Pulmonary oxygen toxicity in professional diving: Scire est mensurare?
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CHAPTER 2 | ASSESSMENT OF PULMONARY OXYGEN TOXICITY: RELEVANCE TO PROFESSIONAL DIVING

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ABSTRACT

When breathing oxygen with partial oxygen pressure ($PO_2$) of between 50 and 300 kPa pathological pulmonary changes develop after 3-24 h depending on the $PO_2$. This kind of injury (known as pulmonary oxygen toxicity) is not only observed in ventilated patients but is also considered an occupational hazard in oxygen divers or mixed gas divers. To prevent these latter groups from sustaining irreversible lesions adequate prevention is required.

This review summarizes the pathophysiological effects on the respiratory tract when breathing oxygen with $PO_2$ of 50-300 kPa (hyperoxia). We discuss to what extent the most commonly used lung function parameters change after exposure to hyperoxia and its role in monitoring the onset and development of pulmonary oxygen toxicity in daily practice. Finally, new techniques in respiratory medicine are discussed with regard to their usefulness in monitoring pulmonary oxygen toxicity in divers.
INTRODUCTION

A bit of history
Pulmonary oxygen toxicity is a medical complication, which can occur during oxygen therapy and in professional conditions such as diving. In 1775 Joseph Priestley reported the injurious character of dephlogisticated air, or oxygen as it is nowadays called:

"Though pure dephlogisticated air might be very useful as a medicine, it might not be proper for us in the usual healthy state to the body"1-3.

However, it took over 100 years before this statement was confirmed beyond doubt. In 1878 Paul Bert described the neurological effects of oxygen toxicity after exposing larks to 100% oxygen at 500 kPa (5 atmosphere absolute) of ambient pressure1,2. In 1899 James Lorrain Smith described the effect of oxygen on the pulmonary system after exposing animals to 100% oxygen at pressures up to 300 kPa3. Post-mortem examinations performed on these animals exposed to oxygen revealed that the lungs were extremely congested4. These pathological changes were confirmed in other animal studies conducted in the following decades1,5,6.

In the mid-1900s, pulmonary oxygen toxicity became a serious clinical problem in ventilated patients. Due to the development of efficient mechanical ventilators these patients were ventilated with much higher concentrations of oxygen than previously used3,7-9. Breathing high concentrations of oxygen led to an increasing number of cases of pulmonary oxygen toxicity, some of which resulted in the death of the patient7,8. However, besides patients, it appeared that healthy subjects were also at risk to develop this kind of oxygen toxicity.

Diving and pulmonary oxygen toxicity
 Particularly in diving medicine pulmonary oxygen toxicity became of daily importance with the introduction of oxygen and mixed gas rebreathing diving systems. With the use of these devices divers were able to breath oxygen for several hours with partial oxygen pressure ($PO_2$) of ≥ 100 kPa10. A drawback of using these devices was the increasing risk of pulmonary oxygen toxicity. Therefore, understanding and dealing with the pathophysiology of diving-related problems was of increasing importance. The first extensive review focussing on the onset and development of pulmonary oxygen toxicity in divers was
published by Bean in 1945\textsuperscript{1}. Until the late 1930s, most of the knowledge on the effects of oxygen in divers was based on deep air dives in which $PO_2$ was increased to levels far above 50 kPa. However, because decompression stress played an important role in these air dives it was unclear whether the respiratory effects were oxygen based, or simply due to decompression\textsuperscript{1}.

A step forward was made by Clark and Lambertsen in the 1960s and 1970s when they exposed divers to 100\% oxygen with $PO_2$ levels of up to 300 kPa\textsuperscript{11-13}. Much of current knowledge on the onset and development of pulmonary oxygen toxicity in divers was derived from these latter landmark studies\textsuperscript{11}.

**Clinical symptoms of pulmonary oxygen toxicity**

As reported by Lorrain Smith, animals exposed to oxygen levels up to about 300 kPa for \geq 6 h eventually died of pathological changes due to oxygen exposure. From the start until the moment the animal died, several 'clinical' symptoms related to pulmonary oxygen toxicity were observed. First, the animals became restless, which was followed by lethargy, anorexia and vomiting. When oxygen exposure was continued the animals became progressively dyspnoeic. The terminal phase was characterised by cyanosis, laboured or gasping respiration with frothy or bloody sputum, after which the animal died\textsuperscript{4,5}. However, it is impossible to extrapolate these findings to the human clinical situation because it is unknown, for example, how 6 h in the lifespan of a rat relates to that in humans.

In humans, the symptoms of pulmonary oxygen toxicity that occur during the early stages are similar to those observed in animals. When humans were exposed to 100\% oxygen at 101 kPa for 24 h the first symptom of pulmonary oxygen toxicity was described as a mild substernal tickling or tracheal irritation\textsuperscript{5,14}. This sensation was accentuated by deep inspirations and often provoked coughing\textsuperscript{5}. When oxygen exposure was continued for another 24 h subjects complained about chest tightness followed by progressively increasing tracheal irritation that eventually led to substernal pain and uncontrollable coughing\textsuperscript{5,15,16}. Dyspnoea during exercise and later at rest developed towards the end of this early stage\textsuperscript{15}.

Nowadays, both clinical symptoms and changes in lung function are used to monitor the onset and development of pulmonary oxygen toxicity. This review presents an overview of the pathophysiological
effects on the respiratory tract when breathing oxygen with partial pressures of between 50-300 kPa (hyperoxia). We also discuss to what extent the most commonly used lung function indices change after exposure to hyperoxia and what role they can play as possible markers of pulmonary oxygen toxicity. Finally, new techniques in lung function analyses are presented and their usefulness for monitoring pulmonary oxygen toxicity is evaluated.

PATHOPHYSIOLOGICAL CHANGES DUE TO PULMONARY OXYGEN TOXICITY

Human studies on pulmonary oxygen toxicity

Only a few histopathological studies have investigated the onset and development of pulmonary oxygen toxicity in humans\textsuperscript{8,17-20}. When neonates or adults were ventilated with 100% oxygen for \( \geq 30 \) h, denuded alveolar type 1 cells, oedematous endothelial capillary cell swelling, necrosis of the respiratory epithelium, and squamous metaplasia of tracheal and bronchial mucosa was found. Furthermore, deposition of eosinophilic slough within the bronchioles, and oedema within the alveola and interstitium was reported\textsuperscript{8,9,20-23}. Consequently, the proteinaceous oedema fluid became organized within the alveoli with the formation of hyaline membranes on the denuded membrane\textsuperscript{9,21,22,24,25}. In addition, fibrin thrombi were formed in the pulmonary capillaries leading to dilatation and cellular infiltration of neutrophils\textsuperscript{9,18,21,26,27}. Due to the presence of exudate, this initial phase was called the exudative phase and was found up to 5.5 days of continued 100% oxygen exposure. If ventilation with oxygen was continued the exudative transformed into a proliferative phase, which was characterised by proliferation of alveolar type 2 cells that replaced alveolar type 1 cells. Due to alveolar replacement, the alveolar lining underwent a cuboidal transformation which led to an increased thickening of the alveolar membrane\textsuperscript{27}. Furthermore, derangement of collagen and elastin, incorporation of hyaline membranes into the septal walls, fibroblastic proliferation, collagen fibre deposition, fibro-proliferative organisation of intra-alveolar exudate and infiltration with inflammatory cells occurred\textsuperscript{9,21,22,24-28}. Eventually, interstitial lung fibrosis or emphysematous alveoli with areas of fibrosis developed as an end stage of pulmonary oxygen toxicity\textsuperscript{7,18,20,21,25}. Although these observations seem to be confirmed, it should be emphasised that the pathological findings in humans were based on post-mortem studies of ventilated patients. This introduces two
problems. First, the underlying disease could influence the pathological changes found. Second, mechanical ventilation itself introduces changes, known as ventilator induced lung injury, which could confound post-mortem results and mimics the effect of hyperoxia. Therefore, fully understanding the development of pulmonary oxygen requires consideration of comparative animal studies.

**Animal models**

Following the study of Lorrain Smith many animal studies were performed, the majority employing rodents like mice, rats and rabbits. Although these animals are suitable, they differ from humans in their anatomical structure of the lung. Of all animals, the baboon is considered the most appropriate model to study pulmonary oxygen toxicity. Similar to humans, baboons also follow a two-stage pattern of pulmonary oxygen toxicity. When baboons were breathing 100% oxygen for 2-4 days the exudative phase developed. Massive exudation of oedema fluid into the alveoli and septal walls occurred, resulting in distension of the lymphatic vessels. After 4 days, resolution started with the formation of hyaline membranes and the influx of inflammatory cells. With the increased number of alveolar type 2 cells the proliferative phase started after 5-7 days of continued oxygen breathing. After 12 days, almost all alveolar type 1 cells were replaced by alveolar type 2 cells. This resulted in a thickening of the blood-air barrier by 4-5 times. In addition, early interstitial fibrosis, and focal or diffuse subacute inflammation of the septa, became evident. When the exposure to oxygen was continued for an additional 10 days, proliferation of alveolar lining cells with severe interstitial inflammation and finally acute exudation, haemorrhage and fibrosis occurred until the animal died.

Taken together, pulmonary oxygen toxicity has a two-stage course; an exudative, early phase when oxygen is breathed for up to 5 days, which is followed by a proliferative late phase if oxygen exposure is continued.
Figure 1 A: Normal alveolocapillary region

Legend: 1 = alveolar type 1 cell; 2 = alveolar type 2 cell; 3 = basement membrane; 4 = interstitium; 5 = capillary endothelial cell; 6 = fibroblast; 7 = alveolar macrophage; 8 = surfactant layer; 9 = red blood cell
Although the exudative phase can be life-threatening\textsuperscript{37} the histopathological changes appear to be reversible when breathing of oxygen is stopped\textsuperscript{38}. 

\textbf{Legend:} 1 = type 1 alveolar cell; 2 = type 2 alveolar cell; 3 = alveolar oedema; 4 = neutrophil; 5 = hyaline membrane; 6 = oedematous interstitium; 7 = fibroblast; 8 = fibrin thrombus; 9 = swollen capillary endothelial cell; 10 = denuded basement membrane; 11 = alveolar fibrin formation; 12 = collagen fibres deposition; 13 = incorporation of hyaline membrane; 14 = fibroblastic proliferation; 15 = interstitial fibrin
CLASSIFICATION OF PULMONARY OXYGEN TOXICITY

Much of what we know today about the development of pulmonary oxygen toxicity in divers is derived from the work of Clark and Lambertsen\textsuperscript{11}. They concluded that the decrease in vital capacity (VC) could best be used as a marker for the onset of pulmonary oxygen toxicity (as discussed later on). Based on those studies, in 1970 Bardin and Lambertsen introduced the Unit of Pulmonary Toxic Dose (UPTD). In this VC-based model, one UPTD was the degree of pulmonary poisoning produced by breathing 100\% oxygen continuously at 101 kPa (1 atmosphere absolute) for 1 min. The total amount of UPTD for a continuous oxygen exposure at a single pressure could be calculated using the equation:

\[
UPTD = t \cdot \sqrt[1.2]{\frac{0.5}{P_{O2} - 0.5}}
\]

where \(t\) is the exposure time in minutes and \(P_{O2}\) the inhaled partial oxygen pressure expressed in atmosphere\textsuperscript{39}. A \(P_{O2}\) below 50 kPa (0.5 atmosphere) was considered not to induce any toxic effect with respect to the lung\textsuperscript{2,3,5,7,11,40,41}. By using a reference table (Table 1) total units of UPTD estimates the median decrease in VC provoked by this oxygen load\textsuperscript{39}. Tracking the onset and development of pulmonary oxygen toxicity was therefore possible using only \(P_{O2}\) and time as variable\textsuperscript{42}.

To minimize the risk of pulmonary oxygen toxicity the US Navy allows their oxygen divers or mixed gas divers a single exposure of 615 UPTD units as the maximum tolerable for an ordinary operational dive whereas the upper limit for a single exposure was set at 1425 units\textsuperscript{39,43}. These loads represent a median decrease in VC of 2\% and 10\%, respectively (Table 1).

Table 1: Corresponding percentage median decrease in vital capacity (VC) related to total amount of UPTDs

<table>
<thead>
<tr>
<th>UPTD</th>
<th>%Median VC decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>615</td>
<td>2</td>
</tr>
<tr>
<td>825</td>
<td>4</td>
</tr>
<tr>
<td>1,035</td>
<td>6</td>
</tr>
<tr>
<td>1,230</td>
<td>8</td>
</tr>
<tr>
<td>1,425</td>
<td>10</td>
</tr>
<tr>
<td>1,815</td>
<td>15</td>
</tr>
<tr>
<td>2,190</td>
<td>20</td>
</tr>
</tbody>
</table>
Although the UPTD method is used worldwide and considered to be an easy to use method it has two important disadvantages. First, the UPTD concept does not take any recovery (e.g. air breathing between oxygen exposures as in repetitive oxygen diving) into account\textsuperscript{44}. Secondly, the UPTD method appeared to be a poor indicator for the onset of pulmonary oxygen toxicity for short oxygen exposures or in-water oxygen dives\textsuperscript{45,46}. Therefore, for the latter types of dives the need for other markers for monitoring became apparent.

**OBJECTIVES OF PULMONARY OXYGEN TOXICITY MARKERS**

The accuracy of a diagnostic test depends on its internal and external validation. The Standards of Reporting of Diagnostic (STARD) Initiative provides guidelines on how to assess the validity\textsuperscript{47}. Topics like sensitivity (ability to truly identify a disease), specificity (ability to truly reject a disease) and their 95% confidence interval (95% CI) are key features that should be reported\textsuperscript{48}.

For divers it is important to know whether or not pulmonary oxygen toxicity is developing. The absence of this disorder will qualify a diver fit to continue oxygen diving. Therefore, for pulmonary oxygen toxicity the specificity of the diagnostic test is important. Unfortunately, no data on the sensitivity and specificity of lung function parameters to characterise pulmonary oxygen toxicity are available. Thus, other means to express the reliability of a diagnostic test such as the 95% CI, relative standard deviation (RSD), coefficient of repeatability (CR) or the coefficient of variation of repeated measurements (CV) should be considered.

**LUNG FUNCTION CHANGES DUE TO PULMONARY OXYGEN TOXICITY**

Figure 2 presents a summary of the pathophysiological changes due to oxygen exposure and to what extent they may influence lung function indices. The following sections describe and discuss the applicability of the indices as a marker for pulmonary oxygen toxicity. The strength and limitations of the lung function parameters can be found in Table 3.
**Spirometry**  
*Vital Capacity (VC)*  

The pathophysiological cause of an oxygen-induced decrease in VC has not yet been fully clarified, but purely pathological changes on the alveolar-capillary level might be responsible. First, thickening of the alveolar-capillary membrane increases its stiffness and might therefore decrease VC\(^7\). Second, the development of alveolar oedema might reduce alveolar volume\(^{49}\). Third, a decrease in VC has been found in high altitude pulmonary oedema as a result of interstitial fluid accumulation\(^{50,51}\). Interstitial oedema is also one of the pathological features in pulmonary oxygen toxicity and could therefore be responsible for a decrease in VC\(^{19}\). Fourth, as the interstitial volume increases a decrease in alveolar volume develops which might account for the reduction in VC\(^{19}\). Fifth, small non-detectable atelectasis might retract the bronchiolar lining of the airways, causing irritative friction and in turn triggering a neural reflex preventing full inspiration\(^{52}\).

Although absorption atelectasis is also mentioned as an explanatory factor for the decrease in VC, this idea has been abandoned as a few deep inspirations will diminish any atelectasis\(^{53}\). Furthermore, it seems that oxygen-induced atelectasis may result from post-mortem rather than from ante-mortem changes\(^3\).

In contrast to the pathological explanations, it is also suggested that clinical symptoms could explain a decrease in VC. Due to perceived pain the subject stops full inhalation that can lead to decreased VC\(^{53-55}\). Furthermore, the reduction in VC seems to occur mainly at the expense of the inspiratory reserve volume, implying that substernal soreness induced by deep inspiration, and muscle weakness due to fatigue and discomfort might be an underlying mechanism\(^2\). For these reasons changes in VC could represent a change in effort rather than an underlying pulmonary disease\(^7\). A combination of symptoms and pathological changes might also be considered as an underlying mechanism. For instance, the initial reduction in VC develops due to pain from tracheobronchitis whereas the subsequent changes arise from pulmonary oedema\(^{56}\).
Table 2: Range of variation of most used lung function parameters and method used for expression of the variation. RSD = relative standard deviation; 95%CI = 95% confidence interval; CV = coefficient of variation; CR = coefficient of repeatability. * = value as displayed in column "Parameter".

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RSD (%)</th>
<th>95%CI</th>
<th>CV (%)</th>
<th>CR</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC (L)</td>
<td>0.58</td>
<td>7%; 0.18*</td>
<td>2.5-5.7</td>
<td>0.33*</td>
<td>57,58,62,160-162</td>
</tr>
<tr>
<td>FEV₁ (L)</td>
<td>0.50</td>
<td>8.2%; 0.15*</td>
<td>2.9-5.8</td>
<td></td>
<td>62,160-162</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>7.37</td>
<td></td>
<td>1.8</td>
<td></td>
<td>62,162</td>
</tr>
<tr>
<td>PEF (L/s)</td>
<td>1.0</td>
<td>16%; 1.01*</td>
<td>3.3-13.0</td>
<td></td>
<td>62,160-162</td>
</tr>
<tr>
<td>FEF₅₀% (L/s)</td>
<td>1.38</td>
<td></td>
<td>6.4</td>
<td></td>
<td>62,162</td>
</tr>
<tr>
<td>FEF₇₅% (L/s)</td>
<td>0.55</td>
<td></td>
<td>8.9</td>
<td></td>
<td>62,162</td>
</tr>
<tr>
<td>Lung Compliance (L/kPa)</td>
<td>2.84</td>
<td></td>
<td>6.6</td>
<td></td>
<td>62,163</td>
</tr>
<tr>
<td>DLₜ,co (mmol/min/kPa)</td>
<td>1.71</td>
<td>3.8-7</td>
<td>1.6*</td>
<td></td>
<td>45,58,62,117,164-167</td>
</tr>
<tr>
<td>DLₜ,NO (mmol/min/kPa)</td>
<td>6.39</td>
<td></td>
<td>6.5*</td>
<td></td>
<td>115,165</td>
</tr>
<tr>
<td>DLₜ,NO/DLₜ,co ratio</td>
<td></td>
<td></td>
<td>6.4</td>
<td></td>
<td>117</td>
</tr>
<tr>
<td>Vₐ (L)</td>
<td>0.70</td>
<td></td>
<td></td>
<td></td>
<td>168</td>
</tr>
<tr>
<td>Parameter</td>
<td>RSD (%)</td>
<td>95% CI</td>
<td>CV (%)</td>
<td>CR</td>
<td>References</td>
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<td>----------------</td>
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<td>--------</td>
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<td>------------</td>
</tr>
<tr>
<td>$D_M$ (mmol/min/kPa)</td>
<td>20.6</td>
<td></td>
<td></td>
<td>169</td>
<td></td>
</tr>
<tr>
<td>$V_C$ (ml)</td>
<td>8.1</td>
<td></td>
<td></td>
<td>169</td>
<td></td>
</tr>
<tr>
<td>$F_{E_{NO}}$ (ppb)</td>
<td>10</td>
<td>2*</td>
<td></td>
<td>94,170,171</td>
<td></td>
</tr>
<tr>
<td>EBT ($^\circ$C)</td>
<td>1.18-2.35</td>
<td></td>
<td></td>
<td>151</td>
<td></td>
</tr>
</tbody>
</table>
Although changes in VC are generally used to monitor pulmonary oxygen toxicity in healthy subjects, some important points need to be stressed. First of all, repetitive measurement of VC shows a variability of 2.5-5% even in healthy individuals\textsuperscript{57,58}. This limits the ability to detect small changes. Only oxygen exposures comparable to a UPTD load of 1,035 UPTD units or more would (theoretically) lead to a change in VC of more than 5% (Table 1). Thus, only after oxygen exposures equal to 18 h of 100% oxygen breathing at 101 kPa would generate this kind of defect. Second, it has shown that the median decrease in VC (like the UPTD concept) was a poor predictor for short oxygen exposures or in-water oxygen dives compared to dry hyperbaric oxygen exposures\textsuperscript{45,46}. Considering the variation in individual responses and its limited use in submerged environment, it can be postulated that the decrease in VC is not the best method to monitor the onset of pulmonary oxygen toxicity in submerged divers\textsuperscript{57}.

**Other spirometric parameters**

Besides VC, the most commonly studied spirometric parameters are forced expiratory volume in 1 second (FEV\textsubscript{1}) and maximal expiratory flow rates (FEF\textsubscript{25,50,75}). Both values decrease after oxygen exposure\textsuperscript{59,60}. However, as with VC, the pathological implication of this decrease is unclear. The decrement in both FEV\textsubscript{1} and FEF\textsubscript{25,50,75} could develop due to the narrowing of the peripheral airways induced by the inhalation of oxygen. As this peripheral narrowing is vagally mediated, an indirect central nervous system effect seems more likely than a direct pulmonary effect\textsuperscript{59}. Whatever the origin of this decrement, both indices are effort dependent\textsuperscript{61} and share the same disadvantages as VC.
Figure 2: Relationship between pathological changes and changes in several of the most used lung function parameters. Straight line: literature based; dotted line: hypothetical.
**Lung compliance**

Compliance provides information about the elasticity of the lung, also called the distensibility. Lung compliance can be divided into static (Cst,L) and dynamic compliance (CL$_{\text{dyn}}$). Cst,L is the change in lung volume per unit change in the transpulmonary pressure without any gas flow into or out of the lung$^{62}$, whereas the CL$_{\text{dyn}}$ represents pulmonary compliance during periods of gas flow (such as during active inspiration) which includes differences in time constants. In healthy subjects both CL$_{\text{dyn}}$ and Cst,L yield similar results, with a mean value of 2.4 L.kPa$^{-1}$ $^{62}$. A decrease in Cst,L is interpreted as increased stiffness of the lung. This can be found in thickening of the visceral pleura due to tuberculosis or exposure to asbestos, respiratory distress syndrome, surfactant protein B deficiency, and following myocardial infarction and lung parenchyma disorders like fibrosis$^{62-64}$. An increase in Cst,L implies a more 'floppy' lung and can be found in emphysema$^{62,64}$. Finally, CL$_{\text{dyn}}$ is reduced in the presence of narrowing of the small airways $^{62}$.

Alveolar and interstitial oedema that develops during pulmonary oxygen toxicity increases stiffness of the lung$^{7}$ and, therefore, a reduction in lung compliance can be expected. Indeed, studies measuring lung compliance after hyperoxic exposure in healthy subjects showed a decrease of lung compliance of up to 22%$^{3,12,28,49,56,59,65}$. In contrast, an increase in lung compliance was found after a dive of 28 days at 250 kPa with a PO$_2$ of 40-75 kPa$^{66}$. It was hypothesised that an increase in compliance developed due to increased surfactant production, or as a result of destruction of the connective tissue constituents collagen and elastin in the lung$^{66}$. However, it remains unclear whether this kind of increase is purely hyperoxia induced or due to the work of breathing during diving itself. Finally, as the measurement of compliance is technically demanding and its reproducibility is less than for most other parameters it is doubtful whether lung compliance is a suitable parameter to monitor pulmonary oxygen toxicity in divers$^{62}$.

**Diffusing capacity for carbon monoxide (DL$_{\text{CO}}$)**

The diffusing capacity of the lung for carbon monoxide (DL$_{\text{CO}}$) estimates the amount of gas uptake by the lungs and can be determined by the diffusing capacity of the alveolocapillary membrane for CO (D$_{\text{MH}}$) and the pulmonary capillary blood volume (V$_C$)$^{67}$. 


Changes in $DL_{r,CO}$ after exposure to oxygen in divers have been studied since the 1960s\textsuperscript{11,13,45,46,49,59,60,65,68-79}. A decrease in $DL_{r,CO}$ of maximally 25\% was found after exposures with a $PO_2$ up to 400 kPa\textsuperscript{19,46,49,59,60,65,74,75}. The origin of this decrease could be a hindered diffusion across the alveolar capillary membrane\textsuperscript{49,74}, a decrease in alveolar surface area\textsuperscript{56} or a decrease in capillary blood volume\textsuperscript{56,59}. In contrast to these findings, no significant changes in $DL_{r,CO}$ were reported following exposure to oxygen with a $PO_2$ 140-300 kPa\textsuperscript{45,60,65,69,78}. This apparent contradiction might be explained by the way $DL_{r,CO}$ is measured. First, the measurement of $D_M$ and $V_C$ itself is not entirely accurate\textsuperscript{80}. Second, as the value of $D_M$ is determined by at least two measurements with high and low oxygen concentrations, the mode of inhalation, the stability of the gas analyser, and whether or not Valsalva or Muller manoeuvres were performed during the breath-hold phase, could be of influence\textsuperscript{67}. Third, although the affinity of CO for Hb is more than 200 times greater than for oxygen, an overload of oxygen diminishes this affinity of CO leading to a decrease in COHb\textsuperscript{81-83}. Supplemental oxygen breathing affects the measurement of $DL_{r,CO}$ and it is recommended to wait at least 10 min before performing a $DL_{r,CO}$ test after using supplemental oxygen\textsuperscript{67}. As changes in $DL_{r,CO}$ can be measured up to days after termination of oxygen breathing, it might be useful as a marker for recovery after oxygen exposure\textsuperscript{45,59}. As $DL_{r,CO}$ has the ability to measure and differentiate between gas transport changes of the alveolocapillary membrane and those of the pulmonary capillary blood volume, $DL_{r,CO}$ is preferred to VC as a marker of pulmonary oxygen toxicity\textsuperscript{16,46,72,84}.

**Fraction exhaled nitric oxide (FE\textsubscript{NO})**

Nitric oxide (NO) is an oxidation product of L-arginine and is synthesized throughout the respiratory tract by nitric oxide synthases\textsuperscript{85,86}. The FE\textsubscript{NO} pathway is often visualized as a two-compartment model with a conductive compartment (airway up to generation 17) and an alveolar one (generation 18 to alveolus). FE\textsubscript{NO} is the net result of flux and diffusion of NO in these compartments\textsuperscript{87,88}. In healthy subjects FE\textsubscript{NO} is solely dependent on height\textsuperscript{89-91} and age\textsuperscript{90}. Furthermore, FE\textsubscript{NO} is not influenced by day-to-day or within-day variations\textsuperscript{91,92}. Normal values for FE\textsubscript{NO} range from 15 parts per billion (ppb) to 50 ppb\textsuperscript{90,92-95}. Under extreme circumstances, such as in respiratory tract infections, induced nitric oxide synthases (iNOS) produces large quantities of NO\textsuperscript{96-98}. There is evidence from animal studies that also hyperoxia
leads to an increase in iNOS activity\textsuperscript{99}. Therefore, it is feasible that due to this upregulation in iNOS activity an increase of FE\textsubscript{NO} can be found as one of the key pathological aspects of pulmonary oxygen toxicity. Unfortunately, conflicting results have been reported. Increases of FE\textsubscript{NO} have been found\textsuperscript{100,101} when healthy subjects breathed oxygen for 30 min with a \textit{PO}_2 of 60 kPa-100 kPa. In contrast, decreases in FE\textsubscript{NO} were reported in patients receiving hyperbaric oxygen therapy (\textit{PO}_2 = 240 kPa for 90 min) for several weeks\textsuperscript{96,102,103} and in healthy subjects breathing 100% oxygen for 90 min at normobaric pressure\textsuperscript{104}. Finally, unchanged FE\textsubscript{NO} levels were reported in submerged divers who breathed oxygen with a \textit{PO}_2 of 126-180 kPa\textsuperscript{105}.

These contradicting results might be explained in two ways. First, although hyperoxia induces iNOS activity, endogenous NO may be reduced either by inhibition of iNOS activity through the oxidization of tetrahydrobiopterin, or endogenous NO was scavenged by proteins and free radicals\textsuperscript{96,99,104}. Secondly, for the measurement of FE\textsubscript{NO} the ATS/ERS guidelines recommend an exhalation flow rate of 50 ml.s\textsuperscript{-1}\textsuperscript{106}. At this flow rate only changes of FE\textsubscript{NO} derived from the conductive compartment are measured and not those from the alveolar compartment\textsuperscript{88,107-109}. To distinguish between the conductive and alveolar compartment the multiple exhalation flow technique must be used with an exhalation flow rate of at least 250 ml.s\textsuperscript{-1} \textsuperscript{87,88,93,110,111}. Caspersen and co-workers measured changes in FE\textsubscript{NO} after oxygen exposure using this multiple exhalation flow technique\textsuperscript{112}. Significant decreases in FE\textsubscript{NO} from solely the conductive compartment were found in healthy subjects (\textit{PO}_2 = 101 kPa for 90 min). However, as oxygen was breathed in a dry environment it remains unclear whether these results also apply for submerged oxygen breathing divers. Although the measurement of FE\textsubscript{NO} seems to be promising, further studies using the multiple exhalation flow technique are needed to estimate the clinical value of FE\textsubscript{NO} as a monitoring method for pulmonary oxygen toxicity in divers.
EMERGING TESTS FOR MONITORING PULMONARY OXYGEN TOXICITY

**Diffusing capacity for nitric oxide (DL\textsubscript{rNO})**

As NO binds up to 1400 times stronger to Hb than CO the diffusing capacity is not influenced by V\textsubscript{C}\textsuperscript{113}. It can be postulated that DL\textsubscript{rNO} determines the true alveolocapillary D\textsubscript{M}\textsuperscript{114}. Therefore, DL\textsubscript{rNO} might be more appropriate than the DL\textsubscript{rCO} method to differentiate between injuries originating from the alveolocapillary membrane and those from the pulmonary capillaries. DL\textsubscript{rNO} is influenced by height, age, gender and exercise\textsuperscript{115,116}. To what extent it is also influenced by food or coffee intake and smoking remains unclear.

The advantage of the DL\textsubscript{rNO} method over the DL\textsubscript{rCO} method is the absence of interaction between NO and oxygen, which makes measurement of acute changes after oxygen exposure possible\textsuperscript{117}. Although high levels of inhaled NO lead to vasodilatation of the pulmonary capillary vessels\textsuperscript{118} inhaled NO levels up to 67 ppm do not affect DL\textsubscript{rNO} measurements\textsuperscript{119}.

Besides the measurement of DL\textsubscript{rNO} and DL\textsubscript{rCO} per se also the DL\textsubscript{rNO}/DL\textsubscript{rCO} ratio is used in respiratory medicine\textsuperscript{120}. This ratio is inversely related to the thickness of the alveolar membrane and capillary sheath\textsuperscript{120}. Any change in either DL\textsubscript{rNO} and/or DL\textsubscript{rCO} will lead to an increase or decrease of this ratio. Thus, DL\textsubscript{rNO}/DL\textsubscript{rCO} ratio can differentiate between subjects with pure membrane disturbances and those with microvascular diseases\textsuperscript{121}. The normal value for this ratio is in the range of 4.3–4.9\textsuperscript{113,117,120}. Increases in this ratio can be found in patients with pulmonary arterial hypertension whereas in hypoxemic patients this ratio is decreased\textsuperscript{120,121}.

At present, only one study has addressed DL\textsubscript{rNO} after oxygen exposure\textsuperscript{69}. In that study no changes in DL\textsubscript{rNO} or in DL\textsubscript{rNO}/DL\textsubscript{rCO} ratio were found after a submerged oxygen dive with a PO\textsubscript{2} of about 150 kPa for 3 h. However, to determine the role of DL\textsubscript{rNO} as a marker for pulmonary oxygen toxicity in divers more studies (preferably with higher oxygen loads or longer exposure times) are required.
Exhaled breath condensate (EBC)

Exhaled breath contains dozens of non-volatile compounds\textsuperscript{122} which can be collected in cooled and condensed exhalate, also known as exhaled breath condensate (EBC). Standard techniques for the detection of the different species of EBC compounds include enzyme-linked immunoassays, pH measurements and fluorometric assay technique\textsuperscript{122-124}. Analysis of EBC by nuclear magnetic resonance spectroscopy (MRS) has been introduced and seems promising in identifying metabolic fingerprints of EBC in different clinical datasets in lung diseases\textsuperscript{125,126}.

Beside the fact that the collection is time consuming, a major drawback is the lack of a standard measurement procedure for EBC\textsuperscript{122,123,127}. Furthermore, most of the non-volatile compounds are in the lower detection range of the assay where the intra- and inter-assay variability is large\textsuperscript{123}. Finally, salivary contamination may influence the levels of several markers detectable in EBC\textsuperscript{92}.

Lipid peroxidation of the lung cell membrane due to oxidative stress produces various aldehydes like F\textsubscript{2}-isoprostanes, 4-hydroxy-2-nonenal, acrolein, malondialdehyde and hydrocarbons like ethane, pentane, and isoprene\textsuperscript{122}. An increase in 8-isoprostane and interleukin-6 was found after a 1 h exposure to 28% oxygen (28 kPa) in healthy subjects. This suggests that even low doses of supplementary oxygen may enhance oxidative stress and inflammation in the airways\textsuperscript{128}. In contrast to these findings, Taraldsøy \textit{et al.} reported no significant changes in EBC after a series of hyperbaric oxygen treatments (\(PO_2 = 240\) kPa for 90 min)\textsuperscript{102}. As these are the only two studies to measure EBC before and after oxygen exposure it remains unclear to what extent EBC will change following inhalation of higher levels of O\textsubscript{2} and which changes of the non-volatile compounds best represent pulmonary oxidative stress. To fingerprint pulmonary oxidative stress changes of EBC after submerged oxygen exposure, future studies should consider the use of MRS EBC.
Exhaled volatile organic compounds (VOC)

After the first publication by Pauling\textsuperscript{129} more than 3000 volatile organic compounds (VOC) have been detected in the breath we exhale\textsuperscript{130}. However, about 1% of these VOC can be found in all subjects and are likely to contain disease specific VOC\textsuperscript{131,132}. Most VOC found in exhaled breath are alkanes, isoprenes, benzene and methylalkanes derivates\textsuperscript{130}. VOC like hydrocarbons and alkanes are produced and exhaled as a representation of oxidative stress leading to lipid peroxidation of cell membranes\textsuperscript{15,40,56,133-137}. The origin of these exhaled VOC is assumed to be mainly alveolar\textsuperscript{138}. Analysis techniques like gas chromatography, mass spectrometry, absorption spectrometry, photoacoustic spectroscopy, and chemical or semiconductor sensors are used to detect VOC in exhaled breath by real-time measurement or by indirect methods\textsuperscript{137,139,140}. Combining different sensors using one of the aforementioned techniques led to the development of artificial olfaction systems. These systems, also known as electronic noses, produce VOC patterns consisting of each of the detected VOC\textsuperscript{131}. This VOC pattern is also referred to as breath print. Currently, breath prints have been obtained for asthma\textsuperscript{136,137,141,142}, COPD\textsuperscript{136,137,141,142}, interstitial lung diseases\textsuperscript{136,137} and lung cancer\textsuperscript{137,143}.

A general disadvantage of measuring exhaled VOC is the contamination of the inhaled air due to VOC present in indoor air and those related to smoking. These contaminating VOC must be determined and taken into account during analyses\textsuperscript{140}. To achieve good qualitative storage silanised metal canisters or thermal desorption tubes must be used\textsuperscript{140}. Although storage up to 14 days does not influence the diagnostic accuracy\textsuperscript{144}, when stored for a longer period it could bear the risk of decomposition of VOC\textsuperscript{140}.

Several studies measured VOC after oxygen exposure. First, Morita et al. found an increase in n-pentane when healthy volunteers breathed 100% oxygen for 30 min indicating the occurrence of lipid peroxidation after a short period of oxygen breathing\textsuperscript{145}. This result is similar to that found in healthy subjects by Loiseaux-Meunier et al. after breathing 100% of oxygen for 125 min\textsuperscript{146}. In contrast, Lemaître et al. found no significant changes in pentane when healthy volunteers were breathing 60% oxygen for 30 min\textsuperscript{101}.

Phillips et al. reported an increase in alveolar gradients of 3-methyltridecane, 3-methylundecane and 5-methylnonane as well as an increase in the mean volume under the curve of breath methylated
alkane contour when healthy volunteers breathed 28% oxygen at 2.0 l.min⁻¹ via nasal prongs for 30 min¹⁴⁷. This result is very interesting. As mentioned before, it is generally accepted that breathing oxygen with levels up to 50 kPa will not lead to signs of pulmonary oxygen toxicity. However, similar to the study of Carpagnano et al. using the EBC method¹²⁸ this study of Philips et al. also implies that supplemental oxygen at levels assumed to be safe might induce pulmonary oxidative stress. As no studies have been performed in oxygen divers it is unknown whether these kinds of VOC changes are also seen in this population. Therefore, more research with different oxygen loads and exposure times in a submerged environment is needed to determine the exact position of breath prints as a marker for pulmonary oxygen toxicity in divers.

**Exhaled breath temperature (EBT)**
One of the major signs of inflammation is *calor* or the increasing temperature of the inflamed area. As inflammation is a key feature in several respiratory diseases it was hypothesised that the temperature of the air coming from this inflicted area (airways) must be increased. Paredi *et al.* and Piacentini *et al.* found increases in the exhaled breath temperature of asthmatic patients, thereby supporting this hypothesis¹⁴⁸,¹⁴⁹. Measurement of EBT may be used as a parameter for the assessment of asthma control¹⁵⁰-¹⁵². Two methods have been proposed to measure EBT. Paredi and co-workers used the rising part of the exhaled breath temperature (ΔeºT) during a complete exhalation to residual volume as EBT¹⁴⁹,¹⁵⁰ while Piacenti and co-workers used the end-expiratory plateau of the exhaled breath temperature (PLET)¹⁴⁸,¹⁵¹. Comparing both methods PLET might be favoured, but further research is required. Exhalation flow rates, the use of antibacterial filters, and apparatus characteristics should be investigated¹⁵¹,¹⁵³. EBT is not related to age, gender or height¹⁵⁴. Nowadays, portable devices make onsite measurement possible¹⁵². Finally, the EBT measurement is non-invasive and easy to use in both adults and children¹⁵⁴.

As inflammation is also one of the components of pulmonary oxygen toxicity one can hypothesise that EBT will also change after oxygen exposure. However, no studies have been performed in which EBT was measured before and after oxygen exposure. Therefore, any application for pulmonary oxygen toxicity remains speculative.
**Blood biomarkers**

Pulmonary oxygen toxicity is likely to be associated with systemic metabolic changes. Although the respiratory tract is considered to be the first organ to manifest oxygen-induced injury\(^3\),\(^4\),\(^12\) other organs like the central nervous system, retina, liver, heart, endocrine system and kidneys can also be affected by the presence of reactive oxidative species (ROS) derived from oxygen originated metabolites\(^15\),\(^29\). To counteract these ROS, organs developed several antioxidant systems like, e.g., superoxide dismutase, catalase, glutathione peroxidase, albumin, ceruloplasmine, ferritin, reduced glutathione (GSH) and methionine\(^155\). Unfortunately, it is difficult to measure each of these antioxidant components separately, while the measurement of only one individual antioxidant is not representative for the overall antioxidant status. Therefore, methods have been developed to assess the total antioxidant capacity\(^156\),\(^157\). The methods most frequently used are oxygen radical absorbance capacity (ORAC), the ferric reducing-antioxidant power (FRAP), the total radical trapping antioxidant parameter (TRAP), and Trolox equivalent antioxidant capacity (TEAC)\(^156\),\(^157\). Interpretation of changes in plasma and serum antioxidant capacity depends on the conditions under which the antioxidant capacity is determined\(^156\). It is important not to rely on a single measurement of antioxidant status, but to use a battery of measurements\(^156\).

Instead of the total antioxidant capacity it is also possible to measure oxidized products. In pulmonary oxygen toxicity lipid peroxidation of cell membranes is a key feature. Lipid peroxidation products that can be determined in blood are lipid hydroperoxides, malondialdehyde and isoprostanes\(^158\).

Blood biomarkers of oxidative stress have been used in only one study with oxygen divers. Although levels of glutathione peroxidase and total antioxidant status were significantly decreased in divers following a 12-week training program in oxygen diving these reductions were considered too low to generate any clinical effect\(^159\).

It must be emphasized that both lipid peroxidation products and total antioxidant status are not solely pulmonary derived but can develop anywhere in the human body. However, a combination of blood biomarkers with other lung function parameters discussed in this review could be of value in monitoring the early onset and development of pulmonary oxygen toxicity.
CONCLUSION

Breathing oxygen with a $PO_2$ of 50-300 kPa for hours or for days may induce a pathological phenomenon known as pulmonary oxygen toxicity. In both human and animal studies the development of pulmonary oxygen toxicity can be divided into an early exudative phase, which is reversible, and a late irreversible proliferative phase. It is important that with pure oxygen or enriched oxygen mixtures, divers stay within the reversible phase. As part of prevention, changes in lung function are used as markers for the onset and development of pulmonary oxygen toxicity. Nowadays, the most frequently used indices are the decrease in vital capacity and diffusing capacity for carbon monoxide. However, both these tests have substantial disadvantages and it is unclear whether they can be used after wet hyperbaric oxygen exposures in the same way as for dry hyperbaric oxygen exposures.

Several novel techniques are being validated for pulmonary oxygen toxicity. Most of these new methods are easy to perform, can be used repeatedly, are non-invasive and might reduce costs. In particular the use of exhaled volatile organic compounds (breath prints), exhaled breath condensate, exhaled breath temperature, fraction of exhaled nitric oxide, and diffusing capacity for nitric oxide could be of interest in monitoring pulmonary oxygen toxicity. However, until now few if any studies have been performed in which these new techniques are applied for the detection of pulmonary oxygen toxicity. Further research is necessary to determine the role of these new techniques as potential biomarkers for the onset and development of pulmonary oxygen toxicity. Until then, we advise to use changes in vital capacity or diffusing capacity for carbon monoxide to monitor the development of pulmonary oxygen toxicity, as these methods are the best documented.

ACKNOWLEDGEMENTS

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Table 3: Strengths and limitations of lung function indices in monitoring pulmonary oxygen toxicity.

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<thead>
<tr>
<th>Indices</th>
<th>Strengths</th>
<th>Limitations</th>
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<tbody>
<tr>
<td>FVC</td>
<td>Relatively easy to use method</td>
<td>Effort dependent implying the need for properly trained lung function technicians</td>
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<tr>
<td></td>
<td>Can be measured on site using a portable spirometer</td>
<td>Can only be measured in cooperative subjects</td>
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<td></td>
<td>It is the most studied lung function index regarding pulmonary oxygen</td>
<td>Not able to detect small changes</td>
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<td></td>
<td>toxicity in diving</td>
<td>Large variation in individual response</td>
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<tr>
<td></td>
<td></td>
<td>Might be inappropriate for short or in-water oxygen exposures</td>
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<tr>
<td></td>
<td></td>
<td>Is influenced by exercise</td>
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<tr>
<td>FEV₁ and maximal expiratory</td>
<td>Relatively easy to use method</td>
<td>Effort dependent</td>
</tr>
<tr>
<td>flows</td>
<td>Can be measured on site using a portable spirometer</td>
<td>Can only be measured in cooperative subjects</td>
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<td></td>
<td></td>
<td>Not able to detect small changes</td>
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<td>Large variation in individual response</td>
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<td></td>
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<td>Might be inappropriate for short or in-water oxygen exposures</td>
</tr>
<tr>
<td>Indices</td>
<td>Strengths</td>
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<tr>
<td>Lung compliance</td>
<td>– Can be measured in both conscious and unconscious subjects &lt;br&gt; – It can differentiate between lung function impairment by emphysema and fibrosis</td>
<td>– Measurement is technically demanding &lt;br&gt; – Can only be performed in hospital or laboratory setting &lt;br&gt; – Measurement is demanding for the subject &lt;br&gt; – Tonic contraction of the oesophagus can delay the measurement &lt;br&gt; – Reproducibility is less compared to other indices</td>
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<tr>
<td>$DL_{CO}$</td>
<td>– It can differentiate between alveolocapillary membrane derived injuries and pulmonary capillary blood volume derived injuries &lt;br&gt; – Can be used to monitor the development as well and the recovery of pulmonary oxygen toxicity &lt;br&gt; – Might be more sensitive than VC &lt;br&gt; – One of the most frequently studied index regarding pulmonary oxygen toxicity</td>
<td>– Cannot be measured directly after oxygen exposure &lt;br&gt; – Is influenced by smoking habits, food intake, coffee intake and exercise &lt;br&gt; – Can only be performed by properly trained lung function technicians &lt;br&gt; – Can only be measured in cooperative subjects &lt;br&gt; – Small changes cannot be detected</td>
</tr>
<tr>
<td>Indices</td>
<td>Strengths</td>
<td>Limitations</td>
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</table>
| $\text{FE}_{\text{NO}}$ | - Easy to use method  
- Not demanding for subjects  
- Can be used directly after oxygen exposure  
- Measurement time is short  
- Not influenced by within-day or day-to-day variations  
- Can differentiate between alveolar and conductive compartment  
- On site measurement is possible | - Recommended exhalation flow of 50 ml.s$^{-1}$ not suitable for measuring changes in the alveolar compartment  
- Multiple exhalation flow technique can only be done in laboratory or hospital settings  
- Can only be measured in cooperative subjects |
| $\text{DL}_{\text{NO}}$ | - See also $\text{DL}_{\text{CO}}$  
- Can be used directly after oxygen exposure  
- Is capable of differentiating between alveolocapillary membrane component and capillary blood volume component that $\text{DL}_{\text{CO}}$ | - Can only be performed in laboratory or hospital settings  
- Must be performed by properly trained lung function technicians  
- Can only be measured in cooperative subjects  
- Relatively high levels of NO can confound the measurement results  
- Not capable of measuring small changes |
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<tr>
<th>Indices</th>
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<th>Limitations</th>
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<tbody>
<tr>
<td>Exhaled breath condensate</td>
<td>- Well tolerated by subjects</td>
<td>- Condensate must be frozen immediately</td>
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<td></td>
<td>- Can be repeated several times within a short interval</td>
<td>- Must be stored at -70°C</td>
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<td>- Collection can be performed on site</td>
<td>- Is influenced by smoking habits, food intake, drink intake and salivary contamination</td>
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<td></td>
<td>- Multiple non-volatile species can be detected</td>
<td>- No standard procedure available</td>
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<td></td>
<td>- NMR technology applicable</td>
<td>- Inter and intra-assay variability is large</td>
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<td></td>
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<td>- Signals close to detection limits of assay</td>
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<tr>
<td>Exhaled volatile organic compounds</td>
<td>- Can be measured real-time as indirect</td>
<td>- Measurements can be confounded by indoor air or smoking habits</td>
</tr>
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<td></td>
<td>- Collection of breath samples is easy to perform</td>
<td>- Decomposition of VOC can confound the results</td>
</tr>
<tr>
<td></td>
<td>- Collection devices are portable and can be used on site</td>
<td>- When stored silanised metal canisters are needed</td>
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<td></td>
<td>- Collection itself is well tolerated by subjects and takes only a few minutes</td>
<td>- How long VOC can be stored is unknown</td>
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<td>- Collection of breath samples can be performed in both conscious and unconscious subjects</td>
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<tr>
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<tr>
<td>Exhaled breath temperature</td>
<td>– Easy to perform and well tolerated by subjects</td>
<td>– New technique: its limitations are still under study</td>
</tr>
<tr>
<td></td>
<td>– Independent of height, gender and age</td>
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<td></td>
<td>– Devices are portable</td>
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<td>– Measurement time is 4-10 min</td>
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<tr>
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<td>– Coefficient of variation is relatively small</td>
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