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Health evaluation of employees occupationally exposed to methylene chloride

Clinical laboratory evaluation

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OTT MG, SKORY LK, HOLDER BB, BRONSON JM, WILLIAMS PR. Health evaluation of employees occupationally exposed to methylene chloride: Clinical laboratory evaluation. *Scand j work environ health* 9 (1983): suppl 1, 17–25. Hematopoietic effects seen in heavy cigarette smokers have been attributed to the carbon monoxide present in smoke. Since methylene chloride is partially metabolized in vivo to form carboxyhemoglobin, the pattern of hematologic changes relative to both the methylene chloride exposure and the cigarette smoking status of employees in two fiber production plants was investigated. Six serum constituents, changes in which could indicate liver injury, were also examined in relation to methylene chloride exposure. The observed increase in red cell counts, hemoglobin, and hematocrit among the women but not among the men exposed to about 475 ppm of methylene chloride was suggestive of a compensatory hematopoietic effect. Similar changes have been observed in cigarette smokers, but not necessarily only in women. Another frequently reported change in the hematology of smokers, increased mean corpuscular volume, was not seen with methylene chloride exposure. These findings suggest that some, but not all, of the hematologic changes observed in cigarette smokers may be explained on the basis of carbon monoxide alone. A dose-related increase in serum bilirubin was observed for both the men and the women exposed to methylene chloride. This appears to be an isolated finding, as no corresponding pattern of dose-related changes consistent with either liver injury or hemolysis was observed for other serum and blood constituents.

Key terms: carboxyhemoglobin, hematopoietic effects, smoking.

This report is the third in a series of five papers on the health of employees in two fiber production plants. Employees of one plant were exposed to methylene chloride ($MeCl_2$), acetone, and methanol, the methanol being present in a ratio of approximately 1 to 10 to methylene chloride. Employees of the reference plant were exposed to similar concentrations of acetone but were not exposed to methylene chloride or methanol.

The health evaluation portion of the surveillance program was primarily undertaken to provide the employee with a general health examination. However, it was recognized that laboratory data obtained as a routine component of the evaluation could be useful in examining hypotheses concerning adaptive or toxic responses related to increased carboxyhemoglobin in methylene chloride-exposed employees. Certain hematologic and blood volume changes seen in heavy cigarette smokers (increased hematocrit, increased red cell volume as measured by chromium[51] labeling, and decreased plasma volume) have been attributed to the carbon monoxide associated with cigarette smoke (11,

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13). Several investigators have reported other hematologic changes in cigarette smokers compared to nonsmokers among clinic patients undergoing routine examinations or in working populations (1, 2, 7). These changes include increased hemoglobin, mean corpuscular hemoglobin, and mean corpuscular volume The incrimination of carbon monoxide as the principle cause of hematologic changes seen in smokers has been disputed, partly on the basis of the multiple chemical ingredients found in cigarette smoke (6, 8).

Experimental studies involving chronic exposure of dogs and monkeys to high concentrations of carbon monoxide (carboxyhemoglobin levels of 32 to 40 %) produced marked erythrocytosis, increased red blood cell mass, and increased hematocrit and hemoglobin (14). However, these carboxyhemoglobin saturations were considerably higher than those associated with cigarette smoking.

Because of the metabolism of methylene chloride to form carboxyhemoglobin, nonsmoking employees in the present study would have experienced elevations of carboxyhemoglobin in the absence of exposure to agents present in cigarette smoke or often attending environmental carbon monoxide exposures (eg, motor vehicle exhaust fumes). Thus carboxyhemoglobin-related effects on the hematopoietic system could be assessed without the interference of many of the substances commonly associated with carbon monoxide. In addition, possible effects of methylene chloride exposure could be assessed in both cigarette smokers and nonsmokers.

Methylene chloride is a chlorinated hydrocarbon that is metabolized by the liver. Therefore serum constituents that could indicate liver dysfunction and were included in the clinical chemistry battery were also examined in relation to exposure.

Methods

The target population and logistical details for the health evaluation are described in a methodological paper covering all aspects of the health surveillance project (9). The plant with methylene chloride exposure was located in Rock Hill, SC, and the reference plant in Narrows, VA. Analyses of blood constituents for both plants were performed by Diagnostic Laboratories in Charlotte, NC. In the plant with methylene chloride exposure the blood samples were drawn with the subjects in a sitting position, whereas in the reference plant the subjects were recumbent. The effect of posture on concentrations of blood constituents has been experimentally examined by several investigators (4, 12). The effect of being in a recumbent position is to lower most serum and blood constituents (3 % for cellular constituents and up to 6.5 % for serum constituents after at least 30 min in that position). This effect thus represents a relatively minor source of bias in the comparisons between plants (12). Elevation differences between the two plants was another factor that could have affected the hematologic parameters. The plant with methylene chloride exposure was located in a flat geographic area at an altitude of 169 m above sea level. The reference plant was located in a mountainous region (altitude of plant site being 483 m with surrounding elevations of 1,280 m). The altitude differentials between the two sites translate into differences of 5–14 % in partial oxygen pressures.

All the blood samples were refrigerated after collection and were delivered to the laboratory daily. Samples obtained from the employees of the reference plant were flown to Charlotte. Cellular constituents were analyzed with a Coulter model S analyzer, carboxyhemoglobin with a co-oximeter IL 182, and serum constituents with a Technicon SMAC biochemical analyzer, except for serum alanine aminotransferase, which was assayed with a Gilford system 3500. The health evaluations were conducted from October 25th through February 13th, 71 % taking place between November 7th and December 3rd. Nearly all of the examinations of the exposed employees (264 of 266) were conducted during the morning and noon hours, whereas about 23 % (58 of 251) of the evaluations of the referents were conducted later in the day to accommodate production schedules. Participation was on a volunteer basis and was estimated to cover about 61 % of the employees in the plant with methylene chloride exposure and 55 % of the employees in the reference plant.

The blood constituents examined were red cell count, hemoglobin, hematocrit,

mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, carboxyhemoglobin, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, total bilirubin, and albumin.

The results of the industrial hygiene monitoring of the work environment are detailed in another report (9). Median time-weighted average concentrations of methylene chloride for an 8-h day were approximately 60 ppm for the 11 employees who worked in jet assembly, 140 ppm for the 162 employees who worked in block I extrusion jobs or on one job in the fiber preparation area, 475 ppm for the 65 employees assigned to block II, cellulose triacetate production, or related activities in the preparation area, and 280 ppm for the 28 employees whose work station was intermediate to the two blocks or whose work assignments involved time in both block I and block II. Acetone exposures were the lowest in block II and in jet assembly (approximately 110 ppm). In block I and many areas of the reference plant the median acetone exposures were a time-weighted average of about 1,000 ppm.

The statistical analyses were based on a multiple regression approach [the software package was SAS 79.5 supplied by the Statistical Analysis System (SAS) Institute Inc, Raleigh, NC]. The covariates used in the analyses were sex, race, age, cigarette smoking history, time of venipuncture, and current intensity of methylene chloride exposure. Additional analyses indicated that the date of examination and the intensity of acetone exposure within the reference plant were not important covariates for any of the blood constituents. Separate analyses were performed for each of the four sex-by-race subgroups of the exposed population. Direct contrasts of laboratory findings between the exposed and reference groups were given less emphasis because of previously outlined differences in the collection and handling of the blood specimens.

Results

The distribution of the health evaluation participants with respect to sex, race, age, and cigarette smoking history is given for both plants in table 1. The considerable disparity in the composition of the total employee populations of each plant, particularly in regard to sex and race, is reflected in the differences seen in the table. The distribution of cigarette smoking varied with sex and race; however, there were no apparent differences between the exposed and reference groups within the sex-by-race subgroups. Among the exposed volunteers only 9 of 266 (3.4 %) had been employed less than one year and 169 (63.5 %) had been employed for more than five years at the time of the examination. In the reference plant, the percentages were 13.9 and 55.1 %, respectively.

In table 2, the mean and standard deviation of each blood constituent is reported by plant, sex, and race. Fisher's method of combining probabilities from tests of sig-

Table 1. Age and smoking history by sex, race, and study group.

	White men				White women							
	Exposed group		Reference group		Exposed group		Reference group		Exposed nonwhite men[a]		Exposed nonwhite women[a]	
	N	%	N	%	N	%	N	%	N	%	N	%
Age group (years)	63		168		107		77		20		76	
< 35	12	19.0	53	31.5	23	21.5	40	51.9	11	55.0	61	80.3
35–44	11	17.5	31	18.5	39	36.4	21	27.3	5	25.0	14	18.4
45–54	29	46.0	23	13.7	41	38.3	12	15.6	4	20.0	1	1.3
55–64	11	17.5	61	36.3	4	3.7	4	5.2	–	0.0	–	0.0
Smoking history												
Never smoked	17	27.0	32	19.0	48	44.9	37	48.1	9	45.0	46	60.5
Former smoker	21	33.3	56	33.3	6	5.6	7	9.1	4	20.0	2	2.6
Current smoker												
< 1 pack/d	2	3.2	5	3.0	10	9.3	6	7.8	1	5.0	15	19.7
≥ 1 pack/d	23	36.5	75	44.6	43	40.2	27	35.1	6	30.0	13	17.1

[a] There were only three nonwhite men and three nonwhite women in the reference population.

nificance (5) was used to compare the means of the blood constituents for the two plants (white men and women only were compared because there were so few nonwhite referents) after stratification by current smoking status, sex, and age (< 45 vs ≥ 45 years). Statistically significant differences between the two plant populations were observed for carboxyhemoglobin ($p < 0.0001$), serum alanine aminotransferase ($p < 0.0001$), total bilirubin ($p < 0.01$), and mean corpuscular hemoglobin concentration ($p < 0.05$).

Separate multiple regression analyses for the four sex-by-race subgroups of the exposed population were performed for

Table 2. Mean and standard deviation of the blood constituents among the health evaluation participants by sex, race, and study group.

	Exposed group								Reference group			
	White men (N = 63)		Nonwhite men (N = 20)		White women (N = 107)		Nonwhite women (N = 76)		White men (N = 168)		White women (N = 77)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	45.3	10.7	34.4	8.7	41.3	9.1	29.6	5.6	43.8	13.9	34.9	11.3
Serum parameters												
Alkaline phosphatase (U/l)	69.7	19.4	66.1	21.9	60.6	18.2	53.1	17.0	70.6	19.5	61.8	17.9
Lactate dehydrogenase (U/l)	191.4	31.2	189.5	29.2	188.1	33.6	189.3	44.0	198.0	39.0	194.6	38.0
Alanine aminotransferase (IU[a])	20.4	13.2	20.5	11.9	11.0	6.0	11.7	11.6	13.1	8.7	7.6	7.2
Aspartate aminotransferase (IU[a])	22.2	12.6	19.9	3.7	16.7	6.3	16.4	6.5	20.6	6.6	16.7	5.9
Total bilirubin (mg/dl[b])	0.7	0.4	0.5	0.3	0.5	0.3	0.4	0.2	0.5	0.2	0.5	0.2
Albumin (g %)	4.2	0.2	4.2	0.2	4.1	0.2	4.0	0.3	4.2	0.2	4.1	0.3
Blood parameters												
Red cell count (10^{12}/l)	5.1	0.3	5.2	0.7	4.6	0.4	4.5	0.3	5.1	0.4	4.6	0.3
Hemoglobin (g/dl[c])	15.8	0.9	15.0	1.0	14.0	1.2	12.9	1.1	16.0	1.1	14.0	1.0
Hematocrit (%)	45.7	2.7	44.7	3.5	41.4	3.3	38.7	2.8	46.8	3.2	41.6	2.8
Mean corpuscular volume (fl)	90.6	4.3	86.3	7.7	90.5	5.8	86.6	5.8	91.7	4.9	91.1	4.5
Mean corpuscular hemoglobin (pg/cell)	31.0	1.6	28.9	3.4	30.6	2.2	29.0	2.3	31.2	1.7	30.8	1.6
Mean corpuscular hemoglobin concentration (%)	34.6	1.0	33.8	1.6	34.1	0.9	33.5	0.9	34.3	0.9	34.0	0.8
Carboxyhemoglobin (saturation %)	4.9	4.1	5.1	4.8	5.2	3.6	4.9	3.8	1.5	2.1	1.1	1.6

[a] 1 IU = 0.48 U/l.
[b] 1 mg/dl = 17.1 μmol/l.
[c] 1 g/dl = 0.155 mmol/l.

Table 3. Summary of associations, based on multiple regression analyses, between the intensity of methylene chloride exposure and 13 blood constituents.

Population subgroup	Blood constituent	Slope[a]	p-Value
Nonwhite men (N = 20)	Carboxyhemoglobin (%)	2.1	0.005
Nonwhite women (N = 76)	Carboxyhemoglobin (%)	1.0	0.003
	Total bilirubin (mg/dl[b])	0.06	0.020
White men (N = 63)	Carboxyhemoglobin (%)	1.0	0.0003
	Total bilirubin (mg/dl[b])	0.08	0.012
White women (N = 107)	Carboxyhemoglobin (%)	0.7	0.0002
	Total bilirubin (mg/dl[b])	0.05	0.0007
	Red cell count (10^{12}/l)	0.06	0.004
	Hematocrit (%)	0.47	0.016
	Hemoglobin (g/dl[c])	0.16	0.028
	Aspartate aminotransferase (IU[d])	0.78	0.046

[a] Units of change in blood constituents per 100 ppm of methylene chloride.
[b] 1 mg/dl = 17.1 μmol/l.
[c] 1 g/dl = 0.155 mmol/l.
[d] 1 IU = 0.48 U/l.

each of the blood constituents. Independent covariates included smoking category, age, and time of venipuncture, in addition to intensity of methylene chloride exposure. A summary of the statistical findings in regard to intensity of methylene chloride exposure is presented in table 3. A positive association between carboxyhemoglobin and methylene chloride exposure was anticipated for all four subgroups. Further analysis indicated that methylene chloride exposure, cigarette smoking history, and time of venipuncture were important predictors of carboxyhemoglobin within the exposed group, but that sex, race, and age of subject were unimportant in predicting carboxyhemoglobin. A more rigorous treatment of carboxyhemoglobin in relation to methylene chloride exposure is available in a separate paper (10).

A consistent positive association between total bilirubin and methylene chloride exposure was also found in three of the four population subgroups. In addition, the mean bilirubin of the exposed group was statistically higher than that of the reference group. These relationships have been depicted in fig 1 via frequency plots of total bilirubin by methylene chloride exposure. The differences in total bilirubin are less apparent among the women than among the men. Among the men, there was a shift in mean total bilirubin and also a flattening of the distribution curve with increased intensity of current exposure.

The findings obtained for alanine aminotransferase were inconsistent, depending on whether results from the reference plant were considered or not. The mean serum alanine aminotransferase was sta-

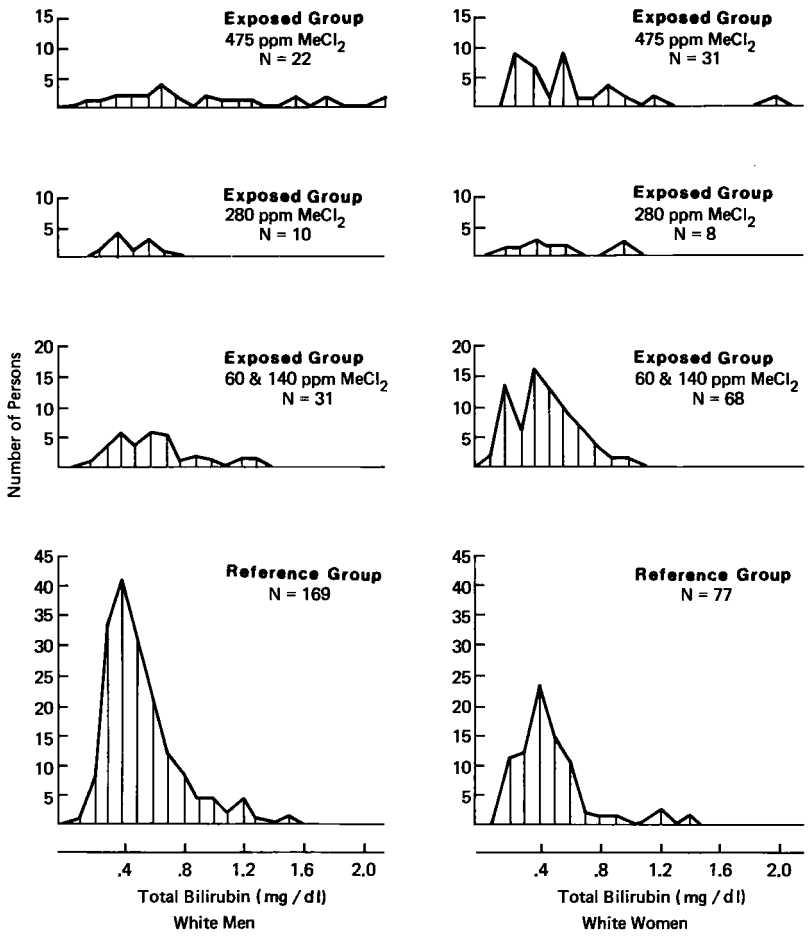

Fig 1. Total bilirubin levels of the white men and women in the exposed group, by exposure level, and in the reference group. ($MeCl_2$ = methylene chloride) (1 mg/dl = 17.1 μmol/l)

tistically higher in the exposed than in the reference group. However, no association with increased methylene chloride exposure was observed when only the exposed group was examined. These relationships are summarized in fig 2.

There was a statistical increase in red cell count with intensity of exposure among the white women of the exposed group, but not among the white men. Parallel increases in hematocrit, hemoglobin, and aspartate aminotransferase were also found among the white women but not among the white men or the nonwhites of either sex. The distributions of red cell counts by methylene chloride exposure are summarized in fig 3. For the women there was a higher mean red cell count and increased spread in the distribution curve with increased exposure. No similar trend was evident for the men. The relative increase in red cell count with exposure was greater among the women who smoked than among those who did not smoke, particularly in the under 45 age group. Based on a fitted regression equation for white women under age 45 who smoked (exposed group only), the mean red cell count was 4.39×10^{12}/l cells for 0 ppm, 4.53×10^{12}/l cells for 140 ppm, and 4.88×10^{12}/l cells for 475 ppm of methylene chloride. The corresponding estimates for nonsmokers were 4.39, 4.44, and 4.55×10^{12}/l cells, respectively.

Discussion

The carboxyhemoglobin concentrations measured in the present study showed the expected rise with both cigarette smoking

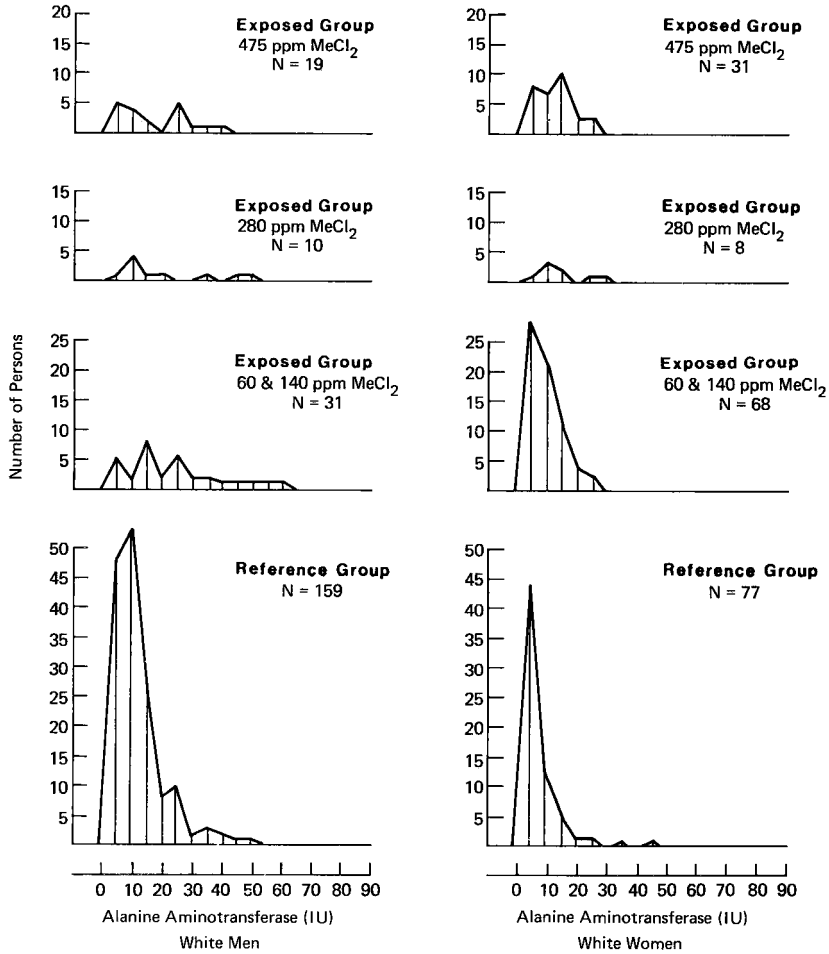

Fig 2. Serum alanine aminotransferase levels of the white men and women in the exposed group, by exposure level, and in the reference group. ($MeCl_2$ = methylene chloride) (1 IU = 0.48 U/l)

and increased methylene chloride exposure. A number of the other associations found in this investigation were not anticipated.

Serum alanine aminotransferase was statistically increased for the exposed employees in comparison to that of the referents, but the increase was not related to intensity of methylene chloride exposure within the exposed population. The shift of between 3.4 and 7.3 IU (1.6 & 3.5 U/l, respectively) (see table 2) was probably due to differences in the collection and handling of the blood specimens from the two plants, particularly since it was not supported by a dose-response relationship among the exposed population. Total bilirubin was positively correlated with methylene chloride exposure among both the men and the women. This finding, if observed in conjunction with other positive indicators, could point to either liver injury or hemolysis. However, concomitant elevations of neither alkaline phosphatase nor the transaminases were found on a group basis or among the six exposed individuals (four white and two nonwhite) with total bilirubin in excess of 1.4 mg/dl (23.9 μmol/l). In addition no employees from the plant with methylene chloride exposure reported a history of cholestasis. Furthermore supporting evidence for increased red cell turnover was also lacking. No dose-response relationship was found for lactate dehydrogerase, and there was no decline in the red cell count, hemoglobin, or hematocrit, either in the group with the highest exposure or among persons with high serum bilirubin. Unfortunately, additional tests which could have clarified the

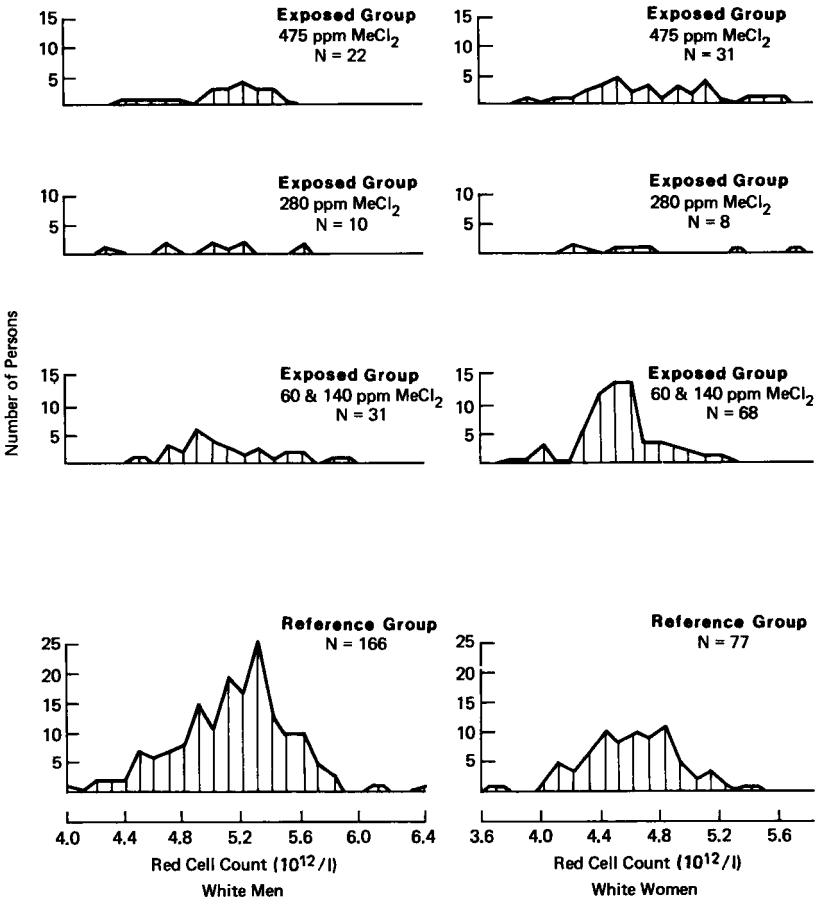

Fig 3. Red cell counts of the white men and women in the exposed group, by exposure level, and in the reference group. ($MeCl_2$ = methylene chloride)

situation further (eg, direct bilirubin and reticulocyte counts) were not performed.

Among the exposed white women red cell count, hematocrit, and hemoglobin were increased in the group exposed to the highest methylene chloride concentrations; however similar results were not obtained for the exposed white men. The findings among the women, especially those who smoked cigarettes, are consistent with a compensatory response similar to that observed with high altitude hypoxia. The partial pressure of oxygen at which hemoglobin is 50% saturated (P_{50}) was measured in 132 referents and 136 exposed employees as part of the present research (10). Interestingly, there was a slight differential sex effect in P_{50} in relation to methylene chloride exposure and cigarette smoking. The difference occurred because nonexposed nonsmoking women had average P_{50} determinations about 0.8 mm Hg (106.7 Pa) higher than nonexposed, nonsmoking men, whereas, among cigarette smokers in the highest methylene chloride exposure group, there was no difference in P_{50} between the men and women. It has been suggested that men have higher baseline hemoglobin, which is at least partly compensated for by a greater oxygen-releasing capacity of the red cells of women under standard conditions (3). The apparent loss of this compensatory advantage, as suggested by the P_{50} measurements, may offer an explanation for the hematologic changes that were observed in the exposed women but not in the men.

The hematologic patterns associated with methylene chloride exposure among the women were similar to those seen with cigarette smoking, except that the mean corpuscular volume and mean corpuscular hemoglobin findings were not elevated with methylene chloride exposure. On the basis of parallel analyses in the two plants in question, the mean corpuscular volume was significantly increased among the white cigarette-smoking men and women in both plants. Hematocrit, hemoglobin, and mean corpuscular hemoglobin were also increased in each subgroup among the cigarette smokers. Our observations in regard to cigarette smoking are consistent with those reported in other studies using the Coulter model S to examine cigarette smokers. Thus there are differences in the hematologic profiles, particularly in regard to mean corpuscular volume and mean corpuscular hemoglobin between cigarette smokers and individuals exposed to methylene chloride.

In conclusion, the expected changes in carboxyhemoblogin were found, but there were also changes in serum and blood constituents, a finding suggestive of a hematopoietic effect, as evidenced by increased red cell counts in the white women exposed to methylene chloride concentrations of a time-weighted average of approximately 475 ppm. The bilirubin findings were statistically significant but were not supported by parallel changes in other serum and blood constituents.

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