Effect of Exercise or Smoking on the Uptake, Metabolism, and Excretion of Methylene Chloride Vapor

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Effect of Exercise or Smoking on the Uptake, Metabolism, and Excretion of Methylene Chloride Vapor. DiVINCENZO, G. D., and KAPLAN, C. J. (1981). Toxicol. Appl. Pharmacol. 59, 141–148. The finding that CH₂Cl₂ is metabolized in vivo to carbon monoxide (CO) prompted us to study the absorption, metabolism, and excretion of CH₂Cl₂ in humans and in experimental animals, and to extend such studies to include the effect of physical exercise and smoking. Controlled exposures to CH₂Cl₂ were carried out at 100 ppm for 7½ hr with exercising or smoking individuals. Exercise was performed on a treadmill at light, moderate, or heavy workloads. Cigarettes were smoked during and after exposure to CH₂Cl₂. The concentration of CH₂Cl₂ in the blood and the end tidal air, of CO in the end tidal air, and of carboxyhemoglobin in the blood were determined during and after the exposure. Physical exercise increased the absorption of CH₂Cl₂, the biotransformation of CH₂Cl₂ to CO₂, blood carboxyhemoglobin saturations and the pulmonary excretion of CO compared to those values obtained from sedentary exposures. It is noteworthy, however, that there was no further increase in blood carboxyhemoglobin saturations in individuals exercising at moderate to heavy workloads. This was apparently due to a marked increase in the pulmonary excretion of CO while performing heavy workloads. The combined effect of smoking and exposure to CH₂Cl₂ produced an additive increase in blood carboxyhemoglobin values. These findings show that smokers or physically active workers exposed to 100 ppm of CH₂Cl₂ vapor may have slightly higher blood carboxyhemoglobin saturations than do sedentary nonsmokers.

The finding that methylene chloride (CH₂Cl₂) was metabolized to carbon monoxide (CO) in humans (Stewart et al., 1972) prompted us to examine workers exposed to CH₂Cl₂ vapor and to conduct controlled experimental human exposures (DiVincenzo and Kaplan, 1981). Since most experimental exposures have been conducted with sedentary nonsmokers, the effect of exercise or of smoking on the uptake, metabolism, and elimination of CH₂Cl₂ has not been fully investigated. Astrand et al. (1975) measured blood carboxyhemoglobin (COHb) values in volunteers exercising on a bicycle ergometer for up to 2 hr while exposed to either 250 or 500 ppm of CH₂Cl₂ vapor. As expected, exercise produced higher blood COHb saturations than found under sedentary conditions.

The National Institute of Occupational Safety and Health (NIOSH) has recommended that the time-weighted average exposure to CH₂Cl₂ should not produce blood COHb saturations that exceed 5.0% (NIOSH, 1976), and on that basis has suggested a biological threshold limit value for CH₂Cl₂. Cigarette smokers, however, frequently have blood COHb saturations that are greater than 5%. To our knowledge, the combined effect of smoking and exposure to CH₂Cl₂ vapor has not been investigated.

This paper describes the pulmonary ab-
sorption, metabolism, and elimination of CH₂Cl₂ by humans experimentally exposed to CH₂Cl₂ vapor while either exercising or smoking.

METHODS

Inhalation exposures were performed with Eastman grade CH₂Cl₂ which was greater than 99% pure as determined by gas chromatography. All the other chemicals were reagent grade and unless stated otherwise were not purified further.

Experimental human exposure and exercise studies. Protocols for experimental human exposures to CH₂Cl₂ were approved by a corporate Human Studies Review Committee. Informed consent was obtained from each volunteer.

Three male employees (nonsmokers), aged 23 to 27 years, volunteered to participate in experimental exposures to CH₂Cl₂. Approval to participate was given following a physical examination, an EKG, and a battery of hematologies and blood chemistries. These included CBC, glucose, blood urea nitrogen, uric acid, sodium, potassium, chloride, cholesterol, triglyceride, ornithine carbamyltransferase and aspartate aminotransferase activities, and serum and urine formaldehyde and formic acid determinations. Both the blood work and the EKG were repeated on the day following the exposure.

Volunteers were stress tested on a four-stage sub-maximal treadmill to determine heart rate and oxygen consumption at different work intensities, and to predict aerobic capacity. Workloads equivalent to 50, 100, and 150 W as reported by Astrand et al. (1975) were determined for each individual. During exposures, heart rate was monitored to permit control of workload and, thereby, of cardiac output. Workloads ranged from 14 to 28 ml O₂ min⁻¹ kg⁻¹ body wt (25 to 70% of predicted aerobic capacity). Because the volunteers exercised for only 5 min of each 15-min period, the average oxygen consumption for the 7½-hr period ranged from 7 to 12-ml O₂ min⁻¹ kg⁻¹, similar to workloads observed in many industrial jobs. Exercise was carried out on an Avionics® treadmill (Model E-10B) during exposure to CH₂Cl₂.

The exposure facility has been described elsewhere (DiVincenzo and Kaplan, 1981). Inhalation exposures were conducted at 100 ppm and consisted of 4 hr of exposure, interrupted by a half-hour period and then followed by 3½ hr of exposure. Sedentary exposures for five volunteers were described in a companion paper (DiVincenzo and Kaplan, 1981).

Smoking studies. Two male smokers, 24 and 26 years of age, were exposed for 7½ hr to 100 ppm of CH₂Cl₂. Both individuals smoked about one pack of cigarettes each day. The experimental protocol included adherence to a smoking schedule and use of the same brand of cigarettes by both individuals (100-mm filter cigarettes). Four cigarettes were smoked during the 2 hr preceding the exposure to CH₂Cl₂; two cigarettes were smoked outside the exposure room at 2 and 6 hr during the exposure, and three cigarettes were smoked during lunch. After the exposure one cigarette was smoked every 30 min for 6½ hr. The combined smoking and CH₂Cl₂ exposure was preceded by a control period in which the same protocol was followed with the exception that the subjects were not exposed to CH₂Cl₂ vapor.

Breath, blood, and urine collections. End tidal air was collected in Saran bags immediately prior to entering the exposure facility, and during and after the exposure. Blood was collected by venipuncture into heparinized containers before, during and after the exposure. Urine was collected at regular intervals for 24 hr and was analyzed for CH₂Cl₂ as described elsewhere (DiVincenzo, et al., 1971). Formaldehyde concentrations in the urine and serum were analyzed by the Hantzsch reaction ( Nash, 1953). Formic acid was determined gas chromatographically (James and Davison, 1965). All urine samples were frozen until the time of analysis.

Breath, blood, and urine analyses. Gas chromatographic procedures used for the analysis of CH₂Cl₂ in the breath, blood, or urine are described elsewhere (DiVincenzo and Kaplan, 1981). Blood COHb saturations were determined by the gas chromatographic procedure of Collison et al. (1968).

RESULTS

Exercise Studies

The effect of exercise on several physiological parameters is summarized in Table I. Work intensity was estimated by monitoring the heart rate and relating it to the equivalent O₂ consumption measured during preexposure aerobic capacity testing. In addition, pulmonary minute ventilation and cardiac output were determined. Oxygen consumption at rest was taken as 4 ml O₂ min⁻¹ kg⁻¹ body wt, based on preexposure testing.

1 Eastman Organic Chemicals, Rochester, N.Y.
2 Avionics Division, Delmar Laboratories, Los Angeles, Calif.
TABLE 1

**Effect of Exercise on Physiological Parameters for Volunteers Exposed to Methylene Chloride Vapor**

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Work intensity (ml O₂ min⁻¹ kg⁻¹)</th>
<th>Percentage aerobic capacity</th>
<th>Heart rate (beats/min)</th>
<th>Estimated average cardiac output (liters/min)</th>
<th>Estimated average alveolar ventilation (liters/min)</th>
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<tr>
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<td>4</td>
<td>25</td>
<td>56</td>
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<td>4</td>
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<td>28</td>
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* Volunteers were stress tested on a four-stage submaximal treadmill to determine heart rate and oxygen consumption at different work intensities. Workloads equivalent to 50 to 150 W were determined. Volunteers exercised during the exposure on an Avionics treadmill for 5 min of each 15-min period. Experimental exposures to 100 ppm of CH₂Cl₂ vapor were carried out for 7½ hr.

The time course of CH₂Cl₂ in the end tidal air for three volunteers exercising at 14 to 28 ml O₂ min⁻¹ kg⁻¹ is illustrated in Fig. 1. Individual values are compared to the time course of CH₂Cl₂ in the end tidal air for five volunteers exposed to CH₂Cl₂ under sedentary conditions (DiVincenzo and Kaplan, 1981). Light work was performed by volunteer 1 and moderate or heavy work was performed by the other volunteers. Breath concentrations of CH₂Cl₂ were consistently higher for exercising individuals.

![Fig. 1. Time course of methylene chloride in the end tidal air for volunteers exposed to 100 ppm of CH₂Cl₂ vapor at light, moderate, and heavy workloads. Individual values from three subjects exercising at different work intensities (broken lines) are compared to the time course of methylene chloride in the end tidal air for five volunteers exposed to methylene chloride under sedentary conditions (solid line).](image-url)
both during and after the exposure than those found at rest. The elimination of CH₂Cl₂ in the end tidal air was similar for both exercising and resting individuals. The concentration of CH₂Cl₂ in the end tidal air was less than the limit of detection (0.1 ppm) at 24 hr after the exposure.

The time course of CH₂Cl₂ in the blood for volunteers exercising at light, moderate, and heavy workloads is shown in Fig. 2. Exercise produced a marked increase in the concentration of CH₂Cl₂ in the blood both during and after the exposure. For volunteers 2 and 3 the blood concentrations of CH₂Cl₂ during the exposure appeared to vary directly with the work intensity. Postexposure elimination curves for CH₂Cl₂ were at first similar to that of sedentary subjects, but by 4 hr after the exposure a trend toward longer elimination times became apparent for exercising individuals.

The time course of blood COHb saturations for volunteers exercising at various work intensities is illustrated in Fig. 3. Each volunteer was compared to the mean blood COHb saturation obtained for five volunteers exposed to 100 ppm of CH₂Cl₂ under sedentary conditions. There was a gradual time-related increase in COHb during the exposure for both sedentary and exercising individuals. Peak blood COHb saturations were reached either at the end of the exposure or shortly thereafter. The time-related increase in COHb occurred more
TABLE 2

EFFECT OF EXERCISE ON THE PULMONARY UPTAKE AND METABOLISM OF METHYLENE CHLORIDE

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Work intensity (ml O₂ min⁻¹ kg⁻¹)</th>
<th>Exposure to CH₂Cl₂ (mmol)</th>
<th>Pulmonary uptake of CH₂Cl₂ (mmol)</th>
<th>Pulmonary uptake (%)</th>
<th>Net pulmonary excretion of CO (mmol)</th>
<th>Metabolism of CH₂Cl₂ to CO (%)</th>
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<tr>
<td>1</td>
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<td>41.87</td>
<td>49</td>
<td>9.82</td>
<td>3.52</td>
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</tbody>
</table>

* Experimental exposures to CH₂Cl₂ vapor were carried out for 7½ hr at 100 ppm. Volunteers exercised during the exposure on an Avionics treadmill for 5 min of each 15 min of exposure. Heart rate was monitored continuously during the exposure.

* The pulmonary uptake of CH₂Cl₂ was estimated by using the following formula: uptake (mg) = (C吸入 - C呼気) × V × t where C吸入 equals the concentration of CH₂Cl₂ in the inhaled air, C呼気 equals the concentration of CH₂Cl₂ in the exhaled air, V is the minute ventilation (liters/min), and t is time (min).

* Mean for five volunteers exposed to CH₂Cl₂ under sedentary conditions.

* The conversion of CH₂Cl₂ to CO was calculated from the following formula: % conversion = amount CO produced/ CH₂Cl₂ uptake - postexposure excretion of CH₂Cl₂.

rapidly with exercising individuals than was seen with sedentary volunteers. Moderate to heavy workloads produced about a two-fold increase in peak blood COHb saturations compared to those of sedentary volunteers. Although postexposure COHb val-

![Diagram](a) The time course of methylene chloride in the end tidal air for two cigarette smokers is compared to the time course of methylene chloride in the end tidal air for five nonsmokers exposed similarly to 100 ppm of methylene chloride. (b) Time course of methylene chloride in the blood for two cigarette smokers exposed to 100 ppm of methylene chloride vapor is compared to the time course of methylene chloride in the blood of five nonsmokers exposed similarly to methylene chloride.
Fig. 5. The combined effect of smoking and exposure to methylene chloride vapor. Two male smokers were exposed for 7½ hr to 100 ppm of CH₂Cl₂ vapor. Cigarettes were smoked before, during, and after the exposure. The combined smoking and CH₂Cl₂ exposure (Volunteer 1; •; Volunteer 2; △) was preceded by a control period (Volunteer 1; ○; Volunteer 2; ▲) in which the same protocol was followed with the exception that the subjects were not exposed to CH₂Cl₂.

Values were substantially higher for exercising individuals, the elimination curves were similar for both groups.

The effect of exercise on the pulmonary uptake and metabolism of CH₂Cl₂ is presented in Table 2. Control values (i.e., 4 ml O₂ min⁻¹ kg⁻¹) were determined for five volunteers experimentally exposed to CH₂Cl₂ vapor under sedentary conditions. Under sedentary conditions about 72% of the CH₂Cl₂ inhaled was absorbed through the lungs. The percentage of CH₂Cl₂ absorbed at light, moderate, and heavy work intensities was 58, 53, and 47%, respectively.

The net pulmonary excretion of CO increased markedly with exercise; i.e., 2.66 mmol of CO was excreted under sedentary conditions compared to 5.12 mmol under conditions of light work and 10.18 to 14.34 mmol for moderate and heavy work. The extent of CH₂Cl₂ converted to CO was about 26% under sedentary conditions and ranged from 28 to 40% with exercise.

The quantity of CH₂Cl₂ excreted in the urine was relatively low (142–318 µg/day) and did not appear to be related to work intensity. There was no net increase in the excretion of formaldehyde in the urine nor was there an increase in the concentration of formaldehyde in the serum. No exposure-related trends were apparent for formic acid either in the serum or urine. The concentration of chloride in the serum was similar for sedentary and exercising individuals. Preexposure clinical values and electrocardiograms were similar to postexposure values.

Smoking Studies

The time course of CH₂Cl₂ in the end tidal air and in the blood for smokers exposed to 100 ppm of CH₂Cl₂ vapor is illustrated in Figs. 4a and b, respectively. The concentration of CH₂Cl₂ in the breath during the exposure was higher for smokers than it was for nonsmokers. No explanation is offered for this observation. The postexposure elimination curves were virtually identical. Blood concentrations of CH₂Cl₂ for smokers appeared to be elevated over those for nonsmokers both during and after the exposure. The disappearance of CH₂Cl₂ from the blood was rapid and by 16 hr after the exposure the concentration of CH₂Cl₂ was below the limit of detection.

The time course of blood COHb saturations for two smokers exposed to CH₂Cl₂ vapor is presented in Fig. 5. Exposure to CH₂Cl₂ vapor produced a gradual increase in blood COHb values above that expected from active smoking. Peak blood COHb saturations were reached toward the end of the exposure period and were sustained during the early phase of the postexposure period. The net increase in COHb at 7½ hr of exposure was comparable to the increase in COHb expected from a 7½-hr exposure to COHb to 100 ppm, i.e., about 3.0%.

The quantity of CH₂Cl₂ excreted in the urine ranged from 138 to 242 µg per day, similar to that reported previously for nonsmokers (DiVincenzo and Kaplan, 1981). The excretions of chloride, formaldehyde,
and formate in the urine were not substantially different from those of nonexposed controls.

DISCUSSION

Previously we reported that controlled experimental human exposures to CH₂Cl₂ vapor at 150 ppm for 7½ hr produced peak blood COHb saturations that approximated the biological threshold limit value recommended by NIOSH, viz., 5%. These studies were performed with sedentary nonsmokers and consequently the effect of smoking or physical exercise on the formation of blood COHb was not known. The findings described herein show that physical exercise led to increases in the absorption of CH₂Cl₂, the conversion of CH₂Cl₂ to CO, blood COHb values, and the pulmonary excretion of CO compared to those values obtained from sedentary exposures. The combined effect of smoking and exposure to CH₂Cl₂ vapor produced an additive increase in COHb.

Compared to sedentary exposures, moderate or heavy workloads produced a marked increase in the absorption of CH₂Cl₂ and an increase in the percentage COHb saturation of the blood. Breath elimination times for CH₂Cl₂ were also prolonged by physical exercise. Heavy workloads, however, did not produce blood COHb saturations that were greater than those levels encountered at moderate workloads. Although Astrand et al. (1975) reported similar results for volunteers exposed to substantially higher concentrations of CH₂Cl₂ for shorter durations, our findings show that exercise was accompanied by an increased pulmonary excretion of CO during the exposure which undoubtedly contributed to the lower than expected COHb values encountered during heavy workloads.

The National Institute of Occupational Safety and Health (NIOSH) has recommended a biological threshold limit value for CH₂Cl₂ based on blood COHb saturation (viz., 5%). Nonsmoking urban dwellers have blood COHb saturations that range from 0.4 to 2.0%, and smokers have blood COHb saturations that usually range from 2 to 8% and in heavy smokers may be over 10%. It has been reported that individuals with cardiovascular disease may suffer discomfort when blood COHb saturations exceed 5%. The finding that heavy workloads did not produce a further increase in blood COHb levels over that of moderate workloads is particularly relevant to the workplace. Although light and moderate work intensities may lead to higher blood COHb saturations over those of sedentary workers, the performance of heavy workloads is unlikely to produce blood COHb saturations that are higher than those found at moderate work intensities. Workers performing physical exercise while exposed to CH₂Cl₂ vapor at the recommended TLV of 100 ppm are unlikely to exceed the COHb biological threshold limit value suggested by NIOSH.

In conclusion, exercise performed during an exposure to CH₂Cl₂ vapor produced both an increased pulmonary uptake and metabolism of CH₂Cl₂. Blood COHb saturations, however, did not increase proportionately with the work intensity. The lower than expected COHb levels were apparently due to an increased pulmonary excretion of CO during the exposure. Changes in the COHb saturation of the blood in smokers exposed to CH₂Cl₂ vapor were additive.

ACKNOWLEDGMENT

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REFERENCES


