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Health evaluation of employees occupationally exposed to methylene chloride: metabolism data and oxygen half-saturation pressures

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Key terms: carboxyhemoglobin; employee; health evaluation; hemoglobin oxygen affinity; metabolism; metabolism data; methylene chloride; occupational exposure; oxygen half-saturation pressure; oxygen pressure; oxygen transport

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Health evaluation of employees occupationally exposed to methylene chloride

Metabolism data and oxygen half-saturation pressures

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OTT MG, SKORY LK, HOLDER BB, BRONSON JM, WILLIAMS PR. Health evaluation of employees occupationally exposed to methylene chloride: Metabolism data and oxygen half-saturation pressures. *Scand j work environ health* 9 (1983): suppl 1, 31–38. The in vivo metabolism of methylene chloride to form carboxyhemoglobin has been demonstrated in a number of experimental studies. The present investigation was undertaken to quantitate the relationships between methylene chloride exposure, the percentage of carboxyhemoglobin saturation, the alveolar carbon monoxide level, and the oxygen half-saturation pressure in an occupational setting. As expected, methylene chloride exposure produced dose-related increases in the level of carboxyhemoglobin and alveolar carbon monoxide and a corresponding decrease in the oxygen half-saturation pressure. The calculated decrease in oxygen half-saturation pressure was between 2 and 4 mm Hg (266.6 & 533.3 Pa, respectively) among persons exposed to ≥ 300 ppm of methylene chloride for an 8-h workday. Additional analyses indicated that this shift in the oxyhemoglobin dissociation curve could be explained on the basis of the known carbon monoxide interactions with hemoglobin.

Key terms: carboxyhemoglobin, hemoglobin oxygen affinity, oxygen transport.

The discovery that methylene chloride ($MeCl_2$) is biotransformed in the body to form carboxyhemoglobin (COHb) has raised questions as to what effect methylene chloride exposure may have on tissue oxygenation (1, 16). Carboxyhemoglobin, produced because of the high affinity of carbon monoxide for hemoglobin, affects oxygen transport in two ways – first by partially replacing oxyhemoglobin with carboxyhemoglobin and second by increasing oxygen affinity for hemoglobin through carbon monoxide, oxygen, and hemoglobin interactions (3).

Methylene chloride is also capable of directly interacting with hemoglobin, as evidenced by its antisickling properties (9, 13). It was first suggested that methylene chloride might increase the affinity of carbon monoxide for hemoglobin and thereby account in part for the increased carboxyhemoglobin seen with methylene chloride exposure (14). However, subsequent in vitro studies using whole blood have shown that low partial pressures of methylene chloride do not influence carbon monoxide binding to hemoglobin (4, 5). The immediate objective of the present study was to quantitate, in an occupational setting, the interrelationships among methylene chloride exposure, oxygen half-saturation pressures, blood carboxyhemoglobin, and alveolar carbon monoxide. The broader objective was to provide an additional perspective to the mor-

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tality and blood constituent findings described in earlier reports (10, 11, 12).

Materials and methods

Two hundred and sixty-eight employees from two fiber production plants voluntarily participated in the present investigation. In one plant, employees were exposed to methylene chloride, acetone, and methanol, the methanol being present in a ratio of about 1 to 10 to methylene chloride. Employees of the reference plant were exposed to similar acetone concentrations but not to methylene chloride or methanol. On the basis of results of an intent-to-participate questionnaire, individuals were entered into the study so as to achieve approximately equal numbers of participants from the two plants among both the men and women. The distribution of the 268 employees by sex, race, smoking habits, and plant is given in table 1. There were more nonwhite men and women in the exposed group, a reflection of the larger numbers of nonwhites at that plant location. The age range of participants was 19 to 62 years in the plant with methylene chloride exposure and 21 to 63 years in the reference plant, the average age being 40.4 and 39.4 years, respectively. There were geographic differences between the two plants, the former being located in flat terrain (elevation of 169 m above sea level) and the latter being situated in a mountainous area (elevation of 483 m with surrounding elevations of 1,280 m above sea level). The altitude differences account for approximately 5 to 14 % lower partial oxygen pressures at the reference plant site.

Table 1. Distribution of the study participants by sex, race, and smoking habits.

Population subgroup	Exposed employees (N = 136)	Referents (N = 132)
Women		
White		
Nonsmokers	29	41
Smokers	39	26
Nonwhite		
Nonsmokers	6	1
Smokers	7	2
Total	81	70
Men		
White		
Nonsmokers	23	27
Smokers	19	33
Nonwhite		
Nonsmokers	8	1
Smokers	5	1
Total	55	62

The design of the study permitted the estimation of possible drift in the measurements over time since about one-fourth of the referents were evaluated first, then all of the exposed participants, and finally the remaining referents. The following variables were determined twice for each participant, immediately preceding and following the workshift: blood carboxyhemoglobin, alveolar carbon monoxide, and the partial oxygen pressure required to keep 50 % of the blood oxygen-carrying capacity saturated with oxygen at pH 7.4 and 37°C (P_{50}). Personal monitoring for the methylene chloride exposure of each employee was carried out during the workshift to coincide with the time period between the two blood samples. The environmental monitoring procedures have been described elsewhere (10).

Alveolar carbon monoxide was analyzed with an Ecolyzer series 2200 carbon monoxide analyzer from the end expiratory air samples collected in a Saran® bag. Carboxyhemoglobin was determined by the Diagnostic Laboratories, Charlotte, NC, with a co-oximeter IL 182 three-wavelength differential spectrophotometer.

Analytical procedure for the determination of the oxygen half-saturation pressures

The measurement of P_{50} requires determination or knowledge of the partial oxygen pressure and the corresponding oxygen saturation percentage and pH. The pH was measured with a PHM73 pH meter supplied by the London Company, Cleveland, OH. Oxygen saturation was measured with the OSM2 hemoximeter (manufactured by Radiometer, Copenhagen). A general procedure for using the OSM2 hemoximeter to determine P_{50} has been presented by Siggaard-Andersen (15). While it is possible to measure actual venous pH, partial oxygen pressure, and oxygen saturation, an equilibration procedure was selected since (i) the partial oxygen pressure calculated from the oxy-

gen concentration of the equilibrating gas is not subject to the variance attending direct measurements of partial oxygen pressure and (ii) after equilibration all samples have approximately the same partial oxygen and carbon dioxide pressures so that oxygen saturation readings are within a narrower range. Three tanks of equilibration gas with a gas mixture of 3.7 % oxygen, 5.6 % carbon dioxide, and the balance nitrogen were supplied by Matheson Gas, Chicago, IL. The oxygen content of each tank was analytically measured to three significant digits. The partial oxygen pressure of the equilibrating gas was corrected for changes in barometric pressure on a twice daily basis.

About 5 ml of blood was drawn from an antecubital vein, half of that amount being used for the determination of total hemoglobin and carboxyhemoglobin, the remainder being used for the P_{50} determination. The measurement of P_{50} was performed in duplicate with two tonometers (model IL 237, Instrumentation Laboratories, Lexington, MA). After a 9-min equilibration period, blood samples were extracted and directly injected into both the hemoximeter and the pH electrode, recording, in order, the oxygen saturation and pH twice. The same procedure was then repeated with a blood sample from the second tonometer. A major concern was the stability of the carboxyhemoglobin during tonometry for 9 min with a carbon monoxide-free gas. However, experiments showed that, at the low oxygen concentration in the equilibration gas, the loss of carbon monoxide was only about 1 to 2 % of the amount present in the sample. Thus the impact of carboxyhemoglobin stability on the P_{50} was considered to be minimal under the conditions of the study.

Computations of P_{50} were based on the equation given by Siggard-Anderson using the single-point principle (15). The extrapolation to 50 % oxygenation was based on Hill's plot with an assumed slope of 2.7. Extrapolation to a constant pH of 7.4 assumed a Bohr coefficient of −0.4. Oxygen saturation was expressed as a percentage of the total available hemoglobin rather than as the percentage of total hemoglobin, a convention followed by Collier (3). The expression for calculating the oxygen saturation percentage was 100 x (hemoximeter reading % − COHb %)/(100 − COHb %). Although the slope in the Hill equation varies slightly depending on the presence of carboxyhemoglobin (3), this variability had little effect on the extrapolation to the P_{50} as the oxygen saturation readings were all between 39 and 70 %.

Collier obtained a measure of the shift in the oxyhemoglobin dissociation curve in the presence of carboxyhemoglobin by first determining the P_{50} with or without carboxyhemoglobin present and then using an empiric correction to remove carboxyhemoglobin effects (3). Estimation of the corresponding carbon monoxide-free P_{50} may be performed with an empiric procedure analogous to that used by Collier or with the equations given by Ledwith (8). Both approaches were used in the present investigation.

Results

The distribution of the individual methylene chloride exposure measurements, as obtained by personal monitoring on the day of the P_{50} determination, is given in fig 1. Monitoring data were available for 135 of the 136 employees who participated in this aspect of the health surveillance program. The bimodal distribution of the methylene chloride exposure was due to differences in the levels of methylene chloride use in two separate blocks of the plant (10).

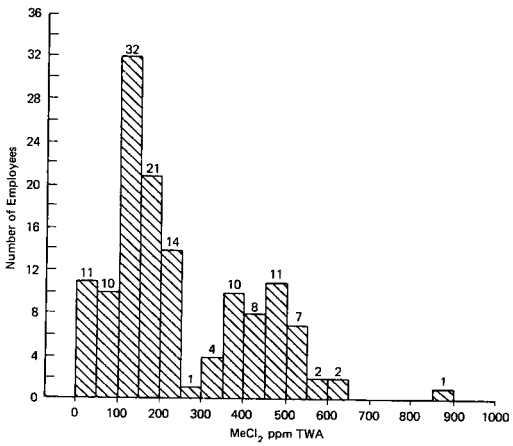

Fig 1. Distribution of employee exposures to methylene chloride ($MeCl_2$) on the day of the oxygen half-saturation pressure (P_{50}) determination. (TWA = time-weighted average)

The relationships between methylene chloride exposure and carboxyhemoglobin percentage saturation before and after the shift are depicted in fig 2. Separate equations were fit for the smokers and nonsmokers; however the smoking contribution to the carboxyhemoglobin level was nearly constant across the methylene chloride exposure concentrations both before and after the shift. The linear association between methylene chloride exposure and the carboxyhemoglobin level before the shift reflects residual carbon monoxide metabolism associated with previous-day exposure to methylene chloride. The significant quadratic fit for postshift carboxyhemoglobin indicates partial saturation of the enzyme system for metabolizing methylene chloride. Very similar results were obtained when alveolar carbon monoxide was related to methylene chloride exposure (see fig 3). This finding would be expected in view of the relationship between alveolar carbon monoxide and carboxyhemoglobin. The correlation between the change in carboxyhemoglobin during the workshift and the corresponding change in alveolar carbon monoxide was 0.79.

Several quality assurance steps were taken to evaluate the possibility of drift in the P_{50} determinations during the course of the study. As previously mentioned, the measurements of P_{50} in the reference plant were made both before and after completion of the measurements among the exposed participants. There was only a 0.2 mm Hg (26.7 Pa) difference in the group mean P_{50} for nonsmoking referents in the November 1977 period in comparison with the January-February 1978 period, adjustments being made for differences in the sex distribution of the participants. In addition, there were no significant associations of P_{50} with change of equilibrating gas tanks or with pressure changes in the tanks.

The means and standard deviations of the methylene chloride exposure, standard P_{50}, and carbon monoxide-free P_{50} deter-

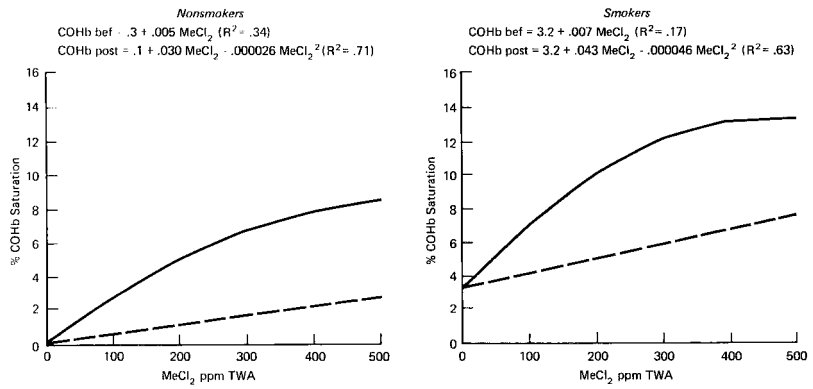

Fig 2. Relationship of the carboxyhemoglobin (COHb) level before (bef) and after (post) the shift to the methylene chloride ($MeCl_2$) exposure of the smokers and nonsmokers. (TWA = time-weighted average, — — — = before-shift values, ——— = after-shift values)

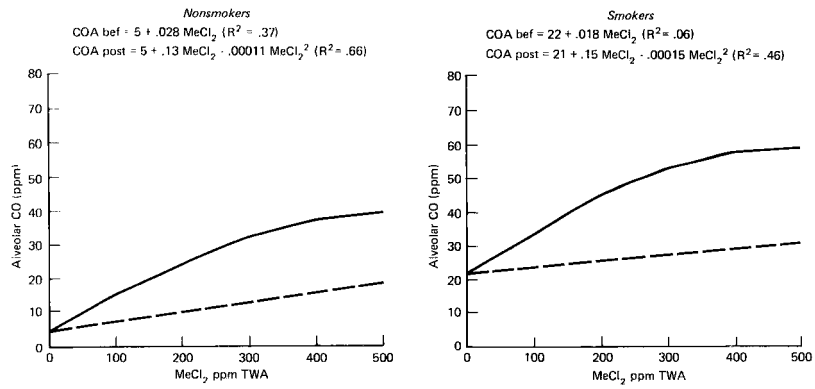

Fig 3. Relationship of the alveolar carbon monoxide (COA) level before (bef) and after (post) the shift to the methylene chloride ($MeCl_2$) exposure of the smokers and nonsmokers. (TWA = time-weighted average, — — — = before-shift values, ——— = after-shift values)

Table 2. Means and standard deviations of the time-weighted average of the methylene chloride ($MeCl_2$) exposure and the standard oxygen half-saturation pressure (P_{50}) and carbon monoxide-free (CO-free) P_{50} before and after the workshift by sex and smoking status.

Population subgroup	$MeCl_2$ (ppm)		Standard P_{50} (mm Hg[a])				Co-free P_{50} (mm Hg[a])			
			Before		After		Before		After	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Reference group										
Women										
Nonsmokers (N = 42)	..		26.9	0.8	27.1	0.7	27.1	0.8	27.2	0.7
Smokers (N = 28)	..		24.8	1.2	24.9	1.0	25.9	1.1	25.9	1.0
Men										
Nonsmokers (N = 28)	..		26.1	0.8	26.4	0.8	26.2	0.7	26.5	0.8
Smokers (N = 34)	..		24.3	0.9	24.6	1.0	25.1	0.8	25.4	0.8
Exposed group										
Women										
Nonsmokers (N = 35)	261	170	25.8	1.3	24.8	1.3	26.1	1.2	26.0	1.2
Smokers (N = 46)	225	151	24.7	1.2	23.6	1.4	26.0	1.0	25.8	1.3
Men										
Nonsmokers (N = 31)	252	201	25.1	1.2	23.9	1.5	25.5	1.0	25.1	1.3
Smokers (N = 24)	220	167	24.3	1.1	23.3	1.4	25.4	1.2	25.5	1.3

[a] 1 mm Hg = 133.3 Pa.

Table 3. Multiple regression analysis of the oxygen half-saturation pressure (P_{50}) and the carbon monoxide-free (CO-free) P_{50}.

Covariate	Dependent variable[a]			
	P_{50} (mm Hg[b])		CO-free P_{50} (mm Hg[b])	
	Slope	p-Value	Slope	p-Value
Before shift				
Sex (female = 2, male = 1)	0.41	0.011	0.41	0.015
Age (years)	0.021	0.0002	0.021	0.0003
Group (reference = 2, exposed = 1)	0.21	0.177	0.35	0.041
Hemoglobin (g/dl[c])	−0.25	0.0001	−0.29	0.0001
Race (nonwhite = 2, white = 1)	0.53	0.006	0.58	0.005
Carboxyhemoglobin (% saturation)	−0.30	0.0001	−0.04	0.071
Acetone (ppm TWA[d])	0.00004	0.772	0.00005	0.761
Methylene chloride (ppm TWA)	−0.0007	0.180	−0.0007	0.176
After shift				
Sex (female = 2, male = 1)	0.40	0.021	0.49	0.010
Age (a)	0.024	0.0001	0.023	0.0006
Group (reference = 2, exposed = 1)	0.52	0.005	0.56	0.003
Hemoglobin (g/dl[c])	−0.23	0.0001	−0.24	0.0001
Race (nonwhite = 2, white = 1)	0.22	0.302	0.26	0.259
Carboxyhemoglobin after-shift (% saturation)	−0.23	0.0001	−0.05	0.064
Acetone (ppm TWA)	−0.0001	0.426	−0.0002	0.226
Methylene chloride (ppm TWA)	−0.0012	0.035	−0.0011	0.066

[a] Significance levels determined based on each covariate being the last entered into the equation.
[b] 1 mmg Hg = 133.3 Pa.
[c] 1 g/dl = 0.155 mmol/l.
[d] TWA = time-weighted average.

Table 4. Means and standard deviations of the before- and after-shift oxygen half-saturation pressure (P_{50}) and carbon monoxide-free (CO-free) P_{50} by sex, smoking history, and methylene chloride ($MeCl_2$) exposure intensity.

Population subgroup	Number of participants	Time-weighted average of $MeCl_2$ exposure (ppm)	P_{50} (mm Hg[a])				CO-free P_{50} (mm Hg[a])			
			Before		After		Before		After	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Nonsmoking men										
0 ppm $MeCl_2$ (referents)	28	..	26.1	0.8	26.4	0.8	26.2	0.7	26.5	0.8
< 100 ppm $MeCl_2$	7	44	25.8	1.1	25.6	1.1	25.8	1.2	25.8	1.5
100–299 ppm $MeCl_2$	14	179	25.3	1.2	23.9	1.3	25.5	1.1	25.2	0.9
≥ 300 ppm $MeCl_2$	10	501	24.4	0.9	22.7	1.0	25.2	0.8	24.6	1.6
Smoking men										
0 ppm $MeCl_2$ (referents)	34	..	24.3	0.9	24.6	1.0	25.1	0.8	25.4	0.8
< 100 ppm $MeCl_2$	9	47	24.7	1.0	24.4	1.0	25.4	1.2	25.9	1.1
100–299 ppm $MeCl_2$	5	172	23.9	1.6	23.1	1.4	25.5	1.5	25.1	0.9
≥ 300 ppm $MeCl_2$	10	400	24.3	1.0	22.5	1.0	25.3	1.0	25.3	1.5
Nonsmoking women										
0 ppm $MeCl_2$ (referents)	42	..	26.9	0.8	27.1	0.7	27.1	0.8	27.2	0.7
< 100 ppm $MeCl_2$	2	25	27.0	1.0	26.2	0.5	27.0	1.0	26.6	0.1
100–299 ppm $MeCl_2$	20	154	26.0	0.8	25.2	0.9	26.2	0.9	26.0	1.0
≥ 300 ppm $MeCl_2$	13	461	25.3	1.7	24.1	1.4	25.9	1.6	26.0	1.4
Smoking women										
0 ppm $MeCl_2$ (referents)	28	..	24.8	1.2	24.9	1.0	25.9	1.1	25.9	1.0
< 100 ppm $MeCl_2$	4	47	26.1	0.8	25.5	0.9	26.9	0.5	26.6	0.7
100–299 ppm $MeCl_2$	29	152	24.9	1.1	23.9	1.3	26.0	0.9	25.9	1.4
≥ 300 ppm $MeCl_2$	13	444	23.8	1.0	22.3	0.8	25.5	1.0	25.3	0.9

[a] 1 mm Hg = 133.3 Pa.

minations are given in table 2. The P_{50} group means were lower among the exposed participants than among the referents, and among the exposed employees there was a decrease in P_{50} during the shift, as one might anticipate in view of the relationship between P_{50} and carboxyhemoglobin. The smokers also had lower P_{50} values than the nonsmokers, and the men had lower P_{50} values than the women.

A multiple linear regression analysis was employed to help sort out the interrelationships among the P_{50} values and other covariates. Both the standard P_{50} and the P_{50} corrected for the presence of carboxyhemoglobin were considered to be dependent variables. Covariates considered in the analysis as either independent factors or stratification variables included sex, race, age, smoking history, before versus after shift, carboxyhemoglobin, hemoglobin, location of plant, and methylene chloride and acetone exposure concentrations. The regression results summarized in table 3 indicate that sex, age, location, hemoglobin, race, and carboxyhemoglobin were each independently associated with P_{50}.

The empirical equation developed by Collier to account for the carboxyhemoglobin-induced shift in P_{50} was based on a slope of −0.3 relating change in carboxyhemaglobin to P_{50} (3). Our findings of a preshift slope of −0.30 and postshift slope of −0.23 for relating change in carboxyhemoglobin to P_{50} agree well with the slope estimates derived by Collier.

Group analysis of the P_{50} values was also undertaken separately for the men and women subcategorized by cigarette smoking status. With the recognition that the P_{50} differences between the exposed and reference populations may be related to geographic location, as well as to methylene chloride exposure, emphasis was placed on comparison among the three methylene chloride exposure categories of the exposed employees. From table 4 a left shift in the oxyhemoglobin dissociation curve is apparent both in the morning (evidently due to the residual percentage of carboxyhemoglobin saturation) and after the workday. Among individuals exposed to ≥ 300 ppm of methylene chloride the P_{50} decreased by 1 to 2 mm Hg (133.3 to 266.6 Pa) during the workday. Thus, in a comparison of the postshift averages for persons exposed to methylene chloride levels of ≥ 300 ppm to preshift averages for persons exposed to < 100 ppm, the calculated decreases in P_{50} were between 2 and 4 mm Hg (266.6 & 533.3 Pa, respectively).

Discussion

The relationship between the percentage of carboxyhemoglobin saturation, alveolar carbon monoxide, and methylene chloride exposure, as determined in this observational study, agree reasonably well with experimental findings reported by DiVincenzo & Kaplan (6). For example, these investigators found that carboxyhemoglobin increased from 1 % at 0 ppm to 3.4 % at 100 ppm, and 6.8 % at 200 ppm of methylene chloride for 14 nonsmokers (single day exposure). With exposures repeated over five consecutive days with a single volunteer, baseline carboxyhemoglobin saturations rose to about 2 % at 200 ppm of methylene chloride. These authors also reported a decline in the percentage of carboxyhemoglobin saturation within 2 h of the last exposure (the decrease was about 2 % of the carboxyhemoglobin level at 200 ppm of methylene chloride). In the present study, carboxyhemoglobin was measured after the employees had finished work and reported to the Medical Department. Since a number of employees were evaluated each day, the interval between the last exposure and venipuncture could have been as much as 1 h. In addition, exposures in the work setting were variable rather than constant. Thus, carboxyhemoglobin saturations of 0.1 % at 0 ppm, 2.8 % at 100 ppm, and 5.1 % at 200 ppm of methylene chloride are in line with the values arrived at experimentally. The low levels of methanol present in this study were insufficient to affect any changes in the carboxyhemoglobin formation rates based on experimental findings by Ciuchta et al (2).

The evaluation of P_{50} and carbon monoxide-free P_{50} revealed an expected dose-dependent effect of methylene chloride on P_{50}, mediated through the presence of carboxyhemoglobin. The magnitude of the effect in employees exposed to over 300 ppm of methylene chloride was on the order of 2 to 4 mm Hg (266.6 to 533.3 Pa). Because of the rapid elimination of methylene chloride from blood (6), the present investigation could not reliably assess the direct effects of methylene chloride on hemoglobin function. However, it has been shown experimentally that low partial pressures of methylene chloride do not influence carbon monoxide binding to hemoglobin (4, 5). In addition regression analyses with carbon monoxide-free P_{50} as the dependent variable did not reveal a significant independent effect for methylene chloride. Thus the presumed effects of methylene chloride on tissue oxygenation would be those projected on the basis of equivalent carboxyhemoglobin saturations derived from carbon monoxide exposure.

Apparent compensatory changes in oxygen transport were observed for one subgroup of exposed employees. Hematologic findings for this employee subgroup, women who smoked cigarettes and were assigned to work areas of high methylene chloride use, included an increase in red cell count, hemoglobin, and hematocrits (12). These observations are supported by the higher carboxyhemoglobin and lower P_{50} findings for these women in the present study.

Because carboxyhemoglobin elevations may reduce oxygen availability to the heart muscle, it has been postulated that the risk of ischemic events could be increased by exposure to methylene chloride. Death rates due to ischemic heart disease in the plant with methylene chloride exposure were not significantly elevated in relation to the corresponding rates of the general population of the United States (14 observed vs 13.2 expected deaths for active and inactive employees and 8 observed vs 7.4 expected deaths among active employees only) (11). Long-term follow-up of another industrial population exposed to 30 to 125 ppm of methylene chloride did not reveal an excess risk for ischemic heart disease either (7). Thus other aspects of the present health surveillance program indicate that physiological adaptations may have occurred, but evidence of adverse cardiovascular outcomes is lacking in the present study and in one other investigation with an occupational cohort exposed to methylene chloride.

Acknowledgments

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