THE MEDICAL COLLEGE OF WISCONSIN

Department of Environmental Medicine

METHYLENE CHLORIDE:

DEVELOPMENT OF A BIOLOGIC STANDARD FOR THE INDUSTRIAL WORKER BY BREATH ANALYSIS

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SYNOPSIS-ABSTRACT

Twenty healthy adults of both sexes were exposed to methylene chloride vapor concentrations of 0, 50, 100, 250 and 500 ppm for periods of 1, 3 and $7\frac{1}{2}$ hours in a controlled-environment chamber. These studies were designed to simulate the type of exposures encountered in the industrial setting and consisted of both steady, non-fluctuating vapor concentrations as well as widely fluctuating vapor concentrations. Exposure resulted in a prompt elevation of carboxyhemoglobin (COHb) which had a biologic half-life twice that of COHb formed as a result of exposure to exogenous carbon monoxide. This solvent-induced COHb was derived from the metabolism of methylene chloride and added to the body burden of carbon monoxide derived from other sources. Repetitive vapor exposure to the current TLV of 250 ppm produced no untoward subjective or objective health responses in the healthy subjects who are able to compensate for the mild anoxic stress of the COHb. However, if a uniform philosophy for the development of TLV's is followed, the TLV for methylene chloride for <u>active</u>, non-smoking workmen should be reduced to 50 ppm if excursion of COHb above 4 to 5% is to be prevented. Post-exposure breath analysis for methylene chloride and its metabolite, carbon monoxide, proved to be a practical screening method with which to determine the body burden of these two compounds and so provides a Biologic Threshold Limit measurement for workmen.

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The discovery by Stewart, et al [1, 2], that the exposure of humans to the vapor of methylene chloride (dichloromethane, CH_2Cl_2) is associated with a significant increase in carboxyhemoglobin (COHb) saturation has initiated a review of the industrial Threshold Limit Value (TLV) for this compound [3]. Since the data from Stewart's laboratory indicated that exposure to the 1971 TLV for methylene chloride of 500 ppm (1,740 mg/M^3) for an eight-hour work day would probably result in a COHb higher than the 8% permitted by the current TLV for carbon monoxide of 50 ppm (55 mg/M^3), the TLV for methylene chloride was lowered to 250 ppm.

Other investigators confirmed that exposure to methylene chloride resulted in elevated COHb in mammals and proved the Stewart conjecture that the carbon monoxide is a metabolite of the solvent [4, 5, 6]. The use of methylene chloride in the workplace was further complicated by the proposal to lower the TLV for carbon monoxide to 25 - 35 ppm. The comparable methylene chloride exposure which would result in the equivalent endogenous carbon monoxide exposure was not known. In fact, the human data base regarding the physiological effects of exposure to low concentrations of methylene chloride was very limited. This study was undertaken to expand this toxicological data base.

This report presents the findings of the investigation conducted for the purpose of studying the effects of methylene chloride upon the health of human volunteers repeatedly exposed to vapor concentrations that included the 1971 TLV and to lower concentrations which may be proposed as the future TLV.

The report includes post-exposure breath concentrations of methylene chloride which can be used to estimate the magnitude of industrial exposure and so provides a "Biologic Threshold Limit" measurement. The endogenous formation of carbon monoxide as a result of exposure to this solvent, and its slow elimination after exposure, is carefully documented.

EXPERIMENTAL

Healthy adults of both sexes were exposed to known concentrations of methylene chloride vapor in a controlled-environment chamber (Figure 1). These studies were designed to simulate the type of exposures encountered in the industrial setting and consisted of both steady, non-fluctuating vapor concentrations as well as widely fluctuating vapor concentrations of methylene chloride.

Exposure Schedule:

The vapor exposure sequence is presented in Table I. The sequence was initiated with male subjects who were exposed to methylene chloride vapor concentrations of 0, 50, 100, 250 and 500 ppm for periods of 1, 3, and $7\frac{1}{2}$ hours. The female subjects were exposed to 250 ppm for periods of 1, 3 and $7\frac{1}{2}$ hours. The vapor concentrations in the controlled-environment chamber were not permitted to fluctuate widely except for the male exposure during week 4 when the wide fluctuation experiment was performed. Figure 2 illustrates the male subject exposure sequence while Figure 3 illustrates the planned daily fluctuations in vapor concentration during week 4.

Figure 1

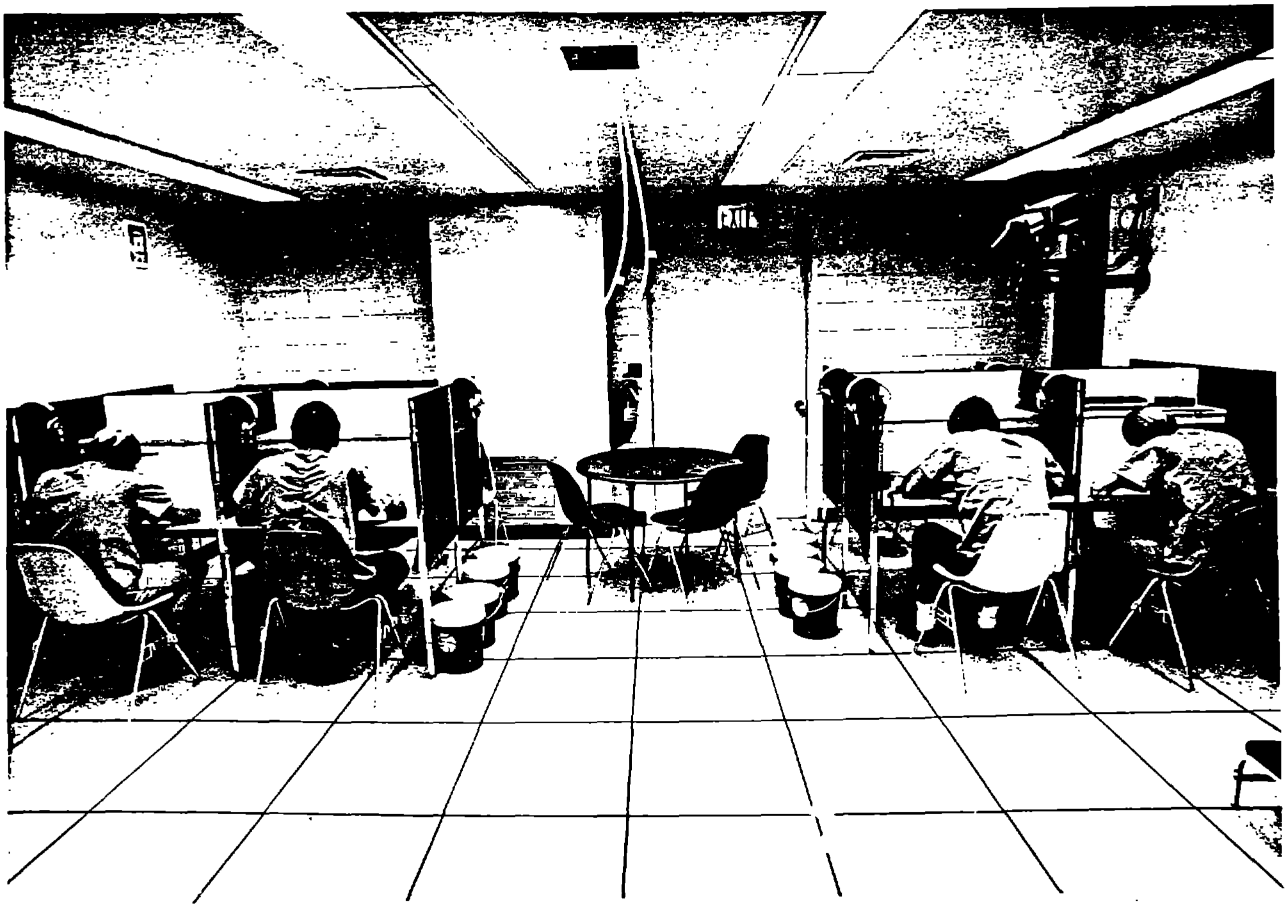


Figure 1: Controlled-environment chamber. This 20' x 20' x 8' room in which eight subjects are performing cognitive tasks features an independent ventilation system and a remotely controlled closed circuit TV camera. The two polyethylene probes in the center of the picture supply chamber air to the two independent analytical systems used to monitor the vapor concentration.

TABLE I

METHYLENE CHLORIDE EXPOSURE SCHEDULE: MALE SUBJECTS

EK	DAY OF WEEK	DESIRED CONCENTRATION, PPM	ACTUAL TIME-WEIGHTED AVERAGE VAPOR CONCENTRATION, PPM					
			7-1/2 Hour		3-Hour		1-Hour	
			MEAN	± S.D.	MEAN	± S.D.	MEAN	± S.D.
1	4	0	0		0		0	
	5	0	0		0		0	
2	1	50 (minimal fluctuation)	49.8	2.3	49.6	2.4	47.4	3.0
	2		48.7	4.6	50.2	2.2	47.1	4.1
	3		50.0	2.6	49.9	1.6	49.6	2.2
	4		50.0	1.4	50.1	1.2	49.9	0.6
	5		50.1	2.1	49.2	1.9	50.3	2.3
3	1	250 (minimal fluctuation)	248.3	15.5	250.3	9.0	248.1	10.0
	2		249.9	8.6	251.4	9.0	251.7	9.9
	3		250.0	9.4	248.9	12.1	243.4	3.1
	4		250.0	7.6	249.9	7.0	251.8	9.3
	5		249.8	9.5	249.7	7.8	249.7	4.8
4	1	250 (wide fluctuation)	249.2	50.3	249.2	52.3	248.7	47.0
	2		247.1	45.3	247.6	50.7	248.6	45.6
	3		250.1	52.2	250.9	50.9	254.2	52.8
	4		250.1	51.0	249.0	51.7	252.6	48.1
	5		250.1	51.2	250.3	47.1	247.8	49.1
5	1	100 (minimal fluctuation)	99.7	4.1	99.4	2.2	101.9	4.3
	2		100.0	4.2	99.0	3.2	100.2	2.6
	3		100.1	9.4	100.4	6.4	103.4	9.4
	4		99.0	5.1	100.6	4.1	99.8	1.5
	5		100.5	5.8	100.2	3.0	101.2	4.2
6	1	0	0		0		0	
	2		0		0		0	
	3	500 (minimal fluctuation)	499.5	15.9	494.2	10.7	495.3	30.1
	4		497.1	11.7	494.1	9.5	498.5	8.4

(Continued on next page)

TABLE I (Continued)

METHYLENE CHLORIDE EXPOSURE SCHEDULE: FEMALE SUBJECTS

WEEK	DAY OF WEEK	DESIRED CONCENTRATION, PPM	ACTUAL TIME-WEIGHTED AVERAGE VAPOR CONCENTRATION, PPM					
			7-1/2 Hour		3-Hour		1-Hour	
			MEAN	± S.D.	MEAN	± S.D.	MEAN	± S.D.
1	5	0	0		0		0	
	1		248.7	7.7	249.7	8.5	246.4	11.1
	2		249.7	6.6	249.5	6.6	250.1	8.0
2	3	250	249.9	5.9	249.3	4.1	248.6	2.9
	4	(minimal	250.1	5.3	251.3	6.0	248.6	5.9
	5	fluctuation)	243.4	6.4	244.9	7.3	248.5	3.9
3	1	0	0		0		0	

FIGURE 2

METHYLENE CHLORIDE EXPOSURE SCHEDULE

MALE SUBJECTS

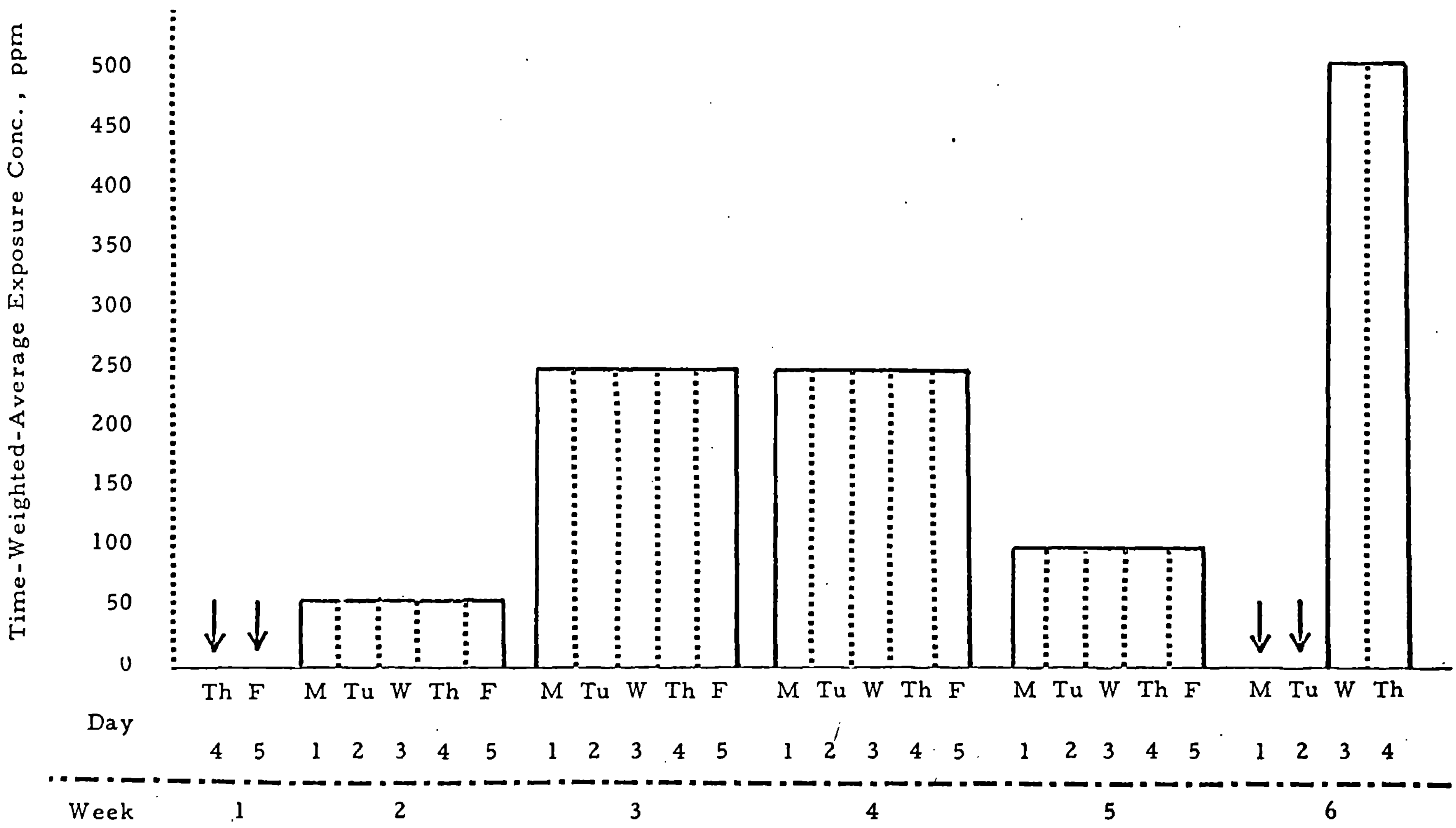
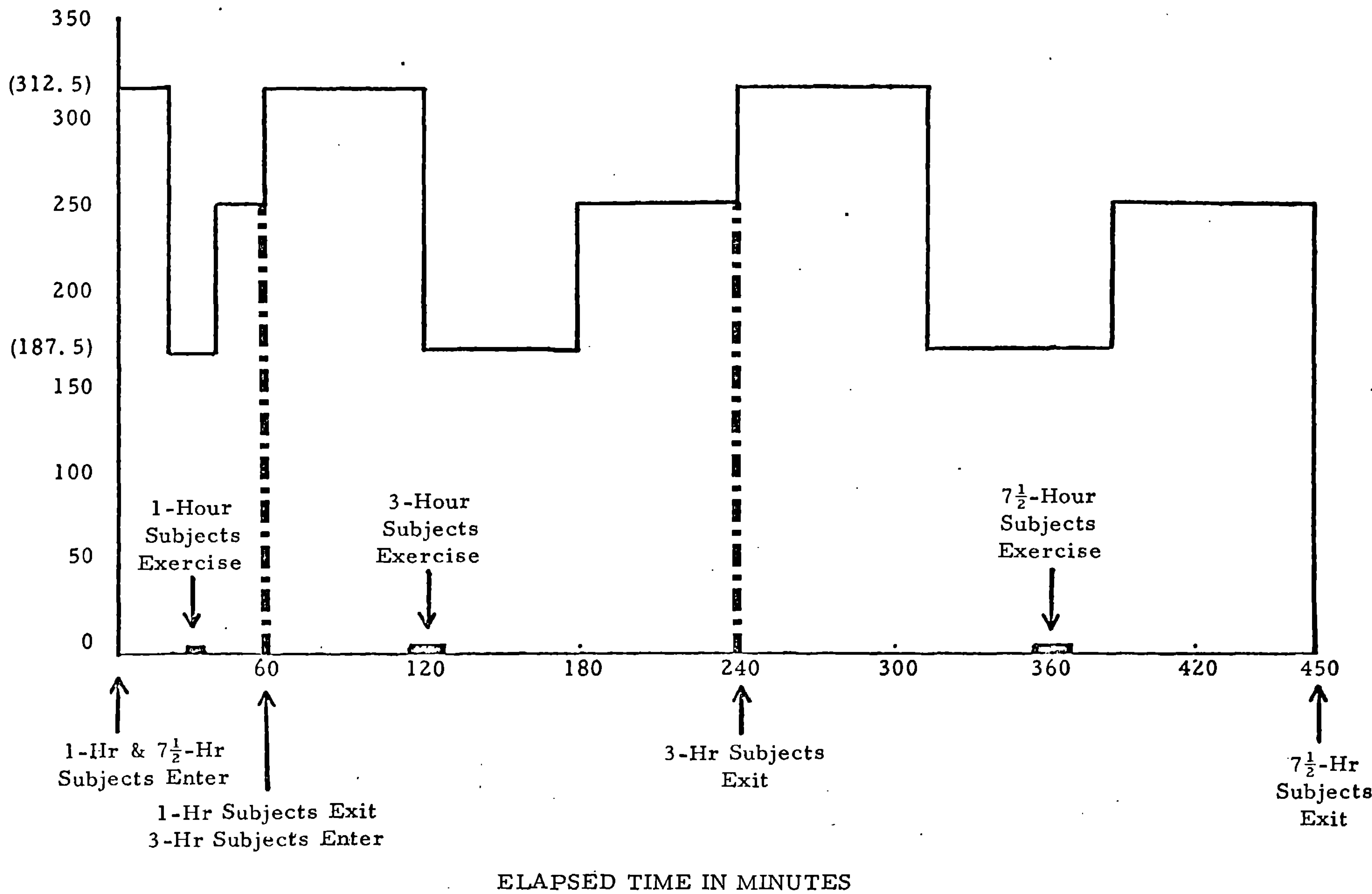


FIGURE 3

FLUCTUATING METHYLENE CHLORIDE VAPOR CONCENTRATION EXPERIMENT: 188-313 PPM

Time-Weighted-Average Concentration: 250 ppm



One-hour and $7\frac{1}{2}$-hr subjects entered the environmental chamber at 9:00 A.M. each day while the 3-hr subjects entered at 10:00 A.M., at which time the 1-hr subjects exited. The 3-hr subjects exited at 1:00 P.M., while the $7\frac{1}{2}$-hr group exited at 4:30 P.M. The latter group was served lunch in the chamber. There were never more than 7 subjects in the chamber at one time.

Subjects:

The subjects were selected from the Caucasian, middle class, working population of the Milwaukee metropolitan area. They were recruited for this study by a private employment agency. Each subject who completed a section of the study received $2.50 per hour spent at the laboratory, plus overtime, with a three-hour minimum payment for the Saturday morning medical surveillance check. After the objectives of the study and the nature of the procedures to be used were fully explained to them, all subjects signed an informed consent form, a copy of which is attached as Appendix I.

Eleven healthy males volunteered. Their ages ranged from 20 to 39 years, height from 170 to 190 cm, and weight from 57 to 93.5 kg. None was obese.

The ages of the nine participating females ranged from 20 to 41 years, their height from 158 to 169 cm, and their weight from 46.3 to 75.0 kg. None was obese.

All subjects were cautioned to abstain from the use of drugs and to limit their use of alcohol to very moderate amounts. Subjects who were smokers were not allowed to smoke during their stay in the controlled-environment chamber. Subjects who underwent behavioral testing (3-hr and $7\frac{1}{2}$-hr) were asked to refrain from consuming any caffeine prior to the end of each day's study (one hour post-exposure).

One male subject from each of the exposure groups smoked cigarettes. All other male subjects were non-smokers. The smokers from the $7\frac{1}{2}$-hr and 1-hr groups smoked one-half to one pack of cigarettes per day, while the smoker from the 3-hr group smoked approximately two packs of cigarettes per day. Another subject from the 3-hr group worked in a garage two to three evenings per week, while a non-smoker from the 1-hr group worked the third shift in an industrial plant.

Most of the subjects had no other wage-earning job during the time of the study, and none experienced any exposure to methylene chloride outside of the laboratory.

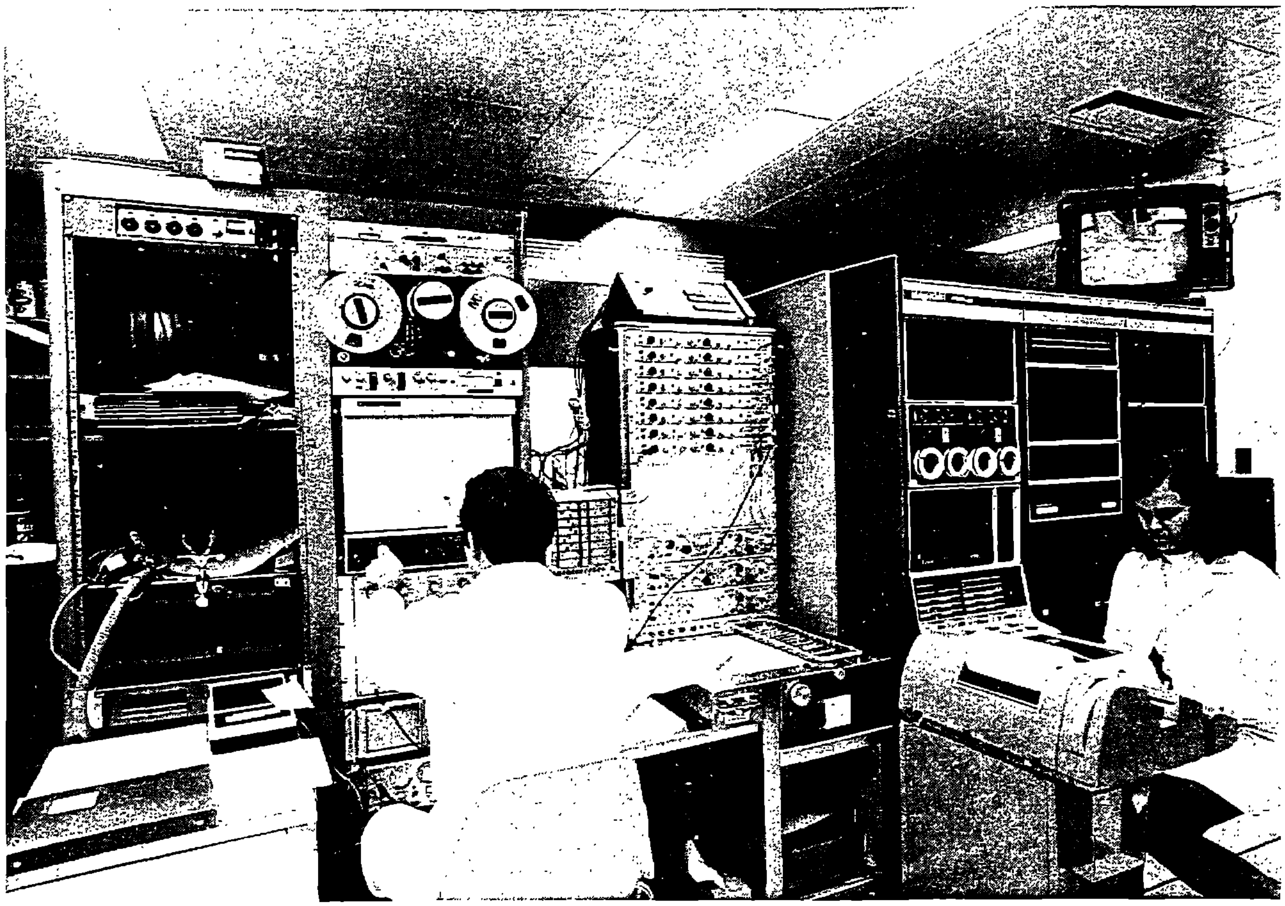
Exposure Chamber:

All exposures to the vapor of methylene chloride were conducted in a controlled-environment chamber 20' x 20' x 8' in size, which contained a 5' x 4' x 8' toilet facility and a 5' x 4' x $6\frac{1}{2}$' commerical audiometric booth. Both the toilet facility and the audiometric booth were ventilated with air from the chamber. Air flow through the chamber was approximately 1500 cu ft/min, and approximately 25% of this flow was exhausted. A slight negative pressure was maintained in the chamber at all times. Air temperature was 72 - 74°F, while relative humidity ranged between 45 - 55%. The methylene chloride was introduced by sweeping the concentrated vapor from a warm flask with a stream of air into the chamber's circulating air. A reciprocal dual-piston pump maintained a steady flow of liquid methylene chloride into the flask.

Analysis of Exposure Chamber Atmosphere:

The methylene chloride (Fisher Scientific Co., Certified ACS, D-37) used in these studies was shown by infrared analysis to be > 99% pure. The vapor concentration in the chamber atmosphere was continuously recorded by an infrared spectrometer (MIRAN I, Wilks Scientific Corporation) equipped with a 20-meter path-length gas cell which was continuously flushed with air drawn from the chamber through $\frac{1}{4}$" diameter polyethylene tubing. The absorbance at 13.3μ through a path-length of 2.25 m was measured. The infrared signal to the recorder was monitored each second by an on-line PDP-12 (Digital Equipment Corporation) computer, which displayed the mean vapor concentration, as compared to standards, for each 30-second time interval of exposure and calculated the daily time-weighted-average exposures. Calibration standards of methylene chloride in purified air were prepared in saran bags and analyzed before and hourly during each daily exposured. A second infrared spectrometer was connected to an independent polyethylene tubing loop which sampled the environmental chamber atmosphere in a manner similar to the first. However, the signal to the recorder was not monitored by computer. This second monitor was used as a back-up and a check on the infrared spectrometer connected to the computer.

The second method of monitoring the chamber atmosphere during the study with female subjects was through use of a gas chromatograph equipped



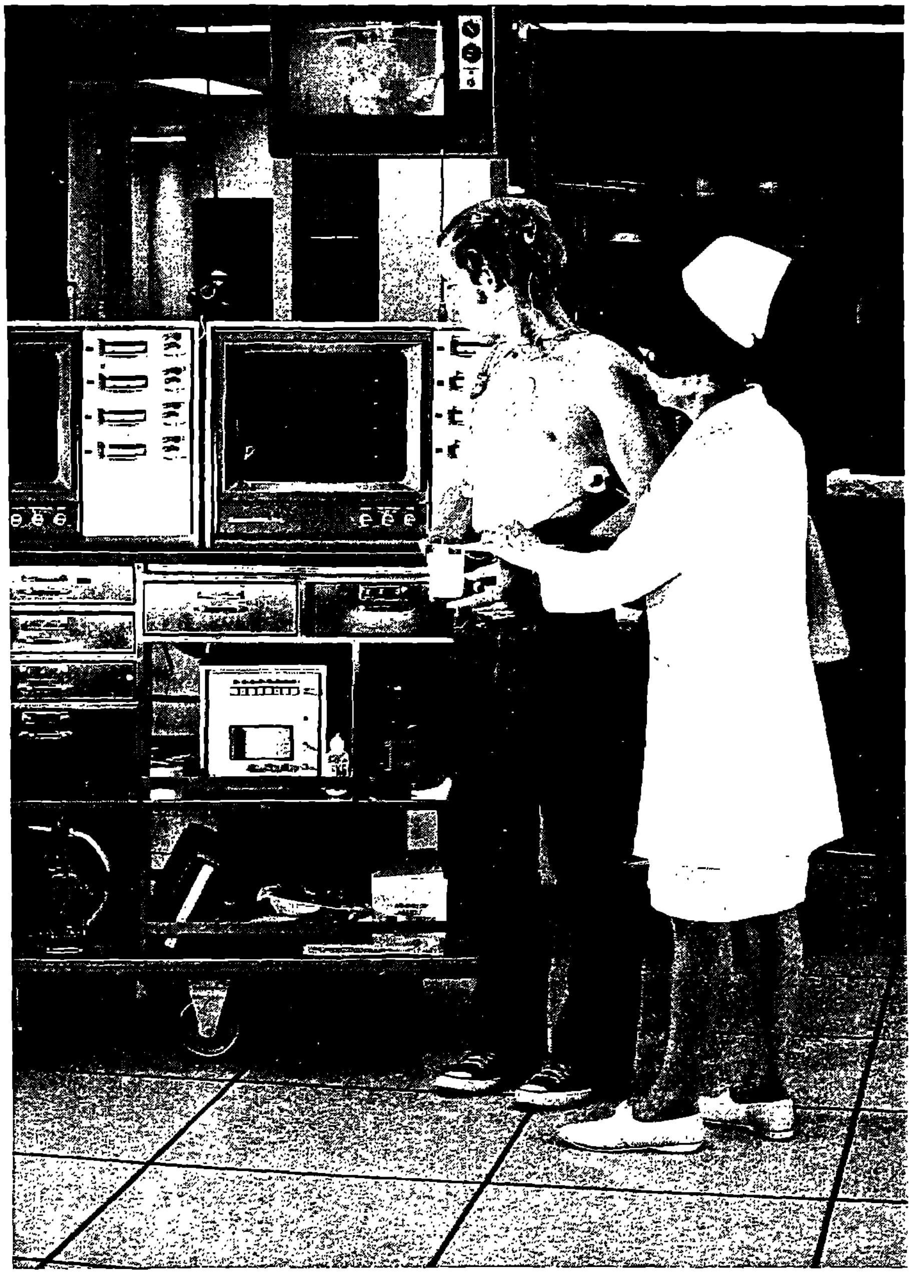
Physiological monitoring center. To the left is the equipment for the computerized measurements of pulmonary function. In the center the laboratory assistant is recording the visual evoked response and spontaneous EEG of a subject within the chamber. To the right is the PDP-12 Digital computer used to collect and analyze the data from tests such as the "clock" test. Above the computer is one of the TV monitors.

with a flame ionization detector and an automatic sampling device. A sample of the chamber atmosphere was automatically swept into the carrier gas (N_2) of the chromatograph every three minutes. The Varian Aerograph Model 2700, Moduline® gas chromatograph (GC) was fitted with a stainless steel column, 14" x 1/8", packed with Poropak Q, 60/80 mesh. The column was pre-conditioned at 200°C for 24 hours prior to its use. Throughout the study, the column was baked at 150°C when it was not in use. The operating conditions of the GC were: carrier gas flow rate of 35 ml/min, column temperature 150°C, injection port 230°C, and detector 200°C.

The ambient concentration of carbon monoxide in the chamber was measured once or twice daily while exposures were in progress. An aliquot from a grab sample of air obtained from the chamber was analyzed by gas chromatography using the method of Porter and Volman [7].

Medical Surveillance:

Each subject was given a comprehensive medical examination prior to the study and after the last exposure day of the study. These examinations included a complete history and physical examination with the following laboratory studies: complete blood count, SMA-12 survey panel of clinical chemistries and a 12-lead electrocardiogram (EKG). A complete blood count and the SMA-12 survey panel of clinical chemistries was repeated at least once per week during the weekly exposures. Triglycerides were added to the clinical chemistries performed on the male subjects during the second week of exposure. Prior to each day's exposure, the subjects were given a brief



Lead II of the electrocardiogram was continuously monitored by telemetry. No abnormalities occurred in any of the subjects during the series of vapor exposures.

medical examination which included blood pressure, temperature, subjective signs or symptoms, and urinalysis (Combistix®). During the time that they were in the environmental chamber, each subject's EKG (lead II) was continuously monitored by telemetry and recorded at hourly intervals. The subjects were under continual surveillance by medical personnel while they were in the study.

Twenty-four hour urine collections were begun daily as each subject entered the chamber. Urobilinogen determinations were carried out on fresh 24-hr urine specimens using the method of Watson, et al, as modified by Henry, et al, in Henry [8]. A Coleman Model 6/20 A Junior® IIA spectrophotometer was used to determine linear absorbance at 562mμ. The PSP dye standard was adjusted in concentration to give an optical density of 0.384 at 562mμ on a Beckman DB spectrophotometer, using water as a blank. Results were reported in Ehrlich units per 24 hours.

<u>Breath Sample Collection and Analysis:</u>

Alveolar breath samples were obtained daily from each subject prior to entry into the environmental chamber, immediately prior to exiting the chamber, and at the following times after exiting the chamber (post-exposure): 1, 15, 30 min; 1, 2, 3 hr. The last two samples were collected in duplicate. A 16-, 21-, and 23-hr post-exposure sample (usually identical to the pre-exposure sample) for group I, II, and III, respectively, was also collected. Breath samples, with the exception noted below, were collected in 35-ml glass tubes fitted at each end with screw caps containing saran liners [9]. One of the caps had a pre-drilled hole through which an aliquot of the alveolar breath samples could be withdrawn. The subjects flushed the tube twice, then after holding the third breath for 30 seconds, exhaled and capped the tube while finishing the exhalation. The alveolar breath sample obtained just prior to exit from the environmental chamber was collected by having the subject breathe through a polyethylene tube which extended through a porthole in the chamber door to a saran bag. Sampling of the saran bag was accomplished by puncturing with the syringe needle.

Fifteen minutes after the end of the exposure on the fifth day of weeks 2, 3, 4 and 5, a second alveolar breath sample was collected from each subject, this one in a 4-liter saran bag. This breath sample was slow-scanned in a Beckman® IR-10 infrared spectrometer with a 10-meter gas cell.

A Varian Aerograph Model 2700 gas chromatograph equipped with a hydrogen flame ionization detector was used to determine methylene chloride in the breath samples. The gas chromatograph (GC) was fitted with two stainless steel columns: 3' x 1/8" and 1' x 1/8", each packed with Poropak Q, 50/80 mesh (Waters Associates, Inc.). The columns were preconditioned at 200°C overnight prior to use. The operating conditions of the GC were as follows: carrier gas (helium) flow rates, 10 and 20/ml min; column temperature, 75°C; injection port, 150°C; and detector, 200°C. Standards at several concentrations to bracket the unknown levels were prepared with purified air as diluent. One-ml aliquots were injected and the concentration of methylene chloride in unknowns was obtained by direct comparison of peak heights to the standards.

The collection of an expired breath sample in a glass pipette. This technique has been previously described in detail [9].

Collection of an expired breath sample in a saran bag. The subject takes a normal breath, holds it for 20 seconds and then exhales into the bag.

On days 1 and 3 of weeks 3, 4, 5 and 6, one of the duplicate alveolar breath samples obtained at two and three hours post-exposure was assayed for carbon monoxide by the gas chromatographic procedure of Porter and Volman[7]. In addition, the carbon monoxide in an additional duplicate breath sample was assayed at 15 minutes post-exposure for one subject in lieu of determining his COHb.

Blood Sampling and Carboxyhemoglobin Analysis:

Approximately 2 ml of blood was withdrawn from an antecubital vein of each subject each morning prior to chamber entry, and the sample was analyzed for baseline COHb saturation. Smokers were not allowed to smoke between the withdrawal of this sample and the last blood sample of the day. Additional blood samples for COHb assay were obtained immediately pre-exit from the chamber, and one hour post-exposure on days 1, 3, and 5 of each week. Fewer samples were obtained from one 3-hr subject who had difficulty with the procedure. His breath sample was analyzed for carbon monoxide in lieu of the assay of blood COHb at some of these times.

Blood COHb was determined directly in a CO-Oximeter (Instrumentation Laboratories, Inc.), an automated instrument which also determined the hemoglobin concentration. The calibration of this instrument has been detailed previously[10].

Neurological Studies:

Within five minutes of entry into the environmental chamber, and within ten minutes prior to exit, each subject performed a modified Romberg equilibrium test which was video taped for later inspection if necessary. The test consisted of standing upon each leg singly with arms at the side for a minimum of three seconds, and walking heel-to-toe in a straight line for approximately five feet. This was first done with the eyes open and then repeated with eyes shut.

Electroencephalograms and visual evoked response (VER) measurements were made each Monday, Wednesday and Friday on the $7\frac{1}{2}$-hr subjects. On each measurement day, four recordings were made, one during the first hour of exposure and three between the fifth and seventh hours of exposure. A modified 10 - 20 international electrode arrangement was used for EEG recordings. One electrode was place 2 to 2.5 cm above the inion, and the VER was recorded from this electrode with the left ear as the reference. The details of the recording arrangements have been presented in a previous publication [11].

The EEG and VER were both analyzed by visual examination. In addition, the amplitude of the 3rd, 4th, and 5th waves of the VER complex were measured (mm ruler). The objective was to ascertain whether changes occurred within a day during a week at each concentration of methylene chloride and from one concentration to another.

<u>Pulmonary-Hematologic Studies:</u>

Four specific functions were studied during the male methylene chloride exposures: (1) functional integrity of airways, (2) alveolar to capillary gas exchange, (3) pulmonary ventilation, and (4) hemoglobin affinity for oxygen. Each of these was assessed at least once on the $7\frac{1}{2}$-hr subjects during each week of the exposure (Thursday or Friday, 5th to 7th hr), and once before and once after methylene chloride exposure.

Functional Integrity of Airways: This function was assessed by having the subject perform a forced maximum expiratory maneuver. The subject was seated erect, and breathing through a mouthpiece connected with wide bore tubing to a Fliesch flow-transducer. The transducer was connected to a Vertek pneumotachograph which sent analog data to the analog-to-digital converter of a PDP-12 computer. Appropriate software was utilized to calculate values for vital capacity (FVC), percent of VC expired in one second (FEV 1) peak expiratory flow rate (PEFR), and flow rate of 50% of FVC (MMEF). This maneuver was performed at least three times, with the data from the two "best" maneuvers being saved on magnetic tape. The mean of the two values was taken as indicative of the function for each specific condition.

Alveolar-Capillary Gas Exhange: This function was assessed by the single breath carbon monoxide diffusion technique (D_LCO) during rest and during the sixth minute of two levels of moderate exercise on a bicycle ergometer[12]. The first exercise level was set to elicit a heart rate of approximately 110 beats/min (350 KPM) while the second elicited a heart rate of approximately 150 beats/min (700 KPM). Computerized systems as noted above were used to calculate inspired, residual, and total lung volume and D_LCO. Neon was used as the inert gas to measure residual volume. Neon and carbon monoxide concentrations in the collected alveolar sample were analyzed using a Quintron Thermoconductivity Chromatograph. Pre-exercise COHb prior to the maneuver were determined. These COHb levels were then used to calculate carbon monoxide "back pressure" from the blood to the lungs so that the true diffusion characteristics of the alveolar-capillary system could be ascertained[13].

Pulmonary Ventilation: Pulmonary ventilation, $PaCO_2$, arterial pH, PAO_2, arterial lactate, and metabolic rate were measured directly or calculated for resting and two conditions of exercise (5th - 6th minute). These parameters were used to assess the effect of methylene chloride exposure on mechanisms regulating breathing. Ventilation was measured using a Parkinson-Cowan gas meter whose output was fed to a Hewlitt-Packard recorder. While ventilation was being measured, 50 ml of the mixed expired air was collected in a glass syringe. This sample was analyzed for CO_2 and O_2 concentration using a Quintron chromatograph. Ventilation and CO_2 and O_2 concentration were used to calculate metabolic rate and PAO_2. For sampling of blood, a 21-gauge needle was placed in a superficial dorsal hand vein. The needle was attached to a tubing-stopcock arrangement which during non-sampling periods was filled with heparinized saline. For five minutes prior to sampling, the entire hand was heated to approximately 42°C. This procedure sufficiently "arterializes" the blood so that the PCO_2, pH, and lactate approximate those of arterial blood[14]. The blood was analyzed within 15 minutes for PCO_2 and pH with the Radiometer electrode arrangement. Blood lactate concentration was determined using a modification of the Barker-Summerson method [15].

Hemoglobin Affinity for Oxygen: Fifteen to 20 ml of blood was drawn from each subject into heparinized syringes. This sample was divided into two-ml aliquots. Each aliquot was equilibrated in a tonometer (Instrumentation Laboraties, Inc., Model 137) with gases of known CO_2 and O_2 concentrations (37°C). The O_2 concentrations were chosen to yield hemoglobin saturation of 35, 45, 55 and 65%, and at least two aliquots were tonometered at each level. The CO_2 concentration was chosen to maintain CO_2 tension near 40 mm Hg. Near the completion of the equilibration, 100 µl of blood was withdrawn and analyzed for pH. If needed, appropriate amounts of $NaHCO_3$ or HCl were then added to bring the pH to the desired level of 7.40. Hemoglobin saturation for oxygen (HbO_2) was measured on an Instrumentation Laboratories, Inc. CO-Oximeter while PCO_2 and PO_2, and pH were measured on a Radiometer electrode system. Using the paired PO_2-HbO_2 values, an O_2 dissociation curve was approximated. An alteration in hemoglobin affinity for oxygen was expressed as the PO_2 required to achieve 50% hemoglobin saturation.

Aliquots of the blood samples were assayed for 2,3-DPG by the Sigma colorimetric method [17]. In this method, the protein-free filtrate from one ml of blood treated with three ml of cold 8% trichloroacetic acid is divided into two fractions. One fraction is incubated with the necessary enzymes to release one phosphorus atom from each molecule of 2,3-DPG present while the second is incubated without the key enzyme. Inorganic phosphorus is determined colorimetrically on both samples and the second, used as the blank, is subtracted from the first. A standard curve using known amounts of inorganic phosphorus was prepared for each daily assay. A Coleman Model 6/20A Junior IIA spectrophotometer was used to determine linear absorbance at 660 mμ. The μ moles of 2, 3-DPG/ml blood were determined from the standard curve and converted to μ moles/gm hemoglobin using the values for hemoglobin obtained on the CO-Oximeter.

Cognitive Testing:

A battery of cognitive tests was performed by the male subjects exposed for 3 and $7\frac{1}{2}$-hr. An alertness test lasting $1\frac{1}{2}$ hours was performed on days 1, 3 and 5 of each week. It began $2\frac{1}{4}$ hours after the start of the $7\frac{1}{2}$-hr exposure and $1\frac{1}{4}$ hours after the 3-hr exposure. No training was required for this test. On days 2 and 4 of each week, the remaining tests in the series were performed beginning at three and two hours after beginning exposure for the $7\frac{1}{2}$ and 3-hr groups, respectively. The subjects were trained to a performance plateau before these tests were used during exposures.

The subjects sat in comfortable chairs at individual carrels to perform the cognitive tests. The subjects were not permitted to talk or have access to watches, food, soft drinks, radios, etc., during the tests. All instructional commands were made from outside of the chamber via an intercom system. The tests are described below in the order in which they were performed.

Alertness Test: This test, called the "clock" test because the subject watches a black clock face from which all numerals and hands have been removed except the sweep second hand (Figure 4), was administered and graded by a PDP-12 computer. It presented a primary and secondary task. For the primary task, the subject pressed a hand-held micro-switch as rapidly as possible whenever he observed a "stop and start" of the sweep second hand. The clock stoppages were for 0.23 seconds or 0.13 seconds occurring 30 times during the 90 minute test. For the secondary task, the subject pressed the hand-held switch whenever a tone was heard through the headphones he was wearing. These auditory signals were presented randomly for one second, three to six times per test. At the completion of the test, the computer printed the results in table form for each subject. The table listed signal duration, time of occurrence, reaction time or miss for each signal, percent correct for the day, and the average reaction times for clock stoppages and for tones.

This was the only cognitive test performed by the female subjects because no training was required.

Ten- and Thirty-Second Time Estimation Test: Each male subject upon verbal signal "ready, begin", depressed a hand-held, silent, push-button micro switch for an interval of time he estimated to be ten seconds. This was repeated two additional times, and then three 30-second estimates were made. The micro-switches were connected to a polygraph whose pen-deflection could be read to the closest ten milliseconds. This test took approximately three minutes to perform.

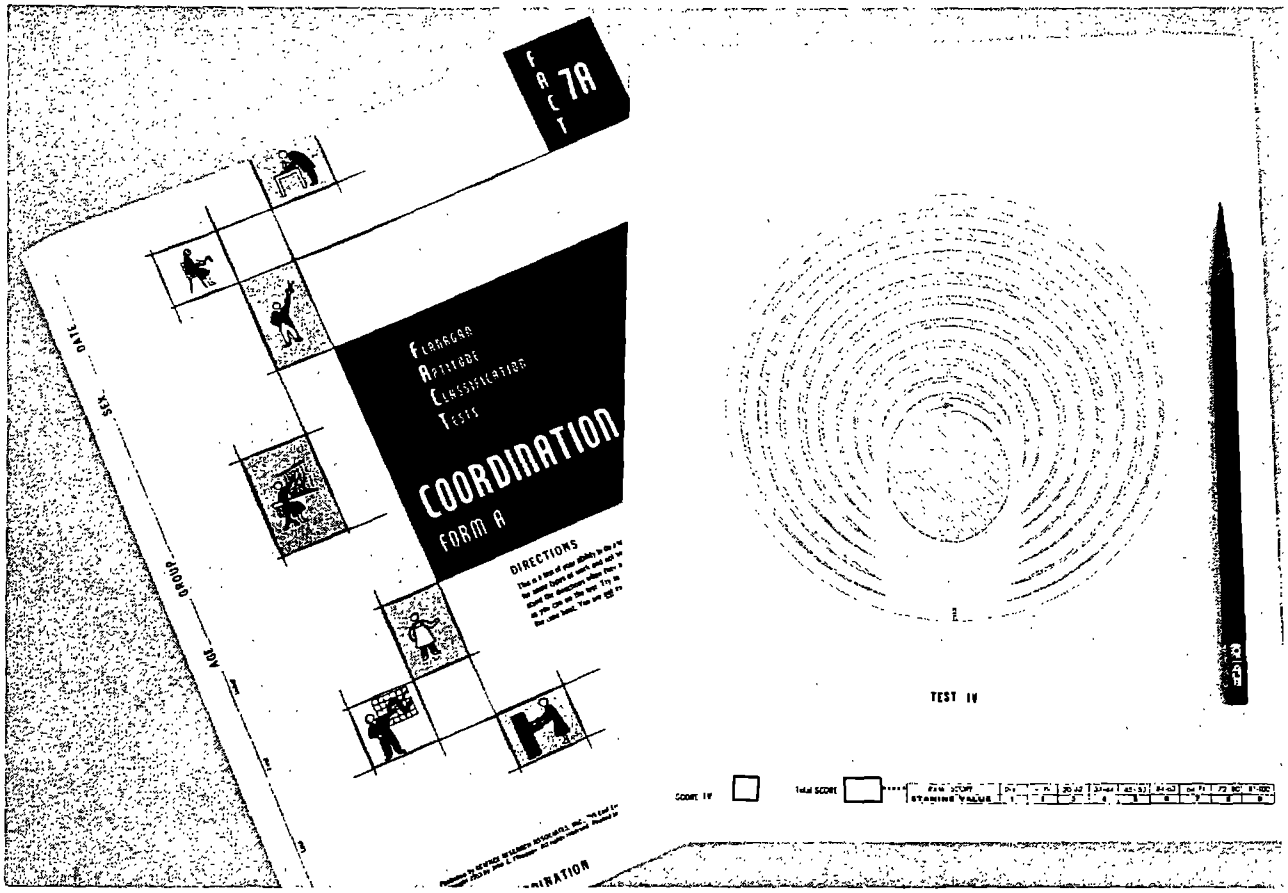
Figure 4

Figure 4: Subject performing the Alertness or "Clock" Test.

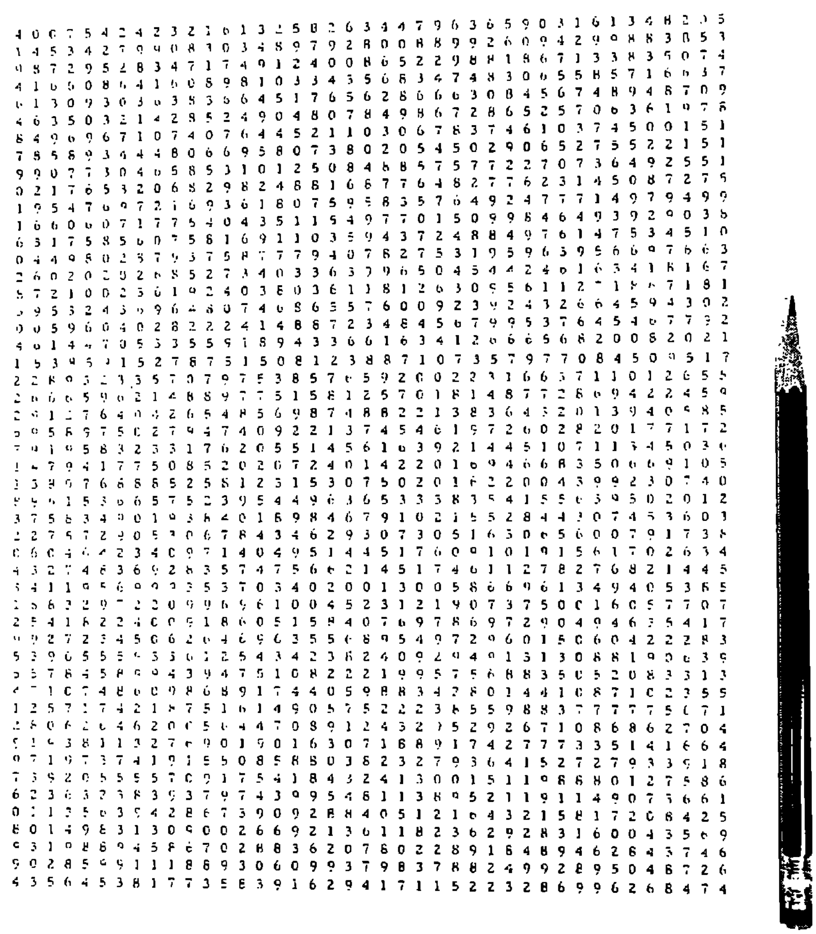
Marquette Time Estimation Test: This test consisted of a series of nine tone stimuli followed by nine light stimuli of approximately one, three or five seconds' duration presented in a random sequence but always with three stimuli of each time interval. At the termination of each stimulus, the subject depressed the push-button for that interval of time he estimated to be equal in length to the original auditory or light stimulus. A detailed description of the test and the instrumentation used to carry it out has been described by Stewart, et al [18]. This test took approximately seven minutes to perform.

Coordination Test: This test was the Flanagan Aptitude Classification Tests, 7A, Coordination, published by Science Research Associates, Inc., 259 East Erie Street, Chicago, Illinois. This test asked the subject to rapidly follow a spiral pathway with a pencil. The subject was allowed 40 seconds to complete each of six spirals. The first two were considered practice and the last four were scored and totaled. The total score depended upon the longest distance attained in each spiral minus the number of times the sides of the spiral pathway were touched with the pencil. This test took approximately five minutes to perform.

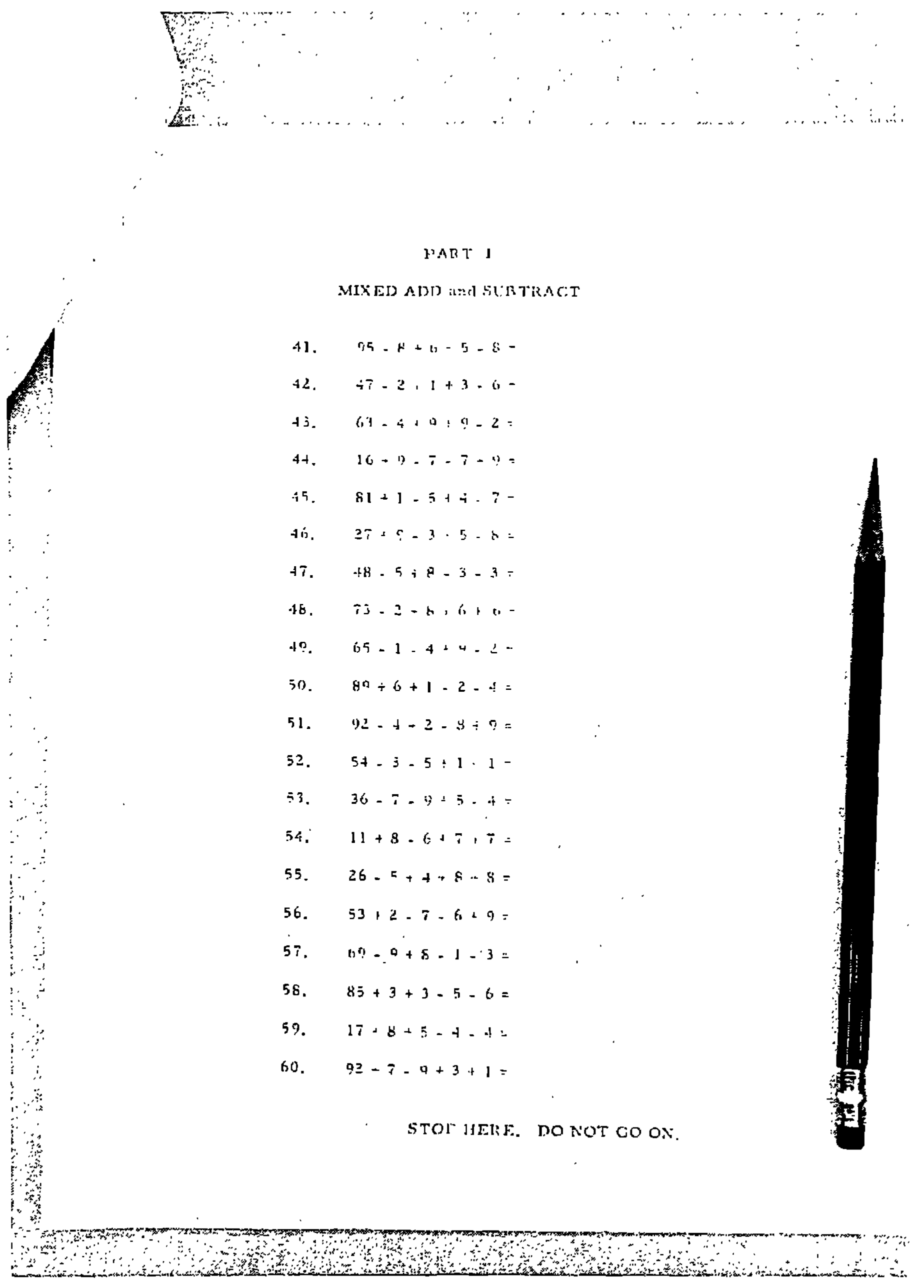
Arithmetic Test: This test, which measured the subjects' ability to work with numbers, was divided into two parts. The first part, lasting five minutes, consisted of simple addition and subtraction problems while the second part, lasting three minutes, consisted of multiplication and division. The maximum score attainable if all answers were correct was 125; however, no subject completed the tests in the allotted time. In order to minimize memorization of answers, four permutations of problem order were used.



Flanagan Coordination Test



Inspection Test



Arithmetic Test

Inspection Test: This test was a measure of the subjects' ability to spot the number "3" in rows of random numbers on an $8\frac{1}{2}$" x 11" page. The subject was asked to scan each row, beginning at the top of the page, and slash out with a red pencil each "3" encountered. The subject was given two minutes to strike out as many as possible. No subject ever finished the entire page. A subject's score was the total number of "3's" struck. Six differing pages with random numbers were utilized so that no subject received an identical number sheet on a given day.

RESULTS

Analysis of Exposure Chamber Atmosphere:

The daily time-weighted-average concentrations of methylene chloride vapor in the chamber atmosphere as determined by continuous infrared spectroscopy are listed in Table I. It is evident from the results that all actual mean exposure concentrations were within 6% of those planned, and usually they were within 1% of planned concentrations.

Analysis of the chamber atmosphere for ambient carbon monoxide revealed concentrations varying from 0.4 to 4.3 ppm. On the majority of days the background carbon monoxide concentration was less than 2 ppm.

Medical Surveillance:

The health of all subjects, with the exception of minor colds and an occasional sore throat, remained excellent throughout the study. The only abnormal laboratory values reported which could be correlated in degree of abnormality with the magnitude of exposure to methylene chloride were venous blood COHb. There were no other consistently abnormal results from any laboratory study, no individual significant abnormality, nor were the occasional abnormal laboratory results more prevalent after the high exposure days than after the four days of zero exposure. For example, the 24-hour urobilinogen values were greater than 10 Ehrlich units in 4 of 29 specimens collected after daily 0 ppm exposures, 1 of 50 specimens after 50 ppm, 5 of 100 after 250 ppm, 3 of 49 after 100 ppm, and 0 of 19 after daily 500 ppm exposures to methylene chloride. The transient urobilinogen elevations did correlate well with ethanol consumption.

Breath Analysis:

The infrared scan of the 15-minute post-exposure breath samples revealed no unusual absorptions except those expected in the methylene chloride and carbon monoxide regions of the spectrum. The values obtained for the carbon monoxide in breath samples analyzed by gas chromatography were converted to COHb [19] and are reported in the next section.

Methylene chloride was readily detected in the expired breath of each of the subjects following exposure to the four vapor concentrations. These post-exposure breath data for male and female subjects are summarized in Table II. Examination of these breath data reveals that a predictable excretion pattern exists for each of the vapor concentrations studied. Furthermore, the rate of excretion of methylene chloride in the breath is seen to be a

TABLE II

CH_2CL_2 BREATH CONCENTRATION OF SEDENTARY MALES

EXPOSED TO 50 PPM

Time, Min	N	Mean	Range	N	Mean	Range	Std. Dev.
	Single Exposure for 1 Hour			5 Daily 1-Hour Exposures			
1 min, pre-exit	3	11.6	11.2 - 12.0	8	11.8	8.4 - 15.7	2.22
1 min, post	2	5.5	5.3 - 5.7	9	4.7	1.5 - 8.5	2.06
15 min, post	3	3.9	0.9 - 7.8	10	2.6	0.9 - 7.8	2.05
30 min, post	3	1.8	1.7 - 2.1	10	1.4	0.3 - 2.7	0.76
60 min, post	2	1.0	0.9 - 1.2	8	0.6	0.2 - 1.2	0.36
120 min, post	2	0.4	0.3 - 0.5	8	0.3	0.2 - 0.5	0.13
180 min, post	2	1.2	1.1 - 1.2	8	0.4	0.2 - 1.2	0.45
	Single Exposure for 3 Hours			5 Daily 3-Hour Exposures			
1 min, pre-exit	3	12.5	11.7 - 13.2	12	13.8	11.3 - 18.1	2.10
1 min, post	3	7.4	5.2 - 9.4	7	5.8	3.0 - 9.4	2.24
15 min, post	3	4.1	3.6 - 4.5	8	4.0	2.4 - 5.0	0.89
30 min, post	3	2.2	1.7 - 2.4	9	1.9	0.8 - 2.8	0.72
60 min, post	2	0.9	0.4 - 1.4	8	1.1	0.4 - 2.1	0.67
120 min, post	3	1.0	0.2 - 2.1	9	0.9	0.2 - 2.1	0.57
180 min, post	2	1.3	0.7 - 1.8	8	0.7	0.3 - 1.8	0.46
	Single Exposure for $7\frac{1}{2}$ Hours			5 Daily $7\frac{1}{2}$-Hour Exposures			
1 min, pre-exit	4	14.2	13.1 - 15.5	16	15.0	12 - 22.6	2.76
1 min, post	4	8.4	5.0 - 11.3	16	7.6	4.5 - 12.9	2.16
15 min, post	4	4.7	4.0 - 5.7	16	5.2	3.2 - 8.1	1.25
30 min, post	4	3.6	2.8 - 5.0	16	3.6	2.1 - 5.5	0.97
60 min, post	4	1.4	0.8 - 1.9	12	1.8	0.8 - 2.4	0.46
120 min, post	4	0.9	0.4 - 1.5	12	1.1	0.4 - 1.7	0.37
180 min, post	3	0.9	0.6 - 1.1	11	0.9	0.6 - 1.4	0.21

(Continued on next page)

TABLE II (Continued)

CH_2CL_2 BREATH CONCENTRATION OF SEDENTARY MALES

EXPOSED TO 100 PPM

	Single Exposure for 1 Hour			5 Daily 1-Hour Exposures			
Time, Min	N	Mean	Range	N	Mean	Range	Std. Dev.
1 min, pre-exit	3	35.6	28.0 - 41.9	14	36.1	28 - 41.9	4.56
1 min, post	3	17.6	13.6 - 21.3	12	19.1	10.5 - 29.5	6.16
15 min, post	2	6.9	4.9 - 8.9	12	7.9	3.4 - 12.8	2.43
30 min, post	3	3.7	3.2 - 4.1	15	5.0	1.7 - 9.0	2.14
60 min, post	3	2.0	1.5 - 2.4	13	3.0	1.4 - 5.1	1.23
120 min, post	3	0.9	0.3 - 1.2	15	0.9	0.3 - 1.7	0.40
180 min, post	3	0.8	0.7 - 0.9	13	0.7	0.2 - 1.6	0.36
	Single Exposure for 3 Hours			5 Daily 3-Hour Exposures			
1 min, pre-exit	3	34.1	26.2 - 41.8	12	36.9	25.3 - 44.5	5.99
1 min, post	3	19.7	18.2 - 21.1	14	19.4	11.3 - 29.2	4.70
15 min, post	3	10.0	9.6 - 10.5	15	9.9	7.4 - 14.6	2.00
30 min, post	3	5.0	2.3 - 6.7	15	6.1	2.3 - 9.4	1.60
60 min, post	3	3.7	3.5 - 3.9	15	3.8	2.4 - 5.9	0.90
120 min, post	3	1.6	0.9 - 2.5	14	1.9	0.9 - 3.0	0.61
180 min, post	1	0.9		8	0.8	0.5 - 2	0.55
	Single Exposure for 7½ Hours			5 Daily 7½-Hour Exposures			
1 min, pre-exit	4	32.9	26.6 - 39.9	20	33.4	17.5 - 49.3	8.27
1 min, post	4	20.3	18.6 - 23.4	20	21.9	12.3 - 32.3	5.22
15 min, post	4	9.5	8.4 - 10.5	19	11.6	8.4 - 17.8	2.54
30 min, post	4	6.1	4.7 - 7.5	20	7.9	4.7 - 13.3	2.36
60 min, post	4	4.0	3.1 - 5.2	20	4.5	2.8 - 6.7	4.45
120 min, post	4	3.7	1.6 - 5.2	18	3.2	1.6 - 5.2	1.12
180 min, post	4	2.4	0.9 - 3.9	16	2.1	0.4 - 3.9	1.05

TABLE II (Continued)

CH_2CL_2 BREATH CONCENTRATION OF SEDENTARY MALES EXPOSED TO 250 PPM

Time, Min	Single Exposure for 1 Hour N	Mean	Range	5 Daily 1-Hour Exposures N	Mean	Range	Std. Dev.
1 min, pre-exit	3	74.7	63.6 - 88.6	15	84.1	63.6 - 103.2	11.92
1 min, post	3	25.4	21.0 - 32.1	14	35.2	21.0 - 51.6	9.39
15 min, post	2	8.2	5.7 - 10.6	14	17.3	5.7 - 31.6	7.21
30 min, post	3	6.8	2.4 - 11.9	14	11.6	2.4 - 19.4	4.43
60 min, post	3	5.9	4.2 - 7.5	14	6.2	3.4 - 9.2	1.91
120 min, post	2	1.5	1.4 - 1.6	13	2.1	1.3 - 3.0	0.56
180 min, post	3	0.9	0.7 - 1.2	12	1.3	0.8 - 2.5	0.47
1350 min, post	0	0.0		6	0.6	0.2 - 1.3	0.38

Time, Min	Single Exposure for 3 Hours N	Mean	Range	5 Daily 3-Hour Exposures N	Mean	Range	Std. Dev.
1 min, pre-exit	3	86.0	79.4 - 95.8	15	103.0	79.2 - 130.0	14.42
1 min, post	3	25.6	22.6 - 29.7	15	54.2	22.6 - 92.8	20.77
15 min, post	3	19.1	11.7 - 26.0	15	30.4	11.8 - 52.9	12.44
30 min, post	3	13.1	6.4 - 16.5	14	21.1	6.4 - 37.8	7.84
60 min, post	3	7.4	6.3 - 9.4	15	10.2	4.7 - 16.2	3.69
120 min, post	3	3.8	2.4 - 4.7	13	4.8	2.4 - 9.3	2.08
180 min, post	3	2.1	1.7 - 2.7	11	2.9	0.9 - 5.3	1.31
1170 min, post	2	0.4	0.3 - 0.6	14	0.3	0.2 - 1.3	0.28

Time, Min	Single Exposure for $7\frac{1}{2}$ Hours N	Mean	Range	5 Daily $7\frac{1}{2}$-Hour Exposures N	Mean	Range	Std. Dev.
1 min, pre-exit	4	100.1	64.2 - 133.2	16	102.9	40.6 - 160.2	35.25
1 min, post	4	63.1	44.9 - 84.6	19	82.0	44.9 - 110.1	18.82
15 min, post	4	37.3	30.2 - 49.7	18	47.2	30.2 - 64.9	11.02
30 min, post	4	28.2	13.8 - 43.1	20	31.4	13.8 - 44.0	7.49
60 min, post	4	12.3	9.2 - 15.7	19	17.9	9.2 - 27.9	5.65
120 min, post	2	5.84	4.5 - 7.1	17	8.8	4.5 - 14.7	2.86
180 min, post	3	5.38	4.6 - 6.4	17	6.4	3.8 - 10.0	1.88
960 min, post	4	0.3	0.1 - 4.8	19	0.7	0.1 - 1.6	0.41

TABLE II (Continued)

CH_2CL_2 BREATH CONCENTRATION OF SEDENTARY FEMALES

EXPOSED TO 250 PPM

	Single Exposure for 1 Hour			5 Daily 1-Hour Exposures			
Time, Min	N	Mean	Range	N	Mean	Range	Std. Dev.
1 min, pre-exit	3	82.3	68.7 - 98.6	13	89.3	68.7 - 108.2	14.11
1 min, post	3	56.7	51.0 - 62.6	15	61.5	42.8 - 78.8	10.62
15 min, post	3	28.6	26.5 - 29.9	15	28.3	23.9 - 31.9	2.19
30 min, post	3	17.9	17.0 - 19.1	15	18.1	12.5 - 26.9	3.19
60 min, post	3	10.7	8.8 - 12.9	15	11.2	8.6 - 15.2	1.83
120 min, post	3	2.8	1.6 - 3.5	13	3.3	1.4 - 5.4	1.27
180 min, post	2	2.6	2.5 - 2.7	12	2.7	1.2 - 3.6	0.68
1350 min, post	3	2.7	1.3 - 4.0	15	1.9	0.7 - 4.0	0.90

	Single Exposure for 3 Hours			5 Daily 3-Hour Exposures			
Time, Min	N	Mean	Range	N	Mean	Range	Std. Dev.
1 min, pre-exit	2	81.3	75.5 - 87.1	9	85.7	72.4 - 102.9	10.14
1 min, post	2	57.8	51.7 - 64.0	9	66.5	51.7 - 89.1	11.32
15 min, post	2	24.2	22.5 - 25.9	10	29.7	22.5 - 44.9	7.35
30 min, post	2	17.4	12.9 - 21.8	8	19.1	12.9 - 32.6	6.49
60 min, post	2	13.8	7.0 - 21.7	9	14.8	10.2 - 21.7	3.87
120 min, post	1	1.2		9	3.6	1.2 - 7.0	1.83
180 min, post	1	1.8		8	2.7	1.6 - 3.9	0.88
1170 min, post	2	1.5	1.30 - 1.6	10	1.8	0.6 - 4.0	0.97

	Single Exposure for 7½ Hours			5 Daily 7½-Hour Exposures			
Time, Min	N	Mean	Range	N	Mean	Range	Std. Dev.
1 min, pre-exit	4	116.7	100.0 - 136.3	19	108.1	77.6 - 141.3	18.33
1 min, post	4	81.9	69.9 - 99.3	20	83.7	55.3 - 119.6	16.37
15 min, post	4	40.1	24.7 - 50.7	19	40.5	18.6 - 60.1	11.31
30 min, post	4	32.2	22.6 - 46.6	20	33.3	19.7 - 55.8	10.18
60 min, post	4	22.1	13.7 - 30.1	20	23.2	13.7 - 37.7	6.14
120 min, post	4	7.0	3.0 - 11.2	18	6.9	3.0 - 13.6	2.98
180 min, post	3	4.5	2.1 - 6.7	19	4.9	1.5 - 9.9	2.10
960 min, post	4	3.4	2.0 - 4.8	20	2.3	0.8 - 4.8	1.15

TABLE II (Continued)

CH_2CL_2 BREATH CONCENTRATION OF SEDENTARY MALES
EXPOSED TO 250 PPM, WIDELY FLUCTUATING

	Single Exposure for 1 Hour			5 Daily 1-Hour Exposures			
Time, Min	N	Mean	Range	N	Mean	Range	Std. Dev.
1 min, pre-exit	3	67.5	61.7 - 73.5	15	77.7	61.7 - 102.8	12.82
1 min, post	2	44.5	40.0 - 49.1	13	44.5	20.0 - 61.9	12.99
15 min, post	3	15.1	12.6 - 17.9	15	18.6	9.5 - 32.2	6.30
30 min, post	3	8.4	7.8 - 8.8	14	11.6	7.8 - 15.6	2.45
60 min, post	2	4.5	4.1 - 5.0	14	6.4	4.1 - 12.9	2.40
120 min, post	3	1.8	1.6 - 2.0	15	2.2	0.4 - 3.6	0.83
180 min, post	3	1.3	1.0 - 1.5	12	1.2	0.4 - 2.0	0.41
1350 min, post	3	0.2	0.1 - 0.3	12	.24	0.1 - 0.5	0.11
	Single Exposure for 3 Hours			**5 Daily 3-Hour Exposures**			
1 min, pre-exit	3	76.3	58.8 - 91.1	15	93.4	58.8 - 114.1	15.59
1 min, post	3	48.3	39.4 - 61.4	15	58.6	39.4 - 76.2	13.28
15 min, post	2	29.6	28.9 - 30.3	14	32.1	18.7 - 46.9	8.40
30 min, post	3	13.4	10.9 - 16.6	15	20.4	10.9 - 31.2	6.53
60 min, post	3	9.2	6.8 - 10.9	15	11.1	5.3 - 21.6	4.11
120 min, post	3	3.4	1.2 - 4.7	12	4.5	1.2 - 7.0	1.40
180 min, post	3	2.5	0.8 - 3.6	11	2.5	0.8 - 4.6	1.16
1170 min, post	3	0.5	0.4 - 0.6	12	0.4	0.2 - 0.7	0.17
	Single Exposure for 7½ Hours			**5 Daily 7½-Hour Exposures**			
1 min, pre-exit	4	91.4	71.4 - 109.4	19	108.3	71.4 - 136.4	14.87
1 min, post	4	71.7	67.6 - 80.6	16	77.3	65.7 - 96.0	9.46
15 min, post	4	30.2	28.2 - 32.9	20	39.0	15.8 - 69.2	10.74
30 min, post	4	21.8	21.1 - 22.5	19	23.6	13.5 - 32.6	4.40
60 min, post	3	12.2	11.2 - 13.0	19	15.4	10.5 - 26.5	4.47
120 min, post	3	8.9	4.4 - 11.8	19	8.8	4.0 - 11.8	2.31
180 min, post	3	7.2	3.7 - 9.2	16	6.0	3.4 - 9.4	1.84
960 min, post	4	0.8	0.4 - 1.3	16	1.1	0.3 - 2.8	0.63

TABLE II (Continued)

CH_2CL_2 BREATH CONCENTRATION OF SEDENTARY MALES EXPOSED TO 500 PPM

	Single Exposure for 1 Hour			2 Daily 1-Hour Exposure			
Time, Min	N	Mean	Range	N	Mean	Range	Std. Dev.
1 min, pre-exit	3	205.5	163.4 - 239.0	6	212.8	163.4 - 242.2	31.89
1 min, post	3	119.7	82.7 - 178.2	6	135.1	82.7 - 208	50.17
15 min, post	3	59.8	31.4 - 90.5	5	71.5	31.4 - 90.5	26.35
30 min, post	3	38.2	30.6 - 43.8	6	39.5	22.5 - 53.5	11.26
60 min, post	3	19.8	17.7 - 21.2	5	21.7	17.7 - 26.8	3.35
120 min, post	3	4.5	3.4 - 5.8	6	6.5	3.4 - 9.1	2.38
180 min, post	3	1.8	0.7 - 2.6	5	2.6	0.7 - 4.2	1.36
1350 min, post	3	0.4	0.2 - 0.5	6	0.4	0.2 - 0.5	0.11
	Single Exposure for 3 Hours			**2 Daily 3-Hour Exposures**			
1 min, pre-exit	3	218.3	182.0 - 260.0	6	247.7	182.0 - 319.0	46.69
1 min, post	3	152.1	80.2 - 190.0	6	193.0	80.2 - 254.8	63.04
15 min, post	3	109.0	98.1 - 116.0	6	120.2	98.1 - 149.0	19.38
30 min, post	3	80.8	72.9 - 89.8	6	88.1	72.9 - 108.0	14.15
60 min, post	3	44.5	41.7 - 48.4	6	47.6	41.7 - 54.9	6.02
120 min, post	2	19.7	19.3 - 20.1	4	16.9	12.0 - 20.1	3.69
180 min, post	3	2.4	1.0 - 4.3	4	2.9	1.0 - 4.3	1.64
1170 min, post	3	0.5	0.3 - 0.7	6	0.4	0.3 - 0.7	0.14
	Single Exposure for $7\frac{1}{2}$ Hours			**2 Daily $7\frac{1}{2}$-Hour Exposures**			
1 min, pre-exit	3	241.0	221.0 - 251.0	7	276.8	221.0 - 333.0	39.8
1 min, post	3	187.7	158.0 - 215.0	7	225.8	158.0 - 280.0	42.77
15 min, post	3	111.2	95.7 - 138.8	7	146.8	95.7 - 201.0	41.70
30 min, post	3	89.1	82.6 - 98.1	7	106.0	82.6 - 145.0	26.15
60 min, post	3	60.43	47.7 - 79.7	7	66.2	47.7 - 94.5	16.47
120 min, post	3	24.9	24.0 - 25.3	7	25.6	18.0 - 30.7	4.20
180 min, post	3	15.1	6.0 - 22.0	6	16.4	6.0 - 22.0	5.41
960 min, post	3	1.3	0.9 - 2.1	6	1.4	0.9 - 2.1	0.55

function of the duration of the exposure. Several of the breath decay curves obtained in this study are graphically presented in Figures 5 - 7.

A "family" of post-exposure breath decay curves can be constructed from the methylene chloride breath data. Such a "family" is presented in Figure 5. These are second degree polynomial curves which can be described by the empirical equation:

$$1/Y = B_0 + B_1 X + B_2 X^2$$

where: Y = breath vapor concentration

X = time after exposure

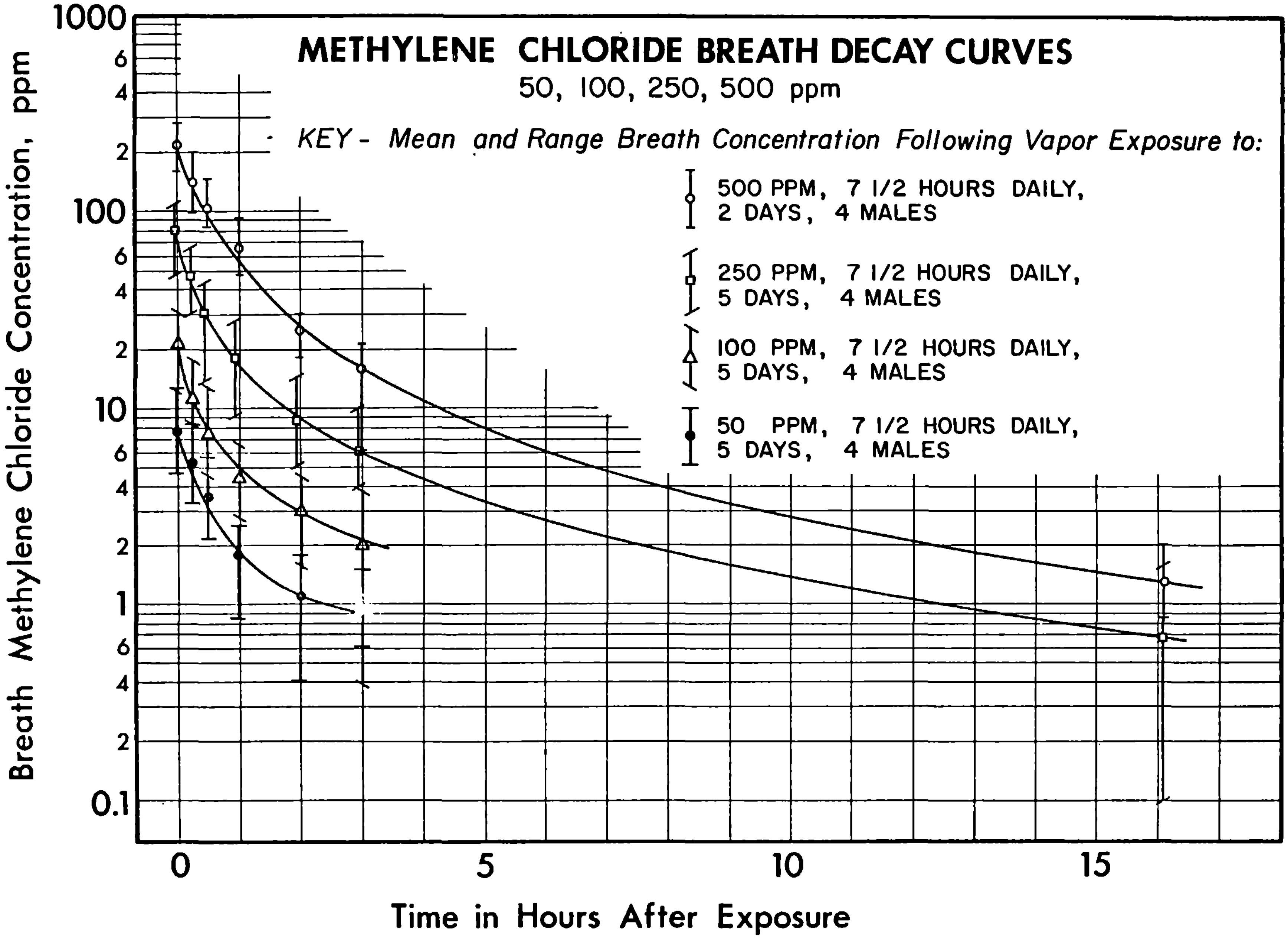
B_0, B_1, B_2 = arbitrary constants found by regression analysis

This equation fits the data points for the 50 ppm post-exposure data rather well as the correlation coefficient $r = 0.997$, and for the 500 ppm data $r = 1.000$.

The concentration of methylene chloride in the breath after exposure is directly related to the time-weighted-average concentration during exposure, the duration of exposure being constant. The variation in breath concentration between male subjects identically exposed, while not great, was large enough to prevent segregation of subjects into one of the four exposure categories on the basis of a single breath sample collected more than two hours after exposure. The variation between subjects was due in part to breath sample collection and analytical error, but also in part to genuine reproducible differences between individuals.

The length of time after exposure in which the methylene chloride could

Figure 5


METHYLENE CHLORIDE BREATH DECAY CURVES
50, 100, 250, 500 ppm
KEY - Mean and Range Breath Concentration Following Vapor Exposure to:
500 PPM, 7 1/2 HOURS DAILY, 2 DAYS, 4 MALES
250 PPM, 7 1/2 HOURS DAILY, 5 DAYS, 4 MALES
100 PPM, 7 1/2 HOURS DAILY, 5 DAYS, 4 MALES
50 PPM, 7 1/2 HOURS DAILY, 5 DAYS, 4 MALES
Breath Methylene Chloride Concentration, ppm
1000
6
4
2
100
6
4
2
10
6
4
2
1
6
4
2
0.1
0
5
10
15
Time in Hours After Exposure

be detected in the breath was related to the magnitude of exposure. The solvent was still detectable 16 hours after exposure to 250 ppm for $7\frac{1}{2}$ hours, but was beneath detectable limits within a few hours after exposure to 50 - 100 ppm. Breath analysis in the first two hours after exposure was the most reliable sampling time for estimating the time-weighted-average vapor concentration for a $7\frac{1}{2}$-hr exposure.

The duration of exposure to a given vapor concentration of methylene chloride was an important variable influencing the rate of excretion of the solvent in the breath. This is shown in Figure 6. In this example, serial breath sample analysis following exposure to 500 ppm would permit the construction of a decay curve which would indicate the probable duration of that exposure.

The influence of widely fluctuating methylene chloride vapor concentrations on the breath decay curves is illustrated in Figure 7. The overall breath decay curve very accurately reflects the time-weighted-average vapor concentration. A breath sample collected within a few minutes after exposure would reflect the most recent vapor concentration while a sample collected one hour after exposure would indicate the time-weighted-average exposure.

The female subjects had breath decay curves very similar to the male curves during the first hours after exposure (Figure 7). The mean breath concentration 16 hours after exposure to 250 ppm was slightly higher than that recorded for similarly exposed male subjects.

In contrast to 1,1,1-trichloroethane and tetrachloroethylene, there

Figure 6

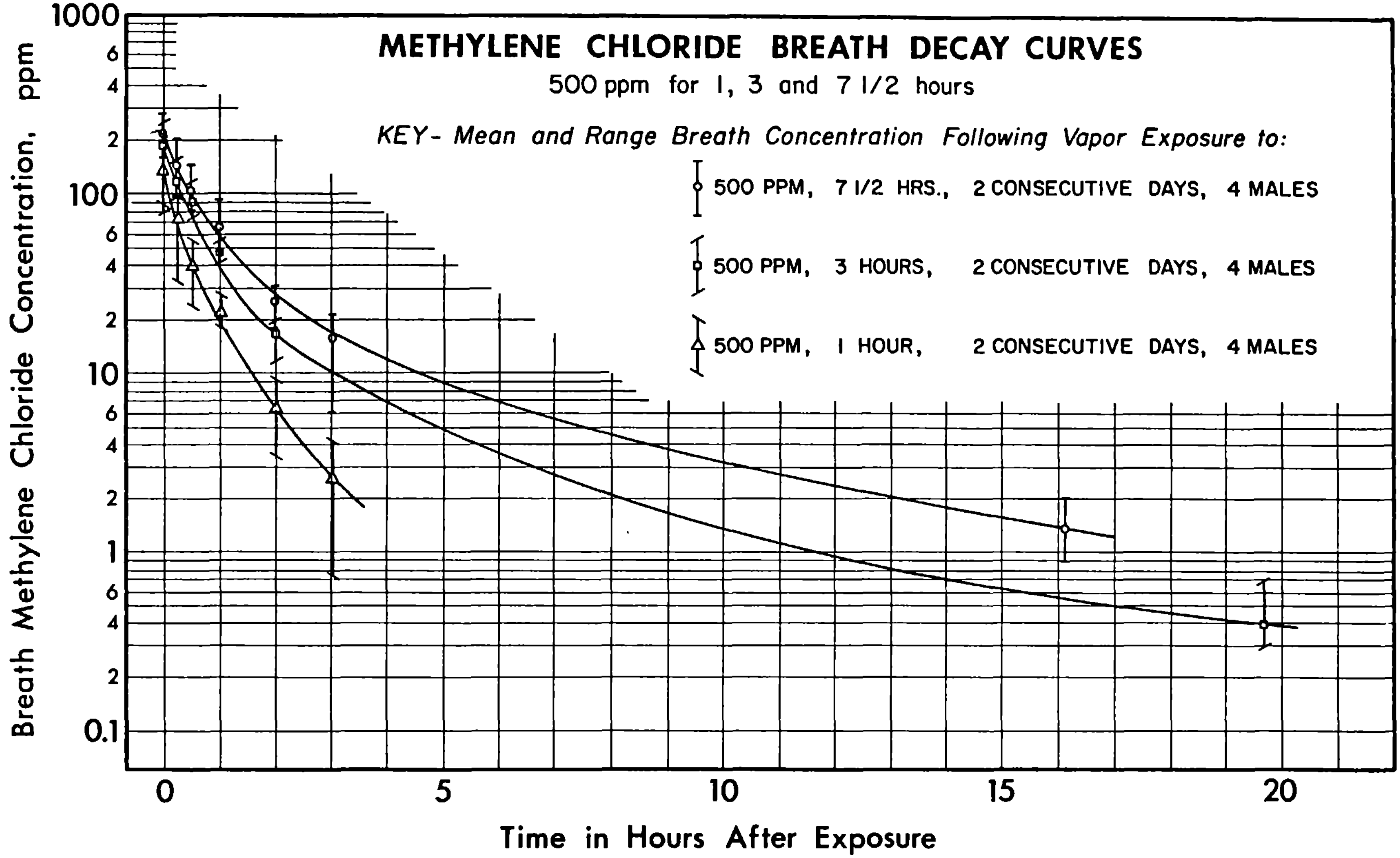
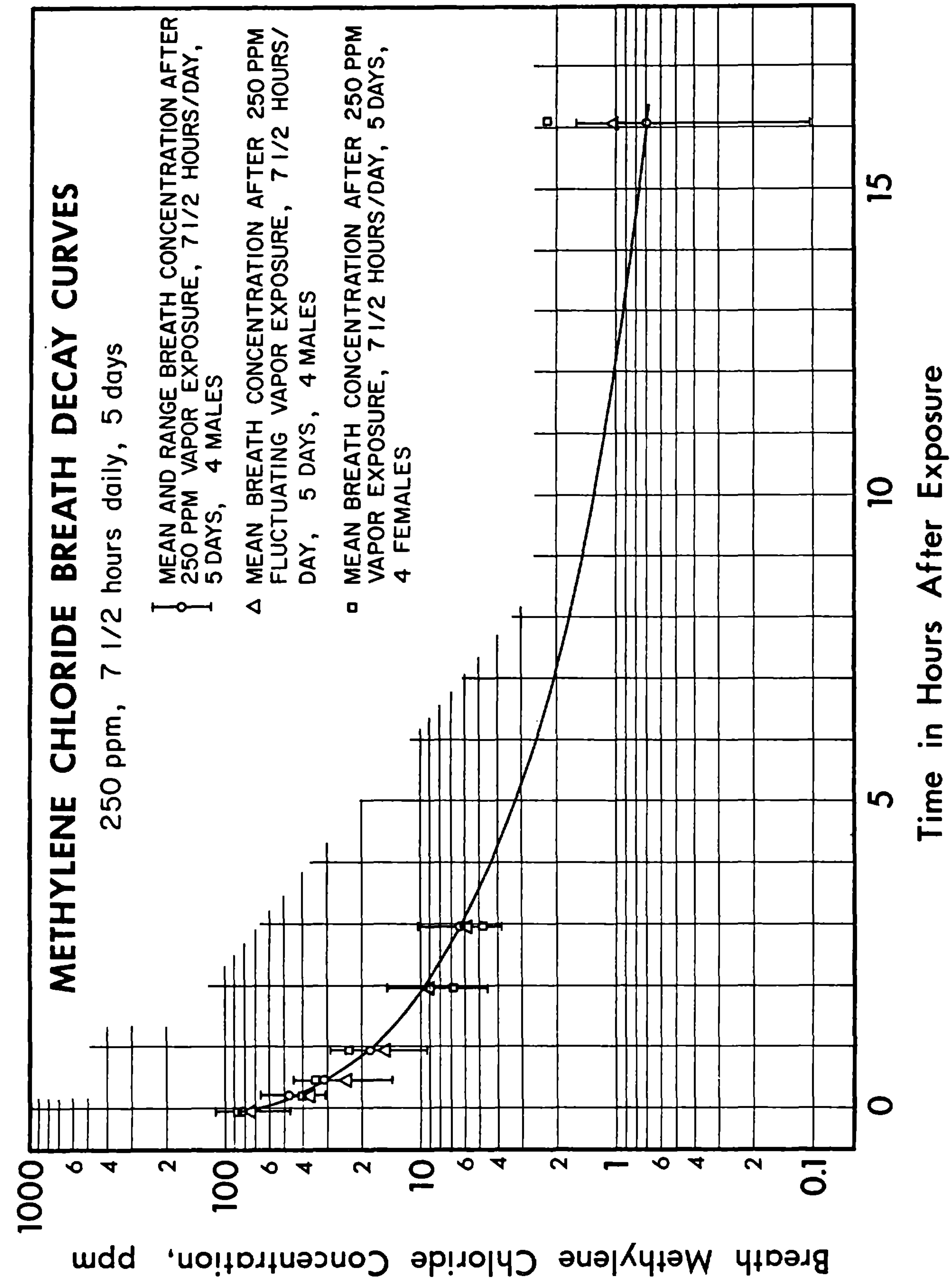

METHYLENE CHLORIDE BREATH DECAY CURVES
500 ppm for 1, 3 and 7 1/2 hours
KEY- Mean and Range Breath Concentration Following Vapor Exposure to:
500 PPM, 7 1/2 HRS., 2 CONSECUTIVE DAYS, 4 MALES
500 PPM, 3 HOURS, 2 CONSECUTIVE DAYS, 4 MALES
500 PPM, 1 HOUR, 2 CONSECUTIVE DAYS, 4 MALES
Breath Methylene Chloride Concentration, ppm
1000
6
4
2
100
6
4
2
10
6
4
2
1
6
4
2
0.1
0
5
10
15
20
Time in Hours After Exposure

Figure 7


METHYLENE CHLORIDE BREATH DECAY CURVES
250 ppm, 7 1/2 hours daily, 5 days
MEAN AND RANGE BREATH CONCENTRATION AFTER 250 PPM VAPOR EXPOSURE, 7 1/2 HOURS/DAY, 5 DAYS, 4 MALES
MEAN BREATH CONCENTRATION AFTER 250 PPM FLUCTUATING VAPOR EXPOSURE, 7 1/2 HOURS/DAY, 5 DAYS, 4 MALES
MEAN BREATH CONCENTRATION AFTER 250 PPM VAPOR EXPOSURE, 7 1/2 HOURS/DAY, 5 DAYS, 4 FEMALES
Breath Methylene Chloride Concentration, ppm
1000
6
4
2
100
6
4
2
10
6
4
2
1
6
4
2
0.1
Time in Hours After Exposure
0
5
10
15

was no significant accumulation of methylene chloride in the body from day to day which would effect the breath decay curves (Table II). Therefore, no correction factor for repetitive exposures need be considered when breath decay curves are used to estimate the magnitude of exposure.

Carboxyhemoglobin Elevation:

Exposure to methylene chloride vapor from 50 to 500 ppm resulted in a prompt elevation of COHb, the height of which was directly related to the magnitude of the exposure. The COHb continued to rise for one to two hours following the exposure and then gradually fell with a biological half-life twice as long as occurs when COHb is produced from an exogenous carbon monoxide source.

The results of all COHb determinations are listed in Tables III, IV, V, VI and VII. Carboxyhemoglobin values estimated from breath carbon monoxide concentration are noted. Table III lists the values for the $7\frac{1}{2}$-hr male subjects. Because three were non-smokers and had no obvious exposure to more than ambient levels of carbon monoxide, their COHb values were averaged. All male subjects in the 1-hr and 3-hr groups are listed individually in Tables IV and V because of either smoking or possible work exposure to carbon monoxide.

The results of the COHb analyses for the female subjects are listed in Table VI. Average values for the four $7\frac{1}{2}$-hr subjects and the three 1-hr subjects are listed, while individual values for the 3-hr subjects are given. The 3-hr subjects were not averaged because the one subject who had been a

TABLE III

BLOOD COHb% SATURATION[a] OF HUMANS EXPOSED DAILY FOR 7 1/2 HOURS TO CH_2Cl_2

	DAY 1					DAY 2	DAY 3					DAY 4	DAY 5			
	pre-exp.	7/2 hr exp.	1 hr post exp.	2 hr post exp.	3 hr post exp.	pre exp.	pre exp.	7 1/2 hr exp.	1 hr post exp.	2 hr post exp.	3 hr post exp.	pre exp.	pre exp.	7 1/2 hr exp.	1 hr post exp.	16 hr post exp.
WEEK 2 (50 ppm)																
3 non-smokers	0.9[b]	2.4	2.3	NA	NA	1.0	1.1	2.5[b]	2.9	NA	NA	1.0	1.0	2.5	2.1	1.4
1 light smoker	1.4	2.9	2.5	NA	NA	1.3	1.5	3.1	2.7	NA	NA	1.3	1.2	2.8	2.5	1.9
WEEK 3 (250 ppm)																
3 non-smokers	0.6	9.4	9.2	10.8[b]	10.0[b]	2.7	2.4	7.4	7.8	8.9	7.0	2.2	2.5	9.2	9.6	3.1
1 light smoker	0.7	7.4	8.5	10.3	9.6	3.0	2.7	8.7	8.6	7.9	7.1	2.8	3.1	10.1	10.6	4.5
WEEK 4 (250 ppm)																
3 non-smokers	0.9	8.9	8.5	7.6	8.9	2.5	2.5	8.3	8.2	8.7[b]	7.3	2.3	1.9	9.3	9.5	2.1
1 light smoker	0.8	7.5	7.6	NA	NA	3.0	3.3	9.4	8.9	7.5	10.1	3.5	2.7	9.9	9.4	3.4
WEEK 5 (100 ppm)																
3 non-smokers	0.8	5.0	4.9	6.1[b]	5.9[b]	1.6	1.9	5.0	4.7	5.4	4.8	1.9	1.6	5.2	5.7	1.6
1 light smoker	1.5	5.3	5.0	7.1	5.8	1.9	2.6	6.1	5.3	6.4	-	2.1	2.5	5.3	6.0	3.1
WEEK 6 (0 & 500 ppm)																
3 non-smokers	0.8	1.0[b]	0.7	NA	NA	1.1	1.2[b]	10.7[b]	11.4[b]	11.7[b]	11.4[b]	3.4[b]	3.1	NO EXPOSURE		
1 light smoker	1.5	1.1	1.3	NA	NA	1.6	1.4	11.9	11.0	12.2	12.1	4.2	5.3	NO EXPOSURE		

[a] All pre-exposure, 7 1/2 hr exposure and 1 hr post-exposure values represent blood analyses by CO-Oximeter, 2 hr and 3 hr post-exposure values calculated from CO concentration by G.C. in breath samples.

[b] Average of values from 2 non-smokers. Other values are averaged from 3 non-smokers.

NA Value not available.

TABLE IV

BLOOD COHb% SATURATION OF HUMANS EXPOSED DAILY FOR 3 HOURS TO CH_2Cl_2

	DAY 1					DAY 2	DAY 3					DAY 4	DAY 5			
	pre-exp	3 hr exp	1 hr post exp	2 hr post exp	3 hr post exp	pre-exp	pre-exp	3 hr exp	1 hr post exp	2 hr post exp	3 hr post exp	pre-exp	pre-exp	3 hr exp	1 hr post exp	21 hr post exp
WEEK 2 (50 ppm)																
non-smoker	0.8	1.6	1.2	NA	NA	0.5	0.8	NA	1.4	NA	NA	1.7[a]	0.6	3.0[a]	1.2	1.7[a]
N-S garageman	1.2	1.9	1.5	NA	NA	1.0	0.9	1.2	1.2	NA	NA	1.3	0.8	1.4	1.3	2.1
heavy smoker	7.0	5.5	5.0	NA	NA	6.3	6.5	5.7	4.7	NA	NA	6.1	6.2	5.1	4.2	7.4
WEEK 3 (250 ppm)																
non-smoker	0.9	5.8[a]	3.6	5.3[a]	5.3[a]	2.2[a]	NA	NA	4.6	6.8[a]	6.6[a]	2.5[a]	1.1	8.3[a]	5.0	NA
N-S garageman	0.7	3.0	3.3	3.7[a]	NA	2.0	1.7	3.8	4.4	6.4[a]	4.4[a]	1.9	1.9	4.1	4.8	2.2
heavy smoker	7.6	8.6	8.0	11.9[a]	11.9[a]	8.0	6.1	8.6	8.1	11.0[a]	6.0[a]	9.0	8.7	9.5	9.9	7.8
WEEK 4 (250 ppm)																
non-smoker	0.8	7.1[a]	4.3	4.9[a]	5.3[a]	1.9[a]	NA	5.3[a]	4.0	5.1[a]	NA	NA	1.2	NA	3.9	NA
N-S garageman	1.3	3.4	4.0	4.4[a]	3.0[a]	2.2	1.9	3.4	4.3	4.9[a]	NA	2.0	1.2	3.2	3.6	1.0
heavy smoker	8.6	8.9	8.7	6.8[a]	9.4[a]	9.0	8.2	8.2	8.2	8.7[a]	10.6[a]	8.2	7.5	8.3	8.6	7.7
WEEK 5 (100 ppm)																
non-smoker	0.5	3.7[a]	NA	3.5[a]	3.0[a]	NA	NA	3.9[a]	2.8	3.2[a]	3.2[a]	NA	1.0	2.0[a]	3.0	1.7[a]
N-S garageman	1.7	2.3	2.2	3.2[a]	NA	1.4	2.3	2.7	2.8	3.7[a]	6.0[a]	3.3	1.0	1.7	2.5	0.9
heavy smoker	7.9	7.3	7.0	9.4[a]	6.6[a]	7.0	9.3	8.0	7.1	9.8[a]	9.8[a]	6.3	7.0	6.9	7.1	8.5
WEEK 6 (0 & 500 ppm)																
non-smoker	1.0	NA	1.2	0.7[a]	1.5[a]	NA	NA	NA	5.6	6.0[a]	7.3[a]	2.2	1.6 (24 hour post)			
N-S garageman	1.2	0.9	0.9	2.2[a]	2.5[a]	1.6	1.2	3.7	4.6	6.0[a]	NA	2.5	2.7 (19 hr post) No Exposure			
heavy smoker	6.2	5.1	4.9	6.0[a]	4.6[a]	7.3	7.0	8.6	8.6	9.2[a]	11.9[a]	7.1	8.5 (24 hr post)			

NA Value not available.

a Value determined indirectly from CO concentration in breath. Other values obtained directly on CO-Oximeter.

TABLE V

BLOOD COHb% SATURATION OF HUMANS EXPOSED DAILY FOR 1 HOUR TO CH_2Cl_2

	Day 1					Day 2	Day 3					Day 4	Day 5			
	pre-exp	1 hr exp	1 hr post exp	2 hr post exp	3 hr post exp	pre-exp	pre-exp	1 hr exp	1 hr post exp	2 hr post exp	3 hr post exp	pre-exp	pre-exp	1 hr exp	1 hr post exp	23 hr post exp
WEEK 2 (50 ppm)																
non-smoker	0.9	1.0	0.9	NA	NA	0.8	0.8	1.1	1.3	NA	NA	1.0	0.6	NA	NA	NA
N-S ind. wrkr	1.3	1.4	1.3	NA	NA	2.1	2.2	2.1	1.9	NA	NA	2.5	2.0	1.9	1.6	1.9
light smoker	2.9	2.7	2.1	NA	NA	4.2	2.5	2.3	2.3	NA	NA	2.6	2.7	2.2	2.1	2.4
WEEK 3 (250 ppm)																
non-smoker	1.1	2.3	2.8	3.7[a]	3.2[a]	1.7	1.2	2.0	2.4	3.2[a]	3.2[a]	1.7	1.4	2.9	3.0	1.9
N-S ind wrkr	1.2	2.0	2.1	NA	1.2[a]	2.3	2.2	3.2	3.2	2.7[a]	NA	2.7	2.1	3.2	3.4	1.9
light smoker	2.1	2.7	3.1	3.7[a]	5.3[a]	3.0	1.5	2.3	2.8	5.1[a]	NA	2.3	3.2	4.0	4.6	4.6
WEEK 4 (250 ppm)																
non-smoker	0.8	1.6	2.0	3.2[a]	2.5[a]	1.5	1.6	2.1	2.5	NA	2.7[a]	1.1	1.1	1.7	2.0	1.2
N-S ind wrkr	2.1	2.4	2.8	2.5[a]	2.7[a]	2.4	2.0	2.6	2.6	1.7[a]	3.2[a]	2.4	1.3	2.2	2.6	1.8
light smoker	2.4	2.7	3.3	3.7[a]	3.5[a]	NA	2.6	3.3	3.6	5.8[a]	4.9[a]	2.9	2.9	4.0	4.1	2.6
WEEK 5 (100 ppm)																
non-smoker	0.8	1.2	1.3	1.7[a]	2.0[a]	1.4	1.1	1.7	1.8	2.7[a]	2.0[a]	1.0	2.5	3.2	2.9	1.0
N-S ind wrkr	1.8	2.0	1.7	2.7[a]	1.5[a]	2.0	1.9	2.3	2.3	2.2[a]	2.7[a]	2.1	1.4	1.8	1.8	0.9
light smoker	1.1	2.0	1.9	2.2[a]	1.7[a]	3.0	2.3	2.7	2.8	3.0[a]	3.0[a]	2.7	2.9	3.5	3.3	3.0
WEEK 6 (0 & 500 ppm)																
non-smoker	0.9	0.7	0.9	1.2[a]	0.9[a]	0.9	1.6	2.6	4.4	4.9[a]	3.5[a]	1.3	2.2 (23 hour post)			
N-S ind wrkr	2.3	1.9	1.8	1.7[a]	1.5[a]	2.2	2.1	2.8	4.2	3.2[a]	2.7[a]	2.8	2.1 (27 hr post) No Exposure			
light smoker	2.0	1.6	1.5	6.6[a]	2.2[a]	2.2	NA	4.1	6.1	5.8[a]	5.8[a]	2.5	4.8 (25 hour post)			

NA Value not available.

[a] Value determined indirectly from CO concentration in breath. Other values obtained directly by CO-Oximeter

TABLE VI

AVERAGE AND RANGE OF BLOOD COHb% SATURATION OF FEMALES EXPOSED DAILY TO CH_2Cl_2

	WEEK 1	WEEK 2: 250 PPM												WEEK 3
Hours of Daily Exposure	Day 5 0 ppm pre-exp	pre-exp	pre-exit	1 hr post	Day 2 pre-exp	Day 3 pre-exp	pre-exit	1 hr post	Day 4 pre-exp	Day 5 pre-exp	pre-exit	1 hr post	16, 21, or 23 hrs post	Day 1 0 ppm pre-exp
7 1/2	1.5	1.4	9.6	9.4	2.7	3.1	10.3	10.6	2.8	2.5	10.0	9.8	2.5	1.9[a]
	(1.1-1.8)	(1.1-2.2)	(7.3-11.7)	(7.5-10.7)	(2.3-3.2)	(2.5-3.5)	(8.8-11.3)	(9.7-11.1)	(2.6-2.8)	(1.7-3.0)	(8.1-11.2)	(8.0-10.9)	(2.4-2.7)	(1.6-2.2)
3	1.2	1.2	4.5	4.7	2.0	2.2	4.9	5.4	1.9	2.1	4.9	5.1	2.2	1.6
	-	2.4	6.8	6.8	2.4	3.1	7.5	7.6	2.8	2.8	6.9	7.2	3.6	3.1
1	1.3	1.3	2.8	3.1	1.7	2.1	3.5	4.0	1.7	1.6	2.9	3.4	1.7	1.6
	(1.2-1.4)	(1.0-1.6)	(2.2-3.2)	(2.6-3.6)	(1.4-2.0)	(2.1-2.2)	(3.3-3.6)	(3.9-4.1)	(1.5-2.0)	(1.6-1.7)	(2.7-3.0)	(3.2-3.5)	(1.5-1.9)	(1.4-1.7)

[a] Value excluded for one subject who had been exposed to CO the previous night.

TABLE VII

BLOOD % COHb SATURATION AFTER EXPOSURE TO
250 PPM CH_2Cl_2 FOR 7-1/2 HOURS

SUBJECT #	PRE-EXP.	PRE-EXIT	HOURS POST-EXPOSURE 0.5	1.0	1.5	2.0	2.5	3.0
1	2.9	7.6	7.6	7.8	7.5	7.2	7.0	6.6
2	2.8	11.7	11.9	11.0	10.6	10.0	9.2	9.0
3	2.8	9.7	10.0	10.0	9.6	8.7	8.6	8.1
4	2.6	9.3	8.7	8.6	8.6	7.9	7.6	7.0
Average	2.8	9.6	9.6	9.4	9.1	8.5	8.1	7.7

moderate cigarette smoker prior to the study had COHb values which were always higher than the other non-smoker subjects. There is a good probability that she was exposed to carbon monoxide outside of the laboratory, perhaps from light cigarette smoking. One of the $7\frac{1}{2}$-hr subjects had an abnormally high (9.6%) COHb saturation on the Monday morning following the last exposure on Friday. She had spent the night in an apartment above a bakery, where air sampling in the apartment by our personnel the next morning revealed a carbon monoxide concentration of nearly 100 ppm in the atmosphere. Fortunately, this was the only time that she had stayed in this apartment during the study.

The results of the study to determine when peak percent COHb saturation occurred are shown in Table VII. Individual values and the means are listed for all samples obtained on this fourth consecutive day of exposure to 250 ppm methylene chloride.

Subjective Responses:

The male subjects were unable to detect the odor of methylene chloride at concentrations of 50 or 100 ppm. The majority of subjects could detect the odor at a concentration of 250 ppm but did not find it objectionable. The male subjects reported the odor to be mild to moderate in intensity at a concentration of 500 ppm and during the first hour of exposure noted mild eye, nose and throat irritation. There were no other untoward subjective responses.

Pulmonary-Hematologic Function Studies:

Functional Integrity of Airways: Presented in Table VIII are the mean

($\pm$ SEM) values of the parameters utilized to assess functional integrity of airways in the male subjects. There was no significant change in any of the parameters during exposure to methylene chloride. All subjects were within the normal range for young adult males [20].

The parameter MMEF is thought to be sensitive to small changes in the diameter of intra-pulmonary airways [21]. These airways supposedly are the first to be affected by the processes responsible for chronic bronchitis and airway diseases of various etiology [22, 23]. Accordingly, if methylene chloride and/or its chief metabolite carbon monoxide has an effect on airway diameter, the parameter MMEF should change. Because this parameter remained normal throughout the exposure, we can conclude that exposure to methylene chloride as in the present study does not affect the functional integrity of pulmonary airways.

The parameter PEFR is determined by the maximum forces generated by contraction of the expiratory muscles and lung elastic recoil [24]. The constancy of this parameter indicates methylene chloride exposure has no effect on these systems.

Alveolar-Capillary Gas Exchange: The single breath carbon monoxide diffusion test was used to assess this function, and the appropriate values are presented in Table IX. There was no consistent change in D_LCO during methylene chloride exposure. Values obtained at rest were normal for young adult males, and the progressive increases accompanying increases in metabolic rate are consistent with published data [24].

TABLE VIII

Parameters Utilized to Assess Functional Integrity of Airways

		FVC-liters	FEV_1%	PEFR l/sec	MMEF l/sec
0 PPM	$\bar{X}$	4.59	81.6	9.87	3.9
	SEM	0.08	0.7	0.3	0.1
50 PPM	$\bar{X}$	4.55	81.0	9.54	3.73
	SEM	0.1	0.6	0.4	0.1
100 PPM	$\bar{X}$	4.41	81.4	9.67	3.84
	SEM	0.1	0.8	0.3	0.1
250 PPM	$\bar{X}$	4.54	81.2	9.57	3.73
	SEM	0.1	0.6	0.2	0.1
500 PPM	$\bar{X}$	4.30	81.3	9.13	3.59
	SEM	0.1	1.04	0.5	0.1
Post 0 PPM	$\bar{X}$	4.45	81.1	9.52	3.71
	SEM	0.2	1.3	0.5	0.2

FVC = The maximum volume of air one can expire following a maximum inhalation.

FEV_1 = The percent of the VC expired in one second.

PEFR = The maximum flow rate achieved during expiration.

MMEF = The flow rate achieved at the mid-point of the VC.

The magnitude of alveolar-capillary gas exchange is dependent primarily upon the distribution of ventilation relative to perfusion (V/Q) and the diffusion characteristics of the alveolar-capillary membranes [4]. In conditions such as the present, V/Q would probably change as a result of a change in V, and this effect would probably be a manifestation of a change in functional integrity of the small airway. A constant D_LCO throughout exposure corroborates the findings outlined in the previous section, and it appears that methylene chloride had no effect on the diffusion characteristics of the alveolar-capillary membranes.

<u>Pulmonary Ventilation</u>: The three parameters, pulmonary ventilation, $PaCO_2$, and PAO_2 should all change in a predictable manner if an experimental condition alters in some way the ventilatory regulatory mechanism. During 0 ppm conditions, all parameters are in the normal range [25], and generally there was no consistent change during methylene chloride exposure (Table IX). Previous studies have shown that a high COHb does not affect ventilation [26].

Arterial pH and lactate were studied because, due to the reduced arterial O_2 content and increased hemoglobin O_2 binding, one might expect a greater utilization of the anaeorbic metabolic pathway. While there was a slightly higher lactate and a lower arterial pH during exercise during exposure to 500 ppm, the differences were not significantly different from control conditions (Table IX). Apparently the demand for oxygen at these metabolic rates could be adequately met by the aerobic mechanisms.

TABLE IX

Data Obtained at Rest and During Work to Evaluate Effect of CH_2CL_2 Exposure on Pulmonary Ventilation and Gas Exchange

Condition	Rest							WORK 1		
	$\dot{V}O_2$ l/min	$\dot{V}_E$ l/min	$PaCO_2$ mmHg	pH	PAO_2 mmHg	Arterial Lactate mg%	D_LCO ml/min/ mmHg	$\dot{V}O_2$	$\dot{V}_E$	$PaCO_2$
0 PPM	.270	8.80	37.3	7.406	106.1		29.0	1.45	40.9	39.3
	.01	0.1	.8	.007	1.5		.9	.19	3.2	0.6
50 PPM	.252	8.72	41.3	7.408	98.7		24.1	1.50	41.6	42.0
	.005	.3	0.6	.007	1.8		2.1	.23	4.0	1.0
100 PPM	.280	9.66	39.1	7.387	100.8	12.1	29.5	1.42	41.8	41.2
	.03	.78	1.1	.005	.7	.6	1.3	.19	2.8	1.2
250 PPM	.254	8.39	41.0	7.392	99.3		29.6	1.37	42.2	41.6
	.02	.15	.45	.008	1.3		1.0	.15	3.8	.9
500 PPM	.315	9.08	38.3	7.387	101.9	10.1		1.87	51.3	38.3
	.04	.74	1.0	.006	4.0	.8		.4	11.6	.9
0 PPM	.267	7.93	40.6	7.397	99.1	12.1	30.3	1.71	41.9	39.0
	.03	.24	1.1	.007	2.2	2.1	0.8	.22	4.9	.6

$\dot{V}O_2$ = Oxygen consumption per minute
$\dot{V}_E$ = Pulmonary ventilation per minute
$PaCO_2$ = Partial pressure of CO_2 in the blood
PAO_2 = Partial pressure of O_2 in the alveolus
D_LCO = Lung diffusing capacity for carbon monoxide

(Continued on next page)

TABLE IX (Continued)

WORK 1							WORK 2			
pH	$P_{A_{O_2}}$	Lactate	D_LCO	$\dot{V}O_2$	$\dot{V}_E$	$PaCO_2$	pH	$P_{A_{O_2}}$	Lactate	D_LCO
7.385	107.9		35.7	1.90	61.3	35.7	7.383	113.8		43.9
.005	0.9		1.1	0.2	3.3	1.0	.006	1.4		1.8
7.377	99.1		35.3	2.11	64.0	37.8	7.372	108.3		40.2
0.12	3.8		3.9	.3	4.7	1.4	.013	1.4		4.7
7.361	103.2	18.3	36.5	2.02	63.6	37.0	7.355	109.1	42.7	40.8
.005	1.8	.7	1.6	.241	6.2	.6	.002	1.0	1.1	1.8
7.353	105.0		34.9	2.29	69.2	39.2	7.335	108.5		42.1
.005	1.5		1.7	.1	5.8	.9	.01	.6		2.1
7.370	106.6	21.0		2.29	67.8	34.9	7.352	113.6	47.3	
.006	3.7	1.5		.17	6.0	1.3	.007	3.5	3.6	
7.380	101.4	19.8	32.6	2.09	58.2	37.6	7.372	109.2	41.5	41.2
.007	2.3	1.5	2.1	.34	9.4	.7	.004	1.4	5.4	3.2

Hemoglobin Affinity for Oxygen: Elevated levels of COHb are known to cause an increased affinity of hemoglobin for oxygen [27]. Hemoglobin that is partially saturated with carbon monoxide does not as readily release oxygen at the tissues and, hence, tissue hypoxemia may result. Other conditions of hypoxemia induce an increased production of red cell 2,3-DPG which reduces the affinity of hemoglobin for oxygen [16,28]. We were thus interested in ascertaining whether an elevated COHb, as in the present study, would induce such an adaptation.

The P50 value is the standard means of expressing hemoglobin affinity for oxygen, and it is the oxygen tension required to maintain 50% hemoglobin saturation. The P50 values observed under 0 ppm conditions (Table X) are normal for young adult sea level residents [16]. The reduced P50 values associated with high COHb levels are as one would predict from the results of earlier studies during acute carbon monoxide studies [27,29].

The values for 2,3-DPG, calculated as μm 2,3-DPG/g hemoglobin are listed for each subject in Table X. All values are within the normal range (12.9 $\pm$ 2.3) for adult males [16], and no pattern of increase or decrease in these values emerged during methylene chloride exposure. Reproducibility of this method is $\pm$ 5% [17]. Chronic elevated COHb as in the present study does not induce an adaptation in red cell 2,3-DPG to restore hemoglobin affinity of oxygen to normal [29].

Neurological Studies:

No significant neurological abnormalities occurred during the

TABLE X

Effect of Exposure to CH_2CL_2 on Hemoglobin Affinity for Oxygen

and Red Cell 2, 3-DPG.

Condition	Subject #117	Subject #118	Subject #119	Subject #120	$\bar{X}$	SEM
Week 1 0 PPM COHb = 0.8	26.8 mmHg 14.2 μm/gHb	26.5 13.4	26.7 14.0	26.7 13.6	26.7 13.8	0.6 0.9
Week 2 50 PPM COHb = 2.0	25.2 14.4	25.8 12.7	27.0 14.1	27.2 13.2	26.3 13.6	0.5 0.4
Week 3 250 PPM COHb = 7.7	24.3 14.8	24.0 12.6	24.0 12.9	25.1 13.0	24.4 13.3	0.3 0.3
Week 4 250 PPM COHb = 7.5	24.3 13.8	23.4 13.2	23.6 14.8	24.6 14.8	24.0 14.2	0.3 0.4
Week 5 100 PPM COHb = 4.1	25.0 12.8	25.0 13.4	24.5 13.7	24.4 13.1	24.7 13.3	0.2 0.3
Week 6 0 PPM COHb = 1.0	25.7	25.5	26.2	26.3	25.9	0.3

Hemoglobin affinity for oxygen is expressed as the oxygen tension (mmHg) required to saturate fifty per cent of the hemoglobin. Red cell 2, 3-diphosphoglycerate (2, 3-DPG) is presented as μm/g. Hb.

observation period. The modified Romberg test and the heel-to-toe test remained normal. During studies at 0 ppm the frequency and wave amplitude of each subject's EEG were within normal limits at each recording site. There were no consistent significant changes during exposure at any of the concentrations. Exposure to methylene chloride did not cause any consistent significant changes in the amplitude, latency, and configuration of the VER. Two minor VER changes did occur, however. The peak-to-peak amplitude of one male subject's 4th and 5th wave of the VER complex tended to decrease slightly as vapor concentration increased. In addition, relative to 0 ppm, the amplitude of another male subject's 3rd, 4th and 5th wave was slightly reduced throughout the exposure.

Cognitive Testing:

No significant changes occured in the battery of cognitive tests which could be attributed to methylene chloride exposure. The analyses of these data using the paired-t test to compare the performance for each day of exposure to the non-exposure performance are found in Tables XI-XV. The graphs illustrating the mean scores plus one standard deviation for each of the cognitive tests performed have been previously reported in NIOSH Report, ENVM - MC - 73 - 7.

Only on two occasions were the alertness test exposure day results significantly different than those on non-exposure days for the $7\frac{1}{2}$-hr male subjects. The reaction times at 250 and 100 ppm were faster. In the 3-hr exposure groups two significantly different results were found. The

TABLE XI

ALERTNESS TEST

Male Subjects, 7½ Hour Exposure

Paired-t Values For Comparison of Control Vs. Exposure Scores

Concentration ppm	% Correct	Reaction Time	
50	.304	.958	Monday
100	-.642	2.312	Monday
250	-.240	2.143	Monday
250	-.058	3.181*	Monday
50	-2.232	1.944	Wednesday
100	1.084	2.913	Wednesday
250	-.031	1.720	Wednesday
250	2.733	1.614	Wednesday
50	.614	2.988	Friday
100	-.751	7.340**	Friday
250	-.284	2.409	Friday
250	-.713	2.693	Friday

* Significant at P = .05

** Significant at P = .01

TABLE XII

ALERTNESS TEST

Male Subjects, 3 Hour Exposure

Paired-t Values for Comparison of Control Vs. Exposure Test Scores

Concentration ppm	% Correct	Reaction Time	
50	.878	-.210	Monday
100	.773	-1.135	
250	2.352	-1.262	
250	2.224	-.849	
50	1.051	.485	Wednesday
100	1.995	-1.459	
250	1.560	-1.403	
250	2.728	-.480	
50	15.254**	-.756	Friday
100	.578	.524	
250	2.200	-.922	
250	9.430*	-1.248	

* Significant at P = .05

** Significant at P = .01

TABLE XIII

ALERTNESS TEST

Female Subjects, $7\frac{1}{2}$ Hour Exposure

Paired-t Test For Comparison of Control Vs. Exposure Test Scores

	250 ppm, Monday		250 ppm, Wednesday		250 ppm, Friday	
	t	df	t	df	t	df
Percent Correct	.567	3	2.324	3	-.517	3
Average Reaction Time	1.486	3	1.701	3	.099	3

Significance Level: None at < 0.05

TABLE XIV

PAIRED-t VALUES FOR COMPARISON OF CONTROL VS. EXPOSURE TEST SCORES

Male Subjects, $7\frac{1}{2}$ Hour Exposure

Test		T - Th 50 ppm t	df	T - Th 100 ppm t	df	T - Th 250 ppm t	df	Th 500 ppm t	df
Marquette Test:	E/S	.687	3	.052	3	.01	3	.797	3
Sound Stimulus	|E-S|	- .181	3	-1.000	3	- .747	3	.399	3
	RxT	.494	3	- .811	3	- .363	3	- .487	3
	E/S	-2.114	3	-2.112	3	-2.118	3	-1.108	3
Light Stimulus	|E-S|	2.277	3	.352	3	1.548	3	- .966	3
	RxT	4.852*	3	3.326*	3	3.770*	3	.046	3
10 Second Estimation		.321	3	- .594	3	- .626	3	.813	3
30 Second Estimation		2.961	3	-3.212	3	-1.156	3	.857	3
Arithmetic Test		- .333	3	.528	3	- .08	3	.233	3
Coordination Test		- .775	3	- .724	3	-1.743	3	.407	3
Inspection Test		.403	3	-2.772	3	.997	3	-9.101	3 **

* Significant at P = .05
** Significant at P = .01

TABLE XV

PAIRED-t VALUES FOR COMPARISON OF CONTROL VERSUS EXPOSURE TEST SCORES

Male Subjects, 3 Hour Exposure

Test		T - Th 50 ppm t	df	T - Th 100 ppm t	df	T - Th 250 ppm t	df	Th 500 ppm t	df
Marquette Test:	E/S	.477	2	- .045	2	- .02	2	- .053	2
Sound Stimulus	|E-S|	1.239	2	1.852	2	- .024	2	- .905	2
	RxT	.59	2	-2.031	2	-1.292	2	.308	2
	E/S	.407	2	- .832	2	- .915	2	1.039	2
Light Stimulus	|E-S|	4.413	2	2.240	2	.855	2	1.993	2
	RxT	1.403	2	.301	2	.752	2	- .571	2
10 Second Estimation		1.348	2	- .598	2	1.400	2	-1.068	2
30 Second Estimation		.699	2	- .677	2	.115	2	- .643	2
Arithmetic Test		-1.960	2	- .819	2	-1.957	2	-2.298	2
Coordination Test		- .577	2	- .839	2	-10.777**	2	- .023	2
Inspection Test		- .871	2	-2.644	2	-1.394	2	-3.120	2

* Significant at P = .05
** Significant at P = .01

percent of correct responses at 50 and 250 ppm, both on Fridays, were lower than controls. Because this finding was not dose related, it was considered to be a Friday fatigue effect. Data from the two 3-hr female subjects, though showing no indication of an effect from the methylene chloride exposure, were too inconsistent to be of value when analyzed.

In the $7\frac{1}{2}$-hr male group, reaction time to the light stimulus improved significantly ($P = 0.05$) at 50, 100 and 250 ppm, but not at 500 ppm, when compared to control days. Also in this group, test scores increased significantly ($P = 0.01$) over control days for the inspection test at 500 ppm methylene chloride. Likewise, the only significant difference in paired t-tests for the 3-hr male group was an improvement in performance ($P = 0.01$) of the coordination test at 250 ppm versus control.

DISCUSSION

Previous studies reporting on well-controlled exposures of human subjects to methylene chloride have not included repeated daily exposures. Therefore, in this sense, this study was unique. Fortunately, this study did corroborate previous single exposure studies in that no deleterious effects upon the health or performance of healthy adults could be detected when they were repeatedly exposed to 250 ppm or less for $7\frac{1}{2}$ hours per day, five days per week, or in the case of the male subjects, to 500 ppm on two consecutive days.

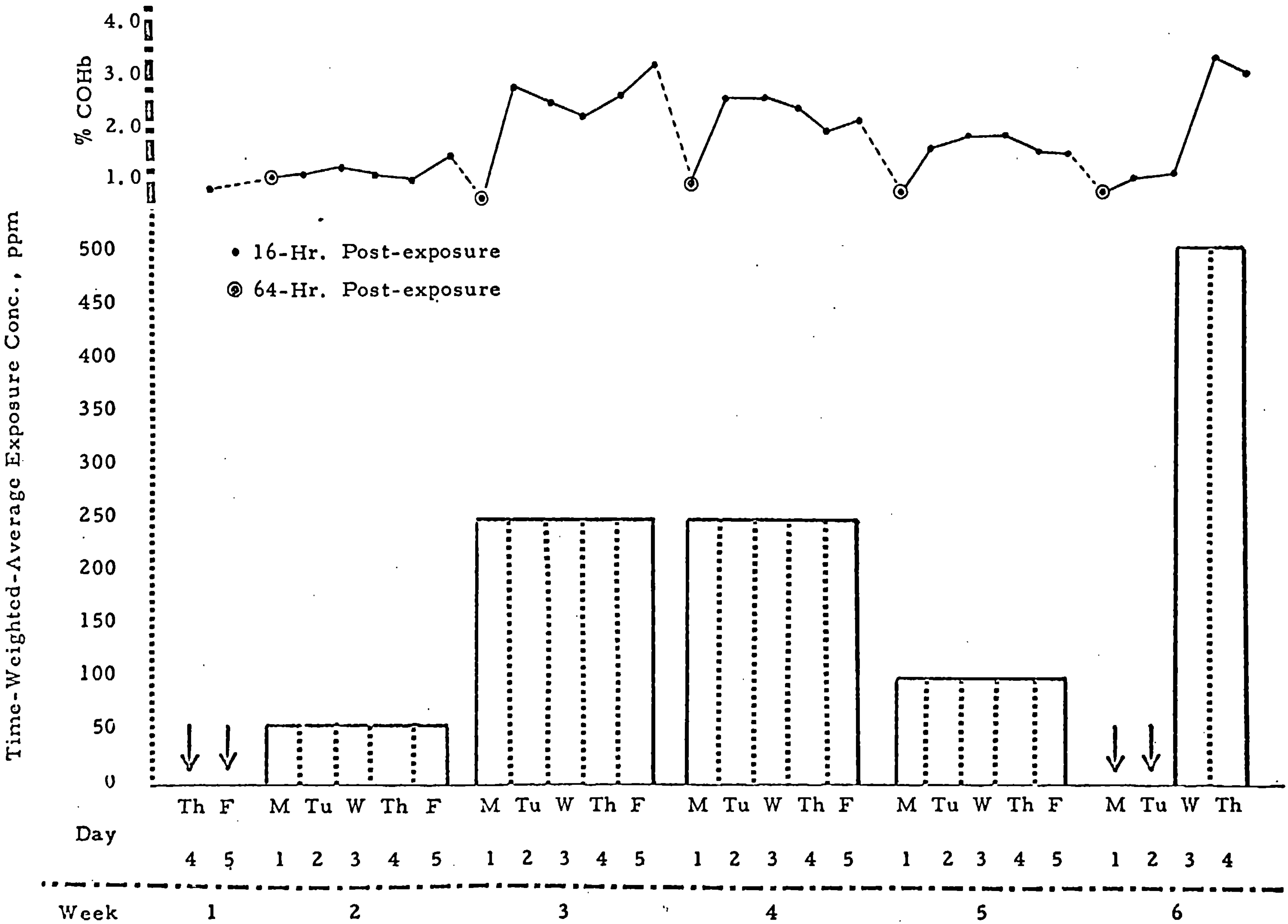
Confirmation of the discovery by Stewart, et al [1,2], that exposure to methylene chloride vapor results in increased COHb levels has come from other laboratories [4,5,6], along with proof that in mammals the carbon monoxide source is the methylene chloride molecule [4]. The work reported herein further confirms this observation and suggests that this metabolic pathway is common to all humans, as all subjects showed increases in COHb when exposed to even the lowest concentrations of the solvent vapor.

Of paramount interest in this study was the magnitude of the increase in COHb occurring as a result of exposure to different concentrations of methylene chloride. The increase in COHb was related to the magnitude of the vapor exposure. Both duration of exposure and vapor concentration were factors. Seven and one-half hour exposures to concentrations as low as 100 ppm resulted in COHb elevations above 5% in non-smokers. Coupled with this elevation was a doubling of the biological half-life of COHb resulting from its endogenous reduction. Thus, exposure to 100 ppm not only resulted in a COHb saturation above 5% but a COHb with a protracted biological half-life too long to permit the body to rid itself of the compound before the next working day which normally would be the case had the carbon monoxide been derived from an exogenous source.

Figure 8 denotes the daily 16 hour post-exposure COHb levels for the $7\frac{1}{2}$-hr male subjects. Sixty-four hours after exposure the COHb had returned to the pre-exposure level. It is obvious from the graph that COHb levels had not returned to baseline the morning following $7\frac{1}{2}$ hours of exposure to 100,

RELATIONSHIP OF POST-EXPOSURE CARBOXYHEMOGLOBIN LEVELS

TO MAGNITUDE OF $7\frac{1}{2}$-HOUR EXPOSURE TO METHYLENE CHLORIDE



250 or 500 ppm methylene chloride. However, on Monday mornings (64 hours after Friday's exposure), a baseline level was always achieved. Carryover was negligible after a daily exposure to 50 ppm methylene chloride.

The group-average blood COHb levels of the male subjects, excluding smokers, as they exited the chamber and for three hours thereafter, are depicted in Figure 9. The levels varied directly with the concentration of the methylene chloride in the chamber and also with the length of exposure.

Although the number of COHb determinations was limited because of the need to conserve blood, it was important to learn when the metabolism of the methylene chloride to carbon monoxide began. These data were obtained from a faculty member who volunteered for serial blood sampling after single three-hour exposures to three levels of methylene chloride. His COHb climbed steadily during exposure and peaked at one hour post-exposure (Figure 10). The half-life from the decay of the COHb after peaking appears to be approximately seven to ten hours, twice the biological half-life of COHb resulting from exogenous carbon monoxide exposure.

The carbon monoxide derived from the in vivo metabolism of methylene chloride was additive to the carbon monoxide body burden derived from environmental sources. This was particularly important in the case of the tobacco smokers who already had significant body burdens. (Tables III - V).

The elevation in COHb observed in these studies occurred in sedentary subjects. Obviously, the COHb would have been further increased had the

FIGURE 9

AVERAGE CARBOXYHEMOGLOBIN LEVELS OF NON-SMOKERS

EXPOSED TO THREE CONCENTRATIONS OF METHYLENE CHLORIDE

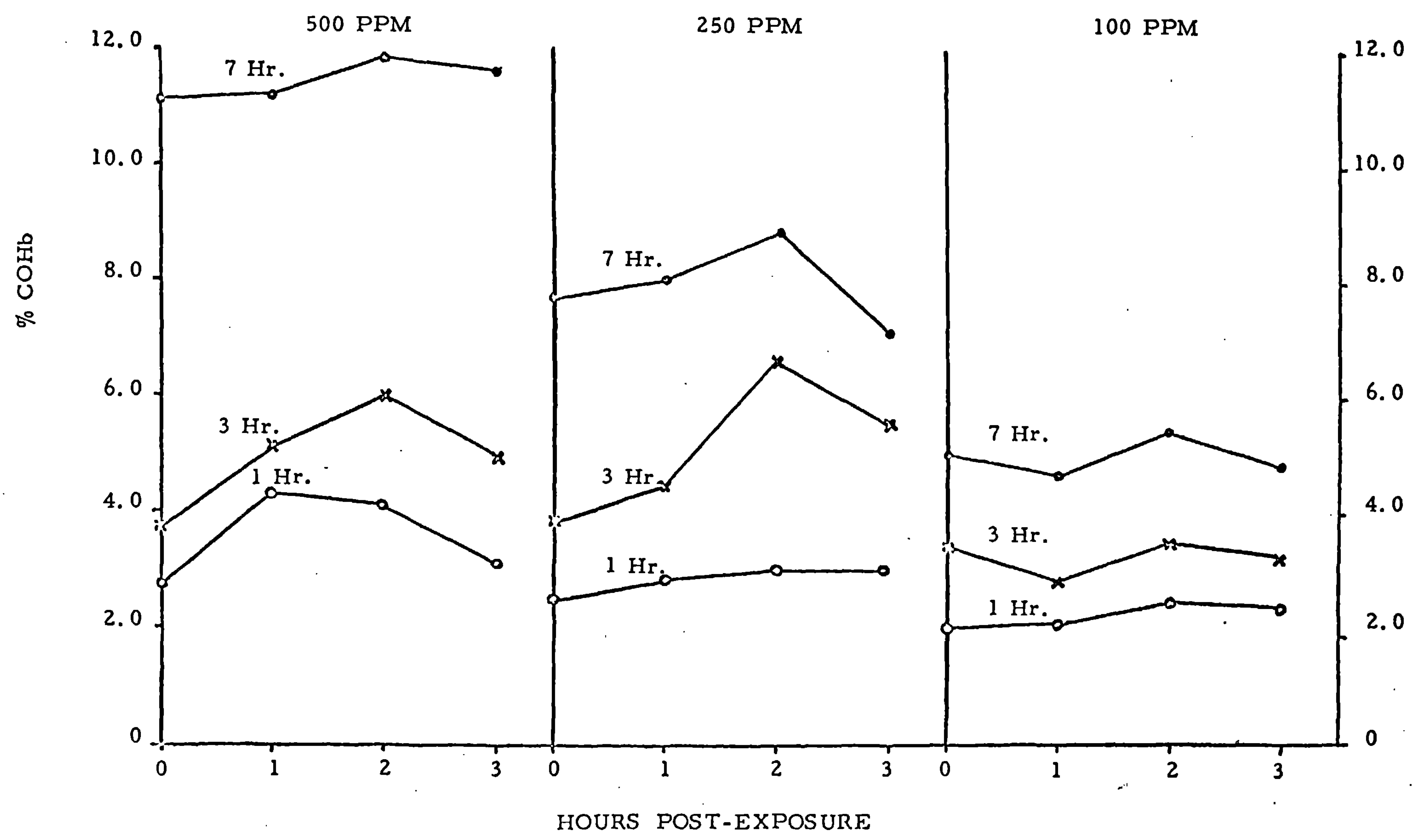
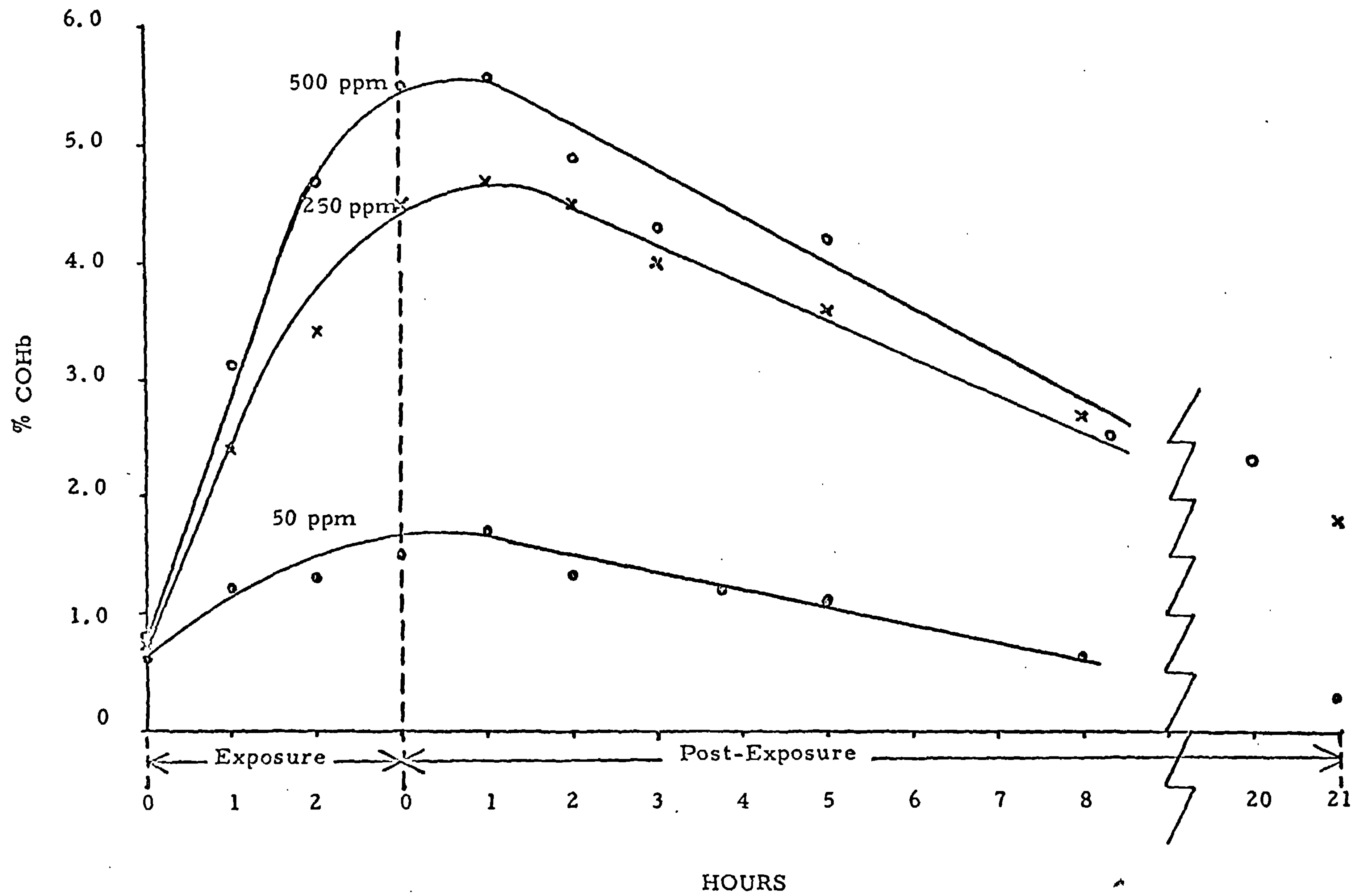


FIGURE 10

CARBOXYHEMOGLOBIN VALUES FROM A SUBJECT EXPOSED FOR
THREE HOURS TO THREE CONCENTRATIONS OF
METHYLENE CHLORIDE ON THREE SEPARATE DAYS



subjects been actively engaged in heavy work resulting in an increase in their minute respiratory volume. It is conceivable that heavy exertion sufficient to increase the minute respiratory volume to three times the resting state could result in the absorption of three times as much methylene chloride which would promptly be metabolized to significantly greater quantities of carbon monoxide.

The importance of this increase in COHb following exposure to methylene chloride vapor is obvious. The untoward effects of carbon monoxide on the cardiovascular system and on cognitive performance have been the subject of intense investigation and have recently been reviewed [30]. Exposure to carbon monoxide sufficient to elevate the COHb to 5% does impose the maximum toxic stress an otherwise healthy workman should be expected to tolerate without experiencing an untoward health effect.

It is interesting to speculate as to where the methylene chloride metabolism was taking place. Though the liver is the most likely place, we ruled out metabolism in the red cells by incubating the blood of an exposed subject overnight at 37°C. There was no increase in COHb even though methylene chloride was present in the blood. Although the site of metabolism is still unproved, it can be said with certainty, contrary to papers published as recently as 1972 [31], that absorbed methylene chloride vapor is not metabolically inert.

The elevated COHb following methylene chloride exposure is a definite physiological stress which must be taken into account when establishing the threshold limit value (TLV) for the solvent. In 1972 the National Institute for Occupational Safety and Health recommended lowering the TLV for carbon monoxide to 35 ppm, following which the Department of Labor considered further lowering the TLV to 25 ppm [32]. Exposure to carbon monoxide at these lowered levels would result in COHb of 5 - 4%, respectively, with a biological half-life of five hours in a sedentary person. If a uniform philosophy for TLV recommendations is followed, the TLV for methylene chloride should not result in a body burden of COHb greater than that permitted for exposure to carbon monoxide itself. Therefore, to prevent excursion of COHb above 4 - 5% would require limiting methylene chloride exposure to 50 ppm for active, nonsmoking adults. A sedentary male with a baseline COHb of 0.6 - 0.9% could reach a peak COHb of 2.5 - 2.9% as a result of exposure to methylene chloride, 50 ppm, for $7\frac{1}{2}$ hours. The same male, heavily exerting, could double or triple the absorption of methylene chloride as a result of the increase in his minute respiratory volume. This could easily result in a doubling of the COHb production.

On the basis of these observations, it is proposed that the threshold limit value for methylene chloride be reduced to 50 ppm to avoid body burdens of COHb greater than those allowed persons exposed to exogenous carbon monoxide.

Results of the previous study [3] suggested that the peak to peak amplitude of the 3, 4, 5 complex of the VER was increased during two hours of exposure at 986 and 700 ppm. In the present study, there was no indication of increased amplitude even at the highest methylene chloride concentration (500 ppm). In fact, exposure at these low concentrations resulted in a slight reduction in amplitude in two of the subjects. The results of these two studies are not incompatible. Anesthetic agents are also known to induce a similar pattern of change in cortical electrical activity[33]. In the initial stage of anesthesia, EEG amplitude is decreased due to a direct excitatory effect of the agent on cortical neurons. As anesthesia is deepened, EEG amplitude increases due to the depressant effect of the agent on the CNS arousal mechanism (reticular activating system).

Behavioral tests on subjects undergoing exposure, and thus under the influence of both methylene chloride and COHb, showed no deleterious effects at the magnitude of exposure studied. Although exact COHb levels at the time of testing are unkown, it can be assumed that the COHb levels were well above 2% saturation when the $7\frac{1}{2}$-hr and 3-hr subjects were tested in an atmosphere of 250 or 500 ppm methylene chloride. This is a most important finding as some previous reports have indicated that carbon monoxide alone at low levels had a deleterious effect upon time perception[34], although other investigators have been unable to confirm this finding[30, 35].

The analysis of expired breath from methylene chloride in the post-exposure period provides an excellent diagnostic test of exposure. The detection of the solvent in the breath by infrared spectroscopy constitutes an unequivocal diagnosis of exposure. When it is known that exposure to the solvent has occurred, gas chromatography can be used, a rapid and very applicable method in the industrial setting.

The breath data obtained in this study are in excellent agreement with human data reported following exposure to methylene chloride vapor 100 - 200 ppm for two or four hours [31].

The analysis of the breath in the first three hours after exposure appears to be an excellent screening test with which to estimate the magnitude of the time-weighted-average exposure. If, however, the methylene chloride vapor concentration has been fluctuating during exposure, a breath sample obtained in the first hour following exposure may not accurately reflect the time-weighted vapor exposure, but will reflect the most recent vapor concentration to which the subject has been exposed. Therefore, the ideal time for the collection of a single breath sample from which to estimate the time-weighted vapor exposure is in the two to four hour post-exposure period.

Experience in this laboratory indicates that it is feasible to allow the subject to collect breath samples in glass pipettes in the post-exposure period while at home [9]. The breath samples should be collected in duplicate pipettes to reduce the troublesome problem of pipette leakage or breakage. In addition, it is imperative that background pipette samples be collected to eliminate the possibility of background contamination yielding a false analytical result.

Ideally, it would be desirable to construct an individualized breath decay curve for each workman following exposure to a known concentration of methylene chloride. This would provide a superior breath decay curve, further reducing the variation due to biological differences and sampling techniques.

In the nonsmoking segment of the work population one can also analyze the breath sample for carbon monoxide and estimate the COHb level [19]. From the data presented, it is apparent that one can quite accurately separate the males exposed to the four concentrations into the appropriate exposure group on the basis of a single COHb determination obtained anytime in the 16 hour post-exposure period. Since this same breath sample can be used for both the analysis of carbon monoxide and methylene chloride, it should be possible to use breath analysis as a practical screening device to determine whether or not a nonsmoker has exceeded the TLV for methylene chloride.

Thus, breath analysis for methylene chloride and carbon monoxide can be used as a practical means with which to determine the body burden of these two compounds, establishing a Biologic Threshold Limit.

CONCLUSIONS

Exposure to methylene chloride results in the prompt elevation of COHb which has a biological half-life twice that of COHb formed as a result of exposure to exogenous carbon monoxide. This solvent-induced COHb is derived from the metabolism of methylene chloride and adds to the body burden of carbon monoxide derived from both hemoglobin metabolism and exogenous carbon monoxide absorption, i.e., automobile exhaust and tobacco smoke.

Control of the magnitude of this COHb elevation is important if untoward anoxic stress is to be avoided. Exposure to the current TLV of 250 ppm results in production of COHb in the non-smoker comparable to that experienced by the heavy tobacco smoker, a COHb body burden well above that capable of decreasing exercise tolerance in those individuals with significant angina pectoris. While the healthy non-smoker can tolerate this mild anoxic stress, solvent-derived COHb is additive to the already increased COHb of the tobacco smoker, setting the stage for clinically significant anoxic stress.

If a uniform philosophy for the establishment of industrial TLV's is followed, the TLV for methylene chloride should not result in a body burden of COHb greater than that permitted for exposure to carbon monoxide itself. Therefore, to prevent the excursion of COHb above 4 or 5% would require limiting methylene chloride exposure to 50 ppm for active, non-smoking adults. A sedentary male with a baseline COHb of 0.6 - 0.9% could reach a peak COHb of 2.5 - 2.9% as a result of exposure to methylene chloride, 50 ppm for 7½ hours. The same male, heavily exerting, could double or triple the absorption of methylene chloride as a result of the proportionate increase in his minute respiratory volume. This could easily result in COHb levels above 5% saturation. Therefore, it is recommended that consideration be given to reducing the TLV for methylene chloride to 50 ppm to avoid body burdens of COHb greater than those allowed persons industrially exposed to exogenous carbon monoxide.

Repetitive vapor exposures to 250 ppm resulted in no untoward subjective or objective health responses in healthy male and female subjects.

The majority of subjects were unable to detect the odor of methylene chloride at a concentration of 100 ppm or lower. Therefore, odor detection cannot be relied upon as a warning property should the TLV be placed at 100 ppm or below.

Analysis of methylene chloride and its metabolite, carbon monoxide, in the expired breath after exposure provides an excellent screening test with which to estimate the magnitude of the solvent exposure. The breath data presented are sufficient to permit breath analysis to be used as a Biologic Threshold Limit measurement for workmen.

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STATEMENT OF VOLUNTARY CONSENT
FOR RESEARCH INVESTIGATION OF
HUMAN EXPOSURE TO:
METHYLENE CHLORIDE

I, ________________________________, hereby agree to participate as a subject, in a program of research investigation under the direction and supervision of Dr. R. D. Stewart.

The general purpose of this research is to determine rates of uptake, excretion and metabolism of methylene chloride.

The studies have been described to me and the known risks involved in this experimental procedure have been explained to me. I understand that the most frequently described known risks are: none known at this level of exposure.

I understand also that it is not possible to identify all potential risks in experimental procedures which involve controlled exposures to the chemical in a specially designed chamber.

I further understand that reasonable precautions and safeguards have been and will be taken to remove and reduce both the known and the potential but unknown risks and to provide for my safety and comfort.

I also understand that, while the program will be under the direction and supervision of Dr. R. D. Stewart, other professional persons who work with him may be designated to assist him or to act for him.

In view of those considerations, I hereby authorize Dr. R. D. Stewart and his representatives to proceed with the investigation on the understanding that I may terminate my service as a subject in this research at any time I so desire. I also authorize Dr. R. D. Stewart to use any type of data, pictures, films etc. for use in any scientific report or publication.

I am offering my service freely, in consideration of similar actions on the part of other subjects involved in like voluntary efforts to improve our society through research.

Witness ____________________ Signed ____________________
Investigator Subject

Date ____________________

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16. Abstract (Limit: 200 words)

Twenty healthy adults of both sexes were exposed to methylene chloride vapor concentrations of 0, 50, 100, 250 and 500 ppm for periods of 1, 3 and 7-1/2 hours in a controlled-environment chamber. These studies were designed to simulate the type of exposures encountered in the industrial setting and consisted of both steady, non-fluctuating vapor concentrations as well as widely fluctuating vapor concentrations. Exposure resulted in a prompt elevation of carboxyhemoglobin (COHb) which had a biologic half-life twice that of COHb formed as a result of exposure to exogenous carbon monoxide. This solvent-induced COHb was derived from the metabolism of methylene chloride and added to the body burden of carbon monoxide derived from other sources. Repetitive vapor exposure to the current TLV of 250 ppm produced no untoward subjective or objective health responses in the healthy subjects who are able to compensate for the mild anoxic stress of the COHb. However, if a uniform philosophy for the development of TLVs is followed, the TLV for methylene chloride for active, non-smoking workmen should be reduced to 50 ppm if excursion of COHb above 4 to 5% is to be prevented. Post-exposure breath analysis for methylene chloride and its metabolite, carbon monoxide, proved to be a practical screening method with which to determine the body burden of these two compounds and so provides a biologic threshold limit measurement for workmen.

17. Document Analysis a. Descriptors

methylene-chloride, biological-effects, neurological-effects, breathing-analysis, carboxyhemoglobin, human-subjects

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

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