TABLE 15-25. (Continued)

Summary of Categorized Current Dioxin Analyses for Endocrine Variables (Ranch Hands and Comparisons)

Variable	All	Adjusted: Unknown versus Background	Adjusted: Low versus Background	Adjusted: High versus Background
Questionnaire				
Current Thyroid Function (Self-Administered) (D)	NS	NS	ns	ns
History of Thyroid Disease (Interviewer-Administered) (D)	NS	NS	ns	ns
Physical Examination				
Thyroid Gland (D)	NS	ns	NS	ns
Testes (D)	0.010	ns	ns	+0.001
Laboratory				
T_3 % Uptake (C)	** (0.005)	** (NS)	** (ns)	** (-0.001)
T_3 % Uptake (D)	** (NS)	** (NS)	** (ns)	** (ns)
TSH (C)	NS*	NS	NS	+0.010
TSH (D)	NS	NS	ns	NS
FSH (C)	NS	NS	NS	NS
FSH[a] (D)	NS	NS	NS	ns
FSH[b] (D)		NS	NS	NS
Testosterone[c] (C)	NS	NS	NS	ns
Testosterone[c,d] (C)	<0.001	+0.001	ns	-0.010
Testosterone (D)	** (NS)	** (ns)	** (ns)	** (NS)
Fasting Glucose (C)	***(<0.001)	*** (ns)	*** (NS)	*** (+<0.001)
Fasting Glucose (D)	<0.001	ns*	NS	+<0.001
2-Hour Postprandial Glucose (C)	NS	ns	ns	NS
2-Hour Postprandial Glucose[d] (C)	0.010	-0.035	ns	+0.041
2-Hour Postprandial Glucose[e] (D)	NS	NS	NS	NS
2-Hour Postprandial Glucose[f] (D)		ns	ns	+0.035
Composite Diabetes Indicator (D)	** (0.003)	** (ns)	** (NS)	** (+<0.001)

[a]Low FSH contrasted with normal FSH for last three columns.

[b]High FSH contrasted with normal FSH for last three columns.

[c]Negative difference considered adverse for this variable.

[d]Adjusted results from models without percent body fat presented for this variable; see Appendix Table N-2 for a detailed description of these analyses.

[e]Impaired contrasted with normal for last three columns.

[f]Diabetic contrasted with normal for last three columns.

C: Continuous analysis.

D: Discrete analysis.

+: Relative risk 1.00 or greater for discrete analysis; difference in means nonnegative for continuous analysis.

-: Difference in means negative.

NS/ns: Not significant ($p>0.10$).

NS*:/ns* Marginally significant ($0.05<p\leq0.10$).

** (NS)/** (ns): Categorized current dioxin-by-covariate interaction ($p\leq0.05$); not significant when interaction is deleted; refer to Appendix Table N-1 for a detailed description of this interaction.

** (...): Categorized current dioxin-by-covariate interaction ($p\leq0.05$); significant when interaction is deleted and p-value is given in parentheses; refer to Appendix Table N-1 for a detailed description of this interaction.

TABLE 15-25. (Continued)
Summary of Categorized Current Dioxin Analyses for Endocrine Variables (Ranch Hands and Comparisons)

*** (NS)/*** (ns): Categorized current dioxin-by-covariate interaction ($p \leq 0.01$); not significant when interaction is deleted; refer to Table I-1 for a detailed description of this interaction.

*** (...): Categorized current dioxin-by-covariate interaction ($p \leq 0.01$); significant when interaction is deleted and p-value is given in parentheses; refer to Table I-1 for a detailed description of this interaction.

Note: P-value given if $p \leq 0.05$.

Appendix Table N-2 contains detailed analyses for models without percent body fat.

A capital "NS" denotes relative risk 1.00 or greater for discrete analysis or difference of means nonnegative for continuous analysis; a lowercase "ns" denotes relative risk less than 1.00 for discrete analysis or difference of means negative for continuous analysis; a capital "NS" in the first column does not imply directionality.

assumptions. The adjusted relative risk was marginally significant for Ranch Hands with an early tour under the maximal assumption. The adjusted analyses of categorized current dioxin found that Ranch Hands in the high current dioxin category were 3.8 times more likely to have an abnormal testes than Comparisons in the background category.

Laboratory Examination Variables

Seven laboratory examination variables were analyzed to assess current endocrine function: T_3 % uptake, TSH, FSH, testosterone, fasting glucose, 2-hour postprandial glucose, and a composite diabetes indicator. Each variable was analyzed in continuous and discrete forms, except for the composite diabetes indicator, which was only analyzed discretely.

Model I: Ranch Hands – Initial Dioxin

Adjusted analyses found that initial dioxin was significantly associated with increases in diabetes, fasting glucose, and 2-hour postprandial glucose; significant decreases were noted in T_3 % uptake and testosterone. Under both the minimal and maximal assumptions, the adjusted initial dioxin analyses found a significant negative relationship with T_3 % uptake in its continuous form. The analyses of discretized T_3 % uptake were not significant. No significant findings were noted for either the unadjusted or adjusted initial dioxin analyses of TSH and FSH.

For the continuous analysis of testosterone, the interaction of initial dioxin and personality type was significant under both assumptions. Stratifying by personality type, a significant negative association was seen between testosterone and initial dioxin for type A Ranch Hands. This contrasted with a nonsignificant positive association for type B Ranch Hands. Excluding the interaction, contrary results arose based on which covariates were used for adjustment. No significant results were found for the maximal analysis when adjusting for percent body fat, age, and race, but a highly significant negative association was found when percent body fat was deleted from the model. The minimal analysis displayed similar results. Despite these findings, the prevalence of abnormally low testosterone levels was not significantly associated with initial dioxin for any of the analyses of discretized testosterone.

The longitudinal analyses found that Ranch Hands with higher levels of initial dioxin had less of a decrease in testosterone between 1982 and 1987 than Ranch Hands with lower levels of initial dioxin. These results are inconsistent with the previously discussed findings, which showed that higher levels of dioxin were associated significantly with lower levels of testosterone, when percent body fat was not in the adjusted model.

The unadjusted initial dioxin analysis of 2-hour postprandial glucose in its continuous form was not significant under the minimal assumption, but the maximal analysis revealed a significant positive association. The adjusted minimal analysis detected a significant interaction between initial dioxin and percent body fat, but stratified results did not show a significant initial dioxin effect for either normal/lean Ranch Hands or for obese Ranch Hands. Ignoring the interaction, adjusted results for both assumptions were not significant when percent body fat was retained in the final model. However, comparable to the testosterone findings, the association between initial dioxin and 2-hour postprandial glucose became significant when percent body fat was removed from the model.

Under both assumptions, the overall initial dioxin effect was not significant for either the unadjusted analysis of discretized 2-hour postprandial glucose or for the adjusted analysis that kept percent body fat in the model. However, these analyses indicated a marginally significant increased risk of diabetic glucose levels for Ranch Hands in the high initial dioxin category relative to the low category. This contrast became significant when percent body fat was deleted from the adjusted model. The overall dioxin effect was of borderline significance in the adjusted model without percent body fat.

All unadjusted and adjusted initial dioxin analyses for fasting glucose and for the composite diabetes indicator were significant.

Model 2: Ranch Hands – Current Dioxin and Time

The association between current dioxin and the laboratory variables did not differ significantly between time since tour strata for most analyses. Under the minimal assumption, the current dioxin-by-time interaction was not significant for all analyses, except for the adjusted analysis of T_3 % uptake treated as a continuous variable (marginally significant results were noted for the unadjusted analysis of T_3 % uptake and for the unadjusted and adjusted continuous analyses of FSH and fasting glucose). For T_3 % uptake, the association with current dioxin was significantly negative for Ranch Hands with more than 18.6 years since tour, but a nonsignificant positive association was seen for Ranch Hands with time since tour of 18.6 years or less.

Under the maximal assumption, the interaction between current dioxin and time was not significant for all variables except for the analyses of FSH. Under the maximal assumption, higher levels of FSH were associated significantly with dioxin among Ranch Hands with a later time since tour. The association between FSH and dioxin was not significant and negative for Ranch hands with an early tour.

However, the adjusted maximal analyses detected significant positive associations between current levels of dioxin and fasting glucose within each time stratum. In addition, the continuous analyses of 2-hour postprandial glucose found a marginally significant positive association with dioxin for Ranch Hands with an early tour; the association became significant when percent body fat was excluded from the adjusted model (adjusted for age and personality type). The discrete analyses of 2-hour postprandial glucose were significant or marginally significant for Ranch Hands with a later tour. Ranch Hands with a later tour in the high current dioxin category had an increase in diabetic glucose levels relative to those in the low current dioxin category. This finding was marginally significant under the minimal assumption and significant under the maximal assumption.

Under both the minimal and maximal assumptions, there was a significant or marginally significant increased risk of diabetes associated with current dioxin levels within each time stratum. However, there was no significant interaction between current dioxin and time.

The adjusted analyses detected significant current dioxin-by-time-by-personality type interactions for TSH and for 2-hour postprandial glucose, but stratified results did not indicate

a dioxin effect for either variable. There was no significant current dioxin-by-time interaction in the longitudinal analyses of T_3 % uptake, TSH, and testosterone.

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

The adjusted analyses of categorized current dioxin found that Ranch Hands in the high current dioxin category (>33.3 ppt) had significantly higher incidences of diabetes and abnormally high levels of fasting glucose relative to the background category. Adjusted analyses also found that these Ranch Hands had significantly higher mean levels of TSH, fasting glucose, and 2-hour postprandial glucose than the background category, and significantly lower mean levels of T_3 % uptake and testosterone. For all laboratory variables, Ranch Hands in the low current dioxin category (15 ppt to 33.3 ppt) never differed significantly from the background group (exclusive of interaction analyses). The unknown versus background contrast was often in the opposite direction of the high versus background contrast. Ranch Hands in the unknown current dioxin category (0 ppt to 10 ppt) had a significantly higher mean level of testosterone and a significantly lower mean postprandial glucose level than the background group.

For T_3 % uptake in its continuous form, the unadjusted and adjusted analyses found a significant overall difference among current dioxin categories, with the mean T_3 % uptake for the high current dioxin category significantly less than the background mean. The interaction between current dioxin and age was significant for both the continuous and discrete adjusted analyses of T_3 % uptake. The interaction for the continuous analysis occurred partly because the difference in mean T_3 % uptake for the low versus background contrast was significantly negative for participants born in or after 1942, but it was marginally positive for individuals born before 1942. This same pattern was seen for the discrete analysis, but neither age-specific contrast was significant.

The mean TSH for the high current dioxin category was significantly greater than the background mean in the adjusted analysis.

The mean testosterone for Ranch Hands in the high current dioxin category was significantly less than the background mean, and the mean for the unknown category was significantly more than the background mean, adjusting for the age-by-race interaction. However, when percent body fat was included in the model, neither of these findings was significant. The unadjusted analysis of discretized testosterone found relatively more abnormally low testosterone levels in the high category than in the other three current dioxin categories, but no significant contrasts were noted. The adjusted analysis for discretized testosterone revealed a significant interaction between personality type and current dioxin. Stratified results showed a significant increased risk of an abnormally low testosterone level for the high current dioxin category relative to the background category for type A participants.

In the adjusted analyses of fasting glucose, there was a significant interaction between categorized current dioxin and lifetime alcohol history for older Ranch Hands. Stratified results showed that the mean difference between the high current dioxin category and the background category increased with levels of lifetime alcohol consumption.

Adjusted for age and personality type, the mean 2-hour postprandial glucose was significantly greater in the high current dioxin category than in the background category. Also, the mean for the unknown category was significantly less than the background mean. Comparable to the testosterone findings, neither of these results was significant when percent body fat was retained in the final model. The adjusted analyses of discretized 2-hour postprandial glucose found a significant increased risk of diabetic glucose levels for Ranch Hands in the high category relative to the background category.

The unadjusted and adjusted analyses for the composite diabetes indicator detected a highly significant increased risk of diabetes for Ranch Hands in the high current dioxin category relative to the background category. The adjusted analyses also detected a significant interaction between categorized current dioxin and age. Older Ranch Hands, those born before 1942, were more than three times as likely to be diabetic than similar-aged Comparisons in the background group. This difference was highly significant. In contrast, younger Ranch Hands were only 1.5 times as likely to be considered diabetic than background Comparisons born in or after 1942, which was not significant. No increase in risk was evident for Ranch Hands in the unknown or low categories.

The longitudinal analyses did not indicate that dioxin was associated with changes in T_3 % uptake, TSH, and testosterone.

CONCLUSION

The endocrine assessment found a strong association between initial dioxin and an increase in the incidence of diabetes and the prevalence of testes abnormalities. However, the analyses of current dioxin levels in Ranch Hands and Comparisons indicated that the increased risk was only apparent for Ranch Hands in the high current dioxin category (>33.3 ppt, n=187). These Ranch Hands also had significantly higher mean levels of TSH, fasting glucose, and 2-hour postprandial glucose than background Comparisons, as well as lower mean levels of T_3 % uptake and testosterone. The discrete analyses of these variables found a significant increase in the prevalences of abnormally elevated fasting glucose levels and diabetic 2-hour postprandial glucose levels. The longitudinal analyses provided no consistent support that changes in T_3 % uptake, TSH, and testosterone between 1982 and 1987 were related to dioxin exposure.

These results must be interpreted with caution. Though the data clearly establish a strong association between glucose intolerance and dioxin exposure, it would be premature to draw conclusions regarding cause and effect. Clinically, obesity is well recognized as the most common cause of adult-onset diabetes mellitus. Data analyzed in Chapter 6 document a strong correlation between serum dioxin levels and percent body fat. Pending further investigation into the pharmacokinetics of dioxin in lean versus obese individuals, a causal relationship between exposure to dioxin and diabetes remains to be proven.

CHAPTER 15

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CHAPTER 16

IMMUNOLOGIC ASSESSMENT

INTRODUCTION

Background

Of the many chemical compounds known to cause immune system dysfunction in laboratory animals, the polyhalogenated aromatic hydrocarbons have been the most extensively studied and, among these, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has proven to be the most toxic. Since TCDD-induced immunotoxicity was first reported in experimental animals in the early 1970's (1, 2, 3), a large body of literature pertinent to this subject has accumulated and has been summarized in previous reports from the Air Force Health Study (AFHS) (4, 5).

In laboratory animals, numerous studies have demonstrated that TCDD has a wide range of toxic effects and is a potent suppressor of both humoral- and cell-mediated immune function (6, 7). In mice, TCDD has been shown to cause myelosuppression (8), impaired lymphoproliferative responses and humoral antibody production (9, 10), thymic atrophy (11), and impaired complement activity (12). More recent research has focused on defining the mechanisms of TCDD-induced immune dysfunction. Some, but not all of the manifestations of TCDD toxicity are clearly related to the presence of the aryl hydroxylase (Ah) receptor that is present in lymphoid tissue and lymphoid cells (13-16). Myelotoxic effects (17, 18), suppression of humoral antibody responses (19), and impaired complement activity (20) are among those that have been proven to be Ah receptor mediated. In contrast, numerous investigators have established that the effects of TCDD on B-cell maturation can occur independent of the presence of the Ah receptor (21, 22, 23). In one study, the primary target for TCDD-induced suppression of IgM antibody production was found to be the B lymphocyte at the level of cell differentiation (24). Further, there is good evidence that the age of the experimental animal is an important determinant of several immune system consequences of dioxin (25), including the responsiveness of thymocytes to Interleukin 1 (26), and the more persistent thymic atrophy and suppression of cell-mediated immunity seen in perinatal versus adult mice (27).

It is difficult to extrapolate the results of these animal studies to humans for a number of reasons. Doses of TCDD administered were extreme by any measure of human dioxin exposure, routes of administration were usually not comparable, interspecies variation is unpredictable, and the period of observation was insufficient to reflect the latency effect that may be required to produce clinical endpoints in humans. As noted in Chapter 15, Endocrine Assessment, there are similarities in the physicochemical properties of the Ah receptor in animals and those that mediate the effects of thyroid and glucocorticoid hormone function in humans. To date, a receptor capable of binding TCDD has been defined in several human tissues (including placenta [28], skin [29], and lung [30]), and an Ah receptor has been identified in cultured human thymic epithelial cells. Initial characterization of its physicochemical properties has been the subject of several reports (31, 32).

In contrast to the active research in animals, relatively few studies have been published describing immune system effects of TCDD in humans and, from these, no consistent evidence for immunologic abnormalities has emerged. An apparent impairment in cell-mediated immunity was found after an environmental exposure (33) but was not confirmed in followup observations (34). A more recent report examining immunologic indices and, for the first time, correlating the results with the body burden of dioxin based on adipose tissue levels, found no evidence for any immune system impairment (35). These findings are consistent with those recently reported in the AFHS (5).

Earlier studies of the effects of TCDD on the human immune system have been limited by unreliable indices of dioxin exposure and/or insufficient followup to reflect a latency effect. Though the severe consequences of advanced immune suppression in humans (overwhelming infection and malignancy) are well established, reliable clinical and laboratory indices to detect more subtle compromise in immune function are not well understood. In this regard, two recent publications have made valuable contributions to consistency in laboratory methodology and quality control (36, 37).

More detailed summaries of the pertinent scientific literature for the immunologic assessment can be found in the report of the previous analyses of the 1987 examination data (5).

Summary of Previous Analyses of the 1987 Examination Data

For the assessment of the 1987 immunologic examination data, composite skin reaction test results and various laboratory examination measurements from cell surface marker studies, three groups of functional stimulation tests, and quantitative immunoglobulins were analyzed. Ranch Hands had a higher frequency of individuals with possibly abnormal reactions on skin testing than the Comparisons. The analysis of the composite skin test results, adjusting for covariate information, contained a significant group-by-lifetime cigarette smoking history interaction. Followup analyses showed that, among those individuals with the heaviest smoking histories, Ranch Hands had a higher frequency of possibly abnormal readings when contrasted with Comparisons. Within the other strata, there were no significant differences. The unadjusted analyses of the laboratory examination data indicated no significant group difference between Ranch Hands and Comparisons. For the adjusted analyses of the natural killer assay measurements with and without Interleukin 2 (IL-2), significant interactions between group and race were present. Exploration of these interactions revealed that the Black Ranch Hands had higher adjusted means than the Black Comparisons for the natural killer assay measures. The adjusted mean values for Black Ranch Hands, non-Black Comparisons, and non-Black Ranch Hands were numerically similar in these analyses. Black Comparisons had lower mean values than the other three groups. The clinical significance of these findings is not apparent and does not point to any known clinical endpoints. In general, the immunologic assessment revealed no medically important differences between the Ranch Hands and Comparisons.

Parameters of the Immunologic Assessment

Dependent Variables

Data from the physical examination and the Scripps Immunology Reference Laboratory (SIRL) were used in the immunologic assessment. Immunologic tests were carried out on a random sample of approximately 40 percent of the participants because of the complexity of the assay and the expense of these tests. Blood was drawn for testing from approximately one-half of these randomly chosen participants on the first day of the physical examination, and blood was drawn from the rest of the selected participants on the second day.

All participants except those chosen to receive the immunologic tests at SIRL on day 2 of the physical examination were scheduled to receive the skin test as a part of the physical examination (approximately 80 percent of the 1987 examination participants). Participants chosen to receive the immunologic blood draw on day 2 of the physical examination were not given skin tests to avoid any effect the skin test antigens might have on the cell counts and functions.

Physical Examination Data

Physical examination data concerning the skin tests were used to evaluate immunologic function. A composite skin test diagnosis variable was constructed based on the response to four separate antigens injected interdermally to measure antigen reactivity or sensitivity. This composite skin test variable was analyzed as a discrete, dichotomous variable: each participant was considered possibly abnormal or normal based on his skin reactivity to the antigens *Candida albicans*, mumps, Trichophyton, and staphage-lysate. The response to each antigen was scored positive (normal) if the maximum diameter of the resulting 48-hour induration was greater than or equal to 5 mm, which indicated intact cell-mediated immunity. If none of the four antigen responses was positive, the composite skin test diagnosis was scored possibly abnormal. If one or more of the four antigen responses was positive, the composite skin test was considered normal.

Participants taking anti-inflammatory (except aspirin) or immunosuppressant medication, or who had recently received x-ray treatment or chemotherapy for cancer (as reported in the 1987 health interview questionnaire and verified by medical records review) were excluded from all analyses of skin test data. In addition, data from participants in examination group 2, except for one participant, were not used in the analysis of the composite skin test diagnosis variable, since they received staphage-lysate at a different dosage than all the other examination groups. One of the two nurses made a dosage error affecting all but the one participant in examination group 2.

Laboratory Examination Data

From the SIRL immunologic tests, the results of cell surface marker studies, total lymphocyte count (TLC), functional stimulation studies, and quantitative immunoglobulins were analyzed. Figure 16-1 presents the immunologic parameters evaluated and describes their medical importance. In the report on the 1987 examination, these data were evaluated to determine whether the natural logarithm scale was more appropriate for use with the statistical procedure(s) than the original scale (5). Appendix Table P-1 of the report on the 1987 examination summarized the statistics used in that assessment. The descriptive

FIGURE 16-1.

Medical Significance of the Immunologic Data

Immunologic Measure	Rationale of the Measurement	Disease/Syndrome/Condition Endpoint
Skin Tests		
Candida Mumps Tricophyton Staphage-lysate	Skin testing measures in vivo hypersensitivity responses to antigens of bacteria, fungi, and a virus to which most persons have previously been exposed. The skin reaction to intradermal injection of these antigens indicates integrity of T-cell memory and ability of effector cells to mount a response.	Antigen reactivity or sensitivity. Lack of response to all antigens indicates anergy which may occur in overwhelming infections, widespread malignancy, immunosuppression, or malnutrition.
Marker Studies		
CD2 (T11)	Measures CD2 cells coincident with sheep rosette receptor on cell surface (most are CD4 and CD8 cells). CD2 positive cells represent total T cells.	Decrease may result in cellular immune deficiency; increased with lymphoproliferative disorders.
CD20 (B1)	Measures peripheral blood B cells; no reaction with T cells, granulocytes, or monocytes.	Decrease may result in humoral immune deficiency with impaired production of antibodies; increased in lymphoproliferative disorders.
CD4 (Leu3a+b)	Measures T cells that exhibit helper/inducer phenotype. CD4 cells initiate an immune response to processed antigens.	Markedly decreased in AIDS due to HIV infection of CD4+ cells; increased in autoimmune diseases.
CD8 (OKT8)	Measures T cells that exhibit suppressor/ cytotoxic functions. Responsible for appropriate down regulation of an immune response after antigen has been cleared.	Variable in autoimmune diseases; increased in some viral illnesses and immunodeficiencies.

FIGURE 16-1. (Continued)

Medical Significance of the Immunologic Data

Immunologic Measure	Rationale of the Measurement	Disease/Syndrome/Condition Endpoint
CD14 (LeuM3)	Measures mature monocytes in peripheral blood. Monocytes take up and process foreign antigens for presentation to CD4+ cells.	Increases with inflammation of many etiologies.
CD25 (IL-2 Receptor)	Present on activated T cells; absent on normal peripheral blood lymphocytes, monocytes, and granulocytes. Stimulation with IL-2 induces more IL-2 Receptor synthesis in activated T cells (positive feedback).	Increased in lymphoproliferative disorders. Also increased with any immune activation (viral infection, organ transplant rejection).
HLA-DR	Measures cells expressing HLA-DR antigen; includes B cells and monocytes. HLA-DR+ cells present antigen to CD4+ T cells.	Decreased in B-cell deficiency; decreased in agammaglobulinemia. Deficiency may reflect ability to mount primary cellular immune response.
CD4/CD8 Ratio	Measures proportional difference between CD4+ cell populations and CD8+ cell populations. Reflects balance between up regulation and down regulation of T cells.	Decreased in immunodeficiencies and viral illnesses. AIDS causes very low ratio as does immunosuppression with cyclosporine.
TLC	Measures absolute number of total lymphocytes circulating in peripheral blood. Major immune mechanism against fungi and viruses.	Decreased in immunodeficiency; increased in lymphoproliferative disorders.

FIGURE 16-1. (Continued)

Medical Significance of the Immunologic Data

Immunologic Measure	Rationale of the Measurement	Disease/Syndrome/Condition Endpoint
Immunoglobulins		
IgG IgA IgM	Each measures ability of specific B-cell subgroup to secrete specific antibody class of molecules. Antibodies normally rise in response to infections or immunizations with bacteria, fungi, and viruses. Major immune mechanism against bacteria.	Increased in hyperglobulinemia or myeloma (monoclonal). Decreased in selective or total B-cell immunodeficiency. Polyclonal increases in chronic inflammation and liver disease (cirrhosis).
Functional Studies		
PHA	Measures functional capability of T cells to become activated by mitogen and undergo proliferation. Relies on integrity and in vitro interaction of several different cell types including macrophages and T-lymphocytes.	Decreased with impaired natural defenses due to stress, surgery, age, malnutrition, burns, uremia, malignancy, some infections.

FIGURE 16-1. (Continued)

Medical Significance of the Immunologic Data

Immunologic Measure	Rationale of the Measurement	Disease/Syndrome/Condition Endpoint
NKCI (with IL-2) NKCA (without IL-2)	Measures natural killer cell lytic activity with and without Interleukin 2 (IL-2) treatment of the natural killer cells. Percent release relates the amount of chromium-51 released when target cells are killed by natural killer cells to the amount of chromium-51 released when all target cells are killed (maximal release of radioactivity). Net response cpm is generated by the release of isotope from target cells killed by natural killer cells minus the cpm generated by spontaneous lysis or isotope leakage of the target cells. NK activity does not require antibody and is independent of antigen specificity.	Decreased with impaired natural defenses. NK cells are responsible for immunosurveillance in the body. They may attack and destroy virus-infected cells as well as tumor cells arising from carcinogens. NK cells may screen and remove early growths of malignant cells.
MLC	Measures reactivity of T cells to foreign histocompatibility class II antigens on cells from different individuals. Defines HLA-D specificities. Must have several cell types functionally intact as in PHA.	Used for cross-matching HLA-D in organ transplantation. PHA stimulation indicates cellular immune response to very strong mitogen, whereas MLC indicates cellular ability to respond to more subtle antigens on surfaces of living cells. Strong correlation between active PHA and MLC responses.

statistics of skewness and kurtosis were used in conjunction with the Kolmogorov D statistic for deciding whether to use the original scale or the natural logarithm scale (38).

Participants taking anti-inflammatory (except aspirin) or immunosuppressant medication, or who had recently received x-ray treatment or chemotherapy for cancer were excluded from all analyses of laboratory data.

Quantitative Studies: Cell Surface Marker (Phenotypic) Studies

Quantification of the different cell populations was carried out with the use of mouse monoclonal antibodies. Seven cell surface markers and a ratio of cell markers were analyzed in the evaluation of the immunologic system. The unit of measurement (for all variables except the CD4/CD8 ratio) was cells/mm^3. These variables were treated as continuous data, and were subjected to the natural logarithm transformation for statistical analysis.

Quantitative Studies: TLC

Statistical analysis on TLC was performed. The unit of measurement was cells/mm^3. A natural logarithm transformation was applied to the TLC data for statistical analyses.

Functional Stimulation Tests

Cell function responses to stimulation by phytohemagglutinin (PHA), mixed lymphocyte culture (MLC), and natural killer cell assays were also analyzed in the immunologic evaluation.

The following three PHA variables were analyzed: unstimulated PHA response for 2 mitogen harvest days, an overall PHA net response (adjusting for 3 mitogen concentrations and 2 harvest day effects), and the maximum PHA net response among the 3 mitogen concentrations and 2 harvest days. Each observation was the result of the averaging of quadruplicate readings.

MLC of donor lymphocytes was also used to stimulate in vitro cell proliferation of participant lymphocytes; the following two MLC variables were analyzed: unstimulated MLC response and MLC net response.

The following four variables from the natural killer cell assays were analyzed:

- Natural Killer Cell Assay (NKCA):
  - (1) NKCA 50/1 net response
  - (2) NKCA 50/1 percent release
- Natural Killer Cell Assay with Interleukin 2 (NKCI):
  - (3) NKCI 50/1 net response
  - (4) NKCI 50/1 percent release.

The unit of measurement for the PHA and MLC responses and the natural killer cell assay net response variables was counts per minute (cpm). These variables were treated as continuous in the statistical analysis. A natural logarithm transformation was applied to the unstimulated PHA response and the unstimulated MLC response.

Quantitative Studies: Immunoglobulins

The immunoglobulins IgA, IgG, and IgM were also analyzed statistically. The unit of measurement was mg/dl. The natural logarithm transformation was used in analyses of the immunoglobulins.

Covariates

Covariates used in the immunologic evaluation for adjusted statistical analyses included age, race, current alcohol use (drinks/day), lifetime alcohol history (drink-years), current cigarette smoking (cigarettes/day), and lifetime cigarette smoking history (pack-years). Further, batch-to-batch (examination group) variation and blood draw day-to-day variation (for each examination group) were also used as covariates for laboratory-dependent variables. Study participants who began their physical examination on the same day formed a batch. For the unstimulated PHA response, day of mitogen harvest was also used as a covariate in the adjusted analysis. For the overall PHA net response, mitogen concentration and mitogen harvest day were also used as covariates in the adjusted analyses.

Relation to Baseline, 1985, and 1987 Studies

For the 1985 examination report, the following variables were analyzed for group differences and associations with the exposure index: CD2, CD4, CD8, CD14, CD20, CD4/CD8 ratio, HLA-DR, unstimulated PHA response, PHA net response, MLC net response, and pokeweed net response. All of these variables, except for pokeweed net response, were also analyzed in this report and the previous 1987 examination report. In addition, statistical analyses were also performed in these reports on the following: CD25, unstimulated MLC, TLC, maximum PHA net response, IgA, IgG, IgM, natural killer cell assays with and without Interleukin 2, and the composite skin test diagnosis. Some of the variables in this report were also analyzed in the Baseline study.

Longitudinal analyses were performed on the CD4/CD8 ratio using the data collected for the 1985 and 1987 examinations.

For the 1987 examination report, the PHA net responses were analyzed for each of the six individual combinations of mitogen harvest day and mitogen concentration. In this report, these six analyses were not performed. Instead, the interactions of dioxin-by-harvest day, dioxin-by-mitogen concentration, and dioxin-by-harvest day-by-mitogen concentration were evaluated to determine whether stratified analyses were needed. As a result of those evaluations, the analyses involving initial dioxin in Ranch Hands and categorized current dioxin in the Ranch Hands and Comparisons were performed for each of the three mitogen concentrations.

Statistical Methods

Chapter 4, Statistical Methods, describes most of the basic statistical methods used in the immunologic evaluation. For both the 1985 and 1987 studies, large variation was expected from batch and blood draw day variability. Because of the variation, these covariates were generally incorporated into the unadjusted and the adjusted models of the respective immunologic assessments for those studies. For the serum dioxin analyses of the Ranch Hand immunologic measurements, these covariates were subjected to a prescreening procedure to determine whether the unadjusted and adjusted models would incorporate batch-to-batch and blood draw day-to-day covariates. The prescreening was performed because of the reduced sample sizes available for the stepwise modeling procedure applied to the models involving only the Ranch Hands. In addition, the batch-to-batch and blood draw day-to-day covariates would absorb many of the available degrees of freedom if routinely forced into a particular analysis model.

To address these data issues, a main effects prescreening model with the following terms was used for each immunologic measurement: $\log_2$ (initial dioxin), batch-to-batch variation, blood draw day-to-day variation, age, race, current alcohol use, lifetime alcohol history, current cigarette smoking, and lifetime cigarette smoking history. The models were used to evaluate the significance of the batch-to-batch and blood draw day-to-day covariates using the data from the maximal cohort (i.e., the larger data set). As a result of that analysis, the batch-to-batch and blood draw day-to-day covariates were used for the unadjusted and adjusted analyses of the following measures: CD14, CD25, HLA-DR, CD4/CD8, unstimulated PHA, PHA net response, maximum PHA net response, unstimulated MLC response, MLC net response, NKCI 50/1 net response, and NKCI 50/1 percent release. The unadjusted and adjusted analyses of CD20 and NKCA 50/1 net response were adjusted only for batch-to-batch variation. Batch-to-batch and blood draw day-to-day variation were not used in the unadjusted and adjusted analyses of CD2, CD4, CD8, TLC, and NKCA 50/1 percent release.

Table 16-1 summarizes the statistical analyses performed for the serum dioxin analyses of the immunologic assessment. The first part of the table describes the dependent variables analyzed. The second part of the table provides a further description of the candidate covariates examined. Abbreviations are used extensively in the body of the table and are defined in footnotes.

Data for four participants (two Ranch Hands and two Comparisons) were judged clinically unreasonable and were excluded prior to analysis. Some participants were excluded from the immunologic evaluation as stated above, and some dependent variable and covariate data were missing for other participants. Table 16-2 summarizes the number of participants excluded for medical reasons and the number of participants with missing data, by assumption and Ranch Hand and Comparison group. Variables used to evaluate skin and immunologic testing are detailed separately in this table, since different subsets of participants received these two types of tests.

Appendix O contains graphic displays of immunology system dependent variables versus initial dioxin for the minimal and maximal Ranch Hand cohorts, and immunology variables versus current dioxin for Ranch Hands and Comparisons. Graphics for dioxin-by-

TABLE 16-1.

Statistical Analysis for the Immunologic Assessment

Dependent Variables

Variable (Units)	Data Source	Data Form	Cutpoints	Candidate Covariates	Statistical Analyses
Composite Skin Test Diagnosis (based on length of four skin test antigen induration measurements)	PE	D	Possibly Abnormal: 0/4 ≥5 mm Normal: ≥1/4 ≥5 mm	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR	U:LR A:LR
CD2 Cells (cells/mm^3)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
CD4 Cells (cells/mm^3)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
CD8 Cells (cells/mm^3)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
CD20 Cells (cells/mm^3)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM

TABLE 16-1. (Continued)

Statistical Analysis for the Immunologic Assessment

Dependent Variables

Variable (Units)	Data Source	Data Form	Cutpoints	Candidate Covariates	Statistical Analyses
CD14 Cells (cells/mm^3)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
CD25 Cells (cells/mm^3)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
HLA-DR Cells (cells/mm^3)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
CD4/CD8 Ratio	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM L:GLM
Total Lymphocyte Count (TLC) (cells/mm^3)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM

TABLE 16-1. (Continued)

Statistical Analysis for the Immunologic Assessment

Dependent Variables

Variable (Units)	Data Source	Data Form	Cutpoints	Candidate Covariates	Statistical Analyses
Unstimulated Phytohemagglutinin (PHA) Response (counts/min [cpm])	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH), DAY	U:GLM A:GLM
PHA Net Response (cpm)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH), CONC,DAY	U:GLM A:GLM
Maximum PHA Net Response (cpm)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
Unstimulated Mixed Lymphocyte Culture (MLC) Response (cpm)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
MLC Net Response (cpm)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM

TABLE 16-1. (Continued)

Statistical Analysis for the Immunologic Assessment

Dependent Variables

Variable (Units)	Data Source	Data Form	Cutpoints	Candidate Covariates	Statistical Analyses
Natural Killer Cell Assay (NKCA) 50/1 Net Response (cpm)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
NKCA 50/1 Percent Release	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
Natural Killer Cell Assay With Interleukin (NKCI) 50/1 Net Response (cpm)	LAB	C	--	AGE,RACE CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
NKCI 50/1 Percent Release	LAB	C	--	AGE,RACE CSMOK,PACKYR, ALC,DRKYR, BATCH, DAY(BATCH)	U:GLM A:GLM
IgA (mg/dl)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR	U:GLM A:GLM
IgG (mg/dl)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR	U:GLM A:GLM
IgM (mg/dl)	LAB	C	--	AGE,RACE, CSMOK,PACKYR, ALC,DRKYR	U:GLM A:GLM

TABLE 16-1. (Continued)

Statistical Analysis for the Immunologic Assessment

Covariates

Variable (Abbreviation)	Data Source	Data Form	Cutpoints
Age (AGE)	MIL	D/C	Born ≥1942 Born <1942
Race (RACE)	MIL	D	Black Non-Black
Current Cigarette Smoking (CSMOK) (cigarettes/day)	Q-SR	D/C	0-Never 0-Former >0-20 >20
Lifetime Cigarette Smoking History (PACKYR) (pack-years)	Q-SR	D/C	0 >0-10 >10
Current Alcohol Use (ALC) (drinks/day)	Q-SR	D/C	0-1 >1-4 >4
Lifetime Alcohol History (DRKYR) (drink-years)	Q-SR	D/C	0 >0-40 >40
Batch-to-Batch (BATCH)	LAB	D	1, 2, 3, ... 80
Blood Draw Day-to-Day (DAY[BATCH])	LAB	D	1, 2 (actual day dependent on batch)
Mitogen Concentration (CONC)	LAB	D	1, 2, 3
Mitogen Harvest Day (DAY)	LAB	D	1, 2

TABLE 16-1. (Continued)

Statistical Analysis for the Immunologic Assessment

Abbreviations

Data Source:	LAB--1987 SIRL laboratory results MIL--Air Force military records PE--1987 SCRF physical examination Q-SR--1987 NORC questionnaire (self-reported)
Data Form:	D--Discrete analysis only C--Continuous analysis only D/C--Appropriate form for analysis (either discrete or continuous)
Statistical Analyses:	U--Unadjusted analyses A--Adjusted analyses L--Longitudinal analyses
Statistical Methods:	GLM--General linear models analysis LR--Logistic regression analysis

TABLE 16-2.

Number of Participants Excluded and With Missing Data for the Immunologic Assessment

Variable	Variable Use	Assumption (Ranch Hands Only) Minimal	Assumption (Ranch Hands Only) Maximal	Categorized Current Dioxin Ranch Hand	Categorized Current Dioxin Comparison
Skin Test Analysis[a]					
Composite Skin Test Diagnosis[b]	DEP	7	12	12	20
Current Alcohol Use	COV	3	5	5	0
Lifetime Alcohol History	COV	6	9	9	1
Chemotherapy	EXC	0	0	0	1
X-Ray Treatment	EXC	1	1	0	2
Anti-Inflammatory or Immunosuppressant Medication	EXC	13	16	13	18
Examination Group 2	EXC	6	6	4	2
Quantitative Immunoglobulins[c]					
Current Alcohol Use	COV	3	5	5	0
Lifetime Alcohol History	COV	6	9	9	2
Chemotherapy	EXC	0	0	0	1
X-Ray Treatment	EXC	1	1	0	2
Anti-Inflammatory or Immunosuppressant Medication	EXC	16	21	19	24

TABLE 16-2. (Continued)

Number of Participants Excluded and With Missing Data for the Immunologic Assessment

Variable	Variable Use	Assumption (Ranch Hands Only) Minimal	Assumption (Ranch Hands Only) Maximal	Categorized Current Dioxin Ranch Hand	Categorized Current Dioxin Comparison
Immunologic Test Analyses[d]					
CD2 Cells	DEP	2	2	3	4
CD4 Cells	DEP	3	3	3	0
CD8 Cells	DEP	3	4	3	0
CD20 Cells	DEP	2	2	2	0
CD25 Cells	DEP	1	1	1	2
HLA-DR Cells	DEP	0	0	0	1
CD4/CD8 Ratio	DEP	4	5	4	0
Unstimulated PHA Response (day 1)	DEP	0	2	2	3
Unstimulated PHA Response (day 2)	DEP	4	5	5	2
PHA Net Response (day 1, conc. 1)	DEP	0	4	4	3
PHA Net Response (day 1, conc. 2)	DEP	0	4	4	3
PHA Net Response (day 1, conc. 3)	DEP	0	4	4	2
PHA Net Response (day 2, all conc.)	DEP	5	6	6	2
Overall PHA Net Response	DEP	5	10	10	4

TABLE 16-2. (Continued)

Number of Participants Excluded and With Missing Data for the Immunologic Assessment

Variable	Variable Use	Assumption (Ranch Hands Only)		Categorized Current Dioxin	
		Minimal	Maximal	Ranch Hand	Comparison
Maximum PHA Net Response	DEP	5	10	10	4
Unstimulated MLC Response	DEP	4	6	7	7
MLC Net Response	DEP	4	6	7	7
NKCA 50/1 Net Response	DEP	6	7	5	11
NKCA 50/1 Percent Release	DEP	6	7	5	11
NKCI 50/1 Net Response	DEP	2	5	5	3
NKCI 50/1 Percent Release	DEP	2	5	5	3
Current Alcohol Use	COV	1	1	1	0
Lifetime Alcohol History	COV	1	1	1	1
Chemotherapy	EXC	0	0	0	1
X-Ray Treatment	EXC	1	1	0	0
Anti-Inflammatory or Immunosuppressant Medication	EXC	6	8	9	9

[a]Scheduled for 702 Ranch Hands and 664 Comparisons who had a quantified serum dioxin assay.
[b]Includes 31 participants who refused and five equivocal results.
[c]Performed on 866 Ranch Hands and 804 Comparisons who had a quantified serum dioxin assay.
[d]Performed on 324 Ranch Hands and 306 Comparisons who had a quantified serum dioxin assay.
COV--Covariate (missing data).
DEP--Dependent variable (missing data).
EXC--Exclusion.

covariate interactions determined by various statistical models are also presented in Appendix O. Chapter 4 provides a guide to assist in interpreting the graphics.

Three statistical analysis approaches were used to examine the association between an immunology dependent variable and serum dioxin levels. One model related a dependent variable to each Ranch Hand's initial dioxin value (extrapolated from current dioxin values using a first-order pharmacokinetic model). A second model related a dependent variable to each Ranch Hand's current serum dioxin value and each Ranch Hand's time since tour. The phrase "time since tour" is often referred to as "time" in discussions of these results. Both of these models were implemented under the minimal and maximal assumptions (i.e., Ranch Hands with current dioxin above 10 ppt and above 5 ppt, respectively). The third model compared the dependent variable for Ranch Hands having current dioxin values categorized as unknown, low, and high with Comparisons having background levels. The contrast of the entire Ranch Hand group with the complete Comparison group can be found in the previous report of analyses of the 1987 examination (5). All three models were implemented with and without covariate adjustment. Chapter 4 provides a more detailed discussion of the models.

RESULTS

Exposure Analysis

Physical Examination Variable

Skin Reaction Test

Model 1: Ranch Hands - Log_2 (Initial Dioxin)

Under both the minimal and maximal assumptions, the unadjusted analysis of the composite skin reaction test displayed a nonsignificant negative association with initial dioxin (Table 16-3 [a] and [b]: p=0.519 and p=0.207, respectively).

Similarly, the adjusted analysis of the composite skin reaction test was not significant for an association with initial dioxin under either the minimal or the maximal assumption (Table 16-3 [c] and [d]: p=0.201 and p=0.207, respectively).

Model 2: Ranch Hands - Log_2 (Current Dioxin) and Time

Under both the minimal and maximal assumptions, the interaction between current dioxin and time since tour was not significant (Table 16-3 [e] and [f]: p=0.474 and p=0.418, respectively); hence, the relative risks were not significantly different between the two time strata. The relative risks for each time stratum were not significant.

Under the minimal assumption, the adjusted analysis contained a significant interaction among current dioxin, time, and age (Table 16-3 [g]: p=0.013). To investigate the interaction, adjusted analyses were performed separately for Ranch Hands born in or after 1942 and those born before 1942. For the younger Ranch Hands, the interaction of current dioxin and time was not significant (Appendix Table O-1: p=0.198). For the older Ranch Hands, the current dioxin-by-time interaction was significant (p=0.024). For older Ranch

TABLE 16-3.

Analysis of Composite Skin Test Diagnosis

Ranch Hands - Log_2 (Initial Dioxin) - Unadjusted

Assumption	Initial Dioxin	n	Percent Possibly Abnormal	Est. Relative Risk (95% C.I.)[a]	p-Value
a) Minimal (n=397)	Low	100	7.0	0.89 (0.63,1.27)	0.519
	Medium	203	7.4		
	High	94	4.3		
b) Maximal (n=570)	Low	139	9.4	0.85 (0.66,1.10)	0.207
	Medium	293	7.2		
	High	138	5.1		

Ranch Hands - Log_2 (Initial Dioxin) - Adjusted

Assumption	Adj. Relative Risk (95% C.I.)[a]	p-Value	Covariate Remarks
c) Minimal (n=394)	0.78 (0.53,1.15)	0.201	CSMOK*ALC (p=0.008)
d) Maximal (n=570)	0.85 (0.66,1.10)	0.207	- -

[a]Relative risk for a twofold increase in dioxin.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.
Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-3. (Continued)

Analysis of Composite Skin Test Diagnosis

Ranch Hands - Log_2 (Current Dioxin) and Time - Unadjusted

Assumption	Time (Yrs.)	Percent Possibly Abnormal/(n) Current Dioxin Low	Medium	High	Est. Relative Risk (95% C.I.)[a]	p-Value
e) Minimal						0.474[b]
(n=397)	≤18.6	7.0 (57)	9.0 (100)	2.5 (40)	0.78 (0.44,1.39)	0.393[c]
	>18.6	4.9 (41)	6.7 (104)	5.5 (55)	1.02 (0.64,1.61)	0.946[c]
f) Maximal						0.418[b]
(n=570)	≤18.6	10.0 (80)	7.8 (142)	6.1 (66)	0.77 (0.52,1.15)	0.202[c]
	>18.6	7.3 (69)	6.5 (138)	5.3 (75)	0.96 (0.68,1.35)	0.814[c]

Ranch Hands - Log_2 (Current Dioxin) and Time - Adjusted

Assumption	Time (Yrs.)	Adj. Relative Risk (95% C.I.)[a]	p-Value	Covariate Remarks
g) Minimal			0.341**[b]	CURR*TIME*AGE (p=0.013)
(n=394)	≤18.6	0.64 (0.35,1.16)**	0.143**[c]	ALC*CSMOK (p=0.004)
	>18.6	0.92 (0.55,1.54)**	0.762**[c]	
h) Maximal			0.418[b]	- -
(n=570)	≤18.6	0.77 (0.52,1.15)	0.202[c]	
	>18.6	0.96 (0.68,1.35)	0.814[c]	

[a]Relative risk for a twofold increase in dioxin.

[b]Test of significance for homogeneity of relative risks (current dioxin continuous, time categorized).

[c]Test of significance for relative risk equal to 1 (current dioxin continuous, time categorized).

**Log_2 (current dioxin)-by-time-by-covariate interaction ($0.01<p\leq0.05$); adjusted relative risk, confidence interval, and p-value derived from a model fitted after deletion of this interaction.

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.
Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.
CURR: Log_2 (current dioxin).
TIME: Time since tour.

TABLE 16-3. (Continued)

Analysis of Composite Skin Test Diagnosis

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Percent Possibly Abnormal	Contrast	Est. Relative Risk (95% C.I.)	p-Value
Background	605	4.6	All Categories		0.331
Unknown	269	7.1	Unknown vs. Background	1.57 (0.86,2.86)	0.143
Low	153	7.8	Low vs. Background	1.75 (0.87,3.53)	0.116
High	141	5.7	High vs. Background	1.24 (0.55,2.78)	0.603
Total	1,168				

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Contrast	Adj. Relative Risk (95% C.I.)	p-Value	Covariate Remarks
Background	604	All Categories		0.332	AGE (p=0.008) DRKYR (p=0.054)
Unknown	266	Unknown vs. Background	1.52 (0.83,2.78)	0.176	
Low	151	Low vs. Background	1.81 (0.89,3.67)	0.099	
High	137	High vs. Background	1.33 (0.56,3.15)	0.519	
Total	1,158				

Note: Background (Comparisons): Current Dioxin ≤10 ppt.
Unknown (Ranch Hands): Current Dioxin ≤10 ppt.
Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.
High (Ranch Hands): Current Dioxin >33.3 ppt.

Hands with time since tour less than or equal to 18.6 years, there was a nonsignificant negative association with current dioxin and for those whose time was greater than 18.6 years, there was a nonsignificant positive association. Without the interaction of current dioxin, time, and age in the model, the adjusted relative risks were not significantly different between the two time strata (Table 16-3 [g]: p=0.341) and the adjusted risks within time strata also were not significant.

Under the maximal assumption, none of the covariates or associated interaction terms were retained in the adjusted analysis; therefore, the unadjusted and adjusted results are the same (as seen in Table 16-3 [f] and [h], respectively).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

In the unadjusted analysis of the relative frequency of participants with a possibly abnormal composite skin test reaction, the overall contrast of Ranch Hands classified in the unknown, low, and high current dioxin categories and Comparisons in the background current dioxin category was nonsignificant (Table 16-3 [i]: p=0.331).

In the adjusted analysis of the composite skin test reaction, the overall contrast for Ranch Hands in the unknown, low, and high current dioxin categories versus Comparisons in the background current dioxin category was also nonsignificant (Table 16-3 [j]: p=0.332). The contrast for Ranch Hands in the low current dioxin category versus the Comparisons in the background current dioxin category was marginally significant (p=0.099, Adj. RR=1.81, 95% C.I.: [0.89,3.67]).

In the 1987 examination report, the composite skin test displayed unadjusted and adjusted relative risks that were greater than 1 for the Ranch Hand versus Comparison analyses. Although the relative risks of the three Ranch Hand versus Comparison contrasts were nonsignificant, each relative risk exceeded 1. The risks, however, were not indicative of a dose-response pattern.

Laboratory Examination Data: Quantitative Studies—Cell Surface Marker (Phenotypic) Studies

CD2 Cells

Model 1: Ranch Hands - Log_2 (Initial Dioxin)

For the unadjusted analyses under both the minimal and maximal assumptions, the associations between CD2 cell counts and initial dioxin were not significant in the adjusted analysis (Table 16-4 [a] and [b]: p=0.747 and p=0.628, respectively).

Under the minimal assumption, the adjusted analysis contained a significant interaction between initial dioxin and current alcohol use (Table 16-4 [c]: p=0.003). Stratifying by current alcohol use (zero to one drink per day, over one drink per day), there was a significant negative association between CD2 cell counts and initial dioxin for Ranch Hands who had more than one drink per day (Appendix Table O-1: p=0.002). For the other current drinking stratum, there was a nonsignificant positive association (p=0.442). Under the maximal

TABLE 16-4.

Analysis of CD2 Cells (cells/mm^3)

Ranch Hands - Log_2 (Initial Dioxin) - Unadjusted

Assumption	Initial Dioxin	n	Mean[a]	Slope (Std. Error)[b]	p-Value
a) Minimal	Low	45	1,699.1	-0.007 (0.021)	0.747
(n=195)	Medium	97	1,662.3		
(R^2<0.001)	High	53	1,614.6		
b) Maximal	Low	65	1,645.5	0.008 (0.016)	0.628
(n=273)	Medium	136	1,628.2		
(R^2<0.001)	High	72	1,647.1		

Ranch Hands - Log_2 (Initial Dioxin) - Adjusted

Assumption	Initial Dioxin	n	Adj. Mean[a]	Adj. Slope (Std. Error)[b]	p-Value	Covariate Remarks
c) Minimal	Low	45	****	****	****	INIT*ALC (p=0.003)
(n=194)	Medium	96	****			AGE (p=0.091)
(R^2=0.125)	High	53	****			CSMOK (p=0.008)
						DRKYR (p=0.036)
d) Maximal	Low	65	1,665.5	0.002 (0.016)	0.874	CSMOK (p<0.001)
(n=272)	Medium	135	1,626.4			AGE*DRKYR
(R^2=0.110)	High	72	1,621.6			(p<0.001)

[a]Transformed from natural logarithm scale.

[b]Slope and standard error based on natural logarithm CD2 cells versus log_2 dioxin.

****Log_2 (initial dioxin)-by-covariate interaction ($p \leq 0.01$); adjusted mean, adjusted slope, standard error, and p-value not presented.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.
Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.
INIT: Log_2 (initial dioxin).

TABLE 16-4. (Continued)

Analysis of CD2 Cells (cells/mm^3)

Ranch Hands - Log$_2$ (Current Dioxin) and Time - Unadjusted

Assumption	Time (Yrs.)	Mean[a]/(n) Current Dioxin Low	Medium	High	Slope (Std. Error)[b]	p-Value
e) Minimal						0.739[c]
(n=195)	≤18.6	1,640.5 (22)	1,602.2 (49)	1,626.4 (22)	-0.019 (0.033)	0.563[d]
(R^2=0.007)	>18.6	1,737.4 (25)	1,697.2 (47)	1,659.0 (30)	-0.005 (0.028)	0.870[d]
f) Maximal						0.880[c]
(n=273)	≤18.6	1,581.1 (39)	1,637.0 (70)	1,556.5 (30)	0.001 (0.024)	0.961[d]
(R^2=0.004)	>18.6	1,630.8 (24)	1,659.9 (67)	1,718.0 (43)	0.006 (0.023)	0.786[d]

Ranch Hands - Log$_2$ (Current Dioxin) and Time - Adjusted

Assumption	Time (Yrs.)	Adj. Mean[a]/(n) Current Dioxin Low	Medium	High	Adj. Slope (Std. Error)[b]	p-Value	Covariate Remarks
g) Minimal						0.448[c]	AGE (p=0.103)
(n=194)	≤18.6	1,679.4 (22)	1,598.4 (49)	1,588.9 (22)	-0.036 (0.033)	0.289[d]	CSMOK (p=0.011)
(R^2=0.090)							DRKYR (p=0.058)
	>18.6	1,742.8 (25)	1,665.6 (46)	1,684.1 (30)	-0.003 (0.029)	0.907[d]	
h) Maximal						0.717[c]	CSMOK (p<0.001)
(n=272)	≤18.6	1,718.6 (39)	1,757.6 (70)	1,654.1 (30)	-0.007 (0.024)	0.773[d]	AGE*DRKYR (p<0.001)
(R^2=0.126)	>18.6	1,828.9 (24)	1,766.0 (66)	1,822.6 (43)	0.005 (0.022)	0.835[d]	RACE*ALC (p=0.050)

[a]Transformed from natural logarithm scale.

[b]Slope and standard error based on natural logarithm CD2 cells versus log$_2$ dioxin.

[c]Test of significance for current dioxin-by-time interaction (current dioxin continuous and time categorized).

[d]Test of significance for slope different from 0 (current dioxin continuous, time categorized).

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.
Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-4. (Continued)

Analysis of CD2 Cells (cells/mm^3)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean[a]	Contrast	Difference of Means (95% C.I.)[e]	p-Value[f]
Background	307	1,615.9	All Categories		0.712
Unknown	130	1,568.6	Unknown vs. Background	-47.3 --	0.405
Low	74	1,636.2	Low vs. Background	20.3 --	0.777
High	76	1,651.5	High vs. Background	35.6 --	0.618
Total	587		(R^2=0.002)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean[a]	Contrast	Difference of Adj. Means (95% C.I.)[e]	p-Value[f]	Covariate Remarks
Background	306	1,687.0**	All Categories		0.825**	DXCAT*AGE (p=0.015)
Unknown	130	1,645.5**	Unknown vs. Background	-41.5 --**	0.470**	DXCAT*DRKYR
Low	73	1,709.3**	Low vs. Background	22.3 --**	0.759**	(p=0.014)
High	76	1,704.9**	High vs. Background	17.9 --**	0.805**	RACE (p=0.112)
Total	585		(R^2=0.106)			CSMOK (p<0.001)

[a]Transformed from natural logarithm scale.

[e]Difference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.

[f]P-value is based on difference of means on natural logarithm scale.

**Categorized current dioxin-by-covariate interaction ($0.01<p\leq0.05$); adjusted mean and p-value derived from a model fitted after deletion of this interaction.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.
Unknown (Ranch Hands): Current Dioxin ≤10 ppt.
Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.
High (Ranch Hands): Current Dioxin >33.3 ppt.
DXCAT: Categorized current dioxin.

assumption, the association between CD2 cells and initial dioxin was not significant (Table 16-4 [d]: p=0.874).

Model 2: Ranch Hands - Log_2 (Current Dioxin) and Time

In the unadjusted analysis relating CD2 cells to current dioxin and time since tour, the models under both the minimal and maximal assumptions did not contain significant current dioxin-by-time interactions (Table 16-4 [e] and [f]: p=0.739 and p=0.880, respectively), indicating that the relationships between CD2 and current dioxin did not differ between time strata. In the adjusted analysis, the models based on the minimal and maximal assumptions also contained nonsignificant current dioxin-by-time interactions (Table 16-4 [g] and [h]: p=0.448 and p=0.717, respectively).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

In the unadjusted analysis of CD2 cell counts, the mean levels of the four current dioxin categories did not differ significantly (Table 16-4 [i]: p=0.712).

The adjusted analysis of the CD2 cell counts displayed a significant interaction between categorized current dioxin and age and a significant interaction between categorized current dioxin and lifetime alcohol history (Table 16-4 [j]: p=0.015 and p=0.014, respectively). To investigate the interactions, age was dichotomized for Ranch Hands and Comparisons born in or after 1942 and those born prior to 1942 and lifetime alcohol history was trichotomized as 0 drink-years, greater than 0 but less than 40 drink-years, and over 40 drink-years. For participants born in or after 1942 with a lifetime alcohol history of zero drink-years, the high versus background contrast was of borderline significance (Appendix Table O-1: p=0.082) with the Comparisons having a higher adjusted CD2 mean than the Ranch Hands. However, the contrast was based on eight Comparisons and three Ranch Hands. For participants born in or after 1942 with a lifetime alcohol history of greater than 0 but less than 40 drink-years, the unknown versus background contrast was significant (p=0.032), with the Comparisons having the higher adjusted CD2 mean. All other contrasts were nonsignificant. A followup model was examined without the two interactions cited above. For that model, the overall contrast was nonsignificant (Table 16-4 [j]: p=0.825).

CD4 Cells

Model 1: Ranch Hands - Log_2 (Initial Dioxin)

Under both the minimal and the maximal assumptions, the unadjusted analyses of the CD4 cell counts were not significant for an association with initial dioxin (Table 16-5 [a] and [b]: p=0.809 and p=0.157, respectively). For the adjusted analyses, the minimal and maximal assumptions also exhibited nonsignificant associations between the CD4 cell counts and initial dioxin (Table 16-5 [c] and [d]: p=0.936 and p=0.324, respectively).

Model 2: Ranch Hands - Log_2 (Current Dioxin) and Time

For the unadjusted analyses of CD4, under both the minimal and maximal assumptions, the interaction of current dioxin and time since tour was not significant (Table 16-5 [e] and [f]: p=0.510 and p=0.453, respectively). Therefore, the associations (i.e., slopes) did not differ significantly between the two time strata.

TABLE 16-5.

Analysis of CD4 Cells (cells/mm^3)

Ranch Hands - Log_2 (Initial Dioxin) - Unadjusted

Assumption	Initial Dioxin	n	Mean[a]	Slope (Std. Error)[b]	p-Value
a) Minimal	Low	45	940.4	0.006 (0.024)	0.809
(n=194)	Medium	96	966.3		
(R^2<0.001)	High	53	920.2		
b) Maximal	Low	65	894.5	0.027 (0.019)	0.157
(n=272)	Medium	136	924.5		
(R^2=0.007)	High	71	941.0		

Ranch Hands - Log_2 (Initial Dioxin) - Adjusted

Assumption	Initial Dioxin	n	Adj. Mean[a]	Adj. Slope (Std. Error)[b]	p-Value	Covariate Remarks
c) Minimal	Low	45	960.6	0.002 (0.024)	0.936	AGE (p=0.097)
(n=193)	Medium	95	952.7			CSMOK (p<0.001)
(R^2=0.126)	High	53	920.6			DRKYR (p=0.063)
d) Maximal	Low	65	921.1	0.018 (0.019)	0.324	AGE*CSMOK (p=0.005)
(n=271)	Medium	135	917.7			AGE*PACKYR (p=0.042)
(R^2=0.155)	High	71	923.6			AGE*DRKYR (p<0.001)
						CSMOK*PACKYR (p=0.039)

[a]Transformed from natural logarithm scale.
[b]Slope and standard error based on natural logarithm CD4 cells versus log_2 dioxin.
Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.
Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-5. (Continued)

Analysis of CD4 Cells (cells/mm^3)

Ranch Hands - Log$_2$ (Current Dioxin) and Time - Unadjusted

Assumption	Time (Yrs.)	Mean[a]/(n) Current Dioxin Low	Medium	High	Slope (Std. Error)[b]	p-Value
e) Minimal						0.510[c]
(n=194)	≤18.6	943.6	917.8	902.2	-0.017 (0.038)	0.657[d]
(R^2=0.008)		(22)	(48)	(22)		
	>18.6	941.2	999.4	959.0	0.016 (0.032)	0.621[d]
		(25)	(47)	(30)		
f) Maximal						0.453[c]
(n=272)	≤18.6	880.9	939.2	865.2	0.008 (0.029)	0.772[d]
(R^2=0.009)		(39)	(70)	(29)		
	>18.6	840.3	935.9	997.9	0.038 (0.027)	0.161[d]
		(24)	(67)	(43)		

Ranch Hands - Log$_2$ (Current Dioxin) and Time - Adjusted

Assumption	Time (Yrs.)	Adj. Mean[a]/(n) Current Dioxin Low	Medium	High	Adj. Slope (Std. Error)[b]	p-Value	Covariate Remarks
g) Minimal						0.213**[c]	CURR*TIME*DRKYR
(n=193)	≤18.6	886.9**	822.8**	781.9**	-0.042 (0.037)**	0.259**[d]	(p=0.038)
(R^2=0.170)		(22)	(48)	(22)			AGE (p=0.053)
	>18.6	864.0**	887.2**	894.1**	0.017 (0.031)**	0.598**[d]	RACE (p=0.135)
		(25)	(46)	(30)			CSMOK (p<0.001)
h) Maximal						0.243**[c]	CURR*TIME*AGE
(n=271)	≤18.6	922.0**	929.8**	845.6**	-0.008 (0.028)**	0.778**[d]	(p=0.024)
(R^2=0.182)		(39)	(70)	(29)			AGE*CSMOK (p=0.003)
	>18.6	876.3**	925.9**	972.3**	0.036 (0.026)**	0.174**[d]	AGE*PACKYR (p=0.038)
		(24)	(66)	(43)			AGE*DRKYR (p<0.001)
							CSMOK*PACKYR (p=0.030)

[a]Transformed from natural logarithm scale.

[b]Slope and standard error based on natural logarithm CD4 cells versus log$_2$ dioxin.

[c]Test of significance for current dioxin-by-time interaction (current dioxin continuous, time categorized).

[d]Test of significance for slope different from 0 (current dioxin continuous, time categorized).

**Log$_2$ (current dioxin)-by-time-by-covariate interaction (0.01<p≤0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deletion of this interaction.

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.
Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-5. (Continued)

Analysis of CD4 Cells (cells/mm^3)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean[a]	Contrast	Difference of Means (95% C.I.)[e]	p-Value[f]
Background	301	907.8	All Categories		0.351
Unknown	127	861.5	Unknown vs. Background	-46.3 --	0.216
Low	72	938.6	Low vs. Background	30.8 --	0.525
High	72	942.2	High vs. Background	34.4 --	0.478
Total	572	(R^2=0.006)			

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean[a]	Contrast	Difference of Adj. Means (95% C.I.)[e]	p-Value[f]	Covariate Remarks
Background	301	907.1	All Categories		0.406	AGE (p=0.018) CSMOK (p<0.001)
Unknown	127	866.5	Unknown vs. Background	-40.6 --	0.259	
Low	72	945.4	Low vs. Background	38.3 --	0.409	
High	72	929.0	High vs. Background	21.9 --	0.637	
Total	572	(R^2=0.095)				

[a]Transformed from natural logarithm scale.

[e]Difference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.

[f]P-value is based on difference of means on natural logarithm scale.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.
Unknown (Ranch Hands): Current Dioxin ≤10 ppt.
Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.
High (Ranch Hands): Current Dioxin >33.3 ppt.

For the adjusted analyses of CD4 cell counts under the minimal assumption, there was a significant interaction among current dioxin, time, and lifetime alcohol history (Table 16-5 [g]: p=0.038). To examine the interaction, Ranch Hands with lifetime alcohol history values were dichotomized into less than or equal to 40 drink-years or greater than 40 drink-years. For the former lifetime alcohol history stratum, the interaction between current dioxin and time was significant (Appendix Table O-1: p=0.013); there was a significant negative association (p=0.035) with current dioxin for time less than or equal to 18.6 years, and a nonsignificant positive association with current dioxin for time over 18.6 years (p=0.200). For the latter lifetime alcohol history stratum, the interaction of current dioxin and time was marginally significant (Appendix Table O-1: p=0.054) with a nonsignificant positive association between CD4 cells and current dioxin for time of 18.6 years or less and a nonsignificant negative association for time over 18.6 years (p=0.191 and p=0.163, respectively). Without the interaction of current dioxin, time, and lifetime alcohol history in the adjusted model, the interaction between current dioxin and time was not significant (Table 16-5 [g]: p=0.213).

Under the maximal assumption, the adjusted analysis contained a significant interaction among current dioxin, time, and age (Table 16-5 [h]: p=0.024). The interaction was explored for Ranch Hands born in or after 1942 and those born prior to 1942. For the older Ranch Hands, the association between CD4 and current dioxin differed significantly between the time strata (Appendix Table O-1: p=0.043); for time of 18.6 years or less there was a nonsignificant negative association (p=0.114), and for time greater than 18.6 years there was a nonsignificant positive association (p=0.207). For the younger Ranch Hands, the interaction of current dioxin and time was nonsignificant for CD4 (p=0.753). An adjusted model without the interaction of current dioxin, time, and age displayed a nonsignificant current dioxin-by-time interaction (Table 16-5 [h]: p=0.243).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

For the unadjusted and the adjusted analysis of CD4 cell counts, the overall contrasts of the four current dioxin categories were not significant (Table 16-5 [i] and [j]: p=0.351 and p=0.406, respectively) and none of the Ranch Hand versus Comparison contrasts were significant (p>0.20 for all).

CD8 Cells

Model 1: Ranch Hands - Log_2 (Initial Dioxin)

For both the minimal and the maximal assumptions, the association between the CD8 cell counts and initial dioxin was not significant in the unadjusted analysis (Table 16-6 [a] and [b]: p=0.934 and p=0.705, respectively).

Under the minimal assumption, the adjusted analysis of the CD8 cell counts contained a significant interaction between initial dioxin and lifetime alcohol history (Table 16-6 [c]: p<0.001). The interaction was investigated by trichotomizing the Ranch Hands into the following lifetime alcohol history strata: 0 drink-years, above 0 drink-years to 40 drink-years, and over 40 drink-years. For Ranch Hands with a lifetime history over 40 drink-years, there was a significant negative association between CD8 cell counts and initial dioxin (Appendix Table O-1: p=0.016). For the nondrinkers, there was a nonsignificant positive

TABLE 16-6.

Analysis of CD8 Cells (cells/mm^3)

Ranch Hands - Log$_2$ (Initial Dioxin) - Unadjusted

Assumption	Initial Dioxin	n	Mean[a]	Slope (Std. Error)[b]	p-Value
a) Minimal	Low	45	494.6	-0.002 (0.029)	0.934
(n=194)	Medium	96	468.9		
(R^2<0.001)	High	53	483.4		
b) Maximal	Low	64	505.7	-0.008 (0.021)	0.705
(n=271)	Medium	135	475.2		
(R^2=0.001)	High	72	486.7		

Ranch Hands - Log$_2$ (Initial Dioxin) - Adjusted

Assumption	Initial Dioxin	n	Adj. Mean[a]	Adj. Slope (Std. Error)[b]	p-Value	Covariate Remarks
c) Minimal	Low	45	****	****	****	INIT*DRKYR (p<0.001)
(n=193)	Medium	95	****			PACKYR (p=0.086)
(R^2=0.142)	High	53	****			AGE*RACE (p=0.047)
						AGE*DRKYR (p=0.001)
						CSMOK*DRKYR (p=0.014)
d) Maximal	Low	64	511.9**	-0.014 (0.022)**	0.518**	INIT*ALC (p=0.041)
(n=270)	Medium	134	473.9**			CSMOK (p=0.011)
(R^2=0.076)	High	72	479.5**			AGE*DRKYR (p=0.003)

[a]Transformed from natural logarithm scale.

[b]Slope and standard error based on natural logarithm CD8 cells versus log$_2$ dioxin.

**Log$_2$ (initial dioxin)-by-covariate interaction (0.01<p≤0.05); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deleting this interaction.

****Log$_2$ (initial dioxin)-by-covariate interaction (p≤0.01); adjusted mean, adjusted slope, standard error, and p-value not presented.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.
Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-6. (Continued)

Analysis of CD8 Cells (cells/mm^3)

Ranch Hands - Log$_2$ (Current Dioxin) and Time - Unadjusted

Assumption	Time (Yrs.)	Mean[a]/(n) Current Dioxin Low	Medium	High	Slope (Std. Error)[b]	p-Value
e) Minimal						0.982[c]
(n=194)	≤18.6	456.7	444.6	518.6	-0.009 (0.045)	0.840[d]
(R^2=0.005)		(22)	(49)	(22)		
	>18.6	529.7	479.2	485.4	-0.010 (0.039)	0.788[d]
		(24)	(47)	(30)		
f) Maximal						0.472[c]
(n=271)	≤18.6	466.7	465.2	464.9	-0.003 (0.032)	0.931[d]
(R^2=0.013)		(39)	(70)	(30)		
	>18.6	590.8	489.1	493.6	-0.035 (0.030)	0.257[d]
		(23)	(66)	(43)		

Ranch Hands - Log$_2$ (Current Dioxin) and Time - Adjusted

Assumption	Time (Yrs.)	Adj. Mean[a]/(n) Current Dioxin Low	Medium	High	Adj. Slope (Std. Error)[b]	p-Value	Covariate Remarks
g) Minimal						0.721[c]	AGE (p=0.047)
(n=193)	≤18.6	472.1	443.1	511.3	-0.029 (0.046)	0.531[d]	PACKYR (p=0.045)
(R^2=0.059)		(22)	(49)	(22)			DRKYR (p=0.107)
	>18.6	523.5	470.2	489.6	-0.008 (0.040)	0.842[d]	
		(24)	(46)	(30)			
h) Maximal						0.513[c]	CSMOK (p=0.013)
(n=270)	≤18.6	476.9	462.8	461.2	-0.013 (0.032)	0.686[d]	AGE*DRKYR
(R^2=0.073)		(39)	(70)	(30)			(p=0.004)
	>18.6	620.1	483.0	480.4	-0.041 (0.030)	0.174[d]	
		(23)	(65)	(43)			

[a]Transformed from natural logarithm scale.

[b]Slope and standard error based on natural logarithm CD8 cells versus log$_2$ dioxin.

[c]Test of significance for current dioxin-by-time interaction (current dioxin continuous, time categorized).

[d]Test of significance for slope different from 0 (current dioxin continuous, time categorized).

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.
Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-6. (Continued)

Analysis of CD8 Cells (cells/mm^3)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean[a]	Contrast	Difference of Means (95% C.I.)[e]	p-Value[f]
Background	301	471.8	All Categories		0.937
Unknown	126	485.2	Unknown vs. Background	13.4 --	0.581
Low	72	469.2	Low vs. Background	-2.6 --	0.930
High	73	481.6	High vs. Background	9.8 --	0.741
Total	572		(R^2<0.001)		

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean[a]	Contrast	Difference of Adj. Means (95% C.I.)[e]	p-Value[f]	Covariate Remarks
Background	301	473.1	All Categories		0.937	AGE (p=0.089) CSMOK (p<0.001)
Unknown	126	485.2	Unknown vs. Background	12.1 --	0.614	ALC (p=0.144)
Low	71	465.3	Low vs. Background	-7.8 --	0.790	
High	73	475.5	High vs. Background	2.4 --	0.934	
Total	571		(R^2=0.037)			

[a]Transformed from natural logarithm scale.

[e]Difference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.

[f]p-value is based on difference of means on natural logarithm scale.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.
Unknown (Ranch Hands): Current Dioxin ≤10 ppt.
Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.
High (Ranch Hands): Current Dioxin >33.3 ppt.

association (p=0.760) and for the 0 drink-years to 40 drink-years stratum there was a nonsignificant negative association (p=0.894).

Under the maximal assumption, the adjusted analysis of the CD8 cell counts contained a significant interaction between initial dioxin and current alcohol use (Table 16-6 [d]: p=0.041). After stratifying the Ranch Hands into two current alcohol use strata (zero to one drink per day, over one drink per day), a significant negative association was found between CD8 cell counts and initial dioxin for Ranch Hands who had more than one drink per day (Appendix Table O-1: p=0.033). The other stratum exhibited a nonsignificant positive association (p=0.844). Deleting the initial dioxin-by-current alcohol use interaction from the model resulted in a nonsignificant association (Table 16-6 [d]: p=0.518) between CD8 and initial dioxin.

Model 2: Ranch Hands - Log_2 (Current Dioxin) and Time

For both the minimal and maximal assumptions, the unadjusted analysis of CD8 cell counts indicated the associations with current dioxin did not differ significantly between time since tour strata (Table 16-6 [e] and [f]: p=0.982 and p=0.472, respectively).

Under the minimal and maximal assumptions, the adjusted slopes for the association between CD8 cells and current dioxin did not differ significantly between time strata (Table 16-6 [g] and [h]: p=0.721 and p=0.513, respectively).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

For the unadjusted and adjusted analyses of CD8 cell counts, the overall contrast of the four current dioxin categories was not significant (Table 16-6 [i] and [j]: p=0.937 for each).

CD20 Cells

Model 1: Ranch Hands - Log_2 (Initial Dioxin)

For the unadjusted analyses under both the minimal and maximal assumptions, the association between CD20 cell counts and initial dioxin was not significant (Table 16-7 [a] and [b]: p=0.102 and p=0.212, respectively).

In the adjusted analysis under the minimal assumption, there was a significant interaction between initial dioxin and age (Table 16-7 [c]: p=0.013). Current cigarette smoking, current alcohol use, and lifetime alcohol history were covariates retained in the adjusted model. To investigate the interaction, the results were examined separately for Ranch Hands born in or after 1942 and those Ranch Hands born prior to 1942. For the older Ranch Hands, there was a significant positive association between CD20 cell counts and initial dioxin (Appendix Table O-1: p=0.002). For the younger Ranch Hands, there was a nonsignificant negative association (p=0.566). Without the interaction of initial dioxin and age in the model, there was a positive association between the CD20 cell counts and initial dioxin that was marginally significant (Table 16-7 [c]: p=0.086).

In the adjusted analysis of the maximal cohort, the association between CD20 and initial dioxin was not significant (Table 16-7 [d]: p=0.363).

TABLE 16-7.

Analysis of CD20 Cells (cells/mm^3)

Ranch Hands - Log$_2$ (Initial Dioxin) - Unadjusted

Assumption	Initial Dioxin	n	Mean[a]	Slope (Std. Error)[b]	p-Value
a) Minimal	Low	45	127.7	0.074 (0.045)	0.102
(n=195)	Medium	97	166.6		
(R^2=0.389)	High	53	169.6		
b) Maximal	Low	65	157.9	0.036 (0.029)	0.212
(n=273)	Medium	136	144.7		
(R^2=0.341)	High	72	164.6		

Ranch Hands - Log$_2$ (Initial Dioxin) - Adjusted

Assumption	Initial Dioxin	n	Adj. Mean[a]	Adj. Slope (Std. Error)[b]	p-Value	Covariate Remarks
c) Minimal	Low	45	142.8**	0.075 (0.043)**	0.086**	INIT*AGE (p=0.013)
(n=194)	Medium	96	155.0**			CSMOK (p=0.064)
(R^2=0.516)	High	53	167.3**			ALC (p=0.055)
						DRKYR (p=0.004)
d) Maximal	Low	65	196.1	0.025 (0.027)	0.363	RACE (p=0.033)
(n=272)	Medium	135	174.3			CSMOK (p=0.001)
(R^2=0.465)	High	72	193.7			ALC (p=0.072)
						AGE*DRKYR (p=0.002)

[a]Transformed from natural logarithm scale.

[b]Slope and standard error based on natural logarithm CD20 cells versus log$_2$ dioxin.

**Log$_2$ (initial dioxin)-by-covariate interaction ($0.01<p\leq0.05$); adjusted mean, adjusted slope, standard error, and p-value derived from a model fitted after deletion of this interaction.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.
Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.

TABLE 16-7. (Continued)

Analysis of CD20 Cells (cells/mm^3)

Ranch Hands - Log$_2$ (Current Dioxin) and Time - Unadjusted

Assumption	Time (Yrs.)	Mean[a]/(n) Current Dioxin Low	Medium	High	Slope (Std. Error)[b]	p-Value
e) Minimal						0.825[c]
(n=195)	≤18.6	122.8 (22)	161.0 (49)	168.3 (22)	0.052 (0.066)	0.436[d]
(R^2=0.388)	>18.6	140.4 (25)	168.7 (47)	175.5 (30)	0.071 (0.063)	0.262[d]
f) Maximal						0.302[c]
(n=273)	≤18.6	162.9 (39)	151.6 (70)	152.3 (30)	0.007 (0.043)	0.877[d]
(R^2=0.345)	>18.6	140.3 (24)	142.5 (67)	170.6 (43)	0.069 (0.042)	0.099[d]

Ranch Hands - Log$_2$ (Current Dioxin) and Time - Adjusted

Assumption	Time (Yrs.)	Adj. Mean[a]/(n) Current Dioxin Low	Medium	High	Adj. Slope (Std. Error)[b]	p-Value	Covariate Remarks
g) Minimal						0.371[c]	CSMOK (p=0.023)
(n=194)	≤18.6	128.2 (22)	165.2 (49)	173.9 (22)	0.047 (0.061)	0.446[d]	ALC (p=0.041)
(R^2=0.485)							DRKYR (p=0.004)
	>18.6	132.7 (25)	155.9 (46)	192.2 (30)	0.121 (0.059)	0.043[d]	
h) Maximal						0.171[c]	RACE (p=0.032)
(n=272)	≤18.6	203.4 (39)	186.5 (70)	186.7 (30)	-0.008 (0.040)	0.845[d]	CSMOK (p=0.001)
(R^2=0.473)							ALC (p=0.051)
	>18.6	187.5 (24)	164.4 (66)	202.2 (43)	0.068 (0.039)	0.083[d]	AGE*DRKYR (p=0.003)

[a]Transformed from natural logarithm scale.

[b]Slope and standard error based on natural logarithm CD20 cells versus log$_2$ dioxin.

[c]Test of significance for current dioxin-by-time interaction (current dioxin continuous and time categorized).

[d]Test of significance for slope different from 0 (current dioxin continuous, time categorized).

Note: Minimal--Low: >10-14.65 ppt; Medium: >14.65-45.75 ppt; High: >45.75 ppt.
Maximal--Low: >5-9.01 ppt; Medium: >9.01-33.3 ppt; High: >33.3 ppt.

TABLE 16-7. (Continued)

Analysis of CD20 Cells (cells/mm^3)

i) Ranch Hands and Comparisons by Current Dioxin Category - Unadjusted

Current Dioxin Category	n	Mean[a]	Contrast	Difference of Means (95% C.I.)[e]	p-Value[f]
Background	301	148.9	All Categories		0.269
Unknown	127	154.3	Unknown vs. Background	5.4 --	0.544
Low	72	161.7	Low vs. Background	12.8 --	0.260
High	73	171.1	High vs. Background	22.2 --	0.066
Total	573	(R^2=0.213)			

j) Ranch Hands and Comparisons by Current Dioxin Category - Adjusted

Current Dioxin Category	n	Adj. Mean[a]	Contrast	Difference of Adj. Means (95% C.I.)[e]	p-Value[f]	Covariate Remarks
Background	301	172.4**	All Categories		0.485**	DXCAT*AGE (p=0.014) RACE (p=0.004)
Unknown	127	176.5**	Unknown vs. Background	4.1 --**	0.670**	CSMOK (p<0.001)
Low	71	183.2**	Low vs. Background	10.8 --**	0.377**	ALC (p=0.008)
High	73	190.8**	High vs. Background	18.4 --**	0.148**	
Total	572	(R^2=0.344)				

[a]Transformed from natural logarithm scale.

[e]Difference of means after transformation to original scale; confidence interval on difference of means not given because analysis was performed on natural logarithm scale.

[f]p-value is based on difference of means on natural logarithm scale.

**Categorized current dioxin-by-covariate interaction ($0.01<p\leq0.05$); adjusted mean, and p-value derived from a model fitted after deletion of this interaction.

Note: Background (Comparisons): Current Dioxin ≤10 ppt.
Unknown (Ranch Hands): Current Dioxin ≤10 ppt.
Low (Ranch Hands): 15 ppt < Current Dioxin ≤33.3 ppt.
High (Ranch Hands): Current Dioxin >33.3 ppt.

Model 2: Ranch Hands - Log_2 (Current Dioxin) and Time

In the unadjusted analysis of the relationship between CD20 cell counts with current dioxin and time since tour, the interaction of current dioxin and time was not significant for both assumptions (Table 16-7 [e] and [f]: p=0.825 and p=0.302, respectively). Under the maximal assumption, Ranch Hands with early tours (i.e., time>18.6 years), displayed a marginally significant positive association between CD20 and current dioxin (p=0.099).

Under the minimal assumption, the adjusted analysis of the association between CD20 cells with current dioxin and time indicated that the interaction between current dioxin and time was not significant (Table 16-7 [g]: p=0.371). Current cigarette smoking, current alcohol use, and lifetime alcohol history were covariates retained in the adjusted model. For Ranch Hands with time over 18.6 years, there was a significant positive association between CD20 cells and current dioxin (p=0.043).

Under the maximal assumption, the adjusted analysis of the CD20 cell counts also indicated that the interaction between current dioxin and time was not significant (Table 16-7 [h]: p=0.171). Therefore, the adjusted slopes for the association between CD20 cells and current dioxin were not significantly different between time strata. For Ranch Hands with time over 18.6 years, there was a positive relationship between CD20 cells and current dioxin that was marginally significant (Table 16-7 [h]: p=0.083).

Model 3: Ranch Hands and Comparisons by Current Dioxin Category

The simultaneous contrast of the CD20 unadjusted means for the four current dioxin categories was nonsignificant (Table 16-7 [i]: p=0.269). The unadjusted means for the background, unknown, low, and high current dioxin categories were 148.9, 154.3, 161.7, and 171.1 cell/mm^3. The CD20 mean for Ranch Hands with high current dioxin was marginally higher than the CD20 mean of the Comparisons (p=0.066).

In the adjusted analysis of the CD20 cell counts using the four categories, there was a significant interaction between categorized current dioxin and age (Table 16-7 [j]: p=0.014). To explore the interaction, the results were examined separately for Ranch Hands and Comparisons born in or after 1942 and those born prior to 1942 (Appendix Table O-1). For the younger participants, the overall contrast of the adjusted CD20 cell means was not significant (p=0.307); however, all Ranch Hand categories had lower adjusted mean CD20 counts than Comparisons and Ranch Hands with unknown current dioxin had a marginally lower adjusted mean count (p=0.069; 170.2 versus 200.4 cells/mm^3). For the older participants, the overall contrast of the adjusted CD20 cell means for the four current dioxin categories was significant (p=0.006). The adjusted means for the background, unknown, low, and high current dioxin were 156.9, 179.9, 186.3, and 215.9 cells/mm^3. The three contrasts were at least marginally significant (unknown versus background, p=0.047; low versus background, p=0.053; high versus background, p=0.002). A followup model without the interaction of age and categorized current dioxin displayed a nonsignificant overall contrast (Table 16-7 [j]: p=0.485) and individual contrasts (p>0.10 for all).

CD14 Cells

Model 1: Ranch Hands - Log_2 (Initial Dioxin)

The unadjusted analysis of the CD14 cell counts exhibited nonsignificant associations with initial dioxin for both the minimal and the maximal assumptions (Table 16-8 [a] and [b]: p=0.842 and p=0.633, respectively).

In the adjusted analysis of the CD14 cells under the minimal assumption, the model contained significant interactions between initial dioxin and lifetime smoking history, and between initial dioxin and current alcohol use (Table 16-8 [c]: p=0.014 and p=0.008, respectively). To investigate these interactions, lifetime smoking history was dichotomized into zero pack-years and over zero pack-years, and current alcohol use was dichotomized into zero to one drink per day, and over one drink per day. For Ranch Hands who smoked and had one drink per day or less, there was a marginally significant positive association between CD14 cells and initial dioxin (Appendix Table O-1: p=0.051). For Ranch Hands who smoked and had more than one drink per day, there was a marginally significant negative association (p=0.078). For the other strata combinations of lifetime smoking and current alcohol use, there were nonsignificant negative associations between CD14 and initial dioxin (p>0.25 for both).

In the adjusted analysis under the maximal assumption, the association between CD14 and initial dioxin was nonsignificant (Table 16-8 [d]: p=0.728).

Model 2: Ranch Hands - Log_2 (Current Dioxin) and Time

In the unadjusted analysis of the relationship between CD14 cell counts with current dioxin and time since tour, the interaction of current dioxin and time was not significant for both the minimal and maximal assumptions (Table 16-8 [e] and [f]: p=0.156 and p=0.300); thus, the association between CD14 cells and current dioxin did not differ significantly between time strata.

In the adjusted analysis of the CD14 cell counts under the minimal assumption, the interaction between current dioxin and time was nonsignificant (Table 16-8 [g]: p=0.174).

In the adjusted analysis of the CD14 cell counts under the maximal assumption, there was a significant interaction among current dioxin, time, and lifetime cigarette smoking history (Table 16-8 [h]: p=0.001). Because of the interaction, the association between CD14 cell counts and current dioxin within each time strata was investigated for Ranch Hands categorized by lifetime cigarette smoking history (0 pack-years, over 0 pack-years but not over 10 pack-years, and over 10 pack-years). For nonsmoker Ranch Hands and Ranch Hands not exceeding 10 pack-years, the current dioxin-by-time interaction was nonsignificant (Appendix Table O-1: p=0.309 and p=0.841, respectively). For Ranch Hands with more than 10 pack-years for lifetime cigarette smoking history, the association between CD14 and current dioxin differed significantly between time strata (p=0.014). Within that lifetime smoking stratum, there was a significant positive association between CD14 and current dioxin for Ranch Hands with time over 18.6 years (p=0.006) and a nonsignificant negative association for the other time strata (p=0.452).

TABLE 16-8.

Analysis of CD14 Cells (cells/mm^3)

Ranch Hands - Log_2 (Initial Dioxin) - Unadjusted

Assumption	Initial Dioxin	n	Mean[a]	Slope (Std. Error)[b]	p-Value
a) Minimal	Low	45	29.7	0.011 (0.054)	0.842
(n=197)	Medium	98	31.1		
(R^2=0.651)	High	54	29.6		
b) Maximal	Low	65	31.2	-0.017 (0.036)	0.633
(n=275)	Medium	137	28.9		
(R^2=0.568)	High	73	27.6		

Ranch Hands - Log_2 (Initial Dioxin) - Adjusted

Assumption	Initial Dioxin	n	Adj. Mean[a]	Adj. Slope (Std. Error)[b]	p-Value	Covariate Remarks
c) Minimal	Low	45	****	****	****	INIT*PACKYR (p=0.014)
(n=196)	Medium	97	****			INIT*ALC (p=0.008)
(R^2=0.728)	High	54	****			RACE (p=0.032)
						CSMOK*PACKYR (p=0.015)
						CSMOK*ALC (p=0.009)
d) Maximal	Low	65	32.4	-0.012 (0.036)	0.728	DRKYR (p=0.147)
(n=274)	Medium	136	28.4			CSMOK (p=0.013)
(R^2=0.596)	High	73	28.2			

[a]Transformed from natural logarithm scale.

[b]Slope and standard error based on natural logarithm CD14 cells versus log_2 dioxin.

****Log_2 (initial dioxin)-by-covariate interaction ($p \leq 0.01$); adjusted mean, adjusted slope, standard error, and p-value not presented.

Note: Minimal--Low: 52-93 ppt; Medium: >93-292 ppt; High: >292 ppt.
Maximal--Low: 25-56.9 ppt; Medium: >56.9-218 ppt; High: >218 ppt.