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A Field Method for Near Real-Time Analysis of Perchloroethylene in End-Exhaled Breath

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The field method for near real-time analysis of perchloroethylene (Perc) in breath is simple, fast, and reproducible for Perc breath analysis in field settings and should prove useful in industrial hygiene practice. The method allows Perc monitoring with good specificity to the sub-part per million (ppm) level within minutes of exposure. A commercially available, portable gas chromatograph with a photoionization detector was used in these analyses. Gas chromatograph settings were optimized in the laboratory for measurement of Perc in TedlarTM bags. Laboratory development of the method included evaluation of the sensitivity, specificity, precision, and speed of analysis for Perc. Replicate aliquots of Perc at concentrations ranging from 0.01 to 100 ppm were used to construct a calibration curve. The mean retention time for Perc was 238 sec. The impact of potential interference by acetone, toluene, isoprene, methanol, ethanol, acetaldehyde, carbon tetrachloride, benzene, or chloroform was evaluated by mixing Perc with each compound and performing analyses. Measurements of Perc in human breath samples collected in Tedlar bags in a workplace setting were made and compared to measurements of the same samples made by an established analytical method using charcoal tubes (National Institute of Occupational Safety and Health [NIOSH] Method 1003). The accuracy, precision, and speed of the gas chromatograph method were determined. Measurements made with the new method were within a margin of $\pm 8.8\%$ (95% CI, $n = 6$) of measurements made according to NIOSH Method 1003 for field samples in the range of 0.9 to 6 ppm. Method precision was determined by calculating the pooled coefficient of variation for all measurements (replicates = 3) made in the field and was found to be 5.8%.

Keywords biological monitoring, exhaled breath, dry cleaning, perchloroethylene, real-time analysis

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INTRODUCTION

Biological monitoring is the “measurement and assessment of workplace agents or their metabolites either in tissues,

secreta, excreta, expired air, or any combination of these to evaluate exposure and health risk compared to an appropriate measure.”⁽¹⁾ In certain circumstances, biological monitoring can be better for assessing potential health risk than ambient air monitoring because it assesses dose from all potential exposure routes including inhalation, ingestion, and dermal absorption.⁽¹⁾

Breath analysis is a useful biological monitoring technique for estimating exposure and uptake of many volatile solvents.^(2,3) In the case of perchloroethylene (Perc), $\text{Cl}_2\text{C} = \text{CCl}_2$, a common dry cleaning solvent, several advantages of breath analysis can be enumerated. First, the biological half-lives of Perc (0.25, 4, and 96 hours)⁽⁴⁾ are such that sampling at the end of the day reflects the exposure for that day. Second, Perc is poorly metabolized; practically all of the Perc absorbed (97–99%) is excreted by exhalation, whereas only a small amount (1–3%) is excreted in urine as the major metabolite, trichloroacetic acid.⁽⁵⁾ This increases the relative concentration of analyte in the exhaled breath, which in turn improves analytical precision at low exposure levels. Third, biological monitoring for internal Perc exposure integrates for dermal absorption. This is important because skin contact with liquid Perc can substantially affect the internal dose.⁽⁶⁾

Measurement of Perc concentration in exhaled air has been used in previous research. It has been found that the portion of a single respiration that best represents the concentration of solvent in venous blood is the end-exhaled air.^(3,7) Usually the analyte is collected and concentrated on a sorbent material from which it is later desorbed and analyzed by chromatography and/or spectroscopy. Wallace collected exhaled air into a spirometer and concentrated it onto TenaxTM. After thermal desorption it was analyzed by high-resolution gas chromatography/mass spectrometry (GC/MS).⁽⁸⁾ Monster and Boersma collected exhaled air in a TedlarTM bag and performed analysis for a metabolite of Perc with a GC and an electron capture detector.⁽⁹⁾

Another collection technique used activated coconut shell carbon as the sorbent.⁽⁷⁾ Techniques that require transporting the collected samples to off-site analytical laboratories produce a lag time on the order of several hours to days or weeks

between exposure and analysis. This lag time diminishes the ability to identify activities that increase exposure and reduce the impact that interventions can make on the worker if he/she were able to get immediate feedback. Thus, real-time analysis of Perc concentrations in breath would be beneficial to worker education and exposure intervention.

Absolute analytical methods are often needed when the identity of the compound is not known; however, when the identity of the compound is known, comparative techniques such as GC can be used.⁽¹⁰⁾ GC is simple and very small amounts of sample are adequate for analysis.⁽¹⁰⁾ Single or repeated breath samples can be collected in a sample bag or other collection device and analyzed within minutes of exposure. Near real-time monitoring for Perc uptake is possible by locating a GC with a photoionization detector in a work site to analyze end-exhaled air. This use requires that the instrument be relatively portable and rugged.

The purposes of this study were twofold. The first objective was to develop a field method to evaluate Perc concentrations in human breath soon after exposure. The second objective was to validate the method in an occupational setting. This research was a pilot study for the National Institute for Occupational Safety and Health (NIOSH), which is investigating techniques to reduce the risk of Perc exposure and other health and safety hazards in small dry-cleaning facilities. The resulting method has been published in the *NIOSH Manual of Analytical Methods* (Method 3704) as a field method for measuring Perc in human breath.⁽¹¹⁾ The data in this article are the basis for that method.

EXPERIMENTAL DESIGN AND MATERIALS

The GC used for development of this method was a Photovac 10S50 portable gas chromatograph containing a photoionization detector (Photovac, Inc., Waltham, Mass.). The instrument was arranged with a 10-m (1-m pre-column, 9-m analytical column) CPSil 5 CB capillary column, with a 2- μ m polymethyl siloxane film coating, internal diameter of 0.53 mm, and low polarity characteristics. Operating temperature was 40°C isothermal with ultrapure air as the carrier gas at a flow between 10 and 20 mL/min. Settings on the GC were adjusted to optimize analysis for Perc in a time frame that would allow measurements near real-time. Gain was set at 20, and the cycle time was adjusted to 400 sec. This time was also sufficient to allow later eluting materials to exit the column in preparation for the next injection. Compressed air was used as the carrier gas because it is the sample matrix and it is recommended by the manufacturer.

The unit weighed approximately 11 kg (25 lb) and was 46 cm (18 in.) wide, 16 cm (6 in.) high, and 34 cm (13 in.) deep. Rechargeable batteries and a small, internal carrier gas cylinder are included in these dimensions. These studies were performed using a small external cylinder of compressed ultrapure compressed air to minimize cylinder refilling in the

field. The column leads to a photoionization detector with a 10.6 eV lamp. Instrument operation and data output were managed through an internal microprocessor.

Both 1- and 5-L Tedlar bags were used as sample containers during these studies. Syringe injection was selected for introduction of analyte to the GC. Syringes used for sample injection were Hamilton Gas Tight syringes from 10- to 1000- μ L volumes equipped with 26S gauge needles (Hamilton Inc., Reno, Nev.). Gas tight syringes were used to assure that the volume removed from the bag was delivered to the instrument.

A laboratory investigation of the method was the first phase of the study, prior to field validation. Concentrations of Perc and other compounds of interest were generated using a static "bag" generation system and analyzed to determine the response of the instrument. Along with the sample stability in the Tedlar bags, the sensitivity and specificity of the method were determined.

The stability of Perc in Tedlar bags was investigated by analyzing and reanalyzing a specific sample at intervals over a period of 24 hours. Three concentrations of Perc in air (5, 25, and 100 ppm) were generated in separate bags and aliquots injected into the GC at 0, 1, 2, 3, 4, 6, 8, 12 (except for 25 ppm), and 24 hours from sample creation. This concentration range includes the biological exposure index (BEI[®]) at 5 ppm, threshold limit value (TLV[®]) at 25 ppm, and short-term exposure limit (STEL) at 100 ppm.^(4,12) Peak areas were noted and concentrations obtained from calibration curves.

Sensitivity of the Photovac 10S50 GC was evaluated by running a series of analyses of known Perc concentrations in Tedlar bags. These samples were prepared by serial dilution of commercially procured 1000-ppm calibration gas (Scott Specialty Gases, Troy, Mich.) to create bags of 0.01 and 0.1 ppm. Sensitivity determinations were essential to instrument selection for this method, since the instrument of choice had to measure contaminant within the expected exposure range. The Perc concentrations in biological samples were expected to be between 0.001–1000 ppm, based on previous studies of Perc exposure.⁽¹³⁾

Instrument response was determined by injecting known Perc concentrations into the GC. Three injections of a single concentration were made in order to determine the response for that concentration. This procedure was followed for 0.001, 0.01, 0.1, 1, 10, 25, and 100 ppm Perc concentrations. Injection volumes ranged between 5 and 1000 μ L. Carrier gas flow was approximately 16 mL/min.

The specificity of the instrument for Perc in spite of interferences was investigated by analyzing nine different compounds. Acetone, toluene, isoprene, methanol, ethanol, acetaldehyde, carbon tetrachloride, benzene, and chloroform were selected as possible interferences due to the probability of their presence in human breath and ambient air in dry-cleaning establishments. To test the specificity, each compound was generated in inert bags at 1000 ppm in combination with Perc at 10 ppm. Triplicate injections were performed with air containing only Perc and then with air containing Perc and one of each of the

nine compounds. The Perc peak areas and retention times were noted for each set.

Drierite™ was used during the collection of samples to remove water vapor in the breath of subjects, thus insuring that Perc was not extracted into water and lost to condensation during sample storage. In addition, it allowed simpler laboratory sample generation. Effects of Drierite were determined by analyzing a synthetic breath sample composed of carbon dioxide, Perc, and water vapor in air, before and after passing through Drierite. Results of pre-Drierite measurements were then compared to post-Drierite measurements.

Field analyses comprised the second component of the study. Measurements of Perc in end-exhaled breath were made in a dry-cleaning shop to validate the method. In general, breath samples were collected in 5-L Tedlar bags and then analyzed by the portable GC or collected onto charcoal tubes that were shipped to another laboratory for analysis. A single informed volunteer was asked to provide breath six times throughout his work shift by exhaling into separate Tedlar bags so that a range of concentrations could be captured. The following procedures were adapted from Echeverria et al.⁽¹³⁾ and performed during collection of end-exhaled breath:

1. Subject moved outside the shop immediately after clothing was loaded into or unloaded from the cleaning machine.
2. The subject then breathed four normal breaths followed by one deep breath.
3. The deep breath was held for 10 sec.
4. After 10 sec, the subject exhaled the first half of the breath into the air and the last half through a tube filled with approximately 10 mL of Drierite into a sampling bag.
5. Steps 2–4 were repeated no more than three times until the 5-L sample bag was full, providing sufficient volume to allow replicate analysis by the portable GC and the collection of multiple charcoal tube samples. Opdam and Smolders⁽¹⁴⁾ reported that there were no significant differences in values when breath was held for more than 10 sec before sampling.

Sample 1 was taken before the shift began, Samples 2 and 4 were taken before loads of clothes were removed from a cleaning machine, Samples 3 and 5 were taken after a load change, and Sample 6 was collected after the shift. Triplicate injections of breath samples collected in the bags were immediately analyzed by GC. Aliquots of air were drawn into three charcoal tubes from each bag for laboratory analysis following NIOSH Method 1003 for collection and analysis.⁽¹⁵⁾ An SKC sampling pump (SKC Inc., Eighty Four, Pa.) was set at a flow of 100 mL/min and allowed to run for approximately 15 min for each bag, collecting the three charcoal tube samples. Three charcoal tubes without sample were added to the sample batch as blanks.

RESULTS

Laboratory Analyses

To determine whether Perc samples would be stable under the defined collection conditions, Tedlar bags were prepared at three concentrations and aliquots were removed at various times and injected into the portable GC. Concentrations of 5, 25, and 100 ppm were injected into the GC at 0, 1, 2, 3, 4, 6, 8, 12 (except for 25 ppm), and 24 hours from sample creation. Data in Table I show loss of Perc as a function of time for 24 hours. Loss of analyte appeared to increase with increased concentration and was somewhat erratic, but mean Perc loss was <4% over 8 hours. The mean coefficient of variation (CV) for these measurements was approximately 4%.

The GC detected and reproducibly quantified Perc from 16 to 810 ng per injection, which corresponds to concentrations from 0.01 to 100.0 ppm. Injection volumes were 1000 μ L for 0.001, 0.01, and 0.1 ppm; 450 μ L for 1.0 ppm; 25 μ L for 10.0 and 25.0 ppm; and 5 μ L for 100.0 ppm. At a gain setting of 20, the GC was not able to detect a response different from background for 16 ng of Perc, or a 1000- μ L injection of 0.001 ppm. CVs were calculated for each triplicate set and were as low as 0.5% and as high as 11.5%.

Co-exposure studies were performed to determine the potential for interference by a hundredfold excess of solvents that might be used with Perc in some settings. Toluene was found to be the only one of the nine compounds investigated with a retention time similar and potentially overlapping with Perc under the chromatographic conditions employed. However, reduction of carrier gas flow rate to approximately 10 mL/min resulted in a good separation of the two compounds. This change did not result in a significant increase in analysis time and still allowed good separation of the other eight compounds as well. The CV for peak area measurements of Perc only and toluene mixed with Perc was found to be 15.7% higher than for all other comparisons. This suggests that even under

TABLE I. Stability of Perc in Tedlar Bags as a Function of Time

Time (hr)	Perc Concentration		
	Low (ppm)	Medium (ppm)	High (ppm)
0	5.0	25.0	100
1	4.7	22.6	95.9
2	4.7	22.2	102
3	4.7	22.6	101
4	5.0	23.6	94.3
6	4.9	23.5	94.3
8	4.9	23.2	97.7
12	5.0	— ^A	103
24	4.8	21.0	79.9

^AData not collected at this time interval.

conditions of good peak resolution, there was some interference by a hundredfold excess of toluene. This should be borne in mind when co-exposure to these two compounds occurs, although at levels far below the 100:1 ratio toluene concentration would become an insignificant issue. The CV for each of the other eight comparisons ranged from 1.4 to 4.0% and the CV for Perc only and Perc plus all nine compounds was 9.8%.

Comparison of Perc measurements made with and without a Drierite tube to remove water vapor indicated a mean 8.3% loss of analyte to Drierite, although no significant change in retention time was found. While work presented here measured and corrected for this loss, work done subsequently by others and discussed below indicates that this is not necessary.

Field Analyses

Precision, accuracy, and speed of analyses in the field were determined while operating in an occupational setting. A calibration curve was created to permit quantification of the concentration of the analyte. The curve related instrument response to amount of Perc injected. Perc at 1 ppm was used as the working concentration for calibration. Triplicate injections of 50, 100, 150, 200, 250, and 300 μL were made from this concentration. These volumes correspond to pure Perc amounts of 0.339, 0.678, 1.017, 1.356, 1.695, and 2.034 ng. Areas

TABLE II. Breath Concentrations Measured by Portable GC and NMAM Method 1003

Sample Number	Average Perc Concentration ^A	
	Portable GC ^A (ppm)	NMAM Method 1003 (ppm)
1	0.94	1.3
2	3.95	3.8
3	4.74	5.0
4	3.83	4.4
5	5.54	6.2
6	4.35	4.8

^AMean difference between Portable GC and NMAM Method 1003 measurements: 0.37, p-value 0.2188, % error 8.8, % validity 91.2.

under the chromatogram peaks measured in volt second were first corrected for Drierite interference (8.3%) and then plotted against pure Perc amounts.

A total of six separate end-exhaled breath samples were analyzed both by the portable GC and also by NIOSH Method 1003. These data are presented in Table II. The relationship between the results obtained by the two methods is shown in Figure 1. The results are highly correlated ($r = 0.98$, $r^2 = 0.97$).

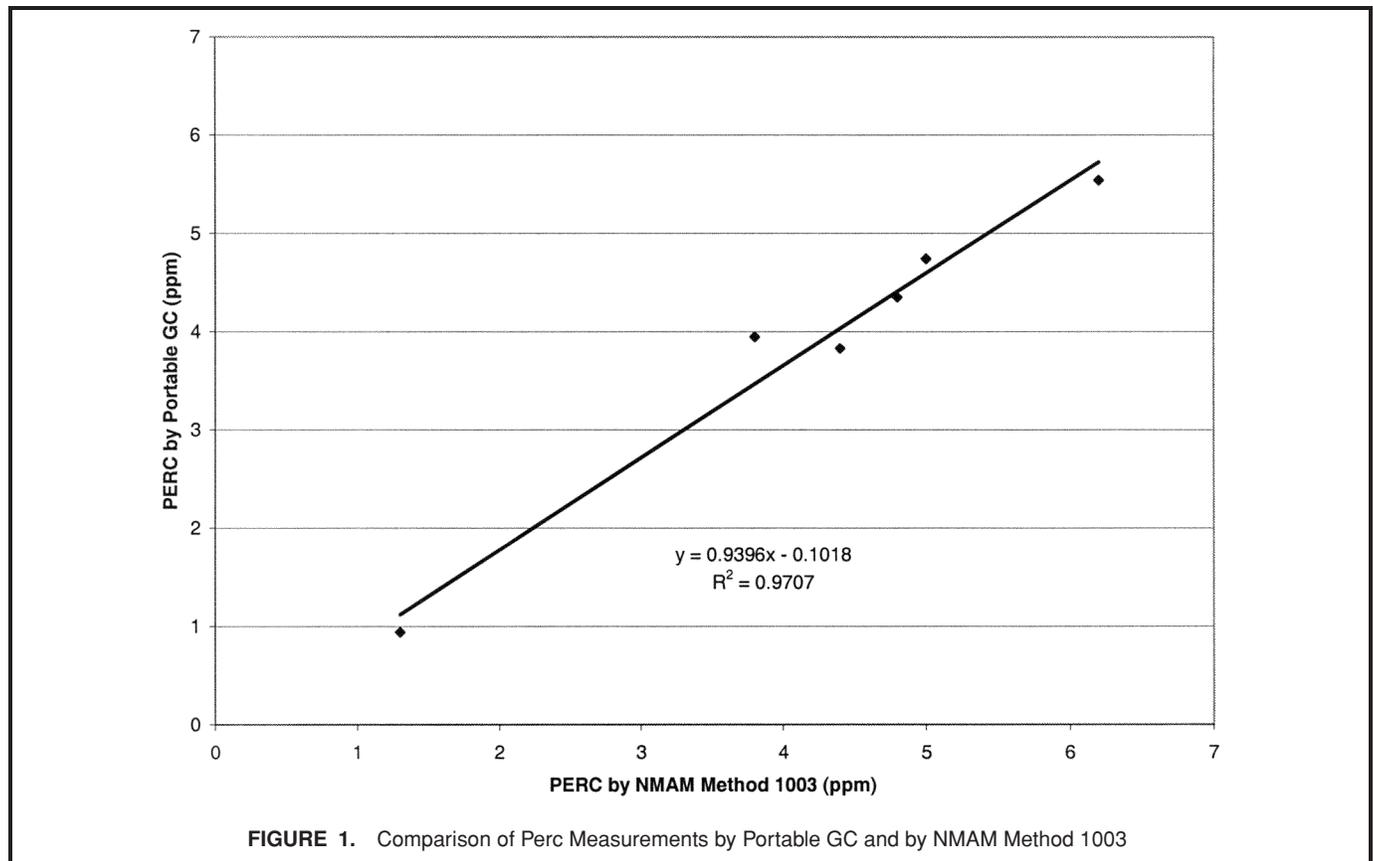


FIGURE 1. Comparison of Perc Measurements by Portable GC and by NMAM Method 1003

On average, results obtained with the portable instrument and direct analysis were slightly lower than those obtained with Method 1003 and later analysis at a contract laboratory (slope 0.94, intercept -0.1). Both sets of results were corrected for loss due to Drierite since the sorbent tube's samples were collected subsequent to the transfer of exhaled breath through this material.

GC measurements were determined to be within a margin of $\pm 8.8\%$ (95% CI, $n = 6$) of measurements made according to NIOSH Method 1003. This was used with a relative standard deviation of 3.4% to produce an estimate of accuracy of $\pm 18\%$ using the NIOSH accuracy nomogram.⁽¹⁶⁾

Carrier gas flow of 10 mL/min yielded a mean retention time of approximately 240 sec for Perc. This potentially allows for 10 or more analyses per hour.

DISCUSSION AND CONCLUSIONS

End expiratory breath samples were collected in a sampling train that consisted of a Drierite-containing tube and a Tedlar bag. Aliquots of the samples in the bags were removed and analyzed with the portable GC. Data for sample stability in Tedlar bags suggest that higher concentrations of Perc result in higher losses. This is most likely due to greater concentration gradients being generated by the higher concentrations. The percent loss at each interval through time was found to be erratic, with fluctuations on the order of ± 5 –6%. This could be due to atmospheric conditions in the laboratory leading to sporadic Perc loss rates from the bags. The error that results from these collection losses was not systematic and therefore not directly correctable.

The GC was used with a gain setting of 20 during field analysis. Gain can be adjusted down to 2 (least sensitive) or as high as 1000 (most sensitive). Informal analyses showed a range of quantification below 0.001 ppm and above 100 ppm. Additional analyses could be performed to optimize measurements beyond the range for a gain setting of 20.

Results of the specificity investigation in the laboratory and findings from field analysis suggest minimal interference in the analysis for Perc in the dry-cleaning setting. No peaks were found from breath measurements to interfere with chromatograph peaks for Perc. Laboratory investigations suggested toluene interference at 1000 ppm due to its retention time; however, this concentration is higher than would be expected in breath of persons not exposed to high concentrations of the compound. Therefore, a faster carrier flow could be used in most situations to decrease retention time and increase the speed of analysis.

Drierite was found to reduce the concentration of Perc in air passing through on an average of 8.3%. This loss can be easily corrected in concentration calculations or by passing calibration gas through Drierite during creation of a calibration curve. Studies performed by Groves and Zellers⁽¹⁷⁾ subsequent to collection of this data set indicate use of Drierite or other desiccant may not be necessary since Perc is only sparingly soluble in water.

Data collected during method validation showed measurements made by the GC were within a margin of $\pm 8.8\%$ of accepted actual concentrations, well within the $\pm 25\%$ criteria for a NIOSH analytical method. The slope of NIOSH Method 1003 measurements vs. measurement obtained with the described method was 0.94; the relationship between measurements was very strong ($r^2 = 0.97$), further suggesting only a slight difference in measurements. Concentrations ranged from 1.0 ppm to approximately 6.0 ppm, close to the ACGIH's BEI of 5 ppm. Further investigation into the accuracy of measurements at higher concentrations would explain the general measurement capabilities of this method and provide insight for ambient air measurements. Furthermore, for simplicity, air temperature was assumed to be 25°C and atmospheric pressure was assumed to be 760 mmHg. Actual air temperature was probably lower than 25°C and could have decreased the mean concentrations measured by NIOSH Method 1003 and thus improve the accuracy of the GC measurements.

Measurement of Perc in the field exhibited an acceptable degree of precision with a pooled CV of 5.8% for nine sets of observations (replicates = 3). This variation was most likely due to inconsistent analyte volumes being injected into the GC. An increased sample set would strengthen the confidence of the reported value.

Results of the research showed retention times to be consistent over several hours, with a maximum CV of 0.7% for the nine sets of field measurements. Laboratory analyses suggested a minute increase in retention time over 8 hours on the order of a couple of seconds, most likely due to a slight decrease in carrier gas flow rate. This change in retention time could be avoided by maintaining a constant carrier gas flow rate during the time analyses are performed.

Setup for monitoring required about 30 min to organize and prepare instrumentation. However, 3–4 hours were necessary for the GC column and detector units to stabilize. Efficient monitoring could be performed if the GC was set up in advance so that the instrument could reach stable operating conditions. Also, one must be prepared to generate a calibration curve in the field setting with approximately 25 injections for about 6 or 7 points.

Field analysis of Perc in end-exhaled breath by portable GC in conjunction with a photoionization detector using a 10.6 eV lamp is within an acceptable error margin and thus deemed accurate. Newer, more portable GCs are now available which should further facilitate implementation of near real-time methods.

An advantage of field analysis by GC is the ability to generate near real-time results, within 15 min of sample collection. This near real-time monitoring capability should (1) allow gathering of Perc exposure data throughout a work shift, (2) aid in characterizing specific dosing events that contribute to the total dose of an individual over an extended period of time, and (3) prove to be an effective intervention tool. These areas could be investigated in the future. The ability to measure air concentrations of Perc in the field with a GC allows quick

and accurate monitoring of work environments in dry-cleaning shops and other industries dealing with Perc.

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