Formation of nitrogen dioxide from nitric oxide and their measurement in clinically relevant circumstances

U. Schedin1*, C. G. Frostell2 and L. E. Gustafsson3

1Division of Anaesthesia and Intensive Care, Karolinska Institute at Danderyd Hospital, S-182 88 Danderyd, Sweden. 2Department of Paediatric Anaesthesia and Intensive Care, Karolinska Hospital, Stockholm, Sweden. 3Department of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden

*To whom correspondence should be addressed

Therapy with inhaled nitric oxide in oxygen requires adequate monitoring of nitric oxide and nitrogen dioxide. The characteristics of chemiluminescence and electrochemical measurement techniques were determined by analysis of continuously flowing gas mixtures and comparisons with traceable gas standards. Gas mixtures were also diluted with mass flow controllers and in addition created in ventilator breathing systems. Factors influencing the formation of nitrogen dioxide were defined. Both techniques accurately measured nitric oxide (10–80 parts per million, ppm) and nitrogen dioxide (0.5–5 ppm) in normoxic and hyperoxic (90% oxygen) gas in the studied ranges. Nitrogen dioxide in hyperoxic gas had three origins: (1) from the pre-mixing point of nitric oxide in nitrogen, (2) as a result of the mixing process, and (3) from post-mixing and time-dependent continuous formation of nitrogen dioxide in oxygen. We conclude that adequate monitoring is possible and that factors affecting nitrogen dioxide generation can be defined.

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Clinical use of inhaled nitric oxide has been increasing since the first articles reporting selective pulmonary vasodilation in animals1 and humans2 with pulmonary hypertension. Inhaled nitric oxide in the range 0.1–100 parts per million (ppm), usually in a hyperoxic gas mixture, is typically administered to intensive care patients suffering from a variety of diseases characterized by pulmonary hypertension and/or hypoxaemia.3–6

Nitrogen dioxide formation

In clinically used gas mixtures of nitric oxide and oxygen, there is spontaneous formation of nitrogen dioxide during delivery of the gas to the patient. Formation of other oxides of nitrogen has also been implied,7 but nitrogen dioxide seems to be of greatest concern as it is probably the dominant species. This time-dependent nitrogen dioxide formation is proportional to oxygen (linear relationship) and nitric oxide (square relationship) concentrations. In air, it takes approximately 7 h to oxidize 50% of nitric oxide 10 ppm to nitrogen dioxide, and approximately 24 s if the nitric oxide concentration is 10 000 ppm (1%).8

Nitrogen dioxide toxicity

Acute effects of higher doses of nitrogen dioxide are hypoxaemia, acidosis, pulmonary oedema and pneumonitis.9–11 Chronic exposure to minute concentrations of nitrogen dioxide (in the low ppm range) may injure the respiratory tract in humans, causing inflammatory conditions and increased sensitivity to irritants, and may possibly lead to increased immune sensitization.12 There is also some evidence of the mutagenicity of nitrogen dioxide.13 Therefore, clinical use of nitric oxide requires adequate monitoring devices for nitrogen dioxide and nitric oxide.

Analysis

For environmental monitoring, nitric oxide and nitrogen dioxide concentrations have been measured by electrochemical (EC), chemiluminescence (CL) and colour indicator techniques. The equipment used has been developed for gases containing 21% oxygen or less. Such gas mixtures are relatively stable over time and therefore an instrument with a response time of 30 s to a few minutes still yields useful data. Other feasible methods for measurement of
nitrogen dioxide are ultraviolet or infrared techniques. In addition, laser diode, gas chromatography–mass spectrometry or differential optical absorption spectroscopy (DOAS) may be used. Several recent reports have questioned the accuracy of at least some commercially available chemiluminescence devices, when applied for clinical monitoring of nitrogen dioxide in oxygen-rich (90–95% oxygen) gases also containing significant amounts of nitric oxide and where rapid fluctuations in nitric oxide and nitrogen dioxide concentrations over time may occur. Even negative values for nitrogen dioxide have been reported. The accuracy of electrochemical cells, and their effective calibration, have been questioned. Also, factors affecting the formation of nitrogen dioxide in clinically relevant conditions are poorly understood.

In this study, we have evaluated the accuracy of chemiluminescence and electrochemical techniques in constantly flowing gas mixtures containing up to 90% oxygen and nitric oxide 80 ppm, using mass flow controllers for accurate gas mixing. We have also determined the rate constant for nitrogen dioxide formation under these conditions, the amount of nitrogen dioxide formed in typical ventilatory conditions, have been questioned. Also, factors affecting the accuracy of electrochemical cells, and their effective calibration, have been questioned. Also, factors affecting the formation of nitrogen dioxide in clinically relevant conditions are poorly understood.

In this study, we have evaluated the accuracy of chemiluminescence and electrochemical techniques in constantly flowing gas mixtures containing up to 90% oxygen and nitric oxide 80 ppm, using mass flow controllers for accurate gas mixing. We have also determined the rate constant for nitrogen dioxide formation under these conditions, the amount of nitrogen dioxide formed in typical ventilatory conditions, and have studied some factors contributing to the concentrations of nitrogen dioxide found in clinically relevant steady flow gas mixtures. Preliminary accounts of these data have been presented.

Material and methods

Experimental set-up

A basic experimental set-up was used for validation of analysers and for kinetic studies, using a ‘Nomius’ (see below) gas metering and mixing device, feeding mixed test gas into a corrugated tubing of varying length (indicated as broken lines in Fig. 1). The mixing point consisted of a narrow Y-shaped connector immediately followed by a small 90° knee. The test gas was moving from left to right at a temperature of 21–24°C. Beyond the mixing point (‘downstream’), preliminary experiments showed that full mixing had occurred and that changes in gas mixture followed expected kinetics. However, when further gas additions were made downstream from the side arms into this tubing, signs of incomplete mixing occurred. This necessitated creating turbulent flow before the downstream addition points by incorporating a stricture proximal to these. The stricture (R in Fig. 1) consisted of a plug with a channel of 3 mm inner diameter and 25 mm in length placed to fit tightly inside the tubing. Thus additions of nitrogen dioxide or nitric oxide could be made into the gas delivered by the device, and nitrogen dioxide and nitrogen were always added immediately downstream of the restriction, in order to mix the added gas into a turbulent gas flow. A small increase in gas pressure (<0.5 cm H2O) was created before the stricture. When the tube between the mixing point and the restrictor was very short, there was still a minimum delay (t0 <0.5 s) between mixing and a sample reaching the measuring points in the analysers; when the tube was long (up to 6.2 m at 340 ml m⁻¹) there was an increased time (up to 25 s) for formation of nitrogen dioxide (aged gas). The residence time or age of the gas was calculated from the gas flow rate and actual tube length.

To the left, outside the figure, the system was connected to gas cylinders containing nitric oxide in nitrogen, and oxygen and nitrogen, or to a supply of charcoal-filtered room air. The regulators for nitric oxide in nitrogen were of 3–16 stainless steel and the high pressure tubing was made from Teflon. The desired oxygen fraction was delivered by a Siemens (Siemens-Elema, Stockholm, Sweden) standard mechanical ventilator gas mixer (indicated as ‘S’). For hypoxic mixtures, the air supply to the mixer was replaced by the nitrogen cylinder. The two gas mixtures (nitric oxide in nitrogen and oxygen or air) passed the dosage unit, ‘Nomius’ (see below under ‘Control of gas flows and mixing’) in separate high-pressure tubes. The pressure reduction to atmospheric conditions occurred at the mass flow controllers in the ‘Nomius’. At the exit from the
‘Nomius’, before the mixing point, pressures did not exceed atmospheric pressure by more than a few centimetres of water (a pressure required for flow through unobstructed tubing).

After the mixing point, the streaming gas had a constant flow rate of 5 litre min⁻¹ if not otherwise stated. A steady flow of nitrogen dioxide or nitrogen could then be added via mass flow controllers (MFC1 for nitrogen dioxide and MFC2 for nitrogen). Finally, the gas mixture was analysed for oxygen, and for nitric oxide and nitrogen dioxide by chemiluminescence (CL) and electrochemical (EC) techniques using side-stream sampling. To the right the system was open to air. In some types of test series (e.g. kinetic studies), tubing of different lengths were inserted between the mixing point and the restriction (R) to increase the residence time of the gas in the system during which a certain amount of nitrogen dioxide was formed (aged gas).

In reality, nitrogen dioxide concentrations were monitored after the gas had passed from the mixing point through the sampling tubes to the true measuring point in the analyser and some nitrogen dioxide formation must be considered in nitric oxide- and oxygen-containing gases during this time (t₀), even though it was short (<0.5 s).

Gases. In addition to charcoal-filtered room air, the following bulk gases were used: nitrogen; oxygen; medical grade nitric oxide in nitrogen from two different stock cylinders certified as nitric oxide 1006 ppm–nitrogen dioxide 0 ppm (called GS1000) and nitric oxide 310 ppm–nitrogen dioxide 1 ppm (GS300), respectively; nitrogen dioxide in nitrogen certified as nitrogen dioxide 95.7 ppm–NOₓ 97.0 ppm (NOₓ is the total amount of nitrogen oxides); and calibration gas certified as nitric oxide 1006 ppm–nitrogen dioxide 0 ppm (GS300), respectively; nitrogen dioxide in nitrogen certified as nitrogen dioxide 83.3 ppm–nitrogen dioxide 6.3 ppm. The certified gases were traceable directly to NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA). All gases were delivered in cylinders from AGA Gas AB, Lidingö, Sweden.

Control of gas flows and mixing. The desired oxygen fraction was delivered by the oxygen-air mixer (Siemens Elema, Stockholm, Sweden; ‘S’ in Fig. 1). Normoxic and hyperoxic mixtures were created by mixing air (charcoal-filtered air; content of nitric oxide and nitrogen dioxide <1 ppb) and oxygen. Hypoxic mixtures were created by mixing oxygen and nitrogen (Nitrogen Plus, AGA Specialgas). In the nitric oxide dosage unit ‘Nomius’ (DanSjö Medical AB, Järfälla, Sweden) two mass flow controllers (Bronkhorst, Ruurlo, The Netherlands) delivered the set flows of nitric oxide–nitrogen and oxygen–nitrogen, respectively. The flow rate of the mass flow controllers used for mixed oxygen–nitrogen ranged from 3 to 30 litre min⁻¹ and for nitric oxide–nitrogen, 0.04 to 2 litre min⁻¹. Their accuracy according to the manufacturer was greater than ±1% of full scale. For the dosage of nitrogen dioxide and nitrogen (added after R in Fig. 1) two other mass flow controllers (same manufacturer) were used with flow rates of 0–1.5 litre min⁻¹ for nitrogen dioxide (MFC1) and 0–15 litre min⁻¹ for N₂ (MFC2). Their accuracy according to the supplier was ±0.5% of the reading and ±0.1% of the full scale.

Tubing. The corrugated ventilator tubing between the mixing point and the restrictor (‘R’ in Fig. 1) was made of clear polyethylene acetate (Smooth-Flo Corr-A-Tube II corrugated tubing, Hudson Oxygen Therapy Sales Co, Temecula, CA, USA) of different lengths (up to 6.2 m in some experiments). In the other tube connections (e.g. sampling tubes to the analysers), the material was polypropene (Furon P-tubing).

Ventilators

To evaluate conditions in a clinical situation, two ventilators, Servo 300 and Servo 900D (Siemens Elema, Stockholm, Sweden), were used, ventilating a balloon instead of a patient.

Servo 300. Servo 300 was equipped with a nitric oxide dosage unit inside the ventilator; air, oxygen and a cylinder of nitric oxide 1000 ppm in nitrogen were connected directly to the ventilator. During expiration, this ventilator had a remaining ‘bypass’ flow with the intention of assisting exhalation and with the same composition as during inspiration. The magnitude of the bypass flow was dependent on the different available settings: 2 litre min⁻¹ in the adult, 1 litre min⁻¹ in the paediatric and 0.5 litre min⁻¹ in the neonatal settings. The ventilator was also equipped with an electrochemical cell placed on the expiration side just before the outlet. Consequently, this sensor measures a gas mixture of delivered bypass gas and expired gas, the latter having a low content of nitric oxide and nitrogen dioxide in the clinical situation as pulmonary uptake is high. These nitric oxide and nitrogen dioxide values were noted, but not evaluated further in this study. When we were simulating an adult patient, the ventilator settings chosen were: adult, 5 litre min⁻¹ total ventilation and 15 bpm, and for children: paediatric, 3 litre min⁻¹ and 30 bpm. Samples were aspirated from the inspiratory limb 13 cm and 17 cm before the Y-piece, by EC and CL, respectively. The displayed total ventilation (delivered volume per minute) and bypass flow had an impact on the dwelling time of the gas in the ventilator before reaching the patient.

Servo 900D. Servo 900D had no internal nitric oxide delivery device and was used together with the Nomius dosage device, connected to the low pressure inlet of the ventilator, with the mixing point of nitric oxide–nitrogen and oxygen–nitrogen downstream of the ‘Nomius’ device just before the ventilator. The ventilator settings and gas sampling points were the same as for Servo 300, with the exception that the 900D does not have bypass flow during expiration.

Tubings. From the ventilators, gas passed through 1.5 m of Hytrel breathing tubing (Anmedic AB, Vallentuna, Sweden) on its way to the Y-piece and balloon. We used different tube sizes for the adult and paediatric studies.
Nitrogen oxides in hyperoxic gas

**Analysers**

**Electrochemical analyser.** The analyser NOxBOX (denoted ‘EC’; Bedfont Scientific Ltd, Upchurch, Kent, UK) had electrochemical sensors for nitric oxide and nitrogen dioxide. We attached an external pump which forced a sample flow of 0.59 litre min\(^{-1}\) of gas through the analyser. According to the manufacturer, analytical sensitivities of 0.2 ppm were possible and the cross sensitivity response of the nitric oxide sensor to nitrogen dioxide was <20–30%, but the nitrogen dioxide sensor did not respond to nitric oxide.

**Chemiluminescence.** The analyser ML 9841A (denoted ‘CL’; Monitor Labs Inc., Englewood, CO, USA) used the chemiluminescence technique for determination of nitric oxide and nitrogen dioxide. A built-in pump, also delivering the necessary vacuum for the chemiluminescence reaction, aspirated a sample flow of 0.64 litre min\(^{-1}\) of gas through the analyser.

**Datex Oscar Oxy.** Datex Oscar Oxy (Instrumentarium OY, Helsinki, Finland), a clinical respiratory monitor, was used for oxygen determinations.

**Procedure**

We elected to study only continuously flowing gas mixtures.

(A) **Daily tests of the system**

Every morning the test system was flushed with carbon-filtered room air and checked for nitric oxide and nitrogen dioxide. The same procedure was followed for every new series of measurements. Measurement performance checks with the calibration gas were also made every morning.

(B) **Addition of nitrogen dioxide after \(t_0\)**

Nitrogen dioxide was added to the main flow to produce added concentrations, which increased in steps of 1 ppm up to 5 ppm (0.5 or 1 ppm up to 2 ppm in hyperoxic nitric oxide-containing gases) to different gas mixtures: nitrogen; oxygen; nitric oxide 80 ppm in nitrogen; and nitric oxide 20 and 80 ppm in 85–90% oxygen. There was no corrugated tubing between the mixing point and restrictor, so gas was analysed at time \(t_0\). Our aim was to evaluate the performance of the two analysers in different gas mixtures, from nitric oxide-free nitrogen to hyperoxic nitric oxide-rich gases, by varying the total nitrogen dioxide content in a known manner and also to obtain information on possible nitrogen dioxide formation before and during mixing (see below).

(C) **Addition of nitrogen dioxide after \(t_0+25\) s**

Twenty-five seconds after gas mixing (i.e. with 6.2 m of corrugated tubing inserted in the test rig), nitrogen dioxide was added in steps of 0.5 or 1 ppm up to 2 ppm to gas mixtures, initially containing nitric oxide 20 or 80 ppm in 85–90% oxygen. We aimed to determine the effect of the increased time for nitric oxide to react with oxygen and whether, again, the additions of nitrogen dioxide gave the expected increases in nitric dioxide concentration.

(D) **Dilution with nitrogen after \(t_0+25\) s**

Twenty-five seconds after gas mixing, nitrogen was added to gas mixtures, initially containing nitric oxide 80 ppm in 90% oxygen and by that time some nitrogen dioxide, to reach calculated dilutions of 75%, 50% and 25% of the original concentrations. Our aim was to evaluate if the concentration of oxygen affected measurement of nitric oxide or nitrogen dioxide.

(E) **Nitrogen dioxide formation up to \(t_0\) using different nitric oxide stock concentrations**

Two different nitric oxide stock gases, containing 300 ppm and 1000 ppm, respectively, were used to produce concentrations of nitric oxide 10, 20, 30, 40, 60 and 80 ppm in 75% and 90% oxygen (\(n=4\) for all combinations) and nitric oxide 80 ppm in nitrogen (\(n=3, n=4\), respectively). The aim was to study how stock cylinder nitric oxide concentration influenced mixing point (nitric oxide in hyperoxic gas) and pre-mixing point (nitric oxide in nitrogen) generation of nitrogen dioxide.

(F) **Kinetics**

The spontaneous formation of nitrogen dioxide (NO\(_2\)) in gas mixtures of nitric oxide (NO) is proportional to the cube of the pressure, the square of the concentration of nitric oxide and is linearly proportional to the concentration of oxygen (O\(_2\)) and, at any given pressure, can be described by the reaction formula:

\[
2\text{NO} (\text{gas}) + \text{O}_2 (\text{gas}) \rightleftharpoons 2\text{NO}_2 (\text{gas})
\]

leading to the kinetics:

\[
\frac{d[\text{NO}_2]}{dt} = k[\text{NO}]^2 [\text{O}_2]
\]

Four estimates of the rate constant were made from the differences in measurements at \(t_0\) and at \(t_0+25\) s (in procedures (B) and (C)) at two different nitric oxide concentrations (20 and 80 ppm in 85–90% oxygen), using two techniques: chemiluminescence and electrochemical. Additional measurements were made at other nitric oxide and oxygen concentrations, and at shorter time delays (\(n=1\) for all combinations) to examine conformity with equation (2).

(G) **Ventilators**

Nitrogen dioxide formation in gas mixtures of nitric oxide 20–80 ppm in 90% oxygen was determined with adult settings on the Servo 300, and with adult and paediatric settings on the Servo 900D.

**Statistical analysis**

Results are given as mean (SD). Descriptive statistics and calculation of regression lines, including slopes and 95% confidence limits, were obtained using the procedures in Sigma Stat (Jandel Corporation, Corte Madera, CA, USA); \(n\) denotes number of measurements.
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Table 1 Data from nitrogen dioxide (NO₂) measurements using electrochemical (EC) and chemiluminescence (CL) techniques. NO₂ was added stepwise to different gas mixtures of nitrogen (N₂), O₂ and nitric oxide (NO). Medical grade NO stocks of two concentrations were used: 1000 parts per million (ppm, GS1000) and 300 ppm (GS300). NO₂ concentrations were measured either immediately after gas mixing (at time \( t_0 \)) or 25 s later (\( t_0 + 25 \)), when the gas had passed through a long corrugated tube (6.2 m). Regression lines were calculated and the y-intercepts (\( y_o \)) and slopes (with lower and upper 95% confidence limits) are given.

<table>
<thead>
<tr>
<th>Series number</th>
<th>NO (ppm)</th>
<th>NO stock gas</th>
<th>( O_2 ) (%)</th>
<th>Time (s)</th>
<th>( y_o ) (ppm) EC</th>
<th>( y_o ) (ppm) CL</th>
<th>Slope (95% CL) EC</th>
<th>Slope (95% CL) CL</th>
<th>n</th>
<th>Fig. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>( b_0 )</td>
<td>0.10</td>
<td>0.01</td>
<td>0.98 (0.97;1.00)</td>
<td>1.01 (1.00;1.03)</td>
<td>4</td>
<td>2A</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>100</td>
<td>( b_0 )</td>
<td>0.07</td>
<td>0.02</td>
<td>1.02 (1.00;1.03)</td>
<td>1.01 (1.00;1.03)</td>
<td>4</td>
<td>2h</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>GS1000</td>
<td>( b_0 )</td>
<td>0.35</td>
<td>0.45</td>
<td>1.07 (1.04;1.09)</td>
<td>1.01 (0.98;1.04)</td>
<td>4</td>
<td>2c</td>
<td></td>
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<tr>
<td>4</td>
<td>80</td>
<td>GS300</td>
<td>( b_0 )</td>
<td>0.28</td>
<td>0.27</td>
<td>1.08 (1.05;1.11)</td>
<td>1.02 (0.97;1.07)</td>
<td>3</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>GS1000</td>
<td>90</td>
<td>0.24</td>
<td>0.25</td>
<td>1.00 (0.97;1.03)</td>
<td>0.99 (0.95;1.04)</td>
<td>6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>80</td>
<td>GS1000</td>
<td>( b_0 + 25 )</td>
<td>1.37</td>
<td>1.38</td>
<td>1.13 (1.07;1.19)</td>
<td>1.02 (0.92;1.12)</td>
<td>6</td>
<td>2b, 3</td>
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</tr>
<tr>
<td>7</td>
<td>20</td>
<td>GS1000</td>
<td>90</td>
<td>( b_0 + 25 )</td>
<td>0.46</td>
<td>0.49</td>
<td>0.99 (0.96;1.03)</td>
<td>0.98 (0.93;1.04)</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>80</td>
<td>GS1000</td>
<td>( b_0 + 25 )</td>
<td>4.66</td>
<td>4.33</td>
<td>1.17 (0.97;1.38)</td>
<td>1.11 (0.94;1.28)</td>
<td>6</td>
<td>3</td>
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</table>

Table 2 Dilution with N₂ after \( t_0 + 25 \) s (see ‘(D) Dilution with N₂ after \( t_0 + 25 \) s’ under ‘Results’) by stepwise addition of nitrogen to deliberately aged nitrogen dioxide (NO₂)-containing gas mixtures of nitric oxide (NO) 80 parts per million (ppm) in 90% oxygen (O₂). NO₂ concentrations were measured by chemiluminescence (CL) and electrochemical (EC) techniques and are given in parts per million (ppm) and dilution % (measured mean value in percent of the initial, undiluted mean concentration). Number of inter-assay observations, \( n = 7 \)

<table>
<thead>
<tr>
<th>Calculated dilution (%)</th>
<th>NO₂ (ppm)</th>
<th>NO₂ (ppm)</th>
<th>Dilution NO₂ %</th>
<th>Dilution NO₂ %</th>
<th>Dilution O₂ %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC</td>
<td>CL</td>
<td>EC</td>
<td>CL</td>
<td>Oscar</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>3.4 (0.1)</td>
<td>3.2 (0.2)</td>
<td>70</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>2.1 (0.1)</td>
<td>1.9 (0.1)</td>
<td>44</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1.0 (0.1)</td>
<td>1.0 (0.1)</td>
<td>21</td>
<td>21</td>
</tr>
</tbody>
</table>

Results

(A) Daily tests of the system

When flushing the test system with carbon-filtered room air, nitric oxide and nitrogen dioxide concentrations <0.1 ppm were always obtained by both analysers. The certified composition of the calibration gas was nitric oxide 83.3 ppm and nitrogen dioxide 6.3 ppm. Measured concentrations of the calibration gas using EC (\( n = 10 \)) were for nitric oxide, 82.9 (1.9) ppm (cv 2.3%) and for nitrogen dioxide 6.1 (0.2) ppm (cv 2.9%); using CL (\( n = 11 \)) values were, for nitric oxide 85.5 (0.8) ppm (cv 0.9%) and for nitrogen dioxide 6.0 (0.3) ppm (cv 4.3%).

(B) Addition of nitrogen dioxide after \( t_0 \)

Results are shown in Table 1 (series numbers 1–6) and Figure 2. Linear relationships between calculated and measured nitrogen dioxide concentrations are apparent (Fig. 2) with good agreement between analysers. The regression lines had slope values of 0.98–1.13 with lower and upper 95% confidence limits of 0.92–1.19. For CL, the line of identity (slope 1.0) was included in the confidence interval in all series. For EC this was not the case at the highest nitric oxide concentration (80 ppm), where the lower confidence limits were slightly >1 (i.e. 1.04–1.07) indicating a small overestimation of nitrogen dioxide concentrations. The regression lines passed through the origin for nitrogen dioxide in nitrogen and oxygen, but crossed the y-axis (y intercept=\( y_o \)) in all nitric oxide-containing gas

Fig 2 (See ‘(B) Addition of NO₂ after \( t_0 \)’ under ‘Results’). Measured vs added nitrogen dioxide (NO₂) concentrations in parts per million (ppm) analysed by both chemiluminescence and electrochemical techniques (mean (SD)). NO₂ was added stepwise to different gas mixtures: (A) 100% nitrogen (N₂) (\( n = 4 \)); (B) 100% oxygen (O₂) (\( n = 4 \)); (C) nitric oxide (NO) 80 ppm in N₂ (\( n = 4 \)); and (D) NO 80 ppm in 85% O₂ (\( n = 6 \)) (\( n \) denotes number of inter-assay observations). Values for slopes and y-intercepts of the regression lines are given in Table 1.
mixtures, indicating the presence of nitrogen dioxide by time \( t_0 \), even with nitric oxide in nitrogen (Table 1, series number 3; Fig. 2c) and more so with nitric oxide in oxygen (Table 1, series number 6; Fig. 2d; Fig. 3).

(C) Addition of nitrogen dioxide after \( t_0+25 \) s
Linear relationships between measured and added nitrogen dioxide content were also apparent in aged, hyperoxic, nitric oxide-containing gases (Fig. 3). The slopes with 95% confidence limits (Table 1, series numbers 7–8) demonstrate that the ‘line of identity’ (slope 1.0) was included in the confidence interval. The intercepts (nitrogen dioxide concentrations in the main gas stream) were larger than after \( t_0 \) (Table 1, series numbers 5 and 6) and much larger with 80 ppm than with 20 ppm (series number 8 vs 7). This indicates the formation of additional nitrogen dioxide in the corrugated tube, in the presence of oxygen, especially at the higher nitric oxide concentration.

(D) Dilution with nitrogen after \( t_0+25 \) s
Dilution of aged nitrogen dioxide-containing gases, rich in nitric oxide and oxygen, to normoxic conditions, led to slightly greater than expected reductions in nitrogen dioxide concentrations (Table 2), although measurement of oxygen concentrations confirmed the calculated dilutions.

(E) Nitrogen dioxide formation up to \( t_0 \) using different nitric oxide stock concentrations
Changing between nitric oxide cylinders with different stock concentrations had relatively small effects on nitrogen dioxide concentrations measured at \( t_0 \) s after gas mixing. At the highest nitric oxide concentration (80 ppm) the observed nitrogen dioxide concentrations for the stock gas GS300 in 75% oxygen were 1.2 (0.1) ppm (EC) and 1.0 (0.1) ppm (CL); and for the stock gas GS1000 in 90% oxygen, 1.4 (0.1) ppm (EC) and 1.4 (0.2) ppm (CL) (n=4).

(F) Kinetics
Measured nitrogen dioxide concentrations appeared to increase linearly with time and oxygen concentration and in proportion to the square of nitric oxide concentration (Fig, 4A, B, C) (i.e. in accordance with the kinetic equation (2)). Measured nitric oxide concentration changed little with measurement delay or oxygen concentration.

The following values for the rate constant \( k \) were obtained:

\[
\begin{align*}
    k_{EC} &= 2.55 \times 10^{-5} \\
    k_{CL} &= 2.18 \times 10^{-5}
\end{align*}
\]

The mean (2.5 \( \times \) 10\(^{-5}\) ppm\(^{-1}\) s\(^{-1}\)) had the same magnitude as those constants reported in the literature (e.g. 2 \( \times \) 10\(^{-5}\) ppm\(^{-1}\) s\(^{-1}\)). Using the above rate constant, we estimated that it would theoretically take approximately 4 min to oxidize 50% of nitric oxide 1000 ppm to nitrogen dioxide in air, and only 45 s in 90% oxygen.

(G) Ventilators
With 90% oxygen, nitrogen dioxide concentration in the inspiratory limbs of the ventilators increased sharply with the set concentration of nitric oxide (Table 3) and was much greater with the Servo 900D, at both adult and paediatric settings, than with the Servo 300.

(H) Variability of results
Coefficients of variation (cv, n=6) were calculated from nitrogen dioxide measurements in hyperoxic gases with and without nitric oxide (Table 1, series numbers 2 and 6; Fig. 5). In nitric oxide-free gases, reproducibility was acceptable with cv <4 % for both analysers; however, this is of minor clinical interest. In hyperoxic gases also containing nitric oxide 80 ppm, the chemiluminescence technique displayed increasing deviations at lower nitrogen dioxide concentrations, whereas the electrochemical cell had the same performance except at the lowest nitrogen dioxide concentration. The values for the lowest nitrogen dioxide concentration were 1.4 (0.1) ppm (cv 7%) for EC and 1.4 (0.3) ppm (cv 18%) for CL. It was not possible to...
Fig 4 Data illustrating the kinetics for the formation of nitrogen dioxide (NO\textsubscript{2}) from oxygen (O\textsubscript{2}) and nitric oxide (NO) (see '(F) Kinetics' under 'Results'). NO and NO\textsubscript{2} concentrations in parts per million (ppm) were measured by chemiluminescence, and NO\textsubscript{2} data in the figure were corrected for the presence of NO\textsubscript{2} before the mixing point. Streaming gas (flow rate 6 litre min\textsuperscript{-1}) was allowed to pass through tubing of different lengths giving varying dwelling times from 0 to 21 s when analysed for NO\textsubscript{2}. NO concentrations were 0, 10, 20, 40, 60 and 80 ppm and O\textsubscript{2} fractions 0, 0.2, 0.4, 0.6 and 0.9. As expected, the relationship between NO\textsubscript{2} concentration and time in addition to O\textsubscript{2} concentration was linear (A–C), but non-linear (square) for NO concentration (D).

Table 3 Nitrogen dioxide (NO\textsubscript{2}) concentrations (mean (SD)), in parts per million (ppm) (n=6) measured by chemiluminescence (CL) and electrochemical (EC) techniques in the inspiratory limb just before the Y-piece in the ventilatory circuits, using clinically relevant gas mixtures and ventilator settings (see '(G) Ventilators' under 'Results'). Nitric oxide (NO) in 90% oxygen (O\textsubscript{2}) was added before the ventilator Servo 900D by an external dosing unit ‘Nomius’ and in the Servo 300 by an internal dosing device. ‘Adult’ setting: minute ventilation 5 litre min\textsuperscript{-1}, 15 bpm; ‘paediatric’ setting: 3 litre min\textsuperscript{-1}, 30 bpm. Servo 300 had a bypass flow of 2.0 litre min\textsuperscript{-1} during expiration. nd=Not determined

<table>
<thead>
<tr>
<th>NO (ppm)</th>
<th>NO\textsubscript{2} (ppm)</th>
<th>NO\textsubscript{2} (ppm)</th>
<th>NO\textsubscript{2} (ppm)</th>
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<tbody>
<tr>
<td></td>
<td>EC</td>
<td>CL</td>
<td>EC</td>
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<tr>
<td>20</td>
<td>0.3 (0.0)</td>
<td>0.2 (0.0)</td>
<td>0.7 (0.1)</td>
</tr>
<tr>
<td>40</td>
<td>nd</td>
<td>nd</td>
<td>1.6 (0.1)</td>
</tr>
<tr>
<td>80</td>
<td>2.7 (0.1)</td>
<td>2.5 (0.2)</td>
<td>4.9 (0.1)</td>
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</table>

Evaluate lower nitrogen dioxide concentrations in nitric oxide- and oxygen-rich gas mixtures with our measuring device as these high nitrogen dioxide values were obtained at the first possible measuring point (t\textsubscript{0} s) after gas mixing. For both analysers there was a higher spread in the data for nitrogen dioxide concentrations >5 ppm.

**Discussion**

**Major findings**
The main findings of this study were: (1) that nitrogen dioxide can be measured with accuracy in oxygen- and nitric oxide-rich gas mixtures using both chemiluminescence and electrochemical techniques; and (2) that mixing point generation of nitrogen dioxide is a major source of nitrogen dioxide in hyperoxic gas mixtures even in rapid delivery systems. This will be evident from the following discussion.

**Measurement**

Despite previous reports to the contrary,\textsuperscript{18} we found that we could measure nitrogen dioxide concentrations reliably with both EC and CL techniques. This was true for measurements of the calibration mixture (procedure A) and
Nitrogen oxides in hyperoxic gas

for the increments in concentration when nitrogen dioxide was added in known amounts to the main gas stream, for up to nitrogen dioxide 5 ppm, even in the presence of up to 80 ppm of nitric oxide and up to 90% oxygen (Figs 2–3, Table 1). In addition, we derived a kinetic rate constant (2.5 (0.1) × 10⁻⁵ ppm⁻¹ s⁻¹) which agreed well with literature values of 2 × 10⁻⁵ ppm⁻¹ s⁻¹.⁸

We conclude that the chemiluminescence device (Monitor Labs, 9841A) and the electrochemical cell (Bedfont, NOxBOX) may both yield accurate readings for nitrogen dioxide concentrations < 5 ppm in up to 90% oxygen and nitric oxide 80 ppm.

Nitrogen dioxide sources

On the basis of the above, it is reasonable to interpret the y-axis intercepts of the regression lines in Table 1 and Figure 2 as evidence of the formation of nitrogen dioxide in the main gas stream, containing nitric oxide (Fig 2c, d). In the absence of nitric oxide, no nitrogen dioxide was formed (Table 1, series numbers 1 and 2; Fig. 2a, b).

In the presence of nitric oxide 80 ppm, even with no oxygen, nitrogen dioxide 0.4 ppm was present at the mixing point (by time t₀) (Table 1, series number 3; Fig. 2c). This might be attributable to trace nitrogen dioxide from the stock cylinder of nitric oxide in nitrogen. However, when we returned the stock cylinders to the manufacturer, their analyses showed no significant amounts of nitrogen dioxide. The analysis protocols stated nitrogen dioxide −0.9 ± 1.4 ppm before, and 0.4 ± 1.4 ppm after use, for the GS300 cylinder, and for the GS1000, nitrogen dioxide −0.2 ± 2.1 ppm before and 1.5 ± 2.4 ppm after use.

Another possibility concerning the presence of nitrogen dioxide in nitrogen is pre-mixing point formation of nitrogen dioxide from the remains of oxygen in the pressure regulator, or oxygen permeation from or through the high-pressure tubing walls. This is plausible because the rate of formation of nitrogen dioxide is proportional to the cube of pressure and the square of the nitric oxide concentration; therefore, at high pressure (at least 2 bar) and high nitric oxide concentration (1000 ppm or 300 ppm) upstream of the mass flow controllers in the ‘Nomius’ (Fig. 1) even very low concentrations of oxygen could lead to an appreciable rate of formation of nitrogen dioxide.

In the presence of nitric oxide 80 ppm and 85% oxygen (Table 1, series number 6), nitrogen dioxide 1.4 ppm had been formed by t₀. However, this is approximately 10 times what would be calculated from the kinetic equation (2) for a duration of 0.5 s. This may be explained as follows. During the mixing process, nitric oxide concentration decreases while oxygen concentration increases, but the rate of formation at each instant is proportional to the square of nitric oxide concentration and only directly proportional to oxygen concentration. Therefore, nitrogen dioxide formation is more rapid during the mixing process than when mixing is complete. A device that produced near instantaneous mixing might reduce this source of nitrogen dioxide.

When the reaction is allowed to continue for 25 s (with nitric oxide 80 ppm and 85% oxygen), the nitrogen dioxide concentration increased to 4.5 ppm (Table 1, series number 8; Fig. 3) in accordance with the reaction kinetics.

The changes in nitric oxide and nitrogen dioxide concentrations with time from the mixing point are illustrated in Figure 6.

After submission of this article, Lindberg and Rydgren²¹ reported data suggesting rapid formation of nitrogen dioxide after mixing nitric oxide in nitrogen with 90% oxygen, suggesting that new rate constants can be calculated for clinically relevant hyperoxic gas mixtures containing nitric oxide. On the basis of our data, we suggest that previously reported rate constants describe well the continuous formation of nitrogen dioxide after full mixing. Furthermore, our data suggest an initial, non-linear formation of nitrogen dioxide during the mixing process, and that this nitrogen dioxide constitutes the large portion of nitrogen dioxide in a system with rapid mixing in hyperoxic gas.

Our conclusion is that there are three origins for nitrogen dioxide reaching the patient during nitric oxide inhalation therapy: (1) pre-mixing nitrogen dioxide, either from the nitric oxide cylinder or from oxygen contamination of the pressure regulator and tubing; (2) fast nitrogen dioxide formation in the mixing process; and (3) post-mixing nitrogen dioxide formation that follows the kinetics during delivery to the patient.

Ventilators

Nitrogen dioxide concentrations up to 5 ppm were measured in nitric oxide 80 ppm and 90% oxygen, with adult and paediatric settings, at the inspiratory limb of a ventilatory breathing system, with the nitric oxide inlet just proximal to the ventilator Servo 900D (Table 3). Nitric oxide concentration was crucial for nitrogen dioxide formation and at a
nitric oxide concentration of 20 ppm, nitrogen dioxide concentration remained less than 1 ppm with both ventilators. The situation was most favourable with the ventilator Servo 300, which has an internal nitric oxide delivery device and most likely a shorter gas residence time in the system. In addition, there may be more effective mixing in the internal 90° knee.

**Practical aspects of using the analysers**

**Chemiluminescence.** In chemiluminescence devices, nitric oxide is determined by oxidation with ozone to excited nitrogen dioxide. The excited nitrogen dioxide spontaneously falls back to its normal energetic state by giving off energy as radiation in the infrared range, which can be detected by photomultiplier technology. All higher nitrogen oxides, including nitrogen dioxide, are first reduced to nitric oxide in a molybdenum converter at high temperature and then measured as nitric oxide. This means that the amount of nitrogen dioxide is calculated as the difference between the total amount of nitrogen oxides (NOx) and nitric oxide. In typical clinical inhalation gas mixtures with high nitric oxide and low nitrogen dioxide, nitrogen dioxide concentration is calculated as a small difference between two large numbers. We noted higher SD values in measurements of small amounts of nitrogen dioxide in nitric oxide-rich gas mixtures by CL than by EC and this may be related to CL determining nitrogen dioxide by difference (Fig. 5). The converter efficiency is very important here, especially in the low nitrogen dioxide range, and at least in theory very aberrant and even negative nitrogen dioxide values may be obtained if the converter efficiency is declining. During the present study period of 6 months there was no need for manual calibration, according to our repeated checks with the calibration gas. However, we have experienced previously permanent loss of converter efficiency by exposing the converter to halogenated anaesthetic gases, when using other chemiluminescence analysers. The effect of anaesthetic gases was not tested in the present analyser. The MonitorLabs analyser was slow: every measurement took approximately 5 min and sometimes longer, especially in gas mixtures with a high nitric oxide and a very low nitrogen dioxide content. We found the analyser more reliable if kept running continuously indicating that the warm-up time given in the manual might be suboptimal (data not shown).

**Electrochemical analyser.** Nitric oxide and nitrogen dioxide were determined independently by two different chemical cells. No correction was made for the cross-sensitivity response of the nitric oxide cell, as nitrogen dioxide concentrations were low compared with nitric oxide. Besides, in a recent study no such cross-sensitivity was found with the same analyser. We note that the company Bedfont Scientific, manufacturer of the NOxBOX, does not at present recommend the regular use of a pump, which may, however, ensure that the monitor heads always measure on fresh gas. In pilot experiments we were able to measure more than three-fold higher concentrations of nitrogen dioxide when the analyser was used without a pump measuring gas with a pressure only slightly above atmospheric (unpublished data). However, when used together with an external pump the analyser behaved surprisingly well. It was faster than the chemiluminescence analyser, with every measurement taking approximately 1 min. We saw no drift in measurements during the 5-min wait for the reading on the other analyser. Because of some degree of zero drift, daily calibrations were necessary. Only once during the study period of 6 months was a manual correction of the span factor necessary.

**Clinical implications.** We have demonstrated formation of up to 5 ppm of nitrogen dioxide in a typical nitric oxide delivery device at clinically used concentrations and also that nitrogen dioxide concentrations were significantly lower for nitric oxide 20 ppm than for 80 ppm. Harmful effects to human lungs from nitrogen dioxide 1 ppm in healthy subjects and from 0.1 ppm in sensitive subjects have been demonstrated clearly. Therefore, the concomitant routine nitrogen dioxide administration to patients with pulmonary diseases is a matter of great concern for the whole concept of nitric oxide inhalation therapy. Good monitoring of nitric oxide and nitrogen dioxide is mandatory and in the clinical situation chemi-luminescence and electrochemical methods could be used. However, it is important to have facilities for calibration (e.g. a certified calibration gas of suitable...
composition). For good performance of the EC, the gas has to be forced through the analyser by an external pump (built-in in the CL), which however, might contaminate the tested gas with room air if there is a leak in the system, leading to false low concentrations. When using chemiluminescence one must remember that the converter, used for measurement of nitrogen dioxide, is consumed leading to declining converter efficiency and false analyses and also that the relative amounts of nitric oxide and nitrogen dioxide may influence the measuring accuracy (see above).

Crucial for the amount of nitrogen dioxide reaching the respiratory system of the patient is how and where nitric oxide is added to the inspiratory gas stream of the ventilatory circuit. As we found unexpectedly high nitrogen dioxide formation at the mixing of nitric oxide and oxygen, dilution of nitric oxide in nitrogen might seem advantageous. However, most patients receiving nitric oxide therapy need very high oxygen concentrations, so there is very little room for nitrogen in such gas mixtures. If nitric oxide is added proximal to the ventilator the gas is mixed thoroughly, but the time for nitrogen dioxide formation is relatively long. Addition of nitric oxide into the inspiratory limb near the Y-piece may result in great variations in nitric oxide and nitrogen dioxide concentrations during the respiratory cycle. The explanation is that nitric oxide is added continuously to the system, even when ventilatory gas flow is low or zero, and plugs of highly concentrated nitric oxide, and perhaps nitrogen dioxide, might be formed and reach the lungs. For nitric oxide there are analysers rapid enough to follow most of these variations. In contrast, all available nitrogen dioxide analysers have long response times (more than 30 s) and consequently miss all tidal variations of nitrogen dioxide concentration. More sophisticated methods of administering nitric oxide just proximal to the patient have been reported, where nitric oxide delivery is microprocessor-controlled and integrated with the ventilatory cycle. Different types of nitrogen dioxide scrubbers have been used, most commonly a soda lime absorber, to diminish pulmonary nitrogen dioxide administration. In a recent study of the current clinical practice in nitric oxide inhalation therapy at 54 intensive therapy units in the UK, a wide variety in indications, delivery, monitoring and use of scavengers was demonstrated and UK guidelines for the use of nitric oxide inhalation therapy have been published recently.

Clinical recommendations

We have confirmed that nitric oxide therapy is inevitably accompanied by administration of nitrogen dioxide at concentrations which are likely to cause pulmonary toxicity. Therefore, the benefits of nitric oxide therapy must be balanced against the risks of nitrogen dioxide. Possible ways to minimize these risks are: (1) minimal inhaled nitric oxide concentration, preferably ≤20 ppm; (2) reliable nitric oxide and nitrogen dioxide monitoring, including regular checks with calibration gas; (3) improved nitric oxide delivery device to minimize formation of nitrogen dioxide in the mixing process and during delivery; and (4) correct handling of nitric oxide cylinders and pressure regulators to avoid oxygen contamination.

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