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Biologic Effects of Nitrous Oxide

A Mechanistic and Toxicologic Review

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Nitrous oxide is the longest serving member of the anesthesiologist's pharmacologic armamentarium but remains a source of controversy because of fears over its adverse effects. Recently, the Evaluation of Nitrous oxide In a Gas Mixture for Anaesthesia (ENIGMA) trial reported that nitrous oxide use increases postoperative complications; further preclinical reports have suggested that nitrous oxide may contribute to neurocognitive dysfunction in the young and elderly. Therefore, nitrous oxide's longevity in anesthetic practice is under threat. In this article, the authors discuss the evidence for the putative toxicity of nitrous oxide, from either patient or occupational exposure, within the context of the mechanism of nitrous oxide's action. Although it would seem prudent to avoid nitrous oxide in certain vulnerable populations, current evidence in support of a more widespread proscription from clinical practice is unconvincing.

NITROUS oxide has been used in clinical anesthetic practice for more than 150 yr, and its longevity should be considered within the context of all the major advances in anesthetic practice over that time. Fifty years ago, concerns were expressed about nitrous oxide's toxicity after anesthesia.[1] This report was followed 10 yr later with concerns over the hazards posed by occupational exposure to the gas.[2] Since that time, the status of nitrous oxide in anesthesia has been under almost continuous challenge because of concerns about hematologic, neurologic, myocardial, and immunologic effects, as well as postoperative nausea and vomiting and expansion of air-filled spaces. More recently, nitrous oxide–induced neurotoxicity has been implicated in the development of long-lasting cognitive defects when administered at either extremes of age.[3,4] Perhaps the most controversial evidence challenging the continued use of nitrous oxide was reported in ANESTHESIOLOGY by the Evaluation of Nitrous oxide In a Gas Mixture for Anaesthesia (ENIGMA) trial group, who found that the use of high concentration of oxygen (80% plus 20% nitrogen) in lieu of nitrous oxide (70% plus 30% oxygen) reduced the incidence of postoperative infection and pulmonary complications.[5] This trial has already generated editorial comment in the Journal,[6] and we will elaborate further in the context of the toxicology of nitrous oxide as a whole.

Although several reviews of the toxicology of nitrous oxide have previously been published,[7–10] none have considered these putative toxicities in the context of the anesthetic and analgesic effects of nitrous oxide. We also seek to comprehensively review the evidence addressing risks to the patient and healthcare personnel involved in the administration of nitrous oxide, in the context of modern-day anesthetic practice; multiple issues confound many of the earlier studies of occupational exposure, making many of these studies largely irrelevant to current clinical practice in which use of effective scavenging equipment is commonplace. Incontrovertible side effects of nitrous oxide, such as diffusion hypoxia, are not dealt with; rather, this toxicologic review concentrates on the consequences of nitrous oxide exposure that are related to nitrous oxide's inhibition on methionine synthase[11,12] and the *N*-methyl-D-aspartate (NMDA) subtype of the excitatory glutamate receptor.[13–15] The review is structured to initially consider the mechanisms of nitrous oxide's actions; next, we will focus on the putative toxicity identified in the ENIGMA trial and then broaden the discussion to cover risks from occupational exposure. The goals of this review are to consider evidence that will allow practitioners to derive maximal clinical benefit from nitrous oxide for their patients while minimizing its potential toxicity to both their patients and themselves.

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Mechanisms of Anesthetic Action

The minimum alveolar concentration (MAC) of nitrous oxide is reported as 104% with the initial study completed in hyperbaric conditions on seven human volunteers exposed to tetanic electrical impulses as the noxious stimulus (rather than the standard incision)[16]; therefore, nitrous oxide is the least potent inhalational anesthetic agent in current practice. The predominant theory of the molecular mechanism to account for nitrous oxide's anesthetic action is noncompetitive inhibition of the NMDA subtype of glutamate receptors.[13] The importance of this receptor has recently been confirmed using sophisticated pharmacogenomic techniques in *Caenorhabditis elegans*[14] and in mice.[15] Whether there is also a role for non-NMDA glutamate receptors remains unclear, but inhibitory effects on these receptors have also been reported.[17] Therefore, inhibition of excitatory glutamatergic neurotransmission is central to the thesis for the anesthetic actions of nitrous oxide. Another potential target contributing to nitrous oxide's anesthetic action are the two pore domain potassium channels, such as the TREK-1 channel, which, when activated, increases potassium conductance and thereby hyperpolarizing neurons away from their firing threshold.[18] Although the role of γ-aminobutyric acid type A ($GABA_A$) receptors in anesthetic states produced by intravenous (propofol, etomidate, and pentobarbital) and halogenated volatile (halothane and enflurane) anesthetics seems well established,[19] nitrous oxide exerts insignificant effects on these inhibitory receptors.[17] Despite expanding knowledge of the molecular mechanisms of nitrous oxide, clarification of the specific neural substrates and supraspinal loci for the hypnotic effect of nitrous oxide is required.

Mechanisms of Analgesic Action

The mechanisms of analgesic action have been recently reviewed,[20] and therefore we will merely summarize and update the findings described elsewhere. Nitrous oxide acts supraspinally to induce analgesia by activation of opioidergic neurons in the periaqueductal gray matter and noradrenergic neurons in the locus ceruleus, A5 and A7 areas of the brainstem.[21,22] Critical to the activation of the locus ceruleus (and potentially the other loci) appears the hypothalamic release of corticotropin-releasing factor[23] (fig. 1), which may be provoked by antagonism of the NMDA receptor.[24] Supraspinal administration of antagonists for the corticotropin-releasing factor and opioid receptors attenuates the antinociceptive effects of nitrous oxide in rats.[23] Opioid release in the brainstem inhibits activity of γ-aminobutyric acid–mediated (GABAergic) interneurons, thereby removing the inhibitory tone on the descending noradrenergic inhibitory pathways (fig. 1).[25,26] Upon activation of these descending pathways, the antinociceptive effects of nitrous oxide are transduced *via* α_1 and α_2 adrenoceptors within the dorsal horn of the spinal cord.[21,27] The identity of these adrenoceptors was determined by intrathecal administration of selective antagonists to attenuate nitrous oxide's antinociceptive action. Depletion of spinal norepinephrine also attenuated nitrous oxide's antinociceptive action.[28] Nitrous oxide was also ineffective as an antinociceptive agent (but not as a hypnotic–anesthetic agent) in transgenic mice lacking functional α_{2B} adrenoceptors, establishing a role of this receptor subtype for its analgesic action.[21] The importance of the descending inhibitory neurons was further established after the findings that nitrous oxide lacks antinociceptive effects when the spinal cord is transected in adult rodents[28] and in young animals[29] at ages when the descending inhibitory neurons lack functional connectivity with their targets. Whether this lack of efficacy in young animals pertains to young humans is currently being investigated.

Is There Overlap between Nitrous Oxide's Analgesic and Anesthetic Actions?

Nitrous oxide is an effective inhalation analgesic in multiple settings; furthermore, we now understand much of how nitrous oxide transduces its analgesic effect. This understanding also informs us further about the paradoxical interaction nitrous oxide displays with agents that potentiate the $GABA_A$ receptor.

Nitrous oxide's analgesic action is dependent on both the inhibition of supraspinal $GABA_A$ receptors as well as activation of spinal $GABA_A$ receptors[26] (fig. 1). Therefore, agents that activate the supraspinal $GABA_A$ receptor may interfere with nitrous oxide analgesia by inhibiting the activation of the descending inhibitory neurons.[26] This may explain the reported antagonism of nitrous oxide's antinociceptive effects by the administration of volatile anesthetics,[30,31] midazolam,[26] and propofol[32] in animals.

Some clinical evidence supports this view; in healthy volunteers inhaling subanesthetic concentrations of sevoflurane and nitrous oxide, the sevoflurane element decreases nitrous oxide's analgesic efficacy[33] (fig. 2). However, the interaction at subanesthetic and anesthetic doses may not be the same. To further dissect the importance of nitrous oxide's analgesic mechanism to nitrous oxide's clinical anesthetic role, we have compared MAC data from humans at time points when descending inhibitory neurons are functional (adults) and when they are not functional (infants). If functional descending inhibitory neuron activation is required for nitrous oxide's additivity with volatile anesthetics, any interaction would be predicted to be subadditive in infants. On the contrary, if descending inhibitory neuron activation is

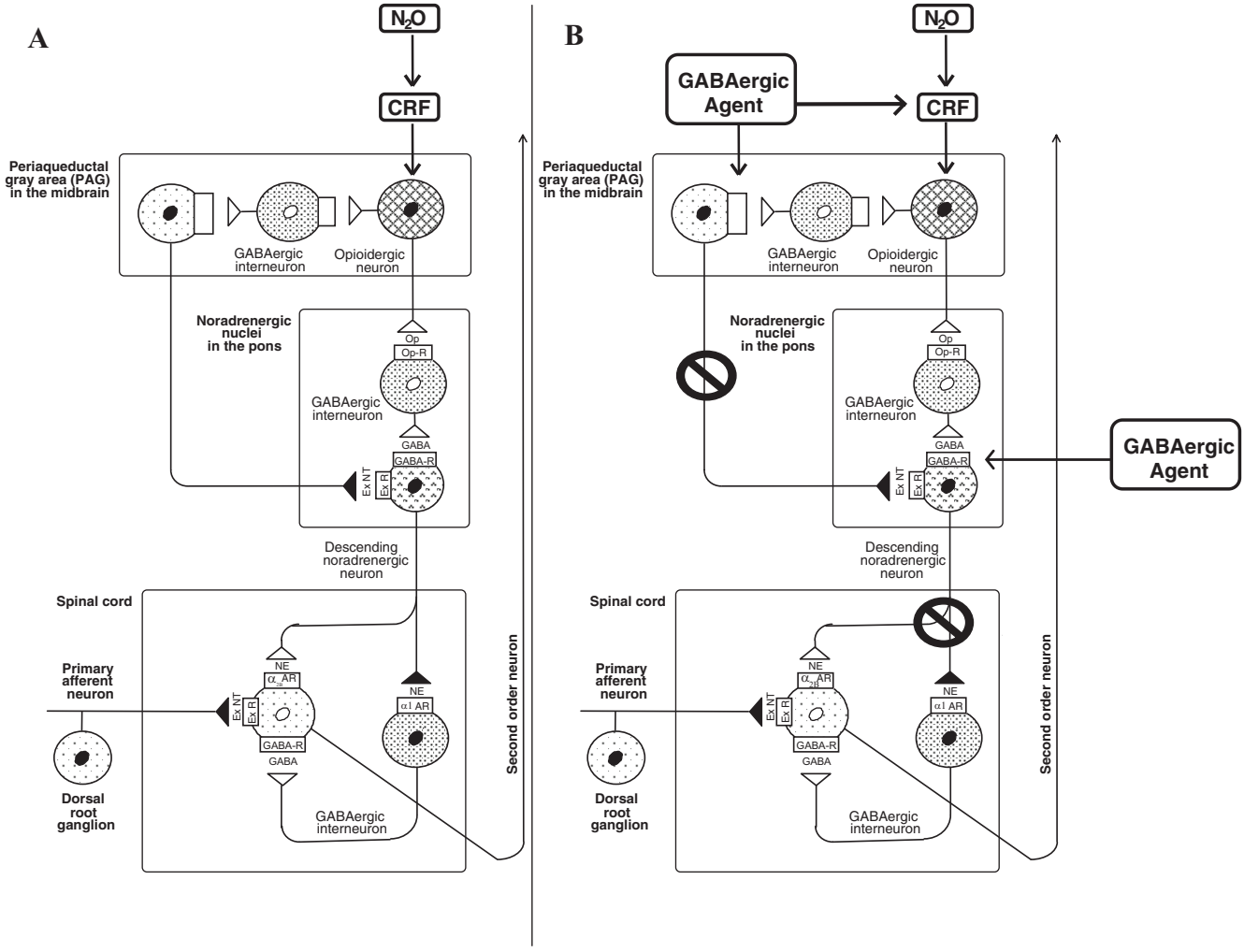

Fig. 1. Putative neuronal pathway for the analgesic effect of nitrous oxide (N_2O). (*A*) Nitrous oxide causes activation of opioidergic neurons *via* hypothalamic release of corticotrophin-releasing factor (CRF), which provokes the release of endogenous opioids at their terminals in the periaqueductal gray region of the midbrain. Opioid receptors on γ-aminobutyric acid–mediated (GABAergic) interneurons are stimulated, resulting in an inhibition of these inhibitory interneurons. In turn, this results in a disinhibition of excitatory neurons of the noradrenergic descending inhibitory neurons in the medulla–pons region. The descending noradrenergic neuron releases norepinephrine at its terminals in the spinal cord, which stimulate at least two species of adrenergic receptors, namely, α_1 subtypes on GABAergic interneurons or α_{2B}-adrenergic receptors located postsynaptically on the second-order neuron. The effect of stimulating these two sets of receptors in the dorsal horn of the spinal cord decreases firing of the second-order neuron and hence results in a reduction in pain impulses ascending into the supraspinal regions. (*B*) The effects of addition of a GABAergic agent, which activates postsynaptic $GABA_A$ receptors, on the pain pathway preventing activation of the noradrenergic descending inhibitory neurons by nitrous oxide (signified by the "no entry" sign). *Black triangle* = excitatory synapse; *white triangle* = inhibitory synapse; *black oval* = nucleus of an active cell; *white oval* = nucleus of an inactive cell. AR = adrenoceptor; ExNT = excitatory neurotransmitters; ExR = receptors for excitatory neurotransmitters; GABA = γ-aminobutyric acid; GABA-R = γ-aminobutyric acid receptor; LC = locus ceruleus; NE = norepinephrine; Op = opioid peptides; Op-R = opioid receptor.

irrelevant to nitrous oxide–volatile anesthetic additivity, an additive interaction in both childhood and adulthood would be expected. In fact, the latter is true; nitrous oxide does interact additively with volatile anesthetics in human infants[34] and adults.[35] This evidence suggests that activation of descending inhibitory neurons is not critical for the anesthetic action of nitrous oxide when coadministered with a volatile anesthetic agent (as occurs in clinical practice).

To verify these extrapolations, we have examined data from a clinical study of 1.66 MAC desflurane with or without 60% nitrous oxide (in the absence of opioids). The authors found a similar effect on blood pressure responses to electrical titanic stimulation between the groups (though heart rate responses were lower in the nitrous oxide group[36]). Because adrenergic responses are frequently used as a measure of pain responses intraoperatively, these findings suggest that the use of nitrous oxide during maintenance of anesthesia as an additional *analgesic* may be confounded and that any analgesic effect, in this context, should be regarded as minimal.

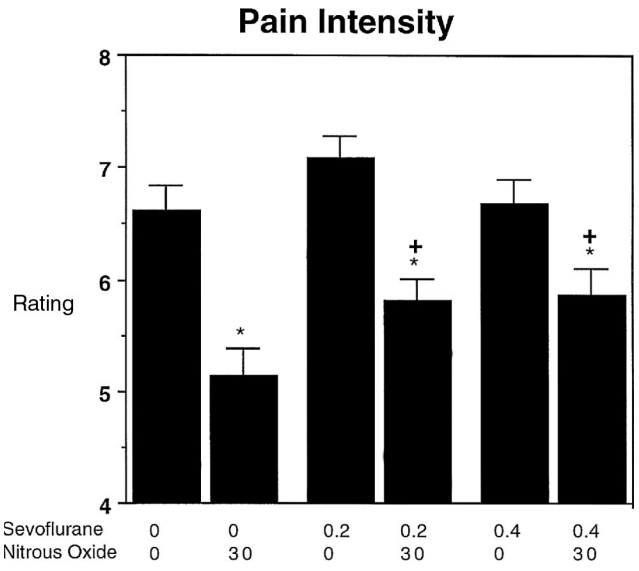

Fig. 2. Effects of sevoflurane and nitrous oxide on ratings of pain intensity to cold. Each *bar* is the mean across all subjects. *Brackets* represent SEM. * Significant decrease from placebo (0% sevoflurane/0% nitrous oxide) ratings. + Significant increase from the 0% sevoflurane/30% nitrous oxide ratings. Reproduced from Janiszewski *et al.*[33]; used with permission.

In hyperbaric conditions, nitrous oxide can induce anesthesia itself,[16] and therefore one would suspect analgesia to play a role in nitrous oxide's ability to induce anesthesia. However, in hyperbaric conditions, nitrous oxide predominantly inhibits ventral horn, rather than dorsal horn, responses to supramaximal noxious stimulus[37] similar to other anesthetics[38] to provoke immobility. If, rather than immobility, sedation is taken as an endpoint, we can also dissect the sedative and analgesic actions of nitrous oxide; in transgenic mice lacking α_{2B} adrenoceptors, antinociceptive responses to nitrous oxide are abolished, but sedative responses are unaffected.[21] Lesioning of the locus ceruleus also attenuated the antinociceptive effects of nitrous oxide without hindering its sedative effects. Therefore, the immobilizing and sedative actions of nitrous oxide should be considered discrete from its analgesic effects.

These preclinical and clinical data suggest that nitrous oxide analgesia is not important for its anesthetic action when administered clinically, *i.e.*, in combination with a GABAergic agent. This is clinically relevant and is supported by the extensive preclinical mechanistic work. The relevance of the mechanism of nitrous oxide's analgesic action to clinical anesthetic practice can be appreciated in different clinical settings. A prime example is its use for the provision of general anesthesia for cesarean delivery (a period of risk of awareness), when clinicians may include nitrous oxide in the anesthetic regimen for extra "analgesia'"; however, there is no evidence that addition of nitrous oxide to a volatile anesthetic enhances the *analgesic* effects of the anesthetic—merely the depth of anesthesia.

What Is the Interaction between Nitrous Oxide and Exogenous Opioids?

Having explored this complicated interaction with anesthetic agents that affect $GABA_A$ receptors, we will discuss the importance of drugs that modulate opioid signaling on nitrous oxide's analgesic effect. Nitrous oxide activates supraspinal opioid receptors (fig. 1) *via* corticotropin-releasing factor.[23] An interaction between nitrous oxide, which indirectly stimulates opioid receptors, and exogenous opioids could be expected. Consistent with this, nitrous oxide reduces the MAC-sparing effect of morphine on isoflurane anesthesia in rats,[39] suggesting overlap between morphine and nitrous oxide's effects. Unfortunately, a dose–response curve for the effect of morphine was not constructed; hence, it is not confirmed that the reported "antagonism" exists between morphine and nitrous oxide[39]; in fact, this effect may represent additivity between opioids and nitrous oxide.

In an attempt to probe this interaction further, we have compared the clinical work of Ghouri and White[40] and Sebel *et al.*,[41] though the reader should note that these were independent studies and therefore we are restricted to a purely qualitative comparison of the data. In these two studies, a MAC value of 2.6% was found when 3 μg/kg fentanyl was coadministered with desflurane (in oxygen without nitrous oxide),[41] and a MAC value of 3.0% desflurane was found when 3 μg/kg fentanyl and 60% nitrous oxide (in oxygen) was given.[40] This comparison suggests that if sufficient opioid is administered, the addition of nitrous oxide would not act to further decrease MAC.

However in another study, addition of 60% nitrous oxide reduced the MAC of sevoflurane from 3.96% to 1.2% during target-controlled analgesia with 1 ng/ml remifentanil, and this sevoflurane-sparing effect was still detectable at 3 ng/ml remifentanil.[42] The apparent discrepancy in the findings with fentanyl and remifentanil may stem from a difference in the actions of fentanyl and remifentanil on the NMDA receptor, because remifentanil potentiates the NMDA receptor.[43] Nitrous oxide, as an NMDA antagonist, may have blocked remifentanil's activating effect on the NMDA receptor and thus continued to be a MAC-sparing drug in the presence of remifentanil. Through a similar mechanism, nitrous oxide may be able to inhibit opioid-induced postoperative hyperalgesia,[44] though further studies are required to elucidate whether this action of nitrous oxide persists in the presence of a volatile anesthetic agent.

We still remain relatively uninformed about the additivity of different anesthetic agents (be they GABAergic, NMDA antagonistic, or opioidergic) on MAC, especially when used in combination, as frequently occurs in clinical practice. We hasten to point out that the clinical data support an additive interaction between nitrous oxide

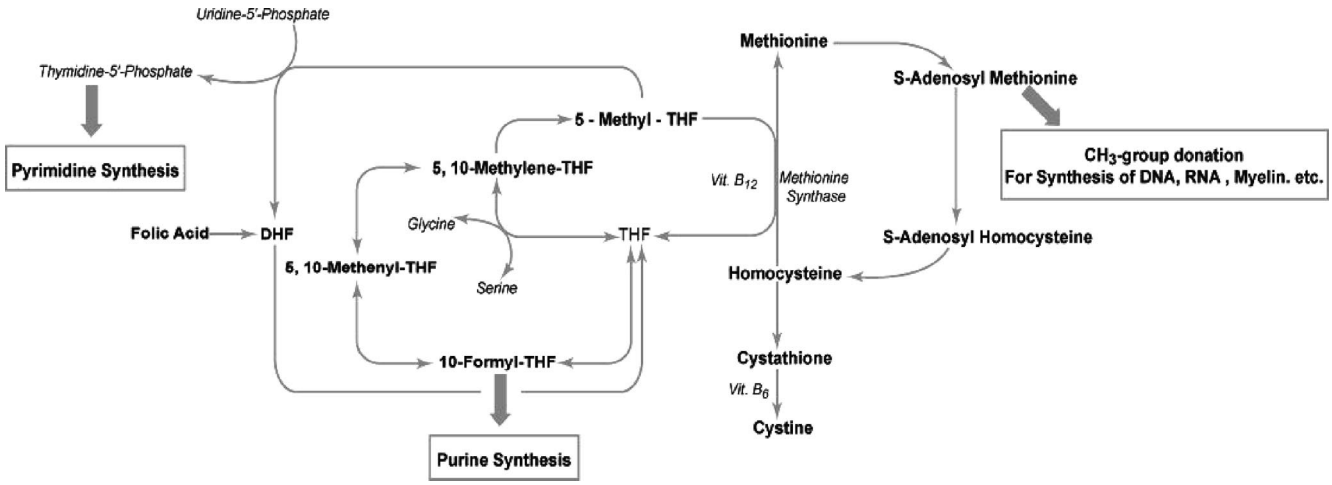

Fig. 3. Methionine synthase is a ubiquitous cytosolic enzyme that plays a crucial role in the generation of s-adenosylmethionine and in the folate cycle. Nitrous oxide inhibits cobalamin (vitamin B_{12}) from acting as a coenzyme for methionine synthase and thus inhibits the cycle. 5-methyl-THF = 5-methyltetrahydrofolate; 5,10-methylene-THF = 5,10-methylenetetrahydrofolate; THF = tetrahydrofolate. Modified from Weimann[9]; with permission from Elsevier.

and halogenated volatile anesthetics; however, the addition of nitrous oxide in this context should not be as an analgesic but for the same indication as other anesthetic agents. The interaction with opioids is complicated and requires further study, but the overlap in their mechanism of action explains both their profound analgesic capabilities and why they may have an interchangeable role in anesthesia. These discussions highlight the importance of studying the mechanisms of anesthesia and consequently understanding the ways we should use current agents and develop new ones.

Mechanism of Inhibition of Methionine Synthase

Aside from the biologic actions of nitrous oxide transducing its anesthetic and analgesic actions, nitrous oxide also affects methionine synthase function. Nitrous oxide oxidizes the cobalt I (Co^+) form of cobalamin (vitamin B_{12}) to Co^{3+} [11]:

$$Co^+ + N_2O + 2H^+ \rightarrow Co^{3+} + N_2 + H_2O$$

A further reaction rapidly ensues:

$$Co^{3+} + Co^+ \rightarrow 2Co^{2+}$$

However, an alternative reaction has also been proposed to account for nitrous oxide's oxidation of cobalamin involving the generation of hydroxyl radicals that irreversibly oxidize cobalamin[12]:

$$Co^+ + N_2O + H^+ \rightarrow Co^{3+} + N_2 + OH^-$$

By either of the above mechanisms, the resulting oxidized cobalt cation prevents cobalamin (vitamin B_{12}) acting as a coenzyme for methionine synthase. Methionine synthase is a ubiquitous cytosolic enzyme that plays a crucial role in the generation of methyl groups (*via* the active intermediary s-adenosylmethionine) for the synthesis of DNA, RNA, myelin, and catecholamines, among other products. After methyl group donation, s-adenosylmethionine is converted to homocysteine, which can then reenter the methionine pathway or be metabolized to cystathione (fig. 3). In addition to cobalamin, methionine synthase requires 5-methyltetrahydrofolate to function as a coenzyme. One carbon transfer by folates plays a crucial role in the biosynthesis of pyrimidines and purines and in serine and glycine metabolism. This latter interconversion between serine and glycine produces the methyl groups to be added to homocysteine to produce methionine. The methyl group is initially bound to tetrahydrofolate (*via* an intermediary) to give 5,10-methylenetetrahydrofolate, which is then reduced to 5-methylenetetrahydrofolate. This is of importance because this pathway represents the only way to produce 5-methylenetetrahydrofolate. The methyl group is then transferred to cobalamin, producing methylcobalamin, the final methyl group donor for methionine synthesis.

This pathway is critical to cellular function, and decreased methionine synthase activity can result in both genetic and protein aberrations.[45] Certain patient groups may be particularly susceptible to reduced methionine synthase activity, including those deficient in cobalamin, such as patients with pernicious anemia or ileal disease, alcoholics, the elderly, and the malnourished[45,46] (table 1).

In preclinical studies, Sharer *et al.*[47] showed that a 24-h exposure to concentrations of 860 parts per million (ppm) or lower of nitrous oxide did not significantly change methionine synthase function in Sprague-Dawley rats. However, at anesthetic concentrations in rats, methionine synthase activity is inhibited rapidly; 50% nitrous oxide exposure decreased methionine synthase activity within 30 min, and the activity was virtually

Table 1. Patients at Risk from Cobalamin Deficiency

Nutritional disorders	Elderly
	Vegans
	Alcoholics
Malabsorption disorders	Prolonged use of proton pump inhibitors or H_2 receptor antagonists
	Pernicious anaemia
	Atrophic gastritis
	Postgastrectomy, Whipple procedure, ileal resection
	Crohn disease
Infection	Bacterial overgrowth, tapeworm

undetectable after 6 h.[48] Because nitrous oxide readily crosses the placenta, this inhibition has been recorded in both the fetus and the dam, with 60 min of nitrous oxide (50%) inhibiting methionine synthase activity to 11% and 18% in maternal and fetal livers in rats, respectively.[49] Extrapolations from these findings to the clinical situation are hindered because of species differences; notably, rats are more sensitive to nitrous oxide–induced methionine synthase inhibition than humans are.[50] Nonetheless, patients anesthetized with nitrous oxide (70%) exhibited a reduction in methionine synthase activity measured in liver biopsies, with a time to half methionine synthase activity of 46 min.[50] After 200 min of nitrous oxide anesthesia, methionine synthase activity approaches zero. Koblin *et al.*[51] demonstrated that nitrous oxide (70%) inhibited methionine synthase activity in liver biopsies with a 50% reduction in activity predicted after approximately 1.5 h. Typically, the enzyme's function will recover within 3–4 days after exposure to nitrous oxide.[48,51] Consistent with this effect, the duration of nitrous oxide exposure is correlated with increased homocysteine levels,[52] which can be prevented by preoperative vitamin B complex therapy.[53]

An important enzyme in the folate cycle, 5,10-methylenetetrahydrofolate reductase (MTHFR), also plays an important role in the conversion of homocysteine to methionine by generating 5-methyltetrahydrofolate (fig. 3). Two single-nucleotide polymorphisms are known (677 cytosine–thymidine and 1298 adenosine–cytosine) and are associated with reduced enzyme activity.[54,55] Mutations in this gene are relatively common, *e.g.*, one homozygous mutation of MTHFR gene (677C–T) is present in 0–3% of African Americans, 9–11% of white Americans, and 32% of Mexicans.[56] In Europe, the highest prevalence seems to be in Southern Europe, with a prevalence of 20–26% in Southern Italy.[55] Homozygosity for many of these mutations is associated with increased homocysteine levels and reduction in MTHFR enzyme activity.[54] Interesting recent data show that patients who receive nitrous oxide anesthesia with a homozygous mutation in MTHFR have a greater postoperative increase in plasma homocysteine levels than heterozygotes or wild type.[57] As yet, the functional consequences of this homocysteine increase are unknown, but it has been hypothesized to play a risk in increased perioperative myocardial morbidity (see section titled Patient Exposure Risk, Myocardial Effects).

Patient Exposure Risk

Immune Effects

Nitrous oxide has been associated with varied effects on the immune system, with decreased proliferation of human peripheral blood mononuclear cells[58] and increased and decreased neutrophil chemotaxis reported.[59,60] Reports such as these have led to concern that nitrous oxide may detrimentally affect immune function. A recent multicenter study of 418 colorectal surgical patients evaluated the effect of nitrous oxide anesthesia (65% nitrous oxide *vs.* 65% nitrogen) on wound infection and healing[61] and found no difference in infection rate, wound healing, hospital stay, or mortality. We must highlight that this trial was powered to find a doubling in the relative risk of wound infection, and therefore a more subtle effect may have been missed.

The ENIGMA trial demonstrated some postoperative benefits of high concentrations of intraoperative oxygen (80%) in nitrogen (20%) when compared with nitrous oxide (70%) in oxygen (30%).[5] Significant differences were noted in the incidences of postoperative complications between the two groups including wound infection, fever, pneumonia, and atelectasis. With 2,050 patients enrolled, this was a very large multicenter randomized controlled trial of patients undergoing major surgery that was powered to show a difference in hospital stay (from 4 days to 3.5 days; β power = 0.9) and a reduction in wound infection from 14% to 10%.

Before further discussion of the results, we wish to highlight a few caveats to the reader regarding the clinical trial design. Structured follow-up occurred on the first postoperative day with a telephone consultation and medical record review at 30 days; in the intervening phase, diagnosis of patient complications was a responsibility of the surgical team, without standardized assessment or stated prospective guidance. Although this may reflect standard medical practice, this may have resulted in underdiagnosis of complications. The study was also not blinded, because those individuals diagnosing wound infection and deciding on discharge from the hospital were aware of the treatment group (because the anesthetic record was available in the notes). Exposure to volatile anesthesia was not standardized, and the nitrous oxide–free group received significantly more propofol to maintain anesthesia. Also, it is unfortunate that biochemical and hematologic monitoring of the patients was not standardized despite the well-known effect of nitrous oxide on methionine synthase. Furthermore, it is also unclear on what criteria the surgeons selected radiologic investigation to establish the pres-

ence of atelectasis, making this a questionable endpoint for the study. Finally, as the authors concede, the large number of comparisons in the trial "increases the chance of a type I error; the secondary, exploratory and subgroup analyses should be treated with caution."

The ENIGMA trial found no difference between the groups for the primary endpoint of the trial; the median duration of hospital stay did not differ between groups, though it did narrowly miss significance (7.0 *vs.* 7.1 day; $P = 0.06$). However, the trial did show a significant reduction for severe nausea or vomiting (which persisted after controlling for increased propofol use in the nitrous oxide–free group) and increased incidences of wound infection (odds ratio [OR], 0.72; 95% confidence interval [CI], 0.52–0.98), pneumonia (OR, 0.51; 95% CI, 0.27–0.97), and atelectasis (OR, 0.55; 95% CI, 0.40–0.75) in the nitrous oxide group. Indeed, the finding of a significant difference in wound infection is remarkable because the incidence was reduced from 10% to 7.7%, with 29 fewer patients getting infected in the nitrous oxide–free anesthesia group. However, these are all secondary endpoints that are vulnerable to type I error.

We consider that the positive results from the ENIGMA trial, with the methodologic and statistical caveats already mentioned, could equally be due to the positive effects of high-dose oxygen therapy or any inherent toxicity of nitrous oxide. The experimental study design of this "pragmatic" clinical trial makes scientific interpretation of the results difficult. The two groups are essentially incomparable because both oxygen concentration and balance gas (nitrogen or nitrous oxide) vary. Therefore, with two variables changing, one cannot definitively know which of the two is the critical factor or whether the abandonment of nitrous oxide plus the adoption of high concentrations of oxygen is necessary to reduce postoperative complications (which has been discussed further[5,6]). In the absence of a clear experimental approach, interpretation of the study becomes speculative as to the critical causative factors involved. Nonetheless, we consider in sum that there is no evidence to suggest that nitrous oxide induces more immunosuppression than other anesthetics. Hence, we have sought to balance these interpretations about the toxicology of nitrous oxide with the available data from the literature in this review.

It is also of interest that in a follow-up trial,§ patients will be randomly assigned to receive either 70% nitrous oxide or nitrogen plus 30% oxygen, which allows more direct comparison between the groups. Unfortunately, a group receiving 70% nitrogen with 30% oxygen was not included in the ENIGMA study because it "is not often used clinically." Nonetheless, we believe that a trial of this nature has the potential to address the association between nitrous oxide and increased postoperative complications.

Hematologic Toxicity

Inhibition of methionine synthase may lead to significant hematologic complications such as megaloblastic anemia.[45] Indeed, even short periods of nitrous oxide exposure (2–6 h) in seriously ill patients can cause megaloblastic bone marrow changes.[62] Another vulnerable group at risk from further methionine synthase suppression may be the elderly, because up to 20% are deficient in cobalamin.[46] Deleu *et al.*[63] followed elderly patients randomly assigned to propofol or nitrous oxide anesthesia. In the nitrous oxide group, postoperative serum but not erythrocyte folate levels were lower than in the propofol group. Hematocrit and hemoglobin levels also decreased, and mean cell hemoglobin levels increased slightly but significantly in the nitrous oxide group (preoperatively *vs.* postoperatively), but no difference was reported between the two anesthetic groups. Three patients experienced neurologic problems such as a sore tongue, mild ataxia, and peripheral polyneuropathy (two were deficient in erythrocyte folate preoperatively), which resolved with folate therapy. In contrast, Koblin *et al.*[64] did not find any changes in two markers of methionine synthase function (urinary formic acid and formiminoglutamic acid urinary excretion) after 3 h of nitrous oxide exposure during hip replacement in elderly patients. A minor increase in these markers was found in younger patients undergoing longer surgery for resection of acoustic neuromas, with a mean duration of anesthesia of 9.3 h. Therefore, whether the elderly represent a population vulnerable to nitrous oxide–induced methionine synthase inhibition requires further investigation, though care should be taken with patients known to be folate or cobalamin deficient.

In another study, Waldman *et al.* did not find hematologic abnormalities in orthopedic and neurosurgical patients exposed to nitrous oxide, apart from a smaller perioperative increase in leukocyte count.[65] Other studies have shown that durations of exposure to nitrous oxide in excess of 6 h are required to provoke hematologic changes that are preventable by preoperative folinic acid supplementation.[66,67] However, patients deficient in cobalamin and substrates for methionine synthase are at potential risk even from exposure to short durations of nitrous oxide. A recent case report showed that after nitrous oxide–based anesthesia, a child with preoperatively unrecognized cobalamin deficiency developed neurologic symptoms and pancytopenia that resolved with vitamin supplementation.[68] Nonetheless, there is no evidence that individuals who are not deficient in cobalamin or folate are vulnerable to hematologic complications of nitrous oxide if exposed for less than 6 h.

§ The ENIGMA II trial: Nitrous oxide anesthesia and cardiac morbidity after major surgery: A randomized controlled trial. ClinicalTrials.gov Identifier: NCT00430989, www.ClinicalTrials.gov. Accessed June 29, 2008.

Neurologic Effects

Reduced methionine synthase function may lead to myelinopathies such as subacute combined neurodegeneration of the spinal cord,[45] which has also been reported to occur with long-term nitrous oxide abuse.[69,70] More severe neurologic complications have been reported after nitrous oxide exposure in patients with rare congenital gene deficiencies.[71,72] Myelin degeneration after chronic nitrous oxide exposure has been observed in monkeys[73] and pigs[74] with some protection afforded by methionine supplementation. Interestingly, in vitamin B_{12}–deficient fruit bats, pretreatment with folates accelerates the development of nitrous oxide–induced neurotoxicity.[75] This has led to a hypothesis that a rapid increase of methyl-folate concentrations in the nervous system with simultaneous inactivation of vitamin B_{12} may produce nitrous oxide's neurotoxic effects.[45]

Neurologic injury has also been noted after a routine nitrous oxide–based anesthetic in patients with cobalamin deficiency, such as those with pernicious anemia, although the injury did not become apparent for many weeks.[76] In another report, a 6-month-old girl developed hypotonia and collapsed with metabolic acidosis, reduced serum cobalamin, and diffuse cerebral atrophy after nitrous oxide anesthesia.[72] The reason for the patient's cobalamin deficiency was unknown, although the mother was noted to also be deficient, having avoided dairy and meat products while breast-feeding.

In contrast, consumption of large quantities of nitrous oxide is required in the nondeficient individual to induce a similar toxicity such as that which occurs in the setting of abuse.[70] Nitrous oxide abusers can present with altered mental status, paresthesias, ataxia, and weakness and spasticity of the legs. A recent case report described a 21-yr-old man who used nitrous oxide at home for 4 months for postoperative analgesia after gastrointestinal tract surgery and developed a demyelinated cervical spinal cord lesion. After treatment with cobalamin and folate, the neurologic symptoms resolved.

Nitrous oxide may also prove toxic in certain rare congenital disorders encountered in pediatric practice. A child exposed twice for elective anesthesia presented 25 days postoperatively with seizures and episodes of apnea.[71] The patient died at 130 days of age, 46 days postoperatively, as a result of respiratory arrest. Postmortem examination showed MTHFR deficiency, which is a rare autosomal recessive disorder characterized by progressive hypotonia, convulsions, and psychomotor retardation.

As described in the section titled Patient Exposure Risk, Hematologic Effects, Deleu *et al.*[63] reported on two patients who presented for ophthalmic surgery as part of a randomized controlled trial with reduced erythrocyte folate levels and subsequently developed neurologic symptoms after nitrous oxide exposure. Furthermore, cobalamin-deficient patients are at risk[71,72,76] from nitrous oxide toxicity, indicating that both should be tested before nitrous oxide use when deficiency is suspected. Because serum cobalamin levels may correlate poorly with tissue levels, a normal serum level does not exclude deficiency. Assessment of methylmalonic acid and homocysteine levels has been suggested to improve the sensitivity of detection of cobalamin deficiency and may be useful in this context.[77] Consequently, we suggest that it is important to replace both cobalamin and folate in the malnourished before nitrous oxide anesthesia to reduce the potential risk of toxicity. Despite being used to treat nitrous oxide neurologic injury previously,[63] folate monotherapy should not be used in this context because of fears that it may exacerbate neurologic injury.[75] However, we stress that nitrous oxide is unlikely to precipitate neurologic injury secondary to demyelination in the majority of cases; this is exemplified both by the prevalence of nitrous oxide use and by the relative scarcity of the case reports reporting this form of injury.

In accord with most other drugs that inhibit the NMDA receptor, nitrous oxide provides both neuroprotection and neurotoxicity.[13] Nitrous oxide is a weak neuroprotective agent against excitotoxic or hypoxic–ischemic brain injury in adult rodent models,[13,78] though there is also evidence disputing this effect.[79] Currently, there is insufficient evidence to suggest an indication of nitrous oxide as a neuroprotective agent. Furthermore, in certain situations where a neuroprotective agent may prove advantageous, such as cardiac and neurosurgery, nitrous oxide is avoided because of effects on cerebral blood flow and the potential to exacerbate air embolism. Nitrous oxide also causes reversible neurotoxicity in the retrosplenial and posterior cingulate cortices with vacuolation of pyramidal neurons in rats.[13] This morphologic neurotoxicity occurs at doses in excess of those normally administered in clinical practice (*i.e.*, in hyperbaric conditions) and resolves within 3 h in rats. However, markers of cerebral injury are detectable at clinical doses when the morphologic injury is not observable.[80] The functional correlate of this injury is thought to be the psychomimetic reactions that can occur with this class of agent. Coadministration of a GABAergic agent, such as isoflurane or propofol, as usually occurs in anesthetic practice, can attenuate this toxicity.[13,81] Female rats seem especially vulnerable to the injury produced by NMDA antagonism, in keeping with the susceptibility of human females to the anesthetic effects of this class of agent.[82] Interestingly, despite both being NMDA antagonists, xenon does not induce the same toxicity as nitrous oxide,[80] which may be related to differences in dopamine release.[83] Corroborating this, nitrous oxide neurotoxicity is also inhibited by the dopamine antagonist haloperidol,[80] further suggesting that the toxicity may be related to nitrous oxide–induced dopamine release.

Recently, exposure to anesthetic agents has been associated with a disturbing pattern of neurodegeneration in the neonatal rat, which has led to concerns regarding the vulnerability of the developing human brain.[3] NMDA antagonists, such as ketamine and MK-801, have been shown to provoke widespread apoptotic neuronal death in the neonatal rat brain[84]; again, it is interesting that xenon does not seem to induce this toxicity.[85] Similar to xenon, nitrous oxide exposure alone does not induce apoptosis in the neonatal rat brain at concentrations up to 75%. However, unlike xenon, it does exacerbate the injury provoked by isoflurane (0.75%).[3,85] Whether equianesthetic dosing of nitrous oxide–isoflurane or sole isoflurane anesthesia provokes the same injury is unknown, and any additivity should be sought to clarify this matter.

Recently, data from 5-day-old rhesus monkeys have been published suggesting that NMDA antagonist–induced injury in the young may be of less clinical importance than initially feared. Whereas exposure to ketamine for 24 h produced robust neurodegeneration in the brain, 3 h of ketamine anesthesia did not.[86] Currently, the evidence of injury from nitrous oxide or other NMDA antagonists is insufficient to condemn them from pediatric anesthetic practice. Indeed, nitrous oxide is a useful agent for gaseous inductions in the young because of its odorless quality and the second gas effect, and there is no evidence currently that its use in this circumstance will negatively affect a child's neurodevelopment.

Another putative neurologic complication of anesthesia is postoperative cognitive dysfunction (POCD). The contribution of general anesthesia to this complication has recently been questioned because there is equal incidence of POCD in patients who have undergone general and regional anesthesia.[87] The direct effect of nitrous oxide on POCD was explored in an animal model[4]; aged rats exposed to 70% nitrous oxide showed impaired choice accuracy in a radial maze (a surrogate of spatial working memory) after exposure (with no difference in error rate and time to complete the maze). This cognitive dysfunction was preceded by a reduction in cortical methionine synthase activity. However, in a clinical study published recently, no association was found between POCD and nitrous oxide exposure, although it was underpowered for this endpoint.[88] No difference in the acute confusional state, delirium (powered primary endpoint), was found either. Data from our recent study suggest that a neuroinflammatory response to surgery is responsible for POCD in an animal model,[89] which would explain the lack of difference in POCD between different anesthetic techniques. On balance, experimental data do not suggest that the phenomenon of POCD is attributable to nitrous oxide; although anesthesia is still a possible risk factor, other factors, such as age and the surgery undertaken, are more likely to be of importance. However, further studies are warranted because hyperhomocysteinemia, as well as reduced folate and cobalamin levels, has been implicated in the pathogenesis of both vascular and Alzheimer dementia; therefore, a role for nitrous oxide remains biologically plausible, especially with high prevalence of cobalamin deficiency in the elderly.[46] Nonetheless, there are currently no clinical data to suggest that nitrous oxide anesthesia is a significant risk factor for the development of POCD.

Myocardial Effects

Nitrous oxide has been associated with increased myocardial risk in the perioperative period.[90] Badner *et al.*[52] initially studied the effects of nitrous oxide administration on plasma homocysteine levels because the conversion of homocysteine to methionine is methionine synthase dependent; they found a significant increase with nitrous oxide administration. As discussed earlier, certain genotypes with point mutations in the MTHFR gene may be at even greater risk of this increase.[57] Increased homocysteine levels are an independent risk factor for cardiac morbidity, potentially through causing endothelial dysfunction and procoagulation.[91,92] In a study of 90 patients undergoing carotid endarterectomy randomly assigned to isoflurane or isoflurane–nitrous oxide anesthesia, a higher incidence of ischemia with significantly longer ischemic events during the first 24 and 48 h was observed in the nitrous oxide group.[90] Unfortunately, the group did not report on enzyme changes or follow-up of the patients, making the true extent of the problem unclear. Although intraoperative ischemia has been noted previously with nitrous oxide administration, this did not result in cardiac morbidity.[93] In an earlier study by Kozmary *et al.*,[94] no effect on enzyme changes was noted with nitrous oxide, though the study may have been underpowered for this endpoint. It should be noted that although plausible, causation between any increased risk from nitrous oxide and increased homocysteine levels remains unproven. A further exploration by Myles *et al.*[95] (in this issue of ANESTHESIOLOGY) showed a correlation between nitrous oxide exposure, increased homocysteine level, and impaired flow-mediated dilatation of the brachial artery. The duration of nitrous oxide exposure further correlated with this marker of endothelial dysfunction, increasing the likelihood of causation. If hyperhomocysteinemia secondary to nitrous oxide exposure indeed contributes to cardiac toxicity, populations such as the elderly with elevated baseline homocysteine levels[45] or those with certain MTHFR polymorphisms[57] may be particularly at risk. Another explanation is that the group that did not receive nitrous oxide was exposed to higher concentrations of the preconditioning agent isoflurane, and so the study has revealed, clinically, the lack of preconditioning effect of nitrous oxide that has been established in animals.[96]

Contrariwise, nitrous oxide exhibits less cardiodepressant properties than other volatile agents and therefore may improve intraoperative hemodynamic parameters.[8] Further studies are required to elucidate the impact of nitrous oxide–based anesthesia on perioperative cardiac outcomes; however, it is interesting that in the recent ENIGMA study, nitrous oxide was not associated with adverse cardiac outcomes,[5] though it was underpowered for this endpoint. As described in the section titled Patient Exposure Risk, Immune Effects, a follow-up study, the ENIGMA II trial, plans to enroll 7,000 patients to evaluate the risk of nitrous oxide anesthesia in patients with coronary artery disease.

Reproductive Effects

Concerns about the reproductive effects of exposure to anesthetic doses of nitrous oxide were founded in part by preclinical studies concentrating on exposure to concentrations of nitrous oxide greater than 50% for 24 h on day 1 of gestation.[97,98] However, the clinical relevance of these studies remains doubtful because of the extremely long duration of exposure and high doses used. Interestingly, in these settings, halothane prevented the nitrous oxide toxicity, including both fetal loss and neuroanatomical abnormalities[99]; isoflurane also prevented fetal losses but not the skeletal abnormalities.[98] These protective effects of volatile anesthetics have been ascribed to enhanced uterine blood flow; however, it remains unknown whether these effects are important clinically.

Some clinical studies have evaluated the risks associated with general anesthesia during pregnancy, but they are also confounded by the surgery undertaken and the pathology necessitating surgical intervention; furthermore, mixed anesthetic gas exposure was used, thereby precluding evaluation of the teratogenicity solely on the basis of nitrous oxide. Obtaining adequate controls is a problem because it would be unethical to randomly assign patients to an anesthesia-only group. However, the effects of clinical anesthesia itself on fetotoxicity have been evaluated with nonexperimental studies, and the results to date suggest that anesthesia is nontoxic.[100–102] Mazze and Kallen[101] addressed this issue in a large registry study of 5,405 patients and found no association between different methods of anesthesia and adverse outcomes. An earlier study by Duncan *et al.*[102] found no teratogenic effect and no difference in the incidence of spontaneous abortion with controls. Subgroup analysis from this study did show a relative risk associated with certain procedures, such as those performed by obstetricians and gynecologists.[102]

In summary, the fetotoxic effects of anesthetic agents remain obscure and require further investigation, but the lack of appropriate control groups continues to confound research in this area. Available clinical data do not suggest an association between anesthesia and fetotoxicity; prospective studies collecting data on patients undergoing standardized (nonobstetric, nongynecologic) surgical procedures with different anesthetic techniques should be considered to help elucidate the safest anesthetic technique. Preclinical work is also needed to ensure that anesthesia is not associated with more subtle neurodevelopmental effects not investigated in earlier studies.[3,85]

Table 2. Time-weighted Average and Short-Term Peak Concentrations for Nitrous Oxide in Different Countries

Country	TWA/ppm	Short-term Peak/ppm
Australia	25	
Austria	100	400 (15 min)
Canada	25 (Ontario) 50 (Quebec) 100 (Alberta)	200 (Alberta)
Denmark	50	
United Kingdom	100	
Estonia	100	
Finland	100	
Germany	100 50 (local recommendations)	200 (15 min)
The Netherlands	80	
Norway	50	
New Zealand	25	
Spain	50 (daily exposure)	
Sweden	100	500 (15 min)
Switzerland	100	200 (15 min)
South Africa	100	
United States of America	25 (NIOSH) 50 (ACIH, California, Washington)	75 (Washington)

ACIH = American Conference of Industrial Hygienists; NIOSH = National Institute for Occupational Safety and Health; ppm = parts per million; TWA = time-weighted average.

Data supplied by BOC Ltd., Guildford, United Kingdom.

Occupational Exposure Risk

Nitrous oxide is used in many types of medical practice, including anesthesia, pain medicine, obstetrics, emergency medicine, and dentistry. The recognition of the potential problems of occupational exposure of anesthetics on health and performance has led to the introduction of occupational exposure limits (OELs; table 2), which are expressed as an 8-h time-weighted average with which scavenging techniques must comply. In addition, in certain countries, a short-term exposure limit to augment the OEL has been adopted to ensure that concentrations beyond a certain threshold are never experienced, even for short periods. The OEL for nitrous oxide of 100 ppm used in the United Kingdom and Sweden has been challenged by the American Conference of Industrial Hygienists, which has advocated an OEL of 50 ppm. The National Institute for Occupational Safety and Health has suggested an OEL of only 25 ppm.

In the prescavenging era, environmental concentrations of nitrous oxide were routinely 1,000–2,000

ppm.[103] Anesthetic waste gas scavenging has significantly reduced these values in the operating room,[104,105] though a recent study showed peak nitrous oxide concentrations in excess of 1,000 ppm recorded for short periods.[106] Some labor ward environments have proved difficult to scavenge efficiently, resulting in midwifery staff being exposed to nitrous oxide levels that exceed the current OELs,[107,108] although safe levels can be achieved in purpose-built facilities.[109]

Table 3. Confounds of Clinical Studies on Occupational Exposure

Reporter bias
Poor response rates
Inadequate controls
Inconsistent results across studies
Uncontrolled confounders: shift work, physical strain and toxins (such as formalin, mercury, alcohol and smoking)
Controlled confounders: age and parity

Reproductive Effects

Inhibition of methionine synthase by nitrous oxide is dose and duration dependent; therefore, it is plausible that environmental nitrous oxide exposure may induce an occupational risk. The majority of animal studies investigating potential effects on reproduction have used very high doses with prolonged administration, limiting the relevance of their results to the clinical setting. Studies of doses of nitrous oxide that are consistent with occupational exposure have shown increased fetal loss and impaired postnatal development with 1,000 ppm but no effect with 500 ppm or lower[110,111] when administered for the duration of pregnancy. These toxic effects are reduced by folinic acid[99] and methionine,[112] indicating the involvement of the methionine synthase pathway. Interestingly, exposure to nitrous oxide up to 10,000 ppm (1%) throughout gestation does not affect the behavioral development of young rats[113]; again higher doses have an effect,[114,115] but the clinical relevance of this is unclear.

Nitrous oxide–induced fertility problems do not occur at 1,000 ppm or lower in animals,[113] although they have been shown to be present at 5,000 ppm in some[116] but not all studies[117] (though these differences may represent interspecies variation). Thus, there is preclinical evidence that nitrous oxide can induce fetotoxicity and fertility defects through inhibition of methionine synthase, but there is no evidence that this toxicity occurs with concentrations below the OELs. The animal data suggest that doses of 500 ppm are a threshold for this toxicity (*i.e.*, 5 times the United Kingdom's or 10 times the United States of America's OELs). Furthermore, because rats seem more susceptible to nitrous oxide's inhibitory effect on methionine synthase than humans do,[50] the preclinical data suggest that the existing OELs for human exposure are appropriate.

Deriving conclusions from the relevant clinical data is primarily hampered by the retrospective nature of the questionnaire-based evidence, which is therefore confounded by reporter bias. For example, in a large study of occupational exposure, the response rate in the control group was 42%, whereas in the exposed group it was 76%.[118] Most of the occupational exposure studies regarding anesthetics also predate modern scavenging and operating room ventilation. These studies also addressed exposure to all anesthetic gases and not specifically nitrous oxide, and often the anesthetic gas exposure levels were not quantified. Therefore, the relevance of many of the studies to current practice is doubtful (table 3).

For example, in one questionnaire study of midwifery staff in Sweden, 1,125 second-trimester pregnancies were analyzed, with approximately half of mothers exposed to nitrous oxide.[119] Nitrous oxide exposure was associated with higher ORs for low-birth-weight (OR, 3.4; 95% CI, 0.9–3.4) and small-for-gestational-age babies (OR, 3.0; 95% CI, 1.2–7.2). However, there were only 43 cases of low birth weight in the whole data set (because of the small sample size). Moreover, the whole data set showed slightly higher birth weight than the national average. Other important confounders include the participation of those with shift work patterns, the lack of quantification of nitrous oxide exposure, and the wide variation in results.[119] This is important because Axelsson *et al.* found an association of spontaneous abortions with night and shift work (OR, 1.63; 95% CI, 0.95–2.81) but not with nitrous oxide exposure (OR, 0.95; 95% CI, 0.62–1.47) involving 1,717 pregnancies in 3,985 midwives.[120] In this latter study, the nitrous oxide exposure was within OELs.

Boivin[121] performed a meta-analysis of 19 studies completed between 1971 and 1995 and found a relative risk of spontaneous abortion with nitrous oxide exposure of 1.48 (95% CI, 1.4–1.58). When the data were refined to use only the studies with the best design, the relative risk increased to 1.9 (95% CI, 1.72–2.09). However, meta-analysis is always limited by the data it seeks to analyze; specifically in this case, the studies suffered from poor response rates, reporter bias, inadequate controls, and lack of nitrous oxide exposure data. We therefore consider this an inappropriate method to analyze these data. Notably, in the most highly rated study included in this meta-analysis, 10 occupations were surveyed in Denmark.[122] In the subgroup exposed to nitrous oxide (dental assistants), a 94% response rate was noted, and the OR for spontaneous abortion was 1.0 (CI, 0.8–1.2) and therefore the group exposed to nitrous oxide was not at increased risk. In another study, Ericson and Kallen[123] analyzed the official birth registry in Sweden to circumvent the problems associated with reporter bias. While again anesthetic exposure was not quantified, this study design did not reveal any differences in the incidence in perinatal deaths or malformations in anesthesiology/operating room nurses.

In a questionnaire study attempting to address concerns about female fertility, data were sought from 7,000 dental assistants and included an estimation of their exposure to nitrous oxide as well as their time to conception.[124] However, only 69% responded, and analysis was possible on only 459 replies because of exclusion through insufficient data or predefined criteria. From this limited data set, the authors concluded that *unscavenged* nitrous oxide exposure (which they estimated to exceed 1,000 ppm) for greater than 5 h per week was associated with reduced ability to conceive. Exposure to nitrous oxide in *scavenged* clinical settings seemed safe. Follow-up data showed that although exposure to nitrous oxide in an *unscavenged* setting was associated with an increased risk of spontaneous abortion, this was attributable to a group of 20 dental assistants exposed to 5–9 h of unscavenged nitrous oxide per week. Interestingly, no greater risk of spontaneous abortions occurred with *unscavenged* exposure beyond 10 h or after occupational exposure in scavenged settings.[125] With the caveat that these are retrospective questionnaire data, these studies confirm the occupational safety of nitrous oxide in the scavenged workplace.

Therefore, concerns about the reproductive toxicity of occupational exposure to nitrous oxide at levels below the OELs have not been substantiated by the available data, which unfortunately do not include prospective epidemiologic studies. Only data from adequately powered prospective studies with quantified nitrous oxide exposure levels and appropriate control groups will finally settle this issue; until then, it seems prudent to ensure that occupational exposure levels do not exceed the stated OELs.

Genotoxicity

Nitrous oxide–induced inhibition of methionine synthase can interfere with transmethylation reactions, folate metabolism, and thereby purine and pyrimidine production (fig. 3). Therefore, genotoxic effects of nitrous oxide are biologically plausible, and there is some *in vitro* evidence to suggest that volatile anesthetics or nitrous oxide (50% for 72 h) can induce sister chromatid exchange, a marker of genotoxicity.[126] Of course, the extreme dose and duration of exposure limit the translatability of these findings to the clinical context. Indeed, there is little clinical evidence of genotoxicity after occupational exposure to nitrous oxide as a sole agent.[127] More recent studies have focused on mixed anesthetic gas exposure, demonstrating an association between occupational exposure and markers of genotoxicity (sister chromatid exchange, DNA strand breaks, and micronuclei formation) in lymphocytes.[128,129] In a study of 50 physicians (25 anesthesiologists and 25 unexposed controls), occupational exposure to sevoflurane (8.9 ± 5.6 ppm) and nitrous oxide (119 ± 39 ppm) was associated with increased levels of sister chromatid exchange.[130] Two months of leave from the operating room resulted in the sister chromatid exchange levels returning to normal, indicating reversibility in the effect. Although the levels exceeded OELs in this study, an earlier study showed a similar effect of occupational exposure to nitrous oxide and isoflurane even when the levels were below the OELs.[127] Therefore, there is a little evidence to suggest that occupational exposure to nitrous oxide alone induces genotoxic effects; however, when anesthesiologists are exposed to mixed gases (nitrous oxide plus a volatile anesthetic), genotoxic effects are apparent. Again, longitudinal cohort studies, with quantified gas exposure, are required to assess the impact of this occupational exposure on the health of anesthesiology staff.

Neurologic Effects

The effect of occupational exposure on the peripheral nervous system has been studied less intensely, but a questionnaire study (with the previously discussed limitations) of 60,000 dentists and their assistants showed that high exposure (greater than 6 h a week for 10 yr) was associated with neurologic symptoms such as tingling, numbness, and weakness (1.5% *vs.* control rate of 0.4%).[131] However, the unknown incidence of nitrous oxide abuse, lack of scavenging, and responder bias confound interpretation of these data.

A few studies in the 1970s attempted to address the potential for occupational exposure to nitrous oxide (with or without halothane) to affect neurocognitive performance. Exposure to 500 ppm for 4 h was associated with decreased performance on 1 cognitive test, the Digit Span Test, out of 12.[132] The same group also showed an effect at 50 ppm on audiovisual reaction time in one study[133] but not at higher concentrations in others. These unblinded results have not been reproduced.[134,135] Subsequent studies have suggested that somewhere in the region of 5–10% MAC (around 52,000–105,000 ppm) of nitrous oxide is required to affect neurocognitive performance.[135]

Hematologic Toxicity

The hematologic toxic effects occur at analgesic and anesthetic doses and not at levels consistent with occupational exposure. There seems to be little risk from occupational exposure to nitrous oxide, with hematologic abnormalities not apparent below 1,800 ppm,[136] no effect at 860 ppm,[137] and some potential effect on lymphocytes at 10,000 ppm (when associated with halothane[138]), although there are conflicting reports on this issue.[139]

Conclusions

Nitrous oxide's role in anesthesiology has again been challenged by the ENIGMA study; unfortunately, the

Table 4. Recommended Indications and Contraindications for Nitrous Oxide Use in Anesthetic Practice

Indications	Inhalational analgesia/sedation
Absolute contraindications	Known deficiency of enzyme or substrate in methionine synthase pathway
	Potential toxicity from expansion of gas filled space, *e.g.*, emphysema, pneumothorax, middle ear surgery, pneumocephalus, air embolus
	Raised intracranial pressure
Relative contraindications	Pulmonary hypertension
	Prolonged anesthesia(> 6 h)
	First trimester of pregnancy*
	High risk of postoperative nausea and vomiting
Putative relative contraindications (requiring further investigation)	Risk of myocardial ischemia

* Based on the theoretical (but unproven) detrimental effect.

study's design prevents clear conclusions being drawn. We support Dr. Hopf's interpretation that the biologic plausibility for the findings rests primarily with a protective effect of high concentrations of oxygen.[6] However, nitrous oxide administration is not without risk. Inhibition of methionine synthase in vulnerable populations anesthetized with nitrous oxide may induce hematologic and neurologic effects. Associated increases in plasma homocysteine levels have been correlated with increased perioperative myocardial ischemia in patients anesthetized with nitrous oxide. Although perioperative myocardial ischemia is associated with an increase in long-term mortality,[140] no study has followed randomized patients receiving either nitrous oxide or nitrogen to determine whether nitrous oxide has a long-term effect on survival. We hope that the ENIGMA II trial is designed to ascertain this information. The reader should also note that we have focused on nitrous oxide's ability to provoke toxicity; however, nitrous oxide may still have a role in some patients, *e.g.*, nitrous oxide does not provoke malignant hyperthermia like other volatile anesthetic agents and may be useful in patients at risk from this disorder.[141]

Further understanding of the anesthetic and analgesic actions of nitrous oxide will help us to identify clinical situations where it has most benefit. As mentioned previously, its use in the very young[29] or in combination with a GABAergic anesthetic agent[26,33] for *analgesia* may be confounded, and therefore we may not realize its profound analgesic effects in these settings. However, nitrous oxide is used by many pediatric anesthesiologists for gaseous induction where its nonirritant quality and the second gas effect are unique attributes. Although attempts are being made to find alternatives to nitrous oxide in the labor ward, it seems that the routine use of nitrous oxide in this setting will continue for the foreseeable future; in these settings, attention to OELs is important.

At the recommended OELs, there is no conclusive evidence for reproductive, genetic, hematologic, or neurologic occupational toxicity from nitrous oxide exposure, though the potential genotoxicity recorded from mixed anesthetic gas exposure needs further attention. Facilities to ensure adequate scavenging and ventilation are imperative to ensure the occupational health of medical staff. Good prospective epidemiologic evidence is required to fully evaluate the risks of different anesthetics in the modern operating/anesthetic room environment.

Nitrous oxide has been used for 150 yr for analgesia and anesthesia and in the main has proven safe and efficacious. Exclusion from clinical practice is not warranted with the current level of evidence. Nitrous oxide currently has a niche role as an inhalational analgesic and sedative (table 4). Whether the contraindications for nitrous oxide should go beyond effects on expanding gas-filled spaces and increasing intracranial pressure is a source for future research that we heartily endorse. Finally, nitrous oxide is cheap and will continue to be used in the developing world where healthcare resources are more limited; therefore, further investigations are indicated to define the future of this drug in anesthetic and medical practice.

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