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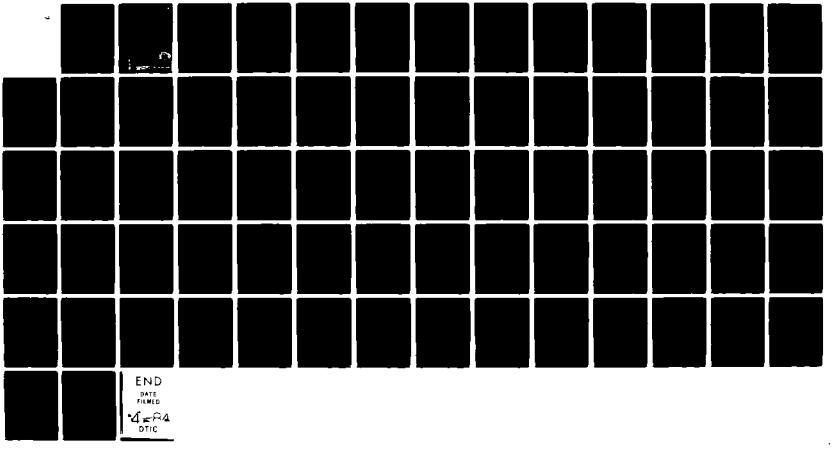

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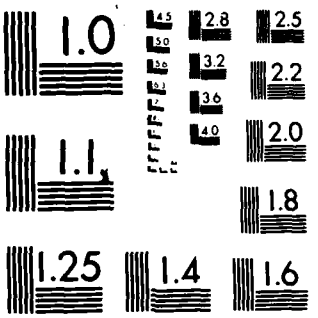

COMBINED EXPOSURE OF METHYLENE CHLORIDE AND CARBON MONOXIDE IN SMOKING AND NONSMOKING PAINT STRIPPERS

by

Ross N. Miller

A thesis submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Master of Science

in

Community Medicine

Department of Family and Community Medicine
The University of Utah

December 1983

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THE UNIVERSITY OF UTAH GRADUATE SCHOOL

SUPERVISORY COMMITTEE APPROVAL

of a thesis submitted by

Ross N. Miller

This thesis has been read by each member of the following supervisory committee and by majority vote has been found to be satisfactory.

12/8/83 — Chairman: Jeffrey S. Lee

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William N. Rom

12-8-83 — Dee W. West
Dee W. West

THE UNIVERSITY OF UTAH GRADUATE SCHOOL

FINAL READING APPROVAL

To the Graduate Council of The University of Utah:

I have read the thesis of Ross N. Miller in its final form and have found that (1) its format, citations, and bibliographic style are consistent and acceptable; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the Supervisory Committee and is ready for submission to the Graduate School.

December 8, 1983
Date

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ABSTRACT

Carboxyhemoglobin (COHb) is formed when carbon monoxide (CO) combines reversibly with the oxygen carrying sites on the hemoglobin molecule. COHb levels above 5% increase the risk of angina pectoris and coronary infarctions by decreasing the oxygen supply in the blood and also in the myoglobin of the heart muscle. Cigarette smoke contains 4% carbon monoxide and a one-pack-per-day smoker exhibits COHb levels of about 5.9% (12). Methylene chloride exposure also results in increased COHb levels (2, 3). Therefore, the combined effect of smoking and methylene chloride exposure may increase the risk of disease to dangerous levels.

This thesis tests the null hypothesis that smokers do not have statistically significant differences in COHb levels following days of exposure to methylene chloride when compared to days of nonexposure.

Subjects were drawn from businesses which strip and refinish furniture in northern Utah. Furniture strippers were solicited as study subjects because methylene chloride is commonly found as a major constituent in paint and varnish stripping products.

The study population contained eight smoking males, eight nonsmoking males and two nonsmoking females. Smoking was defined as any type and quantity of smoking. Daily smoking ranged from six cigarettes to a pack and a half. Blood samples from study subjects

were drawn before and after the work shift and at corresponding times during a nonexposure day. Therefore, controls were self-paired where possible. Four subjects (two smokers and two nonsmokers) did not provide blood samples for the nonexposure period. Controls were selected for these individuals and matched for age, sex and smoking habits. Ages were matched within four years for nonsmokers and smoking habit was matched to within five cigarettes per day for smokers.

Exposures of subjects to methylene chloride were measured with passive organic vapor monitors and charcoal sorbant tubes. Carbon monoxide concentrations in work areas were measured with direct reading instrumentation to ensure that sources of CO other than cigarette smoking were absent.

The data were analyzed for significant differences in COHb levels between periods of exposure and periods of nonexposure. Smokers and nonsmokers demonstrated statistically significant increases in COHb levels during periods of exposure when compared to periods of nonexposure ($P < 0.05$).

Dose response curves for smokers and nonsmokers were estimated. The curve for nonsmokers reached a plateau of about 7% COHb following an eight-hour time-weighted-average (TWA) exposure of approximately 1800 mg/m^3. The smokers dose response curve did not plateau but methylene chloride exposures were lower than those experienced by nonsmokers.

A test of covariance was conducted on the portions of the dose response curves that were comparable. A statistically significant difference in the slope of the curves did not exist. Therefore, this research concludes that the combined effects of smoking and methylene chloride exposure on COHb production are additive following an eight-hour TWA methylene chloride exposure up to 650 mg/m^3.

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INTRODUCTION

Statement of the Problem

Methylene chloride (CH_2CL_2) is a common solvent that has replaced the more toxic carbon tetrachloride and chloroform. Over 500 million pounds are produced annually and the National Institute for Occupational Safety and Health (NIOSH) estimates that 70,000 people are potentially exposed to the chemical in their working environment (1).

Stewart et al. (2, 3), in 1972, were the first to show that humans exposed to methylene chloride vapor exhibited high carboxyhemoglobin (COHb) levels. COHb is formed when carbon monoxide binds reversibly to hemoglobin. Subsequent studies have confirmed the metabolism of methylene chloride to carbon monoxide both in humans and rats. (4, 5, 6, 7, 8, 9, 10, 11, 19, 20)

This metabolic discovery has led to speculation that methylene chloride exposure coupled with exposure to other sources of CO may act additively. Thus exposure standards which are established independently may be inappropriate in situations where combined exposures to CO and methylene chloride exist. Increased COHb levels increase the risk of angina pectoris and coronary infarctions by decreasing the oxygen supply in the blood and also in the myoglobin

of the heart muscle. These effects are aggravated by heavy work and high temperatures due to the body's increased oxygen (O_2) demand. High altitude can also aggravate the effects because oxygen availability is decreased. Smoking also increases the risk because cigarette smoke contains four percent CO and a one-pack-per-day smoker will have a COHb level of approximately 5.9% (12). This concentration alone is sufficient to imply serious threat to health in persons with underlying vascular insufficiency. This level of exposure may account for some of the excess mortality from cardiovascular disease observed among smokers (12). Marginal increments in COHb concentrations necessitate greatly increased cardiac output to supply needed oxygen to the tissues. Carbon monoxide causes functional constriction (ischemia) of blood vessels, necessitating even further cardiac output.

Further studies have shown impairment of time discrimination, visual vigilance, choice response, visual evoked response, and visual discrimination at levels well below five percent COHb (13).

Occupational exposure to methylene chloride vapors at the 1983-84 threshold limit value (TLV) of 100 ppm will result in COHb levels of about three percent (4). A TLV is the time weighted average concentration for a normal eight-hour work day or 40 hour work week of a particular contaminant to which nearly all workers may be repeatedly exposed, day after day, without adverse effect. These values are established by the American Conference of Governmental Industrial Hygienists (ACGIH) and published annually. At a COHb

level of three percent, the nonsmoking healthy worker is at minimum risk of serious health effects. However, persons with a history of coronary disease, anemia, pulmonary heart disease, cerebrovascular disease, thyrotoxicosis, and smokers would be at increased risk of cardiovascular or central nervous system impairment (13).

Since methylene chloride exposure and smoking independently increase COHb levels, the combined exposure may increase the risk of disease or injury to dangerous levels.

DiVincenzo (5) studied the effect of exercising and smoking on the uptake, metabolism, and excretion of methylene chloride vapor. Controlled exposures were accomplished at 100 ppm for 7.5 hours with exercising and smoking men. Physical exercise increased the absorption of methylene chloride and COHb levels. Smoking appeared to have an additive effect because increases in COHb values were similar to increases observed in nonsmokers at comparable concentrations. However, DiVincenzo did not consider daily COHb increases due to smoking in the absence of methylene chloride exposure and since only three subjects were studied the conclusions made are of limited value.

This thesis tests the null hypothesis that smokers do not have statistically significant differences in COHb levels following days of exposure to methylene chloride when compared to days of nonexposure to methylene chloride.

In addition, COHb increases in nonsmokers were statistically evaluated and dose-response curves for both smokers and nonsmokers were estimated. Effects of work load on COHb production were not

evaluated because subjects were all doing similar work at a moderate to heavy work load.

Methylene Chloride

Methylene chloride (CH_2CL_2) is an industrial solvent whose synonyms include dichloromethane, methylene dichloride, and methylene bichloride. Methylene chloride is widely used as a low temperature extractant of substances which are adversely affected by high temperature (14). It is a colorless volatile liquid whose solubility in water is minimal. It is completely miscible with most organic solvents (1). It is nonflammable and has a pleasant aromatic odor noticeable at 300 parts per million (ppm). Physical properties include: Molecular weight - 84.93; Specific gravity - 1.335; Boiling point - 40°C; Freezing point - 95°C; Vapor pressure - 315 mm Hg at 23.5°C (14).

Occupations where exposure to methylene chloride may occur include aerosol packagers, leather finish workers, anesthetic makers, oil processors, bitumen makers, paint remover makers, degreasers, resin makers, fat extractors, solvent workers, flavoring makers, and stain removers (14).

Common operations in which exposure may occur are use as a stripper of paint and varnish with liquid removers; as a cold and ultrasonic cleaner; as a carrier for aerosol products; as an extraction solvent for foods and furniture processing; as a cooling solvent in manufacture of cellulose acetate; as an organic

synthesizer; as a component in the manufacture of plastics; as a vapor degreaser of thermal switches and thermometers; and as a secondary refrigerant in air conditioning and scientific testing.

Exposure to methylene chloride is possible through inhalation of vapors, percutaneous absorption of liquid and ingestion. Methylene chloride is an anesthetic. Inhalation of vapors may cause mental confusion, light-headedness, nausea, vomiting and headache. Continued exposure may cause increased light-headedness, staggering, unconsciousness, and death. Higher concentrations may cause irritation of the respiratory tract and eyes (13).

Acute overexposure may result in pulmonary edema, and severe central nervous system (CNS) depression. One case of overexposure was reported to have resulted in multisystem disorder and was complicated by development of temporary diabetes mellitus (15). This condition may have actually been hyperglycemia. Repeated contact to methylene chloride may cause dry, scaly, and fissured dermatitis. If held in contact with the skin, it may cause burns (13).

Dihalomethanes, the category to which methylene chloride belongs, are unique in that carbon monoxide (CO) is a product of their metabolism by the body (2, 3). This fact has drawn much attention to methylene chloride due to its widespread use.

The current Occupational Safety and Health Administration (OSHA) permissible exposure level (PEL) for methylene chloride averaged over an eight-hour workday (eight-hour time-weighted-average or TWA

exposure) is 500 parts per million (ppm) or 1740 mg/m^3 with an acceptable ceiling level of 1000 ppm which may be exceeded up to a maximum peak concentration of 2,000 ppm for a maximum time duration of five minutes in any two hour period (24). ACGIH has recommended an eight-hour TWA-TLV of 100 ppm or 360 mg/m^3 in the absence of occupational exposure to carbon monoxide with a short-term exposure level (STEL) of 500 ppm or 1740 mg/m^3 averaged over 15 minutes. Where CO and methylene chloride are both present, ACGIH recommends the appropriate formula for exposures to mixtures be used (25). The National Institute for Occupational Safety and Health (NIOSH) has recommended an eight-hour TWA standard of 75 ppm in the absence of occupational carbon monoxide exposure greater than 9 ppm (1). For carbon monoxide exposures greater than 9 ppm, a formula is recommended as follows:

$$\frac{C(CO)}{L(CO)} + \frac{C(CH_2CL_2)}{L(CH_2CL_2)} \leq 1$$

where:

C(CO) = TWA exposure concentration of CO, ppm
L(CO) = The recommended TWA exposure limit of CO = 35 ppm
$C(CH_2CL_2)$ = TWA exposure concentration of methylene chloride
$L(CH_2CL_2)$ = The recommended TWA exposure limit of methylene chloride = 75 ppm

These standards and recommendations were established to prevent interference with delivery of oxygen to tissues and to prevent abnormalities in CNS function.

A discussion concerning the mechanism of methylene chloride metabolism to carbon monoxide, and possible resulting health effects follows.

Methylene Chloride Metabolism

Methylene chloride and carbon tetrachloride are halogenated hydrocarbons which have been widely used as solvents both in industry and commerce. When it is discovered that carbon tetrachloride caused severe liver damage, the use of it was largely discontinued and replaced by methylene chloride. It was orginally felt that methylene chloride was relatively safe with respect to liver damage (8).

Stewart et al. (2,3) in 1972, were the first to show that humans exposed to methylene chloride vapors exhibited high COHb levels. Stewart did not investigate the mechanism of methylene chloride metabolism but suggested that it must metabolize to carbon monoxide.

Kubic et al. (8) determined in 1973 that there was a dose-response relationship for production of COHb after intraperitoneal administration of various doses of methylene chloride to rats. Carbon tetrachloride, chloroform, chloromethane, carbon disulfide, methanol, formaldehyde, freon 11, freon 12, and dimethoxymethane were also administered to rats. These tests produced no elevation in blood COHb levels. This study proved conclusively that methylene chloride exposure resulted in elevated COHb levels by conducting experiments with ^{13}C-labeled methylene chloride. Infrared spectra of blood of rats given the labeled compound showed the presence of absorption bands characteristic of ^{13}C-Carbon monoxide.

These findings by Kubic et al. led to a follow-up in vitro study the following year (9). The purpose of the study was to determine the mechanism for metabolism of methylene chloride to carbon monoxide.

Several dihalomethanes metabolized to carbon monoxide and followed the halide order. That is, compounds with larger molecules yielded greater amounts of CO. Therefore, methylene chloride, having smaller molecules, yields less CO than other dihalomethanes. It was discovered that the mechanism of metabolism in rats was a mixed function oxidase system dependent on a microsomal cytochrome P-450 (9). It was shown that hepatic microsomal fractions in the presence of NADPH converted dihalomethane to carbon monoxide. No other combination of cytosal or microsomal and NADPH yielded CO.

Microsomal preparations from the lung and kidney yielded CO at rates of about 18 and 5% respectively of that found in liver microsomes. Identical tests conducted anaerobically yielded only 30% of the CO content when tested aerobically (9).

Many tests were conducted to establish the relationship of cytochrome P-450 levels with production of CO. These tests resulted in a correlation coefficient, $r = 0.97$, indicating a high degree of dependency of metabolism on cytochrome P-450 content (9).

This study (9) concluded that metabolism of methylene chloride to carbon monoxide, in the rat, is accomplished by a microsomal fraction (cytochrome P-450) requiring both NADPH and molecular oxygen. It also showed that most metabolism is accomplished by the liver and that metabolism is dependent on time, protein content, temperature, and pH.

Hogan et al. (7) conducted inhalation studies on the rat and found that COHb levels plateaued at 7% with a methylene chloride

exposure of 440 ppm over a three hour exposure period. Three hour exposures to levels as high as 2300 ppm resulted in no further increase in this maximum of 7%. Figure 1 illustrates this dose response data together with other inhalation dose response data available in the literature. For exposure periods less than eight hours an eight-hour TWA was computed.

Kubic et al. (8) concluded that COHb levels plateaued at about 8% in the rat following a dose of 3.0 mmoles per Kg of methylene chloride administered intraperitoneally.

Kurppa et al. (10) conducted inhalation exposure studies on rats at methylene chloride concentrations of 500 ppm, 1000 ppm, and 1000 ppm as a time-weighted average (TWA). The 1000 ppm TWA exposure was achieved by fluctuating exposure concentrations between 100 ppm and 2800 ppm for six hours. Continuous exposures to 500 ppm and 1000 ppm were also conducted over a six hour period. This study concluded that COHb levels plateaued at about 8.5% for all concentrations tested. Kurppa was unable to duplicate Kubic's results for metabolic mechanism when rats were exposed to fluctuating concentrations of methylene chloride. In fact, the P-450 content of the liver microsomes in rats was unchanged in Kurppa's study. Kurppa also concluded that the kidney played a larger role in metabolizing of methylene chloride to CO than did the liver. As indicated by these studies, the exact metabolic chain for conversion of methylene chloride to carbon monoxide remains controversial.

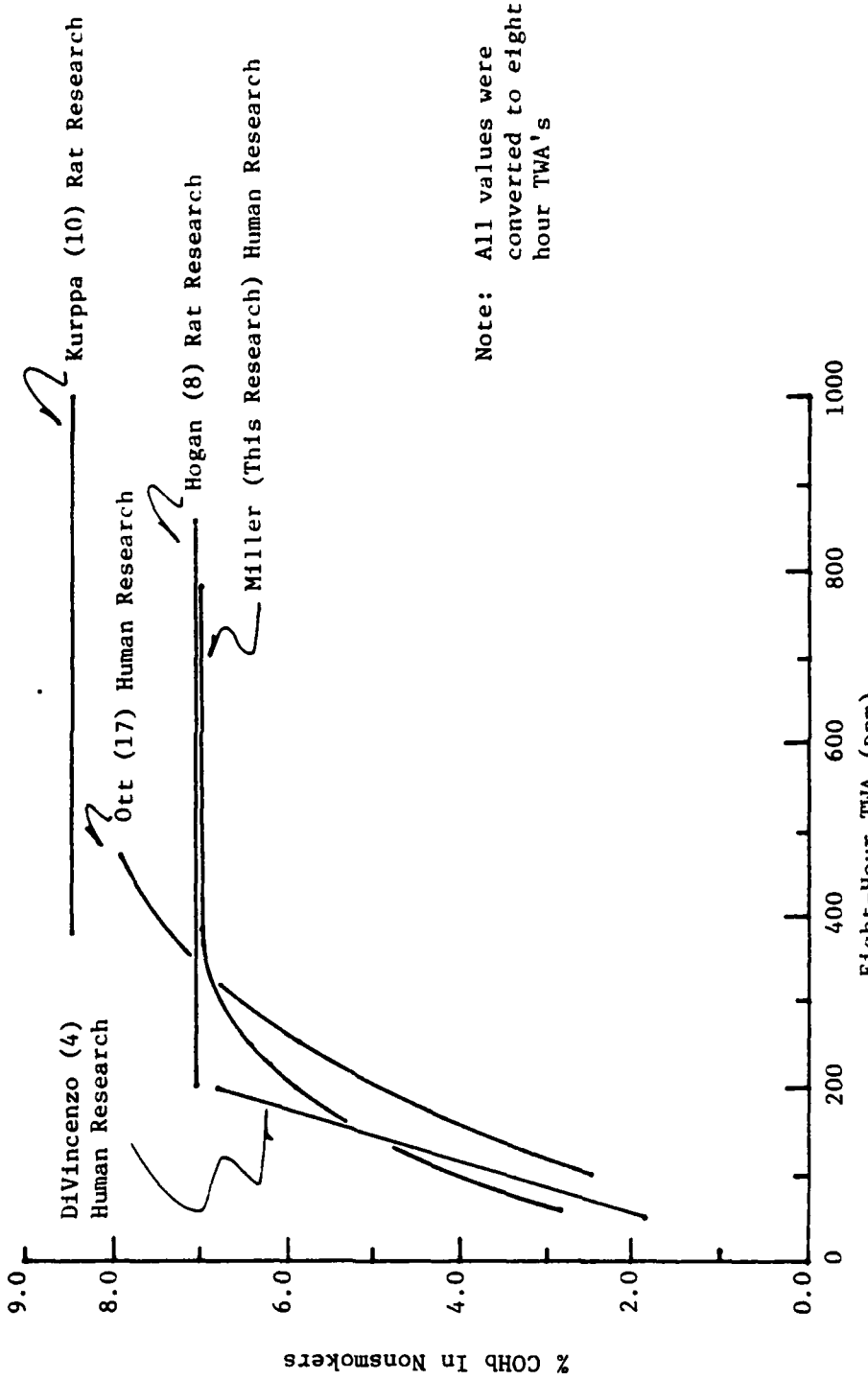

Figure 1 - Comparison of the Dose Response Data Available From the Literature with Results of This Research.

In 1980, DiVincenzo (4) studied the uptake, metabolism, and elimination of methylene chloride. These variables were studied on male and female sedentary nonsmokers with controlled methylene chloride exposures of 50, 100, 150, and 200 ppm. They also studied male nonsmokers exposed occupationally to relatively low concentrations of methylene chloride (33 ppm/eight-hour TWA). The group studied under controlled exposures demonstrated peak COHb levels of 1.9%, 3.4%, 5.3%, and 6.7% respectively. Occupational exposure resulted in COHb levels ranging from 1.6% to 5.4%.

An interesting point concerning the plateau effect in rats is that the same effect may not be demonstrated in man. A 20-year-old art student exposed to a commercial paint remover containing methylene chloride was admitted to the hospital. Upon admission, carboxyhemoglobin concentration was measured at 50% (6). In 1981, methylene chloride was implicated in an accidental fatality suggesting high COHb levels (16). The COHb level was not reported in this case and was merely an assumption based on known methylene chloride exposure. Since rat carboxyhemoglobin levels plateau below 10%, the rat may be a poor animal model for overall toxicity of methylene chloride. In addition, the mechanisms for metabolizing methylene chloride, studied in the rat, may have no relationship to metabolism by man. The literature contains no human metabolism studies or studies on animals other than the rat.

Ott et al. (17) found that the dose-response curve for humans exposed industrially to methylene chloride fit a quadratic equation.

This indicates a partial saturation of the enzyme system required for metabolizing methylene chloride. This researcher made similar findings with COHb levels reaching a plateau of about 7% in nonsmokers, at a concentration of approximately 1800 ppm (eight hour TWA).

This apparent disparity between reported COHb levels and research findings is addressed in the discussion section. Although metabolism mechanisms and COHb maximums are uncertain, it is unquestionable that methylene chloride metabolizes to carbon monoxide. This introduction now proceeds to a discussion of how the resulting carbon monoxide may interfere with the oxygen carrying capacity of the blood.

Carbon Monoxide and Oxygen Transport

Carbon Monoxide impairs the ability of the blood to transport oxygen to the tissues. This impairment is a result of two distinct mechanisms. First, CO combines reversibly with the oxygen-carrying sites on the hemoglobin molecule with an affinity from 210 to 240 times greater than oxygen. The resulting carboxyhemoglobin is unavailable to carry oxygen. Like oxygen, the exact mechanism for reversible binding of carbon monoxide to hemoglobin is unclear. The affinity of carbon monoxide for hemoglobin is best described as resistance to the dissociation of the carboxyhemoglobin complex. Where oxygen disassociates from the hemoglobin molecule in a fraction of a second, carbon monoxide takes minutes. The second mechanism of impairment is interference with the release of oxygen from oxygenated hemoglobin or a shift in the oxyhemoglobin dissociation curve. This

shift leftward results in an increase in the avidity of hemoglobin for oxygen. At high partial pressures this shift in the curve is of minimal importance. However, at tissue level, where oxygen content of capillary blood is reduced, the shift can significantly decrease the oxygen tension supplying the tissues. The effect is most prominent at high carboxyhemoglobin levels and is similar to effects of hypoxic hypoxia or altitude hypoxia (12). Although the above discussion concerning CO and oxygen transport is widely accepted, recent research posed new questions.

Goldbaum et al, (18) demonstrated that route of CO entry was more important to toxicity than COHb levels. Dogs which were transfused with erythrocytes containing 80% COHb and injected (i.p.) with CO gas demonstrated no CO toxicity even though COHb exceeded 50%. Dogs inhaling CO (13 percent in air) for 15 minutes died within 15 minutes to 65 minutes with an average COHb level of 65%. This phenomenon is best explained by the differences in plasma CO concentrations and competition or lack of competition with O_2 for cytochrome a_3 oxidase enzymes. There are several reports of individuals exposed to methylene chloride who demonstrated high levels of COHb but were asymptomatic or mildly sympotmatic for CO poisoning.

Methods for analyzing CO in plasma were investigated by this researcher, with little success. A method for analyzing CO in plasma must be developed to adequately confirm or dismiss the hypothesis that COHb may not be an accurate measure of CO toxicity. This is especially important with CO orginating as a metabolic product rather than an atmospheric gas.

METHODS

Selection of Population

The study described here was a comparative study. Daily COHb increases in workers exposed to methylene chloride were compared to daily COHb increases on nonexposure days.

The subjects were drawn from small private businesses which strip and refinish furniture in northern Utah. There are no large manufacturing or processing plants in the area utilizing methylene chloride and a nearby military installation employing a significant paint stripping work force did not wish to participate in the research.

Furniture strippers were solicited as study subjects because methylene chloride is commonly found as a major constituent in paint and varnish stripping products.

Thirty-one Utah businesses advertising furniture stripping services in the telephone company yellow pages were contacted by telephone to solicit participation. Nine of the businesses listed had disconnected their telephone service. Of the 22 remaining in business, 11 either refused participation in the study or were not currently stripping furniture.

The study consisted of 18 subjects from 11 businesses located between Brigham City on the north and Salt Lake City on the south.

The area from which the subjects were drawn was limited to northern Utah due to time and financial restraints.

The study population contained eight smoking males, eight nonsmoking males and two nonsmoking females. Smoking was defined as any type and quantity of smoking. Table I compiles the smoking habits of the eight smoking subjects and two smoking controls.

Since all subjects included in the study were volunteers, a volunteer bias may exist in the data. This is especially true if characterization of the industry is desired. However, the businesses tendency to accept or reject participation in the study seemed to be influenced by the degree of reluctance to give blood and/or the fear of government regulatory agency intervention into business operations. This conclusions is based on telephone conversations with those refusing participation. The above stated factors, rather than degree of exposure, seemed to be most important in the businesses decision to participate in the study.

Since the purpose of the study is to compare a measured exposure with a measured biological response, volunteer bias is not as significant as it would be if characterization of the industry was the goal.

Sampling Procedure

Each business was contacted and the sampling date scheduled to correlate with a normal stripping day. Upon arrival, participants were asked to sign a release form and complete a questionnaire

Table I

Smoking Habits of Eight Smoking Subjects and Two Smoking Controls

Subject No.	Age	Sex	Type of Smoking	Brand	No. Smoked Per Day	Filtered
11	29	Male	Cigarettes	Marlboro	1 Pack	Yes
12	28	Male	Cigarettes	Unknown	1 1/2 Pack	Yes
13	40	Male	Cigarettes	Tareyton 100	10-15	Yes
14	28	Male	Cigarettes	Carlton 100	1 1/2 Pack	Yes
15	34	Male	Cigarettes	Camel	12-15	No
16	43	Male	Pipe	N/A	N/A	N/A
17	25	Male	Cigarettes	Camel Lights	1 Pack	Yes
18	22	Male	Cigarettes	Drum	6	No
17 Control	32	Male	Cigarettes	Salem	15-20	Yes
18 Control	37	Male	Cigarettes	True Menthol	5	Yes

Note: Subjects 11 throught 16 are self paired controls.
Subjects 17 and 18 were matched with controls for sex and smoking habit but age matching was not adequate.

covering age, sex and smoking information (Appendix I). This was completed prior to drawing blood or collecting atmospheric samples.

Atmospheric concentrations of methylene chloride were measured with 3M 3520 organic vapor monitors (OVM) attached at the lapel. Sampling time for each monitor was approximately four hours. Fifteen side-by-side samples were collected with charcoal sorbent tubes for correlation purposes. Two charcoal tubes (SKC) each containing a 50 mg and 100 mg section of activated charcoal were connected in series with a sampling rate of approximately 0.1 liters per minute (LPM). The sample was collected by drawing air through the charcoal tubes with a low flow sampling pump attached to the workers belt. Charcoal tubes were attached at the workers lapel and connected to the pump with Tygon tubing. Figure 2 is a schematic of the sampling train.

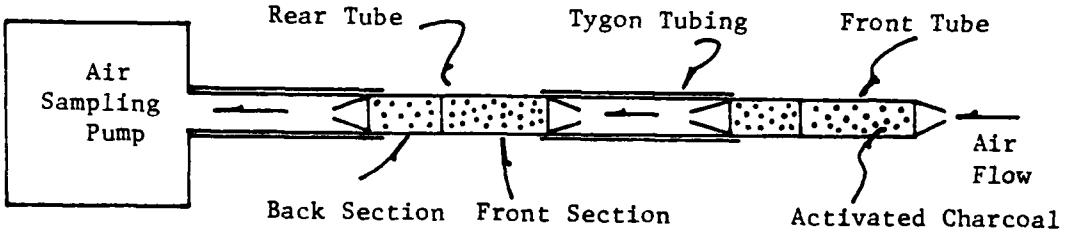

Figure 2

Schematic of the Sampling Train

This sampling method was utilized because methylene chloride has been shown to have a poor affinity or low capacity for charcoal. If only one tube is used, it is impossible to assess whether or not break through has occured because the front and back sections of the tube reach equilibrium rapidly. Break through occurs when the back section of the rear sampling tube (charcoal in this case) contains more than 25% of the contaminant found in the front section of the rear tube. If this occurs, one must suspect that a portion of the contaminant has passed entirely through the sampling tube or "break through" has occurred. By using two tubes in series and capping each following sampling, the second tube acts as a back-up section to assess break through. Appendix II is a summary of total milligrams of contaminant found on individual sections of both charcoal tubes and OVM's. Sampling pumps were calibrated before and after the sampling period with a bubble burette. Relative humidity and temperature were measured and recorded.

Samples were held in a cooler through the end of the day and then frozen until analyzed. All samples were analyzed by a NIOSH certified laboratory located in Salt Lake City, Utah. NIOSH analytical method S329 utilizing gas chromatography was used (26). Organic vapor monitors were analyzed and computations made in accordance with manufacturers instructions. Atmospheric sampling data and comparison of side-by-side sampling is evaluated in the results section.

Carbon monoxide was monitored with an Ecolyzer Series 2000, SN F1646 direct reading instrument. This was necessary to ensure that

sources of CO other than smoking were not contributing to COHb levels. A chemical interference with the ecolyzer was immediately found. With no apparent source of CO, levels up to 300 ppm were unexplainably measured. The instrument manufacturer was contacted to ascertain possible chemical interferences. Although methylene chloride had not been tested by the manufacturer, methanol had been found to have a 4.8 to 1 interference ratio. This means that 1 ppm methanol will appear as 4.8 ppm of CO on the instrument. Methanol like methylene chloride is common to paint and varnish stripping products. To correct this problem an activated charcoal filter was obtained from the manufacturer and monitoring continued with no apparent interference. CO concentrations were subsequently found to be less than 5 ppm.

Blood samples were drawn prior to "morning" and following "afternoon" the work shift with 3 cc heparinized vacutainers. Blood samples were also drawn at corresponding times on a nonexposure day. Blood samples were kept in a cooler or refrigerator at all times prior to analysis. Seventy eight percent of the blood samples were analyzed within 24 hours of the time drawn (Table II). Samples held longer than one day were considered accurate based on the conclusion that unless opened, little COHb deterioration occurs. Appendix III contains deterioration information found over time in eight samples analyzed at various time intervals. Deterioration of COHb in the eight samples is discussed further in the results section. All samples were analyzed with an Instrumentation Laboratory CO-oximeter

Table II

Blood Sample Results and Time Interval Data Between Sample Collection and Analysis

Blood Sample #	Date Drawn	Date Analyzed	% COHb	Blood Sample #	Date Drawn	Date Analyzed	% COHb
1	7-11	7-11	2.3	32	8-7	8-7	9.6
2	7-11	7-11	2.0	33	8-8	8-8	7.2
3	7-11	7-11	3.9	34	8-8	8-8	4.8
4	7-11	7-11	7.5	35	8-8	8-8	6.4
5	7-11	7-11	3.5	36	8-8	8-8	6.1
6	7-11	7-11	4.4	37	8-10	8-10	3.6
7	7-12	7-12	6.0	38	8-10	8-10	6.7
8	7-12	7-12	12.0	39	8-11	8-12	4.0
9	7-14	7-15	4.9	40	8-11	8-12	8.5
10	7-14	7-15	6.5	41	8-10	8-12	4.4
11	7-15	7-15	0.6	42	8-10	8-12	5.4
12	7-15	7-15	4.0	43	8-14	8-15	1.8
13	7-15	7-15	1.2	44	8-14	8-15	1.4
14	7-15	7-15	1.6	45	8-20	8-22	4.0
15	7-15	7-15	0.6	46	8-20	8-22	6.1
16	7-15	7-15	7.4	47	8-20	8-22	1.0
17	7-15	7-15	2.2	48	8-20	8-22	1.2
18	7-15	7-15	4.0	49	8-21	8-22	4.4
19	7-18	7-19	0.8	50	8-21	8-22	2.2
20	7-18	7-19	6.0	51	8-22	8-22	6.3
21	7-18	7-19	0.9	52	8-22	8-22	1.1
22	7-18	7-19	6.2	53	8-22	8-23	7.0
23	7-18	7-19	1.9	54	8-22	8-22	0.9
24	7-18	7-19	9.8	55	8-26	8-30	1.9
25	7-19	7-19	1.3	56	8-26	8-30	2.2
26	7-19	7-19	1.9	57	8-26	8-30	1.4
27	7-27	7-27	6.6	58	8-26	8-30	1.0
28	7-27	7-28	9.8	59	8-28	8-30	2.0
29	8-6	8-7	0.9	60	8-28	8-30	1.8
30	8-6	8-7	5.3	61	8-28	8-30	0.8
31	8-7	8-7	4.6	62	8-28	8-30	0.8

282 located in the pulmonary laboratory of the University of Utah Medical Center in Salt Lake City, Utah. Samples were analyzed at least two times with results averaged. A third analysis was completed if the first two varied by more than 0.4% COHb. The outlying value was disregarded and the average computed on the remaining two values.

Analysis of Data

The data were analyzed for significant differences in COHb levels between periods of exposure and periods of nonexposure. Similar methods were used for both smokers and nonsmokers. For nonsmokers, am and pm COHb levels were considered accurate because am levels were predictable and relatively constant on both exposure and nonexposure days. This was not the case for smokers. Blood samples drawn in the morning on exposure and nonexposure days were not consistent. With one exception, am levels were lower on the nonexposure days for smokers. Several of the subjects were sampled on off days to document nonexposure COHb levels. Although blood was drawn at approximately the same time as during exposure, the subjects had been awake for a shorter period and had smoked fewer cigarettes than on the exposure day. The exception mentioned above had gotten up very early on the nonexposure day and had smoked heavily prior to sampling. This resulted in a higher am COHb during nonexposure than during exposure.

COHb levels appear to rise rapidly in the morning as cigarettes are smoked. Because of the inability to control the number of

cigarettes smoked and the times at which they were smoked it was not possible to assure comparability between morning COHb levels on exposure and nonexposure days for smokers. For this reason, morning COHb readings were rejected for smokers and conclusions were based solely on afternoon blood samples. However, all data is presented in the results section.

The Wilcoxon signed rank statistic for paired samples was used in analyzing the data since this statistical technique allows for non-normally distributed data (21). Subjects were either self-paired or artificially paired by matching for age, sex, and smoking habits. Four individuals, two smokers and two nonsmokers, refused to be resampled during nonexposure and were therefore matched for age, sex and smoking habits with solicited controls. The nonsmokers were matched for age within four years. Smokers were matched to within five cigarettes per day but age matching was not acceptable. For this reason the data is presented in the results section both considering and disregarding the control data for smokers.

Statistically comparing differences in COHb increases between smokers and nonsmokers is inappropriate due to significant differences in exposure dose. Comparing dose response curves for smokers and nonsmokers without adjusting for smoking is also inappropriate because the contribution of smoking to COHb is not considered. To compare the two dose response curves it is necessary to control for the smoking contribution by adjusting COHb increases in accordance with the amount of increase or decrease found during

nonexposure to methylene chloride. In this way it is possible to ascertain the amount of COHb increase attributable to methylene chloride exposure only. This was accomplished by constructing dose response curves with dose being the eight hour TWA methylene chloride exposure and response being ΔCOHb in pm values between exposure and nonexposure days. It is necessary to draw both curves in the same manner so that conclusions can be made concerning statistically significant differences.

RESULTS

Air Sampling

Air sampling results are compiled on Tables III and IV. Table III is data for nonsmokers and Table IV is data for smokers. Exposures to methylene chloride were highly variable ranging from 78 mg/m^3 to 2608 mg/m^3 computed as an eight hour TWA. Table V illustrates the results of the side-by-side sampling conducted with charcoal tubes. Fifteen side-by-side samples were collected with two 150 mg charcoal tubes connected in series (Figure 2). Ten of the fifteen samples collected on charcoal tubes broke through. Break through is assumed when the back section of the rear tube contains more than 25% of the amount of contaminant found in the front section of the rear tube. The five remaining samples, considered valid, were statistically compared to the corresponding concentrations found with OVM's. Table VI statistically compares the five side-by-side samples utilizing the t-test for comparison of paired differences (21). The OVM's yielded an exposure estimate averaging 12% higher than charcoal tubes. However, a statistically significant difference did not exist between the two methods of measurement. A larger sample may have resulted in a statistically significant difference.

Table III

Blood COHb and Methylene Chloride Exposure in Nonsmokers

Exposure Data		Blood COHb%							
			Exposure Day			Nonexposure Day			
Exposure TWA (mg/m^3)	Eight-Hour TWA (mg/m^3)	Subject	AM	PM	ΔE	AM	PM	ΔN	(PM)	ΔE-ΔN
3277	2608	1	0.9	6.2	5.3	1.8	1.4	-0.4	4.8	5.7
218	145	2	0.8	1.9	1.1	1.0	1.2	0.2	0.7	0.9
778	905	3	6.0	12.2	6.2	4.4	2.2	-2.2	10.0	8.4
276	347	3a	4.9	6.5	1.6	4.4	2.2	-2.2	4.3	3.8
428	473	4	0.9	5.3	4.4	1.9	2.2	0.3	3.1	4.1
125	78	5	1.3	1.9	0.6	1.4	1.0	-0.4	0.9	1.0
371	123	6	1.2	2.2	1.0	1.2	1.0	-0.2	1.2	1.2
525	322	7	0.6	4.0	3.4	1.1	0.9	-0.2	3.1	3.6
2595	1757	8	2.0	7.5	5.5	1.3	1.2	-0.1	6.3	5.6
794	634	9	1.5	6.1	4.6	2.0	1.8	-0.2	4.3	4.8
816	650	10	1.0	5.6	4.6	0.8	0.8	0.0	4.8	4.6

Table IV

Blood COHb and Methylene Chloride Exposure in Smokers

Exposure Data			COHb%						
Exposure TWA (mg/m^3)	Eight-Hour TWA (mg/m^3)	Subject	Exposure Day AM	Exposure Day PM	ΔE	Nonexposure Day AM	Nonexposure Day PM	ΔN	(PM)	ΔE-ΔN
174	148	11	4.8	6.1	1.3	3.6	4.4	0.8	1.7	0.5
207	189	12	7.2	6.4	-0.8	6.7	5.4	-1.3	1.0	0.5
319	319	13	6.6	9.8	3.2	4.6	9.6	5.0	0.2	-1.8
936	624	14	6.0	9.8	3.8	4.0	6.1	2.1	3.7	1.7
378	229	15	4.0	7.4	3.4	6.3	7.0	0.7	0.4	2.7
719	533	16	4.0	8.5	4.5	3.7	3.7	0.0	4.8	4.5
207	185	17	3.9	4.4	0.5	-	6.2	-	-1.8	-
171	140	18	2.3	3.5	1.2	-	3.8	-	-0.3	-

Table V

Side-By-Side Sampling Results Conducted with 3M 3520 Organic Vapor Monitors and Two 150 mg Charcoal Tubes Connected in Series

OVM No.	mg/m^3	Charcoal Tube No.	mg/m^3
014	900	006 & 007	* 883
008	654	003 & 004	* 510
011	1350	009 & 010	*1375
003	399	001	327
004	928	005 & 006	764
009	619	008	* 465
1447	319	009	* 171
1255	125	008	116
1443	2920	006	*1306
1366	3654	007	*1525
1434	378	005	323
1370	962	004	* 610
1162	520	003	518
1495	2855	002	*2107
1481	2342	001	*2290

* Break through probable using standard criteria of back section containing more than 25% of front section value.

Table VI

Differences Between Side-By-Side Atmospheric Sampling of Methylene Chloride

Sample No	OVM (mg/m^3)	Charcoal Tube (mg/m^3)	Difference mg/m^3 (d)
1	399	327	72
2	928	764	164
3	125	116	9
4	378	323	55
5	520	518	2

t test for paired samples:

$$\bar{d} = 60.4 \qquad S_d = 65.1 \qquad n = 5$$

$$t_4 = \frac{\bar{d} - \delta_o}{\sqrt{S_d{}^2/n}}$$

$$t_4 = \frac{60.4 - 0}{\sqrt{(65.1)^2/5}}$$

$t_4 = 2.07$ $p > 0.05$: Not statistically significant

Note: Comparison includes only valid samples when judged by break-through criteria.

Conclusion: Based on limited data there is no significant difference between OVM and charcoal tube values.

Blood Samples

Blood samples were analyzed for percent COHb. Tables III and IV summarize COHb levels, measured in nonsmokers and smokers respectively, for both exposure and nonexposure conditions. Figure 3 illustrates the deterioration found in COHb levels in eight samples analyzed at various intervals. Appendix III contains actual sample times and measured COHb levels. Deterioration of COHb was variable with four samples showing significant deterioration and four remaining relatively constant. The only obvious difference was the number of times that the blood sample was analyzed. Repeated opening of the blood sample may be the cause of the deterioration. It appears that a sample could be held in refrigeration for at least 23 days without significant deterioration if not opened.

Statistical Comparisons

The Wilcoxon signed rank test was applied to the data to test for significant differences in COHb levels under exposure and nonexposure conditions (21). The null hypothesis (Ho) for the following comparisons is that no difference in COHb levels or COHb increases exist regardless of methylene chloride exposure conditions.

Table VII presents the data and appropriate test of significance for differences in COHb increases among nonsmokers. The difference in am and pm COHb levels during nonexposure (ΔN) is subtracted from the difference in am and pm COHb levels during exposure (ΔE), to ascertain the desired difference ($\Delta E - \Delta N$). The test concludes that

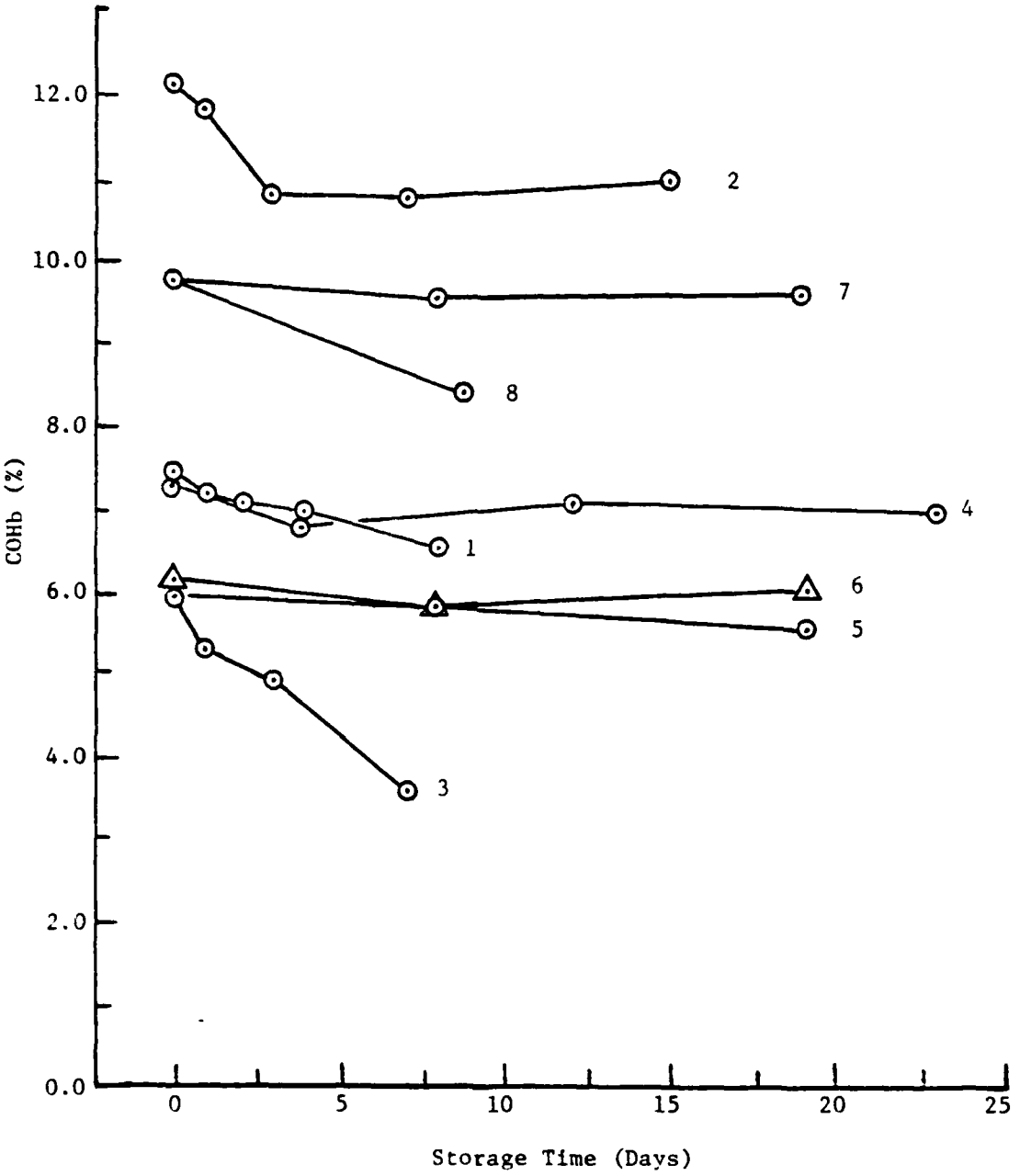

Figure 3

COHb levels over time in eight samples analyzed at various time intervals.

Table VII

Difference Between COHb Increases on Exposure and Nonexposure Days in Nonsmokers ($\Delta E - \Delta N$)

Subject	COHb $\Delta E - \Delta N$	Difference Disregarding Sign	Rank	Signed Rank
1	5.7	5.7	10	10
2	0.9	0.9	1	1
3	8.4	8.4	11	11
3a	3.8	3.8	5	5
4	4.1	4.1	6	6
5	1.0	1.0	2	2
6	1.2	1.2	3	3
7	3.6	3.6	4	4
8	5.6	5.6	9	9
9	4.8	4.8	8	8
10	4.6	4.6	7	7

Wilcoxon signed rank test:

Sum of negative signed ranks: 0
Sum of positive signed ranks: 66
Sample size (n) = 11
$p < 0.01$: Statistical significant

If subject 3 and 3a are disregarded in the analysis p remains less than 0.01. Statistically significant. See discussion section page 45 and 46.

a significant difference in resulting COHb levels does exist among nonsmokers exposed to methylene chloride ($p < 0.01$).

Table VIII also presents the data and test of significance for nonsmokers. This test disregards am COHb and considers only the difference between pm COHb under exposure and nonexposure conditions. This test also concludes significantly ($p < .01$) that nonsmokers demonstrate higher pm COHb levels following exposure to methylene chloride.

Table IX compares differences in COHb increases ($\Delta E - \Delta N$) for smokers. This test does not reject the null hypothesis ($p > .05$), and concludes that a statistically significant difference in COHb does not exist among smokers regardless of exposure to methylene chloride. Significant problems exist when attempting to duplicate am COHb's in smokers. These problems were addressed earlier. The added variability to the data resulted in the conclusion that a statistically significant difference did not exist in COHb levels among smokers when exposed or not exposed to methylene chloride.

The problem with am COHb levels in smokers is eliminated in Table X. Differences in pm COHb levels (Δpm) during exposure and non-exposure conditions are evaluated while disregarding all am readings. The test concludes that a statistically significant difference ($p < .05$) does exist in COHb levels among smokers during exposure and nonexposure.

Table XI displays the data and test of significance when the two additional smoking subjects and their controls are included. Table I

Table VIII

Difference Between pm COHb Levels on Exposure and Nonexposure Days in Nonsmokers (Δ pm).

Subject	COHb Δ PM	Difference Disregarding Sign	Rank	Signed Rank
1	4.8	4.8	8.5	8.5
2	0.7	0.7	1.0	1.0
3	10.0	10.0	11.0	11.0
3a	4.3	4.3	6.5	6.5
4	3.1	3.1	4.5	4.5
5	0.9	0.9	2.0	2.0
6	1.2	1.2	3.0	3.0
7	3.1	3.1	4.5	4.5
8	6.3	6.3	10.0	10.0
9	4.3	4.3	6.5	6.5
10	4.8	4.8	8.5	8.5

Wilcoxon signed rank test:

Sum of negative signed ranks: 0
Sum of positive signed ranks: 66
Sample size (n) = 11
$p < 0.01$: Statistical significant

If subject 3 and 3a are disregarded in the analysis p remains less than 0.01. Statistically significant.

Table IX

Difference Between COHb Increases on Exposure and Nonexposure Days in Smokers ($\Delta E - \Delta N$)

Subject	COHb $\Delta E - \Delta N$	Difference Disregarding Sign	Rank	Signed Rank
11	0.5	0.5	1.5	1.5
12	0.5	0.5	1.5	1.5
13	-1.8	1.8	4.0	-4.0
14	1.7	1.7	3.0	3.0
15	2.7	2.7	5.0	5.0
16	4.5	4.5	6.0	6.0

Wilcoxon signed rank test:

Sum of negative signed ranks: 4.0
Sum of positive signed ranks: 17.0
Sample size (n): 6
$p > 0.05$: Not statistically significant

Table X

Difference Between pm COHb Levels on Exposure and Nonexposure Days in Smokers (Δpm)

Subject	COHb ΔPM	Difference Disregarding Sign	Rank	Signed Rank
11	1.7	1.7	4	4
12	1.0	1.0	3	3
13	0.2	0.2	1	1
14	3.7	3.7	5	5
15	0.4	0.4	2	2
16	4.8	4.8	6	6

Wilcoxon signed rank test:

Sum of negative signed ranks: 0
Sum of positive signed ranks: 21
Sample size (n): 6
$p < 0.05$ = Statistically significant

Note: All subjects self-paired

Table XI

Difference Between COHb Levels on Exposure and Nonexposure Days in Smokers (ΔE-ΔN)

Subject	COHb ΔE-ΔN	Difference Disregarding Sign	Rank	Signed Rank
11	1.7	1.7	5	5
12	1.0	1.0	4	4
13	0.2	0.2	1	1
14	3.7	3.7	7	7
15	0.4	0.4	3	3
16	4.8	4.8	8	8
17	-1.8	1.8	6	-6
18	-0.3	0.3	2	-2

Wilcoxon signed rank test:

Sum of negative signed ranks: 8
Sum of positive signed ranks: 28
Sample size (n) = 8
$p > 0.05$: Not statistically significant

Note: Subjects 11-16 are self-paired.
Subjects 17 and 18 are matched controls.

lists the raw data for smoking subjects and the two smoking controls. Due to the additional variability induced by the control data, the difference in COHb levels during exposure and nonexposure is no longer statistically significant ($p > .05$).

The null hypothesis is rejected for both smokers and nonsmokers when differences in pm COHb levels are considered. However, the differences in nonsmokers are more conclusive ($p < .01$) than those found in smokers ($p < .05$). Nonsmoking data reaches statistical significance regardless of the manner in which it is analyzed. Conclusions for smokers border on statistical significance due to a smaller sample size and inherent variability.

Dose Response Curves

Concentrations of methylene chloride exposure were highly variable across the study population. Nonsmokers were exposed to concentrations of methylene chloride averaging two and one half times greater than those of smokers. The differences in exposure make simple statistical comparisons between the two groups impractical due to such a significant confounding variable. Therefore, comparison of the two groups must be assessed by differences in the dose response curves. COHb levels for the smoking and nonsmoking groups must be compared to assess whether the combined effect of smoking and methylene chloride exposure on COHb levels are compensating, additive or synergistic.

Figure 4 illustrates the difference in dose response curves for exposed smokers and nonsmokers. Response for these curves are

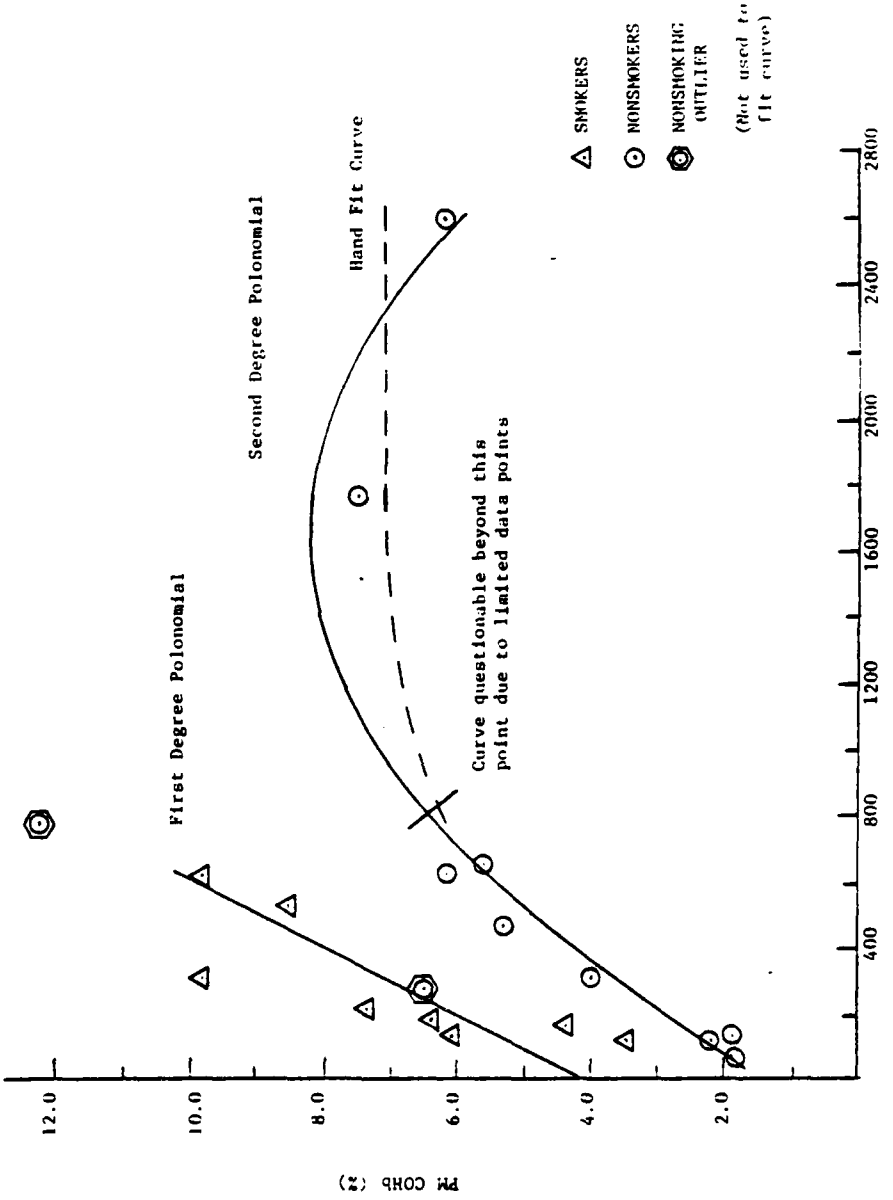

Figure 4 - Dose Response Curves for pm COHb Levels in Smokers and Nonsmokers.

afternoon COHb values on exposure days only. Morning COHb levels and effects of smoking are not considered in the curves. It is obvious from these curves that smokers have higher COHb levels than nonsmokers and therefore may be at higher risk for disease. These curves make no attempt to evaluate the source of elevated COHb levels because nonexposure data (i.e., smoking) is not considered. One nonsmoking subject was studied on two different occasions due to unusually high COHb levels found in the first sampling period. Both points are indicated on Figure 4 as outliers and are not considered in subsequent dose response curves. Potential reasons for the unusual data are evaluated in the discussion section. Smoking data was best fit with a first degree polonomial (straight line). Nonsmoking data, disregarding subject three as an outlier, was best fit with a second degree polonomial. The second degree polonomial may be misleading in that it is based on only two data points at the higher concentrations and indicates a falling off of COHb levels, with increasing exposure concentrations, following the peak. There is no biologic reason for the curve to fall after the peak. It is more reasonable to assume that the enzyme necessary to metabolize methylene chloride to CO reaches a maximum production rate resulting in a sustained plateau of the dose response curve. Based on biologic plausibility, the hand fit curve is probably a better estimation of the true dose response curve. However, at exposure concentrations higher than 800 mg/m^3 (eight-hour TWA) the dose response curve is an estimation at best due to the limited number of data points. This reasoning also holds true for Figures 5 and 6.

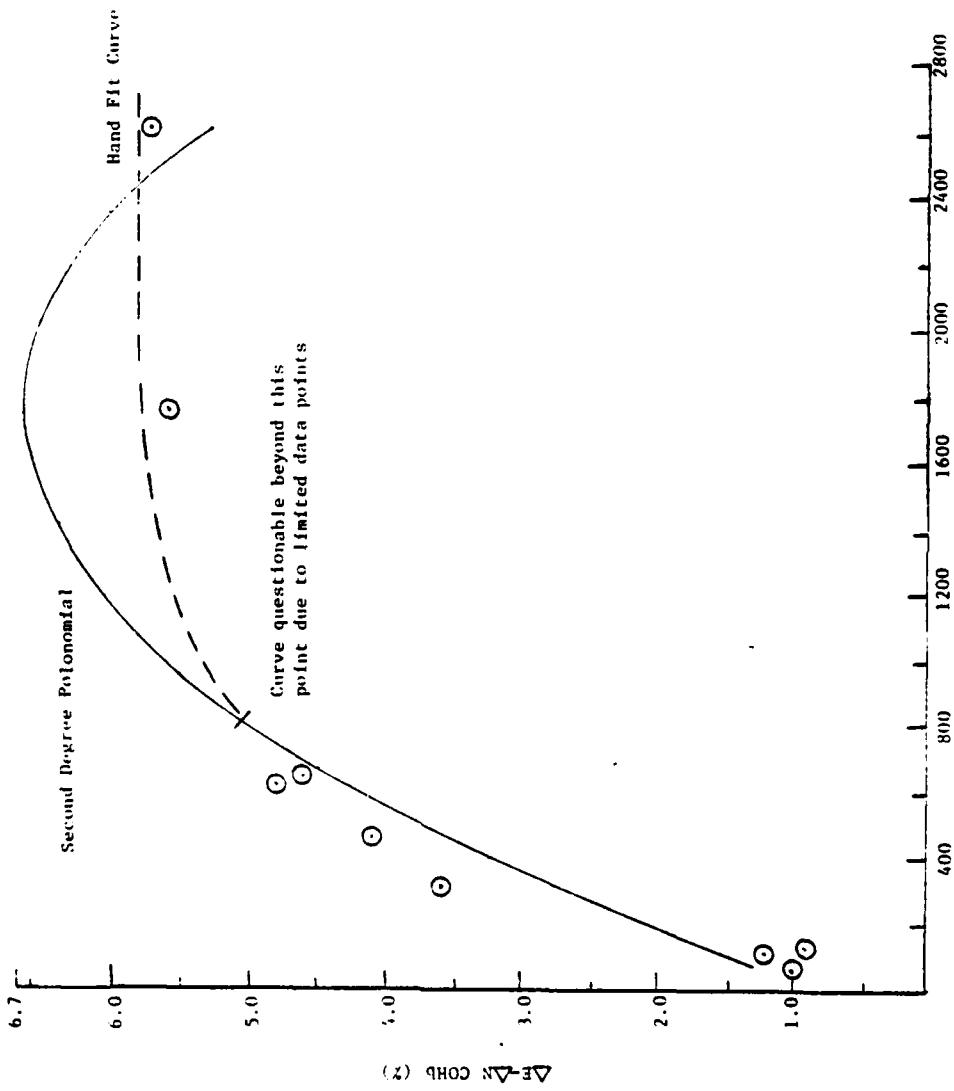

Figure 5 - Dose Response Curve for Nonsmokers Considering Differences in COHb Increases on Both Exposure and Nonexposure Days.

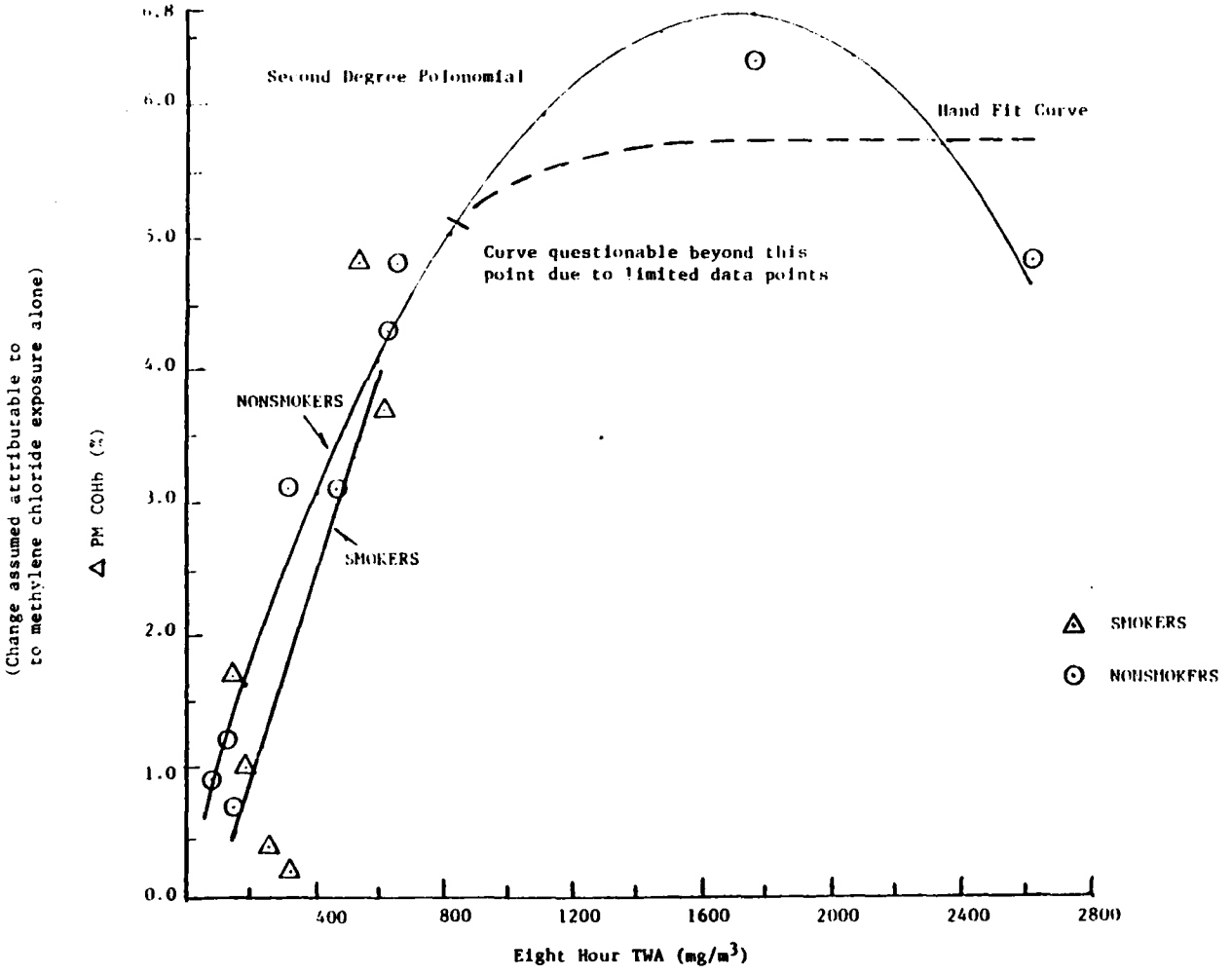

Figure 6 - Dose Response Curves Comparing the Difference in pm COHb (Δpm) Levels in Smokers and Nonsmokers.

Figure 5 is a dose response curve for nonsmokers considering differences in COHb increases on both exposure and nonexposure days (ΔE-ΔN). Both am and pm COHb's are considered in construction of the curve. The polonomial and hand fit curves are similar to Figure 4 and indicate that COHb may start to plateau at about 800 mg/m^3 TWA. The hand fit curve peak is reached at about 2000 mg/m^3 at a ΔE-ΔN COHb of about 5.8%. Adding the average am COHb for nonsmokers of 1.1% to this value yields a maximum of 6.9%. This curve contains all pertinent data for exposure and nonexposure COHb levels and is a good dose response curve for nonsmokers up to methylene chloride concentrations of 800 mg/m^3. It must again be emphasized that at eight-hour TWA concentrations greater than 800 mg/m^3 the dose response curve becomes questionable because of the limited number of data points.

Figure 6 illustrates dose response curves comparing the difference in pm COHb (Δpm) levels in smokers and nonsmokers. By looking at Δpm COHb levels it is possible to control for individual differences in COHb and, most importantly, for smoking. These curves show the contribution that methylene chloride metabolism has on COHb production and eliminates the effects of smoking and other environmental sources of CO.

The nonsmokers hand fit dose response curve peaks at an exposure concentration of approximately 1800 mg/m^3 (eight-hour TWA) and a Δpm COHb of 5.7%. Adding the average am COHb value of 1.1% for nonsmokers yields a peak of 6.8% COHb. The second degree polonomial

curve also peaks at approximately 1800 mg/m^3 (eight-hour TWA) but drops off rapidly. As discussed earlier, it is biologically doubtful that the curve falls off after the peak. Ott (17) found that COHb tends to plateau at approximately the same point shown by this research, but no mention was made of a COHb decrease following the peak.

The smoking dose response curve (Figure 6) extends to an eight hour TWA of 650 mg/m^3. There were no smoking subjects exposed at higher concentrations. The variability in the smoking data is high and can only be reasonably fit with a first degree polonomial. The dose response curve closely parallels that of the nonsmokers curve up to 650 mg/m^3 but is shifted downward. This would lead to the conclusion that the effects on COHb, due to smoking and methylene chloride exposure combined are less than additive up to 650 mg/m^3.

A computer analysis of covariance (22) was conducted on the first degree polonomials of the two dose response curves. The curves were compared only to a concentration of 650 mg/m^3. There was not a statistically significant difference in the two sets of data. Therefore, the conclusion must be made that there is no difference in the two dose response curves making the combined effects of smoking and methylene chloride exposure <u>additive</u> with respect to COHb. This statement is valid to a concentration of 650 mg/m^3 only.

DISCUSSION

This research has served to assess the combined effects of smoking and methylene chloride exposure on COHb levels. It has been shown that smokers and nonsmokers demonstrate statistically significant increases in COHb levels when exposed to methylene chloride. This conclusion for nonsmokers ($p < .01$) is less likely due to chance than the conclusion for smokers ($p < .05$)

The dose response curves for smokers and nonsmokers were estimated using change (Δ) in COHb between exposed and nonexposed days as the measure of response. The curves were compared statistically up to a methylene chloride concentration of 650 mg/m^3. A statistically significant difference in the two curves did not exist. Therefore, this research concludes that the combined effects of smoking and methylene chloride exposure in COHb production are additive up to a concentration of 650 mg/m^3. If CO and methylene chloride acted synergistically the slope of the smoker curve would be significantly steeper. Similarly if CO and methylene chloride acted in less than a combined manner the smoker curve would be significantly flatter than the nonsmoker curve.

The dose response curve for nonsmokers reached a plateau of about 7% COHb at a concentration of approximately 1800 mg/m^3. However,

this conclusion is based on limited data and should be so considered. The smokers dose response curve did not reach a plateau, but the highest exposure observed was 624 mg/m^3 (eight-hour TWA). Although it cannot be proven with this research, the smokers dose response curve may also plateau at higher exposure concentrations.

There exists a confusing disparity in the literature regarding COHb levels resulting from methylene chloride exposure. There are a few reports of extremely high COHb levels (40-50%) in exposed workers (6, 23) and also at least one fatality associated with overexposure to the chemical (16). On the other hand, rat studies indicate a plateauing in the dose response curve (7, 8, 9, 10) at levels of 7-8% COHb. Ott (17) found a plateau effect in humans. He found that the dose response curve was a quadratic fit indicating a partial saturation of the enzyme system required for metabolizing methylene chloride.

Findings of this thesis research tend to support Ott's work. The dose response curve in nonsmokers begins to plateau at a COHb of about 6% and a methylene chloride concentration of around 800 mg/m^3. The curve reaches a peak of about 7% COHb and 1800 mg/m^3 of methylene chloride (eight-hour TWA).

Most of the studies conducted on humans to date have been at eight hour TWA concentrations below 800 mg/m^3 (11). Those studies which have used higher concentrations, have also limited exposure time to one or two hours (2, 3). Since the plateau concentration has not been reached in most human studies, it has been assumed that COHb must continue to climb with exposure dose.

Stewart (2) was able to increase COHb levels to 15% after a two hour exposure of 986 ppm methylene chloride. Langehennig (23) found COHb levels of 26% and 40% in two individuals exposed to methylene chloride while stripping furniture in a large basement room with all doors and windows closed. Actual concentrations were not measured. However, concentrations probably were extremely high based on this researchers observations of conditions and methylene chloride concentrations in the businesses surveyed.

It is possible that the high COHb levels found by Stewart resulted from rapid metabolism of methylene chloride by the body's reserve of the enzyme required for metabolism. It is possible that with extended exposure the enzyme reserve could have been expended with COHb levels declining to a plateau level.

Langehennigs findings (23) could be explained in the same way. If concentrations were extremely high as expected, metabolism could have been rapid, resulting in extremely high COHb levels. It is possible that the COHb peak was in the process of deteriorating when the blood samples were drawn six hours later.

The above ideas serve only as a possible explanation to the disparity in COHb levels reported in the literature, not as arguments to support the findings of this research.

As discussed earlier, there was one obvious outlier in the nonsmoking data (Figure 4). The individual was sampled twice and the exposure to methylene chloride was checked a third time to confirm the original findings. Concentrations were found to be slightly

lower (759 eight hour TWA) when resampled. Morning COHb levels found on the two exposure days were 6.0 and 4.9% respectively. COHb on the nonexposure day declined from 4.4% am to 2.2% pm. This indicates that COHb residual was present from the previous days stripping. For this reason, the data for this individual was eliminated from the statistical analyses found in the results section.

At the levels of exposure found in this particular business, the residual COHb found was unusually high. The only explanation offered is a personal susceptibility to the metabolism of methylene chloride and a reduced rate of CO dissociation.

COHb residual is possible in methylene chloride workers. Ott (17) measured residual in workers and found that it was dose dependent. Stewart (2, 3) monitored COHb excretion and found that residual was negligible 20 hours after exposure and completely absent after 24 hours. By using self controls in this study, COHb residual could act as a significant confounder.

Table XII presents the data and appropriate test of significance for a comparison of am COHb levels in nonsmokers regularly exposed to methylene chloride and nonsmokers never exposed to methylene chloride. The outlier discussed above is not included. There is no statistically significant difference in the two sets of data $p > .10$. The average am COHb is actually slightly higher in the group never exposed to methylene chloride. It is assumed that COHb residual in smokers due to methylene chloride exposure is negligible. The assumption is based on exposure levels which were considerably lower for smokers than nonsmokers.

Table XII

Comparison of am COHb Levels in Nonsmokers Exposed to Methylene Chloride and Nonsmokers not Exposed to Methylene Chloride.

Exposed	Nonexposed
0.9	1.6
0.8	1.2
0.9	1.2
1.3	1.3
1.2	0.6
0.6	1.4
2.0	1.6
1.5	
1.0	
$\bar{X}e = 1.13$	$\bar{X}n = 1.27$
$Se = 0.42$	$Sn = 0.34$
$Ne = 9$	$Nn = 7$

$$\text{Pooled } S^2 = \frac{(N_e-1)\,S_e^2 + (N_n-1)\,S_n^2}{(N_e-1) + (N_n-1)}$$

$$\text{Pooled } S^2 = \frac{8(.42)^2 + 6(.34)^2}{14}$$

$$\text{Pooled } S^2 = 0.15$$

$$\text{Pooled } S = 0.39$$

$$t_{N_e + N_n - 2} = \frac{\bar{X}_e - \bar{X}_n}{\sqrt{S^2\left(\frac{1}{N_e} + \frac{1}{N_n}\right)}}$$

$$t_{14} = \frac{1.13 - 1.27}{\sqrt{0.15\left(\frac{1}{9} + \frac{1}{7}\right)}}$$

$t\ 14 = -0.72$ $p > 0.10$: Not statistically significant

Note A: t Test for the Comparison of Two Independent Means.

Note B: This Test does not Consider Subject 3 and 3a Because the Individual is an Obvious Outlier.

Research by Goldbaum et al. (18) and Langehennig (23) infer that COHb is a poor indicator of CO toxicity. This is especially true when CO is produced as a metabolic product. Plasma CO concentrations may be a better indicator of toxicity. Further research into the significance of plasma CO levels is necessary to assess the real CO toxicity of methylene chloride metabolism.

APPENDIX I

STUDY SUBJECT CONSENT FORM AND QUESTIONNAIRE

ROCKY MOUNTAIN CENTER
FOR OCCUPATIONAL
AND ENVIRONMENTAL HEALTH

COMBINED EFFECTS OF METHYLENE CHLORIDE AND SMOKING

CONSENT FOR PARTICIPATION

This is a study to determine if the combination of exposure to methylene chloride and smoking results in a higher level of carbon monoxide in your blood than would be expected by smoking alone.

As part of the study, 10 cc's of blood will be drawn from your arm at the beginning and close of your work shift, and you will be asked to wear a small, lightweight air sampling device, which will enable us to measure the level of exposure to methylene chloride during your work shift.

All information gathered during this study will be used for scientific purposes, including publication. Only numbers will be reported, and individual reports will be kept strictly confidential.

You will receive a letter with your air and blood sample results. We will also inform you of the standards that have been established for air exposures and the normal levels for blood tests. This study will help us understand the combined effects of exposures to methylene chloride and cigarette smoking, and your participation will be extremely helpful.

In the event you sustain physical injury resulting from the research project in which you are participating, the University of Utah will provide you, without charge, emergency and temporary medical treatment not otherwise covered by insurance. Furthermore, if your injuries are caused by negligent acts or omissions of University employees acting in the course and scope of their employment, the University may be liable, subject to limitations prescribed by law, for additional medical costs and other damages you sustain. If you believe that you have suffered a physical injury as a result of participation in this research program, please contact the Office of Research Administration, phone number (801) 581-6903.

Should any questions arise regarding your participation in this study, please feel free to contact Jeffrey Lee, Ph.D., Rocky Mountain Center for Occupational and Environmental Health, Building 512, University of Utah, (801) 581-7107.

I acknowledge that the nature and purpose of the study have been fully explained to me. I acknowledge that I have had an opportunity to ask any questions I have with respect to my participation and that such questions have been answered fully. Furthermore, I understand that I may withdraw from the study at any time.

Signature _____ Date _____

Witness _____ Date _____

METHYLENE CHLORIDE STUDY

QUESTIONNAIRE AND DATA SHEET

Please complete Part A:

A. Name (please print): _____

Age: _____

Sex: Male ___ Female ___

Company Name: _____

Company Address: Street: _____

City: _____

Zip Code: _____

Company Phone Number: area code ( ) _____

Are you a smoker? Yes ___ No ___

If yes, do you smoke cigarettes ___, cigars ___, pipe ___

If cigarettes, how many do you smoke per day? _____ (Note: If you are not sure, please keep track during today's shift.)

What brand of cigarette do you smoke? _____

Are the cigarettes filtered? Yes ___ No ___

B. Employee identification number: _____

Air Sampling Data

Methylene chloride

Sample Number	mg	Air Volume (liters)	Duration	Concentration (mg/M^3)	TWA (mg/M^3)

Carbon monoxide exposure (ppm) _____

Biological samples:

Blood:

Sample Number	Time Drawn	Time Analyzed	%COHb

Breath:

Sample Number	Time Drawn	Time Analyzed	Conc. mg/M^3

Smoking Habit: Intermittent ___ Continuous ___

Comments:

APPENDIX II

AIR SAMPLING RESULTS

Table XIII

Summary of Total Milligrams of Methylene Chloride Found in Organic Vapor Monitors and Charcoal Tubes.

Organic Vapor Monitors			
	Section (mg)		
Field Number	A	B	Total mg
1851	2.62	2.97	5.59
6302	1.83	0.38	2.21
6150	1.18	0.33	1.51
6121	0.41	0.03	0.44
6254	1.44	0.45	1.89
6194	0.50	0.05	0.55
6030	2.39	0.09	2.48
6230	3.14	0.85	3.99
1447	2.75	2.07	4.82
1255	0.96	0.13	1.09
1143	10.61	3.88	14.49
1366	9.25	6.13	15.38
1144	5.24	0.24	5.48
1260	3.99	0.12	4.11
1264	1.95	0.07	2.02
1235	0.14	0.04	0.18
1411	1.62	0.16	1.78
1434	2.47	0.55	3.02
1374	3.16	0.91	4.07
1283	1.24	0.06	1.30
1541	3.88	0.15	4.03
1162	2.89	0.52	3.41
1370	4.64	2.66	7.30
1500	2.03	0.43	2.46
1481	9.87	1.39	11.26
1495	8.33	3.16	11.49
1229	1.66	0.27	1.93

Charcoal Tubes					
	Front Tube Section (mg)		Rear Tube Section (mg)		
Field No	A	B	A	B	Total mg
1	8.38	5.21	15.54	9.35	38.48
2	9.77	5.78	10.54	6.16	32.25
3	3.63	2.51	4.42	1.57	12.13
4	4.81	3.45	7.22	4.11	19.59
5	2.52	2.55	3.92	0.71	9.70
6	6.74	5.02	8.29	4.77	24.82
7	7.20	5.44	9.52	5.60	27.76
8	1.44	1.63	0.21	0.00	3.28
9	2.04	0.00	3.42	2.55	8.01

APPENDIX III

BLOOD COHb DETERIORATION DATA

Table XIV

COHb Deterioration Measured Over Time

Sample No	Date	Time (pm)	% COHb
1	7-11	5:53	7.5
	7-12	6:48	7.2
	7-13	2:52	7.1
	7-15	4:53	7.0
	7-19	6:22	6.6
2	7-12	6:50	12.2
	7-13	2:52	11.9
	7-15	4:58	10.9
	7-19	6:22	10.9
	7-27	6:30	11.0
3	7-12	6:50	6.0
	7-13	2:55	5.3
	7-15	4:55	4.9
	7-19	6:22	3.6
4	7-15	2:15	7.4
	7-19	6:22	6.8
	7-27	6:30	7.1
	8-7	6:27	7.0
5	7-19	6:04	6.0
	7-27	6:15	5.8
	8-7	6:28	5.6
6	7-19	5:57	6.2
	7-27	6:15	5.8
	8-7	6:25	6.0
7	7-19	6:04	9.8
	7-27	6:15	9.6
	8-7	6:32	9.6
8	7-28	6:05	9.8
	8-7	6:24	8.4

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