Nitrous oxide in modern anaesthetic practice

SM Brown FRCA¹ and JR Sneyd FRCA²,*

¹Registrar in Anaesthesia and Critical Care, Derriford Hospital, Plymouth, UK, and ²Dean and Professor of Anaesthesia, Plymouth University Peninsula Schools of Medicine and Dentistry, The John Bull Building, Research Way, Tamar Science Park, Plymouth PL6 8BU, UK

*To whom correspondence should be addressed. Tel: +44 1752 437 358; Fax: +44 1752 517 842; E-mail: robert.sneyd@pms.ac.uk

Key points

- Nitrous oxide is an N-methyl-d-aspartate receptor antagonist and may reduce the incidence of chronic post-surgical pain.
- Nitrous oxide oxidizes Vitamin B12 and can precipitate sub-acute combined degeneration of the cord with chronic use or in patients with folate/B12 deficiency.
- Nitrous oxide expands air spaces and is contraindicated in patients with pneumothorax or recent (up to 4–6 weeks) ocular surgery using intraocular gas.
- Nitrous oxide has global warming and ozone depletion potential and its concentration in the theatre environment is regulated.
- The ENIGMA-II trial showed that nitrous oxide does not increase the risk of death or cardiovascular complications.

Nitrous oxide (N₂O) was first isolated by Joseph Priestly in 1772 and subsequently recognized for its analgesic properties by Humphrey Davy in 1799. Davy has actually invented a new pleasure, for which language has no name. Oh Tom! I am going for more this evening; it makes one strong, and so happy! . . . Tom, I am sure the air in heaven must be this wonder-working gas of delight!

— Robert Southey, Letter to Thomas Southey, July 12, 1799

Although noted by Davy that it ‘may probably be used with advantage in surgical operations’ and some initial use in dentistry by Horace Wells in 1845, it was firmly established as an anaesthetic agent by Gardner Quincy Colton in the 1860s and promoted around North America and Europe. Because of a diverse range of concerns, the use of N₂O as an anaesthetic is declining in Western countries. It was used in 33% of operations in the USA in 2009 and had reduced to 21% in 2011.¹ Sadly, its recreational use is increasing, outpacing cocaine, ecstasy, and ketamine. In the UK, 470,000 people aged between 16 and 59 used nitrous oxide in the past year compared with 100,000 in 2013.²

This review will examine the physical and pharmacological properties of nitrous oxide and the controversies regarding its current use.

N₂O manufacture and environmental impact

At temperature 170–240°C, ammonium nitrate breaks down in an exothermic reaction to form nitrous oxide and water by the following equation:

\[ \text{NH}_4\text{NO}_3 \rightarrow \text{N}_2\text{O} + 2\text{H}_2\text{O} \]

By-products including nitrogen (N₂), nitrogen dioxide (NO₂), and nitric acid (HNO₃) are removed by scrubbing agents and base/acid gas washes.

Nitrous oxide has a global warming potential (GWP) of 310 (CO₂ is the standard with a GWP of 1) and is regulated under the Kyoto Protocol (1997). It is the third greatest contributor to the greenhouse effect in the UK. However, because of the formation of NOx intermediates under the influence of ultraviolet radiation in the stratosphere, it also has ozone-depleting potential (ODP). As an essential medical gas, it is unregulated by the Montreal Protocol (1987). Nitrous oxide has an ODP 1/60th of the standard chlorofluorocarbon (CFC)-11, but because of the effectiveness of the Montreal Protocol in reducing CFC emissions and the scale of natural and man-made nitrous oxide, nitrous oxide will remain one of the greatest contributors to ozone depletion during the 21st century.

© The Author 2015. Published by Oxford University Press on behalf of the British Journal of Anaesthesia. All rights reserved. For Permissions, please email: journals.permissions@oup.com
**Physiochemistry, storage, and supply**

Nitrous oxide is a colourless gas with a slightly sweet odour and taste at room temperature and pressure (Table 1). It is stored in a cylinder, compressed as a liquid/vapour below its critical temperature (36.5°C). The saturated vapour pressure (SVP) is temperature sensitive (Gay-Lussac’s law). At standard temperature (0°C) the SVP is 35 bar (3500 kPa) and at room temperature (20°C) it is 52 bar (5200 kPa). The filling ratio in the UK is 0.75 (i.e. the weight of nitrous oxide in a full cylinder is three-quarters the weight of water that would fill the cylinder). This is reduced in tropical climates to 0.67 because of the increased vapour pressures exerted at higher temperatures. As nitrous oxide is discharged from a cylinder, it vapourizes, requiring energy in the form of heat (latent heat of vapourization). This process cools the cylinder, reducing the SVP and cylinder pressure. The pressure recovers when the cylinder is closed and it warms back to environmental temperature. Cylinder pressure does not accurately indicate the filling status of the cylinder, as the SVP will only decrease when all the liquid nitrous oxide is consumed and the tank is almost empty.

Hospitals often supply piped nitrous oxide at a pressure of 4 bar to theatre environments. These are supplied by large cylinders (e.g. size-J) in a system that automatically switches cylinder when the previous one is empty.

A 50:50 mixture of nitrous oxide/oxygen (Entonox, British Oxygen Company) is used as an inhalation analgesic. Gaseous oxygen bubbled through liquid nitrous oxide increases the vapour pressure of the mixture to form a gas at pressures far exceeding those capable of liquidizing nitrous oxide alone (Poynting effect). Because of this effect, Entonox has a pseudocritical temperature of −6°C at a cylinder pressure of 137 bar (13700 kPa) and is therefore a compressed gas during storage at room temperature. It must not be stored below its pseudocritical temperature or it will separate under a process called ‘lamination’. This will leave oxygen gas above a layer of liquid nitrous oxide. When used, pure oxygen will be delivered first and the delivered mixture will become increasingly concentrated until pure nitrous oxide is delivered with hypoxic consequences to the patient. Pipeline Entonox at 4 bar has a pseudocritical temperature of −30°C and is safer in this respect.

**Table 1 Properties of nitrous oxide**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular structure</td>
<td>N≡N-(\text{O}^−) (\rightarrow) N≡N-(\text{C}^−)</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>44</td>
</tr>
<tr>
<td>Boiling point</td>
<td>−88°C (309 K)</td>
</tr>
<tr>
<td>Critical temperature</td>
<td>36.5°C</td>
</tr>
<tr>
<td>Critical pressure</td>
<td>72 bar</td>
</tr>
<tr>
<td>Cylinder colour (UK)</td>
<td>Blue</td>
</tr>
<tr>
<td>Cylinder phase</td>
<td>Liquid/vapour at &lt;36.5°C</td>
</tr>
<tr>
<td>Cylinder pressure at 15°C</td>
<td>44 bar (4400 kPa)</td>
</tr>
<tr>
<td>Filling ratio</td>
<td>0.75 (UK) 0.67 (tropical)</td>
</tr>
<tr>
<td>Pipeline pressure</td>
<td>4 bar (400 kPa)</td>
</tr>
<tr>
<td>Mechanism of action</td>
<td>NMDA receptor antagonist</td>
</tr>
<tr>
<td>MAC</td>
<td>105%</td>
</tr>
<tr>
<td>Blood: gas partition coefficient</td>
<td>0.47</td>
</tr>
<tr>
<td>Oil:gas partition coefficient</td>
<td>1.4</td>
</tr>
<tr>
<td>Nitrous oxide: air 50%:50%</td>
<td>White or blue with blue and white shoulders</td>
</tr>
<tr>
<td>Cylinder colour</td>
<td>137 bar (13 700 kPa)</td>
</tr>
<tr>
<td>Cylinder pressure</td>
<td>4 bar (400 kPa)</td>
</tr>
<tr>
<td>Pseudocritical temperature −6°C</td>
<td></td>
</tr>
<tr>
<td>Pipeline pressure</td>
<td></td>
</tr>
<tr>
<td>Pseudocritical temperature −30°C</td>
<td></td>
</tr>
</tbody>
</table>

**Theatre environment**

Nitrous oxide may reduce fertility and increase the rate of spontaneous abortion in female workers with chronic exposure. Rare cases of myelopathy have been recorded in the past from occupational exposure. Therefore, nitrous oxide is controlled under the Control of Substances Hazardous to Health (COSHH) regulations. Exposure is assessed over an 8- or 10-h period and recorded as an average exposure level. The limit in the UK (COSHH) is 100 ppm (parts per million) and in the USA (National Institute of Safety and Health) 25 ppm. Areas that do not use anaesthetic gas scavenging systems or have inadequate ventilation, such as recovery, dental suites, and radiology, are at risk of exceeding these exposure limits.

**Pharmacodynamics**

**Anaesthesia and pain**

**Mechanism of action**

Nitrous oxide acts as an N-methyl-D-aspartate (NMDA) receptor antagonist. This is different from other volatile anaesthetic agents that modulate (usually potentiate) the activity of gamma-aminobutyric acid-A (GABA\(_A\)) receptors and inhibit neuronal potassium channels (TREK-1) among other suggested targets. The NMDA receptor is a glutamate binding, non-selective ion channel involved in synaptic plasticity and memory formation. The GABA\(_A\) receptor is the main inhibitory, chloride-ion selective, ligand-gated channel of the central nervous system (CNS). Through different mechanisms, nitrous oxide and GABA\(_A\) modulators act synergistically to induce amnesia and hypnosis. Thus nitrous oxide is often referred to as a ‘volatile-sparing agent’.

**Awareness and cerebral function monitoring**

The 5th National Audit Project (NAP5) from the Association of Anaesthetists of Great Britain and Ireland estimated the incidence of patient-reported awareness as ~1:19 000 anaesthetics, with a wide variation between settings, the highest being in cardiothoracic (1.8600) and Caesarean section under general anaesthetic (1.670). The NAP5 project found that nitrous oxide was used in 27.7% of ‘Class A’ (certain or probable) awareness cases but this exactly correlated with the frequency of nitrous oxide use in all anaesthetics (28.6%), suggesting that it does not influence the risk of awareness. The use of nitrous oxide was associated with a lower incidence of awareness in Caesarean section under general anaesthesia and its use is recommended in this setting by NAP5.

NMDA antagonists such as nitrous oxide and ketamine do not suppress the cortical electroencephalogram in the same manner as GABA\(_A\) modulators and therefore their effects are not correlated with cerebral function monitoring (e.g. bispectral index). Using cerebral function monitoring in regimes containing nitrous oxide may lead to inappropriately deep anaesthesia.

**Pain**

Nitrous oxide releases proenkephalin in the CNS. While single agent 66–70% nitrous oxide provides an analgesic effect similar to a whole blood concentration of remifentanil of 2 ng ml\(^{-1}\), it seems that volatile anaesthetic agents or strong opioids are not synergistic with nitrous oxide and may in fact negate some of the analgesic effects of nitrous oxide during co-administration. With the advent of short-acting opioids and volatile agents, the role of nitrous oxide in the delivery of analgesia in balanced anaesthesia is of decreasing importance.
However, it seems that the NMDA antagonism of nitrous oxide may offer a significant benefit in the reduction of chronic post-surgical pain and opioid-induced hyperalgesia. NMDA receptors are involved in synapse plasticity and the development of central and peripheral sensitization leading to chronic post-surgical pain. Sub-group analysis of the ENIGMA-I trial showed a significant reduction in the incidence of chronic pain with the use of nitrous oxide.\textsuperscript{5} This effect has been examined by the ENIGMA-II trial and will be reported on shortly.

**Speed of onset, second gas, and concentration effects**

Nitrous oxide has low anaesthetic potency, with a concentration of 105% required for single minimum alveolar concentration (MAC) anaesthesia, a clearly unreasonable proposition at atmospheric pressure. However, its low solubility in blood (blood:gas partition coefficient) leads to a rapid equilibration of partial pressures between blood and inspired gas and rapid onset and offset of action (Fig. 1).

Nitrous oxide also improves the speed of onset of volatile agents by the ‘second gas effect’. Nitrous oxide transfers across the alveolus rapidly because of its high lipid solubility. This leads to concentration of the remaining gases in the alveolus (volatile agent, oxygen, and nitrogen), increasing the driving pressure of volatile anaesthetic into the blood. Also the loss of volume associated with nitrous oxide uptake leads to an augmentation of ventilation. Providing a higher concentration of nitrous oxide or volatile anaesthetic agent further increases this effect. This is referred to as the ‘concentration effect’. The second gas and concentration effects work to increase the speed of onset of anaesthesia when using nitrous oxide (Fig. 1).

**Diffusion hypoxia**

The rapid transfer of nitrous oxide across the alveolus occurs in reverse during wake-up. In the case of low ventilation with air, nitrous oxide will quickly transfer into the alveolus, down its concentration gradient, diluting the concentration of oxygen, and impairing oxygen transfer across the alveolus into the blood, leading to hypoxia. Maintaining adequate minute ventilation and supplementing oxygen during the brief washout phase of nitrous oxide prevent this.

**Diffusion into closed cavities**

Because of the higher blood solubility of nitrous oxide than nitrogen, nitrous oxide transfers faster into closed gas cavities than nitrogen is removed, leading to expansion of air or low-solubility gas-filled cavities. These cavities can be divided into compliant and non-compliant. Compliant cavities, such as pneumothorax, pneumoperitoneum, bowel gas, and air emboli, will increase in volume with transfer of nitrous oxide whereas non-compliant cavities such as the cranium, middle ear, and eye will increase in pressure.

In compliant cavities, the maximum expansion is related to the alveolar percentage of nitrous oxide for equilibration. Therefore, 50% nitrous oxide will lead to a maximum two-fold expansion, and 75% nitrous oxide will lead to a four-fold expansion. In dogs, 75% nitrous oxide led to a two-fold increase in volume over 10 min and a three-fold increase in volume in 30 min.\textsuperscript{6}

Volume expansion can take place over seconds for an air embolus in a circulation containing nitrous oxide. The lethal volume of air in rabbits breathing 75% nitrous oxide is 30% less than in those not receiving nitrous oxide.\textsuperscript{7} The use of nitrous oxide for neurosurgery in the sitting position does not increase venous air embolism (VAE) risk, but if VAE is suspected, nitrous oxide use should be discontinued.\textsuperscript{8} Increases in bowel volume can lead to difficult surgical conditions and an inability to close the abdomen after surgery or high intra-abdominal pressures during laparoscopy.

Nitrous oxide should be avoided during or after eye surgery using intraocular gas (SF\(_6\) or C\(_3\)F\(_8\)). With 75% nitrous oxide a three-fold\textsuperscript{9} increase in size of the bubble can occur within the eye with a resultant increase in intraocular pressure, and a reduction in retinal perfusion pressure and visual loss. SF\(_6\) remains in situ for 7–10 days and C\(_3\)F\(_8\) for 4–6 weeks or more. Pressure changes of 20–50 mm Hg can also occur in the ear, affecting post-operative hearing and worsening surgical conditions.

Nitrous oxide might expand an already present pneumocephaly, converting to a tension pneumocephaly. A recent review suggests waiting 6–8 weeks after open dura surgery before using nitrous oxide, as intracranial air persists for several weeks after intracranial surgery.\textsuperscript{10} However, its use in open brain surgery does not show an increase in intracranial gas post-craniotomy and may in fact reduce intracranial pressure (ICP) because of the rapid washout of nitrous oxide after closure of the dura and wake-up.

Because of these concerns, nitrous oxide is relatively contraindicated in bowel, laparoscopic, middle ear, and eye surgery with caution advised in neurosurgery. Patients with pneumothorax should avoid nitrous oxide and information should be sought from patients before operation to exclude the possibility of recent eye surgery or details of the gas used. Attention must also be paid to the potential effects of increased volume in air-filled spaces such as the tracheal cuff, laryngeal mask, and pulmonary artery catheter cuff if used.

**Physiological systems**

**Metabolic and haematological systems**

Nitrous oxide irreversibly oxidizes the cobalt ion at the centre of Vitamin B12 (cyanocobalamin). Vitamin B12 is required as a co-factor for the enzyme methionine synthetase. This crucial enzyme of one-carbon chemistry transfers the methyl group from S-methyl tetrahydrofolate (THF) to homocysteine, to form THF and methionine. THF is involved in thymidine synthesis and DNA production. After several hours of nitrous oxide anaesthesia, activity levels of methionine synthetase are very low. Mild megaloblastic changes (associated with B12 deficiency) are present after 12 h and are marked after 24 h. After several days, complete bone marrow failure is expected.
Nitrous oxide is required for the methylation of myelin sheath phospholipids. B12 or folate deficiency leads to sub-acute combined degeneration of the cord—presenting as limb weakness, numbness and tingling with imbalance. Patients with subclinical B12 deficiency, because of illness, pernicious anaemia, or nutritional deficiency, and patients with methylene-tetrahydrofolate-reductase deficiency are especially at risk. Preoperative B12 followed by folate supplementation is recommended or nitrous oxide should be avoided.

**Cardiovascular systems**

Nitrous oxide leads to mild adrenergic stimulation with a slight increase in heart rate, venous tone, and pulmonary vascular resistance. It has a mild negative inotropic effect that seems to be offset by the adrenergic stimulation. On balance, when given to reduce the concentrations of volatile anaesthetic agent, nitrous oxide improves haemodynamic performance compared with volatile alone. It is not cardioprotective and has no effects on the coronary circulation.

Nitrous oxide increases plasma homocysteine concentrations, a marker of cardiovascular disease. The ENIGMA-I trial demonstrated a trend to an increased risk of myocardial infarction (MI) in the nitrous oxide group and a statistically significant increase in the risk of MI over a 3.5-yr follow-up. However, sub-group analysis of the PeriOperative ISchaemic Evaluation trial of perioperative β-blockade showed no link between nitrous oxide and cardiovascular death. This has been confirmed by the recent release of the ENIGMA-II trial, a 7112 patient, international, multicentre, randomized controlled trial of nitrous oxide in equivalent oxygen concentrations (Table 2). This showed no increased risk of death or cardiovascular complications with nitrous oxide use.

**Neurological systems**

Increasing concern is being placed on the effects of anaesthesia on the developing brain, using either NMDA antagonists or GABA<sub>α</sub> agonists. A variety of animal experiments demonstrating apoptosis or dendrite growth failure, followed by observational cohort studies of neonates or young children who have received multiple anaesthetics shows a link with poorer neurological outcomes (reviewed in Ref.16). However, from this study design, it is impossible to determine whether children who require multiple anaesthetics are by their nature more at risk of learning difficulties, rather than this being caused by anaesthesia. Supporting this are data suggesting that single anaesthetic episodes do not increase the risk of developing learning difficulties. Finally, brief episodes of nitrous oxide exposure to mothers in the delivery suite or to medical personnel have never been significantly linked with developmental problems. Its use for labour analgesia is therefore considered safe.

The ageing brain is also at risk of postoperative cognitive dysfunction after anaesthesia and the onset of Alzheimer’s disease has been linked to cumulative anaesthetic exposure before the age of 50. The same trial methodology issues exist for these studies as in the paediatric population and nitrous oxide itself has not demonstrated any significant worsening of cognitive decline when compared with volatile agents. Minimizing the depth of anaesthesia in patients who need surgery at the extremes of age seems an obvious suggestion that is already followed by best clinical practice.

Nitrous oxide, when administered alone, increases the cerebral blood flow (CBF), cerebral metabolic rate, and ICP. However, i.v. anaesthetic agents reduce these effects. When administered with volatile agents, an equi-MAC mixture of volatile and nitrous oxide increases CBF when compared with volatile alone. Further discussion of the extensive evidence is outside the scope of this review, but interested parties may wish to read a recent comprehensive review.

**Respiratory systems**

Nitrous oxide is slightly sweet smelling and does not cause airway irritation. It is, thus, an ideal agent for inhalation induction with a suitable volatile agent. It has a minimal effect on minute ventilation and therefore reduces the ventilatory depression induced by volatile agents when used as part of balanced anaesthesia. It does not cause bronchodilation similar to volatile agents.

**Wound infection**

The ENIGMA-I trial demonstrated a significant increase in the risk of wound infection with exposure to nitrous oxide. The summary of the ENIGMA-II trial is as follows:

**Table 2** Summary of the ENIGMA-II trial

<table>
<thead>
<tr>
<th>Design</th>
<th>International multicentre, randomized controlled trial. Intention-to-treat analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusion criteria</td>
<td>Major non-cardiac surgery, ≥45 yr old at risk of cardiovascular complications—history of coronary disease, heart failure, cerebrovascular disease, peripheral vascular disease, ≥70 yr with other comorbidities</td>
</tr>
<tr>
<td>Exclusion criteria</td>
<td>Planned F&lt;sub&gt;O2&lt;/sub&gt; &gt; 30% including thoracic surgery involving one-lung ventilation, patients with substantially impaired gas exchange</td>
</tr>
<tr>
<td>Groups</td>
<td>Intervention: 70% nitrous oxide 30% oxygen after induction and intubation until the end of surgery Control: oxygen and air to achieve F&lt;sub&gt;O2&lt;/sub&gt; of 30%</td>
</tr>
<tr>
<td>Primary outcome</td>
<td>Composite of death and cardiovascular events (non-fatal MI, cardiac arrest, pulmonary embolism, and stroke) within 30 days of surgery</td>
</tr>
<tr>
<td>Selected secondary outcomes</td>
<td>Non-fatal MI, surgical site infection Severe PONV (for others see Ref.17)</td>
</tr>
<tr>
<td>Power calculation</td>
<td>7000 patients for 0.05 type I and 0.1 type II error detection of increased risk of primary outcome from 6 to 8%</td>
</tr>
<tr>
<td>Enrollment</td>
<td>10,102 eligible patients, enrolled 7112</td>
</tr>
<tr>
<td>Primary outcome result</td>
<td>Primary outcome in 283 (8%) of nitrous patients and 296 (8%) of control patients (RR: 0.96, 95% CI: 0.83–1.12, P=0.64)</td>
</tr>
<tr>
<td>Secondary outcomes result</td>
<td>Surgical site infection 321 (9%) nitrous and 311 (9%) control Severe nausea and vomiting 506 (15%) nitrous and 378 (11%) control</td>
</tr>
<tr>
<td>Sub-group analysis</td>
<td>Increased PONV risk with nitrous reduced with prophylactic antiemetics Without antiemetics RR 1.75 (95% CI: 1.43–2.13; interaction P=0.001) With antiemetics RR 1.12 (95% CI: 0.95–1.32)</td>
</tr>
</tbody>
</table>

PONV, post-operative nausea and vomiting; RR, relative risk; CI, confidence interval.
role of hyperoxia in wound infection is controversial and effects may have been caused by the different amounts of oxygen delivered to the trial groups. The ENIGMA-II trial in moderate to high-risk surgical patients delivered equal oxygen fractions with or without nitrous oxide and demonstrated no difference in wound infection rates between the trial groups.15

Postoperative nausea and vomiting

The ENIGMA-I study demonstrated a significantly increased risk of post-operative nausea and vomiting (PONV) with nitrous oxide anaesthesia. This has been confirmed by other non-randomized studies and guidelines suggest avoidance in patients at high risk of PONV. Increased rates of PONV have also recently been shown in the ENIGMA-II trial. However, this trial demonstrated that with prophylactic antiemetic treatment, the relative risk (RR) of severe nausea, and vomiting reduced from RR 1.75 (95% confidence interval [CI]: 1.43–2.13) to RR 1.12 (95% CI: 0.95–1.32).15

Conclusion

Nitrous oxide has been a staple of anaesthetic practice for more than 150 yr. Basic science studies have often questioned its use, but plausible mechanisms of harm have not always proved to be clinically significant. With the arrival of recent studies such as ENIGMA-II, some important controversies regarding its use have been resolved.

There are other anaesthetic agents and methods that increasingly enable us to avoid nitrous oxide. However, with 150 yr of experience, this valuable analgesic and anaesthetic agent should not be discarded.

Declaration of interest

None declared.

References


Nitrous oxide in modern anaesthetic practice