defined for smell and the Babinski reflex.

In summary, the neurological assessment found the prevalence of neurological disease comparable between the Ranch Hand and Comparison groups, and showed no consistent evidence of a dose-response effect with either estimated initial dioxin exposure or current TCDD levels.

## **Psychological Assessment**

Verified psychological conditions and the Symptom Check List-90-Revised (SCL-90-R) inventory of nine primary symptom dimensions and three global indices of distress were examined in the psychological assessment. The SCL-90-R was retained in the 1992 examination because of its effectiveness as a co-measure of variables included in the verified questionnaire as well as to maintain psychometric continuity across the four phases of the AFHS completed to date (Baseline, 1985, 1987, and 1992).

Among the SCL-90-R inventory variables, Ranch Hands exhibited higher psychological distress than Comparisons on the index scores measuring anxiety, obsessive-compulsive behavior, paranoid ideation, somatization, and global severity. A significant group contrast also was exhibited for the verified condition of other neuroses. However, when Ranch Hands were categorized according to serum dioxin levels, significant group differences were revealed only in the contrasts of Ranch Hands with background serum dioxin levels versus Comparisons. The serum dioxin analyses also did not support a dose-response relationship, because there were no significant findings in any of the analyses relating extrapolated initial dioxin and current serum dioxin levels with psychological distress.

indicators. Each of the analyses produced a smaller number of significant results from the adjusted analyses than from the unadjusted analyses due to the adjustment for important confounding effects such as education and occupation.

In conclusion, the differences revealed between the Ranch Hand and Comparison cohorts, together with a lack of any effects attributable to dioxin, suggest that factors other than dioxin exposure continue to influence a relatively small but notable number of abnormalities in Ranch Hand test scores. Previous studies in clinical medicine continue to indicate the need for caution when interpreting the outcome of large statistical studies. The possibility that a small subset of physically or psychologically vulnerable Ranch Hands may have suffered psychological injury in the context of their exposure to dioxin cannot be definitively ruled out at this time.

### **Gastrointestinal Assessment**

The historical, physical examination, and laboratory parameters included in the gastrointestinal assessment are well established in clinical practice as screening tools for investigating digestive disorders in outpatients. There are limitations of reliance solely on data from the patient history and physical examination when diagnosing digestive disorders because digestive symptoms are frequently nonspecific and intermittent. However, data collected in the laboratory can provide early insight into the presence of occult liver disease.

Few of the laboratory analyses revealed any significant differences between the Ranch Hand and Comparison cohorts. Ranch Hands had a slightly higher mean alkaline phosphatase than Comparisons, but the difference in the means cannot be considered biologically significant. Analyzed in the discrete form, which is clinically more relevant, the group difference was not significant.

The serum dioxin analyses indicated that estimated initial dioxin exposure was generally not associated with historical liver disorders or current laboratory measurements. However, the analyses revealed that current dioxin levels were often highly associated with lipid-related health indices. In continuous (but not in discrete) form, two of the four liver enzymes studied, ALT and GGT, revealed highly significant positive associations with current serum dioxin levels. Similar results were noted with serum triglycerides and serum cholesterol, which contributed to a negative association between current serum dioxin and the cholesterol-HDL ratio. These results may be explained in part because the analyses of extrapolated initial serum dioxin were adjusted for differential half-life elimination related to percent body fat, whereas no adjustment was made in the analyses of current serum dioxin.

Analyses of the historical and clinical examination variables revealed no evidence of any overt hepatic disease related to the current body burden of dioxin. Most of the statistically significant associations that occurred in relation to the extrapolated initial level of serum dioxin were limited to laboratory indices. These associations more often were found in the continuous, rather than the more clinically relevant discrete, analyses. While the observed dose-response findings are not accompanied by clinical disease, they may still represent subclinical effects.

Over a decade of observation, the longitudinal analyses yielded significant results in several of the laboratory indices. In particular, ALT, serum triglyceride, and cholesterol levels tended to increase over time in Ranch Hands more than in Comparisons. Although these results are consistent with a subtle effect of herbicide exposure on lipid metabolism, the difference was more pronounced in the enlisted flyer category than it was in the more exposed enlisted groundcrew category.

In summary, the gastrointestinal data confirm observations that would be anticipated in a clinical practice and reflect no apparent increase in organ-specific morbidity in Ranch Hands relative to Comparisons nor do they represent an association with serum dioxin levels. Although a subclinical dioxin effect on lipid metabolism cannot be excluded, some of the results may be related in part to body habitus and percent body fat.

### **Dermatology Assessment**

The dermatologic assessment was based on occurrence of acne, location of acne, other dermatologic abnormalities, and a dermatology index based on the presence of comedones, acneiform lesions, acneiform scars, and inclusion cysts, depigmentation, and hyperpigmentation.

In the study of biological effects of herbicides in humans, the dermatologic examination assumes special importance. Of the organ systems analyzed in this report, only the skin has a clinical endpoint (chloracne) that has been related conclusively to dioxin exposure. Experimental dose-response studies in animals and humans have confirmed that the topical concentrations of dioxin required to produce overt lesions are far greater than the concentrations to which participants in the current study were likely to have been exposed during their times of duty in SEA. It is therefore not surprising that, in the four examination cycles to date, no cases of chloracne have been detected.

In general, the dermatology variables showed no significant differences between Ranch Hands and Comparisons. Although the lifetime occurrence of acne, as self-reported by the questionnaire, was similar in both groups, Ranch Hand enlisted groundcrew, those most heavily exposed to dioxin, appeared to be at increased risk for the development of acne subsequent to time of duty in SEA. There is a possibility of bias associated with self-reporting, however, because no group differences were found in the physical examination indices.

In the analyses of extrapolated initial and current serum dioxin, Ranch Hands with current serum dioxin levels above the background level demonstrated lower occurrence of an abnormal dermatology index than did Comparisons. The dermatology index also exhibited a significant negative association with current serum dioxin in Ranch Hands. Although nonsignificant, all other dermatologic indices displayed negative associations with current dioxin. These results provide evidence against a dose-response effect.

In summary, there is no consistent evidence to suggest an adverse dioxin effect on the dermatologic system at levels received by the Ranch Hand cohort in SEA.

## Cardiovascular Assessment

The cardiovascular assessment examined historical, physical examination, and questionnaire indices, divided into central and peripheral cardiovascular functions used to alert clinicians to the presence of underlying cardiovascular disease.

The verified historical indices (history of heart disease, hypertension, and myocardial infarction) were similar in Ranch Hands and Comparisons, but the analyses employing serum dioxin measurements revealed inconsistent results. In Ranch Hands, an increase in current dioxin levels was associated with a decrease in the prevalence of verified heart disease and an increase in the history of essential hypertension. Although a plausible biologic explanation for this phenomena is lacking, these results are consistent with findings from the 1987 examination.

In general, the analyses of the central cardiac function variables were not positively associated with serum dioxin. Although Ranch Hand enlisted flyers displayed a significantly higher prevalence of bradycardia than did Comparison enlisted flyers, bradycardia exhibited a significant inverse dose-response with initial and current dioxin. Several other electrocardiograph (ECG) indices, including right bundle branch block (RBBB), non-specific ST- and T-wave changes, and arrhythmias, displayed significant positive associations with current serum dioxin levels, but none of these endpoints also displayed a group difference between Ranch Hands and Comparisons to confirm the dose-response relationship.

The analyses of the peripheral vascular function variables displayed significant group differences for a few of the pulse endpoints among enlisted groundcrew personnel (the occupational category with the highest exposure) and between Ranch Hands with the highest current level of serum dioxin and their Comparisons. However, none of these relationships were reinforced by a significant association with initial or current serum dioxin. In the longitudinal analyses of the pulses endpoints, Ranch Hands were slightly more likely than Comparisons to develop peripheral pulse deficits over time. Again, the analyses using extrapolated initial serum dioxin levels as a measure of exposure did not show consistent evidence of a dose-response relationship.

Dorsalis pedis pulse abnormalities were far more prevalent in both Ranch Hands and Comparisons in the 1985 examination than they were in the 1992 examination. The change in results between the two examinations may relate to the use of different and more accurate Doppler instrumentation in the 1992 examinations. During the 10 years of observation, both Ranch Hands and Comparisons have demonstrated a similar reduction in systolic blood pressure and incidence of hypertension. This trend may reflect the beneficial effects of risk factor identification and life-style modification consequent to participation in this study.

In summary, consistent with the results of prior examinations, Ranch Hands were found to be at slightly greater risk than Comparisons to develop selected peripheral pulse deficits, suggesting some effects from dioxin. These findings are based on the 1992 analysis of hypertension and ST- and T-wave changes, taken in conjunction with the 1994 AFHS mortality update showing an increased number of deaths caused by diseases of the circulatory system among Ranch Hand nonflying enlisted personnel. By all other objective and

subjective indices, the development of cardiovascular disease does not appear to be associated with dioxin exposure or current serum dioxin levels.

### **Hematologic Assessment**

The 13 laboratory endpoints analyzed in the hematology assessment provided a comprehensive evaluation of the three peripheral blood lines (erythrocytes, leukocytes, and platelets). These variables are relied upon heavily to reflect disease of the hematopoietic system and also to alert the clinician to the presence of disease in other organ systems.

Of the laboratory variables examined, only platelet count exhibited significant associations with the dioxin exposure indices. Ranch Hands in the enlisted flyer and enlisted groundcrew categories possessed statistically significant higher mean platelet counts than Comparisons, although the differences cannot be considered clinically significant. Ranch Hands with high extrapolated initial dioxin levels also had significantly greater mean platelet count measurements than Comparisons. These results are consistent with those from the 1987 examination, but the biological significance is uncertain.

In the 1987 examination, the mean white blood cell (WBC) counts, platelet counts, and erythrocyte sedimentation rates (ESR) were each higher in Ranch Hands than in Comparisons, raising the possibility of a subclinical inflammatory response associated with prior dioxin exposure. In the current study, no group differences were noted in either the WBC or, as reported in the General Health Assessment (Chapter 9), the ESR. Furthermore, in the current study, current serum dioxin was inversely related to the prevalence of abnormally elevated WBC counts.

In the longitudinal analyses, a gradual reduction was documented in the total platelet count in each group and across all occupations. Ranch Hands continue to have a greater reduction in the total platelet count over time than do Comparisons, but the means from the current examination are nearly equal.

In summary, there is no evidence from the current study to suggest an association between hematopoietic toxicity and prior dioxin exposure. Based on the analyses of WBC, ESR, and total platelet count, there is no longer any evidence that a subclinical inflammatory reaction may be present in Ranch Hands as was thought possible in the 1987 examination.

### **Renal Assessment**

The renal assessment was based on the medical history of kidney disease, physical examination for kidney stones, and five laboratory indices. Pertinent to the interpretation of these analyses is the frequent finding in ambulatory medicine of isolated abnormalities in the routine urinalysis of healthy individuals who, in fact, have no disease of the genitourinary system. No significant group difference or association with serum dioxin was noted in the history of urinary tract disease, as measured by a verified history of kidney disease and the presence of renal calculi detected by plain films of the abdomen.

Although the prevalence of microhematuria (urinary red blood cell counts) was similar in both groups, Ranch Hands with the highest levels of extrapolated initial serum dioxin had a significantly higher prevalence of microhematuria than did Comparisons. These results are similar to those from the 1987 examination. Although not statistically significant, the analyses employing current serum dioxin yielded results consistent with a dose-response effect; however, the longitudinal analyses indicated that the prevalence of microhematuria has decreased in the Ranch Hand cohort since 1985. Clinically, the finding of hematuria can signal the presence of "silent" renal calculi or neoplastic disease; however, the analyses of kidney stones do not support the presence of silent renal calculi.

In the analysis of urinary WBC counts (pyuria), the enlisted groundcrew Ranch Hands—those most highly exposed to dioxin—had twice the prevalence of pyuria than did Comparisons. Longitudinal analyses also showed that the enlisted groundcrew Ranch Hands are twice as likely as the enlisted groundcrew Comparisons to develop pyuria over time, but the similar prevalence of pyuria in Ranch Hands with low and high levels of serum dioxin does not support a dose-response effect.

The analysis of urine specific gravity documented a statistically significant positive association with current serum dioxin, but the magnitude of the association was not clinically significant. Analyses of serum creatinine and proteinuria revealed no differences between the cohorts.

In summary, the renal assessment displayed no consistent evidence for any detriment, with the possible exception of hematuria, related to current body burden of dioxin or to the estimated severity of prior exposure.

### **Endocrine Assessment**

In the endocrine assessment, analyses were performed on 36 historical medical records, physical examination, and laboratory variables—five of which were analyzed separately for diabetics, nondiabetics, and all participants. These indices provide a comprehensive assessment of thyroid, gonadal, and endocrine pancreatic function in the population under study.

Analyses of thyroid functions did not reveal significant differences between the Ranch Hand and Comparison cohorts. Similarly, the prevalence of diabetes mellitus in the two groups was not significantly different, although significant positive associations were found between current serum dioxin levels and the onset of diabetes, specifically in the early stages requiring only dietary intervention or oral hypoglycemic therapy.

In assessing glucose metabolism, along with examining the possibility that dioxin may be a risk factor for the development of diabetes, significant results were limited to the current serum dioxin analyses. Diabetic Ranch Hands with high levels of current serum dioxin had significantly higher fasting glucose levels than those with lower levels of dioxin. Nondiabetics, on the other hand, exhibited an inverse association between fasting glucose and current serum dioxin and a positive association between 2-hour postprandial glucose and current serum dioxin. Although not statistically significant, serum insulin levels in diabetics,

in contrast to nondiabetics, were inversely related to dioxin levels, indicating that serum insulin decreases as dioxin levels increase in diabetics. These results are consistent with a fundamental impairment of islet cell responsiveness to hyperglycemia with compromised insulin production and point to a potential mechanism for an effect of dioxin on glucose metabolism. However, the analyses of serum C peptide and serum proinsulin yielded no significant results and did not reveal the biochemical mechanisms by which dioxin might have an effect on insulin production and glucose metabolism.

Analyses of gonadal function detected a significant inverse dose-response relationship between current serum dioxin and total serum testosterone in Ranch Hands. These results are consistent with those from the 1987 examination, but the clinical significance is uncertain.

The longitudinal analyses yielded results that would be anticipated over time with no significant differences between Ranch Hands and Comparisons. Age-related increases were documented in fasting glucose, 2-hour postprandial glucose, and the incidence of diabetes, while serum testosterone decreased with age.

In summary, after 10 years of observation, the prevalence of endocrine disease remains similar in Ranch Hands and Comparisons. Although cause and effect remain to be established, the current endocrine assessment provides further evidence for an association between glucose intolerance and dioxin exposure. The possibility is raised that, in a subset of individuals predisposed to diabetes, dioxin may impair insulin production.

### **Immunologic Assessment**

Immunologic competence was assessed by analyzing physical examination and laboratory data from skin tests for delayed hypersensitivity response, cell surface marker studies on a randomized subset of the study population, immunoglobulin quantitation, and autoantibody detection. This evaluation went far beyond typical medical examinations employed for general health assessments, and included elements of measurement used frequently to define specific diseases.

Overall, the immunologic assessment did not reveal any relationships that could be considered clinically significant between dioxin exposure and physiologic abnormalities. The MSK smooth muscle antibody, rheumatoid factor, and lupus panel summary index displayed inverse associations with dioxin exposure, but did not support a dose-response relationship; additionally, the magnitude of these associations was small and could not be interpreted as conveying a health risk.

A marginally significant positive association was found between serum IgA concentrations and extrapolated initial dioxin levels. Although the magnitude of this effect was small, its statistical significance coupled with continuity over time suggests a possible relationship that should be evaluated further because elevated IgA may indicate liver disease, chronic inflammation, or selective immune dysfunction.

The longitudinal analyses of the CD4-CD8 ratio did not consistently show significant differences between the 1985 and 1992 measurements in relation to dioxin exposure.

In summary, these findings do not provide evidence of a clinically significant dose-response effect for body burden of dioxin on parameters of immunologic assessment. The minor, statistically significant relationships that do have a small magnitude bear potential for long-term evaluation to identify trends, but currently cannot be interpreted to indicate specific health impairment caused by immune system dysfunction.

### **Pulmonary Assessment**

The pulmonary assessment consisted of three historical variables, physical examination of thorax and lung abnormalities, and six laboratory measurements. Because the lung is often involved secondarily in numerous infectious, inflammatory, and neoplastic disorders, the assessment of lung disease includes a comprehensive multisystem review conducted during the examinations and reported in other chapters. All episodes of pulmonary disease were verified by medical records review.

In the group analyses, Ranch Hands had a significantly higher prevalence of bronchitis and thorax and lung abnormalities. Conversely, pneumonia was less common in Ranch Hands than in Comparisons, though not statistically significant. Of interest, but of uncertain cause, Ranch Hand enlisted flyers appeared to be more at risk than Comparisons, respecting history of bronchitis and thorax and lung abnormalities; however, there was no evidence from the analyses of extrapolated initial and current serum dioxin measurements to confirm a dose-response relationship.

For the laboratory variables, a statistically significant inverse relationship was revealed between percent of predicted forced vital capacity (FVC) and initial and current serum dioxin in Ranch Hands. However, when Ranch Hands were contrasted with Comparisons, no significant differences were detected. The ratio of observed forced expiratory volume in 1 second ( $FEV_1$ ) to observed FVC in Ranch Hands also revealed a significant relationship with initial dioxin, indicating that the ratio increased (became closer to 1) for increasing levels of extrapolated initial dioxin; this effect may be due to the diminishing magnitude of FVC in the denominator of the ratio. Although consistent with a dose-response effect, the changes in the ratio were slight and of doubtful physiologic significance.

In the longitudinal analysis of the ratio of observed  $FEV_1$  to observed FVC, a significant group difference was shown for the enlisted flyers. The Ranch Hand enlisted flyers had a larger decrease in the ratio between 1982 and 1992 than did the Comparison enlisted flyers, but the difference is not physiologically significant, and there was no evidence for any trend in relation to the extrapolated initial or current serum dioxin levels.

In summary, the historical, physical examination, and laboratory data analyzed for the pulmonary assessment revealed no consistent evidence of an increased prevalence of pulmonary disease in the Ranch Hand cohort relative to the Comparison cohort or in relation to body burden of dioxin.

## INTERPRETIVE CONSIDERATIONS

There are certain facts that need to be understood in drawing conclusions from the statistical analysis of the 1992 followup examination results. For example, there are often difficulties associated with multiple testing. With multiple models applied against hundreds of variables, the likelihood of a statistical test indicating some artifactual association is high. But longitudinal comparisons of previous examinations may show a consistent association, supporting a non-artifactual relationship. However, longitudinal tests of the same population are clearly not independent tests. If a chance association was present at the first physical examination, it would tend to persist in subsequent examinations.

Conversely, depending on putative site and mode of action, the association would be expected to increase with time (if latency or other chronic effects predominate) or decrease with time (if current dioxin level predominates in the mechanism). It is also important to note that some conditions do not appear with reasonable frequency until middle age or later, and, in the early years of the study, an eventual significant increase in relative risk in a population easily might be masked by data too sparse for meaningful analysis.

The putative site and mode of action in the body could itself either cause or obscure a relationship. Receptors might be activated only after a certain dioxin threshold value had been exceeded—that is, a value exceeding the body's capability to safely store dioxin. If, on the other hand, dioxin caused a competitive inhibition of receptor actions normally stimulated by other substances, there might be a "no-threshold" effect. Depending on the nature (lipid or non-lipid) and type of function of the hypothetical receptor site, an increase in body fat over time might either cause an increase in dioxin effect because of a greater volume of distribution or a decrease in dioxin effect because of a lesser concentration at the receptor site.

Strength of association is also an issue in a study of a population this size. A study with a population of 2,233 lacks power to determine increases in relative risks for rare events, because rare events are unlikely to occur in a group this small. While certain occupational toxins have truly pathognomonic pathology (e.g., mesothelioma for asbestos, hepatic angiosarcoma for vinyl chloride) virtually non-existent in the absence of the toxin, other toxins merely increase the risk of non-pathognomonic pathology. For example, in the absence of a dioxin-pathognomonic lesion, this study would likely not discern an increase in the relative risk for a rare tumor. By assessing the pathology observed in association with other known environmental risk factors (e.g., tobacco use, alcohol use) it is sometimes possible to provide an upper bound for the magnitude of effect missed. However, this study has inherent limits in detecting modest increases in relative risk for infrequent pathology.

A final difficulty is the presence of a true association that is non-causal. An example might be a condition not caused by dioxin, but resulting in or from an altered biological dioxin half-life. In this case, a correlation might be high in the total absence of causality.

Clearly, there are many issues to be considered in interpreting data for this study. With these issues in mind, certain assessments were made by looking at a number of factors. Among these factors are longitudinal trends, biological plausibility, consistency with animal

toxicology, the presence of a plausible dose-response relationship, and strength of association. But, meeting all of these criteria would not guarantee causality, nor would failing these criteria guarantee the lack of a dioxin effect. It can be argued, however, that the good faith application of these particular filters should be the starting point for generating hypotheses for experimental examination through in vitro and in vivo testing, as well as through further epidemiological analysis of these and other dioxin exposed groups.

## **SUMMARY**

Based on the statistical findings of the 1992 examination, and subject to the qualifications considered above, the principal investigators have drawn the following conclusions.

### **Glucose Intolerance**

The results indicate a statistically and potentially clinically significant association between serum dioxin and glucose intolerance. This association exhibits a dose response relationship, and is present both for non-diabetic individuals (as manifested by elevated insulin levels) and diabetic individuals (as manifested by increased prevalence and severity of diabetes, and decreased age of onset). This association was found with type II diabetes only. This association was also present longitudinally and occurs in other epidemiological studies in addition to the AFHS.

### **Cardiovascular Mortality**

There is a statistically significant increase in cardiovascular mortality in the most heavily exposed subgroup, the enlisted groundcrew. This association persists longitudinally throughout the three examination cycles. Inclusion of this group with lesser exposed Ranch Hand subgroups results in a statistically nonsignificant overall relative risk. Less clinically severe criteria for altered cardiac functions including ECG findings of prior myocardial infarction, non-specific ST- and T-wave changes, and RBBB displayed significant positive associations with dioxin, although these associations did not cause significant group differences between all Ranch Hands and all Comparisons. Peripheral vascular function variables displayed significant subgroup differences for both the enlisted groundcrew and the high current dioxin category in relation to the Comparisons. Both groups had a greater prevalence of new pulse deficits arising since the 1985 followup examination than did their Comparisons.

### **Serum Lipid Abnormality**

There is a highly significant positive statistical association between dioxin and cholesterol, dioxin and triglycerides, and dioxin and the cholesterol-HDL ratio in most models using either current dioxin levels or dioxin levels extrapolated to the end of the tour of duty in SEA. In such models, the correlation between HDL cholesterol and dioxin was highly significant and negative. These lipid findings were consistent with the 1987 findings, but were not consistent with the 1982 examination when serum cholesterol in Ranch Hands was significantly lower than in Comparisons.

## **Liver Enzymes**

Both lipid-adjusted and whole-weight current dioxin showed elevated mean aspartate aminotransferase (AST), ALT, and GGT associations. For ALT and GGT this association was highly significant. This association had not been present in previous examinations. Although these elevations were statistically significant, mean enzyme levels remained well within normal limits and the prevalence of abnormally elevated liver enzymes was not statistically increased. Thus, although this laboratory finding is statistically significant, the AFHS population did not show any clinically adverse outcomes.

## **Increase in IgA**

A marginally significant increase in IgA with increased serum dioxin was found. This paralleled similar findings of increased IgA, first noted in the 1987 followup. Although this elevation was marginally significant, mean IgA levels remained well within normal limits, and the prevalence of significant abnormally elevated IgA was not statistically increased. Thus, although this finding is statistically significant, the AFHS population did not show any clinically adverse outcomes.

## **Decrease in Serum Testosterone**

A statistically significant inverse effect was seen between total serum testosterone and current dioxin in Ranch Hands. This paralleled similar findings first noted in the 1987 followup. Although this decrease was statistically significant, mean serum testosterone levels remained well within normal limits, and the prevalence of abnormally low serum testosterone was not statistically increased. Thus, although this finding is statistically significant, the AFHS population did not show any clinically adverse outcomes.

## **Decrease in MSK and Lupus Panel Positives**

Significant and marginally significant decreases in the prevalence of positive reactions to MSK, lupus, and rheumatoid factor tests in relation to dioxin were seen in the 1992 followup. When present, these tests are indicative of potential autoimmune disorders. Their absence is therefore not normally considered pathologic, but a decreased prevalence could nonetheless indicate some degree of immune suppression. More specific tests of immune suppression were not significantly associated with dioxin.

## **No Significant Difference in Incidence or Prevalence of Neoplastic Disease**

It has been theorized that dioxin can act as either an inducer or promoter of neoplastic disease. A detailed analysis of all forms of neoplastic disease over the course of a decade show no significant group differences in the incidence of benign or malignant neoplasms, including those neoplasms most often associated with herbicide exposure in the Ranch Hand population (e.g., Hodgkin's Disease, non-Hodgkin's lymphoma, soft tissue sarcoma). In the 1992 followup, there was again no significant group differences. The marginally significant differences in site-specific incidence that were found more often favored a decrease in relative risk associated with dioxin exposure rather than an increased risk. As previously

stated, because of its size, this study does lack power to ascertain modest increases in relative risk for uncommon neoplasms. As the population continues to age, the combination of an increase in background rate of neoplastic disease, increased time for latent effects of past exposure, and increased time of total exposure may combine to increase the power of this study to determine neoplastic effects.

In summary, glucose intolerance, serum lipid abnormality, and cardiovascular abnormality and mortality, are areas demonstrating associations that, if causality were established, would represent the most important dioxin-associated health problems seen in the AFHS to date. These three areas appear to have the greatest magnitude of effect in terms of absolute increase in risk, in common areas known to contribute to years of potential life lost and to overall healthcare costs. Clearly, there are biological interrelationships among all three of these variables that will make the task of establishing causality, as well as establishing primary versus secondary causality, challenging. From a public health perspective, these three areas demand the greatest attention.