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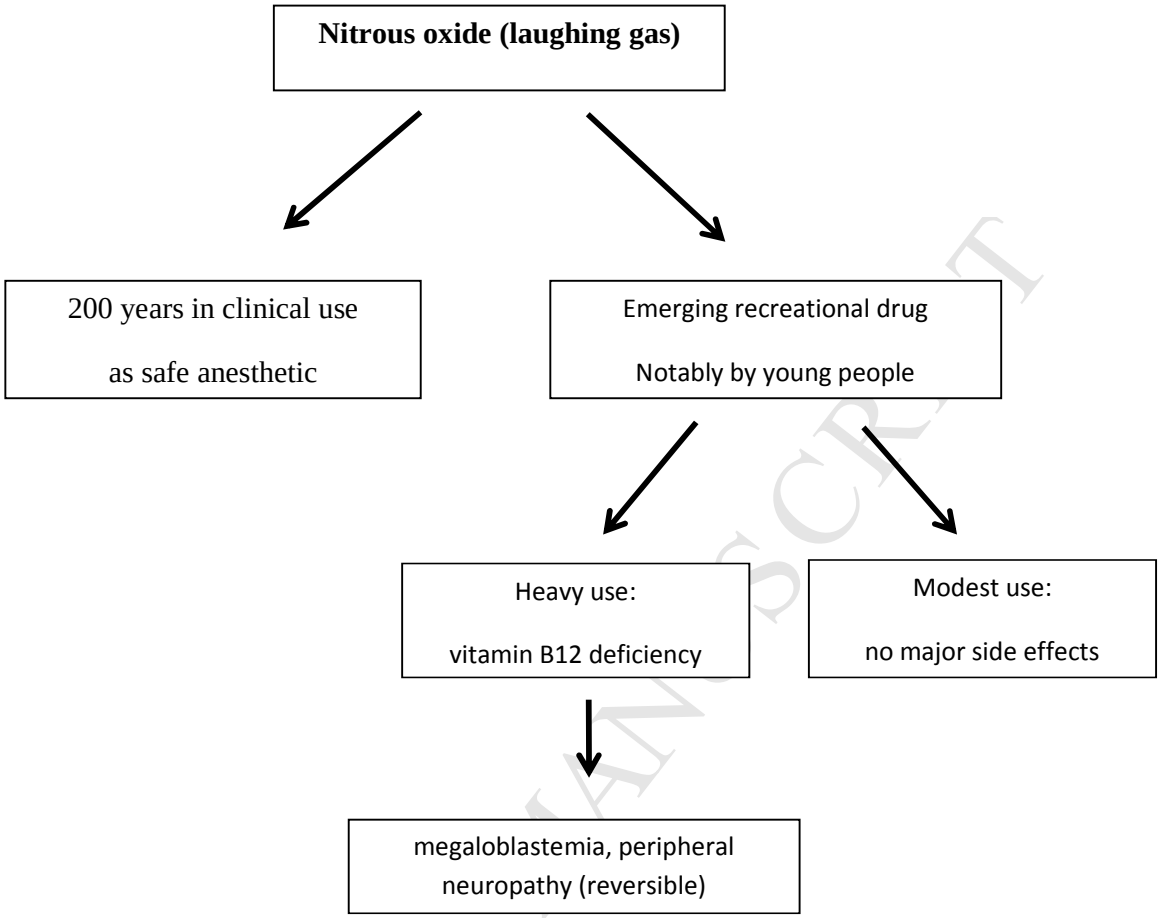

Recreational nitrous oxide use: prevalence and risks

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Abstract

Nitrous oxide (N_2O; laughing gas) is clinically used as a safe anesthetic (dentistry, ambulance, childbirth) and appreciated for its anti-anxiety effect. Since five years, recreational use of N_2O is rapidly increasing especially in the dance and festival scene. In the UK, N_2O is the second most popular recreational drug after cannabis. In most countries, nitrous oxide is a legal drug that is widely available and cheap. Last month prevalence of use among clubbers and ravers ranges between 40 and almost 80 percent. Following one inhalation, mostly from a balloon, a euphoric, pleasant, joyful, empathogenic and sometimes hallucinogenic effect is rapidly induced (within 10 seconds) and disappears within some minutes. Recreational N_2O use is generally moderate with most users taking less than 10 balloons of N_2O per episode and about 80% of the users having less than 10 episodes per year. Side effects of N_2O include transient dizziness, dissociation, disorientation, loss of balance, impaired memory and cognition, and weakness in the legs. When intoxicated accidents like tripping and falling may occur. Some fatal accidents have been reported due to due to asphyxia (hypoxia). Heavy or sustained use of N_2O inactivates vitamin B_{12}, resulting in a functional vitamin B_{12} deficiency and initially causing numbness in fingers, which may further progress to peripheral neuropathy and megaloblastic anemia. N_2O use does not seem to result in dependence. Considering the generally modest use of N_2O and its relative safety, it is not necessary to take legal measures. However, (potential) users should be informed about the risk of vitamin B_{12}-deficiency related neurological and hematological effects associated with heavy use.

Key words: nitrous oxide, laughing gas, bulbs, whippits, peripheral neuropathy, megaloblastoma, recreational use, anesthetic, dependence

Introduction

Nitrous oxide or N_2O, also known as laughing gas, was first synthesized in 1775 by Joseph Priestley. N_2O mixed with 30% oxygen is regularly used as an anesthetic in dental surgery and ambulances, where its short duration of action is an important advantage. Since nitrous oxide is a very stable, chemically inert, and bacteriostatic gas that leaves no taste or odor, it is also widely used in whipped cream charging bottles (steel bulbs containing 10 ml pressurized N_2O). Other applications are found in oil industry to trace leaks and car racing to speed combustion.

Since Victorian times when 'laughing gas parties' were popular, nitrous oxide is also used as an inhalant drug. About two decades ago, N_2O became increasingly known as an inhalant drug, in the scene known as "Hippy Crack", especially in some clubs and music festivals.

Mechanism of action

The precise mechanism of how N_2O induces analgesia or anesthesia is not well understood (Schallner and Goebel, 2013). A number of different receptors have been proposed to mediate the anesthetic effect of N_2O, e.g. dopamine receptors, a_2-adrenergic receptors, benzodiazepine (GABA-A) receptors and glutamatergic N-methyl-D-aspartic acid (NMDA) receptors (Maze and Fujinaga, 2001). The most often proposed mechanism for N_2O-induced anesthesia is inhibition of excitatory glutamatergic neurotransmission via non-competitive inhibition of the NMDA receptors (Sanders et al., 2008, Jevtovic-Todorovic et al., 1998).

With regard to the analgesic effect, rodent studies suggest that N_2O exerts its effects via stimulation of endogenous opioid release in the brain stem (Ohashi et al., 2003). As a result, the opioids stimulate descending noradrenergic inhibitory neurons, modulating inhibition of

pain processing in the spinal cord. Indeed, in humans the N_2O-induced analgesic effect is at least partially blocked by opiate receptor antagonists (Yang et al., 1980).

The mechanism of the euphoric and hallucinogenic effects of N_2O is also not fully known, but these effects are probably due to inhibition of NMDA receptor, i.e. similar to ketamine, another non-competitive NMDA receptor antagonist with anesthetic, antidepressant and hallucinogenic properties.

Medical use

N_2O has a very low solubility in blood and adipose tissue and thus equilibration is rapidly achieved. N_2O has the lowest lipid solubility and the fastest onset of all inhalation agents. N_2O has a long clinical tradition as a relatively safe anesthetic. With a minimum alveolar concentration (the concentration providing anesthesia in 50% of patients) of 105 % (v/v) in oxygen, the potency of N_2O as an inhalation anesthetic is relatively low. In addition to anesthesia, N_2O has been used as an alternative to benzodiazepines to ameliorate craving and withdrawal symptoms from cocaine (Gillman et al., 2006), alcohol (Gillman et al., 2007), nicotine (Daynes and Gillman, 1994), opioids (Gillman and Lichtigfeld, 1985) and cannabis (Daynes and Gillman, 1994). N_2O is also valued for its anxiolytic effects, especially in dental care (Poorsattar, 2010), in adult and pediatric populations (Collins, 2015, Bar-Meir et al., 2006). Although, less effective than epidural analgesia, N_2O is used as an analgesic during labor, because it is safe for the mother, fetus, and neonate, and has no adverse effects on the progress of labor (Rooks, 2011, Likis et al., 2014). Like ketamine, N_2O has recently been proposed for the treatment of patients with treatment-resistant depression with one randomized cross-over study (N=20) showing 20% response in the N_2O condition compared to only 5% in the placebo condition (Nagele et al., 2015).

Recreational use

Nitrous oxide is increasingly popular among recreational drug users for its euphoric effects. It is typically inhaled, sometimes referred to as 'nagging' or "nanging", commonly from bulbs or balloons. The non-refillable steel bulbs (or whippits) contain some 10 ml of nitrous oxide in liquid form under pressure (7-9 bar), which is equivalent to 4 liters of gas under normobaric conditions. For recreational use, the bulbs are mostly released into a balloon using a metal cracker. Larger professional canisters containing 3 to 5 liters of pressurized N_2O are also used to fill balloons. Medical grade nitrous oxide and nitrous oxide in bulbs intended for home use is at least 99% pure, whereas nitrous oxide used in car racing is usually contaminated with toxic hydrogen sulphide and should thus not be consumed. N_2O is inexpensive (5-8 euro for 24 bulbs).

The desired recreational effects include a rush of euphoria, heightened consciousness, disassociation and excitement, which have a rapid onset, peak around one minute after inhaling and then mainly dissipate after two minutes. Users may take many 'hits' over a few hours. However, the number of bulbs inhaled in a session is usually fewer than 5 (Cheng et al., 2013, Ng et al., 2003). However, a small group of heavy users takes 75 to 125 bulbs per session to remain under influence (Sahenk et al., 1978, Garakani et al., 2014). When heavily used, the balloons are filled from larger industrial tanks. In the Global Drug Survey (GDS) – an internet survey in a self-selected sample reporting last year use of N_2O (N=6800) November-December 2014 - overall, 64% used five or less balloons per occasion (Winstock et al., 2015); the number of balloons per session was ≤ 3 (40%), 4-10 (46%), 11-50 (13%), and ≥ 50 (1%). It is, however, difficult to assess the exact quantity of nitrous oxide inhaled, because recreational users will typically inhale a number of small, imprecise volumes from the balloons.

As reported by Advisory Council on the Misuse of Drugs (ACMD) in the UK, the most common method of inhalation among last year users was from a balloon (94%), followed by whipped cream dispensers (5%). Most common sources of N_2O of last year users were friends (60%), followed by the internet (51%), festivals (48%), dealers (14%), head shops (12%), and supermarkets (6%). The most common place of use was at house parties (83%), festivals (74%), at home (50%), at clubs (43%) and at work (2%) (ACMD, 2015). In the GDS internet survey, 37% of the users reported supermarkets as the most common source followed by friends (35%). In the same survey 70% mentioned house parties the most popular place of use followed by festivals (48%), at home (43%) and at clubs (28%) (Winstock et al., 2015). The largest proportion of last year users (78%) used N_2O on less than 10 occasions and only a small minority of users (3%) inhaled N_2O at least weekly (see Table 1 for further details) (Winstock et al., 2015), suggesting that dependence liability is probably low or absent (see later).

In a recent study, Australian regular stimulant users used N_2O on a median of three days in the preceding six months (range 1-130 days); daily use was not reported. Over half (62%) reported using nitrous oxide less than once per month in the preceding six months. Nitrous oxide was nominated by two participants as their drug of choice. Most respondents reported that they used in a heavy session about 10 bulbs (range 0.5-700) (Sindicich and Burns, 2014).

Prevalence of recreational use

Among clubbers in Amsterdam, 71% of Dutch respondents had ever used nitrous oxide (men 75%, women 68%) and 33% had done so in the past month (Nabben et al., 2014). Life time use of nitrous oxide in this group was relatively high among youngsters under twenty (75%) and young adults in their early twenties (78%) compared to other age categories (Nabben et al., 2014). Between 2008 and 2013, N_2O use among clubbers and ravers in The Netherlands

increased 10-fold with a last month prevalence in 2013 of 33% (clubbers 20%, ravers 48%). Another survey among clubbers performed in The Netherland in the same year reported that 40% of the clubbers had ever used N_2O, 26% had done so in the past year and 7% had used N_2O in the last month with most of the users being young adults (Goossens et al., 2014). These figures from The Netherlands are, however, not well comparable, because the respondents to the first survey (Amsterdam) were recruited when going out and the ones responding to the second survey were recruited via internet. On average the respondents of the first survey were visiting clubs more frequently and were using drugs (such as ecstasy) more often than the respondents of the second survey. Finally, a recent study showed that Dutch youngsters in residential care reported a life time prevalence of N_2O that was similar to life time ecstasy use: 14-15 years 18% (ecstasy 9%); 16-17 years 11% (ecstasy 8%) (Benschop et al., 2013).

The latest figures from the worldwide GDS internet survey (November-December 2014) show that 16% of respondents had ever used N_2O and that 7% (6800 people) had done so in the last year (Winstock et al., 2015). Last year prevalence, in Europe ranged from 1% in Portuguese respondents to 24% and 33% in British and Dutch respondents, respectively (GDS, 2015, Winstock et al., 2015). Like in Dutch clubbers, a high past-year prevalence of N_2O use (43%) was reported by regular clubbers in the UK (GDS, 2015). It should be noted, however, that the GDS data are based on a self-selected sample which may results in an over-estimation of prevalence rates.

The most recent Crime Survey for England and Wales (2013/14 CSEW) reported a past year nitrous oxide use by 2% of adults aged 16–59 year and by 8% of 16-24 year olds (375,000; 6% when recorded for the first time in the 2012/13 survey), making N_2O the second most popular recreational drug after cannabis in this population (Home Office, 2014). The use of

N_2O among 330 homosexual men in gay-friendly London clubs was 28% (lifetime use) and 12% (last year use) (Wood et al., 2013, ACMD, 2015).

The National Survey on Drug Use and Health (NSDUH) in the USA estimated that 21% of adolescents that initiated inhalant abuse started with N_2O (OAS, 2009). In the 2015 GDS-survey, last year prevalence in USA responders was 8% (GDS, 2015), whereas the National Survey on Drug Use and Health (NSDUH) from 2013 found that only 4% of a population sample (N=55,160) had ever inhaled nitrous oxide for kicks or to get high (SAMHSA, 2013). Lifetime prevalence of N_2O use in USA adolescents in residential care for antisocial behavior (N= 723; averaged 15.5 years of age) was 16% and last year use 12% (Garland et al., 2009); findings similar to those from the Netherlands for this group.

In 2002, 12% of first-year college students in New Zealand reported incidental or episodic N_2O use, whereas last month' use was 3% (Ng et al., 2003). In the 2013 'Ecstasy and Related Drugs Reporting System' (EDRS) study among regular psychostimulant users in Australia (N=686; mean age 25 years; males 67%), 49% reported lifetime use of nitrous oxide and 25% had used nitrous oxide in the six months before the interview (Sindicich and Burns, 2014).

In summary, there are no data on the use of N_2O in the general population, but N_2O use can be rather high in special populations, including visitors of clubs and festivals and youngsters in residential settings). At least in some countries (e.g. UK, The Netherlands, and Australia) recreational use of N_2O seems to an increasing trend.

Toxicity

Until 1956, when Lassen et al. reported megaloblastic bone-marrow changes following prolonged N_2O exposure (Lassen et al., 1956), N_2O was regarded as completely innocuous and enjoyed the reputation for being the safest general anesthetic (Sund and Berthelsen, 1994). For more than a century it was believed that N_2O was an inert gas and it had been used

without any serious side effect published in literature. This is generally still true as long as nitrous oxide is used for short episodes.

Clinical use

The ENIGMA randomized controlled trial comparing anesthesia with and without N_2O for non-cardiac surgery lasting more than 2 hours showed a borderline relevant increased risk in patients receiving N_2O for myocardial infarction (OR of 1.59; 95% CI: 1.01 to 2.51; p=0.04), but not for stroke or death (Leslie et al., 2011). A recent meta-analysis, however, could not demonstrate robust evidence for the effect of N_2O on cardiovascular complications or mortality in general anesthesia (Imberger et al., 2014).

Nitrous oxide is routinely used in labor and seems not to harm the fetus. However, it has been claimed that anesthesia personnel regularly exposed to anesthetic gases including N_2O show an increased abortion rate, considering that 18 of 31 pregnancies among anesthesiologists exposed to waste anesthetic gases ended in spontaneous abortion (Vaisman, 1967). In the same year, Fink et al. (Fink et al., 1967) showed that nitrous oxide produced adverse reproductive effects in rodents. However, based on a critical review of the available data, a Task Force of the American Society of Anaesthesiologists (ASA, 1999) concluded that 'there are no data suggesting that waste anesthetic gases are a danger to those women who are contemplating pregnancy or who are already pregnant'. Furthermore it was concluded that 'there is no clear evidence that N_2O is mutagenic or teratogenic, or that it produces any organ toxicity'. N_2O is not carcinogenic (O'Donovan and Hammond, 2015).

Due to the low blood:gas partition coefficient of 0.46 (30 times lower than N_2), N_2O displaces nitrogen (and oxygen) from hollow compartments like the lungs (78% nitrogen). When a patient is switched to an anesthetic mixture containing nitrous oxide, the nitrous oxide will enter gas-filled spaces more than 30 times faster than nitrogen can exit the space so that the

volume or pressure in this space will increase. Within obstructed compartments (e.g. bowel tumor, pneumothorax, middle ear with closed Eustachian tube) the pressure can become dangerously high which may result in severe bowel distension, rupture of the tympanic membrane, serous otitis or eye damage due to expanding gas bubbles. Several case studies have reported tympanic rupture following N_2O anesthesia (Ohryn, 1995, Owens et al., 1978, Perreault et al., 1982), but not in recreational users with a narrowed or closed Eustachian tube due to e.g. a common cold.

Accidents in recreational use

The highly pressurized N_2O gas in bubbles, expanding upon release, is extremely cold which, when directly dispensed at the tank tap, can cause hypothermic skin trauma (frost burns) in the mouth, vocal cords and lungs. Occasionally, people try to extend the effects by exhaling into and re-inhale from balloons filled with N_2O. Such maneuvers can easily lead to hypoxia and even asphyxia. N_2O does not induce major respiratory depression, but will inhibit at high concentration (>50%) the normal physiological response to hypoxia. Asphyxia resulting from a bag over the head or opening a tank with nitrous in an enclosed space such as a car (Jay, 2008, Suruda and McGlothlin, 1990, Wagner et al., 1992, Winek et al., 1995) are the main causes of death from recreational nitrous oxide use. Over 30 years, a total of 52 N_2O related fatal cases have been reported due to asphyxiation (Cockery and Schifano, 2015) of which 17 deaths in the UK between 2006-2012 (Cockery et al., 2014). Statistics of the Drug Abuse Warning Network (DAWN) in the USA have shown that only 7 out of 4,678 fatal drug-related cases (0.15%) were associated with N_2O inhalation (Gillman, 1992).

In high dose N_2O becomes, like ketamine, a dissociative anesthetic with less awareness of pain and the environment. Individuals can become dizzy due to lack of oxygen (Sanders et al., 2005), show silly behavior and become disoriented so that accidents like tripping, falling and

collapse are more likely to occur. Concomitant use of alcohol increases this risk. Traffic accidents may occur when N_2O has been used, because driving is impaired of up to 30 min after exposure to N_2O (50% N_2O for 15 min) (Moyes et al., 1979).

Side effects in recreational use

People using subanesthetic concentrations of nitrous oxide (20-30%) may show (Table 2) impaired memory (short-term memory, recall of words), learning difficulties (Zacny et al., 1994a, Ghoneim et al., 1981, Block et al., 1988, Mewaldt et al., 1988), and reduced psychomotor performance (e.g. trail making, reaction time) (Jakovljevic et al., 2012, Duarte et al., 2008). Long-term recall can be impaired even in concentrations as low as 3-15% (Armstrong et al., 1995). However, these unintended cognitive effects gradually subside within 5 minutes post-inhalation (Zacny et al., 1994a).

In 2015, 35% of the N_2O users in the GDS reported hallucinations and/or feeling confused afterwards, 10% reported nausea, 5% reported passing out, and 2% reported falls or injuries (Winstock et al., 2015). Most notably about 5% reported persistent numbness and tingling, lasting days or weeks after their last use of the gas and 30% of them reported related functional impairments in using their phone, walking or typing (Winstock et al., 2015). These symptoms are the first signs of peripheral neuropathy which may progress to more serious symptoms when N_2O use is continued (see below).

Interestingly, 8% reported to be worried about the effects of N_2O on their physical health and 9% was worried about their mental health, which was three-fold higher than the year before (Winstock, 2015, GDS, 2015).

If used chronically i.e. repeatedly, but at very modest dose, N_2O elicits no major side effects. However, the use of high daily doses within a short period or prolonged recreational use of

higher doses of N_2O will lead to vitamin B_{12} deficiency which elicits the neurological signs mentioned below.

Peripheral neuropathy and megaloblastic anemia

Nitrous oxide irreversibly inactivates vitamin B_{12} (cobalamine) by oxidizing the cobalt moiety of the vitamin leading to a functional vitamin B_{12} deficiency. The consequence of vitamin B_{12} is two-fold. First, as vitamin B_{12} inhibits the enzyme methionine synthase, less tetrahydrofolate (THF) and methionine is generated from homocysteine and 5-methyl-tetrahydrofolate (5-methyl-THF). THF is the precursor of thymidine monophosphate (TMP) required for the synthesis of DNA (Figure 2A). In this way, N_2O induced vitamin B_{12} deficiency leads via impaired DNA synthesis to megaloblastic anemia (Nunn, 1987). Secondly, accumulation of methylmalonic acid (MMA) leads via disturbed lipid synthesis (Figure 2B) in demyelization of neurons. The clinical outcome of demyelination and axonal lesions on the peripheral nerves and cervico-thoracic spinal cord is peripheral neuropathy (Richardson, 2010) with numbness in extremities as early symptoms which further progress to acute paralysis of lower limbs, bizarre behavior and delusions. This also explains that acute psychosis following recreational N_2O can be successfully treated with vitamin B_{12} (Wong et al., 2014, Sethi et al., 2006, Garakani et al., 2014). Interference with DNA-synthesis has been shown after as little as 2 hour anesthesia with N_2O (Nunn et al., 1986, Amos et al., 1982). Royston et al. showed that following 3 to 4 hours of nitrous oxide anesthesia (70% in oxygen), methionine synthase activity in liver was zero (Royston et al., 1988), but recovered within 3-4 days via de-novo synthesis of the enzyme (Nunn, 1987). There was, however, a considerable individual variation in the rate of inhibition of methionine synthase, but N_2O exposures of less than 30 minutes are probably harmless (Royston et al., 1988).

In the experiment of Royston et al. (1988) subjects were ventilated during three hours with 70% N_2O. The total volume exchanged during anesthesia (6 L is inhaled and exhaled per minute) was 1080 L of inhalation anesthetic which is equivalent with 750 L of N_2O. From a bulb containing 10 ml of nitrous oxide in liquid form under pressure (7-9 bar) about 4 L of N_2O gas under normobaric conditions is delivered. This comparison implicates that some 180 bulbs must be inhaled within some three days to completely exhaust the vitamin B_{12} capacity. This calculation corroborates the reports of several cases of vitamin B_{12} deficiency (Alt et al., 2011, Brett, 1997, Sethi et al., 2006, Sahenk et al., 1978, Hsu et al., 2012, Alt et al., 2011, Cheng et al., 2013, Miller et al., 2004, Thompson et al., 2015) following repetitive use (50-100 bulbs) of N_2O within three hours or heavy use over prolonged time e.g. more than 10-20 bulbs daily during 10 days (Cartner et al., 2007). For instance, three young people (aged 18-24 years) suffered from a worsening numbness in the limbs and ataxia (impaired coordinated movements of the muscles) in both legs, following recreational use of N_2O since several months. Blood tests established low vitamin B_{12} levels. The diagnosis was degeneration of the spinal cord through abuse of nitrous oxide. Minor effects on the peripheral nervous system following occupational exposure to N_2O have also been reported. A questionnaire study of 60,000 dentists and their assistants showed that high exposure (greater than 6 h a week for 10 yr.) was associated with tingling, numbness, and weakness (1.5% vs. control rate of 0.4%) (Brodsky et al., 1981).

Administration of vitamin B_{12} and cessation of N_2O use quickly leads to improvement of the condition (Lin et al., 2011). Though intramuscular vitamin B_{12} injection is a suitable treatment for nitrous oxide related vitamin B_{12} deficiency (Diamond et al., 2004, Miller et al., 2004, Probasco et al., 2011, Cheng et al., 2013, Richardson, 2010) recovery can take months (Diamond et al., 2004, Stacy et al., 1992). Oral administration of methionine may hasten recovery as this provides an immediate source of the product of the methionine synthase

reaction (Stacy et al., 1992). Parenteral administration of folic acid to restore methionine synthesis is also effective to treat peripheral neuropathy and megaloblastoma (Miller et al., 2004, Butzkueven and King, 2000).

Dependency

Abuse and dependence liability has been a matter of political and scientific debate. Some argued that the gas is known since the 18th century and that abuse was seldom reported (Gillman, 1992). However, others have reported an elevated risk for N_2O misuse, abuse, and dependence in professionals who have ready access to the substance (e.g. hospital staff, dentists, and medical students) (Rosenberg et al., 2015, Blanton, 2006). In the scientific literature, quite a number of cases have been presented suggestive of N_2O-related addiction characteristics, such as psychological dependence and the development of tolerance (gradual increase of nitrous oxide consumption to obtain the same effect).

Like other drugs of abuse, N_2O is self-administrated by animals (Richardson and Shelton, 2015, Wood et al., 1977, Ramsay et al., 2003), and it has positively reinforcing (rewarding) effects for humans. In human studies (Zacny et al., 1994a, Walker and Zacny, 2002, Zacny et al., 1994b), subanesthetic doses of sevoflurane, nitrous oxide, propofol, and ketamine are all associated with feelings of liking and are rated as something the subject “will try again”; they also produce dose-related reinforcement and abuse-related subjective effects. Twelve subjects in an RCT showed individual variation in the degree to which they liked N_2O: eight reported liking the 40% dose (40% in oxygen), one was neutral, and three did not like it (Dohrn et al., 1992). Two years later Zacny et al. showed that the majority of these 12 subjects were either neutral or actually disliked the effects of N_2O (Zacny et al., 1994a); a finding that does not support its abuse potential. The same group, however, showed later that the reinforcing effect of N_2O (10 to 40% in oxygen) was not dose-dependent i.e. the dose liked and the preference

for N_2O vs. placebo varied across subjects. The preferences observed for either N_2O or placebo varied from a monophasic increasing, a monophasic decreasing and a U-shaped to a "flat" (no effects) dose–response relationship (Walker and Zacny, 2003), indicating a considerable within- and between-subject variability in the reinforcing and subjective effects of nitrous oxide (Walker and Zacny, 2001). There is only anecdotal evidence that N_2O induces psychological dependence and cases of (pure) N_2O addiction have not been reported by addiction treatment centers, suggesting that N_2O can hardly be seen as an addictive substance. Furthermore, the rate and amount of N_2O consumption is very modest in most users and abuse of N_2O is limited.

Legal status

Nitrous oxide is a legal drug and relatively easy available. Nitrous oxide can be legally sold for catering and other legitimate reasons, but its sale in gas-filled balloons on festivals and clubs intended for human recreational use is in many countries not allowed. In the UK such practice violates the Medicines Act which has initiated the Medicines and Healthcare Products Regulatory Agency (MHRA) to control the drug's supply under section 52 of the 1968 Medicines Act (ACMD, 2015). Similarly, in the USA it is not illegal to sell or possess nitrous oxide, but the possession of N_2O with the intent to inhale is an offence. In the Netherlands, N_2O is registered as an anesthetic implicating that its sale for non-industrial purposes violates the Medicine Act.

Summary and conclusion

Recreational use of N_2O is emerging in some countries mainly in the club and festival scene. In most cases, N_2O is used very modestly (> 90% use monthly or less) and its use is relative safe. However, neurological and hematological effects may occur following heavy (> 50-100

bulbs per session) or prolonged high dose use due to N_2O induced vitamin B_{12} deficiency. Users should be informed about these potential serious side effect and doctors should be informed about the treatment of these complications. It does not seem necessary to take (further) legal measures to ban the drug.

References

ACMD., 2015. Advisory Council on the Misuse of Drugs (ACMD). Advice on nitrous oxide. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/409403/acmd-advice-on-nitrous-oxide.doc.

Alt, R.S., Morrissey, R.P., Gang, M.A., Hoffman, R.S., Schaumburg, H.H., 2011. Severe myeloneuropathy from acute high-dose nitrous oxide (N2O) abuse. J. Emerg. Med. 41, 378-380.

Amos, R.J., Amess, J.A., Hinds, C.J., Mollin, D.L., 1982. Incidence and pathogenesis of acute megaloblastic bone-marrow change in patients receiving intensive care. Lancet 2, 835-838.

Armstrong, P.J., Morton, C., Sinclair, W., Tiplady, B., 1995. Effects of nitrous oxide on psychological performance. A dose-response study using inhalation of concentrations up to 15%. Psychopharmacology (Berl) 117, 486-490.

ASA., 1999. American Society of Anesthesiologists (ASA). Anesthetic Gases: Information for Management in Anesthetizing Areas and the Postanesthesia Care Unit (PACU). http://ecommerce.asahq.org/publicationsAndServices/wasteanes.pdf.

Bar-Meir, E., Zaslansky, R., Regev, E., Keidan, I., Orenstein, A., Winkler, E., 2006. Nitrous oxide administered by the plastic surgeon for repair of facial lacerations in children in the emergency room. Plast. Reconstr. Surg. 117, 1571-1575.

Benschop, A., Nabben, T., Korf, D.J., 2013. Antenne 2012. Trends in alcohol, tabak en drugs bij jonge Amsterdammers. Amsterdam: Rozenberg Publishers. https://www.jellinek.nl/wp-content/uploads/2013/06/Antenne-2012.pdf.

Blanton, A., 2006. Nitrous oxide abuse: dentistry's unique addiction. J. Tenn. Dent. Assoc. 86, 30-31.

Block, R.I., Ghoneim, M.M., Pathak, D., Kumar, V., Hinrichs, J.V., 1988. Effects of a subanesthetic concentration of nitrous oxide on overt and covert assessments of memory and associative processes. Psychopharmacology (Berl) 96, 324-331.

Brett, A., 1997. Myeloneuropathy from whipped cream bulbs presenting as conversion disorder. Aust. N. Z. J. Psychiatry 31, 131-132.

Brodsky, J.B., Cohen, E.N., Brown, B.W., Jr., Wu, M.L., Whitcher, C.E., 1981. Exposure to nitrous oxide and neurologic disease among dental professionals. Anesth. Analg. 60, 297-301.

Butzkueven, H., King, J.O., 2000. Nitrous oxide myelopathy in an abuser of whipped cream bulbs. J. Clin. Neurosci. 7, 73-75.

Cartner, M., Sinnott, M., Silburn, P., 2007. Paralysis caused by "nagging". Med. J. Aust. 187, 366-367.

Cheng, H.M., Park, J.H., Hernstadt, D., 2013. Subacute combined degeneration of the spinal cord following recreational nitrous oxide use. BMJ Case. Rep. 2013.

Cockery, J., Loi, B., Goodair, C., Schifano, F., 2014. Drug Related Deaths in the UK: Jan-Dec 2012-2013.

Cockery, J. and Schifano, F., 2015. Volatile substance abuse mortality project. http://researchprofiles.herts.ac.uk/portal/en/projects/volatile-substance-abuse-mortality-project%2868e81f06-a692-47d3-a9c3-96b5a93ce34f%29.html.

Collins, M., 2015. A case report on the anxiolytic properties of nitrous oxide during labor. J. Obstet. Gynecol. Neonatal Nurs. 44, 87-92.

Daynes, G., Gillman, M.A., 1994. Psychotropic analgesic nitrous oxide prevents craving after withdrawal for alcohol, cannabis and tobacco. Int. J. Neurosci. 76, 13-16.

Diamond, A.L., Diamond, R., Freedman, S.M., Thomas, F.P., 2004. "Whippets"-induced cobalamin deficiency manifesting as cervical myelopathy. J. Neuroimaging 14, 277-280.

Dohrn, C.S., Lichtor, J.L., Finn, R.S., Uitvlugt, A., Coalson, D.W., Rupani, G., de, W.H., Zacny, J.P., 1992. Subjective and psychomotor effects of nitrous oxide in healthy volunteers. Behav. Pharmacol. 3, 19-30.

Duarte, R., McNeill, A., Drummond, G., Tiplady, B., 2008. Comparison of the sedative, cognitive, and analgesic effects of nitrous oxide, sevoflurane, and ethanol. Br. J. Anaesth. 100, 203-210.

Fink, B.R., Shepard, T.H., Blandau, R.J., 1967. Teratogenic activity of nitrous oxide. Nature 214, 146-148.

Garakani, A., Welch, A.K., Jaffe, R.J., Protin, C.A., McDowell, D.M., 2014. Psychosis and low cyanocobalamin in a patient abusing nitrous oxide and cannabis. Psychosomatics 55, 715-719.

Garland, E.L., Howard, M.O., Perron, B.E., 2009. Nitrous oxide inhalation among adolescents: prevalence, correlates, and co-occurrence with volatile solvent inhalation. J. Psychoactive Drugs 41, 337-347.

GDS., 2015. The Global Drug Survey (GDS) 2014 findings. Last 12 month prevalence of top 20 drugs. http://www.globaldrugsurvey.com/wp-content/uploads/2014/04/last-12-months-drug-prevalence.pdf.

Ghoneim, M.M., Mewaldt, S.P., Peterson, R.C., 1981. Subanesthetic concentration of nitrous oxide and human memory. Prog Neuro-psychopharmacol 5, 395-402.

Gillman, M.A., 1992. Nitrous oxide abuse in perspective. Clin. Neuropharmacol. 15, 297-306.

Gillman, M.A., Lichtigfeld, F.J., 1985. Analgesic nitrous oxide: adjunct to clonidine for opioid withdrawal. Am. J. Psychiatry 142, 784-785.

Gillman, M.A., Lichtigfeld, F.J., Harker, N., 2006. Psychotropic analgesic nitrous oxide for acute cocaine withdrawal in man. Int. J. Neurosci. 116, 847-857.

Gillman, M.A., Lichtigfeld, F.J., Young, T.N., 2007. Psychotropic analgesic nitrous oxide for alcoholic withdrawal states. Cochrane. Database. Syst. Rev. CD005190.

Goossens, F.X., Frijns, T., Hasselt, N.E., van Laar, M.W., 2014. Het grote uitgaansonderzoek 2013. Uitgaanspatronen, middelengebruik en risicogedrag onder uitgaande jongeren en jongvolwassenen. Utrecht: Trimbos-instituut.

Home Office., 2014. Drug Misuse: Findings from the 2013/14 Crime Survey for England and Wales. www.gov.uk: Government, ONS; 2014 15/08/2014].

Hsu, C.K., Chen, Y.Q., Lung, V.Z., His, S.C., Lo, H.C., Shyu, H.Y., 2012. Myelopathy and polyneuropathy caused by nitrous oxide toxicity: a case report. Am. J. Emerg. Med. 30, 1016.

Imberger, G., Orr, A., Thorlund, K., Wetterslev, J., Myles, P., Moller, A.M., 2014. Does anaesthesia with nitrous oxide affect mortality or cardiovascular morbidity? A systematic review with meta-analysis and trial sequential analysis. Br. J. Anaesth. 112, 410-426.

Jakovljevic, M., Vidmar, G., Mekjavic, I.B., 2012. Psychomotor function during mild narcosis induced by subanesthetic level of nitrous oxide: individual susceptibility beyond gender effect. Undersea Hyperb. Med. 39, 1067-1074.

Jay, M., 2008. Nitrous oxide: recreational use, regulation and harm reduction. Drugs and Alcohol Today 8, 22-25.

Jevtovic-Todorovic, V., Todorovic, S.M., Mennerick, S., Powell, S., Dikranian, K., Benshoff, N., Zorumski, C.F., Olney, J.W., 1998. Nitrous oxide (laughing gas) is an NMDA antagonist, neuroprotectant and neurotoxin. Nat. Med. 4, 460-463.

Lassen, H.C., Henriksen, E., Neukirch, F., Kristensen, H.S., 1956. Treatment of tetanus; severe bone-marrow depression after prolonged nitrous-oxide anaesthesia. Lancet 270, 527-530.

Leslie, K., Myles, P.S., Chan, M.T., Forbes, A., Paech, M.J., Peyton, P., Silbert, B.S., Williamson, E., 2011. Nitrous oxide and long-term morbidity and mortality in the ENIGMA trial. Anesth. Analg. 112, 387-393.

Likis, F.E., Andrews, J.C., Collins, M.R., Lewis, R.M., Seroogy, J.J., Starr, S.A., Walden, R.R., McPheeters, M.L., 2014. Nitrous oxide for the management of labor pain: a systematic review. Anesth. Analg. 118, 153-167.

Lin, R.J., Chen, H.F., Chang, Y.C., Su, J.J., 2011. Subacute combined degeneration caused by nitrous oxide intoxication: case reports. Acta Neurol. Taiwan. 20, 129-137.

Maze, M. and Fujinaga, M., 2001. Pharmacology of nitrous oxide. Best Practice Res Clin Anaesthesiol 15, 339-348.

Mewaldt, S.P., Ghoneim, M.M., Choi, W.W., Korttila, K., Peterson, R.C., 1988. Nitrous oxide and human state-dependent memory. Pharmacol. Biochem. Behav. 30, 83-87.

Miller, M.A., Martinez, V., McCarthy, R., Patel, M.M., 2004. Nitrous oxide "whippit" abuse presenting as clinical B12 deficiency and ataxia. Am. J. Emerg. Med. 22, 124.

Moyes, D., Cleaton-Jones, P., Lelliot, J., 1979. Evaluation of driving skills after brief exposure to nitrous oxide. S. Afr. Med. J. 56, 1000-1002.

Nabben, T., Benschop, A., Korf, D.J., 2014. ANTENNE 2013, trends in alcohol,tabak en drugs bij jonge Amsterdammers. Amsterdam: Rozenberg.

Nagele, P., Duma, A., Kopec, M., Gebara, M.A., Parsoei, A., Walker, M., Janski, A., Panagopoulos, V.N., Cristancho, P., Miller, J.P., Zorumski, C.F., Conway, C.R., 2015. Nitrous Oxide for Treatment-Resistant Major Depression: A Proof-of-Concept Trial. Biol. Psychiatry 78, 10-18.

Ng, J., O'Grady, G., Pettit, T., Frith, R., 2003. Nitrous oxide use in first-year students at Auckland University. Lancet 361, 1349-1350.

Nunn, J.F., 1987. Clinical aspects of the interaction between nitrous oxide and vitamin B12. Br. J. Anaesth. 59, 3-13.

Nunn, J.F., Chanarin, I., Tanner, A.G., Owen, E.R., 1986. Megaloblastic bone marrow changes after repeated nitrous oxide anaesthesia. Reversal with folinic acid. Br. J. Anaesth. 58, 1469-1470.

O'Donovan, M.R., Hammond, T.G., 2015. Is nitrous oxide a genotoxic carcinogen? Mutagenesis 30, 459-462.

OAS., 2009. Office of Applied Studies (OAS). The NSDUH Report: Trends in Adolescent Inhalant Use: 2002 to 2007.

Ohashi, Y., Guo, T., Orii, R., Maze, M., Fujinaga, M., 2003. Brain stem opioidergic and GABAergic neurons mediate the antinociceptive effect of nitrous oxide in Fischer rats. Anesthesiology 99, 947-954.

Ohryn, M., 1995. Tympanic membrane rupture following general anesthesia with nitrous oxide: a case report. AANA. J. 63, 42-44.

Owens, W.D., Gustave, F., Sclaroff, A., 1978. Tympanic membrane rupture with nitrous oxide anesthesia. Anesth. Analg. 57, 283-286.

Perreault, L., Normandin, N., Plamondon, L., Blain, R., Rousseau, P., Girard, M., Forget, G., 1982. Tympanic membrane rupture after anesthesia with nitrous oxide. Anesthesiology 57, 325-326.

Poorsattar, S.P., 2010. Recognizing and managing dental fears: anxiolysis from the perspective of a dental student. J. Dent. Educ. 74, 397-401.

Probasco, J.C., Felling, R.J., Carson, J.T., Dorsey, E.R., Niessen, T.M., 2011. Teaching NeuroImages: myelopathy due to B(1)(2) deficiency in long-term colchicine treatment and nitrous oxide misuse. Neurology 77, e51.

Ramsay, D.S., Watson, C.H., Leroux, B.G., Prall, C.W., Kaiyala, K.J., 2003. Conditioned place aversion and self-administration of nitrous oxide in rats. Pharmacol. Biochem. Behav. 74, 623-633.

Richardson, K.J., Shelton, K.L., 2015. N-methyl-D-aspartate receptor channel blocker-like discriminative stimulus effects of nitrous oxide gas. J. Pharmacol. Exp. Ther. 352, 156-165.

Richardson, P.G., 2010. Peripheral neuropathy following nitrous oxide abuse. Emerg. Med. Australas. 22, 88-90.

Rooks, J.P., 2011. Safety and risks of nitrous oxide labor analgesia: a review. J. Midwifery Womens Health 56, 557-565.

Rosenberg, H., Orkin, F.K., Springstead, J., 2015. Abuse of nitrous oxide. Anesthesia & Analgesia 58, 104-106.

Royston, B.D., Nunn, J.F., Weinbren, H.K., Royston, D., Cormack, R.S., 1988. Rate of inactivation of human and rodent hepatic methionine synthase by nitrous oxide. Anesthesiology 68, 213-216.

Sahenk, Z., Mendell, J.R., Couri, D., Nachtman, J., 1978. Polyneuropathy from inhalation of N2O cartridges through a whipped-cream dispenser. Neurology 28, 485-487.

SAMHSA., 2013. Substance Abuse and Mental Health Services Administration (SAMHSA). National Survey on Drug Use and Health.

https://www.icpsr.umich.edu/icpsrweb/SAMHDA/ssvd/studies/35509/datasets/0001/variables/NITOXID?q=NITOXID&series=National+Survey+on+Drug+Use+and+Health+%28NSDUH%29+Series&paging.startRow=26.

Sanders, R.D., Ma, D., Maze, M., 2005. Anaesthesia induced neuroprotection. Best. Pract. Res. Clin. Anaesthesiol. 19, 461-474.

Sanders, R.D., Weimann, J., Maze, M., 2008. Biologic effects of nitrous oxide: a mechanistic and toxicologic review. Anesthesiology 109, 707-722.

Schallner, N., Goebel, U., 2013. The perioperative use of nitrous oxide: renaissance of an old gas or funeral of an ancient relict? Curr. Opin. Anaesthesiol. 26, 354-360.

Sethi, N.K., Mullin, P., Torgovnick, J., Capasso, G., 2006. Nitrous oxide "whippit" abuse presenting with cobalamin responsive psychosis. J. Med. Toxicol. 2, 71-74.

Sindicich, N. and Burns, L., 2014. Australian Trends in Ecstasy and related Drug Markets 2013. Findings from the Ecstasy and Related Drugs Reporting System (EDRS). Australian Drug Trends Series No. 118. Sydney, National Drug and Alcohol Research Centre, UNSW Australia. https://ndarc.med.unsw.edu.au/resource/ecstasy-and-related-drugs-reporting-system-edrs-national-report-2013.

Stacy, C.B., Di, R.A., Gould, R.J., 1992. Methionine in the treatment of nitrous-oxide-induced neuropathy and myeloneuropathy. J. Neurol. 239, 401-403.

Sund, K.H., Berthelsen, P.G., 1994. Risus sardonicus and laughing gas--when nitrous oxide lost its innocence. Acta Anaesthesiol. Scand. 38, 751-752.

Suruda, A.J., McGlothlin, J.D., 1990. Fatal abuse of nitrous oxide in the workplace. J. Occup. Med. 32, 682-684.

Thompson, A.G., Leite, M.I., Lunn, M.P., Bennett, D.L., 2015. Whippits, nitrous oxide and the dangers of legal highs. Pract. Neurol. 15, 207-209.

Vaisman, A.I., 1967. [Working conditions in the operating room and their effect on the health of anesthetists]. Eksp. Khir. Anesteziol. 12, 44-49.

Wagner, S.A., Clark, M.A., Wesche, D.L., Doedens, D.J., Lloyd, A.W., 1992. Asphyxial deaths from the recreational use of nitrous oxide. J. Forensic Sci. 37, 1008-1015.

Walker, D.J., Zacny, J.P., 2001. Within- and between-subject variability in the reinforcing and subjective effects of nitrous oxide in healthy volunteers. Drug Alcohol Depend. 64, 85-96.

Walker, D.J., Zacny, J.P., 2002. Analysis of the reinforcing and subjective effects of different doses of nitrous oxide using a free-choice procedure. Drug Alcohol Depend. 66, 93-103.

Walker, D.J., Zacny, J.P., 2003. Bitonic dose-response functions for reinforcing and self-reported effects of nitrous oxide in humans. Pharmacol. Biochem. Behav. 74, 851-857.

Winek, C.L., Wahba, W.W., Rozin, L., 1995. Accidental death by nitrous oxide inhalation. Forensic Sci. Int. 73, 139-141.

Winstock, A., Ferris, J., Kaar, S., 2015. GDS 2015 findings. Data about nitrous oxide presented by A.R. Winstock on YouTube. https://www.youtube.com/watch?v=T1i0a1onUhY.

Winstock, A.R., 2015. The Global Drug Survey 2015 findings. What did we learn from GDS2015? An overview of our key findings. http://www.globaldrugsurvey.com/the-global-drug-survey-2015-findings/.

Wong, S.L., Harrison, R., Mattman, A., Hsiung, G.Y., 2014. Nitrous Oxide (N2O)-Induced Acute Psychosis. Can. J. Neurol. Sci. 41, 672-674.

Wood, D.M., Measham, F., Dargan, P.I., 2013. Pattern of nitrous oxide use in a men who have sex with men, high-drug using population: how does this compare to the 2011/2012 Global Drug Survey? 2013 Annual Meeting of the North American Congress of Clinical Toxicology (NACCT). Clin Toxicol (Phila) 51, 575-724.

Wood, R.W., Grubman, J., Weiss, B., 1977. Nitrous oxide self-administration by the squirrel monkey. J. Pharmacol. Exp. Ther. 202, 491-499.

Yang, J.C., Clark, W.C., Ngai, S.H., 1980. Antagonism of nitrous oxide analgesia by naloxone in man. Anesthesiology 52, 414-417.

Zacny, J.P., Lichtor, J.L., Coalson, D.W., Apfelbaum, J.L., Flemming, D., Foster, V., 1994a. Time course of effects of brief inhalations of nitrous oxide in normal volunteers. Addiction 89, 831-839.

Zacny, J.P., Sparacino, G., Hoffmann, P., Martin, R., Lichtor, J.L., 1994b. The subjective, behavioral and cognitive effects of subanesthetic concentrations of isoflurane and nitrous oxide in healthy volunteers. Psychopharmacology (Berl) 114, 409-416.

Legends to figures

Figure 1.

Bulbs or whippits commercially available and used in whipped cream charging bottles.

Figure 2.

Panel A. Inactivation of vitamin B_{12} inhibits successively: (1) methionine synthase, (2) the generation of tetrahydrofolate (THF) and methionine, (3) the generation of SAM and 10-formyl-THF, (4) DNA synthesis and (5) the formation of red blood cells. If this routing is defect, the clinical outcome is megaloblastic anemia. SAM: S-adenosylmethionine; THF: tetrahydrofolate. Panel B. * In fact, the co-enzyme involved in this conversion is a*denosyl-cobalamin,* an active form of vitamin B_{12}. Inhibition of this route successively results in: (1) accumulation of methylmalonic acid (MMA), (2) disturbed lipid synthesis, and (3) demyelization of neurons. The clinical outcome is peripheral neuropathy.

Table 1. Last year pattern of use in last year users of N_2O (Winstock et al., 2015).

Number of episodes of use	**Percent**	**Frequency of use**	**Percent**
Less than 10	78	Once or twice	58
Just once	27	Every couple of months	23
51 to 100 occasions	2.3	At least weekly	3.2
More than 100 occasions	0.8	Monthly or less	> 91

Table 2. Intended and unintended (unwanted/ side) effects during recreational N_2O use.

Intended effects	**Unintended/side effects**
Euphoria, pleasant, joyful and empathic, pulsating auditory and visual hallucinations and enhanced experience of other psychedelics, giggling and laughing, deep 'silly' voice (opposite of helium), less pain and less anxiety.	Dizziness, dissociation, disorientation (both spatial and time-based), blurred vision, loss of balance, weakness in the legs, impotence, numbness in fingers, clumsiness of hands, nausea, tight chest after heavy use, headache, vomiting, impaired memory, cognition and psychomotor performance, and learning problems.

Figure 1.

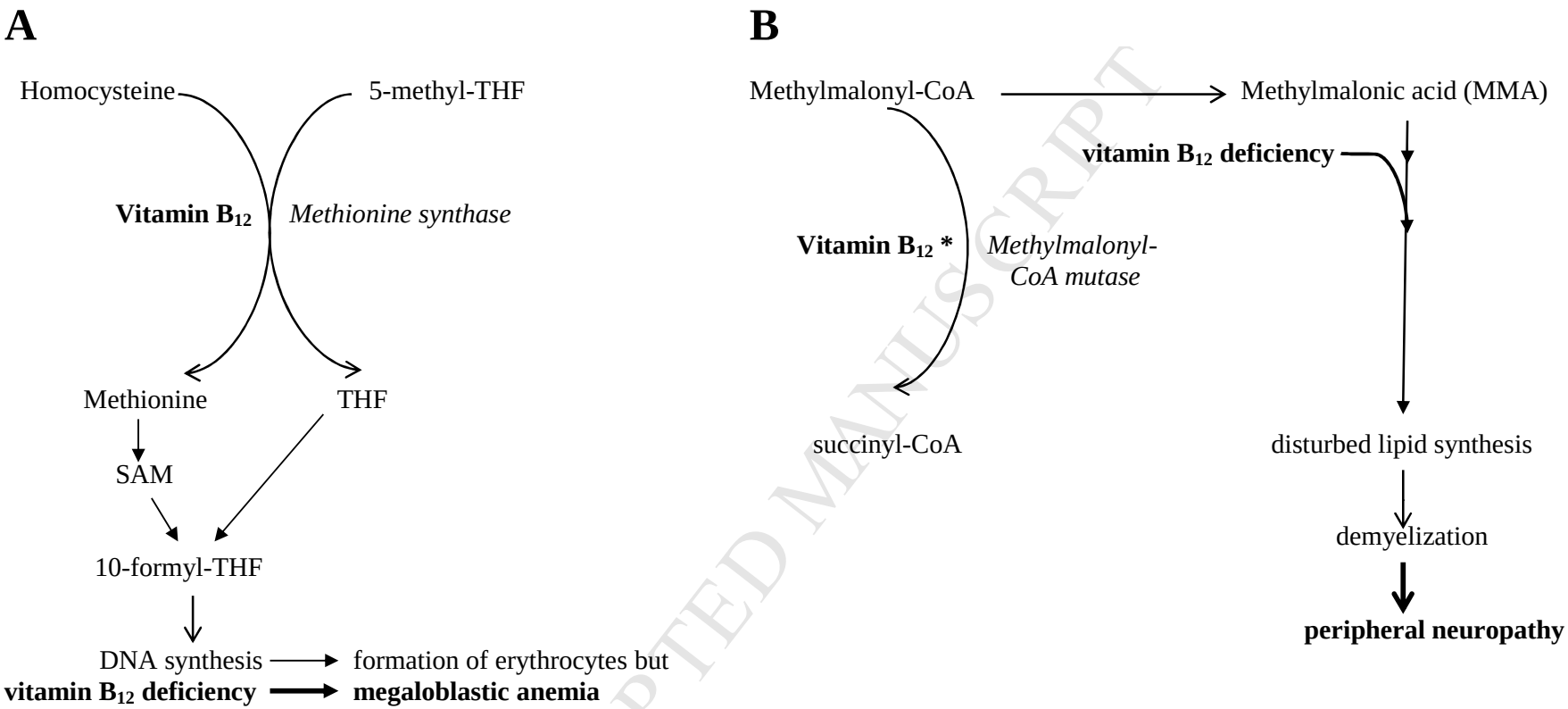

Figure 2.

Highlights

- Recreational use of nitrous oxide is emerging
- No major side effects in moderate use
- Heavy use induces vitamin B12 deficiency leading to peripheral neuropathy
- Early sign of peripheral neuropathy is numbness in fingers
- Users are advised to consult physician when these signs appear