

Breath analysis

Physiological basis and sampling techniques

by H Kerr Wilson, PhD¹

WILSON HK. Breath analysis: Physiological basis and sampling techniques. *Scand J Work Environ Health* 12 (1986) 174—192. This paper examines the physiological basis for breath analysis and draws attention to the factors which may affect the blood:breath ratio. A review of recent breath analysis techniques is presented, and some consideration is given to the use of breath analysis in the biological monitoring of workers exposed to solvents.

Key terms: alveolar air, biological monitoring, mass spectrometry, physiology, sampling methods, solvents.

The history of breath analysis can be traced back over 100 years, when, in 1874, Anstie reported the elimination of alcohol via human breath (8). The techniques established in the early 1900s for use in respiratory physiology permitted the composition of breath and alveolar air to be determined (48). The most well-established use of breath analysis has been for medico-legal purposes, and substantial literature has appeared over the past 50 years on the use of breath alcohol analysis (51, 52, 53, 54, 55, 56, 57, 69, 70). Some progress has been made in the use of breath analysis for the diagnosis of metabolic disorders and diseased conditions, and a wide range of compounds of clinical significance have been detected in breath (24). (See table 1.)

Breath analysis has been proposed and used as a noninvasive method of monitoring workers industrially exposed to solvents (15, 47, 73, 74, 75, 76, 82, 95, 96, 97, 109). However, its use in occupational medicine for routine biological monitoring has been slow to emerge, probably because the available breath analysis techniques are found to be unsuitable for field work.

There is current international interest in setting biological exposure limits for monitoring workers exposed to solvents. The American Conference of Governmental Industrial Hygienists' (1984—1985) list of threshold limit values (TLV) proposes the use of exhaled air for the biological monitoring of ethyl benzene, styrene, toluene, and trichloroethylene without giving any clear guidance on appropriate methodology (6). The Federal Republic of Germany has suggested biological tolerance values for perchloroethylene and methylchloroform based on alveolar air sampling (21). Lauwerys has proposed the use of expired

air for monitoring exposure to cyclohexane, benzene, toluene, dichloromethane, 1,1,1-trichloroethane, trichloroethylene, and tetrachloroethylene and has suggested tentative maximum permissible values (64).

It is claimed that the collection and analysis of breath is a simple, nontraumatic, easily repeated, and useful noninvasive alternative to blood analysis. The apparent simplicity of breath analysis must not be over-stated, and the numerous pitfalls must be identified.

Breath and blood are two physiologically distinct samples, and it may not be appropriate to conclude that a breath sample gives information which equates directly with a peripheral venous blood sample. Under certain circumstances even venous blood samples collected from different arms will give different results. Recent work on the skin absorption of solvents has demonstrated a clear difference between the blood collected from a hand immersed in a solvent for 5 min

Table 1. Some breath components of clinical significance.

Breath component	Use	Reference
Alkanes	Lipid studies	Wendel & Dumelin (106)
Acetaldehyde	Ethanol studies	Jauhonen et al (50), Stowell et al (98)
Acetone	Diabetes, weight loss	Crofford et al (19), Rooth et al (90)
Ammonia	Aminoacid metabolism	Lovett et al (67)
Halothane	Anesthetic monitoring	Eger (28)
Carbon dioxide	Respiratory studies	Fowler & Hugh-Jones (38)
Carbon monoxide	Dichloromethane exposure	Perbellini et al (82)
Dimethylamine	Renal disease	Simenoff et al (92)
Dimethyl sulfide	Hepatic disease	Manolis (68)
Isoprene	Stress studies	Gelmont et al (43)
Hydrogen	Carbohydrate metabolism	Broadbent et al (13), Kneepkens et al (60)

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and the contralateral arm (4). The position becomes more complex when consideration is given to the relationship between local and mixed venous blood, arterial blood, and alveolar air concentrations. If breath analysis is to be considered as an alternative to blood analysis, the following important requirements need to be addressed:

1. The validity of breath as an indicator of venous or arterial blood concentrations should be established.
2. The factors affecting the blood : breath ratio should be identified.
3. Since breath is not a homogeneous sample, the portion of a breath sample which reflects the blood concentration must be identified and collected.
4. The breath sampler per se should have low resistance to normal breathing, be unaffected by water vapor, seal quickly, and enable the sample to be recovered easily and reproducibly for analysis.
5. The analytical technique must be sensitive and specific enough to measure trace levels of solvents reproducibly.

This paper considers these points and reviews the qualitative and quantitative methods which have been reported in the literature for breath analysis.

Physiological basis for breath analysis

Breath analysis for monitoring solvent uptake

Biological monitoring is an established method of estimating the degree of uptake of solvents. Gompertz (44, 45) has argued that variability between workers is reflected in differences in uptake, metabolism, and elimination, and biological monitoring which takes account of these factors enables individual risk assessments to be made. Measures of uptake are available for some solvents, but care must be exercised to adopt the most suitable method for monitoring, and special attention must be given to metabolism and kinetics. In the case of certain solvents there may be good theoretical reasons for considering breath analysis.

The basic assumption underlying breath analysis is that there is gaseous equilibrium between alveolar air and pulmonary blood. Blood takes about 1 s to pass through the pulmonary capillaries; and, although gases or vapors must diffuse across the surface-active layer, the alveolar membrane, the connective tissue, and the capillary endothelium and into the plasma or through the red cell membrane, pulmonary diffusion is very rapid. Diffusion of respiratory gases is known to be complete within 0.3 s (18). During exercise the diffusion capacity rises; since the diffusion coefficient remains unchanged, the increased capacity must reflect

increases in the area of membrane across which diffusion occurs.

After solvent vapor has entered the lungs, equilibration between alveolar air and blood can be considered almost instantaneous, so that the ratio of pulmonary blood to alveolar air concentration is close to the *in vitro* blood/air partition coefficient. During a period of exposure to a solvent, the concentration in arterial blood is always greater than the concentration in mixed venous blood. At steady state there is partial pressure equilibrium between alveolar air, arterial and venous blood, and the body tissues, a condition which rarely exists in the industrial setting. After exposure, pulmonary clearance removes unmetabolized volatile solvents from the systemic circulation. Therefore the concentration in the arterial blood is lower than in the mixed venous blood. During this period (ie, at the end of exposure), when biological monitoring generally takes place, the concentration of solvent in alveolar air will depend on the pulmonary ventilation, the blood flow, the solubility of vapor in blood, and the concentration in mixed venous blood.

Importance of alveolar air sampling

Several types of exhaled air samples have been used for breath analysis. These have included mixed expired air (39, 62, 105), end-tidal air (29, 84), alveolar air (47, 96, 109), and rebreathed air (54). Mixed expired air consists of alveolar air diluted by ambient air retained in the respiratory dead space (mouth, nose, pharynx, trachea, and bronchi). Alveolar air (end-expired air) normally accounts for two-thirds of the tidal volume, so the concentration of solvent in mixed expired air is approximately two-thirds of the concentration in alveolar air. Mixed expired air has been used for qualitative work but is not considered suitable for quantitative assessments because it contains variable proportions of alveolar and deadspace air, unless the total output of breath is collected and the volume and mass are determined. The deadspace air plays little or no part in gas exchange and must be expelled before the alveolar air is reached. However, a significant effect may be encountered with highly water-soluble compounds which may be absorbed and released in the upper respiratory airways (51). Since an exhaled breath sample is not homogeneous, caution must be exercised if quantitative and representative data are required. Examination of the breath profile of solvent elimination is useful in identifying a sampling procedure. The breath profile for carbon dioxide has been demonstrated by the use of mass spectrometry (38) and, more recently by our laboratory, for some industrial solvents (15, 109). The breath profile for solvents, as illustrated in figure 1, may be considered as three phases. At the beginning of expiration, phase I appears as a horizontal line which represents the airway dead space, which contains little or no solvent vapor. This phase is followed by a rapid rise in solvent concentration (phase II), which slows down as the alveolar air is reached (phase

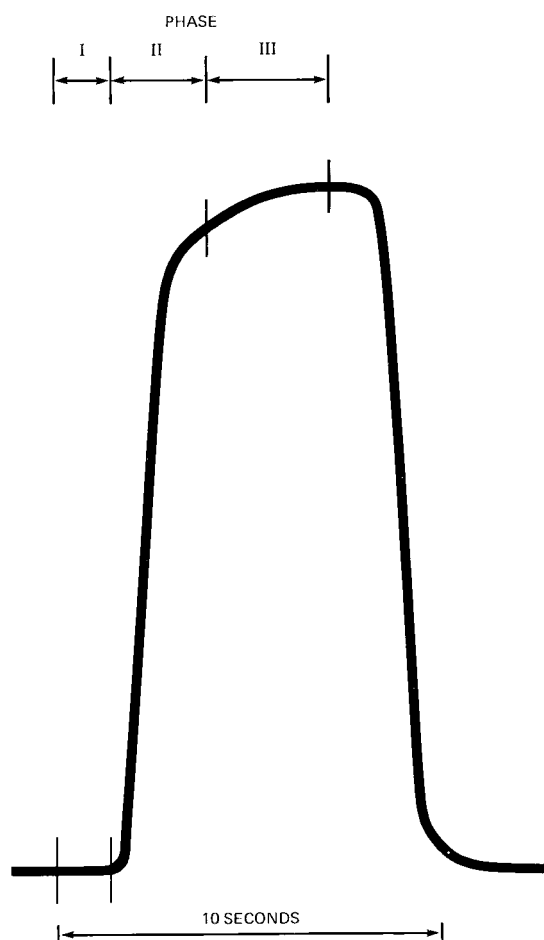


Figure 1. Breath solvent profile for a single expiration.

III), referred to as the alveolar plateau. Phase III has a gentle gradient which can be attributed to the continued liberation of solvent gas from the alveoli. The rapid fall in solvent concentration at the end of phase III occurs when the subject inhales clean air. This model can be a useful basis for designing an alveolar air sampling method. It can be seen that collection of a breath sample too early (ie, during phase II) will not reflect the alveolar concentration (phase III). Collection of the last part of an expiration (about 200 ml) will generally provide a valid alveolar sample.

Factors affecting the blood:breath ratio

The validity of breath analysis as an indicator of solvent concentrations in blood is based on the assumption that equilibrium between the partial pressures of blood and alveolar air has been achieved in the lung and that this equilibrium is reflected in a constant blood:breath ratio. In studies conducted in our laboratory the venous blood:alveolar air ratio has been investigated in two groups of workers occupationally exposed to toluene and 1,1,1-trichloroethane (unpub-

lished observations). Although the correlation (r) between blood and breath toluene was satisfactory ($r = 0.83$) for routine monitoring, it was not as close as might be expected from the known precision of the breath and blood analyses. Paying special attention to the timing of blood and breath collection improved the correlation ($r = 0.91$). The correlation for blood and breath trichloroethane was better for preshift than for postshift samples. This result may be a reflection of the elimination rate of the solvent, which is relatively slow 16 h postexposure. Therefore the sampling time is less critical.

It has been demonstrated that the blood:breath ratio for alcohol varies from person to person and within the same subject with time (51). The theoretical basis of alveolar sampling for solvent analysis has been explored under steady-state conditions (59), and, providing the blood/gas partition coefficient exceeds 10, the partial pressures of mixed venous blood and alveolar air will agree within 10 %. However, with less soluble solvents, it was calculated that alveolar sampling was unlikely to provide a valid index of mixed venous levels.

It should not be assumed that the *in vitro* blood:air partition ratio, as determined by headspace analysis, should be precisely the same as the *in vivo* ratio obtained in the case of alveolar air and peripheral venous blood.

Ventilation-perfusion. Since the uptake and elimination of solvents is primarily dependent on cardiac flow, pulmonary ventilation, and solubility, the effect of ventilation:perfusion ratios is relevant in determining the blood:breath ratio. In a normal subject at rest the alveolar ventilation ($\dot{V}$) is about 4.5 l/min, and the corresponding blood flow ($\dot{Q}$) is about 5 l/min, giving a ventilation:perfusion ratio of about 0.9 (18). This is the average $\dot{V}:\dot{Q}$ for the lung as a whole and ranges from 0.7 to 1.0 from person to person. Variations in the $\dot{V}:\dot{Q}$ ratio are present in different regions of the lung because not all alveoli are ventilated and perfused in ideal proportions, and the ratio may range from 0.5 at the lung base to 3.0 at the apex (107).

Farhi (31) has described the interaction between solubility and ventilation-perfusion relationships in the lungs as:

$$\% \text{ retention} = \frac{P_a}{P_v} = \frac{\lambda}{\lambda + \dot{V}:\dot{Q}}, \quad (\text{eq 1})$$

where P_a and P_v are the partial pressures in the arterial and venous blood, respectively, and λ is the partition coefficient. It is clear that the $\dot{V}:\dot{Q}$ ratio and solubility interact to determine the rate of pulmonary gas excretion. The theoretical retention of solvents in blood with varying partition coefficients for the normal range of $\dot{V}:\dot{Q}$ ratios is given in table 2.

It can be seen in table 2 that the effect of interindividual differences in the $\dot{V}:\dot{Q}$ ratio is unlikely to

affect the results by more than 3 % for the majority of industrial solvents which have partition coefficients greater than 10.

Differences between alveolar air and arterial blood partial pressures are well known in anesthesia. Studies of young healthy volunteers have shown differences in alveolar to arterial partial pressures for the poorly soluble anesthetics halothane (29), nitrous oxide, and isoflurane (28). These differences have been attributed to ventilation-perfusion abnormalities which are present in normal man from the intrapulmonary shunting of blood or from diffusion barriers in the lung (91).

Ventilation-perfusion is more homogeneous during light exercise than at rest. Therefore end-expired air samples taken during exercise may reflect the alveolar concentration better than samples taken at rest (2). Recently, experimental evidence has been reported to illustrate that partial pressures of carbon dioxide in the alveolar air and arterial blood should not be assumed to be equal. It was observed that there was no change in arterial carbon dioxide between rest and heavy exercise. But the mean alveolar carbon dioxide rose significantly during heavy exercise, whereas there was no significant change in the oxygen levels (5).

In the case of very soluble compounds such as ethanol, marked changes in the ventilation-perfusion balance can hardly influence equilibrium at the alveolar-capillary interface (54), but the temperature gradient during passage through the airways has an appreciable effect on the blood:breath ratio.

In a study on the uptake and elimination of acetone in man it has been shown that the ratio of acetone concentration in blood and in alveolar air at the end of exposure was about 30 at rest, and it increased to about 100 with exercise. During the elimination period the ratio was about 400 at rest but fell to 340 in the exercise experiment (108). It would appear that the less soluble compounds are the least affected by exercise. The ratio between arterial blood and alveolar air for the relatively insoluble trichloroethane remained constant at 5 and did not alter with exercise, but for styrene it increased from 15 to 105 with exercise (1).

Diffusion-adsorption-desorption. The rate of diffusion across the alveolar-capillary membrane is influenced by the molecular size of the solvent molecules and the relative partial pressures. The equilibration of insoluble gases such as helium, argon, and sulfur hexafluoride in the lungs is achieved at different times (93). Gaseous diffusion is related to molecular weight as described by Graham's Law. The elimination breath profile for each of these inert gases is slightly different; helium reaches a plateau before argon.

It is not always clear whether slow elimination of a solvent is due to diffusion rate. When the exhaled air of volunteers exposed to styrene was continuously analyzed, the slow diffusion of styrene was considered to be the rate-limiting step in the blood/air partial

pressure equilibration, but other explanations such as solution in the fluids lining the respiratory pathways have been offered (35). Bocek & Jandova (12) noted a 3 % difference in the concentration of trichloroethylene in exhaled air if inhalation of clean air preceded the exhalation rather than solvent-laden air. They attributed this difference to the adsorption and desorption of trichloroethylene in the respiratory airways. The airways and lungs are a dynamic environment with fluctuating temperature and humidity in the upper airways and unpredictable alterations in the solvent concentration in the fluid on the surface of the airways.

Acetone is highly water-soluble, and the relative uptake is 45 %, irrespective of time and exercise. This is a relatively low percentage uptake for a highly soluble compound. It has been suggested that acetone dissolves in the saliva and water of the epithelium of the upper respiratory tract. When alveolar air passes over the epithelium during expiration, acetone is partially desorbed. Consequently, the acetone concentration in expired air will be higher than in alveolar air (3). This phenomenon occurs also with alcohol and may be another source of difficulty in establishing a constant blood:breath ratio (51).

Metabolism. During the study of ethanol metabolism, it has been assumed that breath concentrations of the metabolite acetaldehyde are good indicators of blood concentrations. Recent studies have concluded that acetaldehyde concentrations in expired air do not reflect pulmonary blood acetaldehyde concentrations in a consistent manner (98). They suggested that significant extrahepatic metabolism of acetaldehyde occurs in man. In another study Jauhonen et al (50) demonstrated that, in subjects with less than 5 μ mol of acetaldehyde in their blood, breath acetaldehyde did not correlate with blood levels. It was suggested that this phenomenon was due to the production of acetaldehyde by oropharyngeal microflora and lung microsomes. Long-term cigarette smoking is known to induce microsomal mixed-function oxidases both in pulmonary alveolar macrophages and in lung parenchymal tissues (20). Some evidence is available for the possibility that the microsomal oxidation of ethanol takes place in the lung. Smokers were observed to have higher breath acetaldehyde concentrations than non-smokers in these two studies (20, 50). The possibility exists that, in the case of a metabolized solvent, me-

Table 2. Effect of the ventilation:perfusion ratio (V:Q) and partition coefficient on solvent retention.

Partition coefficient	V:Q	
	0.7 % retained	1.0 % retained
1	58.8	50.0
10	93.5	90.9
100	99.3	99.0

tabolism in the lung may reduce the level of parent compound or increase the level of a volatile metabolite.

Temperature. The pulmonary excretion of gases that readily dissolve in fluids lining the airways is influenced by the temperature gradient during passage through the airways and the consequent changes in the liquid-air partition coefficient during exhalation (110). If the equilibrium of ethanol between pulmonary blood and alveolar air is achieved in the lung at 37°C, that equilibrium is certainly altered during expiration. While the pulmonary-capillary-alveolar temperature probably does not fluctuate ($\pm 0.7^\circ\text{C}$) the temperature of alveolar air is modified on expiration by mixing with dead-space air. The events occurring in the dead space appear to be more important than modest variations in the partial pressure of ethanol in the alveolar spaces of normal subjects due to small temperature fluctuations. Ethanol enters the breath by diffusion from the pulmonary blood at about 37°C during exhalation; alveolar air returns heat to the airways and is cooled to 34.5°C as it leaves the mouth. The lower temperature of expiratory air means that ethanol vapor re-equilibrates with the airways, and its concentration in the expired air is substantially lower than in the alveoli. It has been established that the ethanol blood:breath ratio changes by about 6.5 %/1°C (52).

Breathing technique. A subject's breathing pattern either directly before or during delivery of the breath may influence the blood:breath ratio. When a comparison was made between different breathing techniques in men who had ingested alcohol, breath holding (30 s) increased the ethanol concentration in the alveolar air by 15.7 % when compared with the effect of a control breath (deep inspiration followed by forced expiration). Hyperventilating for 20 s decreased the concentration by 10.6 %. Shallow breathing (by keeping the mouth closed) for 5 min increased the expired ethanol concentration by 7.3 %, and also after a slow exhalation (20 s) the concentration was increased by 2.0 % (53). The author concluded that the changes in concentration were partly caused by the rise and fall in temperature of the breath and partly by the amount of time the breath spends in contact with the mucous membranes of the upper respiratory tract. It was noted that, regardless of the breathing technique, the concentration returned to a normal level immediately after the subjects began normal breathing. With the rebreathing technique the concentration of ethanol in breath rises by about 10 % when compared with the levels in end-expired air (54). The blood:breath ratio is 2 225 for end-expired breath, and this value decreases to 1 947 with rebreathing.

Hypoventilation produces a rapid fall in alveolar carbon dioxide, and breath holding gives higher carbon dioxide levels. Guillemin & Guberan (47) have demonstrated that breath concentrations of 1,1,1-tri-

chloroethane are similarly affected by hyper- and hypoventilation.

Blood composition. The blood/air partition coefficient for some compounds is known to be affected by hematocrit, blood lipid, and protein content (30, 63). Lerman et al (66) has clarified the effect of hematocrit on the solubility of some volatile anesthetics in blood. The effect of variable hematocrit on the blood-gas partition coefficient of enflurane, halothane, isoflurane, and methoxyflurane was measured. The partition coefficient of halothane and methoxyflurane did not change significantly, but a statistically significant effect was observed with the other two anesthetics. As the hematocrit increased, the partition coefficient of isoflurane decreased linearly, whereas that of enflurane increased linearly.

Åstrand (2) has suggested that the higher blood:breath ratio observed during exercise may be caused by a change in the blood composition during exercise. When exercise starts, there is a drop in the concentration of free fatty acids in plasma due to an increased uptake by the working muscles. After a short time the fatty acids move from the adipose tissue and cause a rise in the plasma concentration. This enhanced fatty-acid turnover and plasma glycerol mobilization is needed for the working muscle. However, it is not clear to what extent this change in blood composition affects the solubility of solvents in blood.

The interaction of halothane with five human blood components has been studied by equilibrium dialysis. The data indicated that the solubility of halothane in blood increases with increasing concentrations of albumin, triglyceride, hemoglobin, and hemocrit, but γ -globulin had no effect on solubility (79). It has been demonstrated that the dichloromethane concentration in blood is related to the triglyceride content (82), and Riihimäki and co-workers (88) have shown that the m-xylene concentration in blood is higher after a meal. The postprandial and fasting effects on the solubility of enflurane, halothane, methylene chloride, benzene, trichloroethylene, toluene, and methoxyflurane were investigated in a study on five volunteers. The lipid content in blood was higher postprandially than during fasting, which significantly altered the partition coefficient. The increase and variation in the partition coefficient was larger for the lipid-soluble compounds. However, the effect of this increase on the uptake of solvent was considered to be less than 10 % (37).

Time. Since the uptake and elimination kinetics of a compound are time-dependent, the blood:breath ratio of a solvent may vary with time. The mean blood:breath ratio for 21 men who ingested alcohol was 11 % lower during the active absorption phase in comparison with the peak blood concentrations and was 8.8 % higher during the elimination phase. At 30–90 min after ingestion the blood:breath ratio was less than

2 100:1, which increased to about 2 200:1 after 360 min (51). Martin et al (69) have reported that alveolar ethanol concentrations closely follow arterial blood concentrations during absorption and tend to deviate from venous blood concentrations. During elimination the fall-off of both alveolar and arterial alcohol concentrations tends to change faster than that of venous blood concentrations, and after reaching arteriovenous equilibrium they cross over the venous blood concentrations and thereafter remain lower.

A group investigating experimental human exposure to toluene has shown that there is a curvilinear relationship between toluene concentrations in exhaled breath and peripheral blood during the postexposure period. The breath concentrations decreased much faster than the blood concentrations during early post-exposure and gave a variable blood:breath ratio (102). The in vivo ratio between endogenous acetone in blood and alveolar air averaged 765 compared with the in vitro value of 275. When eight male volunteers were exposed to acetone, a marked shift was noted with time; at the end of exposure the ratio was about 30, which changed to about 400 during the elimination phase (108).

Discussion. Although breath and blood samples are physiologically different, there is clearly a close interaction within the lungs. Therefore, in discussions of the advantages and disadvantages of breath analysis, reference must be made to the relationship between solvent concentration in breath and blood. Both theoretically and practically there are good reasons for establishing the extent to which the blood:breath ratio can vary, and the most appropriate sampling technique for monitoring solvent uptake should be identified.

Normal variation in the ventilation:perfusion ratio is unlikely to have a significant effect on the blood:breath ratio of solvents. However, in the case of certain solvents, the effect of exercise during or after work certainly affects lung uptake and clearance, which in turn may affect the blood:breath ratio. Therefore, before a sampling strategy is established, it may be necessary to ensure that the elimination rate of solvent from the breath is relatively slow. Diffusion of solvents across the alveolar-capillary membrane is relatively fast, and it would be prudent therefore to ensure that the sample concentration is measured at the alveolar plateau.

In the case of poorly metabolized solvents it is obvious that lung metabolism will be insignificant, but metabolism is worthy of consideration in the case of well-metabolized compounds. A significant amount of lung metabolism may affect the blood:breath ratio although with most industrial solvents it is not thought to be a serious problem.

Temperature is an important consideration, especially for water-soluble compounds which are exchanged in the respiratory tract. Many of the difficulties associated with temperature have been observed

with breath alcohol analysis, and suitable procedures, such as rebreathing, have been found to solve the difficulties.

Blood composition is practically impossible to control. However the best estimates appear to suggest that it is unlikely to affect the blood:breath ratio by more than 10 %.

The timing of the sample is critical. Fast elimination phases have a significant effect on the blood:breath ratio. These problems can be minimized by the adoption of a preshift sampling procedure for which the rate of change in concentration is relatively slow.

Breathing technique should be standardized as closely as possible to reduce errors. Normal breathing, followed by a steady expiration until the alveolar plateau is reached, is preferred.

Standardization of breath samples

The difficulties in consistently capturing alveolar air samples have been known for a long time. A standardized, reproducible breath sample is important for quantitative breath analysis. Without efforts to standardize the breathing or collection technique, the proportion of alveolar air to tidal air will vary from sample to sample, as determined by the inspired volume and the type of expiration. From the discussion on the importance of the alveolar sample it is clear that collection of the expired air during the alveolar plateau phase is desirable to establish a reproducible sampling method. Efforts have been made to determine when the alveolar plateau has been reached during a single expiration. These methods have included the monitoring of carbon dioxide (77), oxygen (89), or breath temperature until a constant level is attained (24). A different approach identifying the end-tidal sample has been used for measuring xenon in breath. This system is based on the optical detection of the movement of the inspiratory valve (58).

The assumption that there is a relationship between breath alcohol and carbon dioxide concentrations has been used in the design of some breath analyzers (70). The adjustment of breath alcohol to the carbon dioxide level has been used to calculate the blood concentrations. This standardization has been challenged because the ratio of breath alcohol to carbon dioxide is not constant under all circumstances (57, 94).

Normalization of breath hydrogen to the carbon dioxide concentration has been shown to improve the coefficient of variation in the measurement of hydrogen from 25 to 8 % (77). Robb & Davidson (89) reported similar results for breath hydrogen levels by using respiratory nitrogen as an internal standard and then adjusting the hydrogen levels to a constant oxygen level. Guillemin & Guberan (47) have argued that results from the breath analysis of trichloroethane should be corrected for hyper- or hypoventilation and for dilution of alveolar air with dead-space air by a proportional adjustment of the solvent concentration to the mean alveolar carbon dioxide level or by dis-

regarding samples with a carbon dioxide concentration outside the normal range.

Interference from endogenous compounds

Interference from endogenous compounds may be an important consideration when breath analysis is adopted as a biological monitoring technique for industrial exposure to solvents. Breath is saturated with water vapor, which may present problems in analysis. In addition there is approximately 5 % carbon dioxide and a substantial number of endogenous compounds and volatile components arising from dietary sources and environmental contamination. Approximately 200 compounds from exogenous and endogenous origin have been detected in the breath of normal human subjects (17, 24, 62, 68). The normal output of breath hydrogen is <0.3 ml/min. Evidence to date suggests that breath hydrogen analysis may provide the best method for estimating the degree of carbohydrate malabsorption in adults (78). Endogenous breath acetone concentrations in alveolar air are about 2–3 $\mu\text{g/l}$ (68, 108). Dimethyl- and trimethylamine have been found in the breath of uremic patients. After renal dialysis the amine concentrations in breath and serum return to a normal level (92). Ammonia derived from the catabolism of proteins and amino acids is normally present in breath (1 ppm) but is increased in hepatic disease (67). Isoprene has been reported to be the main hydrocarbon in exhaled human breath and is produced at a rate of about 2–4 mg/24 h (43).

All these breath components are usually present in low concentrations when compared with the level of solvents found in breath following industrial exposure, and they are unlikely to present any major interference problem. However, it would be prudent to be aware of possible interferences when preshift breath samples are being examined, a time at which the solvent concentration is likely to be less than 1 ppm.

Mathematical models

Mathematical models provide a useful approach to the understanding of the kinetics of uptake, distribution, and elimination of solvents in man. A physiologically based model has been developed (7) in which analysis at steady state involves consideration of enzymatic constants, blood flow, and limitations to pulmonary equilibration caused by extensive metabolism. Andersen (7) concluded that neither a simple Michaelis-Menten model, favored by Gehring et al (42), nor the abrupt transition kinetics theory (34) was an entirely adequate explanation. Solvents should be considered as falling between the two extremes, ie, where biochemical steps are rate limiting and where perfusion is rate limiting.

The majority of models is based on a multicompartmental approach in which the organs are characterized by blood flow, organ volume, and partition coefficients. Fernandez et al (33) have adopted the

compartmental analysis approach with perfusion-limited kinetics. In order to simulate the uptake, distribution, and elimination of a solvent, this model assumes that, after the compound enters the body through the pulmonary compartment, it is distributed to three groups of tissues — the blood-rich group (heart, liver, etc), the blood-poor group (skin and muscles, etc), and the adipose tissue. The distribution kinetics depend on the tissue volume, blood flow, perfusion, and partition coefficients of the different groups. Metabolism is assumed to take place in the liver and can accommodate up to two metabolites.

Fiserova-Bergerova and her co-workers (35, 36, 37) have developed an elegant model to predict the uptake, distribution, and clearance of inhaled vapors. The model consists of five compartments with two elimination pathways, breath and metabolism, and is based on first-order kinetics. Tissues are assigned to a compartment according to perfusion, ability to metabolize the solvent, and the solubility of the solvent in the tissues.

These models have been used for simulating the metabolism and kinetics of lipophilic compounds, and they do not accommodate such factors as retention in the respiratory tract, diffusion, and binding to tissue components.

Solvents are conveniently grouped into soluble or poorly soluble compounds and metabolized or unmetabolized solvents. These models can demonstrate that the supply of a poorly metabolized insoluble solvent to the lungs is usually larger than the amount which the arterial blood can remove from the alveoli. Therefore cardiac output controls the equilibration rate. Since tissue solubility is relatively poor, the rise in tissue concentrations is rapid, although uptake is small. However, pulmonary clearance is very efficient.

Tissue uptake of soluble solvents is relatively large because arterial blood has the capacity to remove all the alveolar supply. Thus at the beginning of exposure the equilibration rate is controlled by alveolar ventilation. Eventually the supply by alveolar ventilation exceeds the capability of the arterial blood to remove the solvent, and equilibrium is mainly controlled by solubility and cardiac output. Thus the rise in concentration is flow limited. Pulmonary clearance is relatively inefficient, which explains the rise in adipose tissue concentrations after exposure has ceased.

Metabolism has little effect on the uptake and elimination of insoluble solvents however. In the case of soluble metabolized compounds pulmonary uptake is increased, but clearance is decreased in favor of urinary elimination.

Simulation models have demonstrated that the concentration of solvent in exhaled air and in blood follows the changes in the airborne levels. The less soluble the solvent, the more rapid the response to fluctuation in exposure levels. Concentrations in venous blood are less responsive than the levels in arterial blood or alveolar air. With poorly soluble solvents the

breath concentration mirrors exposure very closely, whereas with the soluble solvents the breath levels reflect exposure but in a more damped way. The concentration of solvent in alveolar air collected in pre-shift samples may be used as indicators of integrated exposure. The pre-shift alveolar concentration of poorly soluble compounds levels off after 2–3 d of exposure, whereas with a soluble solvent the pre-shift level rises throughout the workweek.

Methods used in breath sampling

During the past 20 years the use of breath analysis has been exploited in breath alcohol studies and anesthetics. Steady progress has been made in the use of breath analysis for the clinical diagnosis of disease, but in clinical and forensic toxicology, and in occupational health monitoring, progress has been slow. Each of these areas has relied on the development of suitable sampling methods and measurement techniques.

Qualitative methods

Qualitative methods have been employed to establish the composition of “normal” breath. These base-line data have been used to link environmental exposure from drinking water and air to foreign compounds found in the body (9, 104). The majority of techniques used for this application, for which concentration techniques are required, consists of collecting large volumes of breath in a bag and transferring the contents to an adsorbent tube. The technique described by Wallace et al (104) and Krotoszynski et al (61, 62) consists of collecting 20–45 l of breath in a plastic sampling bag. The breath constituents are preconcentrated as the sample is pumped from the bag onto a Tenax column. The samples are desorbed from the tube into an injector coil cooled with liquid nitrogen and then flash-heated into a gas chromatograph-mass spectrometer. At least 115 volatile compounds have been identified in breath, and eight compounds accounted for 65 % of the total organic content. They were acetone, dichloronitromethane, isoprene, chloroform, 1-propanol, p-dichlorobenzene, 2-methyl-1-butanol, and dichloroacetylene (61, 62). In another study the four chemicals which appeared with the greatest frequency and the highest geometric mean concentrations were tetrachloroethylene, chloroform, 1,1,1-trichloroethane, and trichloroethylene in the range 1–10 $\mu\text{g}/\text{m}^3$ (104).

A multistage cryogenic sampling system was used for the collection of expired air samples from eight male volunteers. Expired air was passed through a three-stage cryogenic trap for about 60 min. Sixty-nine compounds were identified by gas chromatography-mass spectrometry and found to be mainly aliphatic hydrocarbons (17).

Collection of large volumes of breath over an extended period causes fatigue, and this problem has

resulted in an elaborate system being designed to sample only part of the breath (41). The sampling device consists of a fluidics control unit which operates a series of valves which divert a portion of each individual breath onto a Tenax tube. No positive identification of the individual components has been attempted, and the problem of subject fatigue does not appear to have been resolved.

A technique employing a remote collection device and mass spectrometer has been described by Bryant et al (14) for studying metabolic disorders and toxicologic problems. A remote gas filter made from a polyethylene foam wafer is fitted in a face mask directly in the expired air stream. The filter, which provides relative enrichment and storage of the organic components, is transferred into the vacuum system of a mass spectrometer. The high water content is removed by vacuum pumping, and the remaining compounds are passed directly into the ionization chamber. The fragmentation of the compounds is reduced by use of a graphite filament field ionization source which produces strong molecular ions. Results showed that organic substances which were desorbed from the wafer were enriched by a factor of 10^4 to 10^5 . Background data were given on the breath samples, but no results of the application to clinical or toxicologic problems were reported.

Infrared spectrometry has been used in clinical toxicology for the diagnosis of acute exposure to industrial solvents (95). A number of case reports have been described in which the toxic agent has been identified in the breath of patients. This technique appears to have been superseded by gas chromatography and/or mass spectrometry, which is faster, more sensitive, and selective.

An entirely different semi-quantitative approach for the determination of carbon monoxide in expired air using a colorimetric indicator tube (Dräger Multigas) has been developed. The subject inflates a bag with one single exhalation through a resistance tube. The breath is drawn back through a colorimetric tube with a pump. A white indicating layer, which turns brown-green, enables the carbon monoxide in blood to be estimated as the percentage of carboxyhemoglobin. The system may have application as a rapid diagnostic procedure for carbon monoxide poisoning, but no data are available on the relationship of expired carbon monoxide measured by this method to carboxyhemoglobin.

Quantitative methods

The majority of applications of breath analysis for routine monitoring in the toxicologic and occupational health field requires quantitative data. Biological monitoring requires reliable data on the uptake of solvents if it is to be used to estimate individual health risk (45). Quantitative breath measurements depend on a valid, reproducible sampling technique, and Dubowski (24)

has argued that end-expired air (alveolar air) is generally considered to be the most reproducible sample.

Earlier workers have reported quantitative data based on whole breath samples. A method has been described for the assessment of occupational exposure to benzene. The subject is fitted with a mask with a clean-air inlet. The outlet is connected to a 5.5-l aluminum foil bag. The subject is instructed to breathe normally until the bag is full. The contents of the bag are trapped on a silica gel adsorption tube. The tube is desorbed and analyzed with gas chromatography (39). In studies on trichloroethylene, Monster & Boersma (74) have collected exhaled air from normal breathing through a valve into a polyvinylfluoride bag. The exhaled air was analyzed with electron capture gas chromatography. Veulemans et al (103) used samples of mixed exhaled air collected in syringes to study the pulmonary elimination of n-hexane. The conclusion

that n-hexane concentrations in breath at a fixed time after exposure were poor estimates of individual solvent absorption may be attributed in part to the type of breath sample used.

A device for measuring endogenous ethanol in whole breath of healthy abstaining subjects has been reported (56). A 4-l Tedlar bag and breath-inlet tube were maintained at a temperature of 40°C to prevent condensation. The samples of breath were collected after five rebreathings of the initial exhalation. Immediately after the rebreathing procedure was completed, a measured volume of breath was drawn from the bag into a liquid nitrogen trap and then released into a gas chromatograph-mass spectrometer. A deuterium labeled ethanol internal standard was used. Ethanol in rebreathed air was about 10 % higher than in directly analyzed end-expired air. Ethanol vapor is highly soluble in water and can rapidly equilibrate between airway fluids and respiratory air. After about five cycles of rebreathing the concentration of ethanol in respired air approaches steady state. In the case of ethanol and possibly other solvents, the rebreathed sample, although not very practical as a field sampling method, does correlate well with the blood concentration (54).

The described methods are not considered to be simple, practical field sampling devices, and they do not collect alveolar air, which, it has been argued earlier, is probably the most convenient and reproducible sample available for monitoring. A number of techniques have been described for the collection of alveolar air, and some of the more common samplers are listed in table 3.

Table 3. Breath sampling devices capable of collecting alveolar air.

Sampler	Use	Reference
Haldane-Priestley tube	Respiratory gases Hydrogen	Haldane & Priestley (48) Metz et al (72)
Glass pipette	Solvents	Cohr & Stockholm (16), Stewart et al (95), Stewart et al (96), Stewart et al (97), Verhoeff et al (101)
Aluminum tube	Carbon monoxide	
Syringe	Solvents Hydrogen	Imbriani et al (49) Kneepkens et al (60)
Recoil bag	Hydrogen	Gardiner et al (40)
Evacuated container	Ethanol	Principe (85)
Bag sampler	Ethanol	Wright (111)
Glass vial	Ethanol	Dubowski & Essary (25)
Rahn-Otis sampler	Hydrogen	Gardiner et al (40), Mutti et al (76)
Face mask	Hydrogen	Robb & Davidson (89)
Nasopharyngeal catheter	Hydrogen	Perman et al (83)
Indium capsule	Ethanol	Penton & Forrester (81)

Haldane-Priestley tube. The Haldane-Priestley sampling device was first described in 1905 and was used to collect alveolar samples of respiratory gases (48). It consists of a length of rubber tube (120 cm) with a sampling syringe inserted through the wall close to the mouth piece (figure 2). At the end of expiration the tongue is placed over the tube, and a sample of the air is collected from the stationary column of air by it being drawn into the syringe. With some modifications this method has found acceptance in the measurement of breath hydrogen in the investigation of

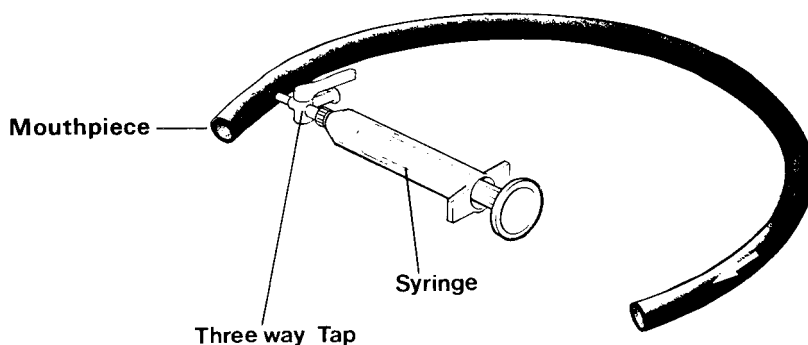


Figure 2. Haldane-Priestley tube.

carbohydrate malabsorption (72). The procedure gave an overall coefficient of variation of 18 %, which accounts for both analytical error and individual variation in hydrogen output over a 10-min period.

Glass pipettes. The glass pipette, developed by Stewart and his colleagues, was derived from the Haldane-Priestley tube (figure 3). It is probably the field-sampling technique which has gained the most acceptance in occupational health monitoring. Alveolar air samples are collected in glass pipettes (length about 25 cm, volume 50 ml) fitted with screw caps containing saran liners. The worker is asked to flush the pipette three times with exhaled breath and then, after holding the fourth breath for 30 s, exhales through the pipette and collects the end portion of breath by replacing the screw caps. The solvent of interest is analyzed by gas chromatography after a sample of breath is removed from the pipette by a gas syringe (95, 96). A number of volunteers were exposed to methylene chloride under controlled conditions, and the individual variation in breath concentrations was wide. The variation between subjects was accounted for by errors in the breath collection, leakage in 8–10 % of the tubes, analytical error, and individual differences (97). It has been reported that there is a 15 % failure rate with these pipettes.

Other workers have used the pipettes to measure breath concentrations of tetrachloroethylene in residents and workers associated with dry-cleaning shops (75, 100), and in cases of environmental exposure to carbon monoxide (101).

Mølhave & Pedersen (73) compared three techniques for the determination of breath toluene, the glass pipette, the gas bag, and a photoionization detector. The results obtained by all three methods did not show any significant difference in the mean values. However, of the three methods, the pipette method was the least precise and showed the greatest interperson variation.

After studying the loss on storage of two solvents, trichloroethane and perchloroethylene, from breath pipettes, Pasquini (80) concluded that water vapor ap-

pears to be linked with losses of up to 65 % within 20 h. The partitioning of a solvent between the air and condensed water vapor on the walls of the pipette appears to be a reasonable explanation to account for this loss. Exhaling into a glass tube at room temperature causes a uniform film of condensate to form, and each purging breath increases the build-up of this water film. The build-up is reduced if the samples are collected and stored at 37°C. Leakage, dilution, and adsorption onto the dry glass surface did not appear to be a significant cause of loss.

Similar storage problems were encountered with pipettes in which alveolar air containing white spirit had been retained. Cohr & Stokholm (16) confirmed that water vapor had contributed to losses, and they circumvented this problem by using a calcium chloride drying filter to remove the water vapor from the breath before it entered the pipette. This drying process and the use of polyamide seals gave a 95 % recovery of white spirit after storage for 24 h. The pipettes were incubated at 90°C for 20 min before being analyzed. The authors commented that further improvements would be necessary if the sampler were to be used in routine practice.

Aluminum tube. An Italian group has described an elaborate version of the pipette sampler (49). The cylindrical sampler (34 ml) is constructed of aluminum with a valve system at each end. The subject is asked to breathe into the sampler three times, followed by a period of apnea, and then exhale through the sampler. When the operator decides to trap the air, activation of the locking device traps the alveolar sample. The samples were collected at 37°C and reheated to 37°C before analysis by gas chromatography. The loss of ethyl acetate, methyl ethyl ketone, and n-hexane from the tubes was about 20 % over 48 h. The precision of the measurements was 10–13 %.

Syringe. Breath samples collected in pipettes or bags usually have to be transferred to a gas chromato-

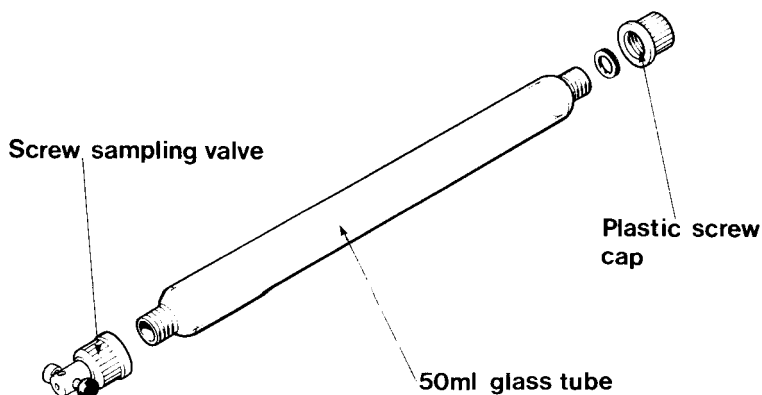


Figure 3. Glass pipette.

graph by syringe with unavoidable losses and poor precision. To improve the precision, attempts have been made to collect the alveolar sample directly into the syringe. The daytime breath hydrogen profile has been reported with the use of a 10-ml syringe with an open side vent at the 9-ml mark. The patient exhales through the barrel of the syringe with the plunger at the 10-ml mark. The plunger is pushed back beyond the outlet, trapping the alveolar sample in the syringe. The mouthpiece is exchanged for a needle, and 3 ml of the breath sample is injected into a vacuum tube. The samples

are analyzed for hydrogen by gas chromatography with a thermal conductivity detector (60). The precision of the analytical method was reported to be about 3 %, and the overall precision, including individual variation, was 16–34 %. This imprecision was thought to be primarily due to inadequate exhalation caused by the restriction in the syringe making a single expiration difficult.

Recoil bag. The recoil bag was adapted from a children's party toy and comprises a mouthpiece with a sample port, a flutter valve, and a spring-loaded nylon recoiling bag (about 100 ml). It behaves as a self-sealing Haldane-Priestley tube. The subject blows through the mouthpiece, and the bag unwinds. As the velocity through the bag decreases, it recoils, trapping an alveolar sample, which is removed through the sample port into a syringe for hydrogen analysis (40).

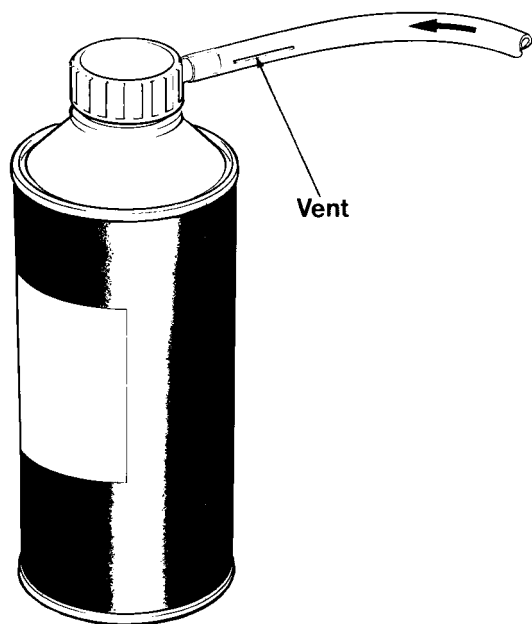


Figure 4. Partially evacuated container.

Partially evacuated containers. A system has been described which is designed to sample alveolar air based on the use of an evacuated container (85). A metal container is partially evacuated to a pressure of 514 mm Hg (68 528 Pa) at 25°C. The subject exhales through a tube attached to the container, and the tidal air vents through a slit (figure 4). At the moment the breath sample is required, depression of the button activator breaks the vacuum, and a predetermined volume of breath is drawn into the can. A sample of air is withdrawn through a septum in the valve cap into a syringe and is analyzed by gas chromatography. Condensation of water on the surface of the container is theoretically unlikely since only one-third of the volume is occupied by breath. It was calculated that condensation would not occur until the temperature of the sampler was below 15.6°C. This technique was applied to breath alcohol analysis, and the results were within 12 % of data analyzed by a breathalyzer. Some solvents have been detected in breath by this device, but no quantitative data have been reported. Although this sampler has the advantage of being portable, unbreakable, and apparently simple to operate, the timing of the collection of the alveolar sample is not obvious. Minimizing the condensation of water is advantageous, but losses on the surface of the container are likely to occur when trace levels of solvent are being measured. Removal of the sample is subject to the problems of transferring gases by syringe.

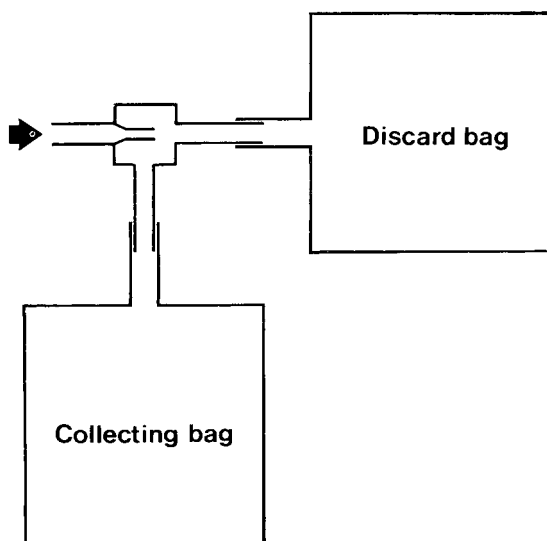


Figure 5. Bag sampler.

Bag sampler. A two-bag system was developed by Wright for collecting end-expired air suitable for breath alcohol analysis. The system consists of two bags connected by an injector "T" mouthpiece (figure 5). The subject blows into the discard bag, and the high velocity through the jet produces a negative pressure which prevents air entering the collecting bag. When the discard bag is full, the pressure in the system rises,

and air enters the collecting bag (111). In a study designed to look at the interaction between toluene and alcohol and drugs, breath samples were collected by blowing the deadspace volume into a rubber bag, and a 1-l teflon bag was used to collect the alveolar sample. The contents of the teflon bag were pumped through a photoionization detector for analysis (23).

In another study using bags, end-tidal breath samples from volunteers exposed to 1,1,2-trichloro 1,2,2-trifluoroethane were collected in saran bags. Each subject breathed the final portion of six consecutive exhalations into the bag (86). A similar technique was adopted for the determination of methylene chloride and carbon dioxide in breath (22). Lauwerys et al (65) collected the last part of an expired air sample through a Pasteur pipette into a polyamide plastic bag after volunteers had been exposed to m-xylene by skin absorption. The bag was closed by welding, and the contents were analyzed for xylene by gas chromatography within 3 h. Under these conditions no losses were observed. Subjects who had been exposed to either toluene, styrene, xylene, trichloroethane, or tetrachloroethylene presented exhaled air samples to polyester-lined polyethylene bags, the alveolar portion remained in the bag. The coefficient of variation was less than 5 % at 0.1 $\mu\text{mol/l}$. The concentration of solvent in expired air was too small for reliable analysis in next morning samples (87).

Glass vial. A breath sampling system reported by Dubowski & Essary (25) consists of a sealed, heated glass vial with two hyperdermic needles penetrating the septum. After normal inhalation the subject exhales through a mouthpiece connected to one of the needles, and the operator removes the vial from the needle assembly after complete exhalation. Two breath samples are collected in rapid sequence and stored for up to 15 d and then analyzed by gas chromatography. The results of this procedure were compared with those of direct alcohol breath measurements using infrared analysis. The authors reported that good agreement was reached between the two methods. This method may create considerable resistance to expiration, and the effect that this resistance may have on the less soluble solvents should be established.

Rahn-Otis sampler. Automatically-timed samples of end-tidal air can be obtained with the Rahn-Otis sampler fitted with a face mask. This device has been used to collect breath samples for hydrogen estimation (40). The same sampler has been used to study the uptake and elimination of hexane. The last 100 ml of each tidal volume was collected for 5 min and taken as alveolar air. The hexane was absorbed in charcoal tubes, desorbed with carbon disulfide, and analyzed by flame ionization gas chromatography (76). There are very little data available on the use of this device in routine monitoring. It has been found to be useful with non-

cooperative patients, and its reliability is reported to be similar to the Haldane-Priestley tube and similar methods.

Face mask. Robb & Davidson (89) designed a face mask sampler for collecting and measuring hydrogen, oxygen, and nitrogen in expired air. The sampler was constructed from a pediatric face mask, two one-way flutter valves which controlled inspiration and expiration, and a three-way stopcock. The mask was fitted over the nose and mouth, and 5-ml samples were aspirated through the stopcock into a syringe. The loss of hydrogen from the syringe was about 4 %/24 h.

In a study in which eight male subjects were exposed to acetone via inspiratory air with a valve and mouthpiece, the alveolar air was analyzed by gas chromatography after sampling with a gas syringe from an outlet in the respiratory valve (108). This technique may be useful in research, but it is not suitable for routine practice.

Nasopharyngeal catheter. The nasopharyngeal catheter was devised for use in pediatrics. The device collects end-expired samples for breath hydrogen with no active patient cooperation. A tube is placed into the anterior nares, and, as the patient breaths normally, the investigator repeatedly collects 1- to 2-ml fractions of each end-tidal sample until 15 ml has been collected. Breath hydrogen was measured by gas chromatography using a microthermistor detector (83). The technique was compared against the modified Haldane-Priestley method and was found to give slightly higher values. A similar device was compared with a valved sampler, and both sets of data were normalized to a constant oxygen level in preference to carbon dioxide adjustment. The authors concluded that the nasopharyngeal catheter gave the best results (13). It is unlikely that this technique would be acceptable to the workforce.

Indium capsule. Indium encapsulated samples have been used for the indirect analysis of breath alcohol. This sampler consists of an indium tube in a heated box (45°C). The subject exhales through the tube, and the final portion of the breath is collected in triplicate by a crimping device. The sample is transferred to a gas chromatograph automatically through a needle which penetrates the capsule and permits the sample to be placed on the column. The technique has a coefficient of variation of 2—3 %, and samples are stable for up to 90 d (81). This system overcomes a number of problems associated with many samplers. Duplicate or triplicate alveolar air samples are collected and analyzed at a temperature which prevents dissolution of the alcohol into a surface film of moisture. The sample is removed by a gas valve system which avoids the disadvantages of removal by syringe. The indium

tube is expensive, and its application to the measurement of low levels of solvent in breath may require much larger sampling tubes.

Discussion. It has been argued that the most appropriate breath sample for use in routine monitoring is the alveolar (end-expired air) sample. Several devices capable of collecting alveolar air samples have been examined, and the mechanism of action and their reliability have been discussed. The principle behind the Haldane-Priestley tube has featured prominently in most samplers and is still the most popular approach. The device is simple and provides an easy method of collecting an alveolar sample without back-pressure on the airways. The difficulty of trapping the alveolar sample without loss in integrity has resulted in several modifications. However, as they become more complex, they lose their appeal as a simple, and inexpensive, sampler.

Almost all the collection devices examined have two common disadvantages. First, the surface of plastic, glass, or metal containers has certain adsorption characteristics, and losses on the surface of the material may be significant, particularly when trace levels of solvent are being measured. The problem is exacerbated when a film of moisture forms on the surface of the container, and losses of solvent may be considerable. The two approaches which have been adopted to minimize condensation are removal of water by a calcium chloride filter (16) or maintenance of the temperature of the sampling device above 40°C. Recovery of the solvent improves, but the modifications required are not very practical for field use.

Second, even if the integrity of the breath sample is maintained, it must be transferred to an analytical instrument, usually a gas chromatograph. Syringes offer an inexpensive and simple method of transfer, but they also contribute to poor precision. Diffusion of ambient air into, and sample out of, the syringe causes loss of sample integrity. Repeatable injections into a gas chromatograph can be difficult with larger volumes (eg, 1–2 ml). The flow of carrier gas from the pressurized injection port of the gas chromatograph into the syringe causes dilution and incomplete mixing with the carrier gas. In addition leakage of the sample past the plunger when under pressure from the carrier gas may cause losses.

All these sample collection devices are relatively expensive and are unlikely to be considered as disposable units. Consequently, they require cleaning for reuse. Removal of solvent and moisture is time consuming, and possible carry-over must be eliminated.

The use of an indium capsule is an attractive possibility for trace solvent analysis. However, the size of sample required and the cost of indium makes this technique unsuitable for monitoring workers.

Direct methods for breath analysis

A number of analytical methods have the advantage of being able to measure breath components directly, avoiding the use of collection and sampling devices.

Infrared spectrometry. Infrared analysis has been established as a reliable technique for monitoring breath alcohol (70), but interference from breath acetone has been observed with some single wavelength instruments (26). Infrared analysis has been used to measure fluorocarbons in breath (99), but is no longer a popular technique for breath solvent analysis, probably because its selectivity and sensitivity cannot compete with those of other analytical methods. It has been used for the determination of carbon dioxide in a study of 1,1,1-trichloroethane exposure (47). Alveolar air samples were collected from eight volunteers. The system consisted of a one-way glass valve, maintained at 40°C to avoid condensation. The sample was continuously aspirated into an infrared spectrometer for carbon dioxide analysis but was then pumped to a gas sampling valve for the gas chromatographic analysis of 1,1,1-trichloroethane.

Gas chromatography. A specialized gas chromatograph with a flame ionization detector has been used for breath alcohol analysis (81). The subject exhaled through a mouthpiece into the instrument, which automatically sampled the last 0.25 ml of alveolar air by a pressure switch. Breath alcohol analyses were performed in 90 s with a precision of 5 %. Alveolar air concentrations of tetrachloroethylene during the absorption and elimination phases have been followed directly by gas chromatography. The alveolar air sample was directed by a specially designed apparatus maintained at 37°C into the gas sampling valve of the gas chromatograph and measured by flame ionization detection (32). A gas sampling valve is the most precise method of introducing a breath sample onto a gas chromatograph column. Whenever an appreciable concentration of water vapor is present, as is the case in breath, the valve should be heated to prevent condensation. The most robust and popular detector is the flame ionization detector, which has adequate sensitivity for monitoring breath solvent concentrations during or immediately after exposure. Sensitivity may not be adequate for preshift monitoring where the concentration in breath may be in the parts-per-billion range. Under these circumstances the excellent sensitivity of the electron capture detector may be exploited provided the solvent is electrophilic. Gas chromatography may be a useful technique for breath analysis, but it cannot be used for the continuous monitoring of exhaled air.

Electrochemical detectors. Electrochemical cells have been used for breath ethanol determinations and have been reported to be reliable for traffic law enforcement

(27). The fuel cell is housed in a breath-sampling chamber maintained at 60°C. The subjects take moderately deep inspirations and immediately exhale through the mouthpiece tube attached to the meter. Breath is sampled from the last part of a prolonged exhalation, and ethanol is oxidized at the fuel cell electrode. The device was found to have a high degree of precision (3.2 %) at a mean blood alcohol level of 50 mg/100 ml (55). The cell does not respond to ketones such as acetone but may respond to other alcohols and aldehydes. The concentration of ethanol in breath vastly exceeds the concentration of possible interfering substances, and therefore the technique has been found to be acceptable for breath alcohol analysis. However, these cells are unlikely to be useful for measuring low concentrations of solvents in breath following industrial exposure because of interference and lack of specificity.

Photoionization detector. The photoionization detector contains an ultraviolet lamp which emits photons with sufficient energy to ionize organic compounds. The current measured across the electrodes is proportional to the concentration. This type of detector has been used for the measurement of alveolar concentrations of toluene during a human volunteer study (73). Sixteen volunteers who were taking part in an exposure chamber study presented breath samples to a photoionization detector with a 1 l/min airflow to the detector cell. Each subject used a nose clip and was instructed to breathe deeply three times. The last exhalation was performed at a normal expiration velocity through the mouthpiece, and the instrument was allowed to reach equilibrium. This technique was compared with the glass pipette and gas bag methods; there was no significant difference between the mean values of the detector and pipette methods, but significantly higher values were obtained with the gas bag method. The major disadvantages with the photoionization detector were its poor specificity, since it responds to most organic compounds, and the detection limit for toluene which was reported to be only 5 ppm.

Mass spectrometry. Since the introduction of the respiratory mass spectrometer by Fowler & Hugh-Jones (38) in 1957, there has been rapid progress in respiratory gas analysis, largely as a result of technical improvements in the design of inlet systems, ionization sources, and electronics. These small quadrupole mass spectrometers have been used in the diagnosis of respiratory disorders and for patient monitoring during anesthesia. The respiratory mass spectrometers are not sufficiently sensitive for fast breath-by-breath monitoring of trace levels of organic compounds. Several approaches have been used to meet these requirements. Tandem mass spectrometry uses a mass spectrometer to fragment the compounds and a second mass spectrometer to separate the resulting molecular ions on

the basis of their mass to charge ratio (71). The compounds in a mixture can be identified by their fragmentation pattern and quantified by measurements of selected daughter ions. This promising technique is extremely sensitive down to the femtogram range, and the response time is very short (approximately 1 ms). But because of the very high cost of these instruments, they are unlikely to be popular as a routine monitoring technique.

Atmospheric pressure chemical ionization-mass spectrometry offers a method for direct, instantaneous analysis of trace compounds in breath. The subject exhales normally into a mouthpiece at a steady flow rate, and the breath sample, which is diluted 300 times with carrier gas, is introduced into the ionization region of the instrument (67). The range of 17–20 mass units has been rapidly scanned during a 30-s exhalation, and the breath profile showed the alveolar plateau for protonated ammonia ($[\text{NH}_4]^+$). Breath ammonia was measured by single ion monitoring for up to 48 h in two volunteers. The breath ammonia concentration appeared to increase as protein catabolism increased, and the mean concentration was 0.95 ppm. Acetone levels in the expired air of normal subjects was found to range from 0.6 to 2 ppm. Pollutants found in the breath of laboratory technicians were styrene ($\text{MH}^+ = 105$) and phthalic anhydride ($\text{MH}^+ = 149$), and naturally occurring metabolites were methanol, acetaldehyde, and ethanol. The sensitivity of the techniques was shown to be in the parts-per-million to parts-per-trillion range.

In this study the direct introduction of breath into the ion chamber produced saturation and interference effects. Although ammonia is not a major breath component, it has a higher proton affinity than the other breath components, the result being a saturated signal at m/z 18 NH_4^+ . This saturation was overcome by a redesigned breath inlet system (10). However, with the fastest scan speed, a complete 200 mass-unit scan could not be obtained during one exhalation. A positive mode breath scan exhibited a prominent peak at m/z 59 corresponding to protonated acetone. Other major ions corresponded to protonated water, ammonia, methanol, and ethanol. Atmospheric pressure ionization-mass spectrometry has been used in exposure chamber studies to determine the uptake and elimination of methanol, toluene, and tetrachloroethylene in human volunteers (11). Green (46) described the use of a Llewellyn-Arnold molecular separator with a water-removing device attached to a mass spectrometer. Breath was introduced into the inlet port of the separator, and real-time monitoring of ethanol in breath was possible. Samples of breath and vapor emanating from the forearm were compared, and the concentration of ethanol was one order of magnitude greater in breath than in vapor from the forearm.

The use of a relatively inexpensive, transportable mass spectrometer has been reported for the direct measurement of some industrial solvents in breath

(109). A quadrupole mass spectrometer of the range of 200 mass units with a specially designed membrane inlet is coupled to a breath sampling system comprised of a modified Haldane-Priestley tube. This procedure permits the entire expiration to be monitored in real-time with no back-pressure on the airways. The analyzer is maintained at 60°C to prevent condensation. The instrument is capable of detecting low levels of organic solvents in breath, typically low parts per billion, with a fast response time to give a breath-by-breath profile. Water vapor was shown to reduce the transmission of organic compounds across the membrane, and a 25 % reduction in response at a concentration of 1 mg/m³ for toluene (10.9 μmol/m³), dichloromethane (11.8 μmol/m³), and trichloroethane (7.5 μmol/m³) was observed. The transmission time for the more polar compounds, such as ketones, was slower, and it was difficult to ensure that the alveolar plateau had been reached. The system can monitor up to eight preselected ions, which improves specificity or permits multiple solvent monitoring. If a correction for carbon dioxide is required, ¹³C carbon dioxide is a suitable marker and may be monitored by one of the channels. The instrument has been transported to several factories and operated under field conditions. It has proved to be reliable, and there has always been an enthusiastic response from the workforce.

The elimination of trichloroethane from the breath of a volunteer who had been exposed to 750 mg/m³ (5.62 μmol/m³) of the solvent for 4 h was followed for 7 d down to parts-per-billion levels. The technique has been used under field conditions to measure both pre- and postshift carbon disulfide in the breath of workers exposed to the solvent (15).

Discussion

Breath analysis is an attractive noninvasive way of monitoring workers who are occupationally exposed to industrial solvents. The technique has been slow to develop and reach acceptance within the field of occupational medicine. There are justifiable reasons for a cautious approach, and two of the main considerations have been addressed in this review. First, a number of physiological and biochemical factors that deserve attention when a breath analysis monitoring strategy is established for occupational exposure to solvents has been identified. The impact of these factors should be examined when sampling methods and a sampling strategy are being devised.

Several types of expired air have been used in breath analysis, but it has been argued that alveolar (end-expired) air is the easiest sample to collect and probably the most reproducible. Experience has shown that the concentration of solvent in end-tidal air is close to that in end-expired air, but the instruction and training of the subjects and the collection of such a sample is difficult. Forced expirations may cause airway closure and, consequently, a sharp rise in

solvent concentration at the end of the expiration. The favored sampling technique is a steady expiration of a normal breath. The validity of such a sample is best established by use of a continuous monitoring breath analyzer.

Blood levels of solvents are considered to be useful indicators of uptake, and, if breath concentrations are to replace blood levels, then the relationship between the blood:breath ratio should be established. In normal healthy workers changes in the ventilation:perfusion ratio will not markedly alter the blood:breath ratio, except in the case of very insoluble compounds.

Identification of the alveolar plateau helps to identify potential problems associated with diffusion, the effect of temperature, and the breathing technique.

Exercise affects not only the uptake, but also the elimination of solvents, and a marked reduction has been observed in breath concentrations of some solvents in workers who regularly exercise outside the workplace. Since the elimination of solvents from the blood-rich compartments is relatively rapid (within a few hours), the blood:breath ratio is more variable. The elimination from the fat compartment is much slower, and the effect of time will be less dramatic. The blood:breath ratio is clearly linked with time, and differences in the ratio may be minimized by the adoption of a preshift monitoring strategy.

Some consideration should be given to the possibility that some solvents may undergo metabolism in the lung, although it is unlikely that this phenomenon is of practical significance. Clearly, it is not possible to control changes in blood composition (hematocrit, lipids), but it has been predicted that such changes would not affect the blood:breath ratio by more than 10 %.

Second, the availability of reproducible, robust, and reliable techniques for collecting and measuring trace levels of solvent in alveolar air has been a limiting factor. If field samples are to be collected prior to analysis, attention should be directed towards collecting a valid alveolar sample and ensuring that losses are minimized. Many of the devices described in this paper are theoretically capable of trapping an alveolar air sample and are suitable for use in field studies. However, the main disadvantage with all these techniques is the difficulty in ensuring that an alveolar sample has been collected and that it was not diluted with tidal air. The samplers should be easy to use, require little training by the operators or subjects, and be user friendly.

Even if a valid alveolar sample is collected, the problems of losses through leakage, adsorption, formation of water film, and transfer to the analytical instrument are common to all methods. The temperature of breath and the high water content cause condensation and recovery problems. The maintenance of the sample at 37°C may overcome this difficulty, but the necessary modifications to the samplers make this technique less attractive as a field sampling device.

The degree of adsorption and loss will depend on the nature of the material used to make the breath collecting device. Almost all surfaces, whether glass, metal, or plastic, will present some problems. Polyvinyl fluoride and polyvinylidene chloride plastics have low permeability to organic vapors in comparison with polythene. But all plastics are permeable to most vapors to some degree, and, when a high water content and trace levels of organic material are being dealt with, recovery can be a problem.

Problems have been encountered in establishing that a valid alveolar sample has been collected without loss or dilution. The use of carbon dioxide levels in breath to endorse the validity of the alveolar sample has advantages. Under some circumstances, correction of the solvent concentration to the carbon dioxide level may improve the precision of sampling (47). However, the carbon dioxide concentration may not always parallel the solvent under investigation. Therefore, a continuous monitoring method which enables the alveolar plateau to be observed overcomes this problem.

Direct measurement of the solvent concentration in the alveolar air is to be preferred. The most satisfactory method of measuring trace solvents in alveolar air is the use of a direct continuous monitoring method in which the alveolar plateau is clearly visible and from which the concentration of solvent can be calculated. The mass spectrometric methods have the speed, specificity, and sensitivity to meet these requirements but are relatively expensive. It would seem important to validate simple field sampling devices against the mass spectrometric or similar methods.

When reliable methods of monitoring alveolar air are available, attention can be directed towards the interpretation of the results. The extensive literature on the blood:breath alcohol ratio has illustrated the problem of extrapolating from breath to blood concentrations (57). Work on the uptake of anesthetics and the use of mathematical models illustrates that breath and blood samples collected during or after exposure will provide data on different aspects of tissue distribution. It must not be assumed that the concentration of solvent in breath samples will reflect the concentration in blood samples under all circumstances. Consideration must be given to the type of breath and blood sample under investigation. During exposure to solvents, equilibration of alveolar to arterial and to brain partial pressures is achieved relatively quickly. During this period the alveolar concentration will reflect the brain concentration. At steady state the partial pressures in alveolar air, arterial blood, tissues, and venous blood are in equilibrium. This is the ideal sampling time. However, this state rarely exists in the industrial workplace. During the postexposure period, when the inspired concentration is zero (the condition most frequently encountered in the industrial setting), the tissue and the venous blood concentrations are higher than the arterial concentration. In the postexposure period after the initial washout from the blood com-

partment the alveolar concentration is closely related to the mixed venous concentration, which is a summation of the tissue levels (body burden).

Recent work on the uptake of solvents through skin absorption has identified a difficulty in the use of peripheral venous blood for biological monitoring (4). The problems associated with using peripheral venous blood sampling when skin absorption is suspected may be overcome by the use of alveolar air, which more closely reflects the mixed venous concentration during the postexposure period and is not subject to local absorption.

There is always a time lag between the concentration of solvent in the peripheral and mixed venous blood and alveolar air. This situation can be explained from a theoretical viewpoint by a multicompartamental model. It becomes clear that blood samples collected from a peripheral vein do not necessarily reflect the body burden of a solvent. Peripheral blood concentrations appear to be related in a complex manner to the partial pressures of mixed venous and alveolar air. Such features as exposure concentration and duration, physical work load, work practice, and time, in addition to all the biological variables, are interrelated.

Conclusions

1. Breath analysis is potentially useful as a biological monitoring technique for estimating the uptake of some solvents in workers. It may have advantages over blood samples if information relating to arterial or brain concentrations is required or if skin absorption is a major route of entry.
2. It has been concluded from theoretical and practical aspects of breath analysis that alveolar air is the most suitable and reliable breath sample for routine monitoring.
3. Mass spectrometry is probably the most versatile and useful technique, and it overcomes all the major disadvantages associated with collection devices.
4. Small-breath collection devices for use in routine practice should collect alveolar air, be easy to use, offer little resistance, minimize condensation, and be amenable to a specific and sensitive analytical technique.
5. A number of physiological and biochemical parameters may affect the concentration of solvent in breath. These effects should be minimized by careful attention to an appropriate sampling strategy.

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