Applied Occupational and Environmental Hygiene

ISSN: 1047-322X (Print) 1521-0898 (Online) Journal homepage: https://www.tandfonline.com/loi/uaoh20

Evaluation of a New Solid Sorbent Sampler for Alveolar Methylene Chloride Used in Tandem with a Bag for Sampling Alveolar Carbon Monoxide

Robert A. Glaser , James E. Arnold , Charles S. McCammon Jr. & Frederick C. Phipps

To cite this article: Robert A. Glaser , James E. Arnold , Charles S. McCammon Jr. & Frederick C. Phipps (1991) Evaluation of a New Solid Sorbent Sampler for Alveolar Methylene Chloride Used in Tandem with a Bag for Sampling Alveolar Carbon Monoxide, Applied Occupational and Environmental Hygiene, 6:5, 380-388, DOI: 10.1080/1047322X.1991.10387899

To link to this article: https://doi.org/10.1080/1047322X.1991.10387899

Evaluation of a New Solid Sorbent Sampler for Alveolar Methylene Chloride Used in Tandem with a Bag for Sampling Alveolar Carbon Monoxide

Robert A. Glaser,[A] James E. Arnold,[A] Charles S. McCammon, Jr.,[B] and Frederick C. Phipps[A]
National Institute for Occupational Safety and Health, [A]4676 Columbia Parkway, Cincinnati, Ohio 45226;
[B]Denver Regional Office, Denver, Colorado

A method for sampling and analysis of methylene chloride and carbon monoxide in alveolar air is described. The method employs a three-section bed of charcoal cloth sorbent for the determination of methylene chloride concentrations and a bag downstream of the solid sorbent sampler for determination of carbon monoxide concentrations. The method was evaluated in a study to assess the level of exposure to and uptake of methylene chloride by 14 furniture strippers. Alveolar breath samples obtained from these workers were analyzed for methylene chloride and carbon monoxide. Venous blood samples also were obtained from the furniture strippers and analyzed for methylene chloride and carboxyhemoglobin.

All methylene chloride samples were analyzed by gas chromatography with flame ionization detection. Breath carbon monoxide was analyzed using an electrochemical detector (Ecolyzer). Blood carboxyhemoglobin levels were determined with a CO-Oximeter. The accuracy of the breath sampling and analytical procedure was assessed by comparing the alveolar breath methylene chloride and carbon monoxide concentrations to venous blood concentrations of methylene chloride and carboxyhemoglobin, respectively. Linear regression analysis showed that the blood and breath concentrations of methylene chloride were reasonably correlated ($r = 0.87$), with a standard error of estimation of 1.37 mg/L (even including two extreme outliers in the data analysis). An *in vivo* blood:gas partition coefficient of 16.7 for methylene chloride was computed from the blood:breath concentration data (excluding the two extreme outliers). Breath carbon monoxide measurements were not as well correlated with blood carboxyhemoglobin ($r = 0.84$) as historically expected, primarily due to two outlying data points. The standard error of the estimate was also relatively large—1.89 percent carboxyhemoglobin. However, the slope of the linear regression equation relating blood carboxyhemoglobin to breath carbon monoxide was comparable to previously reported results. This study has demonstrated that monitoring of these analytes in alveolar breath may be a useful alternative to monitoring them in blood. Recommendations for improvements to the breath and blood sampling and analytical procedures are also given. **Glaser, R.A.; Arnold, J.E.; McCammon, Jr., C.S.; Phipps, F.C.: Evaluation of A New Solid Sorbent Sampler for Alveolar Methylene Chloride Used in Tandem with a Bag for Sampling Alveolar Carbon Monoxide. Appl. Occup. Environ. Hyg. 6:380–388; 1991.**

Introduction

Methylene chloride is the solvent of choice for most furniture-stripping applications. Currently, there are no organic vapor canisters available for respirators to adequately protect the worker against inhalation of the solvent.[1] Since methylene chloride may be applied manually during furniture stripping, there is also considerable opportunity for dermal absorption. Thus, in the absence of adequate exposure control technology, workers engaged in furniture stripping may be at risk to absorb large amounts of methylene chloride.

The most serious effect of acute exposure to methylene chloride is the increased risk of cardiovascular stress due to elevation of blood carboxyhemoglobin levels by the rapid metabolism of the solvent to carbon monoxide.[2] The effects of chronic exposure to methylene chloride are not completely established; however, the National Institute for Occupational Safety and Health (NIOSH)[3] has classified the compound as a potential occupational carcinogen. More recently, concern has been raised that methylene chloride may also be a human reproductive toxin.[4]

In order to further investigate the reproductive toxicity of methylene chloride, NIOSH researchers conducted an industrial hygiene survey of 14 male workers engaged in stripping furniture with the solvent. The study included:

Mention of commercial names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

1) testing of these workers' reproductive function, 2) environmental monitoring of the workers' breathing zones for methylene chloride and an evaluation of the technology in place to control exposure, and 3) postshift analysis of workers' blood for methylene chloride and percent carboxyhemoglobin and pre- and postshift monitoring of alveolar breath for methylene chloride and carbon monoxide. Postshift biomonitoring of these workers was conducted to estimate the maximum body burden of methylene chloride and its toxic metabolite, carbon monoxide. Environmental monitoring cannot estimate the endogenous production of carbon monoxide and ignores differences in solvent uptake due to worker ergonomic differences and absorption via dermal routes.

The reproductive function test results have been reported previously.[5] The results of the industrial hygiene survey, including a comparison of the environmental concentrations of methylene chloride to the concentrations of the biomonitored analytes, are reported in a companion piece immediately preceding this article in this journal.[6]

This article presents evaluations of the solid sorbent sampling and analytical procedure used to determine alveolar methylene chloride and the bag sampling and analytical technique used to determine alveolar carbon monoxide. These evaluations included: 1) studies of the recovery of methylene chloride from the charcoal cloth solid sorbent and 2) a comparison by linear regression of the postexposure blood concentrations of methylene chloride and carboxyhemoglobin, respectively, to alveolar concentrations of methylene chloride and carbon monoxide. An *in vivo* blood:gas partition coefficient for methylene chloride was also computed from this regression analysis. The parameters of the equation determined by linear least squares regression of the carbon monoxide:carboxyhemoglobin data have been compared to those previously reported by other researchers. This extensive analysis of the blood and breath data was made to assess the quality of that data and to demonstrate that noninvasive biomonitoring of solvents in exhaled breath was a useful alternative to invasive biomonitoring of those solvents in blood.

Experimental

Solvents and Sorbent

For the analyses of methylene chloride-in-breath samples, distilled-in-glass grade carbon disulfide and methylene chloride were obtained from MCB Chemicals, Norwood, Ohio. Activated charcoal cloth was obtained from Charcoal Cloth Ltd., Berkshire, England. The cloth was cut into 45-mm diameter wafers and purged of volatile impurities at 250°C with nitrogen, as described previously.[7] For the analysis of methylene chloride-in-blood samples, methylene chloride (HPLC-GC-MS grade) and sodium chloride were obtained from Fisher Chemical Co., Pittsburgh, Pennsylvania. Pesticide-quality tetrahydrofuran was obtained from Burdick and Jackson Co., Muskegon, Michigan.

Evaluation of the Charcoal Cloth

Recovery of Methylene Chloride

The recovery of low levels of methylene chloride from the charcoal cloth was investigated. Each sample was three wafers of the charcoal cloth, spiked with 4 or 8 μg of methylene chloride. All samples were spiked in 10-ml, crimp-cap vials and sealed. The samples were stored at room temperature for periods lasting from 1 to 7 days. The samples were analyzed using the procedures described below.

Migration Studies

Migration of methylene chloride between sorbent sections was investigated. Each sorbent section was comprised of three wafers of charcoal cloth. In separate experiments,

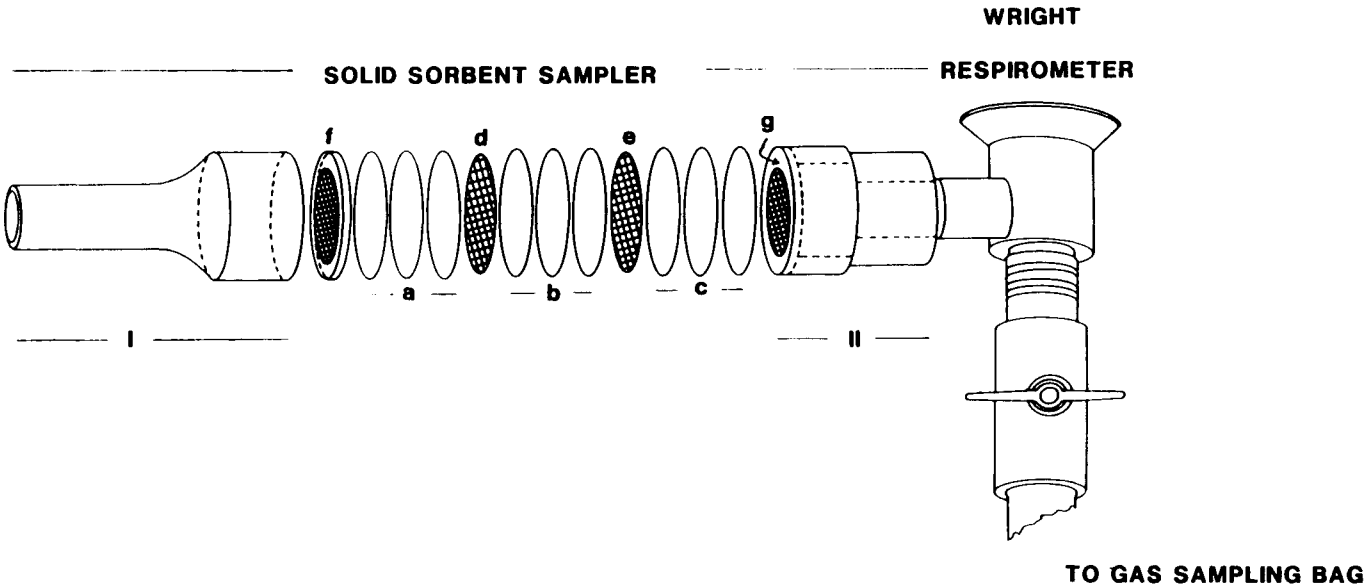


FIGURE 1. System for sampling alveolar breath for methylene chloride and carbon monoxide. See text for description of individual sampler components.

20, 200, or 2000 μg of methylene chloride were metered onto the front sorbent section which had been placed in the bottom of a 60-ml ointment jar. A stainless steel screen was placed over the front section, followed by the middle section, a stainless steel screen, then the back section, and another screen. The sorbent bed was compressed by 44 g of steel washers. After storage of the samples for 2 to 20 hours at ambient temperatures or on prefrozen refrigerant (Blue Ice), the sorbent beds were removed from the ointment jar, placed in 10-ml sample vials, and analyzed using the procedures described below.

Breath Sampling

Apparatus

An exploded view of the assembly used for sampling breath is shown in Figure 1. Methylene chloride was sampled using the solid sorbent sampler which was loaded with three sorbent sections (a, b, and c). Three 45-mm diameter charcoal cloth wafers were used per sorbent section. The sorbent sections were separated by 45-mm diameter stainless steel screens (d and e). The sampler was directly connected to a Wright respirometer for measurement of sample volumes. Carbon monoxide was collected in an aluminized polyester bag placed downstream of the respirometer. A ball valve, located between the respirometer and the bag, was used to seal the bag prior to and after sampling. The solid sorbent sampler was sealed with stoppers before and after use.

The solid sorbent sampler was fabricated as two components: one of glass (I), and the other of Teflon® (II). The glass part served as a sorbent canister and mouthpiece. The Teflon part served as an insert to compress and retain the sorbent bed in place and to provide a connection to the respirometer. The 10-cm long glass part was tapered from the 4.5-cm i.d. by 2.4-cm deep sample chamber to the 5.8-cm long by 2.5-cm o.d. mouthpiece. A 4.0-cm diameter Teflon grating (Savillex Inc, Minnetonka, Minnesota) was press-fit into the 4.0-cm i.d. by 0.5-mm deep by 0.5-mm wide lip of a specially machined, 4.5-cm o.d., Teflon sealing ring to form a front support (f) for the sorbent bed.

The Teflon retainer insert (II) was a 6.4-cm long by 4.5-cm o.d. thick-walled tube. At the exit end, the part was machined with a tapered inside wall (approximately 22 mm in diameter) to fit onto the respirometer. At the entrance end, a 4.0-cm o.d. by 0.5-mm deep by 0.5-mm wide lip was machined along the internal diameter. Another 4.0-cm o.d. Teflon grating (g) was press-fit into this lip to form the rear sorbent bed support. From the base of the lip, a 3.6-cm i.d. cavity was machined along the internal diameter of the Teflon retainer insert for 1.5 cm.

Technique

Both pre- and postshift breath samples were collected. All breath samples were collected outside the furniture stripping shops using a 30-second breath-holding procedure similar to that of Jones *et al.*[8] Each subject was instructed to hyperventilate several times and then to hold his breath for 30 seconds. When the subject exhaled, the first portion (approximately 50%) of the held-breath sample was discarded. The subject then cupped his mouth onto the glass end of the sampler and exhaled the end portion of the sample. The mean volumes of the pre- and postshift samples obtained from the 14 furniture strippers were 2.32 L (relative standard deviation, $S_r = 0.47$) and 2.28 L ($S_r = 0.47$), respectively. At least one methylene chloride field blank sample was obtained at each of the five sampling sites. The blank samplers were opened, exposed to the ambient air, and treated in the same manner as the other samplers except that no breath was sampled with them.

Following sampling, the aluminized polyester bag was immediately sealed by closing the ball valve. The solid sorbent sampler was removed from the respirometer, sealed with stoppers, and placed in a refrigerated, insulated container. The samples were transported back to the laboratory within 2 hours of collection. Upon arrival at the laboratory, the solid sorbent sampler was disassembled, and each of the sorbent sections was placed into separate, 10-ml, crimp-capped containers. Generally, the methylene chloride samples were analyzed the same day as collected. Otherwise, the separated sorbent sections were refrigerated at 4°C; all of the methylene chloride samples were analyzed within 72 hours of collection. All carbon monoxide analyses were completed on the day of collection.

Blood Sampling

Separate blood samples were obtained for methylene chloride and carboxyhemoglobin analyses. For each sample, approximately 10 ml of venous blood were collected in a 20-ml vacutainer from the antecubital vein of each subject. The carboxyhemoglobin samples were preserved with ethylene diamine tetraacetic acid (EDTA). The methylene chloride samples were preserved with heparin. The blood samples were refrigerated on prefrozen refrigerant (Blue Ice) in a portable cooler and transported to the laboratory within 24 hours of collection. The samples were immediately refrigerated and analyzed within 24–48 hours of collection.

Analytical Methods

Breath Methylene Chloride

All methylene chloride samples were analyzed using a Hewlett–Packard Model 5730A gas chromatograph (Palo Alto, California), operated in the flame ionization detection mode. Each three-wafer, charcoal cloth sampling section was desorbed with 4 ml of carbon disulfide in a 10-ml, crimp-capped vial. A 2-μL aliquot was resolved on a 4-mm i.d. by 6.1-m long, 10 percent FFAP on 80/100-mesh Chromosorb, WAW column. The carrier gas (N_2) flow was 27 ml/min. The column temperature was 90°C; the injector, 150°C; the detector, 250°C. All peak areas were measured using a Hewlett–Packard 3357 laboratory data system interfaced

to the gas chromatograph. Calibration curves ranging in concentration from 0.331 to 13.22 μg/ml were used to analyze all samples containing less than 12 μg; all other samples were analyzed using calibration curves ranging in concentration from 0.331 to 130 μg/ml. Limits of detection (LOD), determined using procedures outlined,[9] ranged from 0.7 to 5.4 μg of methylene chloride per three-wafer section of charcoal cloth. Correlation coefficients for all calibration curves ranged from 0.9947 to 0.9999.

The computation of the concentration of methylene chloride was made using the corrected volume of breath (V_{corr}).

$$V_{corr} = V_{exhaled} - V_{void}$$

where: $V_{exhaled}$ = the volume of the breath measured by the Wright respirometer
V_{void} = the void volume of each sampler

The void volume of each sampler was approximately 75 ml. All breath volumes were corrected to breath-temperature-pressure-saturated (BTPS) conditions of 37°C, 760 torr, and 100 percent relative humidity.

Breath Carbon Monoxide

The bag samples were analyzed for carbon monoxide using an Ecolyzer (Energetic Sciences, Hipster Model 2000), which had been calibrated using compressed gas standards containing 20 or 50 ppm carbon monoxide in air. All carbon monoxide concentrations in the bags were corrected for the void volume of the sampling device by multiplying the concentration of carbon monoxide in the bag by a factor corresponding to:

$$\frac{V_{sampled}}{V_{sampled} - V_{void\ of\ assembly}}$$

The void volume of each sampling assembly was approximately 122 ml. All breath concentrations were computed assuming atmospheric-temperature-pressure-saturated (ATPS) conditions of 25°C, 760 torr, and 100 percent relative humidity.

Blood Methylene Chloride

All blood methylene chloride measurements were made using a Hewlett–Packard 5890 gas chromatograph equipped with a 3-m long by 4-mm i.d., 10 percent Carbowax 20M on 60/80-mesh Chromosorb, WAW column and operated in the flame ionization mode. The column temperature was 60°C. The detector and injector were maintained at 225°C and 150°C, respectively. The flow of the carrier gas (N_2) was 15 ml/min. Peak areas were determined using a Hewlett–Packard Model 3390A integrator. Samples were prepared by adding 1 g of sodium chloride, 2 ml of physiological saline solution, and 1 ml of an internal standard solution containing 0.02 mg/ml tetrahydrofuran (in physiological saline) to a 1.0-ml aliquot of heparinized whole blood contained in a 10-ml septum vial. The vial was equilibrated for 1.5–2.0 hours at 55°C in a Dani Automatic Headspace Sampling Unit that was interfaced to the gas chromatograph. A 1.0-ml aliquot of the headspace above the blood was analyzed. A standard curve ranging from 0.1 to 30 μg/ml methylene chloride in water was used to calibrate the gas chromatograph. These standard samples were prepared by adding 1 g of sodium chloride, 2 ml of physiological saline, and 1 ml of the internal standard solution to 1 ml of water contained in a 10-ml septum vial. The limit of detection was 0.1 μg/ml.

Blood Carboxyhemoglobin

All carboxyhemoglobin analyses were performed with a model 282 CO-Oximeter (Instrumentation Laboratories, Lexington, Massachusetts) by a contract laboratory (Metpath Inc., Teterboro, New Jersey).

Results and Discussion

Methylene Chloride—Method Evaluation

Blood

The overall accuracy and precision of the blood sampling and analytical method was not evaluated. However, for replicate analyses of a standard of whole blood containing 1 μg/ml of methylene chloride, the mean percent recovery was 110 percent (relative standard deviation, S_r = 0.29, n = 10).

Breath

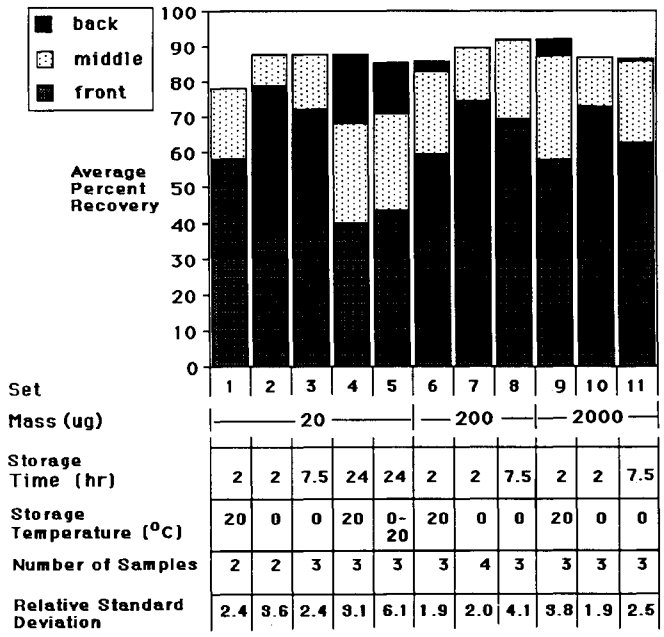
Recovery from the Charcoal Cloth

The percent recoveries of methylene chloride spiked at the 4-μg per sample level and stored for 1 and 7 days at room temperature were 92.7 percent (S_r = 0.036) and 93.6 percent (S_r = 0.048), respectively. The percent recoveries of methylene chloride spiked at the 8-μg per sample level and stored for 1 and 7 days at room temperature were 95.4 percent (S_r = 0.027) and 92.8 percent (S_r = 0.018), respectively. These results demonstrated that trace amounts of methylene chloride such as found in preshift breath samples could be stored on charcoal cloth and quantitatively recovered after storage for 7 days at room temperature.

These recovery experiments were conducted by spiking the analyte onto three wafers of the charcoal cloth and storing the spiked wafers in 10-ml, void-volume vials. When 60-ml, void-volume, ointment jars were used for desorbing 8.7 μg of methylene chloride from a three-wafer sorbent section, the mean percent recovery of methylene chloride after a 1-day storage at room temperature was 70.3 percent (S_r = 0.067, n = 3). This relatively low recovery presumably was due to the volatilization of the analyte into the void volume of the desorbing vessel. For that reason, all methylene chloride field samples were desorbed in vessels of the lowest feasible void volume (10 ml).

Migration of Methylene Chloride Between Sorbent Sections

Figure 2 presents the results of the experiments conducted to determine the extent of methylene chloride migration between sorbent sections during storage. These



Set	1	2	3	4	5	6	7	8	9	10	11
Mass (ug)	20					200			2000		
Storage Time (hr)	2	2	7.5	24	24	2	2	7.5	2	2	7.5
Storage Temperature (°C)	20	0	0	20	0-20	20	0	0	20	0	0
Number of Samples	2	2	3	3	3	3	4	3	3	3	3
Relative Standard Deviation	2.4	3.6	2.4	3.1	6.1	1.9	2.0	4.1	3.8	1.9	2.5

FIGURE 2. Recoveries of 20–2000 μg of methylene chloride from each stage of a three-stage charcoal cloth sorbent bed following storage for 2–24 hours at 0–20°C.

experiments were conducted using 60-ml, void-volume, ointment jars (approximately the same void volume as that of the sampler). The effects of storage time and temperature were investigated. The masses stored, 20–2000 μg, covered the range of masses found in the breath samples. For any spiked sample stored under refrigeration or at ambient temperatures, for periods up to 7.5 hours, a maximum of 4.6 percent of the methylene chloride had migrated to the back section. Sample sets 4 and 5 simulated situations that are likely to be encountered during transportation and storage of field samples, namely storage for

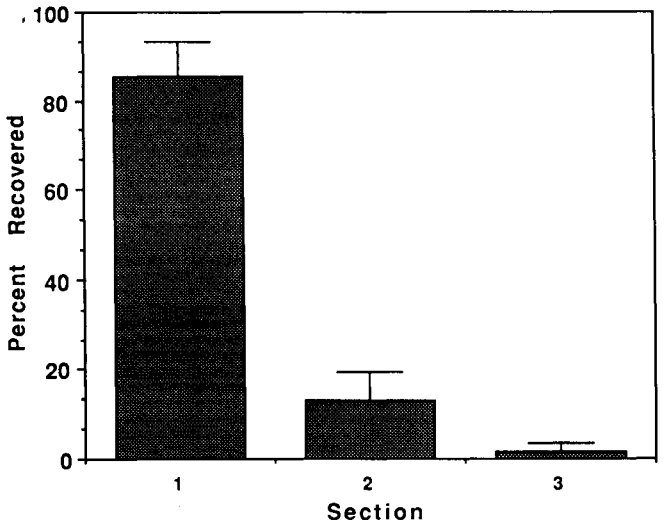


FIGURE 3. Percent of total mass of methylene chloride in postshift samples recovered by each section of the three-section solid sorbent sampler. The standard deviations of the percent of the total mass collected on each section are bracketed.

24 hours at ambient temperatures (set 4) or on prefrozen refrigerant, maintained in a cooler that had warmed to approximately 20°C after storage for 24 hours (set 5). Note that the percent of the total mass migrating to the back section was similar for both sample sets. For the 2-hour storage period under refrigeration, on average, 88 percent of the mass spiked was recovered regardless of the loading level. For this reason, the masses of methylene chloride collected by breath sampling were corrected for a recovery coefficient of 0.88. The precision estimates of replicate analyses after 2 hours' storage (sample sets 2, 6, and 9) were pooled to obtain an overall analytical method precision estimate of 3 percent relative standard deviation.

The degree of migration observed here is likely to be the most serious for a high vapor pressure solvent such as methylene chloride. The authors have conducted similar tests using charcoal cloth sorbent for storage of trace levels of less volatile compounds such as 1,1,1-trichloroethane and found no serious problems with migration, even after 7 days' storage at room temperature.

Breath Methylene Chloride Measurements

The three-section, solid sorbent sampler shown in Figure 1 was used in order to permit a wide range of breath methylene chloride concentrations to be measured from the furniture strippers. A backup section was required in order to assure that no methylene chloride was lost by breakthrough of the sampler. For very low challenge concentrations, as would be found in preshift samples, the first section was to be the primary absorbing bed and the second section, the backup bed. For very high challenge concentrations such as found in postshift samples, the combination of the first and second sections would be the primary bed and the third section, the backup bed.

Of the 14 preshift samples obtained from the furniture strippers, measurable levels of methylene chloride were observed only in samples obtained from subjects 1, 4, 5, 7, 11, and 12. The concentrations of these samples ranged from 1.4 to 10.3 μg methylene chloride per liter of exhaled air. In three of these samples, methylene chloride was found on the second and third sections of the sampler bed. The average amounts of the total mass sampled were 11.2 percent (standard deviation = 38.7%, n = 2), and 5.0 percent (n = 1) for the second and third sections, respectively. Thus, significant loss of analyte was not observed. No measurable methylene chloride was found in the field blanks obtained during preshift sampling.

Figure 3 is a bar graph of the mean percent of the total mass recovered from each of the three sorbent sections of the 14 postshift samples. Although the challenge concentrations in the breath ranged from 7.3 to 540 μg methylene chloride per liter, the distribution of mass collected on each section was similar for each sample. At least 98 percent of the collected mass was trapped by the first and second sections of the sampler. Therefore, sample loss due to breakthrough of the backup sections was not significant in any of the samples. Of the five sites monitored, measurable levels of methylene chloride were only found

in the field blanks obtained during postshift sampling of subjects 8, 9, and 10. The postshift breath methylene chloride concentrations of these subjects were corrected for an average methylene chloride field blank corresponding to 4.7 μg/L.

Comparison of Methylene Chloride Blood and Breath Concentration Data

Figure 4 is a plot comparing the concentrations of methylene chloride in the breath and blood samples obtained from all 14 subjects. The equation of the line regressed through the available data points is

$$C_{Blood} = 13.5\ C_{Breath} + 1.13 \quad (1)$$

with a linear correlation coefficient (r) of 0.87 and a standard error of 1.37 mg/L; the concentration units for both blood and breath are mg/L. For subjects 1–12, the blood-to-breath concentration ratios ranged from 5.03 to 68.3 (mean = 24.5, S_r = 0.70). For subjects 13 and 14, the ratios were 461 and 209, respectively; these two data points have been determined to be gross statistical outliers using a normalized residuals t-test (p = 0.05). The reasons for the gross disparity in the blood-to-breath ratios for these two subjects are unclear.

Estimation of a Methylene Chloride Blood:Gas Partition Coefficient

In Figure 4, the concentration units for methylene chloride are mg/L for blood and mg/L for breath (corrected to BTPS conditions). The slope of a line regressed through the origin of such a plot should correspond to the blood:gas partition coefficient of methylene chloride. Regression of the available blood:breath data, including the two outliers, gave an intercept which was significantly (p = 0.05) greater than zero. However, regression of the data obtained from subjects 1–12 gave an intercept which was not significantly different from zero. Thus, the blood:gas partition coefficient may be estimated by deleting the two outliers and forcing the data obtained from subjects 1–12 through the origin to generate the following equation:

$$C_{Blood}\ [mg/L] = 16.7\ C_{Breath}\ [mg/L] \quad (2)$$

which has a linear correlation coefficient of 0.989 and a standard error of 0.81 mg/L.

Thus, 16.7 is an estimate of the *in vivo* methylene chloride blood:gas partition coefficient and may be compared to the reported *in vitro* blood:gas partition coefficient of 9.7.[10] Such comparisons should be made cautiously. For one thing, the ratio obtained in the present study is based on a very limited amount of data. Furthermore, the blood methylene chloride measurements reflect the concentration in venous and not in arterial blood. For determination of a blood:gas partition coefficient, Andersen recommends that arterial blood concentrations be used since the venous concentration will be reduced by metabolism or absorption into tissues.[11] In addition, in the current study, the blood and breath were only approximately sampled simultaneously. Sampling logistics required that all breath sampling be done outside the furniture stripping shops but just after completion of blood sampling. Although the interval between blood and breath sampling was relatively short, the subjects continued to eliminate methylene chloride via exhalation. Thus, the breath levels were likely lower than had they been obtained simultaneously with the blood samples. A better estimate of the blood:gas partition coefficient would have been obtained if alternate sampling of blood and breath had been feasible.

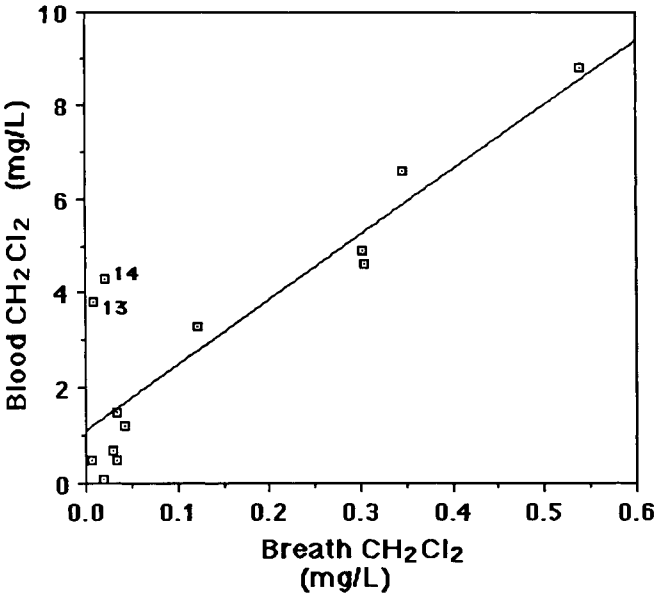


FIGURE 4. Comparison of blood and breath concentrations of methylene chloride. The data points obtained from subjects 13 and 14 have been identified because they represent statistical outliers. See Equation 1 in the text for the regression parameters.

Carbon Monoxide—Method Evaluation

Blood

No formal evaluation of the precision and accuracy of the method used for determination of percent carboxyhemoglobin in the blood was carried out during this study. An overall analytical method accuracy of ± 1 percent and a precision of replicate analysis of standards (Sr) of 0.05 is claimed for the carboxyhemoglobin analytical procedure.[12] It is unknown if the overall sampling and analytical method accuracy and precision have been determined.

Breath

Prior to conducting the field study, tests were conducted to assure that carbon monoxide was stable during storage in the aluminized Mylar bag and was not absorbed by the charcoal cloth. A 20-ppm standard of carbon monoxide was metered into the bag with the ball valve attached and periodically analyzed with an Ecolyzer. No significant decrease in the carbon monoxide concentration was observed for storage periods up to 69 hours. Approximately 1–2 L of a 50-ppm carbon monoxide standard were passed through the sampler at 5 L/min into a gas sample bag both

TABLE I. Summary of Parameters Reported or Computed from Previous Studies for the Linear Regression Equation, %HbCO = M × C_{co}[A] + I, Relating Blood Carboxyhemoglobin (%HbCO) to Alveolar Carbon Monoxide, C_{co}

Ref.	Author (date)	Approximate Range		M	I	r[B]	SE[C]
		C_{co} (ppm)	%HbCO				
13	Haldane (1897)	—[D]	—[D]	0.16[E]	—[D]	—[D]	—[D]
7	Jones (1958)[F]	≤ 100	≤ 17	0.17[F]	0.98[F]	—[D]	—[D]
15	Ringold (1962)[F]	≤ 100	≤ 20	0.20[F]	0.44[F]	0.99[F]	0.59[F]
16	Stewart (1976)	8–50	≤ 8	0.23[F]	0.17[F]	0.99[F]	0.50
17	Ramsey (1967)[F]	≤ 100	≤ 18	0.18[F]	0.24[F]	0.98[F]	1.02[F]
18	Cohen (1970)	≤ 70	≤ 12	0.17	0.43	0.99	—[D]
19	Wald (1981)	≤ 65	≤ 12	0.18	−0.14	0.97	—[D]
20	Hwang (1984)	≤ 100	≤ 30	0.31	4.1	0.83	4.0
21	Tsukamoto (1985)	—[D]	—[D]	0.13	0.0	—[D]	—[D]
22	Peterson (1970)[G]	≤ 250	≤ 30+	0.15	1.35	0.98	1.2[F]
23	Jarvis (1980)[G]	≤ 100	≤ 15	0.18	−0.28	0.98	0.76

[A]M and I are the slope and the intercept of the regression equations, respectively.
[B]r = linear correlation coefficient.
[C]SE = standard error of the estimate in units of %COHb.
[D]The parameter was not reported or could not be estimated from the original data, or was not applicable (reference 13 only).
[E]The Haldane equation: (M) $[pCO/pO_2]$ = $\%HbCO/\%HbO_2$, relates blood carboxyhemoglobin (%HbCO) and blood oxyhemoglobin ($\%HbO_2$) and the partial pressures of carbon monoxide (pCO) and oxygen (pO_2) in the lung; M is an affinity constant of carbon monoxide for hemoglobin. The equation was solved for %HbCO as a function of C_{co} (in ppm) by assuming M = 210, P_{O_2} = 95 torr, and $\%HbO_2$ = 97.[24]
[F]Unless otherwise noted, the parameters have been estimated from authors' original data. For reference 17, only SE was reported. For reference 23, only SE was estimated. For references 16 and 18, all parameters were computed using the original reported data.
[G]References 22 and 23 also reported the following quadratic equations:
$\%HbCO = (109.08 + 7.6C_{co})^{1/2} - 11.89$; r = 0.99; SE = 1.06; range: $C_{co} \leq 250$ ppm, $\%HbCO \leq 33$.[22]
$\%HbCO = 0.222(C_{co}) - 0.0006(C_{co})^2 - 1.63 + 0.018$ age; range: $C_{co} \leq 100$ ppm; $\%HbCO \leq 15$.[23]

with and without the sorbent in place. With no sorbent in the sampler, the concentration of the bag read 49 ppm (S_r = 0.12, n = 2). With the sorbent bed in the sampler, the bag concentration was 48.5 ppm (S_r = 0.06, n = 4). The differences in concentration were not significant (t-test, p = 0.05). These data also provide an estimate that the precision of replicate determination of carbon monoxide was approximately 0.12. Confirmation that the Ecolyzer was unaffected by high humidity was obtained by determining that the concentration of a dry 24-ppm carbon monoxide standard did not significantly change after being humidified to 80 percent relative humidity and stored for 1 hour at ambient temperatures.

Comparison of Blood and Breath Data

Table I is a compilation of the parameters of representative regression equations reported in the literature relating the concentration of alveolar breath carbon monoxide (C_{co}) to blood percent carboxyhemoglobin (%COHb). Although some of these parameters were reported by the authors, some have been recomputed or estimated from the original data. Over the range of concentrations encountered in this study, i.e., below 100 ppm carbon monoxide (below approximately 20 %COHb), a linear equation is satisfactory for estimating blood carboxyhemoglobin from breath carbon monoxide concentrations. Our data on the concentrations of carbon monoxide in the breath and carboxyhemoglobin in the blood are plotted in Figure 5. Note that we have continued to employ the convention of using ATPS conditions to express the concentration of carbon monoxide. The equation of the linear least squares line regressed through the data is

$$\%COHb = 0.15\ C_{co} + 1.31\% \quad (3)$$

The slope of this regression line was comparable to that expected on the basis of the theoretical Haldane relationship[13] and to the empirical linear equations reported by others in Table I. However, the blood:breath data obtained from this study was not as well correlated (r = 0.84), the

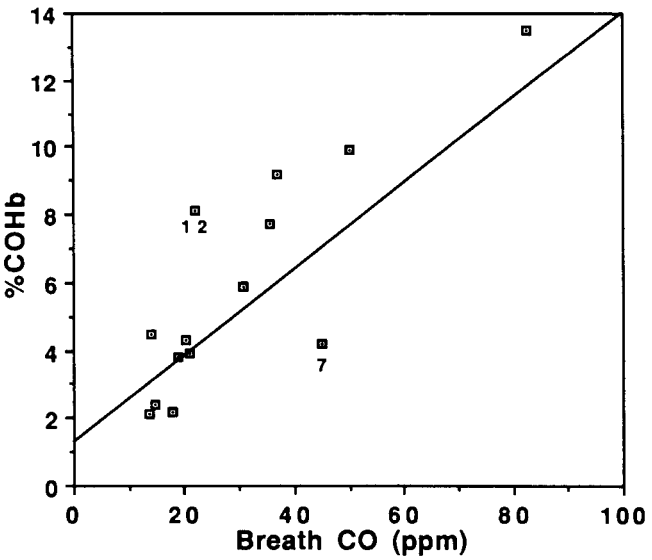


FIGURE 5. Comparison of concentrations of breath carbon monoxide and blood concentrations of carboxyhemoglobin. Data from all 14 subjects have been used for this plot; however, the data from subjects 7 and 12 are outliers. The line is defined by Equation 3 in the text.

intercept of the regression equation was higher, and the standard error of the estimate (1.89 %COHb) was larger than expected historically (reference 20 excepted). The data points from subjects 7 and 12 were statistical outliers using a normalized residuals t-test. If these two points were excluded, the slope and intercept became 0.17 and 0.76, respectively, the blood:breath linear correlation coefficient (r) increased to 0.95 and the standard error decreased to 1.2 %COHb. However, there was no empirical reason to exclude these points from the data analysis.

The authors do not believe the problems with outlying data were due to the breath sampling method. Subjects were closely observed to assure proper delivery of each sample. In addition, the method evaluation experiments described above indicated that measurement of exhaled carbon monoxide should have been unaffected by the solid sorbent sampler. A major source of error was likely the electrochemical detector used for analysis of the breath samples. The instrument was an older model with an electrochemical cell that occasionally responded slowly when breath samples were analyzed. Interference from methylene chloride should not have affected the breath carbon monoxide measurements since there was no evidence of significant breakthrough of the analyte from the back section of the charcoal cloth into the collection bag for any sample. It was known that ethyl alcohol in the breath affected the response of this instrument; however, there was no evidence that any of the subjects consumed ethyl alcohol while at work. In addition, it was likely that any alcohols would have been trapped on the solid sorbent.

The accuracy and precision with which blood carboxyhemoglobin levels were determined in this study may also have been affected by the duration of sample storage. In one study, carboxyhemoglobin levels of refrigerated, EDTA-stabilized blood samples decreased by 6 to 11 percent after storage for 3 days; the carboxyhemoglobin levels were measured by the CO-Oximeter.[14] In the present study, some of the blood samples may have been stored for periods up to 48 hours prior to analysis. Thus, the blood carboxyhemoglobin concentrations for those samples may have been lower than predicted by breath sampling, reducing the correlation between the blood:breath data and increasing the standard error.

Conclusions

Experiments described in this article indicate that a three-section, charcoal cloth sorbent device for sampling alveolar methylene chloride in tandem with a bag for sampling alveolar carbon monoxide is a useful alternative to sampling venous blood for methylene chloride and carboxyhemoglobin.

Postshift alveolar breath concentrations of methylene chloride and carbon monoxide obtained from 14 furniture strippers using the tandem sampler were reasonably correlated with postshift venous blood concentrations of methylene chloride and carboxyhemoglobin, respectively. An *in vivo* methylene chloride blood:gas partition coefficient of 16.7 was higher than the reported *in vitro* value of 9.7. However, this difference was in the direction expected since breath concentrations may have been lower than predicted due to the fact that postshift breath was always sampled after blood.

The slope of the line determined by linear regression of the breath carbon monoxide and blood carboxyhemoglobin data was comparable to that estimated from the Haldane equation or those reported in the literature. However, these blood:breath measurements were not as well correlated, the standard error for estimating blood carboxyhemoglobin from breath carbon monoxide was higher, and the intercept of the regression line was larger than expected based on historical data. Exclusion of two outliers from the data analysis substantially improved the blood:breath correlation and reduced the standard analytical error. A major source of error was the electrochemical detector used for determination of breath carbon monoxide.

The solid sorbent sampler had adequate capacity for postshift breath methylene chloride concentrations ranging from 7 to 540 μg/L. Trace levels of methylene chloride corresponding to 1.4 to 18 μg/L were observed in preshift samples. Recovery of the analyte from the sorbent was quantitative for spiked samples prepared over the range of 4 to 2000 μg per sample. However, migration between sorbent sections was significant even during short-term storage at ambient temperatures. Migration was reduced by refrigerating the samples immediately after collection. Tests showed that carbon monoxide was not absorbed by the charcoal cloth and was stable for extended periods in the bag.

Recommendations

The results of this study indicate that the new solid sorbent breath sampling technique for methylene chloride used in tandem with a bag technique for carbon monoxide was a useful alternative to sampling the corresponding analytes in blood. Although the details of the solid sorbent sampling and analytical procedures are presented in the Experimental section of this article, it should be noted that the device is not commercially available. A new sampler, fabricated from metal to avoid breakage of the glass, is being developed.

The solid sorbent technique permits quantitation of the high concentrations of methylene chloride expected in immediate postshift samples. Based upon limit of detection data, it is estimated that this sampling approach can measure concentrations at least as low as 1.4 ppm in the breath. Thus, the trace levels expected in preshift or 16-hour postshift samples may be quantitated. The analyte migration problem encountered with the technique is minimized by refrigeration of the sampler during storage. The above-described metal sampler should reduce migration even further.

Electrochemical detectors such as used in this study are widely employed for measurement of exhaled carbon

monoxide. With the exception of two outlying data points, the device provided satisfactory results. The outlying data were likely caused by slow instrumental response observed during the measurement of breath carbon monoxide. Such data suggest the necessity for rigorous procedures to assure that the electrochemical cell is functioning properly. In addition to verifying the stability and repeatability of the instrument at the time of calibration, a complete performance check should include a determination that the response time for a calibration gas is comparable to that specified by the manufacturer. If that specification is not met, the cell should be replaced. Periodic checks of the concentrations of standards and samples with an alternative technique, such as with an infrared analyzer, will further assure the user of the quality of the analytical data obtained by such techniques.

References

1. The Bureau of National Affairs, Inc.: Appendix A. In: Guidelines for Controlling Exposure to Methylene Chloride, pp. 21:8765-8778. OSHA Instruction Publication 8-1.2A. BNA, Washington, DC (August 11, 1986).
2. Kubic, V.L.; Anders, M.W.; Engel, R.R.; et al.: Metabolism of Dihalomethanes to Carbon Monoxide, In-Vivo Studies. Drug Metab. and Disp. 2(1):53-57 (1974).
3. National Toxicology Program: Toxicology and Carcinogenesis Studies of Dichloromethane (Methylene Chloride) (CAS No. 75-09-2) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). Technical Report Series No. 306. DHHS (NTP) Pub. No. 85-024. NTP, Research Triangle Park, NC (February 1985).
4. Kelly, M.: Case Reports of Individuals with Oligospermia and Hydrocarbon Exposures, Primarily Methylene Chloride. Society of Occupational and Environmental Health, Washington, DC (April 28, 1986).
5. Wells, V.E.; Schrader, S.M.; McCammon, C.S.; et al.: Letter to the Editor. Repro. Toxicol. 3:1–2 (1989).
6. McCammon, C.S.; Glaser, R.; Wells, V.E.; et al.: Environmental and Biological Monitoring of Furniture Strippers To Determine Exposure to Methylene Chloride. Appl. Occup. Environ. Hyg. 6:000–000 (1991).
7. Glaser, R.A.; Arnold, J.E.: Investigation of Charcoal Cloth as a Sorbent for Integrated Sampling of Solvent Vapors in Mixed Expired Breath Using a New Stainless Steel Sampler. Am. Ind. Hyg. Assoc. J. 50:112–121 (1989).
8. Jones, R.H.; Ellicot, M.F.; Cadigan, J.B.; Gaensler, E.A.: The Relationship Between Alveolar and Blood Carbon Monoxide Concentrations During Breathholding. J. Lab. Clin. Med. 51:553–564 (1958).
9. Hubaux, A.; Vos, G.: Decision and Detection Limits for Linear Calibration Curves. Anal. Chem. 42(8):849–855 (1970).
10. Sato, A.; Nakajima, T.: A Structure Activity Relationship of Some Chlorinated Hydrocarbons. Arch. Environ. Health 34:69–75 (1979).
11. Andersen, M.; Clewell, H.J.; Gargas, M.L.; et al.: Physiologically Based Pharmacokinetics and the Risk Assessment Process for Methylene Chloride. Toxicol. Appl Pharmacol. 87:185–195 (1987).
12. Brown, L.J.: Specifications for IL 282 CO-Oximeter, p. 10–1. Instrumentation Laboratories, Lexington, MA (March 1985).
13. Haldane, J.; Smith, J.L.: The Absorption of Oxygen by the Lungs. J. Appl. Physiol. (London) 22:231 (1897).
14. Goldstein, G.M.; Raggio, L.; House, D.: Factors Influencing Carboxyhemoglobin Stability. Technical Report 1811. U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Dietrick, Frederick, MD (March, 1985).
15. Ringold, A.; Goldsmith, J.R.; Helwig, H.L.; et al.: Estimating Recent Carbon Monoxide Exposures—A Rapid Method. Arch. Environ. Health 5:308–318 (1962).
16. Stewart, R.D.; Stewart, R.S.; Stamm, W.; Seelen, R.P.: Rapid Estimation of Carboxyhemoglobin Level in Fire Fighters. JAMA 235(4):390–392 (1976).
17. Ramsey, J.M.: Potassium Pallado Sulfite Detection of Carbon Monoxide in Exhaled Air as An Estimate of Carboxyhemoglobin. Am. Ind. Hyg. Assoc. J. 28:531–534 (1967).
18. Cohen, S.I.; Goldsmith, J.I.; Permutter; S.: Carbon Monoxide Uptake by Inspectors at a United States–Mexico Border Station. Arch. Environ. Health 22:47–54 (1971).
19. Wald, N.J., Idle, M.; Boreham, J.; Bailey, A.: Carbon Monoxide in Breath in Relation to Smoking and Carboxyhemoglobin Levels. Thorax 36:366–369 (1981).
20. Hwang, S.J.; Cho, S.H.; Yun, D.R.: Post-Exposure Relationship Between Carboxyhemoglobin in Blood and Carbon Monoxide in Expired Air. Seoul J. Med. 25:511–516 (1984).
21. Tsukamoto, M.; Mitsuda, Y.: Relationship Between Smoking and Both the Carbon Monoxide Concentration in Expired Air and the Carboxyhemoglobin Content. Jpn. J. Trans. Med. 39:367–376 (Kotsu Igaku) (1985).
22. Peterson, J.E.: Post-Exposure Relationship of Carbon Monoxide in Blood and Expired Air. Arch. Environ. Health 21:172–173 (1970).
23. Jarvis, M.; Russel, M.A.H.; Salojee, Y.: Expired Air Carbon Monoxide: Simple Breath Test of Tobacco Smoke Intake. Br. Med. J. 281:484–485 (1980).
24. Ross, G.: Essentials of Human Physiology, 2nd ed. pp. 326–329. Yearbook Medical Publishers, Chicago (1982).

Received 4/9/90; review decision 5/25/90; revision 10/11/90; accepted 10/22/90