TABLE 15-18. (continued)

Adjusted Exposure Index Analyses for Peripheral Vascular System Doppler Pulse Reading by Occupation

Variable	Occupation	Contrast	Adj. Relative Risk (95% C.I.)	p-Value	Significant Covariates
Peripheral Pulses	Officer	Medium vs. Low	1.26 (0.71,2.21)	0.430	NONE
		High vs. Low	1.19 (0.66,2.13)	0.562	
	Enlisted Flyer	Medium vs. Low	1.57 (0.63,3.90)	0.327	NONE
		High vs. Low	1.58 (0.63,3.98)	0.327	
	Enlisted Groundcrew	Medium vs. Low	0.90 (0.52,1.57)	0.704	NONE
		High vs. Low	1.02 (0.58,1.79)	0.960	
All Pulses	Officer	Medium vs. Low	1.26 (0.71,2.21)	0.430	NONE
		High vs. Low	1.19 (0.66,2.13)	0.562	
	Enlisted Flyer	Medium vs. Low	1.57 (0.63,3.90)	0.327	NONE
		High vs. Low	1.58 (0.63,3.98)	0.327	
	Enlisted Groundcrew	Medium vs. Low	0.90 (0.52,1.57)	0.704	NONE
		High vs. Low	1.02 (0.58,1.79)	0.960	

--Analysis not performed due to sparse cells.

LONGITUDINAL ANALYSES

Two cardiovascular variables, the index of all pulses (by palpation) and the overall ECG interpretation, were investigated to assess the longitudinal differences between the 1982 Baseline examination and the 1985 followup examination. Both variables are classified as abnormal or normal. As shown in Table 15-19, 2x2 tables were constructed for each group for each variable. These tables show the number of participants who were abnormal at Baseline and abnormal at followup, abnormal at Baseline and normal at followup, normal at Baseline and abnormal at followup, and normal at both Baseline and followup examinations. The odds ratio given is the ratio of the number of participants who were normal at the Baseline and abnormal at the followup to the number of participants who were abnormal at the Baseline and normal at the followup (the "off-diagonal" elements). The changes in normal/abnormal status within each group are contrasted between the Ranch Hand and Comparison groups, and the p-value is derived from Pearson's chi-square test of the hypothesis that the pattern of change in the two groups is the same.

TABLE 15-19.

Longitudinal Analyses of All Pulses Index and Overall ECG's: A Contrast of Baseline and First Followup Examination Abnormalities

Variable	Group	1982 Baseline Exam	1985 Followup Exam		Odds* Ratio (OR)	p-Value (OR_{RH} vs. OR_C)
			Abnormal	Normal		
All Pulses (Manual)	Ranch Hand	Abnormal	50	72	1.44	
		Normal	104	743		
	Comparison	Abnormal	40	63	2.43	0.01
		Normal	153	880		
ECG (Overall)	Ranch Hand	Abnormal	86	192	0.22	
		Normal	43	650		
	Comparison	Abnormal	112	208	0.27	0.42
		Normal	56	763		

*Odds Ratio: $\frac{\text{Number Normal Baseline, Abnormal Followup}}{\text{Number Abnormal Baseline, Normal Followup}}$

The data showed a significant difference (p=0.01) in the pulse index in the two groups between examinations. The percentage of Ranch Hands and Comparisons with abnormalities for the pulse index increased from the Baseline examination to the followup examination; however, the Comparison group showed a larger increase in the proportion of pulse index abnormalities. The greater relative increase in the Comparisons caused the significant result. No significant group differences were detected between examinations for overall ECG abnormalities (p=0.42).

DISCUSSION

In general, the foregoing analyses on a wide range of cardiovascular variables, have shown a lack of significant differences between the Ranch Hands and the Comparisons. The sole exception was the finding of increased verified heart disease in the Ranch Hands versus the Comparisons (24% and 20%, respectively, p=0.054, unadjusted; p=0.036, adjusted). These results were not noted in the Baseline examination (p=0.982, unadjusted). A review of the relative risk patterns, whether or not statistically significant, for all of the other cardiovascular variables showed general equality, with about half of the risks below unity and half above. This rough equivalence suggests that, although the Ranch Hands have slightly more reported heart disease, the finding is not mirrored by substantial and consistent clinical cardiovascular defects at this time. This observation should not be lightly dismissed, and is cause for continued close surveillance.

The most notable cardiovascular finding at the followup examination was the lack of significant peripheral pulse abnormalities, which were unexpectedly found at the 1982 Baseline examination (p=0.05). The primary contributory cause of the change in pulse significance from Baseline to followup was probably the rigid 4-hour tobacco abstinence required prior to Doppler testing (due to the known vasoconstriction effects of nicotine). Tobacco abstinence, however, was not a requirement for the Baseline manual pulse readings. Although tobacco abstinence was not a requirement prior to manual readings at the followup examination, there was general compliance to the smoking prohibition, particularly if a participant's general physical examination preceded the Doppler testing. Therefore it might be expected that the manual readings would show more pulse abnormalities than Doppler testing; in fact, this was the case (see section on Peripheral Pulses).

Whatever the true cause(s), the prevailing fact is that there are no longer significant group differences in pulse abnormalities, as noted by both manual and Doppler techniques, regardless of the poor agreement between the two methods.

The close approximation of the estimated relative risks to unity for practically all of the cardiovascular variables is clearly indicative of equivalent cardiovascular health between the two groups. Furthermore, the general similarity of the unadjusted and adjusted results was suggestive of near equivalence of the important cardiovascular risk factors in the Ranch Hands and Comparisons (see Table 15-13), as well as a balance for unanalyzed or hidden covariates of importance.

These health assessments of the two groups are considerably strengthened by the almost consistent, classical effects of the covariates in this chapter. In particular, the age effect was uniformly profound, affecting

almost all of the dependent variables in the functional categories of reported-verified heart diseases, and central and peripheral vascular function. The covariates of race, percent body fat, and cholesterol (particularly the cholesterol-HDL ratio), and smoking were also generally strong and consistent in their effects. Statistically significant, positive associations were seen between the current level of smoking and posterior tibial, popliteal, and femoral pulses, as well as borderline significant associations between current smoking and other ECG diagnoses, carotid bruits, and reported myocardial infarctions. However, significant negative associations were observed between current smoking and reported and verified essential hypertension. Pack-years of smoking was significantly positively associated with several ECG variables and pulse assessments, although not always in a consistently increasing manner. There was a statistically significant and consistently increasing effect of pack-years of smoking on reported and verified myocardial infarctions, but there was a negative association between pack-years of smoking and verified essential hypertension, with the greatest number of abnormalities in the zero pack-year category. Alcohol was infrequently interactive with the dependent variables, but covariate tests of association generally revealed the classical pattern of more cardiovascular abnormalities in the nondrinking category than in the low drinking category.

Personality score, however, usually failed to demonstrate the "expected" aggregation of cardiovascular abnormalities in the Type A direction. In fact, most associations were in the Type B direction. Generally, only cardiovascular studies ascertaining personality type by the Structured Interview technique have shown an association of Type A personality (Type A-1, in particular) to heart disease endpoints, and conversely, studies using questionnaire techniques to measure personality type have not demonstrated the association. Lastly, the strong association between historical-verified cardiovascular events and the specific dependent variables provides assurance that the overall cardiovascular measurements have been accurate and valid.

SUMMARY AND CONCLUSIONS

The cardiovascular health of both cohorts was assessed by collection of reported and record-verified heart disease events; measurement of central cardiac function by systolic blood pressure, abnormal heart sounds, and electrocardiograph (ECG) findings; and evaluation of peripheral vascular function by diastolic blood pressure, funduscopic examination, presence of carotid bruits, and detailed manual and Doppler measurements of five peripheral pulses. Table 15-20 presents the overall summary of the unadjusted and adjusted results. Where possible, the analyses used the covariates of age, race, occupation, percent body fat, cholesterol, high density lipoprotein (HDL) cholesterol, cholesterol-HDL ratio, smoking history (pack-years and current smoking level), alcohol history (drink-years and current drinking level), personality score, and differential cortisol.

The cardiovascular variables did not reveal significant group differences, with the exception of verified heart disease, for which the proportions of recorded cardiac events were 24 and 20 percent in the Ranch Hand and Comparison groups, respectively, ($p=0.054$ unadjusted, $p=0.036$ adjusted). This finding was not reinforced by results of individual questionnaire or examination variables showing impairment in the Ranch Hands. There was a remarkable balance in relative risks above and below unity between the groups.

TABLE 15-20.

Overall Summary Results of Unadjusted and Adjusted Analyses
Cardiovascular Variables

Variable	Statistical/ Clinical Analysis	Unadjusted	Adjusted
Historical and Verified Heart Disease			
Reported Hypertension		NS	NS
Verified Hypertension		NS	NS
Reported Heart Disease[a]		NS	NS
Verified Heart Disease[a]		NS*	S[b]
Reported Heart Attack		NS	NS
Verified Heart Attack		NS	NS
Central Cardiac Function			
Systolic Blood Pressure	Discrete	NS	NS
	Continuous	NS	****
Heart Sounds		NS	NS
Electrocardiogram (Overall)		NS	****
ECG: RBBB		NS	NS
ECG: LBBB		---	N/A
ECG: Nonspecific T-Wave Changes		NS	NS
ECG: Bradycardia		NS	NS
ECG: Tachycardia		---	N/A
ECG: Arrhythmia		NS	****
ECG: Other Diagnoses		NS	NS

TABLE 15-20. (continued)

Overall Summary Results of Unadjusted and Adjusted Analyses
Cardiovascular Variables

Variable	Statistical/ Clinical Analysis	Unadjusted	Adjusted
Peripheral Vascular Function			
Diastolic Blood Pressure	Discrete	NS	NS
	Continuous	NS	NS
Funduscopic Examination		NS	NS
Carotid Bruits		NS	NS
Radial Pulses	Manual	NS	NS
	Doppler	NS	NS
Femoral Pulses	Manual	NS	NS
	Doppler	NS	NS
Popliteal Pulses	Manual	NS	****
	Doppler	NS	NS
Dorsalis Pedis Pulses	Manual	NS	****
	Doppler	NS	NS
Posterior Tibial Pulses	Manual	NS	****
	Doppler	NS	NS
Leg Pulses	Manual	NS	****
	Doppler	NS	NS
Peripheral Pulses	Manual	NS	****
	Doppler	NS	NS
All Pulses	Manual	NS	****
	Doppler	NS	NS

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

NS*:Borderline significant ($0.05<p\leq 0.10$).

****Group-by-covariate interaction.

[a]Excluding hypertension.

[b]RH>C (Adj. RR: 1.25; 95% C.I.: [1.02, 1.54], p=0.036).

Other related analyses showed an absence of significant group differences in reported or verified hypertension, reported or verified heart attacks, and reported heart disease. There was good correlation between the verified cardiovascular history and the central and peripheral cardiovascular abnormalities detected at the physical examination, supporting accuracy and validity of the cardiovascular measurements.

The adjusted analyses of central cardiac function disclosed a significant group-by-age interaction involving systolic blood pressure in the Black cohort, with a mean systolic blood pressure greater in the Ranch Hands than the Comparisons at younger age levels, but a lower mean pressure at the older ages; the group-by-age interaction was not significant in the nonblack cohort. Additionally, there was a significant group-by-pack-years of smoking interaction for the overall ECG findings, and significant group-by-pack-years of smoking and group-by-percent body fat interactions for arrhythmia, but they all generally pointed to lower adjusted relative risks in the Ranch Hands.

In the analysis of peripheral vascular function, no significant group differences were observed for abnormalities involving radial, femoral, popliteal, posterior tibial, dorsalis pedis, or three anatomic aggregates of these pulses, either by manual palpation or Doppler techniques. This overall finding was in distinct contrast to the 1982 Baseline examination, which by the manual palpation method, showed significant peripheral pulse deficits in the Ranch Hands. This favorable pulse reversal over the two examinations is primarily attributed to the rigid 4-hour tobacco abstinence applied prior to Doppler testing, although other factors may be related. The lack of group differences for pulse abnormalities was noted even though the manual and Doppler techniques differed significantly ($p<0.05$, $p<0.001$ for most) in the detection of abnormalities for all but one of the pulses or pulse combinations.

For manually-determined pulse abnormalities, there was a significant group-by-race interaction for the popliteal pulses, a significant group-by-percent body fat interaction for the leg pulses, and significant group-by-occupation interactions for the posterior tibial, dosalis pedis, and the three pulse aggregates (leg, peripheral, and all pulses). No interactions were encountered in the adjusted analyses of the Doppler results, and none showed significant group differences.

Statistical analyses involving the Original Comparisons also showed no significant differences in the cardiovascular measurements between groups, although slightly different interactions were detected in some of the adjusted analyses.

For the exposure analyses, the only statistically significant effects were those pointing to less bradycardia and less reported and verified heart disease in the medium exposure level category, as contrasted to the low exposure category, among the enlisted groundcrew. In many cases there were too few abnormalities within the occupational categories to permit formal statistical tests. Overall, the exposure analyses were deemed as unsupportive of any meaningful dose-response relationships.

The longitudinal analysis of the pulse index confirmed the significant difference in the change in the pattern of results from the Baseline examination to the followup examination, largely due to a relatively greater

increase of pulse abnormalities in the Comparison group than in the Ranch Hand group. There was no significant change in pattern between the two groups in overall ECG findings between examinations.

There was a similar distribution of the covariates between groups, except for a slightly higher level of current Ranch Hand smoking (also observed at Baseline), and a corresponding slightly lower mean percent body fat. The general covariate effects were strong and showed expected, classical associations with the cardiovascular measurements. However, unexpected effects were consistently noted for personality score, with higher proportions of various cardiovascular abnormalities associated with scores in the Type B direction, a finding possibly attributable to the method of personality determination. Nonetheless, the repeated demonstration of classical covariate associations with cardiovascular pathology lends considerable credence to the quality of the data. Although smoking was positively associated with many of the cardiovascular measurements, negative associations were seen between current smoking and reported and verified essential hypertension and between pack-years of smoking and verified hypertension.

In conclusion, of 27 cardiovascular variables, only one, verified heart disease, showed a significant excess in the Ranch Hands, but this finding was largely unsupported by other cardiac measurements. Both manual palpation and Doppler recordings of five peripheral pulses were similar in both groups, in marked contrast to the 1982 Baseline examination which found significant pulse deficits in the Ranch Hand group. This change at the followup examination was most likely due to required tobacco abstinence prior to the pulse measurements. Exposure index analyses did not support a consistent dose-response relationship for any variable. Overall, there was remarkable similarity in the cardiovascular health between the Ranch Hand and Comparison groups.

CHAPTER 15

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CHAPTER 16

HEMATOLOGICAL EVALUATION

INTRODUCTION

Although direct impairment of the hematopoietic system may result from exposure to chlorophenols or dioxin, marked abnormalities in many of the circulating hematological elements may also be due to the severe and often endstage toxicity observed in other organs or organ systems. Animal experiments have confirmed both direct and indirect hematopoietic effects of TCDD. In a chronic low-dose feeding study of TCDD in eight monkeys, decreased hemoglobin and hematocrit values were noted at the 6-month mark in all animals.[1] Four of these monkeys expired in 7 to 11 months and all had anemia, leukopenia, and thrombocytopenia. Necropsy of three sacrificed animals at 1 year showed multi-organ pathology including bone marrow degeneration, atrophy of lymphopoietic tissue, and numerous hemorrhages in a variety of organs. In another monkey experiment, using single low and high doses of TCDD, early hematological effects included increased neutrophil counts in the low-dose group and lymphopenia and thrombocytopenia in the high-dose group.[2] At the end of the experiment, half the sternal bone-marrow samples revealed a decrease in overall cellularity and an increase in the myeloid-erythroid cell ratio.

Rat experiments with TCDD demonstrated relatively consistent results. One study revealed elevated erythrocyte, reticulocyte, and neutrophil counts with depressed values for the mean corpuscular volume, mean corpuscular hemoglobin, platelet counts, and clot retraction times.[3] The authors attributed most of these effects to terminal dehydration and nonspecific toxicity. Another rat study using gavage doses of TCDD varying from 0.001 to 1.0 µg/kg demonstrated depressed red blood cell counts and packed cell volumes in the high-dose group.[4] In a mixed-dose regimen using rats, mice, and guinea pigs, dose-related decreases in lymphocyte and leukocyte numbers were observed in mice and guinea pigs within 1 week following TCDD administration.[5] Thrombocytopenia and hemoconcentration were found in rats. Because of the lymphopenia in mice and guinea pigs, TCDD was judged to be immunosuppressive.

In general, human observational studies showed fewer and less consistent hematological findings than the structured animal experiments. A case report of 2,4-D intoxication with marked neurological findings described transient bone marrow depression with peripheral leukopenia and granulocytopenia.[6] In two industrial accidents involving significant contamination with TCDD and resulting cases of chloracne, only temporary depression of peripheral leukocyte and lymphocyte formation was observed.[7,8]

Two contemporary indepth morbidity studies[9,10] of the Nitro, West Virginia, accident included routine clinical complete blood counts and differential counts, and hemoglobin and hematocrit determinations. Though these studies shared overlapping study cohorts, they did not report any of the

hematological results in their publications; presumably, there were no significant differences in any of the parameters between the exposed and the unexposed cohorts.

The two pilot studies of TCDD-contaminated residential areas in Missouri also included routine hematological assays of peripheral blood.[11,12] One study paradoxically noted a significantly increased mean platelet count in the high-risk group, although the data were not adjusted for smoking.[11] The Quail Run study, predominantly emphasizing cell-mediated immunity, found significant group differences in the mean leukocyte count, mean absolute granulocyte count, and the mean percentage of monocytes in the differential count.[12] Unfortunately, the authors neglected to identify the group (exposed or unexposed) that had the abnormal hematological findings. However, the finding of a significantly higher proportion of individuals with white blood cell counts exceeding 10,000/mm^3 was in the exposed group.

Baseline Summary Results

A number of statistically significant group differences and interactions emerged in the analysis of the 1982 Baseline examination. The Ranch Hand group had a significantly higher adjusted mean red blood cell corpuscular volume and corpuscular hemoglobin value than the Comparison group ($p=0.05$, $p=0.04$, respectively), although the magnitude of the difference was small in each case. The Ranch Hand adjusted mean values for six other parameters, i.e., red blood cell count, white blood cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin concentration, and platelet count, were nearly identical to the adjusted means of the Comparison group, and all were well within normal range. Similarly, the percent of abnormal values for these eight variables, as established by the upper and lower limits of normal, did not vary by group.

Linear models demonstrated the profound effect of smoking, as measured in pack-years. With increased smoking, white blood cell, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and platelet values increased, whereas the mean corpuscular hemoglobin concentration showed a significant negative association with smoking. The red blood cell count revealed a borderline significant negative relationship to smoking. No statistically significant group-by-smoking interactions were detected.

The exposure index analyses conducted within the Ranch Hand group disclosed two statistically significant exposure-level effects as well as seven significant or borderline-significant exposure-level-by-smoking interactions. In the officer cohort, the percentage of mean corpuscular hemoglobin abnormalities increased with increasing exposure level. The high-exposure group also had the highest percentage of mean corpuscular hemoglobin concentration abnormalities. No significant associations were found, however, in the enlisted flyer or enlisted groundcrew cohort. Five interactions involved a decreasing association (gradient of slopes) between the hematological measure and pack-years of smoking with increasing exposure level, one showed an increasing association with increasing exposure level, and one was uninterpretable. The report concluded that the overall statistical findings were somewhat consistent among themselves, and that medical morbidity was not significant.

Parameters of the 1985 Hematological Evaluation

The 1985 hematological assessment was identical to the 1982 Baseline evaluation. The eight hematological variables were red blood cell count (RBC), white blood cell count (WBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLT); these variables were determined by routine hematological procedures. The normal ranges of the SCRF-determined values differed somewhat from those employed in 1982 by the Kelsey-Seybold Clinic.

As before, the analysis of the hematological data included the covariates of age, race, occupation, and smoking. Updated and more comprehensive smoking data, in terms of pack-years and current smoking (including cigar- and pipe-smoking), were used in most analyses.

Excluded were three individuals with fever at the time of examination (two Ranch Hands and one Comparison). Hematological variables in the continuous form were analyzed by general linear models adjusting for age, race, occupation, and smoking. The hematological data, trichotomized as abnormally low, normal, or abnormally high, were subjected to log-linear (logit) analysis, adjusted for the same covariates. Minor differences in the table totals within this chapter reflect rare missing data for either the dependent variables or the covariates. Parallel analyses using Original Comparisons can be found in Tables N-4 through N-9 of Appendix N.

RESULTS AND DISCUSSION

General

Eight hematological assays were performed on peripheral blood specimens obtained from all participants on the first day of the physical examination. Table 16-1 lists the assays, the abbreviations used in this chapter, the SCRF laboratory normal range for each assay, and the required laboratory coefficient of variation for each assay. The SCRF laboratory norms varied to some extent from the values used at the Baseline examination (see pages XVI-3-1, Baseline Report). The SCRF laboratory coefficients of variation met or exceeded contract requirements and were uniformly achieved due to the precision of the Coulter 5-Plus automated instrument, in conjunction with rigorous FIR CUSUM quality control techniques (see Chapter 6).

The overall precision in the laboratory aspects of the hematological assays is reflected in the analytic ability to discern minute mean shifts between groups. Representative statistical power statements are as follows. Sample sizes were sufficiently large to detect a 0.87 percent mean shift in RBC and a 2.5 percent mean shift in PLT values using an α-level of 0.05 (two-sided) and a power of 0.80. Further, the sample sizes were sufficient to detect a 1.66-fold increase in the frequency of abnormal values for RBC, and a 1.96-fold increase in the frequency of abnormal values for PLT, with 80 percent certainty.

TABLE 16-1.

Laboratory Parameters for
Hematological Test Variables

Hematological Test	Abbreviation	SCRF Laboratory Normal Range	Contract Required Coefficient of Variation (in percent)
Red Blood Cell Count	RBC	4.3-5.9 million/cubic mm	2.0
White Blood Cell Count	WBC	4.5-11.0 thousand/cubic mm	2.5
Hemoglobin	HGB	13.9-16.3 grams/100 ml	1.1
Hematocrit	HCT	39.0-55.0 ml/100 ml	3.0
Mean Corpuscular Volume	MCV	80.0-97.0 cubic micra	2.0
Mean Corpuscular Hemoglobin	MCH	26.0-34.0 micromicrogram	2.0
Mean Corpuscular Hemoglobin Concentration	MCHC	31.0-37.0 percent	2.0
Platelet Count	PLT	130-400 thousand/cubic mm	3.5

The statistical analyses in this chapter are presented in the following order: unadjusted tests, covariate tests of association, adjusted analyses, exposure analyses, and longitudinal contrasts. A variable-by-variable discussion summarizes all of the analyses, and representative exposure analyses are also presented. Group-by-covariate interactions are narratively presented, and illustrated by calculating Ranch Hand-Comparison differences at selected covariate levels. The interaction data tables are found in Tables N-2 and N-3 of Appendix N.

Unadjusted Categorical Analyses

Data from the eight hematological variables were categorized as abnormally low, normal, or abnormally high according to the SCRF laboratory norms cited in Table 16-1. The frequency distribution of these discretized data is presented by group in Table 16-2. As shown, there were no statistically significant, or even marginally significant, differences between the groups. Only one abnormal MCHC value was found among all study participants.

TABLE 16-2.

Unadjusted Categorical Analyses for Hematological Variables by Group

Variable	Group	Abnormally Low		Normal		Abnormally High		Total	p-Value*
		Number	Percent	Number	Percent	Number	Percent		
RBC	Ranch Hand	30	3.0	976	96.2	8	0.8	1,014	0.910
	Comparison	42	3.2	1,239	95.9	11	0.8	1,292	
WBC	Ranch Hand	45	4.4	906	89.4	62	6.1	1,013	0.883
	Comparison	63	4.9	1,149	88.9	80	6.2	1,292	
HGB	Ranch Hand	39	3.8	752	74.2	223	22.0	1,014	0.848
	Comparison	44	3.4	960	74.3	288	22.3	1,292	
HCT	Ranch Hand	11	1.1	1,001	98.7	2	0.2	1,014	0.999
	Comparison	15	1.2	1,274	98.6	3	0.2	1,292	
MCV	Ranch Hand	10	1.0	857	84.5	147	14.5	1,014	0.992
	Comparison	13	1.0	1,094	84.7	185	14.3	1,292	
MCH	Ranch Hand	7	0.7	943	93.0	64	6.3	1,014	0.755
	Comparison	7	0.5	1,211	93.7	74	5.7	1,292	
MCHC	Ranch Hand	1	0.1	1,013	99.9	0	0.0	1,014	--
	Comparison	0	0.0	1,292	100.0	0	0.0	1,292	
PLT	Ranch Hand	5	0.5	987	97.4	21	2.1	1,013	0.828
	Comparison	3	0.2	1,264	97.9	24	1.9	1,291	

*Chi-square test, 2 d.f., except for HCT and PLT which were obtained from continuity adjusted chi-square tests on 1 d.f. (Abnormally high category pooled with normal, and abnormally low category pooled with normal for HCT and PLT, respectively.)

--Only one abnormal MCHC value; p-value not given.

Unadjusted Analyses of Continuous Data

The unadjusted tests of group means from the continuous data for the eight variables are displayed in Table 16-3. The variables WBC and PLT were analyzed in logarithmic units because of their right-skewed original distributions. Antilog values of the means are given for ease of interpretation but their standard error or variance terms are consequently omitted since the relevance of these terms pertains only to the logarithmic scale. The sample sizes were 1,014 for the Ranch Hand group and 1,292 for the Comparisons, except for WBC (Ranch Hands, 1,013; Comparisons, 1,292) and PLT (Ranch Hands, 1,013; Comparisons, 1,291). As shown in Table 16-3, there were no statistically significant group differences between the unadjusted means of each variable.

TABLE 16-3.

Unadjusted Continuous Analyses for Hematological Variables (Contrast of Group Means)

Variable	Group Mean±SE		Difference ±SE	t-Statistic	p-Value
	Ranch Hand	Comparison			
RBC	4.964±0.012	4.982±0.010	-0.019±0.016	-1.19	0.233
WBC[a]	7.003	6.891	--	1.34	0.182
HGB	15.624±0.033	15.626±0.029	-0.002±0.044	-0.05	0.958
HCT	45.904±0.097	45.952±0.083	-0.048±0.127	-0.38	0.703
MCV	92.596±0.150	92.346±0.132	0.250±0.200	1.25	0.210
MCH	31.544±0.055	31.431±0.049	0.113±0.074	1.53	0.125
MCHC	34.040±0.021	34.009±0.017	0.031±0.027	1.17	0.243
PLT[a]	265.2	263.0	--	0.96	0.337

[a]Means transformed from log scale.

--Difference and standard errors (SE) not presented, since variables were analyzed on logarithmic scale.

Dependent Variable and Covariate Relationships

The data from the Ranch Hand and Comparison groups were pooled for each of the eight hematological variables and analyzed independently with the covariates of age (born in or after 1942, born before 1942), race (Black, nonblack), occupation (officer, enlisted flyer, enlisted groundcrew), and smoking history (0 pack-years; greater than 0 to 10 pack-years; and greater than 10 pack-years). These analyses are summarized in terms of statistical significance (p-values) in Table 16-4. As noted, each of the dependent variables was substantially affected by one or more of the covariates. The exact nature of the covariate influence, e.g., directionality, significance, consistency across related variables, is presented in the variable-by-variable discussion section. Covariate effects were also analyzed in continuous form with the use of linear regression models (see Table 16-6 and discussion following). In addition, covariate distributions were examined between groups (see Table N-1 of Appendix N).

TABLE 16-4.

Association Between Hematological Variables and Age, Race, Occupation, and Smoking History in the Combined Ranch Hand and Comparison Groups

Variable	Age	Race	Occupation	Smoking History
RBC	0.010	<0.001	NS	NS
WBC	NS*	<0.001	0.001	<0.001
HGB	NS	0.002	<0.001	0.003
HCT	NS	<0.001	NS	NS
MCV	<0.001	<0.001	0.004	<0.001
MCH	<0.001	<0.001	0.003	<0.001
MCHC	--	--	--	--
PLT	NS	NS	NS	0.004

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

NS*: Borderline significant ($0.05<p\leq 0.10$).

--Not analyzed due to sparse data.

Adjusted Categorical Analyses

Log-linear (logit) models for each of the hematological variables were fit to adjust for age, race, occupation, and smoking history. In addition, all significant group-by-covariate interactions were examined. The covariate of current level of smoking (used in the adjusted continuous analyses described below) was not included in the categorical analyses to avoid problems with sparse cells. Adjusted relative risks for Ranch Hand-Comparison contrasts were calculated for the categories of abnormally low values versus normal values and for abnormally high values versus normal values. Adjusted relative risks were not computed for the abnormally high versus normal categories for HCT, or for the abnormally low versus normal categories for PLT, due to sparse data. The results of these analyses are given in Table 16-5 and were quite similar to the unadjusted results, with no statistically significant or borderline significant associations found.

TABLE 16-5.

Adjusted Categorical Analyses for Hematological Variables (Abnormal Versus Normal), Adjusted for Age, Race, Occupation, and Smoking

	Abnormally Low vs. Normal		Abnormally High vs. Normal	
Variable	Adj. Relative Risk (95% C.I.)	p-Value	Adj. Relative Risk (95% C.I.)	p-Value
RBC	0.93 (0.59,1.47)	0.762	1.04 (0.48,2.28)	0.920
WBC	0.96 (0.66,1.42)	0.854	0.97 (0.69,1.36)	0.852
HGB	1.12 (0.74,1.80)	0.522	0.98 (0.80,1.19)	0.824
HCT	1.02 (0.51,2.06)[a]	0.954[a]	--	--
MCV	1.08 (0.52,2.26)	0.787	0.99 (0.78,1.26)	0.960
MCH	1.33 (0.56,3.17)	0.525	1.10 (0.79,1.54)	0.574
PLT	--	--	1.14 (0.66,1.98)[b]	0.638[b]

[a]Abnormally low versus normal/abnormally high.

--Not analyzed due to sparse data.

[b]Abnormally high versus normal/abnormally low.

Adjusted Analyses of Continuous Data

General linear regression models were performed, adjusting for age (at the Baseline examination), race, occupation (OCC), smoking history (pack-years [PACKYR]), and current level of smoking (cigarettes per day [CSMOK]). The linear models were fit to examine the main effects of group (GRP) membership, the covariates, and two- and three-factor interactions among these variables (only three-factor interactions involving group were considered). The hierarchical modeling approach as described in Chapter 7, Statistical Methods, was performed to arrive at a "best model" containing the group effect and all statistically significant covariate main effects and interactions.

The results of the adjusted analyses for the hematological variables, along with the significance of the adjusting covariates and covariate interactions are summarized in Table 16-6.

These results indicated a lack of significant group differences for RBC, HGB, HCT, MCV, MCH, and MCHC after adjustment for five covariates. Two analyses, WBC and PLT, showed significant group-by-covariate interactions; the statistics of these interactions (along with borderline interactions for RBC) are given in Table N-2 of Appendix N, and the narrative descriptions of these interactions are included in the following variable-by-variable summary presentations.

Discussion

The following variable-by-variable discussion presents the findings for the unadjusted and adjusted results, main covariate effects, group-associated interactions, and when appropriate, Ranch Hand versus Original Comparison contrasts, and comparisons to Baseline results. The results of the covariate effects and covariate interactions (not involving group) for the adjusted analyses are found in Table 16-6; group-by-covariate interactions are given in Table N-2 of Appendix N.

Red Blood Cell Count (RBC)

Both the categorical and continuous unadjusted analyses found no statistically significant differences in RBC values between groups.

The covariate associations for both groups combined showed a significant effect of age (RBC abnormally low in 4.0% of the older cohort versus 1.9% of the younger; $p=0.010$) and race (Blacks having 6.3% and 4.2% in the abnormally low and high categories versus 2.9% and 0.6% in nonblacks, respectively; $p<0.001$).

Continuous regression analyses also detected significant effects of current smoking ($p=0.004$) and an age-by-occupation interaction ($p=0.013$). The adjusted categorical analysis showed no significant group difference, but the adjusted continuous analysis revealed a borderline significant ($p=0.086$) three-factor interaction of group-by-occupation-by-smoking history. Estimated Ranch Hand-Comparison contrasts revealed a significant difference ($p=0.010$) for enlisted groundcrew, 30 pack-years with Ranch Hands exhibiting a slightly lower RBC count than the Comparisons (see Table N-2 of Appendix N).

TABLE 16-6.

Adjusted Continuous Analyses for Hematological Variables, (Ranch Hand-Comparison Group Differences)

Variable	Ranch Hand-Comparison Group Difference ± SE	p-Value	Covariate Remarks*
RBC	-0.021±0.015[a]	0.172	AGE*OCC (p=0.013) CSMOK (p=0.004)
WBC	****	****	GRP*RACE*AGE (p=0.005) GRP*AGE*PACKYR (p=0.004) GRP*RACE*OCC (p=0.004)
HGB	-0.034±0.042	0.410	AGE*OCC (p=0.002) RACE*OCC (p=0.013) CSMOK (p<0.001)
HCT	-0.151±0.121	0.210	AGE*OCC (p=0.004) RACE*OCC (p=0.003) OCC*PACKYR (p=0.035) CSMOK (p<0.001)
MCV	0.108±0.188	0.565	RACE*AGE (p<0.001) RACE*OCC (p=0.015) RACE*CSMOK (p=0.025)
MCH	0.062±0.070	0.378	RACE*AGE (p=0.015) CSMOK (p<0.001) OCC (p<0.001)
MCHC	0.032±0.026	0.226	RACE (p=0.001) CSMOK (p=0.042)
PLT	****	****	GRP*RACE*PACKYR (p<0.001) GRP*RACE*CSMOK (p=0.024) OCC (p=0.039) AGE (p=0.006)

*Abbreviations
OCC: Occupation
CSMOK: Current level of smoking (cigarettes per day)
GRP: Group
PACKYR: Smoking history (pack-years)

[a]Also, borderline significant three-factor interaction (see text).

****Group-by-covariate interaction; group difference, standard error (SE) and p-value not presented.

A similar, but slightly weaker interaction was observed in the analysis of the Original Comparisons versus the Ranch Hands. The general finding of insignificant group differences supported the Baseline observations (despite the use of different statistical procedures), but the followup results differed by the mild three-factor interaction.

White Blood Cell Count (WBC)

The categorical and unadjusted continuous analyses did not disclose any significant differences in WBC levels between the Ranch Hand and Comparison groups.

Covariate tests showed a borderline effect of age (with the older cohort having a slightly lower proportion of abnormally low WBC levels--4.2% versus 5.4% in the younger cohort), and the highly significant effects of race ($p<0.001$), occupation ($p=0.001$), and smoking history ($p<0.001$). Blacks had a much higher proportion of abnormally low WBC counts (15.4%) versus nonblacks (4.0%); higher proportions of enlisted flyers and enlisted groundcrew personnel (9.1% and 7.2%, respectively) had abnormally high WBC counts versus officers (3.6%). Increasing frequencies of leukocytosis were associated with increasing levels of smoking.

The adjusted categorical analysis was nonrevealing with respect to group differences, but the adjusted continuous analysis disclosed three significant three-factor interactions involving group membership: group-by-race-by-age ($p=0.005$), group-by-age-by-smoking history (pack-years; $p=0.004$), and group-by-race-by-occupation ($p=0.004$).

Further analyses were conducted stratifying by race (see Table N-2 of Appendix N). Among Blacks, the best model revealed significant group-by-occupation and group-by-age interactions ($p=0.045$, $p=0.024$, respectively). Group differences for covariate levels corresponding to young officers and young enlisted flyers were statistically significant, with the adjusted mean WBC value considerably lower in the Ranch Hand group than in the Comparison group. Conversely, the adjusted difference for the older enlisted groundcrew was in the opposite direction. The results for nonblacks were more precise: The group-by-age-by-smoking history interaction was highly significant ($p=0.002$), with young heavy smokers having a WBC level approximately 12 percent greater in the Ranch Hands than the Comparisons.

Other differences were small in magnitude and not statistically significant. Ranch Hand and Original Comparison contrasts were similar for nonblacks, but for Blacks, the group-by-occupation and group-by-age interactions did not reach statistical significance ($p=0.077$, $p=0.134$, respectively). The nonsignificance of the unadjusted and categorical adjusted analyses was equivalent to the findings at the Baseline examination. However, possibly due to different model selections, no interactions were noted at Baseline. Race and occupation were not used as covariates at Baseline.

Hemoglobin (HGB)

None of the four analyses, unadjusted and adjusted categorical tests and unadjusted and adjusted tests of mean differences, detected a significant difference between groups.

Covariate tests of association revealed the profound effects of race (8.4% abnormally low in Blacks versus 3.3% in nonblacks; p=0.002), occupation (25.1% and 25.6% abnormally high in enlisted flyers and groundcrew, respectively, versus 16.7% in officers; p<0.001), and smoking history (with proportions of abnormally high HGB levels associated with increases in pack-years of smoking; p=0.003). Continuous analyses detected significant effects of current smoking (p<0.001), occupation-by-age (p=0.002), and occupation-by-race (p=0.013) interactions. No significant group-by-covariate interactions were noted. Analysis of the Ranch Hands and Original Comparisons, however, found significant three-factor interactions of group-by-race-by-age (p=0.030) and group-by-race-by-occupation (p=0.020) (see Tables N-7 and N-8 of Appendix N). For equivalent analyses, the followup results were quite analogous to the Baseline study results.

Hematocrit (HCT)

All of the unadjusted and adjusted categorical tests and analyses of mean differences failed to detect any group differences. Since there were only five abnormally high values, this category was combined with the normal category in the categorical analyses.

The association of race to HCT was highly significant, with 4.9 percent abnormally low values noted in Blacks versus 0.9 percent in nonblacks (p<0.001). Regression analyses also detected significant effects of current smoking (p<0.001) as well as age-by-occupation (p=0.004), race-by-occupation (p=0.003), and occupation-by-smoking history (p=0.035) interactions. In both categorical and continuous adjusted analyses, no significant group-by-covariate interactions were detected. Analyses of data from the Ranch Hands and Original Comparisons, however, detected significant three-factor interactions of group-by-race-by-age (p=0.026) and group-by-race-by-occupation (p=0.011) (see Tables N-7 and N-8 of Appendix N).

Mean Corpuscular Volume (MCV)

No significant group differences were detected for MCV abnormalities or mean values by any of the unadjusted or adjusted analyses.

Main covariate effects were profound for age (p<0.001), race (p<0.001), occupation (p=0.004), and smoking history (p<0.001). The older cohort had a greater frequency of abnormally high MCV values than did the younger age group (18.0% vs. 9.4%, respectively), and Blacks had a far greater frequency of abnormally low MCV values than nonblacks (7.7% vs. 0.6%, respectively). Enlisted groundcrew personnel had a lower percentage of abnormally high values than officers or enlisted flyers (12.5%, 15.5%, and 17.0%, respectively), and increases in pack-years of smoking were associated with increasing percentages of abnormally high levels (0 pack-years: 4.7%; greater than 0 to 10 pack-years: 13.1%; and greater than 10 pack-years: 21.0%).

Continuous analyses detected significant interactions of race-by-age (p<0.001), race-by-occupation (p=0.015), and race-by-current smoking (p=0.025). The analysis of the Ranch Hand and Original Comparisons revealed a significant group-by-race interaction (p=0.031) for the categorical analyses and significant group-by-age-by-smoking history (p=0.041) and group-by-age-by-current smoking (p=0.012) interactions in the continuous

analyses. Various contrasts are given in Table N-8 of Appendix N. No explanations are apparent for these interactions except chance. The followup examination results of MCV (i.e., significant interactions) differed from the Baseline results, which showed a significantly larger adjusted mean MCV value in the Ranch Hands.

Mean Corpuscular Hemoglobin (MCH)

MCH abnormalities and mean values did not differ significantly by group in any of the unadjusted or adjusted analyses.

Main effects were very significant for all of the covariates. The older cohort had a greater frequency of abnormally high MCH values than the younger group (7.9% vs. 3.2%, respectively; $p<0.001$), while Blacks had a greater frequency of low abnormalities than nonblacks (4.9% vs. 0.3%, respectively; $p<0.001$). Enlisted groundcrew had a higher proportion of abnormalities in the lower range than enlisted flyers and officers (1.0%, 0.3%, 0.2%, respectively), but they had a lower proportion of high-range abnormalities compared to the other occupations (4.3%, 7.8%, and 7.3%, respectively). The overall p-value was 0.003. Increasing pack-years of smoking was associated with increasing frequencies of high abnormal MCH results (0 pack-years: 2.1%; greater than 0 to 10 pack-years: 6.0%; and greater than 10 pack-years: 8.3%; $p<0.001$).

Continuous analyses detected a significant race-by-age interaction ($p=0.015$), as well as significant effects of current smoking ($p<0.001$) and occupation ($p<0.001$). The followup findings did not support the Baseline observation of significantly increased MCH in the Ranch Hands, although the mean was still higher (both unadjusted and adjusted) in the Ranch Hand group.

In the analysis of the Ranch Hands and the Original Comparisons, a significant three-factor interaction of group-by-age-by-current smoking emerged ($p=0.026$). Table N-8 of Appendix N presents Ranch Hand-Comparison differences for selected covariate levels corresponding to 35- and 53-year-old nonsmokers, one-pack-per-day current smokers, and two-packs-per-day current smokers. The differences were positive for all contrasts except the 53-year-old smokers, when the differences became increasingly more negative with increasing levels of smoking.

Mean Corpuscular Hemoglobin Concentration (MCHC)

In both groups, only one abnormal MCHC count was recorded for either the abnormally low or abnormally high categories, precluding unadjusted or adjusted categorical tests, and exploration of main covariate effects. No significant group differences were detected by the unadjusted or adjusted tests of MCHC means, although race ($p=0.001$) and current smoking ($p=0.042$) were significantly associated with MCHC (higher MCHC in nonblacks and decreasing MCHC associated with increasing current levels of smoking). Similar findings were noted in the analysis of Ranch Hand and Original Comparisons, and overall, the followup findings were comparable to the 1982 Baseline MCHC results.

Platelet Count (PLT)

Neither the unadjusted nor the adjusted categorical analysis showed statistically significant group differences. Analysis of continuous data disclosed significant effects due to occupation ($p=0.039$), age ($p=0.006$), group-by-race-by-smoking history ($p<0.001$), and group-by-race-by-current smoking ($p=0.024$) interactions, with higher PLT values in the heavily smoking Ranch Hands but similar values for nonsmokers (see Table N-2 of Appendix N).

The significant interactions of group-by-race-by-smoking history ($p=0.011$) and group-by-age ($p=0.040$) were also noted for the analyses involving the Original Comparisons (see Table N-8 of Appendix N). The percentages of abnormally high PLT counts increased with increasing pack-years of smoking (0 pack-years: 0.8%; greater than 0 to 10 pack-years: 2.0%; and greater than 10 pack-years: 2.6%). Other than the interactions encountered in the adjusted analyses, the overall findings at the followup were comparable to the Baseline PLT results.

EXPOSURE INDEX ANALYSES

Exposure index analyses were conducted within each occupational cohort of the Ranch Hand group to search for dose-response relationships (see Chapter 8 for details on the exposure index). Log-linear models were fit to the categorical data to examine the effects of exposure and pack-years of smoking, as well as the interaction between these variables. The normal and abnormally high categories were pooled for the RBC count, and the abnormally low and normal response categories were pooled for MCV, MCH, and PLT due to empty cells in some strata. Because of the small numbers of abnormal values, analyses were not conducted for HCT or MCHC. The results of the unadjusted categorical analyses are presented in Table 16-7, and the counterpart adjusted analyses are given in Table 16-8.

The unadjusted analyses showed only a statistically significant result for the WBC count in the enlisted flyer category, due primarily to an excess of abnormally low values in the high exposure category. The very sparse data support a trend from low to high exposure, and the finding of abnormally low WBC counts associated with exposure is in the direction expected for an herbicide effect. However, the exposure association with abnormally low WBC counts converted to borderline significance ($p=0.082$) in the adjusted analysis. There were no statistically significant exposure level-by-smoking history interactions. Similar analyses in the other occupational strata (with much larger sample sizes) did not produce this pattern.

The unadjusted analysis of means for all eight hematological variables was carried out by a one-way analysis of variance. The results are arrayed in Table 16-9.

These analyses revealed only one statistically significant result ($p=0.038$), the RBC count in the enlisted groundcrew stratum where individuals in the medium exposure category had a higher mean RBC level than those in the low or high exposure categories. Thus, these significant RBC findings did not demonstrate a dose-response relationship. The results for HCT in the enlisted groundcrew stratum were of borderline significance ($p=0.052$) with the highest mean HCT level in the medium exposure category. In contrast to the categorical analyses, mean WBC levels in the enlisted flyers were not significantly different among the three exposure levels.

TABLE 16-7.

Unadjusted Categorical Exposure Index Analyses for Hematological Variables by Occupation

Variable	Occupation	Exposure Index	Abnormally Low		Normal		Abnormally High		Total	p-Value
			Number	Percent	Number	Percent	Number	Percent		
RBC	Officer	Low	3	2.4	123	96.8	1	0.8	127	0.522[a]
		Medium	4	3.1	125	96.2	1	0.8	130	
		High	6	4.9	114	93.4	2	1.6	122	
	Enlisted Flyer	Low	1	1.8	54	98.2	0	0.0	55	0.401[a]
		Medium	1	1.5	64	98.5	0	0.0	65	
		High	3	5.3	54	94.7	0	0.0	57	
	Enlisted Groundcrew	Low	6	3.9	148	96.1	0	0.0	154	0.329[a]
		Medium	2	1.2	158	97.5	2	1.2	162	
		High	4	2.8	136	95.8	2	1.4	142	
WBC	Officer	Low	7	5.5	115	90.6	5	3.9	127	0.919
		Medium	7	5.4	118	90.8	5	3.8	130	
		High	5	4.1	110	90.2	7	5.7	122	
	Enlisted Flyer	Low	0	0.0	51	92.7	4	7.3	55	0.045
		Medium	1	1.6	59	92.2	4	6.2	64	
		High	6	10.5	47	82.5	4	7.0	57	
	Enlisted Groundcrew	Low	4	2.6	139	90.3	11	7.1	154	0.839
		Medium	8	4.9	142	87.6	12	7.4	162	
		High	7	4.9	125	88.0	10	7.0	142	
HGB	Officer	Low	7	5.5	100	78.7	20	15.8	127	0.425
		Medium	2	1.5	106	81.5	22	16.9	130	
		High	6	4.9	92	75.4	24	19.7	122	
	Enlisted Flyer	Low	3	5.4	36	65.4	16	29.1	55	0.350
		Medium	3	4.6	51	78.5	11	16.9	65	
		High	5	8.8	36	63.2	16	28.1	57	
	Enlisted Groundcrew	Low	5	3.2	119	77.3	30	19.5	154	0.352
		Medium	4	2.5	110	67.9	48	29.6	162	
		High	4	2.8	102	71.8	36	25.4	142	

TABLE 16-7. (continued)

Unadjusted Categorical Exposure Index Analyses
for Hematological Variables by Occupation

Variable	Occupation	Exposure Index	Abnormally Low		Normal		Abnormally High		Total	p-Value
			Number	Percent	Number	Percent	Number	Percent		
MCV	Officer	Low	1	0.8	111	87.4	15	11.8	127	0.580[b]
		Medium	1	0.8	111	85.4	18	13.8	130	
		High	0	0.0	102	83.6	20	16.4	122	
	Enlisted Flyer	Low	0	0.0	43	78.2	12	21.8	55	0.764[b]
		Medium	0	0.0	54	83.1	11	16.9	65	
		High	0	0.0	47	82.5	10	17.5	57	
	Enlisted Groundcrew	Low	2	1.3	139	90.3	13	8.4	154	0.091[b]
		Medium	3	1.8	133	82.1	26	16.0	162	
		High	3	2.1	117	82.4	22	15.5	142	
MCH	Officer	Low	1	0.8	117	92.1	9	7.1	127	0.916[b]
		Medium	0	0.0	121	93.1	9	6.9	130	
		High	0	0.0	112	91.8	10	8.2	122	
	Enlisted Flyer	Low	0	0.0	51	92.7	4	7.3	55	0.855[b]
		Medium	0	0.0	60	92.3	5	7.7	65	
		High	0	0.0	54	94.7	3	5.3	57	
	Enlisted Groundcrew	Low	1	0.6	147	95.4	6	3.9	154	0.626[b]
		Medium	2	1.2	151	93.2	9	5.6	162	
		High	3	2.1	130	91.6	9	6.3	142	
PLT	Officer	Low	2	1.6	120	94.5	5	3.9	127	0.487[b]
		Medium	1	0.8	126	97.7	2	1.6	129	
		High	0	0.0	119	97.5	3	2.5	122	
	Enlisted Flyer	Low	1	1.8	51	92.7	3	5.4	55	0.135[b]
		Medium	0	0.0	64	98.5	1	1.5	65	
		High	0	0.0	57	100.0	0	0.0	57	
	Enlisted Groundcrew	Low	0	0.0	152	98.7	2	1.3	154	0.914[b]
		Medium	1	0.6	158	97.5	3	1.8	162	
		High	0	0.0	140	98.6	2	1.4	142	

[a]Normal pooled with abnormally high.

[b]Abnormally low pooled with normal.

TABLE 16-8.

Adjusted Categorical Exposure Index Analyses (Log-Linear Models) for Hematological Variables by Occupation (p-Values)

Variable	Occupation	Exposure Index Effect*	Smoking History Effect**	Exposure Index-by-Smoking History
RBC	Officer	0.593	0.246	0.472
	Enlisted Flyer	0.552	0.364	0.981
	Enlisted Groundcrew	0.310	0.515	0.717
WBC	Officer	0.928	0.001	0.616
	Enlisted Flyer	0.082	0.121	0.971
	Enlisted Groundcrew	0.761	0.009	0.104
HGB	Officer	0.444	0.393	0.424
	Enlisted Flyer	0.413	0.647	0.980
	Enlisted Groundcrew	0.299	0.104	0.143
MCV	Officer	0.718	<0.001	0.334
	Enlisted Flyer	0.619	0.020	0.490
	Enlisted Groundcrew	0.101	0.028	0.574
MCH	Officer	0.852	0.002	0.777
	Enlisted Flyer	0.800	0.168	0.514
	Enlisted Groundcrew	0.681	0.288	0.530
PLT	Officer	0.410	0.099	0.708
	Enlisted Flyer	0.178	0.816	0.976
	Enlisted Groundcrew	0.910	0.363	0.996

*Adjusted for smoking history (no interaction).

**Adjusted for exposure index (no interaction).

TABLE 16-9.

Unadjusted Continuous Exposure Index Analyses for Hematological Variables by Occupation (Analysis of Variance)

Occupation	Variable	Exposure Index Mean ± SE Low	Medium	High	p-Value
		(n=127)	(n=130)	(n=122)	
Officer	RBC	4.904±0.030	4.861±0.029	4.899±0.034	0.560
	WBC[a]	6.488	6.553	6.753	0.512
	HGB	15.468±0.084	15.463±0.087	15.593±0.094	0.507
	HCT	45.379±0.243	45.313±0.255	45.791±0.284	0.380
	MCV	92.648±0.430	93.260±0.365	93.548±0.367	0.252
	MCH	31.606±0.161	31.851±0.134	31.884±0.123	0.314
	MCHC	34.090±0.060	34.123±0.060	34.067±0.059	0.801
	PLT[a]	253.66	255.70[b]	256.72	0.799
		(n=55)	(n=65)	(n=57)	
Enlisted Flyer	RBC	4.972±0.048	4.942±0.037	4.957±0.053	0.894
	WBC[a]	7.531	7.236	6.966	0.378
	HGB	15.785±0.149	15.629±0.110	15.721±0.180	0.744
	HCT	46.345±0.425	45.908±0.315	46.300±0.535	0.717
	MCV	93.269±0.618	92.923±0.501	93.400±0.572	0.817
	MCH	31.782±0.222	31.675±0.187	31.735±0.208	0.933
	MCHC	34.065±0.075	34.058±0.072	33.956±0.072	0.508
	PLT[a]	272.87	275.34[c]	261.13	0.382
		(n=154)	(n=162)	(n=142)	
Enlisted Groundcrew	RBC	4.990±0.032	5.094±0.031	4.999±0.033	0.038
	WBC[a]	7.185	7.236	7.389	0.686
	HGB	15.566±0.099	15.807±0.075	15.685±0.090	0.147
	HCT	45.740±0.284	46.580±0.210	46.086±0.252	0.052
	MCV	91.737±0.399	91.672±0.404	92.376±0.458	0.436
	MCH	31.251±0.154	31.138±0.151	31.468±0.166	0.325
	MCHC	34.032±0.059	33.941±0.051	34.031±0.057	0.409
	PLT[a]	270.97	273.42	268.27	0.748

[a] Standard errors (SE) not presented, since variables were analyzed on logarithmic scale.

[b] n=129.

[c] n=64.

Adjusted analyses of the differences in variable means were performed by regression techniques. As in the unadjusted analysis, RBC and HCT in the enlisted groundcrew stratum presented contrasts of interest for the medium versus low exposure categories ($p=0.059$, and $p=0.041$, respectively). Further, it was noted that smoking history (pack-years) was significantly associated with the RBC and HCT variables in a negative direction, while the current smoking covariate (cigarettes/day) showed a positive trend.

By linear models, the adjusted exposure analyses included the main effects of the covariates, as well as interactions between exposure level and each covariate. No clear dose-response relationships were identified, but eight exposure-by-covariate interactions were noted, and these are reflected in summary form in Table 16-10. Analyses exploring these interactions are presented in Table N-3 of Appendix N.

These data do not disclose any interpretable pattern of occupational predominance in the Ranch Hand group. However, the relative sparseness of the enlisted groundcrew stratum in these interactions is noteworthy, as is the relative representation of race and age as interactive covariates.

Exposure contrasts are shown in Table N-3 of Appendix N. For HGB and HCT, high versus low contrasts were significant at covariate levels corresponding to Black officers, and medium versus low contrasts were significant at covariate levels corresponding to enlisted flyers, age 35. The medium versus low contrast corresponding to Black enlisted groundcrew was also significant for PLT.

In summary, several interactions were detected in the continuous analyses, but only a few of the main effect exposure analyses demonstrated statistical significance. Of these, only one showed a weak linear trend of effects increasing from low to high exposure. No pattern of exposure effects was discernible by occupational category.

TABLE 16-10.

Summary of Exposure Index-by-Covariate Interactions Encountered in Adjusted Continuous Analyses of Hematological Variables (General Linear Models)

Variable	Occupation	Covariate	p-Value
RBC	Officer	Race	0.052
RBC	Enlisted Flyer	Age	0.012
HGB	Officer	Race	0.034
HGB	Enlisted Flyer	Age	<0.001
HCT	Officer	Race	0.048
HCT	Enlisted Flyer	Age	0.002
PLT	Enlisted Flyer	Current Smoking	0.050
PLT	Enlisted Groundcrew	Race	0.044

LONGITUDINAL ANALYSES

The sample data base for the longitudinal analyses was the number of participants who attended both examinations (971 Ranch Hands and 1,139 Comparisons). These variables were analyzed: MCV, MCH, and PLT. The results of these analyses are depicted in Table 16-11.

These analyses showed no statistically significant group differences for the MCV and MCH variables, whereas a highly significant group difference was present for mean PLT counts. Both MCV and MCH counts increased symmetrically from the Baseline examination, a fact likely attributable to an ongoing effect of smoking (see Table 16-4) or to a laboratory technique difference. The highly significant difference (p=0.002) for PLT counts was due to a group-by-examination time interaction, with the Ranch Hands exhibiting a slight decline in the mean PLT value from the Baseline to followup examination, whereas the Comparisons showed a slight increase in the mean PLT value. No biological significance is assigned to the statistically significant group difference in the change in PLT counts. Based upon the results of the longitudinal analysis, there is reasonable equivalence of the hematological status between the two groups.

TABLE 16-11.

Longitudinal Analyses for MCV, MCH, and PLT:
A Contrast of Baseline and First Followup Examination Test Means

			Means				
Variable	Group	Total	1982 Baseline	1985 Followup	Difference (Followup-Baseline)	Error*	p-Value (Equality of Difference)
MCV	Ranch Hand	971	88.89	92.60	+3.71	2.800	0.96
	Comparison	1,139	88.68	92.38	+3.70		
MCH	Ranch Hand	971	30.81	31.55	+0.74	0.879	0.30
	Comparison	1,139	30.65	31.45	+0.80		
PLT	Ranch Hand	971	276.9	271.5	-5.4	35.38	0.002
	Comparison	1,139	266.7	268.2	+1.5		

*Error = $\sqrt{\text{Subj * Time/Group mean squares.}}$

SUMMARY AND CONCLUSIONS

The functional integrity of the hematopoietic system was assessed by the measurement of eight peripheral blood variables: red blood cell count (RBC), white blood cell count (WBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLT). These variables were analyzed in the discrete form to detect differences in the percentages of values outside the designated laboratory range, as well as in the continuous form to detect shifts in mean values between the two groups. A summary of all of these analyses, unadjusted and adjusted for the covariates of age, race, occupation, and smoking, is presented in Table 16-12.

The unadjusted discrete analysis of the percent abnormal values, both low and high, showed no statistically significant differences between the Ranch Hand and Comparison groups for any of the hematological variables. Similarly, the adjusted categorical analysis disclosed that none of the adjusted relative risks was significant for either group, and that no significant group-by-covariate interactions were present.

The unadjusted continuous analysis did not detect any significant differences in group means for any of the eight variables. The adjusted continuous analysis found no significant group differences for HGB, HCT, MCV, MCH, and MCHC, but encountered significant three-factor interactions for WBC (group-by-race-by-age, group-by-age-by-smoking history, and group-by-race-by-occupation), for PLT (group-by-race-by-smoking history and group-by-race-by-current level of smoking), and a borderline interaction for RBC (group-by-occupation-by-smoking history). Ranch Hand versus Original Comparison analyses revealed further significant interactions for HGB, HCT, MCV, and MCH. As no group strata demonstrated consistent patterns of hematologic impairment, biologic relevance was not assigned to the interactions. The covariate effects of age, race, occupation, and smoking history were highly significant for many of the hematological variables.

The effect of race was particularly profound for all variables except PLT. There was fair consistency in the covariate effects upon the RBC-related variables. Generally, decreasing hematologic values were associated with increasing age and the Black race, and increasing hematologic values were associated with increasing smoking. The detection of these classical covariate effects lends credence to the overall finding of nonsignificant group differences for all of the hematological variables. Significant group differences found for MCV and MCH at the Baseline examination were not significant at the first followup. Other differences (e.g., covariate effects, interactions) between the Baseline and followup examinations may be due to small numeric shifts in the cohorts under study (see Chapter 2) and the selection of alternate statistical models, or due to chance.

Unadjusted continuous exposure analyses in the Ranch Hand group revealed only one significant effect (RBC in enlisted groundcrew) and one borderline effect (HCT in enlisted groundcrew), but neither was consistent with a plausible dose-response relationship. The adjusted continuous exposure analyses found only one significant contrast (HCT, medium exposure versus low exposure, enlisted groundcrew). However, seven exposure level-by-covariate interactions were noted for four of the hematological variables. Discrete outcome analyses of the exposure level index revealed a significant result only for WBC in the enlisted flyers.

TABLE 16-12.

Overall Summary Results of Unadjusted and Adjusted Analyses of Hematological Variables

	Unadjusted		Adjusted	
	Mean	Categorical	Mean	Categorical
RBC	NS	NS	NS*	NS
WBC	NS	NS	****	NS
HGB	NS	NS	NS	NS
HCT	NS	NS	NS	NS
MCV	NS	NS	NS	NS
MCH	NS	NS	NS	NS
MCHC	NS	--	NS	--
PLT	NS	NS	****	NS

NS: Not sgnificant ($p>0.10$).

NS*: Borderline significant group-by-covariate interaction ($0.05 \leq p < 0.10$).

--Analysis not performed due to sparse data.

****Group-by-covariate interaction.

Note: Significant group-by-covariate interaction, Ranch Hands versus Original Comparisons only, for HGB, HCT, MCV, and MCH.

The longitudinal analyses of MCV, MCH, and PLT found significant differences only for PLT values between the Baseline and followup examinations, with the Baseline group difference in mean values closing to near equivalence at the followup examination.

In conclusion, none of the eight hematological variables were found to differ significantly between the Ranch Hand and Comparison groups. In fact, group equivalence was more apparent at the followup examination than at the Baseline examination. The classical effects of age, race, and smoking were demonstrated with most of the hematological variables. The longitudinal analyses also suggested that neither group manifested an impairment of the hematopoietic system. Exposure index analyses did not support a plausible dose-response relationship for any of the hematological variables.

CHAPTER 16

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CHAPTER 17

RENAL ASSESSMENT

INTRODUCTION

Renal dysfunction and overt renal disease are not considered to be important clinical sequelae of exposure to phenoxy acids, chlorophenols, or TCDD.

In man and animals, 2,4-D, 2,4,5-T, and TCDD are excreted by the kidney, largely in the unmetabolized state via a first-order kinetic process.[1-5] Excretion of these compounds appears to be a function of the proximal convoluted tubules.[6-8] In experimental animals, renal damage is generally noted only when very high or lethal doses of TCDD have been administered, an observation that reflects the severe systemic toxicity of TCDD as contrasted to a doubtful role of primary nephrotoxicity.[9-12]

A variety of experimental pharmacokinetic studies have been conducted in man using both ingested 2,4-D and 2,4,5-T.[3-5,13,14] Most of these studies suggested an unconjugated excretion of these compounds by first-order kinetics. No acute deleterious effects, as detected by urinalysis or blood chemistries, were either noted or recorded for the volunteer subjects.

In contrast, following significant exposure to a horse arena filled with TCDD-contaminated waste products, a 6-year-old girl developed hemorrhagic cystitis, pyelonephritis, and proteinuria.[15] Horses exposed to this arena and other contaminated arenas also frequently manifested hematuria. A thorough 5-year followup examination of the young girl was essentially normal and did not reveal any renal sequalae.[16]

Most dioxin morbidity studies have only briefly mentioned renal disease and function, and then in the context of routine data collected at physical examination rather than as a specific clinical focus. Some studies of significant occupational exposure have been almost devoid of commentary on renal dysfunction.[17-19] A contemporary study of a residentially exposed cohort showed negative renal findings.[20]

The Times Beach, Missouri, pilot study demonstrated historical "trends" of increased urinary tract disease by questionnaire, along with a compatible pattern of leukocyturia and hematuria manifest at physical examination, but none of the observations was statistically significant.[21] The Monsanto industrial morbidity studies reported essentially negative urinalysis findings, although data were not presented.[22,23]

Baseline Summary Results

The 1982 Baseline examination assessed renal disease and function by questionnaire and basic urinalysis testing.

Based on questionnaire information, the Ranch Hand group reported significantly more kidney disease than the Comparisons ($p=0.039$), but this finding was not substantiated by laboratory test results, even when all abnormalities were summed over the five tests of BUN, creatinine clearance, presence of occult blood, five or more urine WBC's per high-power field (HPF), and the presence of urine protein. The Comparison group manifested a twofold increase in proteinuria ($p=0.055$). The distributions of creatinine clearance levels were similar in both groups, as were the means of the BUN, urine specific gravity, and WBC's/HPF. Difficulty in assessing the degree and significance of hidden noncompliance to the full 24-hour urine collection made the interpretation of the creatinine clearance test results somewhat problematic. Of some interest, known noncompliance to urine collection was observed much more frequently ($p<0.001$) in the elderly participants. Of 18 herbicide exposure analyses, only 1 (enlisted flyer category) was statistically significant vis-a-vis a history of kidney disease, and it did not demonstrate a linear increase from low to high exposure.

The validity of the renal assessment was reinforced by the demonstrated effects of the covariates of age (born in or after 1942, born before 1942) and 2-hour status after postprandial glucose levels (less than 120 mg/dl, greater than or equal to 120 mg/dl). Blood urea nitrogen increased with age and specific gravity decreased ($p<0.001$ for both), while an abnormally high postprandial glucose level indicative of diabetes was associated only with an increasing urine specific gravity, as expected.

Overall, the Baseline renal assessment suggested an excess of historical kidney disease in the Ranch Hand group that was not corroborated by laboratory urinalysis testing.

Parameters of the 1985 Renal Assessment

Because of the essentially negative Baseline results, the fact that kidney disease is not a prime clinical endpoint, and the manifest compliance problems with a 24-hour urine collection, the 1985 examination process did not emphasize further inquiry into renal disease and function.

The onsite NORC questionnaire did not specifically probe for a 1982-1985 interval history of kidney disease, although severe cases may be captured by the generic question, "any other major condition?" or by a detailed extraction of review-of-systems data obtained at the physical examination. Laboratory testing parameters included all the Baseline dependent variables except the creatinine clearance level (omitted because the plasma creatinine assay was deleted from the test battery). Also, the analysis of composite renal abnormalities was deleted. In addition, the 24-hour urine collection was reduced to a 12-hour collection (5:30 a.m. to 5:30 p.m.) to ease participant burden while still maintaining validity for the porphyrin analyses (see Chapter 13). The accuracy of the 12-hour urine collection was not assessed during the 1985 examination.

Renal data analyses paralleled the Baseline analysis except for deleting one of the dependent variables and a composite analysis, adding the covariate of race, and defining the covariate of diabetic class as diabetic, impaired, or normal. No clinical exclusion categories applied to the renal analysis. Minor numerical differences in the tables are due to rare missing dependent

variable or covariate data. Adjusted statistical analyses using the above covariates were based on 1,016 Ranch Hands and 1,293 Comparisons and used logistic regression and analysis of covariance methods. When age was used as a covariate in the logistic regression models, the continuous form was used mathematically, but for summary table purposes, age is displayed as a dichotomy. Parallel analyses using the Original Comparisons can be found in Appendix O (see Tables O-3 through O-5). Tests of association between dependent variables and covariates emphasized Fisher's exact test and Pearson's chi-square test for discrete dependent variables and t-tests and analysis of variance techniques for continuous dependent variables.

RESULTS AND DISCUSSION

Questionnaire Data

History of renal disease was assessed by a self-administered review-of-systems question list at the physical examination. Specific structured questions on renal disease were not incorporated in the NORC questionnaire. The review-of-systems questions, i.e., "kidney trouble?" "kidney stones?" were open-ended with respect to time, and reflected conditions that arose at any time in the past.

These questionnaire data did not show a significant difference between the Ranch Hand and Comparison groups, as reflected by the analysis in Table 17-1.

Tests of association between the historical presence of kidney disease in both groups and the covariates of race, occupation, diabetes, and age are given in Table 17-2.

TABLE 17-1.

Unadjusted Analysis of History of Kidney Disease/Kidney Stones by Group

	History of Kidney Disease/Stones						
	Yes		No				
Group	Number	Percent	Number	Percent	Total	Est. Relative Risk (95% C.I.)	p-Value
Ranch Hand	94	9.3	920	90.7	1,014	0.93 (0.70,1.23)	0.619
Comparison	128	9.9	1,163	90.1	1,291		

TABLE 17-2.

Association Between Kidney Disease/Kidney Stones and Age, Race, Occupation, and Diabetic Class in the Combined Ranch Hand and Comparison Groups

		History of Kidney Disease/Stones					
		Yes		No			
Covariate	Covariate Category	Number	Percent	Number	Percent	Total	p-Value
Age	Born ≥1942	66	6.9	894	93.1	960	<0.001[a]
	Born <1942	156	11.6	1,189	88.4	1,345	
Race	Nonblack	214	9.9	1,949	90.1	2,163	0.106[a]
	Black	8	5.6	134	94.4	142	
Occupation	Officer	83	9.6	781	90.4	864	
	Enlisted Flyer	36	9.3	350	90.7	386	0.969[b]
	Enlisted Groundcrew	103	9.8	952	90.2	1,055	
Diabetic* Class	Diabetic	14	8.0	161	92.0	175	
	Impaired	41	14.5	242	85.5	283	0.011[b]
	Normal	166	9.0	1,677	91.0	1,843	

[a]Fisher's exact test.

[b]Pearson's chi-square test.

*Unable to classify four participants, due to missing 2-hour postprandial glucose level and no historical evidence of diabetes.

These results showed that there was no significant effect due to race or occupation. In contrast, there was a significant effect due to diabetic class (p=0.011), with participants in the impaired diabetic class having a significantly higher proportion of past kidney disease than those in the normal or diabetic classes. Older participants also had a significantly higher history of past renal events than younger participants (p<0.001).

A logistic regression analysis of the history of kidney disease and kidney stones using the above four covariates gave a result very similar to the unadjusted analysis (Adj. RR: 0.95, 95% C.I.: [0.71,1.25], p=0.693). Race and occupation were not significant covariates. However, diabetic class and age were significant covariates (p=0.041 and p<0.001, respectively).

These analyses showed that there was no difference in the history of renal disease between the Ranch Hand and Comparison groups, and that the

proportions of past kidney disease and kidney stones were significantly influenced by age and diabetic class. While these findings are consistent with traditional expectations in renal disease, they were in direct contrast to the findings of the 1982 Baseline examination, which revealed a significant excess of historical kidney disease in the Ranch Hand group, and group data that were not influenced by age or glucose levels.

It is concluded that there were no significant group differences in past renal disease.

Physical Examination Data

No physical examination procedures were used to evaluate the renal system as most procedures are invasive and beyond the scope of this voluntary examination. Accordingly, the renal system was evaluated primarily by laboratory data.

Laboratory Data

Five renal variables were quantitated by general laboratory procedures to assess nonspecific renal system function. The presence or absence of urine protein was determined by standard reagent strip testing. Hematuria and leukocyturia were measured by high-power microscopic examination after centrifugation for 5 minutes. Urine specific gravities were measured by Ames' Multisticks; those urines exceeding normal limits were remeasured by standardized refractometers. BUN levels were assayed by a DuPont Automated Chemical Analyzer, model 500. The SCRF laboratory normal values from these variables are given in Table 17-3.

TABLE 17-3.

Laboratory Norms for Five Renal Variables

Renal Variable	Normal	Abnormal
Urine Protein	Absent	Present
Occult Blood	Absent	$\geq$1 RBC/HPF
WBC/HPF	$\leq$2	>2
BUN (mg/dl)	7-22	$\geq$23
Specific Gravity	1.005-1.03	$\leq$1.004

In this section, urinary protein, hematuria, and leukocyturia were analyzed as discrete variables, whereas BUN and urine specific gravity were analyzed as continuous variables. The number and percent of subjects with abnormal values for the discrete variables are displayed in the summary Table 17-4, along with the number of participants, the unadjusted means, and standard errors of the continuous variables.

TABLE 17-4.

Summary of Renal Laboratory Variables by Group

Renal Variable	Group: Ranch Hand Number Abnormal	Ranch Hand Percent Abnormal	Comparison Number Abnormal	Comparison Percent Abnormal	Unadjusted p-Value
Urine Protein	37	3.6	40	3.1	0.485
Occult Blood	182	17.9	208	16.1	0.239
WBC/HPF	102	10.0	107	8.3	0.145

Renal Variable	Unadjusted Mean (Sample Size)	Standard Error	Unadjusted Mean (Sample Size)	Standard Error	Unadjusted p-Value
BUN (mg/dl)	14.21* (1,016)	--	14.30* (1,293)	--	0.554
Specific Gravity	1.0157 (1,016)	0.0002	1.0152 (1,292)	0.0002	0.082

*Arithmetic mean calculated on square root scale and transformed to original units.

--Standard error not given, since analysis performed on square root scale.

The following statistical power statements apply to several variables displayed in Table 17-4. At a standard α-level of 0.05 and a power of 0.80, the sample sizes were sufficient to detect a 1.28-fold increase in the frequency of percent abnormal values for urinary occult blood, and a 1.43-fold increase in the percentage of leukocyturia, both over that observed in the Comparison group. Further, the sample sizes were adequate to reveal a 2.9 percent mean shift in the BUN value relative to the mean observed in the Comparison group.

Urinary Protein

As displayed in Table 17-4, the Ranch Hand group had a prevalence rate of urinary protein of 3.6 percent versus 3.1 percent in the Comparison group (Est. RR: 1.18, 95% C.I.: [0.75,1.86], p=0.485). This difference was not significant.

Tests of association were conducted with pooled participant data using the covariates of race, occupation, diabetic class, and age. These tests are presented in Table 17-5.

TABLE 17-5.

Association Between Urinary Protein and Age, Race, Occupation, and Diabetic Class in the Combined Ranch Hand and Comparison Groups

		Presence of Urinary Protein					
		Yes		No			
Covariate	Covariate Category	Number	Percent	Number	Percent	Total	p-Value
Age	Born ≥1942	34	3.5	927	96.5	961	0.641[a]
	Born <1942	43	3.2	1,304	96.8	1,347	
Race	Nonblack	65	3.0	2,100	97.0	2,165	0.002[a]
	Black	12	8.4	131	91.6	143	
Occupation	Officer	18	2.1	845	97.9	863	
	Enlisted Flyer	11	2.8	376	97.2	387	0.010[b]
	Enlisted Groundcrew	48	4.5	1,010	95.5	1,058	
Diabetic* Class	Diabetic	20	11.4	155	88.6	175	
	Impaired	18	6.4	264	93.6	282	<0.001[b]
	Normal	39	2.1	1,808	97.9	1,847	

[a]Fisher's exact test.

[b]Pearson's chi-square test.

*Unable to classify four participants, due to missing 2-hour postprandial glucose level and no historical evidence of diabetes.

These results suggested no age effect, but significant associations for the covariates of race ($p=0.002$), occupation ($p=0.010$), and diabetic class ($p<0.001$) were noted. The significant covariate effects were attributable to higher percentages of urinary protein abnormalities in Blacks versus nonblacks, enlisted groundcrew versus officers or enlisted flyers, and diabetes (past history [unverified] or greater than or equal to 200 mg/dl glucose) versus impaired glucose tolerance (at least 140 but less than 200 mg/dl glucose) versus normal glucose tolerance (less than 140 mg/dl glucose).

The prevalence rates of urinary protein abnormalities were adjusted by logistic regression models using the above four covariates. Race and occupation demonstrated significant effects ($p=0.023$ and $p=0.023$, respectively), while age did not ($p=0.294$). Because of a significant interaction between group and diabetic class ($p=0.047$), stratified analyses were conducted to provide further clarification. The results are shown in Table 17-6.

The adjusted relative risk, 95 percent confidence interval, and group p-value for each diabetic class are shown in Table 17-7.

TABLE 17-6.

Frequency of Urinary Protein by Diabetic Class and Group

		Presence of Urinary Protein				
		Yes		No		
Diabetic Class	Group	Number	Percent	Number	Percent	Total
Diabetic	Ranch Hand	7	9.0	71	91.0	78
	Comparison	13	13.4	84	86.6	97
Impaired	Ranch Hand	5	4.7	101	95.3	106
	Comparison	13	7.4	163	92.6	176
Normal	Ranch Hand	25	3.0	807	97.0	832
	Comparison	14	1.4	1,001	98.6	1,015

TABLE 17-7.

Adjusted Relative Risks for Urinary Protein by Diabetic Class

Diabetic Class	Adjusted Relative Risk	95% C.I.	p-Value
Diabetic	0.66	(0.25, 1.77)	0.414
Impaired	0.66	(0.23, 1.93)	0.453
Normal	2.23	(1.15, 4.32)	0.018

This analysis showed that the estimated prevalence of urinary protein is lower in the Ranch Hand group than in the Comparison group for the diabetic and glucose-impaired strata. Conversely, for the normal diabetic class, the Ranch Hand group manifested a significant increased prevalence of positive urinary protein as contrasted with the Comparison group.

These followup examination results were different from the 1982 Baseline examination, which showed significantly more proteinuria in the Comparison group. The prevalence of proteinuria in the followup examination was about 75 percent higher than the prevalence observed in the Baseline study. The interaction of group and diabetic class suggested Ranch Hand increases in proteinuria for normal glucose tolerance participants.

Urinary Occult Blood

Hematuria was determined by microscopic examination. For both groups combined, the frequency distribution of RBC count data was: 0 RBC/HPF, 82.15 percent; 1-2 RBC/HPF, 15.13 percent; 3-5 RBC/HPF, 2.03 percent; and greater than 5 RBC/HPF, 0.69 percent.

As noted in Table 17-4, the prevalence of urinary occult blood in the Ranch Hand group (17.9%) was slightly higher than the rate observed for the Comparison group (16.1%). The unadjusted analysis showed no significant group differences for occult blood (Est. RR: 1.14, 95% C.I.: [0.91,1.42], p=0.239).

Tests of association with the covariates of race, occupation, diabetic class, and age were conducted using combined group data for urinary occult blood, and these results are given in Table 17-8.

TABLE 17-8.

Association Between Urinary Occult Blood and Age, Race, Occupation, and Diabetic Class in the Combined Ranch Hand and Comparison Groups

		Presence of Urinary Occult Blood					
		Yes		No			
Covariate	Covariate Category	Number	Percent	Number	Percent	Total	p-Value
Age	Born ≥1942	148	15.4	812	84.6	960	0.115[a]
	Born <1942	242	18.0	1,105	82.0	1,347	
Race	Nonblack	355	16.4	1,809	83.6	2,164	0.016[a]
	Black	35	24.5	108	75.5	143	
Occupation	Officer	118	13.7	745	86.3	863	
	Enlisted Flyer	76	19.6	311	80.4	387	0.005[b]
	Enlisted Groundcrew	196	18.5	861	81.5	1,057	
Diabetic Class*	Diabetic	33	18.9	142	81.1	175	
	Impaired	52	18.5	229	81.5	281	0.296[b]
	Normal	305	16.5	1,542	83.5	1,847	

[a]Fisher's exact test.

[b]Pearson's chi-square test.

*Unable to classify four participants, due to missing 2-hour postprandial glucose level and no historical evidence of diabetes.

As reflected in Table 17-8, there was no significant effect due to diabetic class or age. However, Blacks had a significantly higher prevalence of urinary occult blood than nonblacks (p=0.016), and significant effects were also due to occupation (p=0.005), with officers having a lower proportion of positive occult blood determinations than enlisted personnel.

An adjusted analysis of urinary occult blood proportions was conducted by logistic regression techniques. Multiple significant three-factor interactions were noted, e.g., group-by-occupation-by-race (p=0.008), group-by-age-by-diabetic class (p=0.045), and group-by-occupation-by-diabetic class (p=0.017). Consequently, a series of analyses stratified by race were performed to determine adjusted relative risks for nonblacks and Blacks separately. The adjusted results for nonblack participants are given in Table 17-9.

TABLE 17-9.

Adjusted Analysis for Urinary Occult Blood for Nonblacks by Group

	Presence of Urinary Occult Blood					
	Yes		No			
Group	Number	Percent	Number	Percent	Total	Summary Statistics
Ranch Hand	166	17.4	789	82.6	955	Adj. RR: 1.13
Comparison	189	15.6	1,020	84.4	1,209	95% C.I.: (0.91,1.42), p-Value: 0.291

The covariates of occupation and age contributed significant effects ($p<0.001$ and $p=0.002$, respectively) to this analysis. Diabetic class was not significant ($p=0.863$), and was consequently not included in the final model. No significant group differences were found ($p=0.291$).

Table 17-10 shows the frequencies for Black participants.

The adjusted analysis of the data on Blacks showed a significant interaction of group and occupation ($p=0.003$). Table 17-11 presents frequencies and percents for the presence of urinary occult blood for each group, stratified by occupation.

This table demonstrates that the group-by-occupation interaction for Blacks was due to the Ranch Hand officers having a lesser prevalence of occult blood abnormalities than Comparison officers, while conversely, Ranch Hand enlisted personnel showed a higher prevalence of abnormalities than enlisted Comparisons. Because of the absence of hematuria in Black Ranch Hand officers, no relative risk was calculated. Consequently, the Black enlisted occupational categories were combined and investigated further through logistic regression techniques. This analysis did not show a difference of urinary occult blood percentages in the Ranch Hand Black

TABLE 17-10.

Frequency of Urinary Occult Blood for Blacks by Group

Group	Presence of Urinary Occult Blood: Yes Number	Yes Percent	No Number	No Percent	Total
Ranch Hand	16	26.7	44	73.3	60
Comparison	19	22.9	64	77.1	83

TABLE 17-11.

Frequency of Urinary Occult Blood for Blacks by Occupation and Group

Occupation	Group	Presence of Urinary Occult Blood: Yes Number	Yes Percent	No Number	No Percent	Total
Officer	Ranch Hand	0	0.0	7	100.0	7
	Comparison	3	42.9	4	57.1	7
Enlisted Flyer	Ranch Hand	3	30.0	7	70.0	10
	Comparison	1	5.9	16	94.1	17
Enlisted Groundcrew	Ranch Hand	13	30.2	30	69.8	43
	Comparison	15	25.4	44	74.6	59

enlisted and the Comparison Black enlisted strata (Est. RR: 1.62, 95% C.I.: [0.73,3.63], (p=0.239). The effects of age (p=0.817), occupation (p=0.171), and diabetic class (p=0.145) were not statistically significant, and were not included in the final adjusted analysis.

In conclusion, both unadjusted and adjusted stratified analyses (by race) did not reveal a consistent and plausible excess of hematuria in the Ranch Hand group. The tenfold or greater increase in the cross-sectional prevalence of hematuria compared to the Baseline examination (1.3% of both groups) to this followup examination may be due to a different sensitivity of the laboratory techniques of reagent-strip testing versus microscopic observation. Nonetheless, an approximate prevalence of 17 percent hematuria merits reevaluation at the next followup examination.

Urinary White Blood Cell Count

Leukocyturia was assessed by microscopic examination. As noted in Table 17-3, more than two white blood cells per high-power field (WBC/HPF) were considered abnormal by the SCRF laboratory. This is in distinct contrast to the cutpoint of five WBC/HPF used at the Baseline examination.

Table 17-4 shows the group frequencies of abnormal urine WBC's. The unadjusted analysis revealed a nonsignificant group effect (Est. RR: 1.24, 95% C.I.: [0.93,1.64], p=0.145).

Tests of association were conducted between the frequency of abnormal WBC counts in both groups and the covariates of race, occupation, diabetic class, and age. The results revealed a significantly higher prevalence of abnormal counts for Blacks than nonblacks ($p<0.001$), an effect due to occupation ($p=0.023$), with a lower prevalence of abnormalities for officers than enlisted personnel and an effect due to diabetic class ($p=0.046$), with a lower prevalence of abnormal WBC counts in the normal diabetic class than in either the impaired or diabetic classifications. Age was noncontributory ($p=0.508$).

Adjusted analyses of leukocyturia by group were performed by logistic regression techniques. A significant three-way interaction for group, age, and race was detected ($p=0.004$), requiring further stratified analyses. A summary of the frequencies for nonblacks is presented in Table 17-12.

TABLE 17-12.

Frequency of Urinary WBC/HPF for Nonblacks by Group

	Urinary WBC/HPF				
	Abnormal		Normal		
Group	Number	Percent	Number	Percent	Total
Ranch Hand	92	9.6	864	90.4	956
Comparison	88	7.3	1,121	92.7	1,209

The logistic regression adjustment of the data for nonblacks showed significant covariate effects for occupation ($p=0.046$) and diabetic class ($p=0.031$), and a significant interaction between group and age ($p=0.018$). Consequently, additional analyses were conducted stratifying by age (born in or after 1942, born before 1942), and are shown in Table 17-13.

TABLE 17-13.

Adjusted Analyses for Urinary WBC/HPF for Nonblacks by Age Category and Group

Age	Group	Urinary WBC/HPF: Abnormal Number	Abnormal Percent	Normal Number	Normal Percent	Total	Summary Statistics
Born ≥1942	Ranch Hand	41	10.8	339	89.2	380	Adj. RR: 2.42
	Comparison	24	4.8	478	95.2	502	95% C.I.: (1.43,4.09)
							p-Value: 0.001
Born <1942	Ranch Hand	51	8.9	525	91.1	576	Adj. RR: 0.99
	Comparison	64	9.1	643	90.9	707	95% C.I.: (0.67,1.46)
							p-Value: 0.956

As depicted by the above table, the adjusted rate of nonblack young Ranch Hands with abnormal urinary white blood cell counts was significantly greater than that for nonblack Comparisons (p=0.001 adjusted for occupation and diabetic class). Demonstrating the interaction involving age and group, the adjusted rate of nonblack older Ranch Hands with abnormal urinary WBC counts was nonsignificant and less than older nonblack Comparisons (p=0.956 adjusted for occupation and diabetic class).

Similar analyses were conducted for Black participants. Rates of abnormal urinary white blood cell count levels were 16.7 percent and 22.9 percent (n=60 and 83) for Black Ranch Hands and Black Comparisons, respectively. Significant interactions involving group and occupation (p=0.002) and group and age (p=0.001) were found. Additional analyses stratified by occupation were performed. Frequencies stratified by occupation are shown in Table 17-14.

This table clearly shows how the proportions of WBC abnormalities vary by group within the various occupational categories. However, because of the lack of abnormalities in the Black Ranch Hand officer stratum, an adjusted relative risk was not calculated for this occupation. Thus, Black enlisted categories were combined and subjected to further logistic regression techniques. The analysis showed yet another interaction, between group and age (p=0.026), requiring an additional stratification by age. Results of these analyses are presented in Table 17-15.

TABLE 17-14.

Frequency of Urinary WBC for Blacks by Occupational Category and Group

		Urinary WBC/HPF Count				
		Abnormal		Normal		
Occupation	Group	Number	Percent	Number	Percent	Total
Officer	Ranch Hand	0	0.0	7	100.0	7
	Comparison	2	28.6	5	71.4	7
Enlisted Flyer	Ranch Hand	3	30.0	7	70.0	10
	Comparison	3	17.6	14	82.4	17
Enlisted Groundcrew	Ranch Hand	7	16.3	36	83.7	43
	Comparison	14	23.7	45	76.3	59

TABLE 17-15.

Adjusted Analyses for Urinary WBC/HPF for Black Enlisted Flyers and Groundcrew by Age and Group

		Urinary WBC/HPF Count					
		Abnormal		Normal			
Age	Group	Number	Percent	Number	Percent	Total	Summary Statistics
Born ≥1942	Ranch Hand	4	13.8	25	86.2	29	Adj. RR: 0.41
	Comparison	13	28.3	33	71.7	46	95% C.I.: (0.12,1.40)
							p-Value: 0.153
Born <1942	Ranch Hand	6	25.0	18	75.0	24	Adj. RR: 2.17
	Comparison	4	13.3	26	86.7	30	95% C.I.: (0.53,8.79)
							p-Value: 0.279