Breath tests are among the least invasive methods available for clinical diagnosis, disease state monitoring, and environmental exposure assessment. In recent years, interest in breath analysis for clinical purposes has increased. This review is intended to describe the potential applications of breath tests, including clinical diagnosis of diseases and monitoring of environmental pollutant exposure, with emphasis on oxidative stress, lung diseases, metabolic disorder, gastroenteric diseases, and some other applications. The application of breath tests in assessment of exposure to volatile organic compounds is also addressed. Finally, both the advantages and limitations of breath analysis are summarized and discussed.

Physical, biochemical, and molecular biological methods for medical monitoring and diagnostics have rapidly developed in recent decades. The major developments in medical monitoring technologies and diagnostic methods have focused on blood and urine analysis for clinical diagnostics. Relatively, diagnostics based on breath analysis are much less developed and not yet widely used in clinical practice (1). Although a few types of breath tests have been successfully used as diagnostic tools in clinical analyses, such as the [13/14C]urea breath test (UBT)1 in the diagnosis of Helicobacter pylori infection (2–4) and the NO breath test in the diagnosis of airway inflammation (5–7), breath analysis could have many more potential applications in the clinical diagnosis of disease and monitoring of exposure to environmental pollutants.

The bulk matrix of breath is a mixture of nitrogen, oxygen, CO2, H2O, and inert gases. The remaining small fraction consists of more than 1000 trace volatile organic compounds (VOCs) with concentrations in the range of parts per million (ppm) to parts per trillion (ppt) by volume (5, 8–10). In terms of their origin, these volatile substances may be generated in the body (endogenous) or may be absorbed as contaminants from the environment (exogenous). The composition of VOCs in breath varies widely from person to person, both qualitatively and quantitatively. Phillips et al. (8) systematically assayed the VOCs and their variations in the breath of healthy humans. Although the number of VOCs found to date in human breath is more than 1000, only a few VOCs are common to all humans. These common VOCs, which include isoprene, acetone, ethane, and methanol, are products of core metabolic processes and are very informative for clinical diagnostics (10). The bulk matrix and trace VOCs in breath exchange between the blood and alveolar air at the blood–gas interface in the lung. One exception is NO, which is released into the airway in the case of airway inflammation.

The endogenous compounds found in human breath, such as inorganic gases (e.g., NO and CO), VOCs (e.g., isoprene, ethane, pentane, acetone), and other typically nonvolatile substances such as isoprostanes, peroxynitrite, or cytokines, can be measured in breath condensate (5). Testing for endogenous compounds can provide valuable information concerning a possible disease state. On the other hand, exogenous molecules, particularly halogenated organic compounds, can indicate recent exposure to drugs or environmental pollutants (5, 11).

Because breath tests are among the least invasive methods for monitoring a person’s disease state or exposure to a drug or an environmental pollutant, interest in breath analysis for clinical diagnosis has increased in recent years. This review is intended to describe the

1 Nonstandard abbreviations: UBT, urea breath test; VOC, volatile organic compound; ROS, reactive oxygen species; MDA, malondialdehyde; ppm, ppt, and ppb, parts per million, parts per trillion, and parts per billion, respectively; GC, gas chromatography; COPD, chronic obstructive pulmonary disease; ARDS, adult respiratory distress syndrome; EBC, exhaled breath condensate; MS, mass spectrometry; THC, tetrahydrocannabinol; and PBPK, physiologically based pharmacokinetic (model).
potential applications of breath tests, including clinical
diagnosis of diseases and monitoring of exposure to
environmental pollutants or drugs.

Depending on the origin of the substances found in
human breath, i.e., endogenous or exogenous, the typical
applications of breath tests fall into two main categories:
diagnosis of disease and assessment of exposure to envi-
ronmental pollutants.

**Diagnosis**

The detection of VOCs in breath for the purpose of
diagnosis has a long history. Ancient Greek physicians
already knew that the aroma of human breath could
provide clues to diagnosis. The astute clinician was alert
for the sweet, fruity odor of acetone in patients with
uncontrolled diabetes; the musty, fishy reek of advanced
liver disease; the urine-like smell that accompanies failing
kidneys; and the putrid stench of a lung abscess (12).
Modern breath analysis started in the 1970s when re-
searchers, using gas chromatography (GC), identified
more than 200 components in human breath [reviewed by
Miekisch et al. (5)]. For more than a decade the main
problems were the effective separation and identification
of exhaled substances, but because of technical advances
in analytical methods in the 1980s and 1990s, this is no
longer the case. Instead, issues concerning the physiologic
meaning of breath substances and correlations of breath
markers with patients’ clinical symptoms have become
increasingly important (5, 13). Since the 1990s, researchers
have worked hard to understand the relationship between
various breath substances and physical condition.

In terms of study methods, breath testing has been the
focus of both cross-sectional and longitudinal studies (5).
Cross-sectional studies have investigated exhaled biomar-
kers as a function of disease, both as biomarkers of disease
state and as predictive markers. In cross-sectional studies,
a control group is compared with a patient or diseased
group, and breath markers are analyzed to identify qual-
itative or quantitative differences between the two
groups. The differences established in this way should be
large enough to enable clinically relevant predictive use of
breath markers. Longitudinal studies of disease progres-
sion involve biomarkers of disease progression, whereas
longitudinal studies of therapeutic intervention involve
biomarkers of therapy. Breath markers are observed dur-
ring the course of a disease or an intervention within one
patient group. This approach is also well suited for
monitoring of pharmacologic interventions.

As a result of extensive studies, a few breath markers
have been discovered and successfully used in diagnosis
of disease, and some of these markers are very promising.
In this section, we reviews the links between substances
measured by breath testing and disease. The breath mark-
ers for different diseases and applications are summa-
rized in Table 1.

### Table 1. Breath markers in certain diseases or applications.

<table>
<thead>
<tr>
<th>Disease or application</th>
<th>Breath marker</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative stress</td>
<td>Lipid peroxidation, Pentane, ethane, H₂O₂, nitrite/nitrate</td>
<td>(9, 25, 26)</td>
</tr>
<tr>
<td></td>
<td>Asthma, COPD, bronchiectasis, ARDS</td>
<td>(19–21, 31)</td>
</tr>
<tr>
<td>Lung diseases</td>
<td>Asthma, NO, CO, H₂O₂, isoprostanes, nitrite/nitrate</td>
<td>(6, 7, 55–57, 60, 61, 66, 69)</td>
</tr>
<tr>
<td></td>
<td>COPD, NO, H₂O₂, eicosanoids (leukotrienes, prostanoids, isoprostanes), isoprostanes</td>
<td>(54, 62, 63, 67)</td>
</tr>
<tr>
<td></td>
<td>Cystic fibrosis, NO, CO, H₂O₂, isoprostanes, nitrite/nitrate</td>
<td>(58, 59, 64, 65, 68, 70, 71)</td>
</tr>
<tr>
<td>Metabolic disorder</td>
<td>Pulmonary allograft dysfunction</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>Lung cancer</td>
<td>NO</td>
</tr>
<tr>
<td>Gastroenteric diseases</td>
<td>Lung transplant recipient with acute rejection</td>
<td>Exhaled carbonyl sulfide</td>
</tr>
<tr>
<td>Assessment of exposure</td>
<td>Diabetes, Disorders of digestion and absorption (lactase deficiency, disorders of di- and monosaccharide malabsorption, starch malabsorption, and small-bowel bacterial overgrowth)</td>
<td>Acetone</td>
</tr>
<tr>
<td>to VOCs</td>
<td>Gastritis, duodenal ulcer, gastric ulcer, and gastric cancer</td>
<td>H₂</td>
</tr>
<tr>
<td>Other applications</td>
<td>Respiratory monitoring, CO₂/O₂ ratio</td>
<td>Isotopes of carbon (¹³C or ¹⁴C)</td>
</tr>
<tr>
<td></td>
<td>Excretion of drugs</td>
<td>Vinyl chloride and cis-1,2-dichloroethene, chloroform and bromodichloromethane, trichloroethene</td>
</tr>
</tbody>
</table>

* BMAC, breath methylated alkane contour.
OXIDATIVE STRESS

Oxidative stress is a condition in which cells are damaged as the result of a chemical reaction with oxidative agents such as oxygen-derived free radicals. Free radicals damage components of cell membranes, proteins, or genetic material by "oxidizing" them—the same chemical reaction that causes iron to rust. Reactive oxygen species (ROS), such as the superoxide anion (O$_2^-$) or the hydroxyl radical (OH$^-$), act physiologically as defense mechanisms against microbial attack (9,14). ROS are generated in large concentrations by activated granulocytes and can potentially damage any cellular structure. Of the oxygen typically exchanged in respiration, 2%-5% is used to produce ROS in the mitochondria (15). Under healthy conditions, ROS activity is restricted to limited regions of external attack or inflammation and is well balanced by antioxidant protection of the body. However, in some diseased states, the balance between ROS activity and protection may be impaired when antioxidant systems are overwhelmed or exhausted (16). Whenever ROS activity takes place in an uncontrolled manner, the organism itself will be damaged by oxidative stress.

Oxidative stress is among the most frequent pathologic conditions in critical illness (17). Multiorgan dysfunction or multiorgan failure, which is among the leading causes of morbidity and mortality in critical care, is a result of oxidative stress (18). The oxidative stress status in humans can be measured through urine, plasma, blood, and breath. Because of the advantages of breath tests, many studies of breath markers related to oxidative stress status have been reported (19–26). Clinical studies have demonstrated a close correlation between clinical conditions with high inflammatory or peroxidative activity and the exhalation of hydrocarbons generated through ROS attack on membrane lipid structures (22). Lipid peroxidation is a chain reaction that is initiated by removal of an allylic hydrogen atom through ROS. The radical generated in this way is conjugated, peroxidized by oxygen, and undergoes further reactions. Eventually, saturated hydrocarbons such as ethane and pentane are generated in this way from $\omega$-3 and $\omega$-6 fatty acids, which are basic components of cell membranes (9, 24). Aldehydes such as malondialdehyde (MDA) are generated along the same pathway (9).

In studies, the concentrations of exhaled pentane and ethane correlated well with other lipid peroxidation markers such as MDA, thiobarbituric acid-reactive substances, and glutathione (25). In vitro studies have also shown that ethane and pentane are generated when cell cultures are exposed to ROS (9). These compounds are therefore regarded as in vitro and in vivo markers of lipid peroxidation. Breath markers are often more sensitive than the serum markers MDA and thiobarbituric acid-reactive substances (26). Hydrocarbons as stable end products of lipid peroxidation show only low solubility in blood and are excreted into the breath within minutes of their formation in tissues. Exhaled concentrations of ethane and n-pentane can therefore be used to monitor the degree of oxidative damage in the body (9,23). In addition, H$_2$O$_2$ measured in breath condensate is considered a breath marker of oxidative stress in lung diseases as well as a marker of oxygen radical–mediated tissue damage in cystic fibrosis (CF) (27–30). H$_2$O$_2$ is formed by inflammatory cells in the upper and lower airways. In general, exhalation of H$_2$O$_2$ appears to increase during unstable disease and is related to the total number of eosinophils in the sputum in asthma and with the total number of polymorphonucleotides in induced sputum in chronic obstructive pulmonary disease (COPD). Increased concentrations have been demonstrated in patients with asthma, COPD, bronchiectasis, and adult respiratory distress syndrome (ARDS). Lases et al. (30) investigated whether oxidative stress occurs after lobectomy and pneumonectomy and evaluated whether markers of oxidative stress might be of value in the assessment of the diagnosis, course, and prognosis of postoperative complications. Their results show increased H$_2$O$_2$ and MDA concentrations in lobectomy patients compared with pneumonectomy patients. A strong correlation was found between H$_2$O$_2$ and MDA concentrations. Antczak (29) found increased H$_2$O$_2$ content in the exhaled breath condensate (EBC) of patients with asthma, COPD, ARDS, CF, and lung cancer and of healthy smokers. H$_2$O$_2$ concentrations in EBC were further increased when COPD and asthma were exacerbated and decreased after steroid treatment and long-term treatment with N-acetylcysteine in COPD patients. Furthermore, Kostikas et al. (28) evaluated the concentrations of H$_2$O$_2$ and 8-isoprostane in the EBC of patients with COPD and assessed the relationship between the above markers of oxidative stress and indices of inflammatory process and disease severity. They concluded that H$_2$O$_2$ and 8-isoprostane concentrations are increased in the EBC of patients with COPD but that H$_2$O$_2$ seems to be a more repeatable and more sensitive index of the inflammatory process and disease severity.

In addition to the breath markers of oxidative stress described above, a group headed by Michael Phillips investigated the breath methylated alkane contour considered it a breath marker of oxidative stress (19–21, 31). They analyzed VOCs in alveolar breath by GC coupled with mass spectrometry (MS) to construct the breath methylated alkane contour, a 3-dimensional display of the abundance of C$_4$ to C$_{20}$ alkanes and monomethylated alkanes as a function of carbon chain length. In a study of 50 healthy individuals, the mean breath alkane profile was negative from C$_4$ to C$_{11}$ and positive from C$_{12}$ to C$_{20}$. The mean age of the older half of the group was significantly greater than the age of the younger half (47.56 vs 29.88 years; P <0.0001), and the mean alveolar gradients of 4 alkanes (C$_5$ to C$_8$) were significantly more positive in the older members of the group (P <0.05). There were no significant differences between males and females. The authors concluded that the spectrum of alkanes in the breath of healthy humans contains apparent markers of...
oxidative stress (27). Furthermore, they found significantly increased concentrations of breath markers indicative of oxidative stress in younger as well as older persons compared with individuals 20–40 years of age (21), in women with uncomplicated pregnancies (20), in women with preeclampsia (20), and in patients with diabetes mellitus (19). However, the biochemical pathways of generation and the physiologic meaning of these compounds remain to be elucidated in sufficient depth (9).

LUNG DISEASES

Many lung diseases, including asthma, COPD, bronchiectasis, CF, interstitial lung disease, and ARDS, involve chronic inflammation and oxidative stress. Because peroxidation and other reactions of ROS are basic mechanisms of inflammatory processes, markers of organic lipid peroxidation should be increased under these conditions. In fact, increased concentrations of pentane and ethane have been measured in the breath of patients with asthma (32), COPD (33), obstructive sleep apnea (34), pneumonia (35), and ARDS (23).

As one of the most important breath marker of lung diseases, exhaled NO has been studied extensively in different lung diseases, such as asthma, COPD, and CF. NO is synthesized by 3 isoforms of NO synthase encoded by 3 distinct genes: nitric oxide synthase 1 (NOS1), nitric oxide synthase 2A (NOS2A), and nitric oxide synthase 3 (NOS3). Genome-wide searches have identified linkages to asthma on chromosomes 7, 12, and 17, where these 3 genes are localized. The increased concentrations of exhaled NO in asthma originate predominately in the lower airway (36) and are most likely attributable to activation of NOS2 by damage to airway epithelial cells and by inflammation (37,38), with a small contribution from NOS1 (39).

Asthma is a chronic inflammatory disorder of the airways that produces airway hyperresponsiveness, reversible airflow obstruction, and symptoms such as wheezing, cough, and shortness of breath. An increase in exhaled NO is not specific for asthma, but an increased concentration may be useful in differentiating asthma from other causes of chronic cough (40,41). The diagnostic value of exhaled NO measurements to differentiate between healthy persons with or without respiratory symptoms and patients with confirmed asthma has been analyzed recently by Dupont et al. (42), who obtained a 90% specificity and 95% positive predictive value when exhaled NO >15 parts per billion (ppb) was used as a cutoff for asthma. The intrairindividual CV of exhaled NO in healthy persons was 15.8% within an interval of 7 days and 16.8% within 23 days, suggesting that a 30%–35% or greater change in exhaled NO within an interval of 1–3 weeks would be abnormal (43). More recent data indicated that fractional exhaled NO at a cutoff of 16 ppb has a specificity for the diagnosis of asthma of 90% and a positive predictive value >90% (44), suggesting that the simple and absolutely noninvasive measurement of exhaled NO can be used as an additional diagnostic tool for the screening of patients with a suspected diagnosis of asthma (41).

COPD is a condition characterized by progressive airflow obstruction and a presumably chronic inflammation (45). In stable COPD, exhaled NO has been shown to be related to the severity of the illness. Concentrations of exhaled NO in patients with stable COPD are lower than concentrations in either smoking or nonsmoking asthmatics (46–48). Patients with unstable COPD, however, have higher NO concentrations than do smokers or ex-smokers with stable COPD (49), which may be explained by increased neutrophilic inflammation and oxidant/antioxidant imbalance (41). Therefore, NO may be considered a useful marker for monitoring clinical instability in these patients (49).

NO can also be a marker of pulmonary allograft dysfunction. Fisher et al. (50) investigated the NO concentration in the exhaled breath of lung transplant recipients and found that exhaled NO was increased in lung transplant recipients with lymphocytic bronchiolitis, early obliterative bronchiolitis, and infection. Their findings suggested that measurement of exhaled NO may have a role as a marker of pulmonary allograft dysfunction.

In addition to pentane/ethane and NO, some other breath markers of different lung conditions have also been reported. Phillips and coworkers (51,52) investigated alveolar gradients (i.e., the abundance in breath minus the abundance in room air) of C4 to C20 alkanes and monoalkylated alkanes in the breath as tumor markers in primary lung cancer. They concluded that a breath test for C4 to C20 alkanes and monoalkylated alkanes provided a rational new set of markers that identified lung cancer in a group of patients with histologically confirmed disease. Studer et al. (53) evaluated the utility of exhaled breath biomarkers in lung transplant recipients with acute rejection. They found increased concentrations of exhaled carbonyl sulfide in patients with acute rejection compared with stable patients, suggesting a diagnostic role for this noninvasive biomarker. Recently, Montuschi (54) reviewed EBC analyses in patients with COPD and listed related breath markers, including H2O2, eicosanoids (leukotrienes, prostanooids, and isoprostanes), NO-derived products (S-nitrosothiols, nitrite, and nitrate), pH, aldehydes, and others. Moreover, CO has also been studied as a breath marker of asthma (55–57) and CF (58,59); H2O2 as a breath marker of asthma (60,61), COPD (62,63), and CF (64,65); isoprostanes as breath markers of asthma (66), COPD (67), and CF (68); and nitrite/nitrate as breath markers of asthma (69) and CF (70,71).

As described above, there are several breath markers of lung disease, most of which need further study before

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2 Human genes: NOS1, nitric oxide synthase 1 (neuronal); NOS2A, nitric oxide synthase 2A (inducible, hepatocytes); NOS3, nitric oxide synthase 3 (endothelial cell).
they can be used for routine monitoring. Rosias et al. (6) compared exhaled markers of inflammation, including NO, CO, and the acidity of breath condensate. They found that, in patients with mild to moderate persistent childhood asthma, exhaled NO correlated better with indicators of lung function and asthma control than did exhaled CO and the acidity of EBC. Kharitonov (41) has also summarized the progress of these breath markers toward clinic applications. He concluded that the technique for exhaled NO analysis is simple, reproducible, and acceptable for patients and is ready for use in routine testing as a noninvasive means of monitoring airway status, particularly in childhood and adult asthma. However, measurement of some of the other exhaled markers, such as hydrocarbons, is much more difficult with present technologies, but it may also be possible to develop much smaller and less expensive detectors that would make these measurements more readily available.

Metabolic Disorder

Metabolism is the sum of all the chemical reactions involved in the continual process of breakdown and renewal in the body (72). Metabolic processes lead to growth, produce energy, eliminate wastes, and control other body functions that distribute nutrients in the blood after food is digested (73). Metabolism maintains homeostasis, or a steady state in the body. A metabolic disorder is any problem in the body that causes loss of metabolic control of the body’s steady state (73); i.e., it is anything that interferes with how food is processed in the body to keep the body healthy. The typical types of metabolic disorders include diabetes, phenylketonuria, metabolic syndrome, sodium metabolism disorders, calcium metabolism disorders, hyper- and hypocalcemia, potassium metabolism disorders, hyper- and hypokalemia, phosphate metabolism disorders, magnesium metabolism disorders, and acid–base metabolism disorders. Among these metabolic disorders, diabetes is most prevalent. The WHO reported that globally, for the year 2000, the number of persons with diabetes was 171 million and estimated that by the year 2030, that number would be 366 million. Therefore, diabetes mellitus is a large and growing problem throughout the world’s developed and developing nations.

From a medical point of view, diabetes mellitus is regarded as a disease in which an absolute or relative inadequacy of the effect of insulin gives rise to a complex disturbance of metabolism. It is dominated by two metabolic changes: increases in glucose concentrations and intensive lipolysis (74, 75). Because glucose is not readily available as an energy source, ketone bodies are produced by the liver and used peripherally as a substitute energy source. Ketone bodies consist of acetocetate, 3-β-hydroxybutyrate, and acetone. They are always present in the blood, and their concentrations increase during fasting and prolonged exercise. They are also present in the blood of neonates and pregnant women. Diabetes is the most common pathologic cause of increased blood ketones. The gas-phase acetone in the blood equilibrates with alveolar air (end-expired air) through the alveoli. Therefore, the concentration of acetone in breath can reflect metabolic products of diabetes. In healthy individuals, breath acetone concentrations are in the range of a few hundred ppb (by volume) (76). The breath acetone concentration slowly increases in diabetes or the fasting state (77, 78). Compared with healthy individuals, persons with diabetes have a broader range of breath acetone concentrations, which depend on the characteristics of the individual (e.g., age, sex, and ethnic group) and mainly on the blood glucose concentration. Diabetic ketoacidosis, which is a serious complication of diabetes, can lead to high glucose concentrations, progressive dehydration, and collapse. It also causes uncontrolled breakdown of fat stores, which overwhelms the liver, leading to large amounts of ketone bodies, including acetone, being released into the circulation. The acetone concentration can therefore be as high as 25 μmol/L (560 ppm) (79) or even >1000 ppm. For this reason, breath acetone may be a useful candidate as a breath marker in diabetes. In fact, it has been studied extensively and shown to be correlated with blood glucose (76, 79, 80).

Currently, breath acetone testing is carried out by GC followed by flame ionization detection (77, 81), ion mobility spectrometry (77), and MS detection (8). These methods need bulky instrumentation and skilled operators. Sample collection/preconcentration involving a complicated procedure is required before introduction of the collected sample into a gas chromatographic column. Furthermore, some or all of the breath acetone may be lost during these time-consuming procedures. Because of these limitations, these methods are not suitable for use in diabetes diagnosis and monitoring applications outside the laboratory. To meet the need for clinic applications, a relatively inexpensive, portable instrument capable of providing nonintrusive, real-time, sensitive, and accurate analysis of breath gases for medical diagnosis is highly desirable.

Gastroenteric Diseases

Breath hydrogen measurements have been applied in clinical medicine for the detection of carbohydrate malabsorption (82). Theoretically, H2 in expired air occurs when dietary sugars escape absorption in the small intestine, thereby becoming available for bacterial fermentation. H2 produced by bacterial metabolism of carbohydrates is absorbed into the portal circulation and excreted in the breath. H2 breath tests can be used to diagnose clinical disorders of digestion and absorption, including lactase deficiency and other disorders of di- and monosaccharide malabsorption, starch malabsorption, and small-bowel bacterial overgrowth. Breath H2 testing has repeatedly been demonstrated to be the most accurate indirect indicator of lactase deficiency, and breath H2 measurements...
have been widely applied in digestion studies on the entire spectrum of dietary carbohydrates (82–84).

In addition to H₂ breath tests, the UBT can be used for the diagnosis of gastroenteric diseases. UBT is one of the most successful breath tests used in clinical applications. The range of diseases that can be identified include H. pylori infection, lactose and fructose intolerance, bacterial overgrowth, bile salt wastage, pancreatic insufficiency, liver dysfunction, and abnormal small-bowel transit (4). Among these diseases, H. pylori has been studied extensively, and testing has been widely applied for clinical diagnosis. We will focus on H. pylori testing in this subsection.

The discovery of H. pylori has revolutionized the pathophysiologic and clinical approaches to gastric and duodenal ulcers (85). Since the first report identifying H. pylori was published only 20 years ago (86), subsequent studies have indicated that this bacterium is probably the most frequent cause of infection in humans. Numerous published reports have confirmed causal relationships between H. pylori infection and gastritis, duodenal ulcers, gastric ulcers, and gastric cancer. Almost all of the routine diagnostics and treatments for gastritis, gastric ulcers, and gastric cancer have been replaced by studies focusing on the epidemiology, isolation, and eradication of this single bacterium (85). The UBT is based on the ability of H. pylori to breakdown urea, a chemical composed of nitrogen and carbon. Urea usually is produced by the body from excess (“waste”) nitrogen and then eliminated in the urine. For the UBT, patients swallow a capsule containing urea labeled with ¹³C or ¹⁴C. If H. pylori is present in the stomach, the bacterium metabolizes the urea into nitrogen and carbon (as CO₂). The CO₂ is absorbed across the lining of the stomach and into the blood. It is then excreted from the lungs in the breath. The study of pharmacokinetics is essential for understanding the fates of the carbon isotopes in the breath test. Irving and coworkers (87–89) have exhaustively investigated the bicarbonate kinetics and developed a pharmacokinetic model. Their model consists of 3 pools of freely exchangeable bicarbonate: a central vascular pool, a “heart–brain–other” pool, and a muscle pool (87). CO₂ production from the heart–brain–other pool and the muscle pool were 127.5 and 25.5 μmol·kg⁻¹·min⁻¹, respectively, which yielded a CO₂ output rate of 153 μmol·kg⁻¹·min⁻¹. The vascular bicarbonate exchanges with the large sink of bone bicarbonate (72 066 μmol/kg) at a rate of 23.9 μmol·kg⁻¹·min⁻¹. The latter value was estimated by use of a skeletal blood flow rate equal to 5% of the cardiac output and a 30% extraction ratio of bicarbonate between bone and blood. The total flux of freely exchangeable bicarbonate is 176.9 μmol·kg⁻¹·min⁻¹, of which 86.5% is accounted for by respiratory losses.

Samples of exhaled breath are collected, and the isotopically labeled carbon in the exhaled CO₂ is measured. If isotopically labeled CO₂ is detected in the breath, it indicates that H. pylori is present in the stomach. When the H. pylori is treated effectively (eradicated) by antibiotics, the test changes from positive (isotope present) to negative (isotope absent). The UBT is also a good method for monitoring the success of antibiotic therapy because it does not require endoscopy and can avoid the false-negative results that can occur with biopsies as a result of focal colonization of bacteria (90).

Currently, two breath tests are available to detect H. pylori: the ¹⁴C UBT (radioactive) and the ¹³C UBT (nonradioactive, stable). Although the risk from the radioactivity in ¹⁴C UBT is real, it was estimated that one UBT with ¹⁴C gives an exposure that is 1/100th of the bone marrow exposure in a standard upper (gastrointestinal) series (91). In fact, in one study (92), the more recent microdose tests, which use 1 μCi of ¹⁴C, produced a radiation exposure equivalent to the natural background exposure for 1 day and could be completed within 10 min. Nevertheless, they are still contraindicated in children and in pregnant women. The sensitivity and specificity were reported to be 90.2% and 95.8%, respectively, in a comparison of diagnostic tests for H. pylori (93). Compared with ¹⁴C UBT, the ¹³C UBT does not have the risk of radioactivity because ¹³C is a stable, nonradioactive isotope. For this reason, it is becoming the test of choice for young children (94) and pregnant women (3). The sensitivity and specificity for the ¹³C UBT were reported to be in the range of 90%–100% (3, 95, 96).

The major disadvantage of the ¹³C UBT is the need for gas isotope ratio MS to analyze the breath samples and to calculate a ratio of ¹²C to ¹³C. These instruments are quite expensive (4). However, because the breath samples are nonradioactive and the isotope is stable, samples can be collected and sent safely to a central processing laboratory. Newer technologies, including nondispersive, isotope selective, infrared spectroscopy and laser-assisted ratio analysis, are less expensive alternatives for these applications.

**OTHERS**

In addition to the diagnosis of diseases, breath tests have been applied to monitor respiratory processes in medical care (97–101) and excretion of drugs (12, 13, 102–105).

A healthy adult human has a respiratory rate of 12–15 breaths/min at rest, inspiring and expiring 6–8 L of air per minute. O₂ enters the blood and CO₂ is excreted through the alveoli. When the end-tidal concentrations of O₂ or CO₂ in healthy persons are measured, the relative change in concentrations between expired and inhaled air for oxygen is less specific (~21% for inhaled air and 15.3% for expired air) than that for CO₂ (~0.03% for inhaled air and 3.6% for expired air) (97). In addition, oxygen is delivered in the inspiratory air, and when the oxygen fraction is high, respiratory depression could lead to hypercapnia without a disturbance in arterial oxygen saturation (SaO₂). The measurement of CO₂ concentrations is therefore preferable.

A miniature sensor could be placed near, but not
attached to, the nasal/oral region because attached gas collectors can affect respiratory activity or disturb the patient. Such placement is possible with a CO₂ sensor because the CO₂ concentration is increased in expired air even at a short distance from the airway. Miniaturization of the sensor would eliminate the pump and sampling line (97). With this miniature sensor, the change of CO₂ in respiration that could lead to hypoxemia can be monitored.

A study has shown that the difference between arterial and end-tidal CO₂ concentrations was smaller when a divided nasal cannula was used than when a facemask was used, when patients were given oxygen at a rate of 4 L/min (106). Separation of the nostrils can be achieved by blocking the passage between the nasal prong at the cannula (107). Problems with the use of cannulae include that mouth breathing is not monitored and that water vapor and phlegm can occlude the tube. Breath analysis for respiratory monitoring is very useful for (a) monitoring unstable, nonintubated patients; (b) reducing the number of arterial blood gas determinations; (c) monitoring patients who are being weaned off a ventilator; (d) monitoring patients in acute respiratory failure, with CO₂ retention, to detect rapid decreases in arterial CO₂ when these patients are placed on a ventilator; and (e) monitoring patients in other cases when there is danger of hypoxia or hyperventilation (108).

Drug monitoring is a neglected but potentially fruitful field because most prescribed and illicit drugs have low molecular weights. Their vapor pressures at body temperature may be sufficiently high so that they or their metabolites are propelled into the alveolar air in measurable quantities (12). Disulfiram (Antabuse), a drug used to render alcoholics averse to alcohol, is converted to CS₂. CS₂ has not been detected in the blood of patients taking disulfiram, but it has been readily detected in breath (102). Δ⁹-Tetrahydrocannabinol (THC), detected in the breath by a few groups (103–105), shows a rapid decay to below the limits of detection after 10 to 15 min. This disappearance is not attributable to its conversion to 11-hydroxy-THC, because THC remains unchanged at high concentrations in the blood for considerably longer periods after smoking. The THC in the breath therefore probably is derived from the mouth or lung surface (13).

Assessment of Exposure to VOCs

According to the National Academy of Sciences, exposure is defined as "an event that occurs when there is contact at a boundary between a human and the environment with a contaminant of specific concentration for an interval of time" (109). Environmental chemicals enter the body by one or more of 3 exposure routes: ingestion, inhalation, and dermal contact. The chemicals can either be absorbed into the systemic blood supply or they can pass through the body not absorbed and be excreted directly in the feces. The chemicals that are absorbed into the blood are distributed within the body and can be excreted; metabolized and excreted; stored, equilibrated with the concentrations in blood, and slowly excreted; or undergo a combination of these processes.

The primary matrices used in biomonitoring are blood and urine; other possibilities are the excretion and secretion matrices of expired air, saliva, sweat, and milk and the storage matrices of fat (generally adipose tissue) and bone. In general, absorbed chemicals with short biological half-lives (nonpersistent chemicals) are eliminated in the urine or, if they are volatile, are eliminated principally in the expired air. Therefore, exhaled breath is a preferred matrix for measuring exposure to VOCs. Moreover, although either blood or breath measurements can be used to assess the exposure of the human body to VOCs, for most VOCs, the preferred sensitive method for determining body burden is the measurement of exhaled breath. Because of steady improvements in analysis techniques, detection limits using Tenax sorbents or evacuated canister samples are usually well below 1 µg/m³, whereas blood measurements would have to detect concentrations as low as 10 ng/L for the same purpose, a sensitivity that was not achievable until recently (110). With the advent of improved sorbents, such as Tenax, and more specific and sensitive detection methods, such as GC with electron capture detection and GC-MS, it has become possible to detect and quantify hundreds of VOCs and in the breath of persons exposed to normal environmental concentrations (111, 112).

VOCs enter the body mainly through inhalation and transfer to the blood through the breath–blood interface in the lungs. Pharmacokinetic models assume that inhaled air resides in the alveoli for sufficient time to allow VOCs to reach equilibrium with arterial blood. Provided that equilibrium is reached, it is governed by the partition coefficient, which determines for each VOC the relative concentration in the blood-breath interface. Partition coefficients have been calculated for ~50 VOCs (113, 114) by laboratory methods and have been validated on humans at occupational (ppm) concentrations. However, there is some evidence that the blood/breath ratio increases with decreasing concentration to environmental concentrations in the ppb range (115). For example, a benzene blood–breath ratio of ~20 was observed for unexposed nurses, compared with a ratio of less than one half that for workers exposed to higher concentrations of the chemical (116). Furthermore, the rate of blood flow through the alveoli is not linear with the breathing rate; therefore, different degrees of exertion will lead to different partition rates. All of these factors must be taken into account when interpreting the results of field and chamber studies (117).

Having a reasonable estimate of the partition coefficient allows estimation of the arterial blood concentration from the breath measurement. Furthermore, if the blood concentration is known, the use of a model of the distribution of the chemical in the body could allow estimation of concentrations in other body tissues.
Pharmacokinetic models are all based ultimately on mass-balance considerations. Differential equations for different compartments (e.g., liver, other organs, muscle, and fat) have been developed and solved, usually numerically. Several simplifying assumptions have been made that allowed analytic solution of the equations for certain simple inputs (a single bolus or a constant exposure at high concentrations); these solutions were generally in the form of exponential functions with different parameters for each compartment \( \text{eq. } (118) \). Wallace et al. \( \text{eq. } (119) \) developed a linear pharmacokinetic model relating VOC concentrations in exhaled breath to personal exposures at environmental (ppb) concentrations in a chamber study of 4 human volunteers. Assuming that someone without previous exposure to a particular VOC is suddenly exposed to a constant high concentration \( c_{\text{air}} \), the alveolar breath concentration \( c_{\text{alv}} \) is given by \( \text{eq. } (119) \):

\[
c_{\text{alv}} = f c_{\text{air}} \sum a_i \left[ 1 - \exp\left( -t / \tau_i \right) \right]
\]

where \( f \) is the fraction of parent compound exhaled at equilibrium; \( \tau_i \) is residence time in the \( i \)th compartment; \( a_i \) is the fraction of breath concentration contributed by the \( i \)th compartment at equilibrium \( (t = \infty) \); \( t \) is the time of exposure \( (t = 0 \text{ at start of exposure}) \); and \( \sum a_i = 1 \).

This model allows estimation of VOC concentrations in the body from measurements of exposure or, conversely, estimation of previous exposure from a measurement of exhaled breath. It differs from previous models in considering long-term inhalation at low or moderate concentrations rather than instantaneous intake (as in drug administration) or intermittent exposure at high concentrations (as in occupational situations). Wallace et al. \( \text{eq. } (119) \) derived the basic equations for the 1-, 2-, 3-, and \( n \)-compartment cases and provided solutions to these equations for the cases of a sudden constant high exposure, a sudden constant low exposure, and a linearly increasing exposure. These solutions can be readily applied to more complex exposure scenarios. The chamber study by Wallace et al. \( \text{eq. } (119) \) suggested residence times on the order of a few minutes in the blood and 1–2 h in vessel-rich groups of tissues.

With the aid of powerful computers, Ramsey and Andersen \( \text{eq. } (120) \) took into account more realistic considerations of physiologic processes and developed a class of models known as physiologically based pharmacokinetic (PBPK) models \( \text{eq. } (120) \). Using PBPK models, Soldat and Thrall \( \text{eq. } (121) \) developed a real-time, field-portable system to analyze undiluted exhaled air from experimental animals and humans. To evaluate the system in actual work environments, they conducted field tests with volunteers providing exhaled breath samples before and after each specific job task. In the field studies, several volunteers had posttask breath concentrations higher than pretask concentrations. The results of the field studies illustrated the utility of monitoring workers for exposures at numerous times throughout the day, particularly when job-specific tasks may indicate a potential for exposure \( \text{eq. } (121) \).

Although PBPK models are powerful and have many successful applications, they require knowledge of a much larger number of variables, some of which are difficult to obtain quantitative information \( \text{eq. } (122) \).

Since the mid-1980s, several major studies have been conducted in which the US Environmental Protection Agency used the Total Exposure Assessment Methodology in an effort to monitor VOC, semi-VOC, and particulate matter concentrations. Similar studies were run throughout Europe and Asia, and the results found were very much the same as those of the Total Exposure Assessment Methodology studies in the United States. Perhaps the common finding of most studies of human exposure is that the most important sources of exposure are small and close to the person (generally readily apparent) \( \text{eq. } (110) \). Several studies of the cancer risks of VOCs ranked 3 chemicals as the highest risks: benzene, chloroform, and \( \text{para} \)-dichlorobenzene. The dominant sources of benzene exposure are smoking for smokers and environmental tobacco smoke, driving, filling fuel tanks, and parking hot cars in attached garages for nonsmokers \( \text{eq. } (110, 123) \). The dominant sources of chloroform and \( \text{para} \)-dichlorobenzene exposure are chlorinated water \( \text{eq. } (124) \) and moth cakes/room air deodorizers \( \text{eq. } (125) \), respectively.

It is interesting to note that none of these sources is from chemical companies, hazardous waste sites, urban air pollution, or the other usually suspected sources.

**Advantages and Limitations of Breath Analysis**

Breath tests provide a unique window to the blood composition. Human alveolar breath typically contains many biomarkers derived from the blood by passive diffusion across the pulmonary alveolar membrane. As reviewed here, breath testing might offer a new approach to the diagnosis of diseases, evaluation of several common disorders, and assessment of exposure to VOCs. The advantages of breath analysis over existing serum or urine analysis include the following \( \text{eq. } (10, 13) \):

- Breath testing is noninvasive, easily repeated, and does not have the discomfort or embarrassment associated with blood and urine tests.
- Breath samples closely reflect the arterial concentrations of biological substances and may obviate the collection of arterial blood samples, which is much more difficult. Breath analysis could be particularly advantageous when many arterial blood samples are required, e.g., in monitoring a patient.
- Breath is a much less complicated mixture than serum or urine and is amenable to complete analysis of all compounds present. No work-up of a breath sample is required, in contrast to many analyses performed on serum or urine samples.
- Breath analysis provides direct information on respiratory function that is not obtainable by other means.
• Breath analysis can dynamically real-time monitor the decay of volatile toxic substances in the body.

However, breath testing also has limitations:

• A considerable problem hampering the use of breath tests in clinical practice is the lack of standardization of analytical methods and the wide variation in results obtained in different studies. Sample collection and preconcentration are usually necessary because most substance concentrations in exhaled breath fall in the nmol/L to pmol/L (ppb to ppt by volume) range. Preconcentration can be achieved by adsorption on sorbent traps, coated fibers (solid-phase microextraction), or by direct cryofocussation. However, standardization of breath sample collection and preconcentration is more difficult than for serum. Different analytical methods may give very different results.

• Another critical issue is the high water content of breath samples, which may affect preconcentration, separation, and detection of single compounds. This is especially true for mechanically ventilated patients if active humidifiers are used in the respiratory circuit.

• Compared with the simple chemical tests widely used in serum and urine analysis, instruments for breath analysis are expensive. At present, the most commonly used method, GC-MS, requires bulky instrumentation, is time-consuming, and needs skilled operators.

• The lack of established links between breath substances and disease is also a problem. As explained in Ref. (10), “Take ethane for example. Ethane is produced as a result of lipid peroxidation, but ethane in breath could also be derived from environmental sources; another source of ethane in breath could be bacteria in the gastrointestinal tract. So I cannot, in all conscience, say that all the ethane in your breath is produced by lipid peroxidation. You don’t know the complete origin of it.” Therefore, in some cases, the breath test is not a conclusive diagnostic tool but can be used as a part of a range of diagnostics.

In conclusion, although use of breath tests for diagnosis of diseases and assessment of exposure to VOCs still has some problems of standardization, defining markers, and sampling methods, these tests have the potential to become useful tools for rapid, noninvasive diagnosis of disease and assessment of exposure to VOCs. In fact, breath analysis has already been shown to be useful in some areas, such as the UBT for identification of H. pylori infection.

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