European Monitoring Centre
for Drugs and Drug Addiction

Recreational use of nitrous oxide: a growing concern for Europe



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Methods and information sources

This review is based on information from the scientific and medical literature. Initial searches were developed around the following terms and areas: nitrous oxide, laughing gas, N_2O, chemistry, analytical methods, manufacture, pharmacology, pharmacodynamics, pharmacokinetics, toxicology, recreational, dependence, abuse, addiction, epidemiology, behaviour, health risks and social risks. PubMed was used as the main literature database for retrieving information; Web of Science was also used. Results from searches were screened for relevance and selected publications were reviewed. Additional articles were identified from a review of the references cited in retrieved publications. Selected medical specialty societies and international, national and local government agency websites were searched to identify relevant grey literature. When preparing the different sections of the review, further searches were performed using additional terms to identify additional information. As well as this, colleagues within our scientific network were contacted to obtain further information.

For the case study of the Netherlands, public data available within the national early warning system network was used as well.

In addition, selected national focal points provided case studies on the situation with nitrous oxide at the national level based on the information collected within their national networks.

For the case study of the United Kingdom, open-source information published in the scientific and medical literature were used alongside grey literature, such as public government reports.

Preface

Since around 2010, some countries in Europe have seen an increase in the recreational use of nitrous oxide. This has become a particular concern from around 2017, as both supply and use of the gas have increased. In part, this is linked to the recent availability of larger cylinders of the gas that deliberately target the recreational market — making nitrous oxide significantly cheaper and promoting broader, more regular and heavier use. Most use is in young people, including teenagers.

As the number of people using nitrous oxide has grown, so too has the number of poisonings. While still relatively small in number, they typically involve neurotoxicity from more frequent or heavier use of the gas. Other concerns include severe burns and lung injuries, typically caused by larger cylinders. Meanwhile, car accidents involving the gas have also significantly increased in at least one country.

The purpose of this report is to examine the current situation, risks and responses to the recreational use of nitrous oxide in Europe. To support this, the report also provides a state-of-the-art review of the chemistry, pharmacology and toxicology of the gas. It is intended for policy makers and practitioners.

The report is structured as follows.

- Section 1 provides a summary of the current situation, established risks and responses.
- Section 2 is a technical review of the chemistry, pharmacology, toxicology, individual risks and legitimate uses.
- Section 3 provides an overview of the epidemiology and social risks. In addition, it includes detailed country case studies from Denmark, France, Ireland, Lithuania, the Netherlands and Portugal. A case study is also provided on the United Kingdom, where the use of nitrous oxide has been established in young people for a longer period, and these experiences, including the response, may be helpful in informing responses in other countries (even though the UK is no longer a member of the EMCDDA, having left the EU on 31 December 2020).

Section 1
Recreational nitrous oxide use in Europe: situation, risks, responses

Background

For almost 250 years, nitrous oxide, commonly known as laughing gas, has been inhaled for its rapid but short-lived feelings of euphoria, relaxation, calmness and a sense of detachment.

Despite this long-standing use, the popularity of the gas has varied greatly, with perhaps three notable periods of interest as a recreational drug.

The first was shortly after its discovery in 1772, when it was used by the British upper class at 'laughing gas parties' and as a source of amusement at fairs and music halls.

The second started in the late 1960s. An important driver was the growing use of the gas in dentistry to relieve pain and reduce anxiety, which spilled out into the emerging drug cultures. This is perhaps best documented in the United States. Only a few people used the gas, as it was relatively difficult to obtain in anything other than large cylinders. It was mostly inhaled from a face mask or after filling a plastic bag that was placed over the head — a cumbersome and dangerous way of using that may also have played a role in limiting its initial spread. Soon, injures from this method led to the use of party balloons that were filled from cylinders, making nitrous oxide easier to use and more appealing.

By the early 1970s, it was being used by university students and at music festivals for 25 cents a balloon. Students sourced it from cylinders, cans of whipped cream, or — perhaps most importantly — small cartridges of the gas that were intended to be used to make whipped cream but were obtained from head shops. A study of around 500 medical and dental students at one US university between 1976 and 1978 found that 16 % (84) had used nitrous oxide recreationally. Of these, around 30 % had used cylinders and almost 50 % had used whipped cream cans or cartridges.

Since then, the use of nitrous oxide bubbled away in the background as a 'cheap' legal high. The occasional report of injury or death — usually caused by asphyxiation as a result of using a face mask or plastic bag — sometimes led to concerns and discussions about regulatory responses.

The third period began in around 2010, although its origins likely date to the 1990s, where interest was (re)kindled first in the party scene and at raves, and then at music festivals and clubs.

What distinguishes the current period from the first two is that nitrous oxide is now widely available. It is cheap and easier to buy and to use. Key to its growing popularity has been the widespread availability of the small, inexpensive cartridges of nitrous oxide used to make whipped cream. These are used to fill party balloons, from which the gas is then inhaled. There is also a perception that nitrous oxide is safe. It is easy to buy the cartridges from legitimate sources, including convenience stores, supermarkets and online suppliers. In addition, a profitable and expanding supply chain has developed, with specialised internet stores directly promoting the gas for its recreational use or offering it under the guise of its use to make whipped cream. It is also the availability of cartridges in large quantities that is responsible for increase in use. In some areas, social media plays an important role in advertising and selling the drug. Use by more young people, including teenagers inexperienced with drug use, also characterises this current period.

Most users inhale small quantities of nitrous oxide occasionally, perhaps one to three balloons in a session, a few times a year. Although it is not possible to define a 'safe' level of use, and this kind of consumption will not be risk free, it appears to pose limited health risks in comparison with more intensive patters of use. There is also a small, but significant, increase in the number of people who use greater quantities of the gas more frequently and for longer periods of time. Some develop problematic use as a result. The short-lived effects of the gas are often cited as a reason for further use in the same session. It is unclear what dose causes chronic toxicity, although the greater the amount used, the greater the risk. Most cases of poisoning involve regular or heavy use, at least over a few months (see box on page 7).

Defining frequent and heavy use of nitrous oxide

Categorising the different levels of use of nitrous oxide is difficult. In response to an increase in poisonings, attempts have been made to distinguish between 'regular use', 'heavy use' (or chronic use), or 'occasional'.

The Dutch Poison Centre classes 'heavy use' as the use of 50 or more balloons in a single session or use from a cylinder, while 'frequent use' is classed based on information from the treating physician and patient history, such as 'uses frequently', 'daily use' or 'weekly use'. If the frequency of use is unknown, this may be classed as 'not heavy' or 'not frequent' to avoid overestimation. Similarly, the Danish Poison Centre classes 'heavy use' as the use of more than 50 balloons in one session and classes 'frequent use' if mentioned by the doctor or patient.

This increased use became a particular concern from around 2017-18. Greater visibility and awareness is partly responsible for this. Suppliers also started selling larger cylinders of the gas, deliberately targeting the recreational market — making the gas significantly cheaper and promoting broader, more regular and heavier use.

Mirroring this is a small but significant increase in reports of poisonings to poison centres. In Denmark, cases rose from 16 in 2015 to 62 in 2019, 90 in 2020 and 73 in 2021. In France, 134 cases were reported in 2020 — up from 46 in 2019 and 10 in 2017. Meanwhile, in the Netherlands, cases rose from 13 in 2015 to 128 in 2019, 144 in 2020 and 98 in 2021.

More generally, the renewed interest in nitrous oxide in Europe and elsewhere has led to an increase in cases reported in the medical literature (Figure 1).

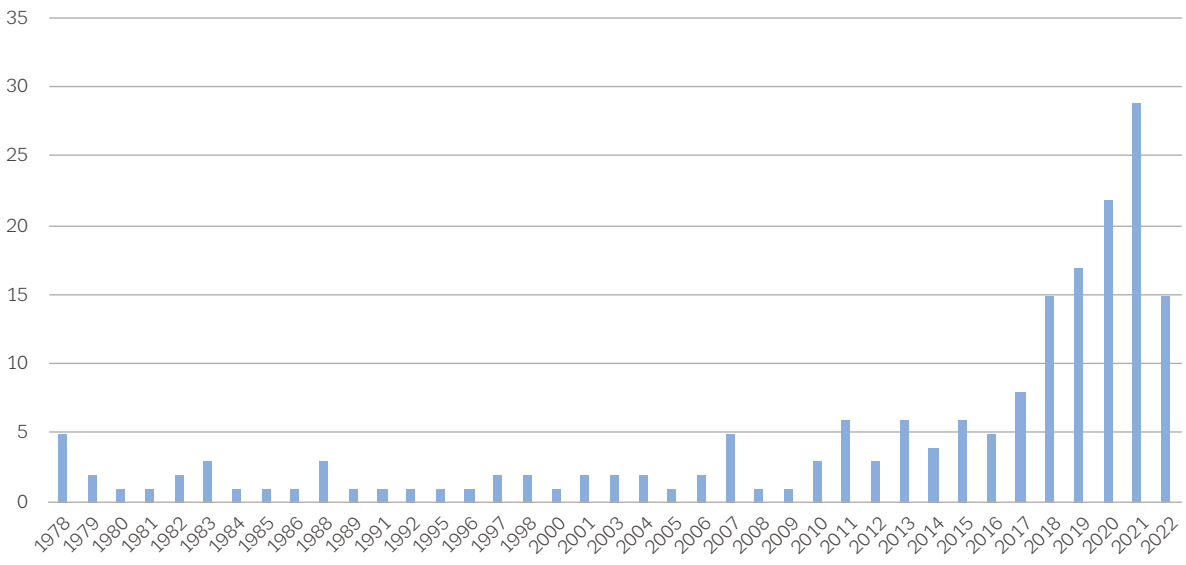
In both cases, many reports are of neurotoxicity, often related to regular or heavier use. Large cylinders can also cause severe frostbite (burns caused by exposure to freezing) and lung injuries due to their high pressure. In addition, car accidents caused by driving while either intoxicated or trying to fill balloons have significantly increased, at least in the Netherlands. Littering of used cartridges and balloons has also been highlighted as an issue.

Despite this, our understanding of use, risks and effective responses is limited, partly because this level of recreational use is relatively new.

Finally, nitrous oxide is a potent greenhouse gas and major cause of the destruction of the ozone layer. The contribution from recreational use is minor compared with other sources, but requires research.

FIGURE 1

Number of reports related to serious harms involving nitrous oxide use in the PubMed database, 1978-2022 (August). Increased awareness of the chronic harms of nitrous oxide may have contributed to this increase



Situation and risks

Use and acute effects

Most people get their nitrous oxide from small cartridges of the gas called 'whipped cream chargers'. Other English names for the cartridges include 'canisters', 'bulbs' and 'whippets' (after one of the original major brand names from the 1930s) (Figure 2). These are small, pressurised metal cartridges containing 8 grams of liquid nitrous oxide that release around 4 litres of gas when opened. They are intended for use with whipped cream dispensers in the home and by the food industry to make whipped cream, desserts and foams, as well as to infuse flavours into drinks.

For recreational use, the cartridges are opened using an empty whipped cream dispenser. This has a holder for the cartridge, and screwing the holder into the dispenser causes a sharp pin to pierce the foil cap at the narrow end of the cartridge, releasing the gas into the dispenser (causing a hissing sound). A balloon is placed over the end of the nozzle of the dispenser. Pressing the lever of the dispenser releases the gas into the balloon. The gas is then inhaled from the balloon, and either exhaled directly to the air or rebreathed into the balloon for extra effect.

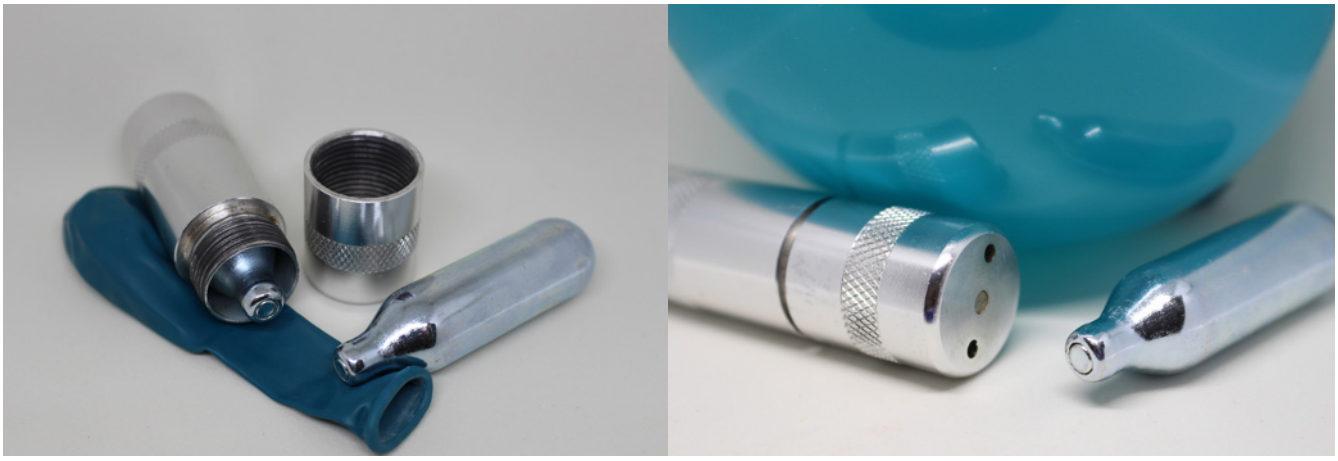
A cheaper, portable and more discreet way to use the gas without a whipped cream dispenser is to use a 'cracker'. This is a pocket-sized cylindrical metal device composed of a holder for the cartridge and a lid. Inserting a cartridge into the holder and screwing on the lid clockwise pushes a pin into the foil cap, piercing it — or 'cracking' it open. A balloon is placed over the lid, and turning the lid anti-clockwise releases the gas into the balloon (Figure 2). Crackers get freezing cold when the nitrous oxide is released into the balloon and may cause cold burns to the hands. A rubber insulating sleeve around the cracker or wearing gloves can prevent this.

Most users use one 8-gram cartridge per balloon, and perhaps 1 to 3 cartridges in a session. Some regular and heavier users may add two cartridges to a balloon or use much greater quantities in a session, either from cartridges or cylinders. In the Netherlands, the sale of 'extra-large balloons' (30 cm) may lead to larger amounts of the gas being used compared with the usual balloon size (22 cm).

Rarely, users may connect a cylinder of nitrous oxide to a tube from which they inhale, or use a face mask or plastic bag which is placed over the head to give a longer-lasting supply of the gas. In some cases, 8-gram cartridges have also been used with bags. This poses an extremely high risk of life-threatening hypoxia and death from asphyxiation. Similarly, release of the gas into an enclosed space without adequate ventilation, such as a car, can also be fatal. While these are extremely uncommon ways of using the gas, they are a frequent feature of the deaths from accidental asphyxiation reported in the medical literature.

A few people inhale the gas directly from whipped cream dispensers, crackers, cartridges or cylinders. This poses an extremely high risk of severe cold burns and lung injury. The gas is freezing when it is released from these containers (–40 to –55 °C). Within seconds, it can burn the nose, lips, mouth, throat, vocal cords and lungs. In some cases, the swelling can obstruct the airway, which can be life-threatening and require urgent medical

FIGURE 2
A 'cracker' used to open nitrous oxide cartridges without the need for a whipped cream dispenser, and balloon



Source: drugsinfo.nl – Trimbos-Instituut.

treatment to prevent suffocation. The gas is also under high pressure and can rupture lung tissue when inhaled directly. Cylinders are under much greater pressure than cartridges and therefore pose a higher risk of pressure injuries. Releasing the nitrous oxide into a balloon first helps to warm the gas and normalise the pressure before inhaling. Even so, rare cases of burns to the throat have been reported after inhaling from a balloon.

According to the Danish Poison Centre, the recent switch from cartridges to larger cylinders has led to an increase in frostbite and lung injuries.

The effects of the gas are very rapid, but short-acting. They start almost immediately, peak at about 10-30 seconds after inhalation, and end within 1-5 minutes.

The subjective effects combine feelings of euphoria, relaxation, calmness and distortions of perception, such as sensation, time and space. The euphoria may be accompanied by giggling or laughter. The distortions may affect hearing and sight. The effects are described as a 'dreamy' state, 'psychedelic-like', or as a general feeling of detachment ('dissociation'). Occasionally, hallucinations are reported, particularly with longer periods of exposure to the gas.

Common adverse effects from using small amounts include dizziness, light-headedness, disorientation, headache and a generalised tingling sensation. Nausea and fainting may also occur, as may temporary loss of coordination and balance. In some cases, users may vomit, which poses a risk of aspiration (breathing the vomit into the lungs) if consciousness is reduced. Some of the effects are from hypoxia caused by a temporary lack of oxygen, which may also cause seizures.

The adverse effects are generally minor and resolve within a short period of time after the user stops breathing the gas. However, some effects, such as light-headedness, dizziness and general impairment, can continue for 30 minutes or so. Using larger amounts of gas in a single session causes a higher number of these adverse effects.

Acute poisoning requiring medical treatment is relatively uncommon. Typically, it involves short-lived disorientation, and injuries from falls caused by fainting or loss of coordination and balance while intoxicated. Occasionally, hallucinations may also require treatment.

Due to disorientation and general impairment, people using nitrous oxide should not drive, ride bikes or scooters or operate machinery. Some people do not view using nitrous oxide while driving as dangerous. In the Netherlands, the number of incidents involving nitrous oxide and driving increased by 80 % between 2019 and 2021 (2 652 to 4 860 incidents). Some incidents relate to driving while intoxicated; others to filling balloons while driving. However, proving use of the gas is difficult in these cases.

Deaths involving nitrous oxide are rare. In most cases, the cause is accidental asphyxiation from breathing the gas using a mask or plastic bag over the head without sufficient oxygen. Deaths may also occur from using the gas in a confined space, such as a car.

More frequent, heavier use of nitrous oxide increases the risk of serious harms such as neurotoxicity. Larger cylinders also pose a greater risk of severe frostbite injuries, typically a result of clamping the cylinder between the thighs while filling the balloons. As the gas is released from the tank to fill the balloon, the cylinder walls can cool down to freezing, especially if the gas is used rapidly. This causes the skin and underlying tissue in contact with the tank to freeze. Users may not be aware of the injury because of the analgesic effect of the nitrous oxide and possibly the cold itself. They are also unlikely to be aware of the severity of the burn, as, initially, the wounds may be like first-degree burns involving mild redness or second-degree burns with blisters. Over the next few days, they can progress to severe third-degree burns.

Early assessment and treatment is essential and may require referral to specialist burn centres. In some cases, treatment may involve multiple surgeries and skin transplantation. Over a five-month period between January and June 2019, 19 patients requiring specialist care for burns caused by cylinders were reported in the Netherlands. Doctors have highlighted issues such as a delay in presentation and referral, which may be partly due to unfamiliarity with this new type of injury and embarrassment on the part of the patient.

The gas may also be used with other substances, such as alcohol, cannabis and MDMA, to enhance their effect or produce different effects. Using other drugs may impair judgement in terms of the amount of nitrous oxide used or how to use it without causing injury.

There is a risk of additive depressant effects when nitrous oxide is used with drugs having a central depressant action. These include alcohol, benzodiazepines and opioids. The extent to which nitrous oxide is used with other depressants is unknown.

The gas causes fires to burn hotter, faster and more intensely. People must not smoke when using the gas and must avoid other sources of ignition.

Chronic effects

Nitrous oxide causes dose-dependent chronic toxicity, with regular and heavy use posing the greatest risk. Overall, how the gas causes these effects is not fully understood. The irreversible inactivation of vitamin B12 in the body plays an important role. It has also been suggested that effects on the *N*-methyl-D-aspartate (NMDA) glutamate receptor may be involved. Other contributing factors may include hypoxia and acidosis (overproduction of acid, which builds up in the blood and other parts of the body) from using the gas without oxygen.

Vitamin B12 is an essential vitamin, meaning that the body cannot make it itself and it must come from the diet. Sources include meat, fish, dairy or use of a vitamin supplement. Among other functions, vitamin B12 is needed for healthy nerve functioning and for making DNA. Some vegetarians and vegans in particular may have subclinical vitamin B12 deficiency, which may predispose them to a greater risk of chronic toxicity.

The most significant chronic effect of nitrous oxide is neurotoxicity, discussed below. In addition, psychiatric symptoms such as altered mental state, hallucinations, psychotic episodes and mood disturbances have been reported, typically in patients with neurotoxicity. The gas may also cause disorders of the blood, such as anaemia. Finally, rare cases of thrombosis (where a blood clot forms) and embolism (where a clot blocks normal blood flow) as well as heart attacks have been reported recently in heavy users. These and other less common adverse effects are discussed in Section 2.

In addition to the effects caused by nitrous oxide itself, frequent, repeated hypoxia may also cause a range of harms. These include brain damage that can result in cognitive impairment that affect a person's everyday life, such as difficulty in concentrating, remembering, learning new things, or making decisions.

Neurotoxicity

Nitrous oxide damages the peripheral and central nervous system. How it causes this neurotoxicity is not fully understood, but it involves inactivation of vitamin B12 and is dose dependent. Cases were first described in the late 1970s in dentists who frequently used the gas recreationally or were exposed to it through work.

The signs and symptoms of neurotoxicity can be highly variable and sometimes vague and subtle. Patients may say their legs 'feel funny' or are 'clumsy' (uncoordinated). Initially, symptoms usually include paraesthesia — which means abnormal sensations, typically tingling or pricking ('pins and needles'), in the hands, arms, legs or feet, and which can also occur in other parts of the body. This may be caused by damage to the peripheral sensory nerves (nerves responsible for transmitting sensations, such as pain and touch, to the brain) and can progress to numbness. Damage may also involve nerves that are responsible for controlling muscles, leading to muscle weakness, loss of balance and difficulty in walking. Reflex responses may be decreased or absent. The damage can involve both the peripheral nervous system and central nervous system, especially the spinal cord. Some cases involve urinary incontinence or retention, constipation and sexual dysfunction. Damage may progress to an inability to walk. In rare cases, the damage is severe enough to cause paraplegia (paralysis of the lower body and legs).

Many of the cases reported to poison centres from 2017 onwards involve neurotoxicity of varying severity. For example, in France during 2020, 58 % (n = 73) of 126 cases had sensory or motor problems, particularly paraesthesia but also problems with balance and walking. Most were in heavy users, who had been using the gas for periods ranging from a few weeks to several years. Use varied from 50 cartridges in an evening to more than 600 cartridges per day. Some reported using more than one 0.56 kg cylinder a day. Five were hospitalised for neurological issues. Meanwhile, in the Netherlands, 64 young adults were treated for a partial spinal cord injury caused by nitrous oxide use between 2018 and 2019.

Usually, the damage is at least partially reversible, especially if identified and treated early. Some individuals may be left with sensory or functional damage. Rare cases of permanent paralysis have been reported. Sometimes, patients stop treatment, so the long-term outcome is unknown.

There are no established treatment guidelines. Treatment involves stopping nitrous oxide use, supplementation with vitamin B12 and methionine, and supportive therapy including physiotherapy. However, studies are needed to identify the most effective treatments. If nitrous oxide use is not stopped, vitamin B12 supplementation may not prevent further damage or improve outcomes.

Prevalence

Information on the prevalence of nitrous oxide use in Europe is limited. Most general population surveys ask about volatile substance use, rather than nitrous oxide specifically. However, recent representative surveys have

examined this issue in response to increased use in some countries, including France, Denmark and the Netherlands. Information is also available from England and Wales in the United Kingdom. Targeted surveys of clubbers, for example, typically find higher levels of regular use than in the general population.

In the Netherlands, the 2020 general population survey for adults aged 18 and older found that nitrous oxide use in the last 12 months was highest among young adults aged 18-19 years (14.5 %) and 20-24 years (12.1 %). This is six times higher than in the entire adult population (2.1 %). Meanwhile, use by 12- to 16-year-olds in the last 12 months was 6.7 %, with 11.7 % of 15- and 16-year-olds using the gas. In Denmark, a 2019 survey found that lifetime use in young people aged 15-25 years was 13.5 %, while 6.5 % used in the last 12 months. In France, a 2021 survey of students aged 14-15 found a 5.5 % reported lifetime use of the gas.

The level of use often varies within a country. For example, in Denmark, lifetime use was four times greater in the Copenhagen area than in North Jutland.

Separately, use in young people in England and Wales appears to have been established for a longer period than in other countries in Europe, with information on prevalence dating back to 2013. In that year, 7.6 % of those aged 16-24 years used in the last 12 months, while in 2019-20, 8.7 % reported using nitrous oxide in the last 12 months — equivalent to just over half a million people. This makes nitrous oxide the second most commonly used drug after cannabis, with use in that age group 3.5 times higher than in the entire adult population (2.4 %). Use had remained at the same level for the previous four years.

Typically, surveys find that more males than females use the gas, with some estimates suggesting the rate is around 30 to 50 % higher.

Nitrous oxide is typically used with friends, but it may also be used alone, especially with heavier use. It is used in a range of settings, including outside in public spaces (such as parks), in parked cars (so-called car parties), at home, at private parties, in nightclubs and at music concerts and festivals.

In some areas, the use of nitrous oxide outside has raised concerns over littering from discarded used cartridges and balloons. Noise nuisance has also been highlighted; this may be from the hissing sound from the release of gas from a cylinder and from a relatively large number of people gathering. These issues can have social, environmental and financial impacts — for example, the costs associated with cleaning up, while loud gatherings may intimidate some people.

Availability and supply

The availability and supply of the nitrous oxide used recreationally in Europe is not well understood, nor is the size and scale of the market.

Much of the gas is sourced from the small 8-gram cartridges that are used to make whipped cream. These can be bought from shops on the high street, such as supermarkets, convenience stores (night shops) and kiosks, as well as online. In France, the increased availability of nitrous oxide from 2017 coincided with the sale of cartridges in convenience stores, bars and nightclubs. In Denmark, until recent legislation, cartridges were sold in boxes of 10-100 in kiosks. It is unlikely that this is solely for making whipped cream.

Larger cylinders, also intended for food preparation, can be purchased from legitimate suppliers, although some companies may limit sales to registered businesses. Medicinal nitrous oxide is a prescription-only medicine, normally only administered by health care professionals.

Other companies specifically supply nitrous oxide to the recreational market. Some do this under the guise of supplying for culinary uses, typically whipped cream. The source of the nitrous oxide is unclear, but in some cases it appears that it was intended for food preparation.

In the Netherlands, suppliers openly advertise and promote the gas for recreational use, describing it with terms such as ‘laughing gas’ or ‘party gas’. This includes distributing adverts through letterbox drops and handouts, as well as advertising online. They sell 8-gram cartridges, larger cylinders and related equipment, including crackers, balloons and fruit flavourings for them, and pressure regulators for larger cylinders. Some sell ‘starter packages’ of 10 cartridges with balloons and a cracker. Orders can be made online or by phone; payment is by card or cash. Delivery in discreet packaging can be on the same day (in some cases within 30 minutes) or the next day depending on location. Some sites offer delivery to other countries in Europe. A risk assessment in the Netherlands in 2019 found that there is some level of criminal involvement in the trade.

A recent innovation in the recreational market, since around 2017, is the sale of larger cylinders. These range from 0.58 to 15 kg, supplying almost 300 litres to just over 5 000 litres of the gas respectively. This has made the gas

TABLE 1
Cylinder sizes and cost of nitrous oxide offered by retailers in the Netherlands. Volume of gas is calculated based on the density of gas 1.799 g/L at 25 °C and 1 bar (Haynes, 2014)

Cylinder size (kg)	Volume of gas (litres)	Cylinder price (€)	Price per litre (€)	Number of balloons	Price per balloon (€)
0.008	4	0.50	0.13	1	0.50
0.58	322	28	0.09	80	0.35
2	1 112	40	0.04	278	0.14
10	5 559	160	0.03	1 390	0.12

cheaper to use; it may also promote wider use, as well as more frequent and prolonged, heavy use (Table 1). The greater availability of cylinders may also promote riskier ways of using the gas and lead to more burns and lung injuries.

The 0.58 and 2 kg cylinders appear to be the most popular with users. Of particular note are the 0.58 kg cylinders that contain more than 300 litres of the gas. This is sufficient for around 80 balloons and is 25 % cheaper than using 'traditional' 8-gram cartridges. The cylinders are relatively discreet and portable, providing an 'all in one' solution for filling large numbers of balloons rapidly. The balloon is placed over the nozzle, and turning the nozzle opens the gas valve, allowing the required volume to be dispensed (Figure 3). In addition, unlike larger cylinders, the 0.58 kg cylinders are disposable and retailers do not require a security deposit, making them more attractive to younger users. During 2022, retailers have introduced similar disposable 2 kg cylinders.

Two large-scale seizures involving such cylinders have recently been reported by police in France. In December 2021, seven tonnes were seized (3.5 million litres), while in August 2022, almost 15 tonnes were seized (7.6 million litres).

In the Netherlands and the United Kingdom, at least, the financial interests of those selling nitrous oxide for recreational use appear to play an important role in promoting the use of the gas.

In addition, illicit markets have developed in some countries, with street-level dealers selling nitrous oxide. Social media are also used to promote and sell the gas. In some cases, the supply has moved from shops to social media following measures to restrict the supply of the gas.

The social supply of the gas between friends and other close social networks also plays an essential role in the distribution of the gas.

FIGURE 3
Disposable 0.58 kg nitrous oxide cylinders discarded in the street – Liverpool, United Kingdom, September 2022. Photos were taken on a Sunday morning and the cylinders were presumably discarded after being used on the previous Saturday night.

Source: Michael Evans-Brown and Harry Evans-Brown.

The safety and quality of nitrous oxide products inhaled for recreational use in Europe has not been assessed. In most cases, sellers use products intended for food preparation. Those supplying the recreational market claim it is 'food grade' or 'medical grade', although these claims have not been checked. Food-grade nitrous oxide is not intended to be inhaled. Concerns have been raised about the possible presence of oils used as coatings or lubricants during the manufacture of cartridges. Similarly, there is the potential risk of metal particles breaking off from cartridges when they are opened, which could then be inhaled. Such injuries have not been reported to date.

COVID-19 pandemic

The effect of the COVID-19 pandemic on nitrous oxide use is unclear. When lockdown measures closed nightlife venues, some people attending these venues may have reduced their use. Conversely, it appears that some users started to use nitrous oxide more frequently at home. Possible reasons for this include disruptions to the illicit drug market as well as boredom, anxiety and stress experienced during the pandemic. Ease of availability from brick-and-mortar shops and online suppliers may be linked to this. Home delivery despite the lockdown measures appears to have played an important role in maintaining or increasing use in some cases. A report from the French Poison Centres notes that many of the 134 cases of poisoning during 2020 started or increased their use during the first lockdown, as the gas was easy to buy and get delivered.

Pharmacology

How nitrous oxide produces its effects is complex and not fully understood. The gas affects several networks in the central nervous system (brain and spinal cord), such as those regulating pain, perception, anxiety, mood and emotion, behaviour and reward. These involve glutamate, opioid, noradrenaline and γ-aminobutyric acid (GABA) neurotransmitters, among others. Importantly, some effects from the recreational use of nitrous oxide arise from hypoxia, caused by inhaling the gas and the displacement of oxygen — unlike medical nitrous oxide, which is always given as a mixture with oxygen. Nitrous oxide is not metabolised (broken down) by the body, but instead exhaled unchanged by the lungs.

Many of the major effects of nitrous oxide, such as analgesia, anaesthesia, dissociation and reward and behavioural effects, appear to involve blocking the actions of the NMDA glutamate receptor. This receptor plays a role in many processes that modify sensations and perceptions of pain, in euphoria and in the effects of dissociative anaesthetics in general.

The analgesic effects of nitrous oxide are also believed to involve the opioid system, including the endorphins. The gas causes endorphins to be released in certain brain regions. This in turn activates pathways for other neurotransmitters, including noradrenaline, which is thought to reduce the receipt of pain messages originating from the body.

The anxiolytic effect of nitrous oxide has some similarities with the effects of benzodiazepines, and likely involves activation of the gamma-aminobutyric acid type A ($GABA_A$) receptor through the benzodiazepine binding site, which has a calming effect on many parts of the brain.

The abuse liability and dependence potential of nitrous oxide is not well understood. The gas does have reinforcing properties, which may involve blocking the NMDA receptor. Some users engage in frequent and heavy use that meets the criteria for substance dependence and substance use disorder. Despite our limited understanding of the mechanisms, some users may develop drug dependence and problematic use. The short-lived effects of the gas are often cited as a reason for repeated use in the same session.

Legitimate uses

Nitrous oxide has important, wide-ranging medical, industrial, commercial and scientific uses. It is widely used as an analgesic and anaesthetic in medicine. The gas is classed as a dissociative anaesthetic, with the World Health Organization listing it as an essential medicine. It is a prescription-only medicine, administered by inhalation. Mixed with oxygen, nitrous oxide is used for short-term pain relief and to reduce anxiety during childbirth, dental procedures, emergency treatment of injuries and as part of end-of-life care. It is also used as a surgical anaesthetic.

In addition, nitrous oxide is extensively used as a food additive, and particularly as an aerosol propellant used to make whipped cream. It is also used as a refrigerant, leak detecting agent, oxidising agent, chemical reagent, in semiconductor manufacturing and as an additive to fuels in car racing, as well as to make electrical, electronic and optical equipment.

Environmental concerns

Littering of used cartridges, balloons and cylinders has been highlighted as an issue in some areas. The cartridges and cylinders are steel, which can be recycled. Used cylinders pose a risk of explosion during waste processing if disposed of in general waste. Discarded balloons break down slowly in the environment and can be eaten by wildlife, who can suffocate.

Nitrous oxide is a potent greenhouse gas, 300 times more powerful than carbon dioxide. It is also a major cause of the destruction of the ozone layer. Global emissions of the gas are increasing as a result of human activities that stimulate its production, especially large-scale farming with synthetic fertilisers and cattle ranching. It is the third most important greenhouse gas, after carbon dioxide and methane. Currently, the impact from increased recreational use on the environment is unknown. While its contribution is minor compared with other sources, as nitrous oxide is exhaled unchanged from the lungs into the atmosphere, the environmental impact requires research.

Responses

This final part of Section 1 examines some of the response measures taken to reduce the availability and use of nitrous oxide and the harms caused by the gas.

The use of a substance in a new geographical area or in new groups of users is always a cause of concern for public health. This is because the population will have little or no experience with its effects and how to use it. Similar concerns apply to new ways of using a substance, new products or new patterns of use. While some risks might be known, others are unknown, and some are unknowable until larger numbers of people have been exposed to the substance. These issues all apply to the increased recreational use of nitrous oxide seen in some parts of Europe, especially since 2017.

Developing and implementing responses to nitrous oxide, whether at EU, national, local or individual level, involves three basic steps.

1. Identify the nature of the problems to be addressed.
2. Select potentially effective interventions to tackle these problems.
3. Implement, monitor and evaluate the impact of these interventions.

General information on these steps, and advice for designing, targeting and implementing effective responses, are available in the EMCDDA publication *Health and Social Responses to Drug Problems: a European Guide*.

Nitrous oxide can be considered a new psychoactive substance. However, its widespread legitimate uses and availability makes monitoring it through the Early Warning System difficult. In addition, its use as a medicine precludes risk assessment at EU level. No guidelines on responses exist at European level.

Countries experiencing issues with nitrous oxide have typically strengthened monitoring in order to better understand the nature of the problem and the risks. This includes studying the prevalence and use in the general population and 'at risk' user groups, as well as their perspectives. It also includes studying the markets and harms.

In some cases, formal risk assessment to identify, describe and estimate the magnitude of the public health and social risks from nitrous oxide has been used to inform the type of responses that are likely to be most effective. In addition, continued monitoring of the situation and evaluation of response measures have been used to inform the need for changes to the existing approach as well as additional responses.

Monitoring systems, including early warning systems, may need to be developed or adapted to monitor nitrous oxide use and harms. Standardised case definitions and classification systems may need to be developed. Better clinical coding is also needed. This allows better diagnosis and treatment, as well as understanding and quantification of the issue. It also allows the number of cases or rate of events identified in one time or place to be compared with the number or rate from another time or place.

Poison centres, hospital emergency departments, specialist neurology and burns centres, and the police all play a key role in identifying, monitoring and responding to the increase in harms caused by nitrous oxide — as do outreach and street-work agencies, and drug prevention and harm reduction services. Working with these agencies and with people who use nitrous oxide improves understanding of use and harms, and helps inform the development of effective responses.

It is important to recognise that the vast majority of people do not use nitrous oxide. Those that do typically use relatively small amounts infrequently. Use may also vary significantly within a country. Most use of nitrous oxide is by young people, including teenagers. It is important to

avoid normalising and unintentionally promoting its use. Therefore, targeted and environment-based interventions should be considered rather than general information or warning campaigns.

Targeted health promotion, including risk communication, should provide timely, clear, credible and consistent evidence-based messages that raise awareness and understanding and offer practical actions that can be taken. This may include communication with users as well as parents and guardians, and should come from trusted sources.

Simple, evidence-based harm reduction advice may help prevent both common adverse effects and the more serious risks linked to nitrous oxide. It can also be used to inform people of what to do in an emergency, and how to seek additional information and help. Existing resources commonly:

- explain what the gas is, how it is used, its effects, unwanted adverse effects and other risks;
- explain why inhaling from a balloon, rather than a cartridge or cylinder, reduces the risk of burns, lung injuries and asphyxiation;
- advise that people sit in a safe environment before inhaling the gas, as this helps prevent injuries from falls caused by fainting or loss of coordination and balance while intoxicated;
- highlight the dangers of driving, or riding a bike or scooter, while intoxicated — the apparent perception that people can drive safely while using the gas may also need to be addressed;
- highlight the dangers of using other drugs, including alcohol, at the same time;
- explain the need for urgent medical care for burns;
- highlight the risks of chronic toxicity from frequent and heavy use — especially important are messages on identifying the early signs of nerve damage and the need to obtain medical care as soon as possible;
- advise what to do in an emergency;
- direct people to further information, treatment and services.

In addition, awareness and understanding of the issue may need to be developed, as well as evidence-based training materials for healthcare professionals, drug workers, social workers and the police.

Any response to nitrous oxide needs to consider the widespread legitimate uses of the gas by industry, healthcare and consumers. Currently, there are few, if any, alternatives to the gas for these uses. Awareness of this issue, discussions and consultation will be required with these partners.

In some countries, the availability of nitrous oxide to consumers has been restricted. Although this may be an approach to limiting availability and use, the impact of these measures needs to be carefully monitored to assess their effectiveness and avoid the risk of unintended negative consequences.

Responses taken include:

- Restricting the maximum quantity of cartridges that can be supplied at any one time;
- Age-restricted sales, typically to those aged 18 and over, including requiring the display of a notice on premises that details the offence of selling to under 18s, and requiring age verification in both brick-and-mortar shops and online;
- Restricting sales of nitrous oxide products during the night (such as between 10pm and 5am), which may include online sales;
- Preventing nitrous oxide products from being visible or accessible to the public in retail stores;
- Prohibiting the sale of nitrous oxide by bars and clubs, or in shops selling alcohol, tobacco products or electronic cigarettes;
- Requiring warning labels on nitrous oxide products, including the 8-gram cartridges that are the most common source of the gas. Warnings may also provide contact details for poison centres;
- Requiring sellers to keep records of sales to verify that sales have been legal;
- Prohibiting sale of crackers and balloons when intended to be used with nitrous;
- Strengthening legislation around safe transport and storage of nitrous oxide.

In some cases, existing legislation and voluntary measures may help reduce the availability and harmful use of the gas for recreational purposes.

This may include EU and national legislation, such as the REACH and CLP Regulations that require nitrous oxide products to be appropriately packaged and labelled with information about their hazards. It may also include food

additive legislation and consumer protection and product safety legislation. Separately, medicines containing nitrous oxide are regulated under medicines legislation and are classed as prescription-only medicines.

Most recreational use is from 8-gram cartridges. These are typically associated with relatively low levels of use and limited harms. Restricting the availability of these cartridges may cause a switch to larger volume cylinders. This may lead to greater levels of harm overall from more frequent and heavy use, burns and the risk of asphyxiation. The recent introduction of disposable larger cylinders to the recreational market highlights this potential. As such, some countries have restricted the sale of cylinders to consumers. Simultaneously, measures to prevent the diversion and theft of cylinders from the legitimate supply chain, as well as from healthcare facilities and other sites such as restaurants, have also been taken.

Similarly, the risk of substitution with more harmful drugs should be considered, including drugs that are more readily available to this age group, especially teenagers. These may include deodorants, spray paints or other sources of volatile substances.

Responses should also consider how some retailers currently exploit the existing legislation (through loopholes). One particular issue dating back to the 1970s is selling the gas under the guise of being used to make whipped cream.

In the United Kingdom, prohibiting the sale of nitrous oxide for recreational use appears to have had no impact on prevalence, which has remained stable and at higher levels for almost a decade. Recently, as in other counties, larger cylinders such as the 0.58 kg cylinders are also sold on the recreational market.

In some countries, the market for recreational nitrous oxide is lucrative. This appears to play an important part in driving its availability and 'innovations' such as larger cylinders.

Restricting supply may lead to the involvement of criminal organisations. This may increase theft and diversion from the legitimate supply chain. It may also lead to homemade or illicit production of nitrous oxide. Methods available on the internet, including do-it-yourself videos, pose a high risk of explosion and contamination with nitrogen oxides, which cause lung toxicity that could be life-threatening.

Many of the chronic effects caused by nitrous oxide are linked to the irreversible inactivation of vitamin B12. More people, particularly young people, are choosing vegetarian and vegan diets that are poor in this vitamin. Low levels of the vitamin increases the risk of chronic toxicity, particularly nerve damage. Due to this, the level of vitamin B 12 deficiencies in this group and the risk of chronic toxicity may need to be assessed. Supplementation with vitamin B12 while continuing to use nitrous oxide does not appear to stop chronic toxicity.

Littering of used cartridges, balloons and cylinders has been highlighted as an issue in some areas. Importantly, canisters and cylinders are steel which can be recycled, although not all areas recycle steel. Evidence-based interventions to reduce littering exist, although their application to nitrous oxide litter will need to be assessed. Among other factors, interventions that motivate a sense of responsibility to the community may help.

Finally, our understanding of use, harms and effective responses is limited, partly because this level of recreational use is relatively new. Research is needed in areas such as epidemiology, supply, pharmacology and toxicology, as well as the effectiveness of treatments and response measures.

Section 2
Technical review of the chemistry, pharmacology, toxicology, health risks and legitimate uses of nitrous oxide

Chemistry

Molecular structure

Nitrous oxide is a simple molecule containing two nitrogens and one oxygen atom (N_2O).

FIGURE 4
Molecular structure of nitrous oxide

Chemical names and identifiers

Common name
- Nitrous oxide

Chemical names
- Nitrogen protoxide
- Nitrous-oxide
- 2-Oxodiazen-2-ium-1-ide
- Diazooxidane
- 1,2-Diazaethyne1-oxide
- Diazyne 1-oxide
- Dinitrogen monoxide
- Dinitrogen oxide
- Hyponitrous acid anhydride
- Nitrious oxide
- Nitrogen hypoxide
- Nitrogen oxide
- Nitrogenium oxydulatum
- Oxodiazen-2-ium-1-ide
- Oxidodinitrogen(N--N)

Other names
- Laughing gas
- Factitious air
- NNO
- NITRAL
- R 744A

[German]
- Stickdioxyd
- Stickstoff(I)-oxid
- Distickstoffmonoxid

[French]
- Oxyde nitreux
- Protoxyde d'azote
- Gaz hilarant

[Spanish]
- Oxido nitroso

[Dutch]
- Distikstofmonoxide
- Lachgas

[Portuguese]
- Óxido nitroso
- Droga do riso

Chemical Abstracts Service (CAS) registry number:
- 10024-97-2

IUPAC International Chemical Identifier Key (InCHI Key)
- GQPLMRYTRLFLPF-UHFFFAOYSA-N

IUPAC International Chemical Identifier String (InChI string)
- *InChI=1S/N2O/c1-2-3*

Simplified Molecular-Input Line-Entry System (SMILES)
- *[N-]=[N+]=O*

Food additive code (E number)
- E942

US Flavor and Extract Manufacturers Association (FEMA) number
- 2 779

Other identifiers

PubChem CID 948

The following terms have been used as 'street names' or slang names for various forms of nitrous oxide: bulb, buzz bomb, cartridges, fall down, gas, going to the dentist, grocery store high, hippy crack, hysteria, laughing gas, nang, nie, nigh, nitro, nitrogen, nitrous, NOS, pan, shoot the breeze, sweet air, tanks, whippet, whippets, wippets.

Physicochemical properties

Nitrous oxide is a colourless, sweet-tasting gas. It is non-combustible but it will accelerate the burning of combustible material in a fire (Cameo Chemicals, 2022a).

It is slightly soluble in water. At 20 °C and at a pressure of 101 kPa, 1 volume dissolves in about 1.5 volumes of water (EDQM, 2020).

Its vapours are heavier than air (Cameo Chemicals, 2022b).

Nitrous oxide refrigerated liquid appears as a colourless liquid. Density is 1.22 g/cm^3 at its boiling point of –89 °C. Vapour pressure is at about 745 psig at 21 °C (Cameo Chemicals, 2022b).

TABLE 2
Physicochemical properties of nitrous oxide

Molecular weight	44.013 g/mol
Boiling point	–88.48 °C (Haynes, 2014)
Melting point	–90.8 °C (Haynes, 2014)
Density of gas at 25 °C	1.799 g/L
Vapour density (air = 1)	1.53 (O'Neil, 2013)
Vapour pressure at 25 °C	51.3 atm (NIOSH, 2019)

Methods for identification and analysis

In its gaseous state, nitrous oxide can be measured using several techniques. In research settings, alongside spectroscopic methods, gas chromatography (GC) has been in use for many years (Heusler, 1985; Uyanik, 1997). To monitor occupational exposure to nitrous oxide among medical professionals, several techniques have been developed. The most widely used technique reported in operating theatre settings is respiratory gas analysis using infrared absorption spectroscopy or photoacoustic spectroscopy (Langton and Hutton, 2009; Navas et al., 2012). Other methods make use of silicone rubber and piezoelectric absorption, refractometry, Raman scattering and mass spectrometry (Langton and Hutton, 2009). In one study using photoacoustic infrared spectroscopy, the limit of detection of nitrous oxide in the breathing zone of surgeons was 0.03 parts per million (ppm = 0.03 µL/L or 0.03 µmol/mol, equivalent to approximately 55 µg/m^3 at 20 °C) (Wiesner et al., 2001). More recently, it was proposed that nitrous oxide could be measured in urine and exhaled air collected immediately after exposure, as part of nitrous oxide occupational exposure monitoring for medical professionals, but sampling and interpreting the results proved to be difficult (Marillier et al., 2020).

The detection of nitrous oxide in biological samples taken from living subjects is challenging due to its short half-life and rapid elimination. While inhaled nitrous oxide can be detected in blood and urine immediately after exposure (Brugnone et al., 1995), routine screening tests are unable to detect nitrous oxide. Detection of nitrous oxide in post-mortem biological samples has been reported using headspace gas chromatography with an electron capture detector (HS-GC-ECD) (Poli et al., 2010) and headspace gas chromatography coupled to mass spectrometry (HS-GC-MS) with hydrogen sulfide as an internal standard (Giuliani et al., 2015). The biological matrices used for post-mortem analysis of nitrous oxide are mainly blood, but also tissues such as lungs, liver and kidneys. The sampling conditions (sample early, use airtight containers, fill containers to the maximum) and storage (freeze if rapid analysis is impossible) must be rigorous in order to avoid partial or total loss of the volatile compound and its incorrect analysis (Marillier et al., 2020). It has been demonstrated that nitrous oxide can be detected in biological samples up to one month after death, in the case of fatal abnormal exposure, using headspace GC (Poli et al., 2010).

Nitrous oxide is found in the ambient air (0.5 ppm), with soils and oceans representing a natural source of this gas, while human activities, mainly agriculture, are another important source of nitrous oxide in the atmosphere. Nitrous oxide is a potent greenhouse gas — 300 times more potent than carbon dioxide (Pascale et al., 2017) — and is considered a substantial contributor to global warming (Tian et al., 2020). Consequently, further development of sensitive analytical methods for the detection and monitoring of nitrous oxide emissions is an ongoing area of research. For example, one GC method has been reported using a barrier discharge detector system (GC-BID) for simultaneous high-precision

measurements of greenhouse gas emissions from wastewater treatment plants, with a limit of detection of 0.062 ppm (Pascale et al., 2017).

A previous study evaluated the performance of both a GC method with electron capture detector (ECD) and a photoacoustic spectroscopic (PAS) method to analyse emissions from fossil fuel combustion facilities (Kang et al., 2014). Linearity and reproducibility were well within established standards. The detection limits were 0.074 ppm and 0.025 ppm for GC and PAS, respectively. Accuracy expressed as relative standard deviation was 0.37 % and 1.04 % for GC and PAS, respectively. The study concluded that the GC method had higher accuracy, but a lower detection limit, shorter response time, greater convenience and higher mobility are advantages of the PAS method (Kang et al., 2014).

Synthesis and preparation

The discovery and first synthesis of nitrous oxide can be traced back to the18th century (Buslov et al., 2018; Smith, 1972). Present-day industrial production involves the thermal decomposition of a hot solution of ammonia nitrate (Denisova et al., 2019). Controlling the temperature of the reaction governs the final yield (optimal temperature is 265-278 ºC) and the occurrence of side reactions, which can generate a number of side products including N_2, NO_2, N_2O_3, NO, NH_3 and HNO_3 (Denisova et al., 2019). These can be removed by a number of industrial or medical purification processes (Austin, 1967; Denisova et al., 2019; EDQM, 2020).

For medical purposes, nitrous oxide is sold in preparations that should not exceed 2 ppm (V/V) NO and NO_2 (EDQM, 2020), whereas food-grade nitrous oxide (also known as E942) should not contain more than 10 ppm (V/V) NO and NO_2 (European Commission, 2012). Given its commercial availability, it is likely that the nitrous oxide available to the drug market originates in the food industry, as ready-to-use cartridges originally intended for whipping cream, or in the medical industry, as larger cylinders for technical or medical use.

The clandestine or home production of nitrous oxide is more expensive, difficult and unsafe, but some methods are documented and available online (Messina and Wynne, 1982; Helmenstine, 2019, 2020; Elementary Productions, 2008; NileRed, 2017). In some, hydroxylammonium chloride and sodium nitrite are mixed together, and controlled addition of sodium nitrite and/or cooling are used to minimise the generation of other compounds.

The final products will invariably contain NO/NO_2 in concentrations that will typically be unknown.

Although somewhat unlikely, the home production of nitrous oxide for the drug market carries serious risks of explosion and may generate considerable amounts of noxious side products, depending on the method used (Austin, 1967; Denisova et al., 2019). The individuals involved in the synthesis may also develop lung damage due to exposure to NO and NO_2.

Route of administration and dosage

Nitrous oxide is a gas and can only be administered by inhalation. The most frequent method of administration is using small cartridges, for example in whipped cream dispensers, or balloons filled with gas discharged from a cartridge or a tank. In the Global Drug Survey (GDS) 2014-2016, 82 % of users indicated that they use a balloon for administration of the gas. 12.8 % indicated that they use a whipped cream dispenser (Winstock and Ferris, 2020).

One cartridge contains 10 mL liquid nitrous oxide under pressure (7-8 bar). Under normobaric conditions, this amount is equivalent to 4.3 litres of nitrous oxide gas, enough to fill one balloon. For anaesthesia, nitrous oxide is administered in a mixture with oxygen (50 %/50 %). Assuming ventilation of 6 l/min, inhalation of five balloons would be equivalent to approximately 7 minutes of nitrous oxide anaesthesia (van Amsterdam et al., 2015; RIVM, 2016). Combined with the use of large tanks, some users prefer large balloons (skippy balls) (Nabben and Bahara, 2020; Nabben et al., 2021). A 2 kg tank would be equivalent to 1 112 litres of nitrous oxide or more than 6 hours of anaesthesia.

Data from the GDS 2014-2016 surveys indicate that for the 16 513 respondents indicating a dose per session, the median number of doses was around five but this ranged from one dose (n = 1 344; 8.1 %) to 100 or more doses (n = 130; 0.8 %) (Winstock and Ferris, 2020). Further information on doses reported by users in national surveys is presented in the national case studies (Section 3).

More recently, there are indications that nitrous oxide is increasingly being used from large cylinders or tanks, rather than small cartridges or balloons. In some cases, users administer nitrous oxide directly from the cylinder. This might result in excessive intake, due to difficulty in dosing, and in additional risks such as severe burns (see 'Health risks' below).

Pharmacology

While the mechanism of action of nitrous oxide is currently not fully known, several targets have been hypothesised to be involved in its action. The main molecular target for nitrous oxide's anaesthetic action is considered to be non-competitive inhibition of the NMDA subtype of glutamate receptors. These receptors are present throughout the brain and are active in many neural networks, including the brain reward networks. The neural network relevant to the anti-nociceptive and analgesic action of nitrous oxide includes opioidergic, GABAergic and noradrenergic neurons in the midbrain and α1- and α2B-adrenoceptors in the spinal cord. The anxiolytic effect of nitrous oxide involves activation of the $GABA_A$ receptor through the benzodiazepine binding site.

The psychoactive effects following a brief inhalation of nitrous oxide occur within 30 seconds of inhalation and subside within 5 minutes. The pharmacological properties of nitrous oxide vary depending on the concentration of nitrous oxide. In clinical settings, an equimolar mixture of oxygen and nitrous oxide (EMONO) is used for analgesic purposes. A concentration of 25 % nitrous oxide is usually adequate to provide a marked reduction in pain, while concentrations above 60 % produce hypnotic effects. In order to produce unconsciousness, a concentration of about 70 % is required (BOC Ltd., 2019). The subjective effects of nitrous oxide may include euphoria, distortions in perception (auditory and visual), numbness, sedation, confusion, changes in mental thought processes and self-awareness, reduced motor control and changes in time perception.

Pharmacokinetics

Unlike solutions, gases are absorbed and distribute throughout the body as the result of differences in pressure (tension) between the inspired gas and the different tissues to which it diffuses. Gases such as nitrous oxide, with low blood solubility, will equilibrate faster and thus diffuse quickly into other tissues — including the brain, where their anaesthetic action occurs. This low solubility and high diffusibility of nitrous oxide gives it the fastest onset of action among inhalation agents (Becker and Rosenberg, 2008).

The blood-gas partition coefficient of nitrous oxide is low (0.46) and >99 % of it is rapidly eliminated unchanged through the lungs, with small amounts diffusing through the skin (Buckingham, 2020; Long, 2019). As nitrous oxide is not metabolised and merely partitions in the body based on its physicochemical properties, its blood concentration is closely related to the concentration of nitrous oxide in inspired air and shows little intraindividual variability (Hopkins, 2005).

Due to the low blood-gas solubility of nitrous oxide, onset and offset of action is rapid and there is usually complete recovery from its main effects within a few minutes of exposure, although lingering behavioural effects may persist for up to half an hour.

The partition coefficients of nitrous oxide for various compartments are provided in Table 3. Although nitrous oxide partitions to fat to some extent, the lipophilicity of nitrous oxide is low compared with volatile anaesthetics. For desflurane, isoflurane and sevoflurane, these figures are 29, 50 and 52, respectively (Kreuer et al., 2007).

TABLE 3
Partition coefficients of nitrous oxide for various compartments (Kreuer et al., 2007)

Tissue	Partition coefficient
Blood/gas	0.46
Brain/blood	1.07
Heart/blood	1.02
Muscle/blood	1.15
Fat/blood	2.39

Nitrous oxide can also diffuse into closed cavities such as obstructed parts of the bowel and the pleural cavity. The difference in the blood-gas partition coefficient of nitrous oxide (0.46) and nitrogen (0.014) results in the preferential transfer of nitrous oxide into such cavities 30 times faster than nitrogen can exit (Eger and Saidman, 1965; Reinelt et al., 2002). This can lead to an increase in the volume of soft structures and an increase in the intra-cavity pressure of structures with rigid walls, which is the origin of some of its contraindications in medicine. Further experiments in dogs showed that direct diffusion of nitrous oxide may also occur from the alveoli across the visceral pleural surface (Kaur et al., 2001). Indeed, this increase in volume can be of concern for recreational users in whom inhaled nitrous oxide can worsen an asymptomatic pneumothorax (Garbaz et al., 2007; McDermott et al., 2015).

Pharmacodynamics

Mode of action

For over a century, the most popular theory explaining the mechanism of action of anaesthetics was the Meyer-Overton rule, which states that the potency of an anaesthetic is proportional to its lipid solubility. Based on this rule, traditional hypotheses of anaesthetic action portray anaesthetics as non-selective agents that act by perturbation of the neuronal cell membrane. These theories have become increasingly marginalised, and it is now widely accepted that lipophilic sites in the proteins forming membrane-crossing ion channels and receptors are the most likely targets for anaesthetic agents. The binding of anaesthetics such as nitrous oxide to these sites may affect the conformation of these proteins and, consequently, activate or block receptors and open or close ion channels (Alkire and Gorski, 2004; Bovill, 2000; Dong et al., 1994; Solt and Forman, 2007).

The mechanism of action of nitrous oxide is not fully elucidated. Several targets have been hypothesised to be involved.

- The glutamatergic system is believed to play a key role in explaining the pharmacological action of nitrous oxide. In particular, it acts as an antagonist at the NMDA and AMPA receptors, which are involved in the transmission of nociceptive messages and in hyperalgesia (Georgiev et al., 2008; Rosen, 2002).
- The kappa-opioid system may be important in the analgesic action of nitrous oxide. The analgesic effects of nitrous oxide were supressed by an opioid antagonist that is selective for the kappa receptor subtype (Koyama and Fukuda, 2010), and genetically modified mice that did not express the kappa-opioid receptor did not experience analgesia when exposed to nitrous oxide (Fukagawa et al., 2014).
- The noradrenergic system may play a role via the descending inhibitory pathways. This effect would involve activation of these pathways and probably also the release of noradrenaline in the dorsal horn. In rats, researchers have described modulation of receptors of the periaqueductal grey matter and activation of the descending adrenergic inhibitory pathways (Zhang et al., 1999).
- Dopaminergic neurons by stimulating the release of dopamine (Sakamoto et al., 2006; Koyanagi et al., 2008).
- The GABAergic system may be involved, with activation of the $GABA_A$ receptor directly or indirectly via the benzodiazepine binding site (Emmanouil and Quock, 2007, Sanders et al., 2008).

The following sections discuss the targets for several important neuropharmacological activities of nitrous oxide. Aspects relating to its dependence-producing potential will be discussed in the section on dependence potential and abuse liability.

Anaesthesia

General anaesthesia is characterised by the dose-dependent development of amnesia, analgesia (for some anaesthetics), unconsciousness and immobility.

The potency of inhalational anaesthetics is often expressed in terms of minimum alveolar concentration (MAC), which is the concentration of gas at 1 atm that will produce immobility in 50 % of exposed subjects. A value in excess of 100 % implies that the MAC value is reached only under hyperbaric conditions (Buckingham, 2020). Note that the definition of the MAC relates to only one aspect of anaesthesia, namely immobility. Amnestic concentrations are usually lower or close to those required to produce loss of consciousness (Eger, 2001). The MAC of nitrous oxide (causing immobility) is approximately 1.5 times higher than the concentration causing hypnosis (the so-called MAC-awake) (Solt and Forman, 2007). The amnestic potency of nitrous oxide is relatively large. The ED_{50} in an aversive footshock ([1]) model in rats was only 6.2 % (95 % CI: 4.0-9.4 %) of the MAC (Alkire and Gorski, 2004).

The mechanism by which nitrous oxide exerts its anaesthetic action has been unknown for many years. But currently, non-competitive inhibition of the NMDA subtype of glutamate receptors is considered the main molecular target for nitrous oxide's anaesthetic action (Jevtović-Todorović et al., 1998; Nagele et al., 2004; Sanders et al., 2008; Sato et al., 2005; Solt and Forman, 2007). Non-NMDA-type glutamate receptors (i.e. AMPA and kainate receptors) are only mildly inhibited by nitrous oxide (Yamakura and Harris, 2000).

Furthermore, the anaesthetic action of nitrous oxide might also be mediated by the activation of potassium channels (Gruss et al., 2004) located in the brain and spinal cord, which would decrease neuronal excitability and thus slow

([1]) Footshock is often employed as an aversive stimulus in studies of learning and memory, allowing for studies that link physiological and behavioural responses (Pacak and McCarthy, 2007).

down the transmission of electrical impulses (Patel and Honoré, 2001; Solt and Forman, 2007).

In addition, studies in *Xenopus* oocytes have suggested that nicotinic acetylcholine (nACh) receptors composed of beta2 subunits are likely targets for the anaesthetic effects of nitrous oxide (Yamakura and Harris, 2000).

Although nitrous oxide was shown to slightly activate glycine receptors and could inhibit $GABA_A$ and $_C$ receptors (Mennerick et al., 1998; Yamakura and Harris, 2000), these receptors are generally not ranked as relevant targets for the anaesthetic action of nitrous oxide (Solt and Forman, 2007).

Nitrous oxide also reduces the required dose of co-administered anaesthetics by improving the speed of onset and the duration of effect (the so-called second gas effect) (Banks and Hardman, 2005).

Anti-nociception and analgesia

Anti-nociception is the action or process of blocking the detection of a painful or injurious stimulus by sensory neurons (Merriam-Webster, 2022a). Analgesia is insensibility to pain without loss of consciousness (Merriam-Webster, 2022b.). The mechanisms by which nitrous oxide leads to anti-nociception and analgesia have been well studied, although not every detail has been uncovered. It is now established that opioid receptors play an important role in rats, mice and humans (Emmanouil and Quock, 2007; Fujinaga and Maze, 2002; Sanders et al., 2008). In brief terms, blocking these receptors in the periaqueductal grey (PAG) area of the midbrain ablates nitrous oxide analgesia. This hypothesis is supported by clinical evidence, as naloxone has been shown to partially reverse the effect of nitrous oxide (Rosen, 2002). Opioidergic neurons in the PAG may be activated by corticotropin-releasing factor, which is released from the hypothalamus under the influence of nitrous oxide. Endogenous opioids released by the PAG neurons, such as dynorphins (DYN), block inhibitory GABAergic neurons in the pons. Subsequently, the reduced activity of the GABA neurons increases firing of pontine noradrenergic neurons with descending pathways to the spinal cord, where α_1-adrenoceptors on inhibitory GABAergic neurons and α_{2B}-adrenoceptors on second-order neurons are activated. 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 reduces pain impulses ascending into the supraspinal regions. More recent studies have questioned whether mu-opioid receptors are involved in the analgesic effects of nitrous oxide, as genetically modified mice without this receptor still experienced analgesia and anaesthesia when exposed to the gas (Koyama et al., 2009). Indeed, it was later shown that the analgesic effects of nitrous oxide are in fact mediated by kappa-opioid receptors — as the effects were supressed by an opioid antagonist selective only for the kappa receptor subtype (Koyama and Fukuda, 2010), and genetically modified mice without the kappa-opioid receptor did not experience analgesia when exposed to nitrous oxide (Fukagawa et al., 2014).

Anxiolytic effect

Nitrous oxide is valued for its anxiolytic properties, especially in dentistry (Buhre et al., 2019; Jastak, 1989). The anxiolytic properties of nitrous oxide have also been demonstrated in a range of behavioural models in animals, such as the mouse staircase test, the mouse elevated plus maze, the mouse light/dark exploration test, the mouse hole board, the rat social interaction test and the rat conditioned defensive burying test (Emmanouil and Quock, 2007; Li et al., 2004). Pharmacological studies in such models have revealed that the anxiolytic effect of nitrous oxide involves activation of the $GABA_A$ receptor through the benzodiazepine binding site, although whether nitrous oxide acts directly or indirectly upon the latter target remains uncertain (Emmanouil and Quock, 2007). *In vitro* studies have indicated that nitrous oxide has little effect on $GABA_A$ receptor-mediated currents (Mennerick et al., 1998; Yamakura and Harris, 2000).

Antidepressant effect

The potential use of nitrous oxide in the treatment of depression has been recently explored (Gillman, 2019), though further studies are needed to confirm the relevance of this effect in clinical settings. Because nitrous oxide is a non-competitive inhibitor of NMDA receptors, it is hypothesised that it may have a rapid-acting antidepressant action similar to ketamine (Nagele et al., 2015). So far, two clinical trials and some case reports have suggested that nitrous oxide can indeed have antidepressant effects in humans (Desmidt et al., 2021; Guimarães et al., 2021; Nagele et al., 2015, 2020). However, these trials had some limitations such as small sample size, limited effect size, large placebo effect and short follow-up period. In addition, the administration procedures and the target populations varied. It therefore remains to be seen if nitrous oxide will prove to have clinical value as an antidepressant treatment. It is likely that the duration of any antidepressant effect seen will be short-lived, as is the case for ketamine (Kalmoe et

al., 2020). While it is thought that the antidepressant effect of nitrous oxide is primarily mediated by its NMDA antagonistic action, there are numerous other neuronal targets of nitrous oxide whose significance for depression is unknown (Kalmoe et al., 2020; Nagele et al., 2018; Zarate and Machado-Vieira, 2015). It has been hypothesised that synaptic plasticity and synaptogenesis play a role in antidepressant effects whereas stress and depression are associated with neuronal atrophy and loss of synapses in the brain areas implicated in mood regulation. Brain-derived neurotrophic factor (BDNF) synthesis and signalling is important for synaptic plasticity, synaptogenesis and neurogenesis (Castrén et al., 2007; Rantamäki and Yalcin, 2019). Nitrous oxide readily regulates BDNF synthesis, while its receptor tropomyosin receptor kinase B (TrkB) is activated upon gas withdrawal (Rantamäki, 2019). Nitrous oxide can also increase cell proliferation in the dentate gyrus (Chamaa et al., 2018). These effects could potentially contribute to antidepressant activity from nitrous oxide. However, as indicated before, such an effect may be short-lived and would require repeated exposure to nitrous oxide, which poses additional safety issues.

Safety pharmacology

Nitrous oxide may produce euphoria, disorientation, sedation, nausea, vomiting, dizziness and generalised tingling. These events are frequent, generally minor and rapidly reversible. Considering that nitrous oxide passes into all gas-containing spaces in the body faster than nitrogen comes out of them, prolonged exposure may result in bowel distension, middle ear damage and rupture of the ear drums. In addition, repeated administration of nitrous oxide or exposure to the gas may lead to addiction, and prolonged or frequent use may result in neurological toxicity, as well as megaloblastic anaemia and agranulocytosis (BOC Ltd., 2019).

Nitrous oxide can decrease the amount of air that moves into the lungs and at the same time increase the respiratory rate, thus having thus minimal to no effect on net ventilation capacity (Becker and Rosenberg, 2008). Arterial partial pressure of carbon dioxide is not affected at 1 MAC, but the ventilatory response to carbon dioxide is blunted, as is common with other inhaled anaesthetics (Fee and Thompson, 1997).

Nitrous oxide may increase intracranial pressure by increasing cerebral blood flow. Given alone, mixed only with oxygen, it appears to increase cerebral oxygen requirements (Fee and Thompson, 1997).

Some authors have described a degree of myocardial depression caused by nitrous oxide, and there are case reports on arrhythmias (Eisele and Smith, 1972; Roizen et al., 1987). Nevertheless, mean arterial pressure, cardiac output, systemic vascular resistance and heart rate are judged not to be affected by nitrous oxide to any considerable extent, especially in comparison with flurane anaesthetics (Becker and Rosenberg, 2008; Fee and Thompson, 1997).

Due to the absent or minimal effects on cardiovascular and respiratory function, the clinical use of nitrous oxide is generally regarded as safe as an analgesic and anxiolytic in labour and in dentistry, and as a mild anaesthetic in emergency medicine (Becker and Rosenberg, 2008; Jastak, 1989; Rooks, 2011; Vallejo and Zakowski, 2019). In clinical practice, nitrous oxide is always used as a mixture with oxygen to prevent hypoxia.

Interactions

Use of nitrous oxide with other central nervous system depressants (such as opioids, benzodiazepines and alcohol) might result in increased sedation, and consequently have effects on respiration, circulation and protective reflexes (BOC Ltd., 2019).

Nitrous oxide has a profound effect on vitamin B12 function and potentiates the effects of methotrexate on folate metabolism (BOC Ltd., 2019).

Psychological and behavioural effects

Subjective effects

Onset of action and duration

After brief inhalation of 40 %, 60 % or 80 % nitrous oxide, the psychoactive effects occur within 30 seconds and subside within 5 minutes (Zacny et al., 1994a). After exposure to 20 % or 40 % nitrous oxide for 20 minutes, ratings for subjective effects remain elevated for five or even up to 30 minutes, while levels of fatigue, anxiety and depression increased during recovery; the latter are still elevated after 60 minutes, suggesting a possible delayed or lingering effect construed as aversive (Dohrn et al., 1992).

Effects experienced

The effects sought by people using nitrous oxide for recreational purposes are mostly euphoria and distortion of perception.

In an early study in 50 male and female college students (average age 21.5 years), the most typical subjective effects reported (>50 %) were physical tingling sensations, dreaminess, difficulty in concentration, euphoria, unsteadiness of finer motor movements, a sense of impaired control over the whole situation, auditory disturbances, memory disturbances, dizziness and numbness. For 45 % of the subjects, the experience was described as pleasant, whereas 33 % indicated it was unpleasant (Steinberg, 1956). Atkinson et al. (1977) studied the subjective effects in 80 male adults (21-30 years of age) who were nitrous oxide-naïve but had variable experience of other recreational drugs, using a questionnaire developed to compare LSD effects with those of amphetamine (Katz et al., 1968). They grouped the effects into Barber's psychedelic effect categories, not to imply that nitrous oxide is similar to LSD, but to explain that pharmacologically different drugs may produce common subjective phenomena. Most effects fell into categories describing changes in body awareness and body image; experiences of a detached, dream-like state; a sense of diminished cognitive-motor proficiency; alterations of time perception; and mood changes of a happy, euphoric nature. Subjects with experience of other recreational drugs most often indicated that nitrous oxide was most similar to LSD (30 %) or cannabis (30 %). The authors characterise the recorded subjective effects as an 'incomplete' psychedelic experience, arguing that besides common effects, some effects characteristic of LSD — notably visual and other special sensory effects and sympathetic-autonomic effects — are lacking (Atkinson et al., 1977).

Regular users of nitrous oxide, when asked for their experiences, mention a dreamy state, feeling comfortable, a rush sense of being away for a moment, a cosmic feeling, changed perception of time and space, a slow motion effect, a downer, feeling introspective, feeling chill, going into pause mode, and feeling a little dizzy and a little giggly (CAM, 2019; Nabben, 2010). Distortion of sounds is another effect regularly mentioned.

According to the numbers from the 2014 Global Drug Survey, the unwanted effects of nitrous oxide most often reported by regular recreational users are hallucinations (27.8 % of cases), confusion (24 %), headache, nausea (5.8 %), fainting (4.4 %), tingling in the hands or feet (4.3 %), falls and associated accidents (1.2 %), vomiting, reduced communication and orientation, and blurry vision (CAM, 2019; Kaar et al., 2016; Nabben et al., 2017). Users reporting multiple unwanted effects are likely to have used more nitrous oxide in a single session, with accidents and fainting associated with the heaviest use (Kaar et al., 2016).

Gender variability

While most studies report subjective effects of 'tingling of the extremities' and 'spaciness', there is a great deal of variability in how the drug is perceived by volunteers, both within and across a number of studies (Dohrn et al., 1992; Walker and Zacny, 2002, 2003). The conditions under which the subjects are using nitrous oxide is one of the factors affecting their experience (Block et al., 1990; Cho et al., 1997). It is possible that gender may influence the outcome: in a study of 50 medical students, 81 % of male volunteers reported the nitrous oxide experience as pleasant, whereas only 50 % of the women did (Rosenberg, 1974). Although a retrospective analysis of nine previous studies could not find a gender-specific difference in subjective ratings (Zacny and Jun, 2010), the results should be taken with caution, because of the differences in design between the examined studies, their imbalance in gender and the retrospective nature of the approach. Sex differences in sensitivity to the anaesthetic effects of nitrous oxide have been reported: when 75 %/25 % nitrous oxide/oxygen was administered, significantly deeper levels of anaesthesia were acquired by females than by males during the initial 10 minutes of inhalation (Barth and Büchel, 1975). A higher susceptibility to the effects of another NMDA antagonist — xenon — has been shown in human females as well (Goto et al., 2002).

Neurocognitive and psychomotor function

Inhalation of nitrous oxide at recreational doses can acutely affect neurocognitive and psychomotor function in a dose-dependent way. These effects fade quickly after administration ceases. Such effects have been demonstrated in numerous studies, as well as in reports of traffic accidents, cases of driving under the influence and falls linked to nitrous oxide use.

In a study where bolus administration of 80 % nitrous oxide was used to mimic recreational use of a single balloon, the acute effects showed a trend towards psychomotor impairment, as measured by the digit symbol substitution test (DSST), with peak decrease in performance about one minute after inhalation. These results suggest that there are acute, albeit brief, adverse effects on psychomotor

function after inhaling nitrous oxide (Zacny et al., 1994a). Sedation by nitrous oxide in human volunteers reduced DSST dose-dependently in the 20-40 % concentration range, but auditory reaction time was not affected (Dohrn et al., 1992). In another study, the effects of nitrous oxide (0 %, 5 %, 10 %, 20 % and 40 %) were measured using a comprehensive battery of performance tests. Performance in DSST, tapping rate and a continuous attention task were already decreased at 10 % concentrations of nitrous oxide. Latency and total time in a choice reaction test, body sway, decision making and visual vigilance were affected at 20 % nitrous oxide, while Gibson spiral maze and paired word learning were only affected at 40 % (Fagan et al., 1994). Early studies suggested that working memory (digit span test) and audiovisual reaction time would be affected by nitrous oxide concentrations as low as 50 ppm, but these could not be confirmed by several independent laboratories, and further research suggested that the threshold at which nitrous oxide affects audiovisual reaction performance is more likely in the range of 8-12 % (Allison et al., 1979; Smith and Shirley, 1978). Indeed, nitrous oxide reduces performance on both short-term and long-term recall memory tests at a concentration of 30 %, but not significantly at 15 % or 25 %. The effect was less than with an equivalent MAC of sevoflurane. In a working memory test, 25 % nitrous oxide did show an impairment that was similar to an ethanol dose calculated to produce a blood ethanol concentration of 80-100 mg/100 ml (Duarte et al., 2008; Galinkin et al., 1997). In an earlier study, nitrous oxide at 20 % and 40 % concentrations was shown to dose-dependently impair both short-term and long-term recall memory. These findings are generally in line with other studies that have also documented the amnestic properties of nitrous oxide, indicating that verbal memory was inhibited at concentrations of 20-30 % but not in the 10-20 % range (Smith and Shirley, 1978; Zacny et al., 1994b).

Recovery of psychomotor function (multiple-choice reaction time, hand-eye coordination and letter deletion tests) was studied in patients undergoing colonoscopy with 50 % nitrous oxide used for sedation (n = 12). Recovery, as judged by a return to baseline in psychomotor function tests, was complete within 30 minutes in all patients (Trojan et al., 1997). Full recovery within half an hour was confirmed in another study in colonoscopy patients using a letter cancellation test (Maslekar et al., 2009). In another study, where volunteers were exposed to 30 % nitrous oxide for two periods of 40 minutes, 45 minutes apart, there was complete recovery of mental and psychomotor skills after 22 minutes, as measured by free recall, tapping board, arithmetic and flicker fusion tests (Korttila et al., 1981).

Abuse liability and dependence-producing potential

The psychotropic properties of nitrous oxide were first described by Humphrey Davy in 1800 (Cartwright, 1972; Gillman, 2019; Smith, 1965b), including references to its abuse liability and dependence-producing potential (Yagiela, 1991):

> *"The desire of some individuals acquainted with the pleasure of nitrous oxide for the gas has been so strong as to induce them to breathe with eagerness, the air remaining in the bags after the respiration of others."*

Neuropharmacology and the dependence-producing potential of nitrous oxide

It has been suggested that the reinforcing effects of nitrous oxide may be primarily mediated by its NMDA antagonistic properties, similar to ketamine (Jevtović-Todorović et al., 1998). Indeed, glutamate homeostasis mediated by the NMDA receptor in the nucleus accumbens (NAcc) is important in the establishment and maintenance of drug-seeking behaviour (Carter et al., 2009; Scofield et al., 2016), and it has been shown that genetic variability in the NMDA receptor may affect susceptibility to developing nitrous oxide dependence (Walsh et al., 2017; Zacny et al., 2008). Considering that administration of NMDA antagonists may reduce drug-seeking behaviour, it could be possible that nitrous oxide has value in the treatment of substance use disorder (Daynes and Gillman, 1994; Gillman and Lichtigfeld, 1990, 1991; Gillman et al., 2007). Nonetheless, considering the complexity of glutamatergic neural networks in the brain regions involved in reward mechanisms, such as the NAcc and the ventral tegmental area (VTA) (Morales and Margolis, 2017; Scofield et al., 2016), more research may be needed in order to fully understand the relevance of nitrous oxide in modulating dependence to other substances. As an example, for ketamine, it is hypothesised that it blocks NMDA receptors on GABA neurons inside the thalamic reticular nucleus, which leads to disinhibition of dopaminergic neurons and increased release of dopamine (Liu et al., 2016). Recent studies in mice suggest that ketamine's antidepressant properties are mediated by strengthening a recurrent neural circuit between the VTA and the medial prefrontal cortex (mPFC), enhancing firing of VTA dopaminergic neurons (Marcus and Bruchas, 2021; Wu et al., 2021). Whether and how nitrous oxide acts by similar mechanisms, ultimately leading to the establishment of drug dependence, remains to be discovered by further research.

It is possible that the dopamine system may be involved in the reinforcing effects of nitrous oxide. Indeed, it has been shown that nitrous oxide activates dopamine neurons in the VTA and that its anti-nociceptive activity can be attenuated by blocking D_2-like receptors in the NAcc of rats (Sakamoto et al., 2006; Koyanagi et al., 2008). Dopaminergic signalling in these pathways is pivotal in reinforcement and reward, and overactivation of this network may lead to addictive behaviours (Carter et al., 2009).

Nitrous oxide can also prevent the amphetamine-induced increase in carrier-mediated dopamine release in the NAcc and block locomotor sensitisation to amphetamine (David et al., 2006) ([2]). Furthermore, in mice, nitrous oxide impaired the acquisition of cocaine- or morphine-induced conditioned place preference (CPP), and in drug-free rats and mice, it was able to suppress morphine-induced CPP and the associated increase in dopamine release in the NAcc (Benturquia et al., 2007, 2008) ([2]), which suggests that nitrous oxide could be effective in the treatment of drug withdrawal syndrome.

In addition, it could be that the GABAergic system is involved in some of the reinforcing effects of nitrous oxide, as supraclinical doses of the benzodiazepine antagonist flumazenil attenuated some of the subjective effects of nitrous oxide in volunteers (Zacny et al., 1995).

After the discovery of the endogenous opioid system in the 1970s, it was initially proposed that the abuse potential of nitrous oxide may be mediated through this system (Gillman, 1986). While there is evidence of opioid receptor involvement in the analgesic effects of nitrous oxide, the role of the opioid system in its other effects, including those effects more directly related to its dependence-producing potential, is not so clear. Indeed, the subjective and psychomotor effects of nitrous oxide in humans are not antagonised by naloxone even at high doses, and nitrous oxide does not have morphine-like discriminative stimulus effects in rats (Balster, 1998).

Finally, considering that administration of 60 % nitrous oxide to nitrous oxide-naïve male Long-Evans rats significantly increases circulating levels of corticosterone, adrenaline and noradrenaline in rats (Al-Noori et al., 2018), it may be that some of the reinforcing effects of nitrous oxide are mediated via its action on the hypothalamic-pituitary-adrenal axis pathway. Indeed, some addictive substances have been shown to activate this axis, causing increased plasma levels of glucocorticoids (Koob and Le Moal, 2001).

([2]) Research supported by a grant from Air Liquide (a nitrous oxide manufacturer) and/or NNOXE Pharmaceuticals.

Animal behavioural studies

Animal studies investigating the reinforcing and rewarding properties of nitrous oxide are relatively scarce, possibly because of the challenges in constructing a model where the self-administration paradigm can be tested. Nonetheless, a straightforward approach was undertaken by Wood et al. (1977), who showed that squirrel monkeys will self-administer nitrous oxide when seated with a gas delivery helmet secured over their head. After learning to press a lever to administer 60 % nitrous oxide for one minute, subjects administered as many as 200 fifteen-second doses of nitrous oxide during a 1-hour session. Self-administration of nitrous oxide was also demonstrated in rhesus monkeys (Walker and Zacny, 2003).

Furthermore, Ramsay et al. (2003) modified a CPP model to investigate nitrous oxide self-administration in rats. There was conflicting evidence on the abuse potential of nitrous oxide, as some rats trained in a CPP paradigm for 8 days displayed conditioned place aversion (CPA), while others displayed CPP or no apparent conditioning. In a free-choice self-administration paradigm, two rats self-administered nitrous oxide, one avoided nitrous oxide and one rat had no preference. In subsequent studies, these researchers built a self-administration apparatus where rats were trained to self-administer nitrous oxide by entering a side chamber where the gas was delivered. Researching interindividual differences in vulnerability to developing self-administration, they showed that rats that initially had a low pharmacological response to nitrous oxide, as measured by hypothermia, eventually developed a stronger pattern of self-administration than rats that were initially sensitive to the pharmacological effects of nitrous oxide (Ramsay et al., 2015).

Tracy et al. (2014) explored the use of an intracranial self-stimulation (ICSS) model in mice to study the rewarding effects of nitrous oxide. Their results suggest that, while nitrous oxide showed some reinforcement-enhancing effects on brain reward systems, these were much weaker than those with toluene or diazepam.

Using a drug-discrimination model in mice, a number of site-selective NMDA antagonists were tested for their ability to substitute for nitrous oxide. The results showed that the discriminative stimulus effects of nitrous oxide are at least partially mediated by NMDA antagonist effects. However, as none of the drugs tested fully mimicked the stimulus effects of nitrous oxide, other mechanisms may also be involved (Richardson and Shelton, 2015). An older study showed that nitrous oxide generalised in a concentration-dependent manner in rats trained to discriminate the

partial κ-opioid agonist ethylketocyclazozine from saline (Hynes and Hymson, 1984).

In addition to behavioural experiments, studies have also demonstrated the phenomenon of tolerance in mice (Dzoljic et al., 1994) and cross-tolerance with alcohol or morphine, as well as withdrawal (Belknap et al., 1987, 1993; Berkowitz et al., 1979; Koblin et al., 1980).

Human data

Underlying interindividual differences

Similar to other drugs or addicting behaviours, some pre-existing individual vulnerability factors might increase the risk of nitrous oxide abuse.

Interestingly, depressive and impulsivity traits affected self-reported rewarding (liking and wanting) in volunteers exposed to nitrous oxide. Whereas those with depressive symptoms indicated less rewarding effects, those with high scores for impulsivity reported more rewarding effects (Kamboj et al., 2021), a finding that complements a larger body of research on the relationship between impulsivity and problematic drug use (Verdejo-García et al., 2008).

Heavy drinkers selected nitrous oxide more frequently in a drug choice procedure and liked inhalation of nitrous oxide more than inhalation of air relative to light drinkers (Zacny et al., 2008). Studies with heavy drinkers and non-dependent healthy individuals with a family history of alcohol use disorder (AUD) showed differences in the degree of stimulating versus sedative effects of nitrous oxide when compared with non-AUD individuals without an AUD family history. It was suggested that underlying interindividual differences in NMDA receptor regulation are behind these differences (Walsh et al., 2017).

Intra- and interindividual variability in drug-liking and reinforcing effects

As already mentioned, the effects of nitrous oxide can vary between individuals, with some experiencing pleasant effects while others dislike the experience. Further laboratory studies with volunteers showed that the reinforcing effects of nitrous oxide are dose dependent and show considerable interindividual as well as intraindividual variation. Some individuals show a bitonic-like dose-response curve for drug liking and/or drug preference (10 % to 50 % nitrous oxide concentration) — that is, increasingly choosing nitrous oxide over placebo when progressing from small to medium doses, with a decreasing trend when progressing from medium to large doses. However, other volunteers showed monotonic increases in nitrous oxide choice as a function of dose, and some did not show evidence of reinforcing effects from nitrous oxide. These studies failed to demonstrate a strong correlation between drug liking and euphoria on the one hand and between drug liking and drug choice on the other, which in turn underscores the large between- and within-subject variability in terms of subjective effects in non-drug-using volunteers (Walker and Zacny 2001, 2002, 2003). An earlier study in non-drug-using volunteers, using a different setup without a 'drug-free choice', showed a decreasing trend in nitrous oxide preference in the majority of volunteers when progressing from 30 % to 40 % nitrous oxide. In that study, there was a clear correlation between drug liking and drug preference (Dohrn et al., 1993). Together, these studies on the reinforcing effects of nitrous oxide in non-drug-using volunteers point to intra- and interindividual variability in drug liking and reinforcing effects with nitrous oxide and suggest that higher doses evoke aversive effects in the majority of volunteers.

Substance use disorder

In 2021, the French National Agency for the Safety of Medicines and Health Products (ANSM) published the results of nitrous oxide monitoring carried out by the national addictovigilance system. In terms of effects reported in the notifications, those related to substance use disorders were the most common: 72 % of the 119 cases involved abuse, dependence, daily use and/or consumption of doses exceeding 20 cartridges per session or per day (CEIP-Addictovigilance de Nantes, 2021).

Fidalgo et al. investigated the dependence-producing properties of nitrous oxide by reviewing the literature and cases collected by the French Monitoring Centre for Addiction (CEIP-A) network on EMONO use disorder (Fidalgo et al., 2019). EMONO is an equimolar mixture of oxygen and nitrous oxide, which is used in France for medical purposes. The predominant DSM-5 criteria for substance use disorder were present in 59 cases identified in the literature and in 17 cases reported to the CEIP-A network. These were 'Taking the substance in larger amounts or over a longer period than was intended' and 'A great deal of time is spent in activities necessary to obtain the substance, use the substance or recover from its effects'. In addition, for the CEIP-A cases, a cluster of three additional criteria were identified: 'Recurrent use of the substance resulting in a failure to fulfil major role obligations at work, school, or home', 'Continued use of the substance despite having persistent or recurrent social

or interpersonal problems caused or exacerbated by the effects of its use', and 'Important social, occupational, or recreational activities are given up or reduced because of use of the substance'. For the majority of cases reporting social consequences, these involved interpersonal problems with hospital staff. This study identified that besides recreational users, a separate group of users exists who start taking nitrous oxide for medical reasons, but subsequently develop a substance use disorder.

More recently, case reports have been published describing chronic abuse and dependence (Sun et al., 2019), tolerance (Marotta and Kesserwani, 2020; Selvaraj and Wong, 2017), loss of control over consumption and excessive preoccupation with the product as well as signs of withdrawal and difficulties in stopping use (Selvaraj and Wong, 2017) and relapse after stopping (den Uil et al., 2018).

Mancke et al. (2016) reported on a polysubstance-abusing patient for whom two months' daily use of up to 50 cartridges nitrous oxide per day met the following DSM-IV-TR criteria for substance dependence: tolerance, continued use despite deleterious consequences, use over a longer period of time than intended, significant time spent obtaining, administering or recovering from the effects of the product, and reduced social, occupational or recreational activities. In addition to tolerance with the anxiolytic effects, Berger-Vergiat et al. (2019) described withdrawal symptoms leading to difficulty in stopping the product in a medical student who started using nitrous oxide at parties and then as an analgesic self-medication for irritable bowel syndrome.

In qualitative research among recreational nitrous oxide users, it was observed that it is common for users to initially increase their use to some extent, only to later reduce it or completely stop using the gas. Heavy users in this study scored low on a severity of dependence scale: only 20 % indicated that they had considered stopping and 95 % thought it would not be difficult to stop (Nabben et al., 2017). Nevertheless, in an exploratory study that interviewed Moroccan-Dutch young adults (Nabben et al., 2021), the problematic users of nitrous oxide reported continued use that qualifies for a diagnosis of substance use disorder under the DSM-5 criteria, considering the physical, mental, social and financial problems that they described. Excessive use among some users, craving, the desire to continue use even when the tank is empty, and spending large amounts of money on nitrous oxide has been reported in another study among young adults with a non-Western migration background, suggesting the development of substance use disorder in a subgroup of problematic users (Spronk et al., 2020a, 2020b). A case of compulsive continuous nitrous oxide use associated with aggressive behaviour was recently reported by the Dutch Poisoning Information Centre (Nugteren-van Lonkhuyzen et al., 2021). Although there have been anecdotal reports of difficult discontinuation symptoms in heavy nitrous oxide users in the media, the scientific evidence to suggest this is currently limited. However, the persistent and extreme patterns of use in problematic users (Nabben and Bahara, 2020; Nabben et al., 2021; Spronk et al., 2020a, 2020b), and the continuation of nitrous oxide use by a user already suffering from ataxia (Blair et al., 2019), show that significant substance dependence can develop following heavy nitrous oxide use. Considering that very heavy users may consume large quantities of nitrous oxide in a single sitting, it may be possible for tolerance to develop. Indeed, tolerance to the analgesic effects of nitrous oxide was noted after 150 minutes of continuous exposure to the gas (Rupreht et al., 1985), though it is unclear whether this tolerance might be quickly reversed upon discontinuation.

In conclusion, an important primary pharmacological target of nitrous oxide is the NMDA receptor. This receptor is present throughout the brain and active in many neural networks, including the brain reward networks. As such, nitrous oxide may be susceptible to abuse, but may also reduce drug-seeking behaviour for other substances. Other neurotransmitters, including dopamine, endogenous opioids and GABA, may be involved as well. The reinforcing properties of nitrous oxide have been demonstrated in self-administration studies in animals and laboratory studies in humans. The reinforcing properties may drive the continuation of nitrous oxide use. However, interindividual differences in drug liking versus drug aversion may affect susceptibility to the reinforcing properties of nitrous oxide. Furthermore, social and cultural factors are also relevant in determining whether people initiate and continue use. Some users engage in very frequent and heavy use and their behaviour qualifies for a diagnosis of substance use disorder. While information on the abuse liability and dependence potential of nitrous oxide is currently limited, some users do develop drug dependence, and their nitrous oxide use can become problematic with serious social and health consequences.

Health risks

Historically, the inhalation of nitrous oxide has been perceived as a rather harmless practice, which has contributed to a general perception among users that nitrous oxide is safe. While occasional use of nitrous oxide might have relatively low associated risks, regular use or use of large quantities of nitrous oxide may be linked

to significant health harms, as reported in the scientific literature. In addition, improper administration of nitrous oxide (i.e. directly from a tank) might be linked to a range of additional risks such as severe burns.

There are many factors that may affect the health risks involved in nitrous oxide use. These include the frequency of use, dosage regime, patterns of use, method of administration, ingestion of any additional substances, and circumstances under which the substance is used. In addition, interindividual differences may impact the outcome of nitrous oxide use. These include genetic differences, tolerance, overall state of health, underlying medical conditions, concurrent medication, age and gender. Individuals with pre-existing health conditions, in particular cardiovascular or respiratory diseases, or in overall poor health might be at higher risk of experiencing adverse effects than healthy individuals.

Acute adverse effects following recreational use of nitrous oxide can be related to its immediate pharmacological effects when inhaled, the physicochemical properties of the gas, or the manner of administration (large tank vs balloon). Usually, these effects are mild and transitory, although loss of coordination and fainting can result in falls. More serious acute toxicity has been reported occasionally, such as hypoxia, a condition where the tissues receive less oxygen. While in clinical practice nitrous oxide is always mixed with oxygen to prevent hypoxia, for recreational purposes the gas is used in its pure form. When it is inhaled, there is temporarily no oxygen entering the lungs, which decreases the oxygen saturation in the blood and causes general hypoxia. Although this is usually a transient condition, frequently repeated hypoxia may lead to brain damage. In addition, a medically serious condition can occur: pneumomediastinum or pneumothorax, where gas enters the chest outside of the lungs. The inhalation of pressurised gas from a balloon may cause spontaneous rupture of the lung tissue and air may leak to surrounding tissues. Tingling and numbness in the hands or feet can occur acutely and transiently and is not considered an issue unless it is persistent, which may signal peripheral neurotoxicity.

Other risks are related to improper administration of the gas, such as direct administration from a tank. Pressurised gas drops sharply in temperature when it expands. Direct administration of nitrous oxide from tank to mouth may lead to frostbite (burn wounds) as the skin is exposed to the freezing cold gas. Even the nitrous oxide tank itself may become so cold that it can be dangerous. In some cases, users hold a tank between their thighs in order to secure it while filling balloons. Due to the analgesic action of the nitrous oxide being consumed, the user may not feel the cold and serious wounds may be the consequence. Furthermore, in order to prolong the effects of nitrous oxide, some users connect a nitrous oxide tank to a face mask or a plastic bag which is pulled over the head, or they use nitrous oxide in enclosed spaces. This constitutes a high risk of asphyxia and might lead to death.

When used alone, nitrous oxide has limited respiratory depressant effects, but it may potentiate the respiratory depressant effects of other sedatives, hypnotics or opioids.

Driving a vehicle while under the influence can be dangerous as a result of the greatly diminished neurocognitive and psychomotor capacities. Behavioural, neurocognitive and psychomotor tests indicate that most functions have normalised 30 minutes after nitrous oxide administration. However, subjects may still experience fatigue, which may persist 1 hour after cessation of nitrous oxide use. In some regions, an increase in traffic incidents involving nitrous oxide has been reported.

Furthermore, nitrous oxide inactivates the enzyme methionine synthase and depletes vitamin B12 stores, which is the basis for many of the health risks associated with chronic use of nitrous oxide. These health risks include haematological, neurological and cardiovascular disorders. The risks increase with longer duration of use, higher frequency of use and greater intake of nitrous oxide. Recreational users of nitrous oxide with a previous suboptimal vitamin B12 or folate status may be at greater risk of these effects. It should be noted, however, that supplementation with vitamin B12 alone is not sufficient to prevent the disorders in question unless nitrous oxide use is also discontinued.

Haematological disorders may occur following repeated exposure to nitrous oxide. Such effects, including megaloblastic anaemia, are associated with the use of large quantities of nitrous oxide and/or long-term frequent use.

One of the most serious consequences from chronic use of nitrous oxide is myeloneuropathy, which resembles the subacute combined degeneration (SCD) of the dorsal columns of the spinal cord seen with classic vitamin B12 deficiency. A milder neurological symptom, which nonetheless signals that some neural damage has occurred, is persistent paraesthesia (numbness and/or tingling) of the hands or feet. This has been reported by 3.3 % of recreational users of nitrous oxide.

Chronic and frequent use of nitrous oxide is also associated with cardiovascular disease and, more specifically, thromboembolic events.

Psychiatric disorders, mostly diagnosed as a psychotic episode, have been reported in nitrous oxide users. Use of other drugs is common and some patients may have a history of mood disorders. It is therefore not always evident that the psychiatric symptoms are attributable to the use of nitrous oxide. However, frequent use of nitrous oxide may contribute to the precipitation of symptoms in vulnerable individuals. When nitrous oxide use is discontinued and vitamin B12 stores are replenished, most patients recover rapidly from psychotic symptoms. Chronic users and those taking large doses have complained of cognitive deficits such as memory impairment, difficulty concentrating and poor responsiveness.

Nitrous oxide-induced oxidative stress may lead to oxidative DNA damage. Minimal oxidative DNA damage following short-term and incidental use is likely to be repaired in individuals who are otherwise healthy and are not genetically or environmentally predisposed to an increased risk of cancer. However, an increased risk of genotoxicity is likely and an increased risk of cancer cannot be ruled out in those maintaining frequent use patterns.

Regarding the effect of nitrous oxide on fertility, the available evidence is limited and conflicting, particularly for recreational users, for whom no studies are available. If such an effect exists, it is most likely reversible upon discontinuation.

However, the available evidence indicates that nitrous oxide has embryofoetal developmental toxicity, which may lead to decreased survival of the conceptus. These effects also occur after intermittent exposure. Based on animal studies, congenital malformations should be considered a potential risk for embryos carried by recreational users who consume nitrous oxide repeatedly and/or in large quantities during early pregnancy. In humans, there is evidence suggesting an increased risk of pregnancy loss.

Concerns about neurocognitive development have been expressed in connection with anaesthetic exposure. The significance of these findings to recreational users of nitrous oxide is unclear, but they are probably of minor concern since the association is less clear with nitrous oxide. Theoretically, exposure would need to take place during late pregnancy and/or early childhood, and co-exposure to GABA-mimetic compounds would probably be required before brain development could be potentially affected.

Nitrous oxide might also pose a risk of immunosuppressive effects, which may potentially relate to reduced immune cell production as a consequence of extended exposure, or to direct modifications of the function of these cells.

It is important to note that for most of the above health risks, there is a dose-effect relationship showing that the harmful effects of nitrous oxide increase in frequency and severity in line with increasing doses and frequency of use.

Acute toxicity

Acute adverse effects following recreational use of nitrous oxide may be related to its immediate pharmacological effects when inhaled, as well as to the physicochemical properties of the gas or the method of administration (large tank vs balloon). Usually, these side effects are mild and transient, although more serious acute toxicity has been reported occasionally.

Hypoxia

For medical use, nitrous oxide is always administered as a mixture with oxygen to prevent hypoxia, but recreational use of nitrous oxide involves balloons filled with pure nitrous oxide. When pure nitrous oxide is inhaled, no oxygen will enter the lungs, thus decreasing the oxygen saturation in the blood and causing general hypoxia. This is a transient condition, because usually the user intermittently breathes from the balloon and takes air. In cases where the hypoxia is maintained for too long, the user may develop purple-coloured lips and eventually faint. Frequently repeated hypoxia may lead to brain damage (Araki et al., 1990; Park et al., 2021). Nonetheless, it is possible that the NMDA antagonistic property of nitrous oxide could have some protective effect in this respect (Kato et al., 1990). However, heavy users report that they experience cognitive deficits such as memory loss, difficulty concentrating and poor responsiveness, suggesting brain damage may have occurred (Spronk et al., 2020a, 2020b). This is particularly concerning considering the widespread use among young people whose brains are still developing.

Additionally, diffusion hypoxia may occur (Brodsky and Cohen, 1986), also referred to as the Fink effect (Fink, 1955). When larger quantities of nitrous oxide have entered the bloodstream and have been distributed to the tissues, upon cessation of nitrous oxide administration, the gas makes its way back to the lungs, crossing the blood-lung barrier. In the alveoli, the oxygen, nitrogen and carbon dioxide are diluted by nitrous oxide and their partial

pressure drops, causing their saturation in blood to drop as well. In Fink's experiment, the post-anaesthesia drop in arterial oxygen saturation was approximately 8 %. In addition, decreased carbon dioxide pressure, combined with a blunted hypoxic response (Yacoub et al., 1976), prevents a compensatory increase in ventilation, and thus maintains the hypoxic condition for up to 10 minutes until most of the nitrous oxide has been exhaled. In anaesthetic practice, this phenomenon is compensated by administration of oxygen after nitrous oxide exposure. There are no data describing to what extent diffusion hypoxia will occur in recreational users of nitrous oxide, but it may be that taking several balloons in a row could increase the likelihood of occurrence.

The most serious acutely occurring consequence of hypoxia is transient loss of consciousness, which may lead to falls or accidents. Other less serious side effects associated with hypoxia may be headaches, dizziness, confusion and disorientation. It is possible that headaches can also be attributed, to some extent, to increases in intracranial blood pressure (Fee and Thompson, 1997).

Falls

Voluntary participants in studies and recreational users indicate that they experience diminished control over their body. Increases in body sway are also noted by observers. This reduced motor coordination may lead to tripping or falling and subsequent injuries. Loss of consciousness as a result of hypoxia further increases the risk of falls.

Nausea

Nausea is a frequently mentioned side effect in voluntary participants in laboratory studies, as well as among recreational users. Nausea may or may not be accompanied by vomiting.

Burns (frostbite)

Pressurised gas drops sharply in temperature when it expands. Direct administration of nitrous oxide from a tank to the mouth may lead to frostbite (cold burns) as the skin is exposed to the freezing cold gas (Chan et al., 2018; Garakani et al., 2016; Hwang et al., 1996). Even the nitrous oxide tank itself may become so cold that it can be dangerous. When a tank is held between the thighs, and the user does not notice the cold due to the analgesic action of the nitrous oxide being consumed, serious wounds may be the consequence. Because the skin abnormalities are often minor in the first few days after freezing, the severity of this injury is not always immediately recognised (van Munster et al., 2020; Quax et al., 2022). In the first half of 2019, in specialised burns clinics in the Netherlands, 19 cases were reported of persons with burn injuries related to holding a nitrous oxide tank between the thighs, and these were serious enough to require specialised care (CAM, 2019).

Pneumomediastinum and pneumothorax

A medically serious condition may occur, known as pneumomediastinum or pneumothorax, when gas enters the chest outside of the lungs (Eger and Saidman, 1965; Garbaz et al., 2007; Jeddy et al., 2016; Kaur et al., 2001; McDermott et al., 2015; Tavare et al., 2018). The inhalation of pressurised gas from a balloon may cause spontaneous rupture of lung tissue and air may leak to the surrounding tissue. When air-filled spaces are formed, subsequent administration of nitrous oxide may aggravate the condition, since nitrous oxide may enter the space faster than air can leave it due to their different blood/gas partition coefficients. The same mechanism may also reveal pre-existing asymptomatic pneumomediastinum following administration of nitrous oxide.

Death

Reported deaths associated with the use of nitrous oxide appear to be caused by secondary effects, most notably asphyxiation, and are not a direct toxic effect of the gas. If inappropriate methods are used to administer nitrous oxide for recreational purposes, this can lead to death. Users may connect a nitrous oxide tank to a face mask or a plastic bag that is pulled over the head in an attempt to prolong the effects, achieving a continuous flow of nitrous oxide which might cause severe hypoxia. A blunted hypoxic response and sedation prevents the victim from removing the mask or bag, eventually leading to asphyxiation (Garakani et al., 2016; Long, 2019; Yacoub et al., 1976; Schwark et al., 2022). A number of deaths in the United Kingdom involved the use of nitrous oxide in an enclosed space (ACMD, 2015). Motor vehicle accidents secondary to nitrous oxide use have also been mentioned as a potential cause of fatalities (Long, 2019), but no specific cases are reviewed in the literature (Long, 2019; Garakani et al., 2016).

Chronic toxicity

Chronic exposure to high doses of nitrous oxide might lead to poor vitamin B12 function and to a range of haematological, neurological, cardiovascular and psychiatric consequences.

Nitrous oxide, one-carbon metabolism, hyperhomocysteinaemia and vitamin B_{12}

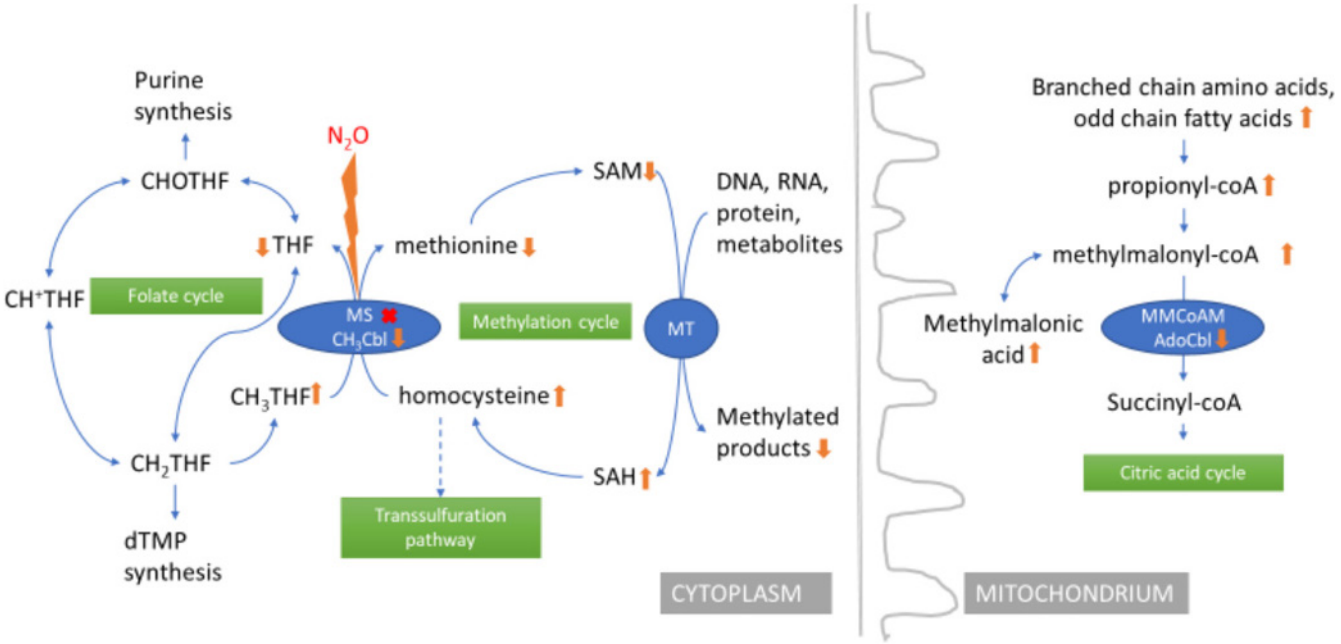
In biochemical terms, most of the toxicity observed after repeated exposure to nitrous oxide is considered to result from oxidative inactivation of the enzyme methionine synthase by nitrous oxide and the subsequent loss of vitamin B12 and changes in one-carbon metabolism.

Vitamin B12 is the common name for several forms of cobalamin, two of which are essential coenzymes: methylcobalamin and adenosyl-cobalamin. For the purpose of this review, the terms vitamin B12 and cobalamin are used interchangeably.

Administration of nitrous oxide has profound effects on cobalamin function. Nitrous oxide exposure rapidly reduces the activity of methionine synthase with a half-life of 5.4 minutes in rat liver and 46 minutes in human liver (Koblin et al., 1982; Kondo et al., 1981; Royston et al., 1988). Methionine synthase binds methylcobalamin as a cofactor and converts homocysteine and N^5-methyltetrahydrofolate (CH_3THF) to methionine and tetrahydrofolate (THF). This is a crucial step in one-carbon metabolism, fuelling the formation of S-adenosylmethionine (SAM), the most important cellular methyl-donor (Froese et al., 2019). Methionine synthase is also the only enzyme that can free THF from CH_3THF, the dominant circulating form of folates (Pfeiffer et al., 2015). THF is needed for the folate cycle, which is essential for purine and thymidine synthesis. The latter are building blocks for DNA (Figure 5).

In particular, nitrous oxide reacts with methionine synthase by oxidising the cobalt atom, which is at the core of the cobalamin coenzyme in its most reduced form, Co(I). In this reaction, nitrous oxide is transformed to nitrogen and a radical is released, which causes oxidative damage to the enzyme and renders it inactive (Drummond and Matthews, 1994a; Frasca et al., 1986). The methionine synthase can be replenished by *de novo* synthesis, but this takes time. In turn, when nitrous oxide exposure continues, a deficiency in vitamin B12 gradually develops, since part of the cobalamin coenzyme is transformed into cobalamin analogues. These analogues are inactive

FIGURE 5
Simplified scheme representing the metabolic functions of vitamin B12 and the effect of nitrous oxide.



Note: Nitrous oxide inactivates methionine synthase, and methylcobalamin, the active form of vitamin B12 in this enzyme, diminishes. The substrates homocysteine and methylTHF rise and the products methionine and THF go down. Both methylation and the folate cycle are affected. Loss of vitamin B12 also reduces adenosyl-cobalamin, the active coenzyme for methylmalonyl-CoA mutase. Methylmalonic acid rises and earlier substrates cannot be catabolised. Diagram based on Froese et al. (2019). AdoCbl: adenosyl-cobalamin; CH+THF: 5,10-methenyltetrahydrofolate; CH2THF: 5,10-methylenetetrahydrofolate; CH3Cbl: methylcobalamin; CH3THF: N5-methyltetrahydrofolate: CHOTHF: 10-formyltetrahydrofolate; dTMP,: deoxythymidine monophosphate; MMCoAM: methylmalonyl-CoA mutase; MS: methionine synthase; MT: methyltransferases; SAH: S-adenosyl-homocysteine; SAM: S-adenosyl-methionin

or less active as a cofactor when bound to the enzymes and will be excreted from the cells (Drummond and Matthews, 1994b; Froese et al., 2019; Kondo et al., 1981; Riedel et al., 1999). In addition, this deficiency also affects the function of a mitochondrial enzyme, methylmalonyl-CoA mutase (MMCoAM) (Kondo et al., 1981). MMCoAM binds adenosyl-cobalamin as coenzyme, converts methylmalonyl-CoA to succinyl-CoA, and thus certain odd chain fatty acids and branched chain amino acids can be catabolised and enter the citric acid cycle (Froese et al., 2019). Deficiencies in methionine synthase and MMCoAM activity cause an accumulation of their substrates, which is reflected in hyperhomocysteinaemia, homocystinuria and methyl malonic acidaemia. As such, serum vitamin B12 status is an imperfect measure of cobalamin function, and homocysteine and methyl malonic acid levels are important indicators of deficiencies in cobalamin function as well (Hannibal et al., 2016; Marsden et al., 2022; Oussalah et al., 2019). Homocysteine may be catabolised through the transsulfuration pathway, leading to the synthesis of cysteine and glutathione. However, many tissues lack a complete transsulfuration pathway, which is only fully functional in liver, kidney, small intestine and pancreas (Finkelstein, 1998).

Haematological disorders

Inhibition of methionine synthase may lead to significant haematological complications such as megaloblastic anaemia (Reynolds, 2006). The earliest report on bone marrow suppression and haematological disorders was presented by Lassen et al. (1956), when tetanus patients were exposed to nitrous oxide for days and developed severe anaemia. Nonetheless, based on a review of reports of bone marrow suppression in patients who have been exposed to nitrous oxide in a clinical setting, the data suggest that exposure times of less than 6 hours in a medical setting are safe with regard to bone marrow changes. However, there are no data available determining the risks and consequences of providing general anaesthesia with nitrous oxide to patients with a pre-operatively undetected deficiency in vitamin B12 or folate (Weimann, 2003). In a meta-analysis of individual patient data from patients exposed to nitrous oxide (in recreational or medical settings) and for whom at least one health outcome was reported, individuals had a higher risk of macrocytic anaemia if they had a median mean corpuscular volume (MCV) of 100 fL (interquartile range (IQR): 94-103) and median haemoglobin values of 12.8 g/dL (IQR: 10.8-14.2) and 10.7 g/dL (IQR: 8.3-12.4) in males and females respectively. At least one haematological abnormality was found in 71.7 % of cases (59.9-83.4 %). The proportions of patients with low haemoglobin level, low haematocrit level and MCV >100 fL were 55.8 %, 52.4 % and 41.8 % respectively (Oussalah et al., 2019).

As some recreational users of nitrous oxide may have suboptimal B12 or folate levels that they are not aware of, it appears likely that some will develop haematological disorders at a faster rate. Such abnormalities will usually then be revealed at the time medical help is sought and haematological parameters are investigated. It also needs to be considered that repeated exposure to nitrous oxide depletes the vitamin B12 stores and that replenishing methionine synthase takes time, thus increasing the risks of haematological disorders with repeated exposure.

Neurological disorders

Tingling and numbness in the hands or feet (paraesthesia) are common side effects of nitrous oxide, also reported by nitrous oxide-naïve volunteers after a single dose. Where this effect is short-lived, it is of little concern. However, paraesthesia persisting for hours or weeks is a sign of peripheral neuropathy. In the GDS covering the years 2014-2016, 3.3 % of users reported persistent paraesthesia (Winstock and Ferris, 2020).

This neurotoxicity can further develop to one of the most serious consequences of chronic nitrous oxide use: myeloneuropathy resembling the subacute combined degeneration (SCD) of the dorsal columns of the spinal cord as caused by vitamin B12 deficiency. Different symptoms reflect the varying involvement of the posterior columns, the corticospinal tracts and the peripheral nerves. In users with neurological disorders, numbness and tingling of the distal extremities is the most common complaint (80 %), followed by gait disturbances or ataxia (70 %), weakness (43 %), falls or equilibrium disorders (24 %) and Lhermitte's sign, an electric shock sensation from the back into the limbs with neck flexion (15 %). Physical examination reveals diminished sensation to pinprick and light touch, vibratory sensation and proprioception, hyperreflexia, spasticity, urinary and faecal incontinence and reduced extensor plantar response. Among reported patients with nitrous oxide-associated neurotoxicity who had documented concentrations of vitamin B12, approximately 50 % were deficient in the vitamin. Results were normal in the few patients who underwent a Schilling test, which evaluates the capacity to absorb vitamin B12 from the bowel using radiolabelled vitamin B12. Elevated concentrations of methylmalonic acid and homocysteine are often reported (Marsden et al., 2022), even when vitamin B12 concentrations are normal. Magnetic resonance imaging of the spinal cord may reveal symmetric enhancement and oedema of

the dorsal columns, referred to as the inverted V-sign. Nerve conduction studies and electromyography typically reveal distal, axonal sensorimotor polyneuropathy (Long, 2019; Oussalah et al., 2019). Common diagnoses have included myeloneuropathy, SCD, peripheral neuropathy or polyneuropathy and myelopathy. Most patients were treated with cyanocobalamin (a pharmaceutical form of vitamin B12) and showed improvement of symptoms upon treatment (Garakani et al., 2016; Lan et al., 2019). However, when nitrous oxide use is not discontinued, cobalamin supplementation may not improve outcomes (Blair et al., 2019).

Despite the apparent association between nitrous oxide exposure, poor cobalamin function and myeloneuropathy, the precise mode of action is still a matter of debate (Hathout and El-Saden, 2011). Early on, it was thought that MMCoAM deficiency was the culprit, leading to sequestration of abnormal fatty acids and phospholipids in cellular membranes and eventually disrupting normal formation of the myelin sheath of nerves. However, no compelling evidence could be found for increased odd chain and branched chain fatty acids in the neural tissues. Furthermore, patients with methylmalonic acidaemia or aciduria from inherited defects in adenosyl-cobalamin synthesis or the MMCoAM enzyme develop lethargy, failure to thrive, muscular hypotonia and intellectual disability, but no subacute combined degeneration. Thus, the MMCoAM hypothesis was abandoned in favour of the methylation hypothesis whereby deficiency in methionine synthase is the culprit (Hathout and El-Saden, 2011; Manoli et al., 2005; Metz, 1992).

Regarding the latter hypothesis, a clear consensus has not been fully reached: Initially, it was thought that diminished capacity of the methylation cycle would reduce the methylation of myelin proteins and lipids and thus interfere with normal myelin formation. However, subsequent research showed that this hypothesis could not be upheld and other possible explanations needed to be explored (Hathout and El-Saden, 2011), considering the wide number of genes, enzymes and molecular pathways implicated in the homeostasis of neural tissue, axonal integrity and myelin formation (Froese et al., 2019). In turn, a potential explanation is that toxicity may be caused by elevated homocysteine levels (Bleich et al., 2004; Savage and Ma, 2014). The effect of homocysteine on neural tissue is influenced by the absence within this tissue of two of the major metabolic routes for eliminating homocysteine: betaine-mediated conversion and transsulfuration (Finkelstein, 1998). Homocysteine is, like glutamate, an excitatory amino acid and markedly enhances the vulnerability of neuronal cells to excitotoxic and oxidative injury *in vitro* and *in vivo*. Elevated homocysteine levels could cause neurotoxicity by activating the NMDA receptor, causing an influx of Ca^{2+}, formation of reactive oxygen species and subsequent cell apoptosis. Furthermore, not only may neuronal cells be at risk for homocysteine-induced toxicity, but the supporting glial cells can also be disrupted (Wyse et al., 2021). As is noted with other pathologies associated with hyperhomocysteinaemia, it remains a challenge to disentangle cause and consequence and to fully understand the modes of action that explain the association between methionine synthase inhibition and pathogenesis (Smulders and Blom, 2011).

Other studies have shown that TNF-α (a pro-inflammatory cytokine), a soluble dyad of CD40:CD40 ligand (a member of the TNF superfamily) and nerve growth factor (NGF) are elevated in spinal tissue in an animal model of B12 deficiency-induced myeloneuropathy. These cytokines and growth factor aggravate myeloneuropathy and antibodies against them can ameliorate the B12 deficiency-induced myeloneuropathy. Inversely, IL-6 (an anti-inflammatory cytokine) and epidermal growth factor show myeloneuroprotective effects (Hathout and El-Saden, 2011; Scalabrino et al., 1999, 2000, 2004, 2006, 2007; Veber et al., 2006). These data show that, at some point, inflammatory processes indeed contribute to the myeloneuropathy induced by vitamin B12. Of note in this respect is a recent report showing a favourable outcome from combining the anti-inflammatory drug methylprednisolone with vitamin B12 supplements in an SCD patient with a history of recreational nitrous oxide use (Zhang et al., 2021).

Some clinical data hint at additional nitrous oxide neurotoxicity driven by cobalamin deficiency (Tani et al., 2019). When vitamin B12-deficient myeloneuropathic patients (n = 6) were compared with nitrous oxide users with myeloneuropathy (n = 8), the latter showed more prominent motor superexcitability changes and less prominent sensory superexcitability changes. In addition, spinal MRI revealed more prevalent T2 hyperintensity in the nitrous oxide group. Clinical evaluation of muscle strength and neuropathy of the lower limbs showed greater impairment in the nitrous oxide group. The functional effects observed in both groups probably reflect a combined effect of axonal loss and focal demyelination. These results should be interpreted with caution, as the study is small and vitamin B12 levels in the nitrous oxide users were lower than in the vitamin B12-deficient group (111 vs 236 pg/ml), although plasma homocysteine levels were comparable. Similarly, a recent retrospective study compared nitrous oxide-related SCD patients (n = 50) with nitrous oxide-unrelated SCD patients (n = 48) (Gao et al., 2021). In this study, the nitrous oxide-related SCD patients

also had more obvious MRI changes, but less severe clinical presentations. In contrast to the previous study, nitrous oxide-related SCD patients had higher vitamin B12 levels than nitrous-oxide-unrelated SCD patients (218 vs 96 pg/ml), but homocysteine levels were comparably elevated in approximately 80 % of each group.

All the above would suggest that the reduction in methionine synthase activity is integral to the myeloneuropathy associated with vitamin B12 deficiency and nitrous oxide exposure. However, it still needs to be determined how reduced methionine synthase activity leads to myeloneuropathy and to what extent homocysteine toxicity and hypomethylation may play a role. In addition, nitrous oxide may have its own toxicodynamic role interfering with or modulating vitamin B12 deficiency-driven pathogenesis.

A number of papers published in the scientific literature have described nitrous-oxide-associated neurological disorders (Chiew et al., 2021; Largeau et al., 2022; Redmond et al., 2022).

Cardiovascular disease

Mild hyperhomocysteinaemia has been firmly established as an independent predictor of cardiovascular disease (CVD). The normal range of total plasma homocysteine in humans is 5-15 µmol/L and values >13 µmol/L may be considered elevated in adults. A meta-analysis of studies published before 2002 concluded that a 3 µmol/L increase in fasting plasma homocysteine is associated with an 11 % increase in the incidence of ischaemic heart disease and a 19 % increase in the incidence of stroke. There is still debate as to whether elevated homocysteine levels are a causal factor in the pathophysiology of CVD, or whether homocysteine is merely a biomarker reflecting aberrant functioning of the folate and methionine/methylation cycles, and thus it remains unclear whether correcting plasma homocysteine levels is a truly preventive action or not (Hannibal et al., 2016; Smulders and Blom, 2011). While exposure to nitrous oxide does raise plasma homocysteine levels, it could be questioned whether elevation of plasma homocysteine following nitrous oxide exposure constitutes a CVD risk or whether nitrous oxide could pose a CVD risk in other ways by disrupting the cobalamin-dependent one-carbon metabolism. In an individual meta-analysis of patients who were exposed to nitrous oxide in a recreational or medical setting, the mean plasma homocysteine level was 55 µmol/L, which is considered slightly elevated (Oussalah et al., 2019).

However, a meta-analysis of clinical trials where nitrous oxide was used for anaesthesia found that the data were far too sparse to draw any conclusions. There were insufficient data to perform meta-analyses for stroke, myocardial infarction, pulmonary embolus or cardiac arrest. Thus, it was concluded that there is no robust evidence regarding how nitrous oxide used as part of general anaesthesia may affect mortality and cardiovascular complications (Imberger et al., 2014).

In two clinical trials, ENIGMA and ENIGMA-II, the safety of use of nitrous oxide was studied in the setting of anaesthetic practice. The first trial included 2 050 patients having non-cardiac surgery lasting for more than 2 hours and receiving nitrous oxide-based or nitrous oxide-free anaesthesia. Nitrous oxide was found to increase the risk of myocardial infarction in the long-term follow-up, but patients were not selected on the basis of cardiovascular event risk and the event rates were low. In the second study, in 7 112 non-cardiac surgery patients at risk of perioperative cardiovascular events, exposure to nitrous oxide was not found to increase the risk of myocardial infarction (odds ratio: 0.97; 95 % CI: 0.81 to 1.17; $p = 0.78$) or stroke (odds ratio: 1.08; 95 % CI: 0.74 to 1.58; $p = 0.70$) (Leslie et al., 2011, 2015).

Nonetheless, in recent years, cases of thromboembolic events in recreational nitrous oxide users have been reported. In all but one, high or very high plasma homocysteine levels have been detected, including in the presence of normal vitamin B12 levels. Discontinuation of nitrous oxide use and treatment with vitamin B12 reduced these elevated homocysteine levels (Bajaj et al., 2018; Bär et al., 2021; Indraratna et al., 2017; Liu et al., 2020; Oomens et al., 2021a, 2021b; Pratt et al., 2020; Sun et al., 2019; den Uil et al., 2018).

Based on the available evidence, it appears that incidental use of nitrous oxide in the clinical setting is not a risk factor for CVD, but recreational users of nitrous oxide may be at risk. It seems likely that the extended duration of exposure has contributed to this increased risk. Possibly, homocysteine levels need to be elevated (or one-carbon metabolism needs to be disrupted) for extended periods of time to induce CVD.

Psychiatric disorders

Disruption of one-carbon metabolism and associated hyperhomocysteinaemia have been implicated in a range of neurodegenerative and psychiatric disorders, including Alzheimer's disease, intellectual disability and depression. It is recommended to correct metabolic

imbalances with specific B vitamin supplementation (Herrmann et al., 2007; Sangle et al., 2020; Smith and Refsum, 2016; Yu et al., 2020). As chronic use of nitrous oxide causes similar disruption of one-carbon metabolism and hyperhomocysteinaemia, it is possible that nitrous oxide use might precipitate similar disorders. Indeed, case reports have been published on psychiatric disorders associated with nitrous oxide. The reported symptoms include delusions (often grandiose and/or paranoid), confusion, visual hallucinations, bizarre behaviour, panic attacks, anxiety, depression, mania, chronic memory and attentional problems, cognitive impairment, perseveration, lability, verbal aggression, suicide attempt, violent behaviour, and also more subtle personality changes (Chen et al., 2018; Chien et al., 2020; Cousaert et al., 2013; Garakani et al., 2016; Hew et al., 2018; Mancke et al., 2016; Nugteren-van Lonkhuyzen et al., 2021; Roberts et al., 2020; Shen et al., 2021; Spronk et al., 2020a, 2020b; Yu et al., 2020; Zheng et al., 2020). It may be that these effects are also related to the direct pharmacological action of nitrous oxide, particularly on the NMDA receptors.

In some cases, a lack of laboratory data for vitamin B12, homocysteine and methylmalonic acid hinders the possibility of evaluating vitamin B12 and folate status and homocysteine levels. Where available, the relationship between psychiatric disorders and vitamin B12 is unclear since reported levels are sometimes low but in many cases may be normal or even elevated. Malfunction of one-carbon metabolism is usually reflected by hyperhomocysteinaemia, if measured, and 'functional' B12 deficiency may also be evident from methylmalonic acidaemia.

Psychotic disorder is a dominant diagnosis among the cases of nitrous oxide abuse that have been described. The use of other drugs is common and some patients may have a history of mood disorders, which makes it difficult to attribute the psychiatric symptoms to the use of nitrous oxide. For instance, the use of amphetamine or cannabinoids may precipitate psychosis as well. However, in one case, the patient's escalating nitrous oxide use directly preceded psychotic symptoms, suggesting a potential correlation between the two, at least in already vulnerable individuals (Roberts et al., 2020). It appears that patients who discontinue the use of nitrous oxide and start treatment for their vitamin B12 deficiency usually recover rapidly from psychotic symptoms (Zheng et al., 2020).

Exposure-response relationships

Nitrous oxide exposure rapidly reduces the activity of methionine synthase with a half-life of 5.4 min in rat liver and 46 min in human liver (Koblin et al., 1982; Kondo et al., 1981; Royston et al., 1988). In mice, the hepatic enzyme recovers within 1 day, but in rats, there is approximately 70 % recovery in two days. In aged rats, cortical methionine synthase has recovered fully within two days (Culley et al., 2007). In human glioma cells exposed to 50 % nitrous oxide for 96 hours, 50 % recovery was observed after 24 hours, which is consistent with other reports indicating recovery over several days in mice, pigs and rats (Riedel et al., 1999).

Generally, myeloneuropathy is associated with long-term frequent use of nitrous oxide, or use of vast quantities in a shorter period of time (van den Hoven et al., 2022; Lan et al., 2019; Lewis et al., 2021; Marsden et al., 2022; Samia et al., 2020). However, individuals with pre-existing vitamin B12 deficiency may develop deficits sooner (McNeely et al., 2000; Singer et al., 2008). Risk factors for low vitamin B12 levels include advanced age; veganism; vegetarianism; alcoholism; prolonged use of proton pump inhibitors, histamine type 2 receptor inhibitors or possibly metformin; genetic anomalies or autoimmune conditions affecting cobalamin metabolism, such as pernicious anaemia; atrophic gastritis; gastrectomy; Whipple procedure; ileal resection; Crohn's disease; bacterial overgrowth; and tapeworm (Hannibal et al., 2016; Sanders et al., 2008).

Considering the potential neurotoxicity of elevated levels of homocysteine, individuals at risk of hyperhomocysteinaemia may be more susceptible to nitrous oxide-induced myeloneuropathy. Besides those with vitamin B12 deficiency, this would include people with low folate levels (Savage and Ma, 2014; Selzer et al., 2003).

Several reviews of case reports consider that measurements of nitrous oxide exposure are too variable to make an assessment of dose-response relationship (Garakani et al., 2016; Lan et al., 2019), and a meta-analysis that evaluated data on nitrous oxide exposure in 28 patients did not find any significant association with the most frequently reported diagnoses (subacute combined degeneration, generalised demyelinating polyneuropathy and myelopathy), the most commonly reported clinical findings (paraesthesia in extremities, numbness, tingling; unsteady gait, walking difficulty; weakness; and falls or balance disorders), or the presence of T2 signal hyperintensity in the spinal cord (Oussalah et al., 2019).

The dose-dependency of peripheral neuropathy was studied using data from the Global Drug Survey (2014-2016) (Winstock and Ferris, 2020). Data were used from 16 124 participants who had used nitrous oxide in the

last 12 months and had provided responses on age, dose, gender and paraesthesia. Of these, the number of respondents reporting persistent numbness or tingling (paraesthesia) in their hands or feet was 537 (3.3 %). The data were modelled in a multivariate logistic regression model, which predicted a 1.8 % probability of reporting paraesthesia in respondents who consumed smaller quantities and an 8.5 % probability in participants who consumed larger quantities, indicating a clear dose-dependency for reports of paraesthesia by nitrous oxide users. In addition, the multivariate analysis indicated that the association between dose and paraesthesia was influenced by gender and age. The gender effect went in opposing directions depending on dose, with a significantly lower probability for women at the lower end of the dose range and a substantially higher point estimate of probability for women at the higher end of the dose range; however, the latter was not statistically significant. This mixed gender effect is not further explained. The analyses indicate a higher probability of reporting paraesthesia at a younger age than at an older age. Nonetheless, the important limitations of the survey data should be borne in mind, as consumption of nitrous oxide was self-reported and is subject to recall bias. None of the neurological symptoms or functional disturbances reported by users were confirmed by clinical examination and there is no supportive clinical biochemistry data.

Genotoxicity and carcinogenicity

The genotoxic and carcinogenic potential of nitrous oxide has been a topic of debate for many years.

Nitrous oxide has been investigated using established bacterial mutagenicity tests (Ames methodology), although the tests deviated from current standard OECD protocol as a limited set of tester strains was employed (TA1535, TA98 and TA100) (OECD, 2020). These studies did not show evidence of increases in revertants, suggesting a lack of direct DNA reactive mutagenicity (Baden et al., 1979; Baden and Monk, 1981). *In vitro* studies in mammalian cells did not show mutation at the *hprt*-locus in Chinese hamster lung fibroblasts or sister chromatid exchange in Chinese hamster ovary cells (Sturrock, 1977; White et al., 1979).

In a chronic study, Fischer 344 rats were exposed for 104 weeks to a mixture of halothane and nitrous oxide at concentrations of 0 and 0 ppm, 1 and 50 ppm or 10 and 500 ppm for 7 hours per day, 5 days per week, which did not reveal any carcinogenicity (Coate et al., 1979b). Conversely, a study in Sprague-Dawley rats, exposed for 52 weeks with the same gas concentrations and exposure frequencies, showed chromosomal damage in the bone marrow at the highest dose regimen of 500 ppm nitrous oxide and 10 ppm halothane (Coate et al., 1979a). Note that halothane displays mutagenic potential in a fruit fly model, but not in a bacterial mutagenicity test (Baden and Kundomal, 1987). However, chromosomal aberrations or sister chromatid exchange has been reported for halothane in human peripheral lymphocytes (Schifilliti et al., 2011).

In male and female Swiss Webster mice treated with nitrous oxide at concentrations of 0 %, 10 % or 40 % for 4 hours per day, 5 days per week over 78 weeks, the incidence of tumours was not increased (Baden et al., 1986). In another study, ICR mice were exposed to a nitrous oxide concentration of ½ MAC during the second half of pregnancy (4 exposures) and after birth (24 exposures) for 2 hours per day, 3 days per week. There was no indication of nitrous oxide-induced tumour formation after 15 months (Eger et al., 1978). These pre-clinical data indicate that nitrous oxide is not a direct DNA reactive mutagen and that, in rodents, no increased tumour formation is observed after chronic exposure.

Despite the pre-clinical findings indicating a lack of genotoxic and carcinogenic potential, debate about the genotoxic and carcinogenic potential of nitrous oxide still persists (Hogan, 2013; Koblin, 1990; O'Donovan and Hammond, 2015; Vallejo and Zakowski, 2019). This debate has been fuelled by occupational studies among medical or dentistry staff chronically exposed to low concentrations of inhalation anaesthetics, including nitrous oxide, showing genetic damage as evidenced by micronuclei, chromosomal aberration or Comet assays (Braz et al., 2020; Fodale et al., 2008; Karelová et al., 1992; Lewińska et al., 2005; Neghab et al., 2020; Schifilliti et al., 2011; Shouroki et al., 2019; Wrońska-Nofer et al., 2009, 2012). In addition, short-term exposure of patients to nitrous oxide during surgery has increased the frequency of DNA strand breaks as evidenced by a Comet assay (Chen et al., 2013). Similarly, neurosurgery patients exposed for three hours to a combination of nitrous oxide and isoflurane showed an increase in DNA single strand breaks in peripheral lymphocytes, comparable to 0.2-0.5 Gray following x-ray radiation of lymphocytes *in vitro* (Reitz et al., 1993). Not all studies showed an association between inhalation anaesthetic exposure and genetic damage. Important limitations of occupational studies are confounding lifestyle factors such as smoking habit, co-exposure to multiple anaesthetics or other drugs used in an operating theatre, and an unknown or limited actual level of exposure. However, in recent studies, the concentrations in the workplace were quantified, and a dose-response relationship could be established for nitrous oxide but

not for the halogenated anaesthetics that were used (Neghab et al., 2020; Wrońska-Nofer et al., 2009, 2012). In a multiple regression model, it was demonstrated that nitrous oxide is dose-dependently associated with oxidative stress markers and oxidative DNA damage by an indirect mechanism involving reactive oxygen species (Braz et al., 2020; Wrońska-Nofer et al., 2009, 2012). Polymorphisms in glutathione-*S*-transferase genes, coding for enzymes that have an important role in free radical scavenging and protecting DNA against oxidative stress, affect susceptibility to micronucleus formation associated with nitrous oxide exposure (Shouroki et al., 2019).

Oxidative stress and oxidative damage to DNA is a major source of the mutation load in living organisms. Over 100 oxidative DNA adducts have been identified. The estimated frequency of oxidative DNA damage is 10^4 lesions/cell/day in humans. Free radicals can react with both purine and pyrimidine bases, as well as the deoxyribose backbone. ROS-induced DNA damage includes single- or double-strand breakage, base modifications, deoxyribose modification and DNA cross-linking. If DNA damage is not properly repaired before or during replication, it may result in cell death, mutation or induction of transcription, induction of signalling pathways, replication errors and genomic instability, all of which have been associated with the carcinogenesis process (Klaunig et al., 2011). Indeed, in mice, it has been shown that exposure to nitrous oxide can enhance development of metastases in tissues where these normally do not arise spontaneously (Shapiro et al., 1981). On the other hand, in healthy mice exposed chronically to nitrous oxide, tumour incidence was not increased above the background level (Baden et al., 1986). Although healthy rats, when exposed chronically to low exposure levels of nitrous oxide and halothane, did show an increase in chromosomal aberrations in the bone marrow, they did not develop more tumours (Coate et al., 1979a, 1979b). In humans, 3-hour exposure of patients to nitrous oxide increased the frequency of DNA strand breaks, but this elevation was no longer detectable one day later, showing rapid repair of DNA damage (Reitz et al., 1993). Furthermore, no increased recurrence of cancer was observed in patients who were anaesthetised with nitrous oxide during colorectal surgery (Fleischmann et al., 2009).

There is currently no strong evidence indicating that nitrous oxide exposure causes cancer in humans. However, the evidence is limited in several ways. Animals that have been exposed chronically in carcinogenicity assays are healthy animals and the study designs do not consider situations where DNA repair mechanisms are compromised by polymorphisms in DNA repair genes or antioxidant genes or where co-exposure to other oxidative stressors exists. Data on exposure in humans is based on either short-term exposure in a medical setting, or occupational exposure at a low level. A genotoxic risk for recreational users displaying a pattern of problematic use may be possible. Minimal oxidative damage to DNA following short-term and incidental use is likely to be repaired in individuals who are otherwise healthy and are not predisposed to an increased risk of cancer by genetic and environmental factors.

Reproductive and developmental toxicity

Even though nitrous oxide is not classified as reprotoxic by the EU and much data is still under debate, several countries have implemented regulations to limit occupational exposure. For example, in France, Circulaire DGS/3A/667 bis of 10 October 1985 sets the exposure limit during the maintenance phase of anaesthesia to 25 ppm in the vicinity of the patient and staff, with lower values not to be exceeded in the periconceptional period and during pregnancy.

The information in this section is based on *in vitro*, animal and human studies among workers exposed chronically to low levels of nitrous oxide, or patients exposed to anaesthetic doses for a short period during surgery. There is no information on fertility or pregnancy outcomes in recreational users of nitrous oxide.

Fertility

When male mice were exposed to up to 50 % nitrous oxide for 4 hours per day over 9 weeks, and then mated nightly for seven nights with untreated females, they showed no reduced capacity to impregnate those females (Mazze et al., 1982). In a similar experiment where mice were exposed to up to 50 % nitrous oxide for 14 weeks, no sperm or testicular abnormalities were seen (Mazze et al., 1983). Female mice in the latter study did not exhibit destruction of primordial oocytes. When male Wistar albino rats were exposed to 0.5 % nitrous oxide/air mixtures (v/v) for 30 days, and after the exposure period were allowed to mate untreated females for one night, litter sizes were reduced. After these males were kept for 6 months of recovery, an effect on litter sizes was no longer detectable (Vieira et al., 1983). It is not known if the differences between these experiments are due to species differences, or if the longer mating period in the study with mice allowed for recovery. Reversibility was clearly established for rats.

Medical staff working in operating theatres or in dental practices have reported reduced fecundability following

occupational exposure to nitrous oxide. However, the retrospective studies have been criticised and the effect of nitrous oxide alone is unclear due to its use in mixtures with other volatile anaesthetics (Buhre et al., 2019; Buring et al., 1985; Burm, 2003; Mazze and Lecky, 1985). Dental assistants who were exposed to unscavenged nitrous oxide for more than 5 h/week in their work environment had apparently reduced fecundability (Rowland et al., 1992). It has been suggested that such an effect might be caused by changes in gonadotropin hormone release, but could also reflect unnoticed loss of the conceptus in early pregnancy (Gray, 1993).

The available evidence for an effect on fertility is limited and conflicting. Nonetheless, an effect on fertility in recreational users of nitrous oxide cannot be ruled out, but if it exists, it is likely a reversible effect (van Amsterdam and van den Brink, 2022).

Pregnancy loss

Most experimental studies in rats have shown that nitrous oxide reduced the number of implantations (pre-implantation loss), increased foetal resorption (post-implantation loss), increased foetal death rate, decreased foetal weight and reduced ossification of the fetuses, signifying the embryofoetal toxicity of nitrous oxide (Corbett et al., 1973; Fink et al., 1967; Lane et al., 1980; Pope et al., 1978; Vieira et al., 1980). These effects not only occur at anaesthetic dosages, but also at subanaesthetic concentrations, starting at 1000 ppm when exposure was continuous on multiple days, or at 5000 ppm when exposed 6 hours per day, 5 days per week. When exposure was limited to a single 24-hour period (on day 9 of gestation), both early and late resorptions increased at a concentration of 75 %, but not at 25 % (Mazze et al., 1984).

Spontaneous abortion is the most often reported finding in studies evaluating occupational exposure to inhalation anaesthetics. Meta-analyses of these studies showed a relative risk of 1.3 (95 % CI: 1.2-1.4) and 1.48 (95 % CI: 1.40-1.58). However, the underlying studies have their limitations (Burm, 2003).

The available evidence indicates that nitrous oxide shows signs of embryofoetal developmental toxicity which leads to decreased survival of the conceptus. These effects also occur after intermittent exposure.

Congenital malformations

Although embryofoetal death is the most common deleterious effect of nitrous oxide when pregnant rats are exposed, malformations have also been observed, including abnormal ribs and vertebrae, altered body laterality, encephalocele, hydrocephalus, anophthalmia, microphthalmia, gastroschisis and gonadal agenesis (Fink et al., 1967; Fujinaga et al., 1989; Lane et al., 1980). The most susceptible period of gestation in rats was day 8 to 9, when the embryo is in its earliest stage of organogenesis, corresponding to the third week after fertilisation of the ovum in humans (Fujinaga et al., 1989). The minimal nitrous oxide concentration needed for 24 hours to induce malformations was 50 %, when exposed on gestational day 9, but no teratogenic effects were observed with 35 % nitrous oxide (Mazze et al., 1987). *In vitro* experiments using rat whole embryo culture showed that methionine could protect against nitrous oxide-induced malformations but folinic acid could not, suggesting that nitrous oxide-induced inhibition of methionine synthase causes a deficit in methionine which leads to the adverse outcome (Fujinaga and Baden, 1994). Both maternal and foetal methionine synthase are rapidly inhibited by nitrous oxide and recovery is slow (Baden et al., 1984, 1987; Hansen and Billings, 1985). Methionine synthase is expressed and active in embryos during the susceptible period for nitrous oxide-induced malformations and the relevance of adequate methionine supplies for normal embryonic development has been further supported in experiments using whole embryo culture (van Aerts et al., 1994, 1995; van Aerts, 1995a, 1995b). *In vivo*, additional mechanisms may possibly contribute to the embryofoetal developmental toxicity, such as reduced uterine blood flow following nitrous oxide administration (Fujinaga et al., 1987).

The evidence for an association between occupational anaesthetic exposure (including nitrous oxide) and congenital malformations is inconsistent and a meta-analysis showed only a weak association of borderline statistical significance (relative risk: 1.2 [CI: 1.0-1.4]) (Burm, 2003). In addition, an association between surgery involving the administration of nitrous oxide during early pregnancy and congenital malformations could not be found (Aldridge and Tunstall, 1986; Crawford and Lewis, 1986).

The available evidence suggests that occupational exposure to low levels of nitrous oxide or incidental exposure during surgery in early pregnancy are not associated with an increased risk of congenital malformations. However, based on the findings of rat studies, congenital malformations should be considered

a potential risk for embryos carried by recreational users of nitrous oxide who consume this substance repeatedly and/or in large quantities during early pregnancy, as nitrous oxide inactivates maternal and foetal methionine synthase.

Neurodevelopmental toxicity and neurobehavioural and neurocognitive development

Studies in rats have shown that administration of NMDA antagonists — depending on specific conditions such as the developmental window, level and duration of exposure, and co-administration of other substances such as GABA-mimetic agents — can trigger excitotoxic neurodegeneration or neuronal apoptosis (Olney, 2002). These effects have also been shown with nitrous oxide and can cause persistent neurocognitive deficits (Jevtovic-Todorovic et al., 2003). The most sensitive window in rats appears to be the first 7 days after birth, a period where, due to apoptotic processes, many neurons die and synaptogenesis occurs. This period in rats is comparable to the last trimester of pregnancy and the first 6 months after birth in humans. However, the co-administration of other anaesthetics appears to be a prerequisite for nitrous oxide to affect neurodevelopment, or nitrous oxide levels need to be administered under hyperbaric conditions to reach a neurotoxic level (Jevtović-Todorović et al., 1998; Savage and Ma, 2014). Although concerns remain that general anaesthesia in children or in late pregnancy may affect neurobehavioural and/or neurocognitive development, nitrous oxide is not usually considered as one of the anaesthetics of concern (Bilotta et al., 2017; Houck et al., 2019). For example, the FDA has issued a safety warning about the use of general anaesthetics in children and in late pregnancy, but nitrous oxide was not listed as one of the drug substances of concern (FDA, 2017). The Pharmacovigilance Risk Assessment Committee (PRAC) of the European Medicines Agency (EMA) only supported an update to the non-clinical data in general anaesthetic product labels, with reference to pregnancy, but did not support an update on use in children because the information remains unclear and no firm recommendations can be given. Nitrous oxide was not listed as one of the substances of concern (PRAC, 2018a, 2018b).

These findings are probably of minor concern to recreational users of nitrous oxide since the association is currently unclear. Exposure would need to take place during late pregnancy and/or early childhood. Also, co-exposure to GABA-mimetic compounds is likely required before brain development could be potentially altered.

Immunotoxicity

In vitro, nitrous oxide was found to affect chemotaxis of leukocytes, impair the generation of reactive oxygen derivatives in neutrophiles, and reduce the proliferation of human peripheral blood mononuclear cells, suggesting that the gas may have some immunosuppressive properties (Fröhlich et al., 1998; Moudgil et al., 1984; Nunn and O'Moráin, 1982; Schneemilch et al., 2005). Bone marrow suppression following prolonged nitrous oxide exposure is a well-known toxic effect of nitrous oxide (Weimann, 2003). A case has been reported of a patient treated with 50 % nitrous oxide for days, who developed septicaemia and died despite cessation of treatment (Lassen et al., 1956).

In CD-1 mice, exposure to 0.5 % nitrous oxide for 6 hours per day, 5 days per week over 2 weeks caused bone marrow suppression. Moreover, after 13 weeks, even 0.005 % nitrous oxide resulted in leukopenia and animals exposed to 0.5 % showed a diminished immune response as evidenced by a lower response in the sheep red blood cell assay, a conventional test for T-cell dependent antibody response (Healy et al., 1990).

Nitrous oxide exposure during surgery has been associated with an increased risk of wound infection (Chen et al., 2013; Myles et al., 2007). However, this appears to be an inconsistent finding (Fleischmann et al. 2005; Myles et al. 2014), and when nitrous oxide is only administered for a limited duration in a medical setting, the clinical significance of its effects on bone marrow and the immune system is debatable or judged to be limited (Buhre et al., 2019; Sanders et al., 2008; Weimann, 2003).

No specific studies on the immune effects of recreational nitrous oxide use are available, and in turn it is unclear to what extent the potential immunosuppressive properties of nitrous oxide are relevant to light recreational users of the substance.

Legitimate uses

Medical use

Nitrous oxide is included on the WHO 22nd Essential Medicines List (EML) (WHO, 2021a) and the 8th Essential Medicines List for Children (EMLc) (WHO, 2021b) as an inhalational general anaesthetic.

Nitrous oxide is one of the oldest substances still in use in medicine today. In clinical practice, nitrous oxide is administered by inhalation as a mixture with oxygen (at concentrations of 30-65 % (Messer, 2017)) and it is best known for its anaesthetic and analgesic properties. While the analgesic properties of nitrous oxide were first described by Humphry Davy in 1800 (Gillman, 2019; Smith, 1965a), its use as an anaesthetic in dentistry was only documented 44 years later by Horace Wells (Gillman, 2019; López-Valverde et al., 2011).

Besides dentistry, where nitrous oxide is valued for its anxiolytic and analgesic properties (Buhre et al. 2019; Jastak, 1989), nitrous oxide is also used as procedural sedation and analgesia in minor or moderate surgical procedures in both adults and children, where it may be combined with local anaesthetics (Buhre et al., 2019). Nitrous oxide is also commonly used in emergency departments and ambulances (Becker and Rosenberg, 2008). For procedures with moderate or severe pain intensity, requiring general anaesthesia, it needs to be combined with other anaesthetics, sedatives, muscle relaxants and analgesics (Becker and Rosenberg, 2008; Buhre et al., 2019; Kreuer et al., 2007).

Nitrous oxide has also been used safely for labour analgesia for over 100 years (Vallejo and Zakowski, 2019). In Europe, the practice varies widely between countries. Although nitrous oxide does not seem to have an adverse influence on the delivery process and on extrauterine adaptation as measured by the Apgar score, there is still significant and ongoing controversy as to whether intrauterine exposure to anaesthetics has persistent adverse effects on neurocognitive function and psychosocial development (Buhre et al., 2019).

Another medical use under investigation is the action of nitrous oxide as a putative antidepressant (Buhre et al., 2019; Desmidt et al., 2021; Guimarães et al., 2021; Nagele et al., 2015, 2018). Additionally, a wide variety of neuropsychiatric indications have been explored. As early as 1928, the use of nitrous oxide for depression, schizophrenia and movement disorders was studied by Zador. In 1944, Rogerson investigated nitrous oxide for psychotherapy and in 1970 MacDonald explored its use in psychoanalysis (Gillman, 2019). In 1972, a case was reported where a women was successfully weaned from her pentazocine addiction using nitrous oxide (Kripke and Hechtman, 1972). More extensive exploration of nitrous oxide for the treatment of substance use disorder was undertaken by Gillman and co-workers (Daynes and Gillman, 1994; Gillman and Lichtigfeld, 1990, 1991; Gillman et al., 2007). This group coined the term PAN (psychotropic analgesic nitrous oxide) (Gillman and Lichtigfeld, 1994) and studied its application in the treatment of anxiety, depression, schizophrenia, akathisia, Tourette syndrome and torticollis (Gillman, 2019). Recently, the potential use of nitrous oxide in the treatment of obsessive-compulsive disorder has gained attention (Grassi et al., 2021).

Gastronomic use

Nitrous oxide is registered in the EU as a food additive (E942) (European Commission, 2012). It is well known for its use as a propellant gas to produce light, fluffy whipped cream and is available either as a ready-to-use aerosol can containing a pressurised mixture of pasteurised cream and dissolved gas, or as small bulb-shaped replaceable cannisters ('whippets', which are one of the main sources of nitrous oxide for recreational users) that are used in a device for whipping the cream. The same device can also be used to produce foams from other foods, provided the liquid contains sufficient stabilising molecules (McGee, 2004).

Industrial use

Nitrous oxide is a REACH registered substance (EC/List no. 233-032-0). It is manufactured in or imported to the European Economic Area at quantities ranging from 1 000 to 10 000 tonnes per annum. Nitrous oxide is used by professional workers for widespread uses in formulation or re-packing, at industrial sites and in manufacturing. At industrial sites, the substance is used in semiconductors and laboratory chemicals, for formulation of mixtures and/or re-packaging and in the manufacture of electrical, electronic and optical equipment (ECHA, 2021).

Nitrous oxide's property as an oxidising gas is put to use in automotive sports, specifically in drag races (Dutch Government, 2020b). When one mole of nitrous oxide decomposes, it releases half a mole of oxygen gas, and one mole of nitrogen gas. This decomposition allows an oxygen concentration of 36.36 % to be reached, which is higher than the oxygen concentration of air (21 %) and allows a higher fuel combustion rate (Formula 1 Dictionary, date unknown).

Section 3
Epidemiology, social risks and national case studies

Epidemiology

Nitrous oxide has been used for its brief psychoactive effects for over 200 years. During the 19th century, laughing gas parties were popular among the members of the British upper class. At that time, nitrous oxide was an interesting novelty limited to a small user group. However, in the last decade there has been a large increase in its recreational use in many regions in the world, including Europe. The growing popularity of nitrous oxide might be explained to some extent by its easy availability, low price, short-lived effects and general perception by users as a relatively safe and socially acceptable drug. Nitrous oxide is not monitored systematically in Europe, and it is also not monitored as a new psychoactive substance by the EU Early Warning System of the EMCDDA. While there is no comparable information on the prevalence of nitrous oxide use, some information is available from targeted surveys. The Global Drug Survey (GDS) is a targeted, internet-based, anonymous, self-reported survey across 35 countries worldwide. Respondents are not representative of the general population and typically use drugs and psychoactive substances. GDS is not a general population survey and cannot be used to determine the prevalence of drug use in a population. However, the GDS can provide quantitative insight on aspects such as drug preference. In GDS 2021, 22.5 % of respondents had ever used nitrous oxide (lifetime use) (compared with 23.6 % in GDS 2019). Current use (use in the last month) was 9.7 % (compared with 13.1 % in GDS 2020 and 11.9 % in GDS 2019). As such, nitrous oxide took 14th place for popularity, ranked according to current use, just after ketamine and prescription opioids and before poppers (Winstock et al., 2019).

When examining data on prevalence at national level, the situation seems to differ between countries with some countries reporting much wider use than others (see the national case studies below).

Nitrous oxide use is particularly widespread among young people (Bethmont et al., 2019; Kaar et al., 2016; van Laar et al., 2021; Nabben et al., 2021; Xiang et al., 2021). Of special concern are reports of nitrous oxide use among school-aged children, as the gas may have a negative impact on the developing brain.

Most users report occasional use of nitrous oxide; however, in some regions, there are signs of increasingly heavy and frequent use linked to the use of large tanks.

The available data suggest that nitrous oxide is often used in recreational settings, such as festivals, pre-clubbing parties or at home. More frequent users also participate in so-called car parties, where nitrous oxide is used in a car together with friends in a parking lot (Nabben et al., 2017). Nitrous oxide is also used outdoors in public spaces (Kaar et al. 2016; Nabben et al., 2017).

While in most cases nitrous oxide is used in a social context, problem use has also been reported where users tend to inhale nitrous oxide on their own, which might lead to social isolation. Using nitrous oxide alone potentially places users at risk of experiencing serious acute toxicity, asphyxia and, ultimately, death, since there is no one around to call for medical assistance.

Impact of COVID-19 pandemic

The available information seems to suggest that the coronavirus disease (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has had some impact on the use of nitrous oxide. The closure of nightlife venues, as a result of public health measures, seems to have resulted in a (temporary) decrease in nitrous oxide use among nightlife users. On the other hand, some people have started to use the gas more frequently. This increase in use might be related to the ready availability of nitrous oxide (from supermarkets and online suppliers) as compared with the disrupted illicit drug market, as well as to boredom, anxiety and stress experienced during the pandemic (Cape et al., 2021; Dufayet et al., 2022; Einsiedler et al., 2022). The COVID-19 pandemic also affected schooling and some case studies have reported that there was a perceived increase in use by school-aged children during this time.

Social risks

While there is limited information on the social risks of nitrous oxide, it may be that the social risks share some similarities with those associated with other drugs.

Psychological effects associated with the long-term use of nitrous oxide in general include, but are not limited to, development of cognitive deficits (concentration, memory and responsiveness), dependence, fatigue and hallucinations. Financial problems and social isolation are mentioned as negative consequences of nitrous oxide use by professionals as well as frequent users. These psychological and social consequences may negatively impact education or career and family or other personal and social relationships, and may result in marginalisation (Spronk et al. 2020a, 2020b). It is often not evident whether nitrous oxide use has caused the psychosocial problems that users are struggling with, and these problems may be pre-existing and have contributed to the development of problematic use (Nabben et al., 2021; Spronk et al. 2020a, 2020b).

It appears that nitrous oxide is currently used by some vulnerable groups including migrants and minors (Ehirim et al., 2018; Nabben et al., 2021; Xiang et al., 2021), who may be more susceptible to the social risks of this substance. In particular, the situation might be aggravated because nitrous oxide is often incorrectly perceived by young people as a harmless substance (Kaar et al., 2016; Huizink, 2022).

Driving a vehicle while under the influence of nitrous oxide can be dangerous as a result of the greatly diminished neurocognitive and psychomotor capacities. Cases of driving under the influence of nitrous oxide, including driving while inhaling it, have been reported (van Laar et al., 2021; Spronk et al., 2020a, 2020b; Team Alert, 2020; VIAS, 2021). This indicates a potential wider risk to public safety as it may result in injury or damage to the health of the user or other people, or in damage to property. In Belgium, in a survey on unsafe driving, 6 % of the respondents indicated driving a car after having used nitrous oxide, at least monthly. Among young male drivers (18-34 years), the prevalence was 20 %, and in Brussels this figure was as high as 34 % (VIAS, 2021). These figures contrast with the prevalence of nitrous oxide use as reported by visitors to clubs, dance and music events in 2018, where last-year prevalence was 3.4 % and regular use (several times a month) was 0.1 % (Rosiers, 2019; VAD, 2021). In the Netherlands, the number of traffic incidents that are connected with nitrous oxide is on the rise (see 'Case study 1: Netherlands' below). However, in most cases it is difficult to prove exposure to the substance while driving, because the direct effect of nitrous oxide on humans is short-lived and its use is generally not detectable in the driver's blood, urine, breath or saliva.

The use of nitrous oxide in public is often associated with causing a public nuisance through littering of public spaces with balloons and cartridges and the hissing sound made when filling balloons. Nevertheless, littered places also provide clues as to where users gather, making outreach possible (Nijkamp, 2020).

There is limited evidence on the involvement of criminal networks in the distribution of nitrous oxide. While legislative approaches to controlling the sale of illicit nitrous oxide have been discussed in a number of regions, control measures may also bring unintended consequences. These may include the emergence of criminal distribution networks for nitrous oxide (EUCPN, 2021).

Availability and supply

Due to the legitimate uses of nitrous oxide, it is quite easy to purchase the substance. For legitimate use, nitrous oxide is available in bulb-shaped non-refillable cartridges ('whippets') used to froth whipping cream. These are available together with a whipped cream dispenser that can be used to release the gas and fill a balloon.

At the same time, easy availability results from the increasing demand for nitrous oxide. Its growing popularity has created a profitable and expanding market with a range of specialised internet shops offering the gas under the disguise of selling 'party accessories'. Specialised internet stores offer the cartridges together with so-called crackers used to open the cartridge so the gas can be released into a balloon. Alternatively, a whipped cream dispenser can be used. Other paraphernalia such as balloons, flavouring solution, dampers (to reduce the hissing sound) and antifreeze holders (to prevent freezing of fingers when repeatedly refilling balloons) can be purchased as well as complete starter packages. The price of the cartridges varies depending on the quantity ordered. In some regions, large cylinders and tanks have also been offered for sale.

In some regions, distribution via social media channels (such as TikTok, WhatsApp, Instagram and Snapchat) seems to play an important role, especially among young people. Distribution among users, so-called social supply, has also been reported.

Increased supply of nitrous oxide has also been reported by some law enforcement agencies (see 'Case study 5: Ireland' and 'Case study 6: Portugal' below). While products containing nitrous oxide may have legitimate uses, law enforcement agents can seize them in instances where there are reasonable grounds to believe that they are intended for recreational purposes.

While medical nitrous oxide is distributed through the appropriate channels for medicinal products, diversion for recreational purposes has been reported (ACMD, 2015).

National case studies

Case study 1: Netherlands

Epidemiology

In the mid-1990s, nitrous oxide gained some popularity in the Netherlands when it was offered at large parties and in clubs, as well as in smartshops and on the street (Nabben, 2010). In a study among attendees of 10 parties in 1996, 5 % indicated they had used nitrous oxide that night. Less than 1 % of the people who sought help at the first aid facility at the party indicated that they had used nitrous oxide (de Bruin et al., 1999; van de Wijngaart et al., 1997). Among clubbers in Amsterdam, lifetime prevalence was 45 % and last-month prevalence was 8 % (Nabben, 2010). After a short period, nitrous oxide disappeared from the scene, except for sporadic reports of use at underground parties, during private parties and in other private settings (Nabben et al., 2005).

Nitrous oxide remained freely available in the form of cannisters used to froth cream and, for those with the right connections, large cylinders could be ordered through the internet or from wholesale traders. Lifetime prevalence among clubbers in Amsterdam had dropped from 45 % in 1998 to 24 % in 2008, and last-month prevalence in this scene decreased from 8 % to 3 % (Nabben, 2010). However, towards the end of the first decade of this century, increased use in the home environment was noted in Amsterdam. This use was mainly experimental. At national level, members of regional expert panels were interviewed, consisting of professionals and other members who were able to provide inside information on drug use in the scenes where they operate. A quarter of the expert panels observed nitrous oxide use in their networks. It was estimated that in half of these networks, 1-10 % used nitrous oxide, and in the other half, 10-25 % used nitrous oxide (Doekhie et al., 2010).

Around 2012, this gradual increase accelerated and in 2013 in Amsterdam on Queen's Day, a national holiday, it was noted that people were carrying larger nitrous oxide cylinders at many places where the gas is available, and that at some places near performance stages, big cylinders were placed and nitrous oxide balloons were being sold. Also, some areas were littered with cannisters and balloons. This reflected the increased casual use of nitrous oxide, not only at parties and raves — as before — but also in the wider nightlife scene (Nabben et al., 2014). Among visitors to cafés in Amsterdam, lifetime prevalence of nitrous oxide use increased from 10.8 % to 31 % between 2010 and 2014, and last-month prevalence jumped from 1.9 % to 10.9 % (Benschop et al., 2011, 2015; van Laar et al., 2021; Trimbos-instituut, 2022).

In 2015, according to panel members of trendsetters in Amsterdam, the use of nitrous oxide at parties stabilised. However, nitrous oxide is now more widely offered. Not only do regular shops such as larger food stores sell nitrous oxide, but it is also sold by specialised stores, night stores, web shops and delivery services. The web shops and delivery services actively promote their merchandise through the internet and social media networks. Due to this wide availability and because nitrous oxide is generally perceived as a harmless substance, many people take nitrous oxide as an add-on. Two thirds (15/23) of the members of an expert panel providing inside information on drug use in the Amsterdam clubbing and party scene indicate they have observed the use of nitrous oxide. In 2015, last-year prevalence among coffeeshop visitors in Amsterdam was 31 % (Nabben et al., 2016, 2017).

As the use of nitrous oxide was becoming mainstream, questions on its use were included in more widely dispersed monitoring tools as of 2015.

Biennial monitoring of the use of nitrous oxide among school-aged children (12-16 years) started in 2015. Among boys, lifetime prevalence increased from 9 % to 11 % between 2015 and 2019 and among girls it rose from 6.5 % to 8.7 % (in total, from 7.8 % to 9.9 %) (Rombouts et al., 2020). Last-month prevalence did not change in this period (2.5 % to 2.8 % for boys and 2.3 % to 2.2 % for girls; the total remained 2.5 %) (van Laar et al., 2022). According to the most recent data, in 2021 the total last month prevalence dropped to 1.3% (Boer et al., 2022) suggesting a recent decrease in nitrous oxide use among young people.

Nitrous oxide use was also monitored among young people and young adults aged 16 to 35 who had visited a party, festival, club or disco at least once in the past year (Monshouwer et al., 2021). In this population, use of nitrous oxide was examined in 2016 and 2020. Last-year prevalence was 37.3 % and 35.2 % respectively. In 2020, the COVID-19 pandemic did affect partying behaviour and substance use. Among previous nitrous oxide users, 38.2 % used less nitrous oxide and 25 % used more nitrous oxide compared with the same period in 2019 (van Beek et al., 2021).

Nitrous oxide use in the general population (>18 years of age) is described in the National Drug Monitor based on two separate surveys, which shows a gradual increase in nitrous oxide use between 2016 and 2019 (van Laar et al., 2021). Between 2016 and 2018, the LSM-A survey indicated that lifetime prevalence increased, especially in the age groups 18-19 years (from 11 % to 17.6 %) and 20-24 years (from 20.8 % to 25.2 %). The Health Survey showed that lifetime prevalence in men increased from 7.9 % to 9.5 % between 2018 and 2019, while in women this figure decreased from 5.9 % to 5.7 % (the total changed from 6.9 % to 7.6 %). Last-year prevalence in this period increased from 3.3 % to 4.3 % in men and from 2.0 % to 2.1 % in women (total: 2.7 % to 3.2 %). The last-month prevalence figures were 0.1 % to 1.5 % for men and 0.7 % in both years for women (total: 0.8 % to 1.1 %). Recent data indicated that last-year prevalence was 2.1 % in 2020 (men: 2.3 %; women: 1.9 %) and 1.6 % in 2021 (men: 2.1 %; women: 1.2 %) (van Laar et al., 2022). This would suggest a decrease over the last two years. However, data collection for the Health Survey was disrupted by the COVID-19 pandemic in 2020. The pandemic and its measures may also have influenced the lifestyle of the respondents, as was shown for nightlife attendees (van Beek et al., 2021).

The education level of users varies, and all levels of education are represented. However, a higher level of education appears to be overrepresented. Lifetime use and recent use among users that attended higher education was 11.6 % and 2.3 % respectively, whereas among those with lower levels of education, lifetime and recent use were 1.8 % and 0.5 % respectively (van Laar et al., 2022).

Overall, the predominant pattern of use for nitrous oxide is occasional use at the weekends (van Laar et al., 2021). Among respondents who reported use of nitrous oxide in the last year, 35.6 % took nitrous oxide once, and 54.1 % used it a few times but less than monthly. One in ten (10.3 %) took nitrous oxide monthly or more often (van Laar et al., 2022). In a study among young adults aged 16 to 35 who had visited a party, festival, club or disco at least once in the past year, most users of nitrous oxide indicated that they use the drug occasionally, 15.6 % use it monthly or a few times a month and 2.8 % use it weekly (Monshouwer et al., 2021). On average, nightlife attendees reported using four balloons on a night out and five balloons on a non-night out. Although these figures seem relatively low, there is also a group of individuals in this sample that uses nitrous oxide frequently and in large quantities. For example, 5 % of last-year users get through more than 10 balloons on average at a time, and amounts of 100 to 200 balloons are also reported (Monshouwer et al., 2021).

Frequent users (monthly use or more frequent) reported average use of eight balloons on an average use day and 17 balloons on a maximal use day. Non-frequent users (several times in the last year, but less than monthly) used five balloons on an average use day and eight balloons on a maximal use day. These data indicate that more frequent use is associated with greater intake per occasion (Nabben et al., 2017).

Recent school-aged users (12-16 years) used variable amounts per occasion. Typically, 19 % use 1 balloon, 41 % use 2-4 balloons, 20 % use 5-9 balloons and 20 % use 10 or more balloons (Rombouts et al., 2020). Further in-depth research on the use of nitrous oxide confirmed that often the use is occasional and typically limited to one or a few balloons of nitrous oxide per occasion. But there are also people and groups who, for a shorter or longer period of time, use frequently and/or use a lot of nitrous oxide (Nabben et al., 2017).

In recent years, tanks of 2, 4 or 10 kg nitrous oxide have been introduced to the market. These cylinders are sold by web shops and delivered to the buyer's home (CAM, 2019; van Laar et al., 2021; Trimbos-instituut, 2022). The price for a 2 kg tank is approximately EUR 35, excluding a deposit of EUR 30 for the tank. Additional hardware such as a dispenser or a complete system including regulator, tube and pistol are also available. These devices can alternatively be rented. Besides the reusable tanks that require a deposit, smaller disposable tanks of 0.6 or 1.1 kg are offered for approximately EUR 30 and 45 respectively. The source and quality of the nitrous oxide sold in these tanks is unknown, although it is often claimed that the tanks contain medical nitrous oxide. It is noted that members of marginalised groups of vulnerable young people, who may be using nitrous oxide themselves, may act as runners for the delivery of nitrous oxide tanks. Although the trade in nitrous oxide is legal, the same groups may also be involved in illicit drug traffic (Nabben, 2020). These observations would confirm information from criminal intelligence networks suggesting that there is also nitrous oxide trade within or between criminal groups (CAM, 2019).

Observational studies in the Netherlands suggest that the availability of large (2-10 kg) tanks of nitrous oxide is associated with an increase in heavy use in recent years (Nabben, 2020; Spronk et al., 2020a, 2020b). Having such large volumes readily available and the ease of repeatedly refilling a balloon using a tank with a suitable nozzle has facilitated the use of large quantities of 100 balloons or more per occasion (CAM 2019; van Laar et al., 2021; Nabben, 2020).

The increase in more excessive use is reflected in an increase in nitrous oxide-related poisonings. The number of information requests at the National Poisoning Information Centre (NVIC) increased from 13 in 2015 to 128 in 2019 and 144 in 2020. Among 272 poisonings linked to nitrous oxide and reported to NVIC in 2019 and 2020, 79 % of the patients indicated heavy use, frequent use or both. Heavy use occurred in 59 % of cases (50 % in 2019, 67 % in 2020), frequent use in 64 % (59 % in 2019, 69 % in 2020), and both heavy and frequent use (by the same patient) in 44 % (33 % in 2019, 53 % in 2020). Use from cylinders (2 to 10 kg) was reported in 42 % of the inquiries (31 % in 2019, 51 % in 2020) (van Riel et al., 2022). The number of exposures to nitrous oxide decreased from 144 in 2020, to 98 in 2021. However, just like in previous years, in 2021 many inquiries involved frequent use of laughing gas and/or the use of large quantities (50 balloons or more during one session). In addition, more than a third of the patients experienced nitrous oxide-related neurological symptoms, like paresthesias or numbness of the arms and/or legs, indicating (chronic) abuse of large quantities (NVIC, 2022).

The Drug Incidents Monitor showed an increase in drug-related health incidents from 29 (0.5 % of all reports) to 114 (1.7 % of all reports) between 2017 and 2019 (van Laar et al., 2021). Of particular concern are reports of severe neurological harm. According to the Dutch Neurologists' Association, at least 64 young adults (average age: 22 years) were treated for a partial spinal cord injury following nitrous oxide use in 2018-2019 (van Laar, 2022).

The use of nitrous oxide in a car has also gained popularity in the Netherlands. In a survey of young people who have used nitrous oxide in a car, 45 % of drivers admitted using nitrous oxide while driving, arguing that it does not affect their ability to drive. The number of police reports about driving under the influence, car accidents or dangerous driving that mention nitrous oxide have increased sharply (from 2 652 in 2019 to 5 102 in 2020 and 4 860 in 2021) (van Laar, 2022). However, no figures are available on fatalities or injuries (van Laar et al., 2021).

Prevention

Various prevention campaigns, both local and national, have been launched on social media and in the form of posters to inform users about the harms associated with nitrous oxide use (van Laar, 2022).

The Trimbos Institute has been asked to focus on information and prevention materials for young people around the risks of using nitrous oxide (Dutch Government, 2020a). Multiple information materials have been developed. Additional emphasis has been placed on developing information and prevention materials on nitrous oxide use while driving (van Laar et al., 2021; Spronk et al., 2020a, 2020b).

The following options for prevention have been recommended (Spronk et al., 2020a, 2020b).

- Outreach youth workers have an important role in signalling nitrous oxide-related problems, counselling young people with problematic use and involving other professionals where needed.
- Information on nitrous oxide use should be distributed in a targeted manner and not broadly to all, to avoid raising interest instead of discouraging its use. Awareness is needed of the promotional role social media can have. Social media can be used to get in contact. Spreading warnings by social media is likely not effective.
- People in the direct environment of the problematic user should be supported to get in touch. Awareness should be raised of where information can be found and where help is offered, especially where the threshold is low.
- Awareness should be raised among first-line professionals such as physicians, emergency department workers and physiotherapists to make sure specialised help is offered when needed.
- The role of parents can be strengthened by increasing their knowledge about nitrous oxide and their skills in recognising the signs of (problematic) nitrous oxide use and addressing this by talking to their children. The threshold for discussing nitrous oxide can be lowered by including nitrous oxide use in a broader context such as healthy eating, gaming and other parenting issues.

Furthermore, the authors of the Amsterdam study emphasise that outreach professionals need to work in a culture-sensitive way, having knowledge of the cultural background of problematic users and how they view their world. Influencers associated with the community need to

be chosen carefully and should understand and support the preventive action fully (Nabben and Bahara, 2020; Nabben et al., 2021).

The Ministry of Transport, Public Works and Water Management has initiated a campaign to warn against the use of nitrous oxide in traffic (Team Alert, 2020).

Policy response

In the early days, medicines legislation was used as a legal basis to prohibit the sale of nitrous oxide. However, it is uncertain to what extent restrictive action by the authorities affected the limited duration of the nitrous oxide hype in the mid-1990s. Party organisers were not pleased by the introduction of nitrous oxide to the scene and soon stopped admitting sellers to the party scene (Nabben, 2010). For more than a decade, prevalence of use was low among participants in Amsterdam night life, and the use of nitrous oxide was limited to underground parties, private parties and other private settings. Although the legal status had not changed, an increase in use was observed starting in around 2008 and accelerating in 2012 (Nabben et al., 2005, 2014).

In July 2014, the European Court of Justice ruled that a substance being used solely for recreational purposes should not be classified as a medicinal product (European Court of Justice, 2014). Consequently, nitrous oxide used for recreational purposes was no longer governed by the Medicines Act, but instead subjected to the Commodities Act. Although the Netherlands Food and Consumer Product Safety Authority (NVWA) requested the National Institute of Public Health and the Environment (RIVM) to provide a risk assessment for nitrous oxide (RIVM, 2016), the report was not followed up by restrictive actions regarding the sale of nitrous oxide. The Commodities Act only regulates the quality of a product related to its proper use and is not suitable for regulating or restricting the recreational use of nitrous oxide. Nor does it restrict possession.

As concerns on nitrous oxide use and its health consequences increased, in 2019, the State Secretary for Health, Welfare and Sport requested the Coördinatiepunt Assessment en Monitoring nieuwe drugs (Coordination Point for Assessment and Monitoring of New Drugs; CAM) to perform a risk assessment on nitrous oxide with the support of the Committee on Risk Assessment of New Drugs, according to established procedures (van Aerts and Niesink, 2012; Dutch Government, 2019a).

Based on its risk assessment, CAM recommended that the use of nitrous oxide should be discouraged and its availability should be limited. The Committee considered existing legislation in chemical and dangerous substances and the extension of municipal bylaws as potential instruments to serve this goal. Another measure to discourage use would be to educate users and potential users. This information should make them aware of the possible negative health effects of use. The Committee also advised that additional research should be carried out to determine the extent of excessive use of nitrous oxide, investigate its addictive properties when used in large amounts and further study the neurological harm caused by the gas. Finally, the Committee recommended continuing to monitor the use of nitrous oxide and its health effects (CAM, 2019).

Following the risk assessment by CAM, an integral approach was developed by the Ministry of Public Health and Sports. Besides a proposal for legislation to restrict the inappropriate use of nitrous oxide by placing it under the powers of the Opium Act, further preventive actions and development of educational material were announced (Dutch Government, 2019b).

A Nitrous Oxide Decision was drafted and opened for public consultation in 2020 and submitted to parliament in autumn 2021 (Dutch Government, 2020c). The Decision would place nitrous oxide under Annex II of the Opium Act. This would imply prohibition of import and export, preparing, handling, selling, delivering, providing, transporting, storing and manufacturing (Dutch Government, 2020d). However, the proper use of nitrous oxide for culinary purposes and technical use, as well as its medical use, would be exempted from the ban. In this way, licit use of nitrous oxide would not be disrupted and consumers could still purchase nitrous oxide for culinary purposes, but only in small quantities in the form of the cannisters used for frothing cream and only above the age of 18 years. The sale of large tanks to consumers would no longer be legal. In this way, the ready availability of large quantities of nitrous oxide is expected to be curtailed (Dutch Government, 2020b). Although on 18 July 2022 the Council of State advised against a general prohibition because of enforcement issues and the necessity to make many exemptions to allow for industrial and technical applications of nitrous oxide ([3]), on 14 November 2022 the Dutch Government announced that nitrous oxide will be a scheduled substance under Annex II of the Opium Act (Schedule II of the Opium Act) as of 1 January 2023 ([4]). The

([3]) https://www.raadvanstate.nl/actueel/nieuws/juli/advies-toevoeging-lachgas-aan-opiumwet/

([4]) https://www.rijksoverheid.nl/actueel/nieuws/2022/11/14/per-1-januari-2023-verbod-op-lachgas#:~:text=Het%20lachgasverbod%20wordt%20van%20kracht,te%20verkopen%20of%20te%20hebben

professional use of nitrous oxide will still be allowed as long as producers and suppliers are in the possession of a so-called opium act waiver.

Faced with public nuisance such as littering of cannisters and balloons and the sale of nitrous oxide in public places, alongside growing concerns related to potential health effects and use in traffic, municipalities have been led to explore and apply restrictive measures by means of municipal bylaws. An increasing number of municipalities have specific provisions to prevent the sale and improper use of nitrous oxide. Advice on this approach was provided by the Vereniging van Nederlandse Gemeenten (Dutch Society of Municipalities). For example, there may be a ban on selling in public places that is specifically aimed at selling nitrous oxide. Another example is designating specific areas where it is prohibited to use and/or sell nitrous oxide (VNG, 2020). By the end of March 2021, more than half of the Dutch municipalities had implemented restrictions on the sale and/or use of nitrous oxide (NOS, 2021).

A guide has been developed to support municipalities (Nijkamp, 2020). This guide, which provides a multifaceted stepwise approach, should support law enforcers and prevention workers alike in dealing with the sale and use of nitrous oxide. In its decentralised approach, municipalities are pivotal in directing the action by first assessing nitrous oxide problems in their municipality, then prioritising these problems politically and administratively, ensuring that all relevant local parties are involved in tackling them, and finally creating a plan together with local stakeholder, in which both the sale and the use of nitrous oxide are dealt with. Sales can be restricted either voluntarily or with the help of legal instruments such as local bylaws and regulations relating to event permits, operation of a public establishment, closing times, restrictions for catering establishments and liquor companies and permits for use of a public place (Nijkamp, 2020; VNG, 2020). Local stakeholders can approach nitrous oxide use cooperatively by exchanging knowledge and increasing capacity, by using legal instruments such as local bylaws, through targeted educational and prevention activities, and by dealing with problematic use, strengthening the social environment and educating first-line professionals (Nijkamp, 2020).

Case study 2: France

In France, nitrous oxide has forcefully established itself on the drug scene over the last five years. An increase in the number of Google searches on nitrous oxide suggests a growing interest in this subject, while the multiplicity of published articles demonstrates that the issue is drawing broader media attention. Media reports reflect how the phenomenon has evolved over the years. Whereas at the beginning they mostly mentioned the presence of cartridges on public roads, soon the focus of reports shifted to alerts on the possible dangers, and more recently the serious consequences have been emphasised. In response to the phenomenon of nitrous oxide, the French Monitoring Centre for Drugs and Drug Addiction (OFDT; Observatoire Français des Drogues et des Tendances addictives) has recently released a report on the recreational use of nitrous oxide (OFDT, 2022).

Epidemiology

According to the 2019 TREND report on recent trends and new drugs there has been 'increasing use of nitrous oxide from the 2000s, during alternative party events, and then, from the mid-2010s, in more general party spaces: student parties organised in nightclubs or festivals with an eclectic musical programme. Gas is sold in the form of a low-priced balloons (1-2 euros). The users are rather young (18 to 25 years old), socially integrated, and the other substances consumed are mainly alcohol and cannabis. Since 2017, use by groups of high school students, in public spaces or at home, has been recurrently reported, first by the Lille site, then by those in Bordeaux and Paris' (OFDT, 2019, our translation).

The TREND report published in 2020 further reports that 'in 2019, the sale of balloons containing the gas (between 1 and 2 euros per unit), in or near party events (free parties, student parties in nightclubs, festivals, bars, etc.), is more frequently observed in Ile-de-France, Brittany, Auvergne, Rhône-Alpes, PACA and Nord-Pas-de-Calais' (OFDT, 2020, our translation).

In the cross-sectional descriptive and analytical COSYS study (Batisse et al., 2021), which investigated the consumption rates of psychoactive substances among individuals aged 18-25 years in France, a questionnaire was mailed to students between January and June 2017. This study recorded 46 203 respondents, mostly in universities (>60 %) and mostly women (63.4 %), with an average age of 21.4 years. The overall prevalence of nitrous oxide use was around 6 % for males and 3 % for females.

In the first quarter of 2021, 1 972 ninth grade students (aged 14-15 years) responded to the national middle and high school survey of adolescents on health and substances (EnCLASS). In the survey, 5.5 % of students reported having ever used nitrous oxide, with boys being twice as likely as girls to have done so (7.3 % vs. 3.7 %) (OFDT, 2021).

At the regional level, studies have been conducted to assess the extent of use and consequences among young people. In particular, several studies showed a relatively high prevalence of use in student populations, although the results vary depending on the study. For example, the i-Share ([5]) study investigated the prevalence of substance use and overlap between various psychoactive substances in students (Perino et al., 2022). This cross-sectional study was conducted in 10 066 students included in the i-Share cohort between 1 January 2015 and 31 December 2017. The psychoactive substances of interest were cannabis, cocaine, amphetamines, nitrous oxide, poppers and MDMA. Most participants were female (75 %), and their average age was 21 years. Lifetime use of at least one substance was reported by 65.5 % of participants. Nitrous oxide was the third most frequently used substance over lifetime (26 % of students) after cannabis and poppers (57 % and 28 % respectively). Among polydrug users (n = 1 242), 65 % used only nitrous oxide and poppers, which demonstrates a strong link between these two substances.

Monitoring

France has a health monitoring system for abuse of and dependence on psychoactive substances. This system consists of a network of 13 centres throughout the country, coordinated by the French National Agency for the Safety of Medicines and Health Products (ANSM). The main remit of the monitoring system is to identify substances that give rise to abuse or dependence, to assess the risk and consequences in terms of public health, and to provide information. In order to fulfil its remit, the network has developed a number of epidemiological tools in addition to the analysis of reports sent by health professionals. In France, it is a regulatory obligation for health professionals to report cases of abuse or dependence to the Addictovigilance (addiction monitoring) centres.

Furthermore, some medicinal products are subject to monitoring mandated by the ANSM. This is the case for the medicinal product EMONO (equimolar mixture of oxygen and nitrous oxide), which has a risk management plan.

([5]) Internet-based Students' Health Research Enterprise, http://www.i-share.fr/

The Addictovigilance Centre of Pays de la Loire carries out this national monitoring, regularly assessing all the data collected by the national network of Addictovigilance centres.

In 2012-2013 EMONO survey, there were five reported cases of use of pure nitrous oxide in the form of cartridges for whipped cream dispensers using balloons. During the subsequent period (October 2013 to August 2016), 9 reports of pure nitrous oxide use were retrieved. The information reported in these cases indicated an increase in doses used and growing evidence of health consequences over the years (CEIP-Addictovigilance de Nantes, 2021).

As a result, monitoring of pure nitrous oxide was set up by the ANSM under the responsibility of the Addictovigilance Centre of Pays de la Loire. Until 2018, the number of reported cases collected by the network remained low (below 10 cases per year). However, since 2019 there has been a large increase in the number of reports with 37 cases in 2019, 120 in 2020 and 358 in 2021 ([6]) (CEIP-Addictovigilance de Nantes, 2021).

In parallel to the health monitoring system described above, a toxicovigilance system is in place consisting of a network of poison control centres coordinated by the French Agency for Food, Environmental and Occupational Health Safety (ANSES). Within this system, the poison control centres have also observed an increase in the number of calls on nitrous oxide received. In 2020, 134 calls were reported, compared with 46 in 2019 and 10 in 2017 and 2018 (ANSES, 2021).

Available data from the Addictovigilance network ([4])

The data collected by the Addictovigilance network allow the consequences of nitrous oxide use reported by health professionals to be analysed. Certain characteristics were found regardless of the study period: the subjects involved were rather young (average age: 22 years), the doses consumed were variable, and daily consumption was reported in nearly half of the cases (47 % of cases reported in 2021 versus 34 % in 2020). At the beginning of monitoring, most cases mentioned the use of small cartridges, but over the years a shift towards larger cylinders has been observed. In some cases, consumption of up to 24 bottles per day and 48 bottles per occasion has been reported. There were very few associated substances reported. The observed effects were mainly euphoria and anxiolysis as well as feeling 'high'. The notion of potentially fatal accidents was increasingly present, including reports of road accidents associated with nitrous oxide use. Risk-taking behaviour was also reported. In 2021, the most commonly reported consequences were substance use disorder and/or use of a high dose (≥20 cartridges or the equivalent in bottles) and/or daily use (in 89 % of the 339 cases analysed). Following substance use disorder and heavy/frequent use, neurological complications came second (in 80 % of patients). Of particular note are spinal cord syndrome and/or peripheral neuropathy, with vitamin B12 deficiency and hyperhomocysteinaemia reported in some cases. Psychiatric symptoms (mainly anxiety, psychosis and behavioural disorders) and cardiac effects were mentioned in about 10 % of cases, including particularly concerning thrombotic events associated with hyperhomocysteinaemia. In addition, coma, loss of consciousness, asthenia, road accidents, burns, falls and so on were reported.

Regarding the data from the poison control centres, the majority of individuals were male with a median age of 20 years. Ile-de-France was the most affected region with a quarter of cases. The way the substance was consumed has also changed over time with bottles becoming more common. The doses consumed were high, with some callers reporting use for more than a year. Of the 134 cases, 126 were symptomatic. Serious neurological disorders were observed, particularly in regular users.

Prevention

In France, a number of actions have been taken both at the regional level and at the national level in response to growing concerns around nitrous oxide. Some of the main communications from health authorities and Addictovigilance centres are:

- an issue of the *Addictovigilance* newsletter dedicated to nitrous oxide, produced by the French Association of Addictovigilance Centres in January 2019 (Addictovigilance, 2019);
- a newsletter on nitrous oxide published by the MILDECA (Inter-Ministerial Mission for the Fight against Drugs and Addictive Behaviours) in July 2019;
- communications to highlight the increase in serious health complications linked to non-medical use of nitrous oxide in France, issued by the French Association of Addictovigilance Centres in November 2019 (French Association of Addictovigilance Centres et al., 2021) and June 2022 (French Association of Addictovigilance Centres, 2022).

([6]) The 2021 data are internal data from the Addictovigilance network.

The DGS (General Directorate of Health) also issued a communication on 19 November 2019. Following this, the Ministry of Solidarity and Health, together with the MILDECA and the OFDT, published a press release with information on the increase in serious health cases related to the diverted use of nitrous oxide. In July 2020, the ANSM and the ANSES respectively published a summary of the Addictovigilance (addiction monitoring) expert report and a toxicovigilance report on nitrous oxide. These publications were supported by a press release from the DGS (DGS, 2020). Subsequently, in November 2021, the ANSM and the ANSES published new reports on cases of misuse reported to the Addictovigilance centres and poison control centres (ANSES, 2021; CEIP-Addictovigilance de Nantes, 2021).

Various prevention campaigns, both local and national, have been launched on social media and in the form of posters to inform users about harms associated with nitrous oxide use ([7]).

Policy response

At the local level, municipal bylaws have been issued by the mayors of various municipalities aiming to restrict access to minors.

On 5 April 2019, a proposal to protect minors from dangerous uses of nitrous oxide was registered ([8]). This bill was passed by the Senate in December 2019 and then sent to the Social Affairs Committee of the National Assembly. The bill has been amended since its introduction in the Senate in December 2019, expanding the text to include adults and to protect minors from psychoactive substances in general. It was adopted unanimously on 17 March 2021 by the Social Affairs Committee of the National Assembly, and was then presented and adopted in a first reading before the National Assembly on 25 March 2021. On 25 May 2021, the law was adopted in a second reading by the Senate. It was signed on 1 June and was published in the Official Journal of the French Republic on 2 June 2021 ([9]). The maximum quantity authorised for sale to individuals will soon be set by joint order of the ministers for the economy and health.

The law to prevent dangerous uses of nitrous oxide now prohibits the sale or offer of nitrous oxide to any minor, states that retailers may require proof of age from customers purchasing nitrous oxide cartridges, and requires that for internet purchases, sites must mention the prohibition of sales to minors before proceeding with any nitrous oxide transaction. It also prohibits the sale or offer of this product in bars or clubs, at student parties or similar and in tobacco shops. Information about the dangers of inhaling the gas will appear on the packaging of all forms of the product, and the wording will be determined by a decree currently in preparation. More recently, in March 2022, a proposal for legislation to ban all sales of nitrous oxide in any form of packaging was introduced ([10]).

([7]) The last campaign was in 2021: https://www.drogues.gouv.fr/lusage-detourne-du-protoxyde-dazote-une-pratique-risques-de-plus-en-plus-repandue

([8]) *Sénat n°438*, http://www.senat.fr/leg/ppl18-438.html

([9]) Law No 2021-695 of 1 June 2021 aiming to prevent the dangerous uses of nitrous oxide: www.legifrance.gouv.fr/jorf/id/JORFTEXT000043575111

([10]) https://www.assemblee-nationale.fr/dyn/15/textes/l15b5174_proposition-loi#

Case study 3: Denmark

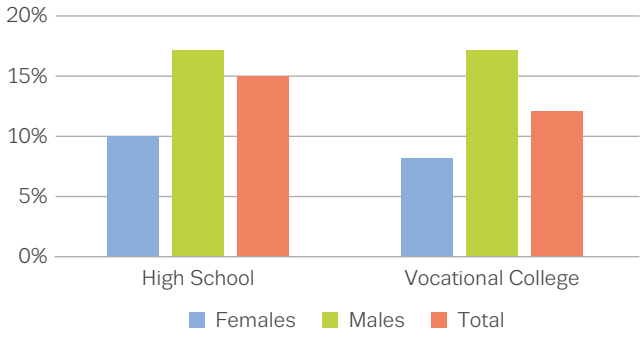
Epidemiology

Recent years have shown that the use of nitrous oxide — namely, inhaling laughing gas from cartridges for the purpose of intoxication — has become a trend among young people in some environments.

In 2019, a representative survey was conducted for the first time on the prevalence of laughing gas from cartridges in Danish secondary schools ([11]). The results from this study show that more boys and young men have tried to inhale laughing gas from cartridges at least once in their lifetime (lifetime prevalence, LTP) than girls and young women. Figure 6 shows that among males aged 15-25, 17 % of those in vocational schools and 17 % of those in secondary schools have tried to inhale laughing gas from cartridges at some point. For females, the figures are 10 % and 8 % respectively.

At the same time, the figures show that laughing gas is most common in the Copenhagen area, where four times as many secondary school students have tried to inhale laughing gas from cartridges (19 %) as in the North Jutland region (4 %).

FIGURE 6
Lifetime prevalence of inhaling laughing gas from cartridges among students in high school (n = 26 599) and vocational college (n = 4 237) aged 15-25 years



Source: Danish Health Authority (2019).

The survey also asked students whether they have inhaled laughing gas from cartridges in the past year and thus have more recent use (last-year prevalence, LYP). The results show that this applies to 7 % of young people in secondary schools and 6 % in vocational schools.

Harms associated with use

There are few systematic sources describing the extent of harms associated with laughing gas from cartridges in Denmark. However, it is possible to obtain information from the Danish National Poison Centre, which reports recorded requests from the public or health professionals.

An overview of the number of contacts with the poison centre shows that these have increased over the years (see Table 4). However, there is a possible impact from the 2020 regulatory framework (see the section on the June 2020 legislation below). With the implementation of this law in 2020, there was a slight decrease in contacts. However, at the end of 2021, the trend started rising again with an increase in contacts. It should be noted that there is uncertainty behind the figures, given the small numbers involved.

The poison centre reports that the vast majority of harmful effects related to inhalation of laughing gas from cartridges in the past few years relate to nerve damage and freezing of the mouth and throat.

Prevention

Drug prevention initiatives targeting young people are mostly at municipal level and are supported by national interventions. In relation to recreational use of laughing gas, the focus is on informing young people about the consequences and harms of inhaling laughing gas from cartridges, as part of other drugs education and community action initiatives.

TABLE 4
Changes in number of requests to the Danish National Poison Centre involving laughing gas over the years

Year	2021	2020	2019	2018	2017	2016	2015	2014	2013	2012	2011	2010
Number	73	90	62	39	18	22	16	N/A*	N/A*	N/A*	N/A*	N/A*

* Data not shown due to sample size < 5.

([11]) Laughing gas from gas cartridges, prevalence on secondary education. Danish Health Authority 2019

The initiatives are often carried out through established partnerships in the municipalities between schools, social services and police. Healthcare workers or specialist counsellors in the municipalities closely monitor the use of laughing gas as an intoxicant among local youth and can intervene with more specific and extensive interventions if needed.

To support the municipal initiatives, the Danish Health Authority prepares counselling materials and other media that can be used locally in dialogue with young people and parents, for example. The Danish Health Authority has produced the altomstoffer.dk website for young people, which, among other things, provides information on recreational laughing gas including the consequences and harmful effects of use. In addition, the Danish Health Authority is also responsible for the website snakomlattergas.dk targeting parents and other adults involved with young people. This site, created in 2019, aims to encourage parents and others to have conversations with young people about laughing gas. The Danish Health Authority regularly assesses developments in relation to the new legislation to consider whether further initiatives are needed.

When preventing drug use in general and use of laughing gas in particular, it is essential that initiatives at national and local level take into account the relatively low prevalence rates among young people. The Danish Health Authority considers that it would not be appropriate, for example, to launch large and wide-ranging campaigns targeting all young people on the use of laughing gas from cartridges, as this could make the practice seem more mainstream than it is and inadvertently support and normalise use. Instead, the focus should be on targeted communication to young people in relevant arenas and groups, while making the information available to young people or adults involved with young people who search for it.

Policy response

In June 2020, a number of restrictions were introduced in Danish legislation to prevent use of laughing gas as an intoxicant among young people. The legislation can be summarised as follows.

- Laughing gas must not be sold to customers under 18 years of age.
- Laughing gas must not be brought into Denmark by persons under 18 years of age.
- Retailers may only sell two small cartridges of laughing gas per day to each private customer.
- Laughing gas cartridges must not be sold in places selling alcohol, tobacco products or electronic cigarettes.

The law prohibits the import and sale of large cartridges of laughing gas.

Businesses can still sell nitrous oxide in cartridges to business customers. The Danish Safety Technology Authority must check compliance with the regulations on selling laughing gas in physical shops as well as online shops and will be given more resources for monitoring compliance. Moreover, it will be possible to apply more stringent penalties if regulations are violated regarding the sale of laughing gas to private customers.

Evaluation of the effects of the control measures

The Ministry of Industry, Business and Financial Affairs has evaluated the new law of 2020 in terms of its effect on abuse. Their evaluation found that it has become more difficult for consumers to buy laughing gas cartridges for recreational purposes, but also showed that laughing gas trading continues — partly because some sales have shifted onto social media. Following the results of the evaluation, it has been agreed between the Danish Government and its contracting parties that the legislation will be further clarified and strengthened to prevent abuse and to ensure that fewer Danes end up with health harms as a result of inhaling of laughing gas from cartridges.

In early 2022, work began on proposed legislation that includes the following four specific actions.

1. **Punishment after anonymous regulatory control:** In future, it should be possible to punish entities before the courts if undercover checks by the Danish Safety Technology Authority find that an offence has been committed.
2. **Social media sales:** Some of the illicit sales of laughing gas have moved from, for example, kiosks to social media, specifically including Snapchat. The Danish Safety Technology Authority has therefore started working with Snapchat, Facebook and Instagram to regularly close profiles where illicit laughing gas sales take place. Efforts have already borne fruit as a large number of profiles have been closed. In order to further investigate sites where laughing gas cartridges are sold and which do not cooperate with the authorities, social media companies may be ordered to close profiles or

sites in future. If social media companies do not comply with this, they will be fined.

3. **Cheating with CVR (VAT) numbers:** In future, companies selling laughing gas cartridges to traders will have to check that the CVR numbers being entered are correct. The company can do this by cross-checking that the CVR number entered matches the details of the person buying (e.g. via the CVR register). This will ensure that laughing gas does not fall into consumers' hands above the permitted limits.
4. **Possession of laughing gas:** The possession of laughing gas for recreational purposes — for example, in nightlife settings and upper secondary schools — will be prohibited in quantities above the limit of two small cartridges. In the past, only sales were prohibited. In addition, it will be prohibited to sell laughing gas cartridges for recreational purposes, irrespective of volume.

The law was not passed due to the general election on 1 November 2022. It is not yet known when the proposed legislation and the new rules on laughing gas cartridges for recreational purposes will be submitted to the Danish Parliament.

Case study 4: Lithuania

Epidemiology

Although the data from the General Population Survey (GPS) have not indicated that nitrous oxide use is particularly high in Lithuania, the trends over 2016-2021 show an increase in the use of inhalants from 2.2 % to 5 % (Table 5). It should also be noted that there is only limited data on nitrous oxide use and there have been no previous specific surveys on nitrous oxide use in Lithuania.

TABLE 5
The use of nitrous oxide in Lithuania (General Population Survey, 2016; 2021)

	2016	2021
Have you ever tried inhalants?	2.2 %	5 %
Have you ever tried nitrous oxide?		
Lifetime prevalence	0.2 %	0.6 %
Never	1.9 %	4.4 %

Another trend observed in the GPS is that lifetime prevalence of nitrous oxide use has increased threefold in Lithuania. Although the number of nitrous oxide users increased significantly, the number of those who have never tried nitrous oxide has also more than doubled. However, it is difficult to understand whether these changes might have been influenced by COVID-19 pandemic.

Policy response

In the period of 2019-2020, there was observed to be a growing interest in nitrous oxide from society, especially from retailers who wanted to buy or distribute nitrous oxide. State authorities such as the Ministry of Health and the Drug, Tobacco and Alcohol Control Department received many inquiries from the public and businesspeople about the possibilities of distributing nitrous oxide for recreational use and the conditions under which nitrous oxide could be legally purchased in Lithuania. In addition, more and more new e-shops selling nitrous oxide were established and there was observed to be growing interest and increasing use at summer music festivals. The information related to growing nitrous oxide use in other countries was also analysed. All of this pointed to increasing interest in the recreational use of nitrous oxide and resulted in state institutions beginning to debate the need to think about controlling nitrous oxide. At that time, the sale and use of nitrous oxide was legal and not specifically controlled in Lithuania.

It is important to mention that the Ministry of Health and the Ministry of the Environment expressed their concern about growing public interest in importing, using and distributing nitrous oxide and called upon other institutions to take action. In response to these concerns, in 2019, the Drug, Tobacco and Alcohol Control Department — which is the national competent authority for drug control and prevention — sent an official enquiry to industry bodies and retail companies about their need to use nitrous oxide in their legal activities. The main purpose of this enquiry was to evaluate the need for legal nitrous oxide for industrial purposes in Lithuania. The data collected showed that there was no significant demand from industry to use this substance (only two companies claimed to use nitrous oxide for their industrial needs). The survey results indicated that nitrous oxide is quite widely used as a food additive (E942), but the responses received from representatives of the food industry gave no sign that there would be difficulties if it were banned for industrial use.

Considering data collected from legal entities and the concerns of state authorities, a proposal to introduce restrictive measures on nitrous oxide was submitted to the Ministry of Health. After the notification procedure to the European Commission, nitrous oxide was included on the List of Narcotic and Psychotropic Substances, Schedule IV 'Narcotic and psychotropic substances allowed for medical purposes and/or used for non-pharmaceutical industrial purposes'. This means nitrous oxide may be used only for medical and industrial purposes, and only by companies. Companies must obtain authorisation before starting their activities. Following this authorisation, only wholesale to other authorised companies is permitted and retail trade is prohibited. In addition, sending nitrous oxide by mail is also prohibited and selling nitrous oxide to individuals is not allowed either. These requirements do not apply to healthcare, scientific and state institutions. The legal amendments were adopted on 1 October 2020.

However, after these legal requirements came into force, there was huge pressure from the food industry and the Association of Lithuanian Restaurant Chefs and Confectioners, who have expressed their concern about restrictions on the accessibility of nitrous oxide. After discussions with the food industry and highlighting the issues, a compromise was reached between state authorities and food industry representatives. The regulation on nitrous oxide remains the same, but an exception has been made. Since 25 December 2020, it is permitted to use nitrous oxide when marketed, used

and/or consumed as a food additive (E942) as defined in Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. No permits or authorisations are required for this exemption.

As a result, no new e-shops selling nitrous oxide have appeared since that time, and the existing ones have closed. No more phone calls or inquiries have been received regarding the recreational use, import and distribution of nitrous oxide.

Case study 5: Ireland

Control status of nitrous oxide

At present, nitrous oxide is not a controlled substance under the Misuse of Drugs Act 1977. It can be legally sold for catering and industrial purposes. The Criminal Justice (Psychoactive Substances) Act 2010 prohibits the sale, importation or exportation of psychoactive substances; under this legislation, it is illegal to sell nitrous oxide for its psychoactive properties.

Epidemiology

Nitrous oxide use among adults

Prevalence of nitrous oxide use is not routinely collected in Ireland's National Drug and Alcohol survey. The only source of data on adult nitrous oxide use is the 2021 European Web Survey on Drugs (EWSD), in which Ireland participated and which included a module on nitrous oxide. Only adults aged 18 and over and who had used illicit drugs were included in this survey. Data were collected from March-May 2021.

Table 6 presents the most recent use of nitrous oxide among EWSD respondents; of the 4 398 respondents who answered this question, 1.1 % reported last month use and a further 3.7 % had used nitrous oxide in the last year. In total, 23.3 % had ever used nitrous oxide. Respondents aged 18-24 years were most likely to have used nitrous oxide in the last year.

The module on nitrous oxide was completed by 142 respondents; 39 % of respondents stated that they had first used nitrous oxide in the last year and 32 % had first used it 1-2 years ago (Table 7). Due to the low number of respondents aged 35 years and over who have used nitrous oxide, results are presented for just two age groups — 18-24-year-olds and 25 years and over.

TABLE 6
Most recent use of nitrous oxide among EWSD respondents by sex and age group (%)

	All	Male	Female	18-24 years	25-34 years	≥35 years
Last month	1.1	1.2	1.1	1.9	0.8	0.2
Last year (but not last month)	3.7	3.8	3.2	5.5	2.9	1.5
More than 12 months ago	18.5	20.2	15.1	12.5	23.0	22.1
Never	76.7	74.8	80.6	80.1	73.4	76.2

TABLE 7
Length of time since first use of nitrous oxide by sex and age group (%)

	All	Males	Females	18-24 years	>25 years
	n=139	n=96	n=39	n=95	n=44
In the last year	38.9	36.5	43.6	42.1	31.8
1-2 years ago	31.7	32.3	33.3	35.8	22.7
≥3 years ago	29.5	31.3	23.1	22.1	45.5

Seventy-one per cent of respondents first used nitrous oxide in Ireland, while 28% first used it as a tourist in Europe (Table 8). Those aged 25 years and over were more likely than 18-24-year-olds to first use nitrous oxide in Europe (37 % versus 24 %).

TABLE 8
Location of first use of nitrous oxide, by sex and age group (%)

	All	Males	Females	18-24 years	>25 years
	n=132	n=94	n=34	n=91	n=41
In Ireland	71.2	72.3	67.7	75.8	61.0
As a tourist in Europe	28.0	27.7	29.4	24.2	36.6
As a tourist in Asia	0.8	0.0	2.9	0.0	2.4

In the last year, 89 % of those who had used nitrous oxide reported infrequent use (1-11 days) while 11 % reported occasional use (12-51 days); there were no sex- or age-group differences. Ninety-one per cent stated that they had used nitrous oxide by inhaling from a balloon, while 11 % had inhaled it from a canister. On a typical day that nitrous oxide was used, 21 % used no more than one canister while 26 % used at least 10 (Table 9).

TABLE 9
Number of canisters typically used on a day that nitrous oxide is used, by sex and age group (%)

Number of canisters	All	Males	Females	18-24 years	>25 years
	n=133	n=96	n=33	n=90	n=43
≤1	21.1	16.7	27.3	18.9	25.6
2-3	25.6	26.0	27.3	24.4	27.9
4-5	18.1	19.8	15.2	18.9	16.3
6-9	9.0	10.4	3.0	8.9	9.3
≥10	26.3	27.1	27.3	28.9	20.9

Context of nitrous oxide use

The most common settings for using nitrous oxide were at a domestic party (63 %) and at home (59 %) (<Table> Table 10). The low proportion using nitrous oxide in a club or bar (14 %) may be explained by the closure of these settings as a result of COVID-19 restrictions.

Fifty-three per cent of respondents stated that people usually share nitrous oxide with them or give it to them for free (Table 11), while 30 % usually buy it from a shop online.

TABLE 10
Settings in which nitrous oxide has been used in the last year, by sex and age group (%)

	All	Males	Females	18-24 years	>25 years
	n=142	n=98	n=40	n=95	n=47
Domestic party	62.7	66.3	55.0	64.2	59.6
At home	58.5	62.2	50.0	60.0	55.3
Public space (street, park, etc.)	16.2	19.4	10.0	19.0	10.6
Club or bar	14.1	16.3	10.0	15.8	10.6
Illegal rave	12.0	11.2	15.0	10.5	14.9

Note: Respondents could select more than one option.

TABLE 11
How nitrous oxide is usually sourced, by sex and age group (%)

	All	Males	Females	18-24 years	>25 years
	n=131	n=91	n=36	n=90	n=41
People share it with me or give it to me for free	53.4	48.4	61.1	53.3	53.7
Buy it from a shop online	29.8	36.3	16.7	27.8	34.2
Buy it from a drug dealer	9.9	9.9	11.1	10.0	9.8
Buy it from a drug dealer on social media	6.1	4.4	11.1	7.8	2.4
Buy it from internet encrypted markets (darknet)	0.8	1.1	0.0	1.1	0.0

Note: Respondents could select more than one option.

Nitrous oxide use among young people

The only available data on nitrous oxide use among young people (<18 years) is a Planet Youth survey conducted among post-junior certificate students in schools in North County Dublin in 2021. The questions on nitrous oxide were answered by 2 384 respondents. The main results were:

- 6.2 % of young males and 5.3 % of young women have ever used nitrous oxide;
- there were no significant differences in use by gender in the overall sample;
- there was a gender x grade interaction, with males attending fifth year in the sample having significantly greater lifetime prevalence of use at 11.9 %;
- being a male in fifth year in this sample increased the odds of lifetime use by a factor of 3.33 (95 % CI: 1.86-5.94);
- heavy use (i.e. more than 40 lifetime uses) was low, at 1 % of males and 0 % of females.

Complete results will be available at a later stage in 2022.

Nitrous oxide use in festival settings

A 2019 online survey of 1 193 Irish festival attendees aged 18 and over found that 28 % had used nitrous oxide while attending music festivals in Ireland in the last year. Of those who had attended music festivals abroad (n = 619), 38 % had used nitrous oxide. Respondents to this survey typically used stimulant 'club drugs' mainly as part of a polydrug use pattern (Ivers et al., 2022).

Availability of nitrous oxide

To assist with this technical report, Merchants Quay Ireland undertook a short survey of 15 member organisations of the National Voluntary Drug and Alcohol Sector. Of these, eight provide services in Dublin only, four provide services in Dublin and other locations and three are based solely outside Dublin.

None of the respondents had robust data on prevalence. However, 12 stated that nitrous oxide was available in their area — 8 believed it had increased in popularity in the last year, with four believing its popularity had remained the same. The sporadic nature of its popularity was also highlighted. Respondents reported that it can be very prevalent for a number of months at a time, and that it is particularly prevalent on weekends, mid-term breaks and bank holidays.

Regarding availability, one Dublin respondent noted that it is available in large blue bottles for EUR 100 per bottle and also in the smaller capsules that cost EUR 50 a box. Young people arrange to buy it from a local nitrous oxide dealer, as most shops will not sell it to them even though it is available in some of the discount shops. Another respondent reported a difference in cost between online purchases, where it costs 30 cents for a canister, and street purchases, where it can cost EUR 2-5 a canister.

Respondents viewed nitrous oxide as a drug that was primarily used by younger people who also use other drugs. Two respondents identified a couple of distinct groups and contexts — early teens who use nitrous oxide in parks and wastelands, and older teens who use it at house parties. It was noted that there is a growing trend for people in their early 20s to use it at parties or as 'pre-loading' before going out.

Seizures of nitrous oxide

A request was submitted to An Garda Síochána (the national police service of Ireland) regarding seizures of nitrous oxide. They replied that there have been a number of significant seizures of canisters containing nitrous oxide in Ireland over the past couple of years. However, information on the number and size of the seizures is not published as there are still issues in relation to the forensic analysis of nitrous oxide, which they are currently in the process of advancing.

Harms associated with nitrous oxide

Requests for information were submitted to a number of sources in order to assess the extent of nitrous oxide-related harm in Ireland: the National Drug Treatment Reporting System (NDTRS), the National Drug Related Deaths Index (NDRDI), the Hospital Inpatient Enquiry (HIPE) scheme and emergency departments.

- National Drug Treatment Reporting System (NDTRS): In mid-2020, in response to anecdotal reports of increased use, the NDTRS added nitrous oxide to its system. In 2020, fewer than five episodes of treatment were reported. Preliminary data from 2021 indicate that 10 episodes of treatment were reported. The majority of these cases were male and the mean age was 16 years. All were new cases, i.e. they had never received treatment before, and most were polydrug users who also reported problem use of cannabis.
- National Drug Related Deaths Index (NDRDI): The NDRDI recorded no drug poisoning deaths due to nitrous oxide for the period 2004-2017 inclusive. Data for 2018 onwards are not yet available.

FIGURE 7
Seizure of over 59 000 nitrous oxide canisters worth over EUR 1.9 million in Meath and Dublin by Revenue in December 2021

Source: https://revenue.ie/, https://twitter.com/RevenueIE) ® Revenue.

- Hospital Inpatient Enquiry (HIPE) scheme: HIPE records information on discharges from hospitals in Ireland. Poisoning by nitrous oxide falls under the ICD-10-AM code T41.0 'Poisoning by Inhaled anaesthetics'; as this code is used for poisoning by any inhaled anaesthetic, it is not specific to nitrous oxide. However, analysis of discharges from 2018-2020 inclusive indicate that, in this 3-year period, there were fewer than five discharges with a T41.0 diagnosis.
- Emergency departments: A case report was published in 2022 describing the presentation of two young males to the emergency department of a large urban university hospital in Dublin with progressive neurological dysfunction related to nitrous oxide use (McCormick et al., 2022). A case with subacute combined degeneration of the spinal cord secondary to nitrous oxide use has also been reported by a hospital in Dublin.

Impact of the COVID-19 pandemic on nitrous oxide use in Ireland

In 2020, the Health Service Executive surveyed the Local and Regional Drug and Alcohol Task Force areas to identify patterns and emerging harms and included a set of questions on nitrous oxide. Most areas reported the use of nitrous oxide in their area and considered that this trend was emerging pre-COVID-19, with notable increases in use during this period. During the same period, there was increased media attention and significant concern relating to use in public spaces and drug-related litter in some areas, as a result of young people congregating during COVID-19 lockdowns. As a result, a number of local education webinars were hosted and new resources were developed to inform concerned parents and to provide harm reduction advice for people who use nitrous oxide.

Case study 6: Portugal

The Portuguese authorities have been monitoring the situation concerning the recreational use of nitrous oxide. Most of the data presented in this case study are from 2021.

Availability

Reports from law enforcement authorities mention the distribution, retail sale and consumption of nitrous oxide either through commercial establishments (catering and drinking establishments), through street distribution or at spontaneous parties held in the street or in private residences, as well as on websites or through contacts via social networks.

- During 2020, there were no occurrences or seizures of nitrous oxide (bottles or balloons).
- During the year 2021, about 93 occurrences or seizures of nitrous oxide (bottles or balloons) were recorded, namely in Lisbon, Setúbal and Faro. Around 300 to 400 units of several sizes of canisters and bottles were seized.
- During the year 2022, to date, there have been around 35 occurrences or seizures of nitrous oxide (bottles or balloons).

The bottles of nitrous oxide seized by law enforcement in various settings were inscribed with the code 'UN1070', which, according to the Manual of Intervention in Emergencies with Hazardous Chemical, Biological and Radiological Materials, corresponds to nitrous oxide.

Control status

Nitrous oxide is not listed as a prohibited substance in the legal regime applicable to the trafficking and consumption of narcotics and psychotropic substances, Decree-Law 15/93 of 22 January.

Following the seizures made during 2021, and to address the threat to public health resulting from the recreational use of nitrous oxide, its ease of access and the need to defend the 'principle of health precaution', the General Directorate for Intervention on Addictive Behaviours and Dependencies (SICAD) submitted a proposal to the Ministry of Health to regulate the sale and consumption of nitrous oxide. The proposal was approved and since 8 September 2022, nitrous oxide is classified as a prohibited psychoactive substance and included in the NPS list under Order No 232/2022 of 7 September 2022 ([12]) (update to the Decree-Law No 54/2013 of 17 April).

FIGURE 8
Examples of seizures of nitrous oxide during 2021-22 by Portuguese law enforcement

Source: www.gnr.pt ® Guarda Nacional Republicana.

([12]) https://dre.pt/dre/detalhe/portaria/232-2022-200734331

Prevalence of use

In the most recent studies carried out by SICAD (ECATD-CAD 2019, ESPAD 2019, National Defence Day 2019, European Web Survey 2021), there are no references to the use of nitrous oxide.

Responses at national level

In 2021, law enforcement authorities prepared a report on the growing use of nitrous oxide in a recreational context, advising of increasing evidence of the sale of nitrous oxide balloons for human consumption, as confirmed by the number of seizures during that year. Its objective was to raise awareness, to alert the various competent authorities responsible for addressing the issue and, if necessary, to review the legal framework for the substance.

An informal inter-ministerial working group was created in 2021. Considering the data available and the harms that recreational nitrous oxide use may cause, this group decided to propose regulatory measures to reduce the general public's access to the substance and control trade, with particular attention to minors.

Case study 7: United Kingdom

Epidemiology

Nitrous oxide has become one of the most commonly abused substances in England and Wales, particularly among young people. The Home Office has published drugs misuse data from the Crime Survey for England and Wales, including data on the prevalence of nitrous oxide use, starting in the year 2012/13. These are summarised in Table 12 below. In the years 2014/2015 and 2015/2016, questions on the use of nitrous oxide were not included in the survey.

TABLE 12
Last-year prevalence of nitrous oxide use in England and Wales (UK Home Office, 2013, 2014a, 2017, 2018a, 2019, 2020)

Year	16-59-year-olds	16-24-year-olds
2012/2013	2.0 %	6.1 %
2013/2014	2.3 %	7.6 %
2016/2017	2.6 %	9.3 %
2017/2018	2.3 %	8.8 %
2018/2019	2.3 %	8.7 %
2019/2020	2.4 %	8.7 %

The 2019/20 Crime Survey for England and Wales indicated that 2.4 % of adults aged 16 to 59 years and 8.7 % of 16- to 24-year-olds reported using nitrous oxide in the last 12 months. This is equivalent to around 796 000 and 549 000 individuals respectively. It makes nitrous oxide the second most prevalent drug among young adults aged 16 to 24 years (after cannabis) and the third most prevalent for adults aged 16 to 59 years (after cannabis and powdered cocaine) (UK Home Office, 2020).

An alternative source of data on prevalence of nitrous oxide use is the Global Drug Survey (GDS), which gathers data from global participants in an online survey. The survey uses a non-probability method and has a self-selected sample, consisting of people who are more likely than average to be male, young and to use drugs (in the 2018 survey, 54 % of the global sample reported that they had used illicit drugs in the previous year). The data are therefore not suited to providing estimates of prevalence in the overall population but can be used to assess trends in drug use among this specific drug-using sub-population. For nitrous oxide, UK respondents in the GDS reported last-year prevalence of 27.2 %, 20.5 % and 24.7 % in the years 2011/2012, 2013 and 2014, respectively. The lifetime prevalence reported by UK respondents in the GDS was 49.6 %, 38.6 %, 38 %, 38 % and 31 % for the years 2011/2012, 2013, 2014, 2016 and 2017, respectively. The reported last-month prevalence in the year 2013 was 7.7 % (ACMD, 2015; Global Drug Survey, 2015; Kaar et al., 2016; UK Home Office, 2018b).

In a survey among 330 homosexual men in gay-friendly London clubs, lifetime prevalence was 28.1 % and last-year use was 11.9 % (ACMD, 2015; van Amsterdam et al., 2015).

In a survey among 18-25 years old in the United Kingdom in 2017, 77.1 % (n = 108) had heard of the drug hippy crack and 27.9 % (n = 39) had taken hippy crack in the past 12 months. Of the 39 participants reporting previous experience of using hippy crack, the majority (n = 27) took it on more than one occasion during the past year. Notably, only seven users had taken it on 10 or more occasions. The amount taken on each occasion varied, with 46.2 % (n = 18) partaking once or twice in one sitting but the majority having ≥3 intakes and some even more than 20 intakes. Most of the users indicated preferring to use it with friends and not alone (Ehirim et al., 2018).

Based on GDS 2014 data for the UK, among last-year users, the most common method of inhalation was from a balloon (94.2 %), followed by whipped cream dispensers (4.8 %). The most common source of nitrous oxide for last-year users was friends (60.2 %), followed by the internet (50.7 %), festivals (48.3 %), dealers (14.1 %), head shops (12.1 %) and supermarkets (6 %). The most common place of use was at house parties (82.5 %), followed by festivals (73.7 %), at home (49.7 %), at clubs (42.5 %) and at work (2.2 %) (ACMD, 2015).

In the 2016 NHS Digital survey 'Smoking, Drinking and Drug Use among Young People in England', 9 % of the schoolchildren aged between 11 and 15 years old reported that they had been offered nitrous oxide, and 4 % responded they had used in the last year (UK Home Office, 2018b).

A one-off data release from the Office for National Statistics shows the number of deaths associated with nitrous oxide between 1993 and 2017. Although the numbers are very small, there are signs of an increasing trend. If we examine the rolling average number of deaths for 5-year periods, the average number peaked at just below five deaths per annum on average for 2013-2017 (Office for National Statistics, 2018; Rough and Brown, 2020).

According to the Office for National Statistics, between 2001 and 2020, there were 56 registered deaths involving nitrous oxide in England and Wales, with 45 of those having been registered since 2010 (Office for National Statistics, 2022).

Policy response

Concerns on the increase in recreational use of nitrous oxide were expressed in a letter from the Advisory Council on Misuse of Drugs (ACMD) to the Home Secretary and the Secretary of State for Health in 2015 (ACMD, 2015). At that time, the sale of nitrous oxide to under-18-year-olds was already illegal through the Intoxicating Substances (Supply) Act 1985. In 2011, the British Compressed Gases Association published a leaflet on the dangers of industrial gas abuse, including that of nitrous oxide, and have since been raising awareness of gas abuse by meeting stakeholders. In 2014, the Home Office published guidance on restricting the supply of nitrous oxide for recreational use (UK Home Office, 2014b) and, in its letter to festivals organisers in 2013 and 2014, the Minister for Crime Prevention also highlighted the risks around the recreational use of gases, including nitrous oxide. The same year, the Local Government Association issued a national public health warning about nitrous oxide in a press release (ACMD, 2015).

In its letter of 2015, the ACMD recommended the Government work with industry and retailers to better understand the supply chain, including areas of vulnerability, and to increase awareness with major retailers about the recreational market to identify key features of misuse, such as bulk purchases and the combined sale of 'crackers'. The ACMD also recommended that local councils consider addressing antisocial behaviour (litter) associated with use of nitrous oxide through local court orders, and encouraged NHS PROTECT to ensure that NHS Trusts and associated medical facilities were fully informed as to the issue of misappropriation of medical gas cylinders using the recently published guidance. Finally, the ACMD recommended that the Department of Health outline current audit processes in place that counter diversion and misuse in hospitals and other relevant medical settings (ACMD, 2015).

As of 2016, nitrous oxide is covered by the Psychoactive Substances Act (PSA) and is illegal to give away or supply for its psychoactive effect. It is not illegal to possess nitrous oxide unless it is with intent to supply. Possession of nitrous oxide in prison is also an offence (Rough and Brown, 2020).

In 2018, the PSA was reviewed by the Home Office. The report concludes that the use of nitrous oxide (among all adults) does not appear to have been affected by the Act, although there are limited time-series data that could be used to draw comparisons (UK Home Office, 2018b).

In September 2021, the UK Home Secretary issued a letter to the chair of ACMD asking the council to conduct an updated assessment of the health and social harms of nitrous oxide, and, following the assessment, advise on whether nitrous oxide should be controlled under the Misuse of Drugs Act 1971 (UK Home Secretary, 2021).

References

Addictovigilance (2019), 'Protoxyde d'azote', *Bulletin de l'association des centres d'addictovigilance*, No 9 (https://addictovigilance.fr/wp-content/uploads/spip/pdf/bulletin_addictovigilance9_site.pdf).

ACMD (Advisory Council on the Misuse of Drugs) (2015), *Letter to the home secretary and secretary of state for health on nitrous oxide abuse* (https://www.gov.uk/government/publications/acmd-advice-on-nitrous-oxide-abuse).

van Aerts, L. A. (1995a), 'Investigation, using rat embryo culture, of the role of methionine supply in folic acid-mediated prevention of neural tube defects', *Toxicology In Vitro*, Vol. 9, No 5, pp. 677-684 (doi:10.1016/0887-2333(95)00070-o).

van Aerts, L. (1995b), 'Embryotoxicity studies on cyclophosphamide and homocysteine', PhD thesis, Radboud University (http://hdl.handle.net/2066/146092).

van Aerts, L. A., Blom, H. J., Deabreu, R. A., Trijbels, F. J., Eskes, T. K., Copius Peereboom-Stegeman, J. H. and Noordhoek, J. (1994), 'Prevention of neural tube defects by and toxicity of L-homocysteine in cultured postimplantation rat embryos', *Teratology*, Vol. 50, No 5, pp. 348-360 (doi:10.1002/tera.1420500506).

van Aerts, L. and Niesink, R. (2012), 'Netherlands — early warning system', in Gallegos, A. and Sedefov, R. (eds.), *Early warning system national profiles*, Publications Office of the European Union, Luxembourg, pp. 105-112 (https://www.emcdda.europa.eu/thematic-papers/ews_en).

van Aerts, L. A., Poirot, C. M., Herberts, C. A., Blom, H. J., De Abreu, R. A., Trijbels, J. M., Eskes, T. K., Peereboom-Stegeman, J. H. and Noordhoek, J. (1995), 'Development of methionine synthase, cystathionine-beta-synthase and S-adenosyl-homocysteine hydrolase during gestation in rats', *Journal of Reproduction and Fertility*, Vol. 103, No 2, pp. 227-232 (doi:10.1530/jrf.0.1030227).

Aldridge, L. M. and Tunstall, M. E. (1986), 'Nitrous oxide and the fetus. A review and the results of a retrospective study of 175 cases of anaesthesia for insertion of Shirodkar suture', *British Journal of Anaesthesia*, Vol. 58, No 12, pp. 1348-1356 (doi:10.1093/bja/58.12.1348).

Alkire, M. T. and Gorski, L. A. (2004), 'Relative amnesic potency of five inhalational anesthetics follows the Meyer-Overton rule', *Anesthesiology*, Vol. 101, No 2, pp. 417–429 (doi:10.1097/00000542-200408000-00023).

Allison, R. H., Shirley, A. W. and Smith, G. (1979), 'Threshold concentration of nitrous oxide affecting psychomotor performance', *British Journal of Anaesthesia*, Vol. 51, No 3, pp. 177-180 (doi:10.1093/bja/51.3.177).

Al-Noori, S., Cimpan, A., Maltzer, Z., Kaiyala, K. J. and Ramsay, D. S. (2018), 'Plasma corticosterone, epinephrine, and norepinephrine levels increase during administration of nitrous oxide in rats', *Stress*, Vol. 21, No 3, pp. 274-278 (doi:10.1080/10253890.2017.1402175).

van Amsterdam, J., Nabben, T. and van den Brink, W. (2015), 'Recreational nitrous oxide use: prevalence and risks', *Regulatory Toxicology and Pharmacology: RTP*, Vol. 73, No 3, pp. 790-796 (doi:10.1016/j.yrtph.2015.10.017).

van Amsterdam, J. and van den Brink, W. (2022), 'Nitrous oxide-induced reproductive risks: should recreational nitrous oxide users worry?', *Journal of Psychopharmacology* (doi:10.1177/02698811221077194).

ANSES (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail) (2021), 'Protoxyde d'azote. Bilan des cas rapportés aux Centres antipoison en 2020', *Rapport d'étude de toxicovigilance* (https://www.anses.fr/fr/system/files/Toxicovigilance2021AST0027Ra.pdf).

Araki, T., Kato, H. and Kogure, K. (1990), 'Neuronal damage and calcium accumulation following repeated brief cerebral ischemia in the gerbil', *Brain Research*, Vol. 528, No 1, pp. 114-122 (doi:10.1016/0006-8993(90)90202-m).

Atkinson, R. M., Morozumi, P., Green, J. D. and Kramer, J. C. (1977), 'Nitrous oxide intoxication: subjective effects in healthy young men', *Journal of Psychedelic Drugs*, Vol. 9, No 4, pp. 317-328 (doi:10.1080/02791072.1977.10472063).

Austin, A. T. (1967), 'The chemistry of the higher oxides of nitrogen as related to the manufacture, storage and administration of nitrous oxide', *British Journal of Anaesthesia*, Vol. 39, No 5, pp.345-350 (doi:10.1093/bja/39.5.345).

Baden, J. M., Kelley, M., Mazze, R. I. and Simmon, V. F. (1979), 'Mutagenicity of inhalation anaesthetics: trichloroethylene, divinyl ether, nitrous oxide and cyclopropane', *British Journal of Anaesthesia*, Vol. 51, No 5, pp. 417-421 (doi:10.1093/bja/51.5.417).

Baden, J. M., Kundomal, Y. R., Luttropp, M. E., Jr, Mazze, R. I. and Kosek, J. C. (1986), 'Carcinogen bioassay of nitrous oxide in mice', *Anesthesiology*, Vol. 64, No 6, pp. 747-750 (doi:10.1097/00000542-198606000-00012).

Baden, J. M. and Kundomal, Y. R. (1987), 'Mutagenicity of the combination of a volatile anaesthetic and nitrous oxide', *British Journal of Anaesthesia*, Vol. 59, No 6, pp. 772-775 (doi:10.1093/bja/59.6.772).

Baden, J. M. and Monk, S. J. (1981), 'Mutagenicity and toxicity studies with high pressure nitrous oxide', *Toxicology Letters*, Vol. 7, No 3, pp. 259-262 (doi:10.1016/0378-4274(81)90078-3).

Baden, J. M., Serra, M. and Mazze, R. I. (1984), 'Inhibition of fetal methionine synthase by nitrous oxide', *British Journal of Anaesthesia*, Vol. 56, No 5, pp. 523-526 (doi:10.1093/bja/56.5.523).

Baden, J. M., Serra, M. and Mazze, R. I. (1987), 'Inhibition of rat fetal methionine synthase by nitrous oxide. An in vitro study',

British Journal of Anaesthesia, Vol. 59, No 8, pp. 1040-1043 (doi:10.1093/bja/59.8.1040).

Bajaj, D., Agrawal, A., Gupta, S. and Bajaj, S. (2018), 'Recreational nitrous oxide abuse causing ischemic stroke in a young patient: a rare case report', *Cureus*, Vol. 10, No 12, e3761 (doi:10.7759/cureus.3761).

Balster R. L. (1998), 'Neural basis of inhalant abuse', *Drug and Alcohol Dependence*, Vol. 51, No 1-2, pp. 207-214 (doi:10.1016/s0376-8716(98)00078-7).

Banks, A. and Hardman, J.G. (2005), 'Nitrous oxide', *Continuing Education in Anaesthesia Critical Care & Pain*, Vol. 5, No 5, pp.145-148 (doi:10.1093/bjaceaccp/mki039).

Bär, S., Praz, F. and Räber, L. (2021), 'Plaque erosion causing ST-elevation myocardial infarction after consumption of cannabis and N(2)O in a 27-year-old man: a case report', *BMC Cardiovascular Disorders,* Vol. 21, No 1, p 147 (doi:10.1186/s12872-021-01953-3).

Barth, L. and Büchel, C. G. (1975), '[Clinical studies on the narcotic effects of nitrous oxide (author's transl.)]', *Der Anaesthesist,* Vol. 24, No 2, pp. 49-55.

Batisse, A., Leger, S., Vicaut, E., Gerbaud, L. and Djezzar, S. (2021), 'COgnitive enhancement and consumption of psychoactive Substances among Youth Students (COSYS): a cross-sectional study in France', *Public Health*, Vol. 194, pp. 75-78 (doi:10.1016/j.puhe.2021.02.036).

Becker, D. E. and Rosenberg, M. (2008), 'Nitrous oxide and the inhalation anesthetics', *Anesthesia Progress*, Vol. 55, No 4, pp. 124-132 (doi:10.2344/0003-3006-55.4.124).

van Beek, R., van Miltenburg, C., Blankers, M. and van Laar, M. (2021), *Uitgaansgedrag en middelengebruik tijdens de coronapandemie van maart tot september 2020 [Partying behaviour and substance use during the corona pandemic from March to September 2020]*, Utrecht (https://www.trimbos.nl/aanbod/webwinkel/product/af1860-uitgaansgedrag-en-middelengebruik-tijdens-de-coronapandemie-van-maart-tot-september-2020).

Belknap, J. K., Laursen, S. E. and Crabbe, J. C. (1987), 'Ethanol and nitrous oxide produce withdrawal-induced convulsions by similar mechanisms in mice', *Life Sciences*, Vol. 41, No 17, pp. 2033-2040 (doi:10.1016/0024-3205(87)90477-2).

Belknap, J. K., Metten, P., Helms, M. L., O'Toole, L. A., Angeli-Gade, S., Crabbe, J. C. and Phillips, T. J. (1993), 'Quantitative trait loci (QTL) applications to substances of abuse: physical dependence studies with nitrous oxide and ethanol in BXD mice', *Behavior Genetics*, Vol. 23, No 2, pp. 213-222 (doi:10.1007/BF01067426).

Benschop, A., Nabben, T., and Korf, D. J. (2011), *Antenne 2010 trends in alcohol tabak en drugs bij jonge Amsterdammers [Antenne 2010. Trends in alcohol, tobacco and drug use amongst young people in Amsterdam]*, Amsterdam (https://dare.uva.nl/search?identifier=9464787d-7b80-4826-ad17-375e555f204e).

Benschop, A., Nabben, T. and Korf, D. J. (2015), *Antenne 2014 trends in alcohol tabak en drugs bij jonge Amsterdammers [Antenne 2014. Trends in alcohol, tobacco and drug use amongst young people in Amsterdam]*, Amsterdam (https://www.hva.nl/binaries/content/assets/subsites/etalage/antenne/pdfs/antenne-amsterdam-2014.pdf).

Benturquia, N., Le Guen, S., Canestrelli, C., Lagente, V., Apiou, G., Roques, B. P. and Noble, F. (2007), 'Specific blockade of morphine- and cocaine-induced reinforcing effects in conditioned place preference by nitrous oxide in mice', *Neuroscience*, Vol. 149, No 3, pp. 477-486 (doi:10.1016/j.neuroscience.2007.08.003).

Benturquia, N., Le Marec, T., Scherrmann, J. M. and Noble, F. (2008), 'Effects of nitrous oxide on dopamine release in the rat nucleus accumbens and expectation of reward', *Neuroscience*, Vol. 155, No 2, pp. 341-344 (doi:10.1016/j.neuroscience.2008.05.015).

Berger-Vergiat, A., Pellereau, K., and Boucher, A. (2019), 'Severe nitrous oxide use disorder: a case-report', *Toxicologie Analytique et Clinique*, Vol. 31, No 2, p. S78 (doi:10.1016/j.toxac.2019.03.124).

Berkowitz, B. A., Finck, A. D., Hynes, M. D. and Ngai, S. H. (1979), 'Tolerance to nitrous oxide analgesia in rats and mice', *Anesthesiology*, Vol. 51, No 4, pp. 309-312 (doi:10.1097/00000542-197910000-00006).

Bethmont, A., Harper, C. E., Chan, B. S., Dawson, A. H. and McAnulty, J. (2019), 'Increasing illicit use of nitrous oxide in presentations to NSW emergency departments', *The Medical Journal of Australia*, Vol. 211, No 9, pp. 429-429.e1 (doi:10.5694/mja2.50377).

Bilotta, F., Evered, L. A. and Gruenbaum, S. E. (2017), 'Neurotoxicity of anesthetic drugs: an update', *Current Opinion in Anaesthesiology*, Vol. 30, No 4, pp. 452-457 (doi:10.1097/ACO.0000000000000482).

Blair, C., Tremonti, C., Edwards, L., Haber, P. S. and Halmagyi, G. M. (2019), 'Vitamin B12 supplementation futile for preventing demyelination in ongoing nitrous oxide misuse', *The Medical Journal of Australia*, Vol. 211, No 9, pp. 428-428.e1 (doi:10.5694/mja2.50371).

Bleich, S., Degner, D., Sperling, W., Bönsch, D., Thürauf, N. and Kornhuber, J. (2004), 'Homocysteine as a neurotoxin in chronic alcoholism', *Progress in Neuro-psychopharmacology & Biological Psychiatry*, Vol. 28, No 3, pp. 453-464 (doi:10.1016/j.pnpbp.2003.11.019).

Block, R. I., Ghoneim, M. M., Kumar, V. and Pathak, D. (1990), 'Psychedelic effects of a subanesthetic concentration of nitrous oxide', *Anesthesia Progress*, Vol. 37, No 6, pp. 271-276.

BOC Ltd (2019), *Entonox summary of product characteristics* (https://mhraproducts4853.blob.core.windows.net/docs/8f91dba66e7427965bf1654d81f314cc4bd56e4f).

Boer, M., Dorsselaer, S., de Looze, M., de Roos, S., Brons, H., van den Eijnden, R., Monshouwer, K, et al. (2022), *HBSC 2021*

Gezondheid en welzijn van jongeren in Nederland (https://hbscnederland.nl/nieuw-nederlands-rapport/).

Bovill J. G. (2000), 'Mechanisms of anaesthesia: time to say farewell to the Meyer-Overton rule', *Current Opinion in Anaesthesiology*, Vol. 13, No 4, pp. 433–436 (doi:10.1097/00001503-200008000-00006).

Braz, M. G., Carvalho, L., Chen, C. O., Blumberg, J. B., Souza, K. M., Arruda, N. M., Filho et al. (2020), 'High concentrations of waste anesthetic gases induce genetic damage and inflammation in physicians exposed for three years: a cross-sectional study', *Indoor Air*, Vol. 30, No 3, pp. 512-520 (doi:10.1111/ina.12643).

Brodsky, J. B. and Cohen, E. N. (1986), 'Adverse effects of nitrous oxide', *Medical Toxicology*, Vol. 1, No 5, pp. 362-374 (doi:10.1007/BF03259849).

Brugnone, F., Perbellini, L., Cerpelloni, M., Soave, C., Cecco, A. and Giuliari, C. (1995), 'Nitrous oxide in blood and urine of operating theatre personnel and the general population', *International Archives of Occupational and Environmental Health*, Vol. 68, No 1, pp. 22-26 (doi:10.1007/BF01831629).

de Bruin, D., Maalsté, N. and van de Wijngaart, G. (1999), *Goed fout gaan. Eerste hulp op grote dansevenementen [Really going bad. First aid at large dance events]*, Utrecht.

Buckingham, R. (2020), *Martindale: the complete drug reference*, 40th ed., ed. Robert Buckingham, Pharmaceutical Press.

Buhre, W., Disma, N., Hendrickx, J., DeHert, S., Hollmann, M. W., Huhn, R., Jakobsson, J. et al. (2019), 'European Society of Anaesthesiology task force on nitrous oxide: a narrative review of its role in clinical practice', *British Journal of Anaesthesia*, Vol. 122, No 5, pp. 587-604 (doi:10.1016/j.bja.2019.01.023).

Buring, J. E., Hennekens, C. H., Mayrent, S. L., Rosner, B., Greenberg, E. R. and Colton, T. (1985), 'Health experiences of operating room personnel', *Anesthesiology*, Vol. 62, No 3, pp. 325-330 (doi:10.1097/00000542-198503000-00018).

Burm A. G. (2003), 'Occupational hazards of inhalational anaesthetics', *Best Practice & Research. Clinical Anaesthesiology*, Vol. 17, No 1, pp. 147-161 (doi:10.1053/bean.2003.0271).

Buslov, A., Carroll, M. and Desai, M. S. (2018), 'Frozen in time: a history of the synthesis of nitrous oxide and how the process remained unchanged for over 2 centuries', *Anesthesia and Analgesia*, Vol. 127, No 1, pp. 65-70 (doi:10.1213/ANE.0000000000003423).

CAM (Coördinatiepunt Assessment en Monitoring nieuwe Drugs) (2019), *Risicobeoordeling lachgas [Risk assessment nitrous oxide]* (https://www.rivm.nl/sites/default/files/2019-12/risicobeoordelingsrapport lachgas 20191209 beveiligd.pdf).

Cameo Chemicals (2022a), *Nitrous oxide* (https://cameochemicals.noaa.gov/chemical/8909).

Cameo Chemicals (2022b), *Nitrous oxide refrigerated liquid* (https://cameochemicals.noaa.gov/chemical/4093).

Cape, M. C., Kiyokawa, M. and Haning, W. F. (2021), 'Astronomical use of nitrous oxide associated with stress from the COVID-19 pandemic and lockdown', *The Primary Care Companion for CNS Disorders*, Vol. 23, No 5, 21cr03022 (doi:10.4088/PCC.21cr03022).

Carter, A., Hall, W. and Nutt, D. (2009), 'The neurobiology of addiction', in EMCDDA, *Addiction neurobiology: ethical and social implications*, EMCDDA Monographs 9, Office for Official Publications of the European Union, Luxembourg, pp. 29-52 (https://www.emcdda.europa.eu/publications/monographs/neurobiology_en).

Cartwright, F. F. (1972), 'Humphry Davy's researches on nitrous oxide', *British Journal of Anaesthesia*, Vol. 44, No 3, pp. 291-296 (doi:10.1093/bja/44.3.291).

Castrén, E., Võikar, V. and Rantamäki, T. (2007), 'Role of neurotrophic factors in depression', *Current Opinion in Pharmacology*, Vol. 7, No 1, pp. 18-21 (doi:10.1016/j.coph.2006.08.009).

CEIP-Addictovigilance de Nantes (2021), *Bilan d'addictovigilance protoxyde d'azote connées 2020, rapport d'expertise* (https://ansm.sante.fr/uploads/2021/11/16/20211116-rapport-anonymise-protoxyde-dazote-sans-annexe-donnees-2020.pdf).

Chamaa, F., Bahmad, H. F., Makkawi, A. K., Chalhoub, R. M., Al-Chaer, E. D., Bikhazi, G. B., Nahas, Z. and Abou-Kheir, W. (2018), 'Nitrous oxide induces prominent cell proliferation in adult rat hippocampal dentate gyrus', *Frontiers in Cellular Neuroscience*, Vol. 12, No 135 (doi:10.3389/fncel.2018.00135).

Chan, S. A., Alfonso, K. P. and Comer, B. T. (2018), 'Upper aerodigestive tract frostbite from inhalation of automotive nitrous oxide', *Ear, Nose, & Throat Journal*, Vol. 97, No 9, pp. E13-E14 (doi:10.1177/014556131809700903).

Chen, Y., Liu, X., Cheng, C. H., Gin, T., Leslie, K., Myles, P. and Chan, M. T. (2013), 'Leukocyte DNA damage and wound infection after nitrous oxide administration: a randomized controlled trial', *Anesthesiology*, Vol. 118, No 6, pp. 1322-1331 (doi:10.1097/ALN.0b013e31829107b8).

Chen, T., Zhong, N., Jiang, H., Zhao, M., Chen, Z. and Sun, H. (2018), 'Neuropsychiatric symptoms induced by large doses of nitrous oxide inhalation: a case report', *Shanghai Archives of Psychiatry*, Vol. 30, No 1, pp. 56-59 (doi:10.11919/j.issn.1002-0829.217084).

Chien, W. H., Huang, M. C. and Chen, L. Y. (2020), 'Psychiatric and other medical manifestations of nitrous oxide abuse: implications from case series', *Journal of Clinical Psychopharmacology*, Vol. 40, No 1, pp. 80-83 (doi:10.1097/JCP.0000000000001151).

Chiew, A. L., Raubenheimer, J. E., Berling, I., Buckley, N. A., Becker, T., Chan, B. and Brett, J. (2021), 'Just 'nanging' around - harmful nitrous oxide use: a retrospective case series and review of Internet searches, social media posts and the coroner's database', *Internal Medicine Journal*, 10. 1111/imj.15391 (doi:10.1111/imj.15391).

Cho, A. M., Coalson, D. W., Klock, P. A., Klafta, J. M., Marks, S., Toledano, A. Y., Apfelbaum, J. L. and Zacny, J. P. (1997), 'The effects of alcohol history on the reinforcing, subjective and psychomotor effects of nitrous oxide in healthy volunteers', *Drug and Alcohol Dependence*, Vol. 45, No 1-2, pp. 63-70 (doi:10.1016/s0376-8716(97)01346-x).

Coate, W. B., Kapp, R. W., Jr and Lewis, T. R. (1979a), 'Chronic exposure to low concentrations of halothane-nitrous oxide: reproductive and cytogenetic effects in the rat', *Anesthesiology*, Vol. 50, No 4, pp. 310-318 (doi:10.1097/00000542-197904000-00006).

Coate, W. B., Ulland, B. M., and Lewis, T. R. (1979b), 'Chronic exposure to low concentrations of halothane-nitrous oxide: lack of carcinogenic effect in the rat', *Anesthesiology*, Vol. 50, No 4, pp. 306-309 (doi:10.1097/00000542-197904000-00005).

Corbett, T. H., Cornell, R. G., Endres, J. L. and Millard, R. I. (1973), 'Effects of low concentrations of nitrous oxide on rat pregnancy', *Anesthesiology*, Vol. 39, No 3, pp. 299-301 (doi:10.1097/00000542-197309000-00007).

Cousaert, C., Heylens, G. and Audenaert, K. (2013), 'Laughing gas abuse is no joke. An overview of the implications for psychiatric practice', *Clinical Neurology and Neurosurgery*, Vol. 115, No 7, pp. 859-862 (doi:10.1016/j.clineuro.2013.04.004).

Crawford, J. S. and Lewis, M. (1986), 'Nitrous oxide in early human pregnancy', *Anaesthesia*, Vol. 41, No 9, pp. 900-905 (doi:10.1111/j.1365-2044.1986.tb12912.x).

Culley, D. J., Raghavan, S. V., Waly, M., Baxter, M. G., Yukhananov, R., Deth, R. C. and Crosby, G. (2007), 'Nitrous oxide decreases cortical methionine synthase transiently but produces lasting memory impairment in aged rats', *Anesthesia and Analgesia*, Vol. 105, No 1, pp. 83-88 (doi:10.1213/01.ane.0000266491.53318.20).

Danish Health Authority (2019), *Lattergas fra gaspatroner* (https://www.sst.dk/-/media/Udgivelser/2019/Lattergas/Lattergas-fra-gaspatroner---Udbredelsen-blandt-elever-paa-ungdomsuddannelser.ashx?sc_lang=da&hash=0DF932DBCBE9FCBB6C3A177F546BFA3F).

David, H. N., Ansseau, M., Lemaire, M. and Abraini, J. H. (2006), 'Nitrous oxide and xenon prevent amphetamine-induced carrier-mediated dopamine release in a memantine-like fashion and protect against behavioral sensitization', *Biological Psychiatry*, Vol. 60, No 1, pp. 49-57 (doi:10.1016/j.biopsych.2005.10.007).

Daynes, G. and Gillman, M. A. (1994), 'Psychotropic analgesic nitrous oxide prevents craving after withdrawal for alcohol, cannabis and tobacco', *The International Journal of Neuroscience*, Vol. 76, No 1-2, pp. 13-16 (doi:10.3109/00207459408985987).

Denisova, K. O., Ilyin, A. A., Rumyantsev, R. N., Ilyin, A. P., and Volkova, A. V. (2019), 'Nitrous oxide: production, application, and protection of the environment', *Russian Journal of General Chemistry,* Vol. 89, No 6, pp. 1338–1346 (doi:10.1134/S107036321906032X).

Desmidt, T., Gissot, V., Dujardin, P. A., Andersson, F., Barantin, L., Brizard, B., Arlicot, N. et al. (2021), 'A case of sustained antidepressant effects and large changes in the brain with a single brief exposure to nitrous oxide', *The American Journal of Geriatric Psychiatry*, Vol. 29, No 12, pp. 1298-1300 (doi:10.1016/j.jagp.2021.01.138).

DGS (Direction générale de la Santé) (2020), *De nouveaux chiffres sur l'usage détourné de protoxyde d'azote (« gaz hilarant ») pour éclairer les autorités sanitaires* (https://solidarites-sante.gouv.fr/actualites/presse/communiques-de-presse/article/de-nouveaux-chiffres-sur-l-usage-detourne-de-protoxyde-d-azote-gaz-hilarant).

Doekhie, J., Nabben, T. and Korf., D. J. (2010), *NL.Trend-Watch. Gebruikersmarkt uitgaansdrugs in Nederland 2008-2009 [NL. Trend-Watch. Recreational drug market in the Netherlands 2008-2009]*, Amsterdam.

Dohrn, C. S., Lichtor, J. L., Finn, R. S., Uitvlugt, A., Coalson, D. W., Rupani, G., de Wit, H. and Zacny, J. P. (1992), 'Subjective and psychomotor effects of nitrous oxide in healthy volunteers', *Behavioural Pharmacology*, Vol. 3, No 1, pp. 19-30 (doi:10.1097/00008877-199203010-00005).

Dohrn, C. S., Lichtor, J. L., Coalson, D. W., Uitvlugt, A., de Wit, H., and Zacny, J. P. (1993), 'Reinforcing effects of extended inhalation of nitrous oxide in humans', *Drug and Alcohol Dependence*, Vol. 31, No 3, pp. 265-280 (doi:10.1016/0376-8716(93)90009-f).

Dong, A., Huang, P., Zhao, X. J., Sampath, V. and Caughey, W. S. (1994), 'Characterization of sites occupied by the anesthetic nitrous oxide within proteins by infrared spectroscopy', *The Journal of Biological Chemistry*, Vol. 269, No 39, pp. 23911-23917.

Drummond, J. T. and Matthews, R. G. (1994a), 'Nitrous oxide degradation by cobalamin-dependent methionine synthase: characterization of the reactants and products in the inactivation reaction', *Biochemistry*, Vol. 33, No 12, pp. 3732-3741 (doi:10.1021/bi00178a033).

Drummond, J. T. and Matthews, R. G. (1994b), 'Nitrous oxide inactivation of cobalamin-dependent methionine synthase from Escherichia coli: characterization of the damage to the enzyme and prosthetic group', *Biochemistry*, Vol. 33, No 12, pp. 3742-3750 (doi:10.1021/bi00178a034).

Duarte, R., McNeill, A., Drummond, G. and Tiplady, B. (2008), 'Comparison of the sedative, cognitive, and analgesic effects of nitrous oxide, sevoflurane, and ethanol', *British Journal of Anaesthesia*, Vol. 100, No 2, pp. 203-210 (doi:10.1093/bja/aem369).

Dufayet, L., Caré, W., Laborde-Casterot, H., Chouachi, L., Langrand, J. and Vodovar, D. (2022), 'Possible impact of the COVID-19 pandemic on the recreational use of nitrous oxide in the Paris area, France', *La Revue de Médecine Interne*, Vol. 43, No 7, pp. 402-405 (doi:10.1016/j.revmed.2022.06.004).

Dutch Government (2020a), *Lachgas voorlichting en preventie [Nitrous oxide education and prevention]* (https://www.

tweedekamer.nl/kamerstukken/brieven_regering/detail?id=2020Z19658&did=2020D42317).

Dutch Government (2020b), *Ontwerp nota van toelichting en bijlage lachgasbesluit [Draft note of explanatory notes and attachment to the nitrous oxide decision]* (https://www.internetconsultatie.nl/lachgasbesluit/document/5898).

Dutch Government (2020c), *Ontwerpbesluit lachgas [Draft decision nitrous oxide]* (https://www.internetconsultatie.nl/lachgasbesluit).

Dutch Government (2020d), *Opiumwet [Opium Act]* (https://wetten.overheid.nl/BWBR0001941/2020-11-17).

Dutch Government (2019a), *Drugbeleid [Drug policy]. Letter to the Parliament* (https://zoek.officielebekendmakingen.nl/kst-24077-426.html).

Dutch Government (2019b), *Integrale aanpak lachgas [Integral approach nitrous oxide]* (https://www.tweedekamer.nl/kamerstukken/amendementen/detail?id=2019Z24503&did=2019D50555).

Dzoljic, M., Rupreht, J., Erdmann, W., Stijnen, T. H., van Briemen, L. J. and Dzoljic, M. R. (1994), 'Behavioral and electrophysiological aspects of nitrous oxide dependence', *Brain Research Bulletin*, Vol. 33, No 1, pp. 25-31 (doi:10.1016/0361-9230(94)90046-9).

ECHA (European Chemicals Agency) (2021), *Dinitrogen oxide* (https://echa.europa.eu/nl/substance-information/-/substanceinfo/100.030.017).

EDQM (European Directorate for the Quality of Medicines & Healthcare) (2020), 'Nitrous Oxide', *European pharmacopoeia* (https://pheur.edqm.eu/app/10-5/search?q=nitrous oxide).

Eger, E. I., 2nd (2001), 'Age, minimum alveolar anesthetic concentration, and minimum alveolar anesthetic concentration-awake', *Anesthesia and analgesia*, Vol. 93, No 4, pp. 947-953 (doi:10.1097/00000539-200110000-00029).

Eger, E. I., 2nd and Saidman, L. J. (1965), 'Hazards of nitrous oxide anesthesia in bowel obstruction and pneumothorax', *Anesthesiology*, Vol. 26, pp. 61-66 (doi:10.1097/00000542-196501000-00011).

Eger, E. I., 2nd, White, A. E., Brown, C. L., Biava, C. G., Corbett, T. H. and Stevens, W. C. (1978), 'A test of the carcinogenicity of enflurane, isoflurane, halothane, methoxyflurane, and nitrous oxide in mice', *Anesthesia and analgesia*, Vol. 57, No 6, pp. 678-694.

Ehirim, E. M., Naughton, D. P. and Petróczi, A. (2018), 'No laughing matter: presence, consumption trends, drug awareness, and perceptions of "hippy crack" (nitrous oxide) among young adults in England', *Frontiers in Psychiatry*, Vol. 8, 312 (doi:10.3389/fpsyt.2017.00312).

Einsiedler, M., Voulleminot, P., Demuth, S., Kalaaji, P., Bogdan, T., Gauer, L., Reschwein, C., Nadaj-Pakleza, A., de Sèze, J., Kremer, L., Schroder, I. and Bigaut, K. (2022), 'A rise in cases of nitrous oxide abuse: neurological complications and biological findings', *Journal of Neurology*, Vol. 269, No 2, pp. 577-582 (doi:10.1007/s00415-021-10702-7).

Eisele, J. H. and Smith, N. T. (1972), 'Cardiovascular effects of 40 percent nitrous oxide in man', *Anesthesia and Analgesia*, Vol. 51, No 6, pp. 956-963.

Elementary Productions (2008), 'Nitrous oxide synthesis', *YouTube* (https://www.youtube.com/watch?v=TGDNm3ENnf8).

Emmanouil, D. E. and Quock, R. M. (2007), 'Advances in understanding the actions of nitrous oxide', *Anesthesia Progress*, Vol. 54, No 1, pp. 9-18 (doi:10.2344/0003-3006(2007)54[9:AIUTAO]2.0.CO;2).

EUCPN (European Crime Prevention Network) (2021), *Recommendation paper: preventing the misuse of nitrous oxide*, Brussels (https://eucpn.org/document/recommendation-paper-preventing-the-misuse-of-nitrous-oxide).

European Commission (2012), 'Commission regulation (EU) No 231/2012 of 9 March 2012 Laying down Specifications for Food Additives Listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council', *Official Journal of the European Union* L 83: 1-295 (https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:083:0001:0295:en:PDF).

European Court of Justice (2014), *Judgement of the Court ECLI:EU:C:2014:2060* (https://curia.europa.eu/juris/document/document.jsf?text=&docid=154827&pageIndex=0&doclang=EN).

Fagan, D., Paul, D. L., Tiplady, B. and Scott, D. B. (1994), 'A dose-response study of the effects of inhaled nitrous oxide on psychological performance and mood', *Psychopharmacology*, Vol. 116, No 3, pp. 333-338 (doi:10.1007/BF02245337).

FDA (US Food and Drug administration) (2017), 'FDA Drug Safety Communication: FDA review results in new warnings about using general anesthetics and sedation drugs in young children and pregnant women', *Drug safety and availability* (https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-fda-review-results-new-warnings-about-using-general-anesthetics-and).

Fee, J. P. and Thompson, G. H. (1997), 'Comparative tolerability profiles of the inhaled anaesthetics', *Drug Safety*, Vol. 16, No 3, pp. 157-170 (doi:10.2165/00002018-199716030-00002).

Fidalgo, M., Prud'homme, T., Allio, A., Bronnec, M., Bulteau, S., Jolliet, P. and Victorri-Vigneau, C. (2019), 'Nitrous oxide: what do we know about its use disorder potential? results of the French monitoring centre for addiction network survey and literature review', *Substance Abuse*, Vol. 40, No 1, pp. 33-42 (doi:10.1080/08897077.2019.1573210).

Fink, B. R. (1955), 'Diffusion anoxia', *Anesthesiology*, Vol. 16, No 4, pp. 511-519 (doi:10.1097/00000542-195507000-00007).

Fink, B. R., Shepard, T. H. and Blandau, R. J. (1967), 'Teratogenic activity of nitrous oxide', *Nature*, Vol. 214, No 5084, pp. 146-148 (doi:10.1038/214146a0).

Finkelstein J. D. (1998), 'The metabolism of homocysteine: pathways and regulation', *European Journal of Pediatrics*, Vol. 157, Suppl 2, pp. S40-S44 (doi:10.1007/pl00014300).

Fleischmann, E., Lenhardt, R., Kurz, A., Herbst, F., Fülesdi, B., Greif, R., Sessler, D. I., Akça, O. and Outcomes Research Group (2005), 'Nitrous oxide and risk of surgical wound infection: a randomised trial', *Lancet*, Vol. 366, No 9491, pp. 1101-1107 (doi:10.1016/S0140-6736(05)67422-3).

Fleischmann, E., Marschalek, C., Schlemitz, K., Dalton, J. E., Gruenberger, T., Herbst, F., Kurz, A. and Sessler, D. I. (2009), 'Nitrous oxide may not increase the risk of cancer recurrence after colorectal surgery: a follow-up of a randomized controlled trial', *BMC Anesthesiology*, Vol. 9, 1 (doi:10.1186/1471-2253-9-1).

Fodale, V., Mondello, S., Aloisi, C., Schifilliti, D. and Santamaria, L. (2008), 'Genotoxic effects of anesthetic agents', *Expert Opinion on Drug Safety*, Vol. 7, No 4, pp. 447-458 (doi:10.1517/14740338.7.4.447).

Formula 1 Dictionary (date unknown), *NOS nitrous oxide (laughing gas)* (https://www.formula1-dictionary.net/nos.html).

Frasca, V., Riazzi, B. S. and Matthews, R. G. (1986), '*In vitro* inactivation of methionine synthase by nitrous oxide', *The Journal of Biological Chemistry*, Vol. 261, No 4, pp. 15823-15826.

French Association of Addictovigilance Centres (2022), *Augmentation des complications sanitaires graves associees a l'usage non medical du protoxyde d'azote en France*

(https://addictovigilance.fr/2022/06/communique-augmentation-des-complications-sanitaires-graves-associees-a-lusage-non-medical-du-protoxyde-dazote-en-france/).

French Association of Addictovigilance Centres, Micallef, J., Mallaret, M., Lapeyre-Mestre, M., Daveluy, A., Victorri-Vigneau, C., Peyrière, H. et al. (2021), 'Warning on increased serious health complications related to non-medical use of nitrous oxide', *Therapie*, Vol. 76. No 5, pp. 478-479 (doi:10.1016/j.therap.2020.01.002).

Froese, D. S., Fowler, B. and Baumgartner, M. R. (2019), 'Vitamin B12, folate, and the methionine remethylation cycle-biochemistry, pathways, and regulation', *Journal of Inherited Metabolic Disease*, Vol. 42, No 4, pp. 673-685 (doi:10.1002/jimd.12009).

Fröhlich, D., Rothe, G., Wittmann, S., Schmitz, G., Schmid, P., Taeger, K. and Hobbhahn, J. (1998), 'Nitrous oxide impairs the neutrophil oxidative response', *Anesthesiology*, Vol. 88, No 5, pp. 1281-1290 (doi:10.1097/00000542-199805000-00020).

Fujinaga, M. and Baden, J. M. (1994), 'Methionine prevents nitrous oxide-induced teratogenicity in rat embryos grown in culture', *Anesthesiology*, Vol. 81, No 1, pp. 184-189 (doi:10.1097/00000542-199407000-00025).

Fujinaga, M., Baden, J. M. and Mazze, R. I. (1989), 'Susceptible period of nitrous oxide teratogenicity in Sprague-Dawley rats', *Teratology*, Vol. 40, No 5, pp. 439-444 (doi:10.1002/tera.1420400505).

Fujinaga, M., Baden, J. M., Yhap, E. O. and Mazze, R. I. (1987), 'Reproductive and teratogenic effects of nitrous oxide, isoflurane, and their combination in Sprague-Dawley rats', *Anesthesiology*, Vol. 67, No 6, pp. 960-964 (doi:10.1097/00000542-198712000-00014).

Fujinaga, M. and Maze, M. (2002), 'Neurobiology of nitrous oxide-induced antinociceptive effects', *Molecular Neurobiology*, Vol. 25, No 2, pp. 167-189 (doi:10.1385/MN:25:2:167).

Fukagawa, H., Koyama, T. and Fukuda, K. (2014), 'κ-Opioid receptor mediates the antinociceptive effect of nitrous oxide in mice', *British Journal of Anaesthesia*, Vol. 113, No 6, pp. 1032-1038 (doi:10.1093/bja/aeu254).

Galinkin, J. L., Janiszewski, D., Young, C. J., Klafta, J. M., Klock, P. A., Coalson, D. W., Apfelbaum, J. L. and Zacny, J. P. (1997), 'Subjective, psychomotor, cognitive, and analgesic effects of subanesthetic concentrations of sevoflurane and nitrous oxide', *Anesthesiology*, Vol. 87, No 5, pp. 1082-1088 (doi:10.1097/00000542-199711000-00012).

Gao, H., Li, W., Ren, J., Dong, X., Ma, Y. and Zheng, D. (2021), 'Clinical and MRI differences between patients with subacute combined degeneration of the spinal cord related vs. unrelated to recreational nitrous oxide use: a retrospective study', *Frontiers in Neurology*, Vol. 12, 626174 (doi:10.3389/fneur.2021.626174).

Garakani, A., Jaffe, R. J., Savla, D., Welch, A. K., Protin, C. A., Bryson, E. O. and McDowell, D. M. (2016), 'Neurologic, psychiatric, and other medical manifestations of nitrous oxide abuse: a systematic review of the case literature', *The American Journal on Addictions*, Vol. 25, No 5, pp. 358-369 (doi:10.1111/ajad.12372).

Garbaz, L., Mispelaere, D., Boutemy, M. and Jounieaux, V. (2007), 'Pneumothorax et inhalation volontaire de protoxyde d'azote [Pneumothorax following recreational inhalation of nitrous oxide]', *Revue des Maladies Respiratoires*, Vol. 24, No 5, pp. 622-624 (doi:10.1016/s0761-8425(07)91130-4).

Georgiev, S. K., Kohno, T., Ikoma, M., Yamakura, T. and Baba, H. (2008), 'Nitrous oxide inhibits glutamatergic transmission in spinal dorsal horn neurons', *Pain*, Vol. 134. No 1-2, pp. 24-31 (doi:10.1016/j.pain.2007.03.026).

Gillman M. A. (1986), 'Nitrous oxide, an opioid addictive agent. Review of the evidence', *The American Journal of Medicine*, Vol. 81, No 1, pp. 97-102 (doi:10.1016/0002-9343(86)90189-0).

Gillman M. A. (2019), 'Mini-review: a brief history of nitrous oxide (N2O) use in neuropsychiatry', *Current Drug Research Reviews*, Vol. 11, No 1, pp. 12-20 (doi:10.2174/1874473711666181008163107).

Gillman, M. A. and Lichtigfeld, F. J. (1990), 'The drug management of severe alcohol withdrawal syndrome', *Postgraduate Medical Journal*, Vol. 66, No 782, pp. 1005-1009 (doi:10.1136/pgmj.66.782.1005).

Gillman, M. A. and Lichtigfeld, F. J. (1991), 'Placebo and analgesic nitrous oxide for treatment of the alcohol withdrawal state', *The British Journal of Psychiatry: the Journal of Mental Science*, Vol. 159, pp. 672-675 (doi:10.1192/bjp.159.5.672).

Gillman, M. A. and Lichtigfeld, F. J. (1994), 'Opioid properties of psychotropic analgesic nitrous oxide (laughing gas)', *Perspectives in Biology and Medicine*, Vol. 38, No 1, pp. 125-138 (doi:10.1353/pbm.1994.0026).

Gillman, M. A., Lichtigfeld, F. J. and Young, T. N. (2007), 'Psychotropic analgesic nitrous oxide for alcoholic withdrawal states', *The Cochrane Database of Systematic Reviews*, 2, CD005190 (doi:10.1002/14651858.CD005190.pub2).

Giuliani, N., Beyer, J., Augsburger, M. and Varlet, V. (2015), 'Validation of an analytical method for nitrous oxide (N2O) laughing gas by headspace gas chromatography coupled to mass spectrometry (HS-GC-MS): forensic application to a lethal intoxication', *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, Vol. 983-984, pp. 90-93 (doi:10.1016/j.jchromb.2014.12.034).

Global Drug Survey (2015), *GDS 2015 findings: nitrous oxide* (https://youtu.be/T1i0a1onUhY).

Goto, T., Nakata, Y. and Morita, S. (2002), 'The minimum alveolar concentration of xenon in the elderly is sex-dependent', *Anesthesiology*, Vol. 97, No 5, pp. 1129-1132 (doi:10.1097/00000542-200211000-00015).

Grassi, G., Cecchelli, C., Vignozzi, L. and Pacini, S. (2021), 'Investigational and experimental drugs to treat obsessive-compulsive disorder', *Journal of Experimental Pharmacology*, Vol. 12, pp. 695-706 (doi:10.2147/JEP.S255375).

Gray, R. H. (1993), 'Nitrous oxide and fertility', *The New England Journal of Medicine*, Vol. 328, No 4, p. 284.

Gruss, M., Bushell, T. J., Bright, D. P., Lieb, W. R., Mathie, A. and Franks, N. P. (2004), 'Two-pore-domain K+ channels are a novel target for the anesthetic gases xenon, nitrous oxide, and cyclopropane', *Molecular Pharmacology*, Vol. 65, No 2, pp. 443-452 (doi:10.1124/mol.65.2.443).

Guimarães, M. C., Guimarães, T. M., Hallak, J. E., Abrão, J. and Machado-de-Sousa, J. P. (2021), 'Nitrous oxide as an adjunctive therapy in major depressive disorder: a randomized controlled double-blind pilot trial', *Revista Brasileira de Psiquiatria*, Vol. 43, No 5, pp. 484-493 (doi:10.1590/1516-4446-2020-1543).

Hannibal, L., Lysne, V., Bjørke-Monsen, A. L., Behringer, S., Grünert, S. C., Spiekerkoetter, U., Jacobsen, D. W. and Blom, H. J. (2016), 'Biomarkers and algorithms for the diagnosis of vitamin B12 deficiency', *Frontiers in Molecular Biosciences*, Vol. 3, 27 (doi:10.3389/fmolb.2016.00027).

Hansen, D. K. and Billings, R. E. (1985), 'Effects of nitrous oxide on maternal and embryonic folate metabolism in rats', *Developmental Pharmacology and Therapeutics*, Vol. 8, No 1, pp. 43-54 (doi:10.1159/000457020).

Hathout, L. and El-Saden, S. (2011), 'Nitrous oxide-induced B_{12} deficiency myelopathy: perspectives on the clinical biochemistry of vitamin B_{12}', *Journal of the Neurological Sciences*, Vol. 301, No 1-2, pp. 1-8 (doi:10.1016/j.jns.2010.10.033).

Haynes, W.M. (ed.) (2014), *CRC Handbook of Chemistry and Physics*, 95th edition, CRC Press LLC, Boca Raton, pp. 4-78.

Healy, C. E., Drown, D. B. and Sharma, R. P. (1990), 'Short term toxicity of nitrous oxide on the immune, hemopoietic, and endocrine systems in CD-1 mice', *Toxicology and Industrial Health*, Vol. 6, No 1, pp. 57-70 (doi:10.1177/074823379000600105).

Helmenstine, A. (2019), 'How to make nitrous oxide (laughing gas)', *ThoughtCo.* (https://www.thoughtco.com/make-nitrous-oxide-or-laughing-gas-608280).

Helmenstine, A. (2020), 'How to make nitrous oxide or laughing gas', *Science Notes* (https://sciencenotes.org/how-to-make-nitrous-oxide-or-laughing-gas/).

Herrmann, W., Lorenzl, S. and Obeid, R. (2007), 'Hyperhomocysteinämie und B-Vitaminmangel bei neurologischen und psychiatrischen Erkrankungen--Aktueller Kenntnisstand und vorläufige Empfehlungen [Review of the role of hyperhomocysteinemia and B-vitamin deficiency in neurological and psychiatric disorders--current evidence and preliminary recommendations]', *Fortschritte der Neurologie-Psychiatrie*, Vol. 75, No 9, pp. 515-527 (doi:10.1055/s-2007-980112).

Heusler, H. (1985), 'Quantitative analysis of common anaesthetic agents', *Journal of Chromatography*, Vol. 340, pp. 273-319 (doi:10.1016/0378-4347(85)80200-0).

Hew, A., Lai, E. and Radford, E. (2018), 'Nitrous oxide abuse presenting with acute psychosis and peripheral neuropathy', *The Australian and New Zealand Journal of Psychiatry*, Vol. 52, No 4, p. 388 (doi:10.1177/0004867417748752).

Hogan, K. (2013), 'Nitrous oxide genotoxicity', *Anesthesiology*, Vol. 118, No 6, pp. 1258-1260 (doi:10.1097/ALN.0b013e31829106cd).

Hopkins, P. M. (2005), 'Nitrous oxide: a unique drug of continuing importance for anaesthesia', *Best Practice & Research. Clinical Anaesthesiology*, Vol. 19, No 3, pp. 381-389 (doi:10.1016/j.bpa.2005.03.002).

Houck, P. J., Brambrink, A. M., Waspe, J., O'Leary, J. D. and Ko, R. (2019), 'Developmental neurotoxicity: an update', *Journal of Neurosurgical Anesthesiology*, Vol. 31, No 1, pp. 108-114 (doi:10.1097/ANA.0000000000000557).

van den Hoven, C., Lambrechts, S. and Reynders, T. (2022), 'Neuro-image: nitrous oxide-induced myelopathy due to vitamin B12 deficiency', *Acta Neurologica Belgica*, Vol. 122, No 1, pp. 203-205 (doi:10.1007/s13760-021-01616-2).

Huizink, A. C. (2022), 'Trends and associated risks in adolescent substance use: e-cigarette use and nitrous oxide use', *Current Opinion in Psychology*, Vol. 45, 101312 (doi:10.1016/j.copsyc.2022.101312).

Hwang, J. C., Himel, H. N. and Edlich, R. F. (1996), 'Frostbite of the face after recreational misuse of nitrous oxide', *Burns: Journal of the International Society for Burn Injuries*, Vol. 22, No 2, pp. 152-153 (doi:10.1016/0305-4179(95)00090-9).

Hynes, M. D. and Hymson, D. L. (1984), 'Nitrous oxide generalizes to a discriminative stimulus produced by ethylketocyclazocine but not morphine', *European Journal of Pharmacology*, Vol. 105, No 1-2, pp. 155-159 (doi:10.1016/0014-2999(84)90660-5).

Imberger, G., Orr, A., Thorlund, K., Wetterslev, J., Myles, P. and Møller, A. M. (2014), 'Does anaesthesia with nitrous oxide affect mortality or cardiovascular morbidity? A systematic review with meta-analysis and trial sequential analysis', *British Journal of*

Anaesthesia, Vol. 112, No 3, pp. 410-426 (doi:10.1093/bja/aet416).

Indraratna, P., Alexopoulos, C., Celermajer, D. and Alford, K. (2017), 'Acute ST-elevation myocardial infarction, a unique complication of recreational nitrous oxide use', *Heart, Lung & Circulation*, Vol. 26, No 8, pp. e41-e43 (doi:10.1016/j.hlc.2017.01.019).

Ivers, J. H., Killeen, N., and Keenan, E. (2022), 'Drug use, harm-reduction practices and attitudes toward the utilisation of drug safety testing services in an Irish cohort of festival-goers', *Irish Journal of Medical Science*, Vol. 191, No 4, pp. 1701-1710 (doi:10.1007/s11845-021-02765-2).

Jastak, J. T. (1989), 'Nitrous oxide in dental practice', *International Anesthesiology Clinics*, Vol. 27, No 2, pp. 92-97 (doi:10.1097/00004311-198902720-00005).

Jeddy, H., Rashid, F., Bhutta, H., Lorenzi, B. and Charalabopoulos, A. (2016), 'Pneumomediastinum secondary to barotrauma after recreational nitrous oxide inhalation', *Case Reports in Gastrointestinal Medicine*, Vol. 2016, p. 4318015 (doi:10.1155/2016/4318015).

Jevtovic-Todorovic, V., Hartman, R. E., Izumi, Y., Benshoff, N. D., Dikranian, K., Zorumski, C. F., Olney, J. W. and Wozniak, D. F. (2003), 'Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits', *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, Vol. 23, No 3, pp. 876-882 (doi:10.1523/JNEUROSCI.23-03-00876.2003).

Jevtović-Todorović, V., Todorović, S. M., Mennerick, S., Powell, S., Dikranian, K., Benshoff, N., Zorumski, C. F. and Olney, J. W. (1998), 'Nitrous oxide (laughing gas) is an NMDA antagonist, neuroprotectant and neurotoxin', *Nature Medicine*, Vol. 4, No 4, pp. 460-463 (doi:10.1038/nm0498-460).

Kaar, S. J., Ferris, J., Waldron, J., Devaney, M., Ramsey, J. and Winstock, A. R. (2016), 'Up: the rise of nitrous oxide abuse. An international survey of contemporary nitrous oxide use', *Journal of Psychopharmacology*, Vol. 30, No 4, pp. 395-401 (doi:10.1177/0269881116632375).

Kalmoe, M. C., Janski, A. M., Zorumski, C. F., Nagele, P., Palanca, B. J. and Conway, C. R. (2020), 'Ketamine and nitrous oxide: the evolution of NMDA receptor antagonists as antidepressant agents', *Journal of the Neurological Sciences*, Vol. 412, 116778 (doi:10.1016/j.jns.2020.116778).

Kamboj, S. K., Zhao, H., Troebinger, L., Piazza, G., Cawley, E., Hennessy, V., Iskandar, G. and Das, R. K. (2021), 'Rewarding subjective effects of the NMDAR antagonist nitrous oxide (laughing gas) are moderated by impulsivity and depressive symptoms in healthy volunteers', *The International Journal of Neuropsychopharmacology*, Vol. 24, No 7, pp. 551-561 (doi:10.1093/ijnp/pyab009).

Kang, S., Kim, S., Kang, S., Lee, J., Cho, C. S., Sa, J. H. and Jeon, E. C. (2014), 'A study on N2O measurement characteristics using photoacoustic spectroscopy (PAS)', *Sensors*, Vol. 14, No 8, pp. 14399-14410 (doi:10.3390/s140814399).

Karelová, J., Jablonická, A., Gavora, J. and Hano, L. (1992), 'Chromosome and sister-chromatid exchange analysis in peripheral lymphocytes, and mutagenicity of urine in anesthesiology personnel', *International Archives of Occupational and Environmental Health*, Vol. 64, No 4, pp. 303-306 (doi:10.1007/BF00378289).

Kato, H., Araki, T. and Kogure, K. (1990), 'Role of the excitotoxic mechanism in the development of neuronal damage following repeated brief cerebral ischemia in the gerbil: protective effects of MK-801 and pentobarbital', *Brain Research*, Vol. 516, No 1, pp. 175-179 (doi:10.1016/0006-8993(90)90916-y).

Katz, M. M., Waskow, I. E. and Olsson, J. (1968), 'Characterizing the psychological state produced by LSD', *Journal of Abnormal Psychology*, Vol. 73, No 1, pp. 1-14 (doi:10.1037/h0020114).

Kaur, S., Cortiella, J. and Vacanti, C. A. (2001), 'Diffusion of nitrous oxide into the pleural cavity', *British Journal of Anaesthesia*, Vol. 87, No 6, pp. 894-896 (doi:10.1093/bja/87.6.894).

Klaunig, J. E., Wang, Z., Pu, X. and Zhou, S. (2011), 'Oxidative stress and oxidative damage in chemical carcinogenesis', *Toxicology and Applied Pharmacology*, Vol. 254, No 2, pp. 86-99 (doi:10.1016/j.taap.2009.11.028).

Koblin D. D. (1990), 'Nitrous oxide: a cause of cancer or chemotherapeutic adjuvant?', *Seminars in Surgical Oncology*, Vol. 6, No 3, pp. 141-147 (doi:10.1002/ssu.2980060304).

Koblin, D. D., Deady, J. E., Dong, D. E. and Eger, E. I. (1980), 'Mice tolerant to nitrous oxide are also tolerant to alcohol', *The Journal of Pharmacology and Experimental Therapeutics*, Vol. 213, No 2, pp. 309-312.

Koblin, D. D., Waskell, L., Watson, J. E., Stokstad, E. L. and Eger, E. I., 2nd (1982), 'Nitrous oxide inactivates methionine synthetase in human liver', *Anesthesia and Analgesia*, Vol. 61, No 2, pp. 75-78.

Kondo, H., Osborne, M. L., Kolhouse, J. F., Binder, M. J., Podell, E. R., Utley, C. S., Abrams, R. S. and Allen, R. H. (1981), 'Nitrous oxide has multiple deleterious effects on cobalamin metabolism and causes decreases in activities of both mammalian cobalamin-dependent enzymes in rats', *The Journal of Clinical Investigation*, Vol. 67, No 5, pp. 1270-1283 (doi:10.1172/jci110155).

Koob, G. F. and Le Moal, M. (2001), 'Drug addiction, dysregulation of reward, and allostasis', *Neuropsychopharmacology,* Vol. 24, No 2, pp. 97-129 (doi:10.1016/S0893-133X(00)00195-0).

Korttila, K., Ghoneim, M. M., Jacobs, L., Mewaldt, S. P. and Petersen, R. C. (1981), 'Time course of mental and psychomotor effects of 30 per cent nitrous oxide during inhalation and recovery', *Anesthesiology*, Vol. 54, No 3, pp. 220-226 (doi:10.1097/00000542-198103000-00009).

Koyama, T. and Fukuda, K. (2010), 'Involvement of the kappa-opioid receptor in nitrous oxide-induced analgesia in mice, *Journal of Anesthesia*, Vol. 24, No 2, pp. 297-299 (doi:10.1007/s00540-010-0886-5).

- Koyama, T., Mayahara, T., Wakamatsu, T., Sora, I. and Fukuda, K. (2009), 'Deletion of mu-opioid receptor in mice does not affect the minimum alveolar concentration of volatile anaesthetics and nitrous oxide-induced analgesia', *British Journal of Anaesthesia*, Vol. 103, No 5, pp. 744-749 (doi:10.1093/bja/aep246).
- Koyanagi, S., Himukashi, S., Mukaida, K., Shichino, T. and Fukuda, K. (2008), 'Dopamine D2-like receptor in the nucleus accumbens is involved in the antinociceptive effect of nitrous oxide', *Anesthesia and Analgesia*, Vol. 106, No 6, pp. 1904-1909 (doi:10.1213/ane.0b013e318172b15b).
- Kreuer, S., Bruhn, J., Wilhelm, W. and Bouillon, T. (2007), 'Pharmakokinetische/pharmakodynamische Modelle für Inhalationsanästhetika [Pharmacokinetic-pharmacodynamic models for inhaled anaesthetics]', *Der Anaesthesist*, Vol. 56, No 6, pp. 538-556 (doi:10.1007/s00101-007-1188-7).
- Kripke, B. J. and Hechtman, H. B. (1972), 'Nitrous oxide for pentazocine addiction and for intractable pain: report of case', *Anesthesia and Analgesia*, Vol. 51, No 4, pp. 520-527.
- van Laar, M. (2022) *Laughing gas in the Netherlands: current situation and responses,* 10th Extended Reitox Network Meeting, 11 May 2022.
- van Laar, M., Beenakkers, E. M. T., Cruts, A. A. N., Ketelaars, A. P. M., Kuin, M. C., Meijer, R. F., van Miltenburg, C. J. A., Mujcic, A. and Strada, L. (2021), *Nationale drug monitor 2020 [National drug monitor 2020]*, Utrecht/Den Haag (https://www.trimbos.nl/aanbod/webwinkel/af1862-jaarbericht-nationale-drug-monitor-2020/).
- van Laar, M., van Beek., R. J. J., Beenakkers, E. M. T., Cruts, A. A. N., Kuin, M. C., Meijer, R. F., Mujcic, A. et al. (2022), *Nationale drug monitor 2021 [National drug monitor 202*1], Den Haag (https://www.trimbos.nl/aanbod/webwinkel/af1911-nationale-drug-monitor-2021/).
- Lan, S. Y., Kuo, C. Y., Chou, C. C., Kong, S. S., Hung, P. C., Tsai, H. Y., Chen, Y. C. et al. (2019), 'Recreational nitrous oxide abuse related subacute combined degeneration of the spinal cord in adolescents - A case series and literature review', *Brain & Development*, Vol. 41, No 5, pp. 428-435 (doi:10.1016/j.braindev.2018.12.003).
- Lane, G. A., Nahrwold, M. L., Tait, A. R., Taylor-Busch, M., Cohen, P. J. and Beaudoin, A. R. (1980), 'Anesthetics as teratogens: nitrous oxide is fetotoxic, xenon is not', *Science (New York, N.Y.)*, Vol. 210, No 4472, pp. 899-901 (doi:10.1126/science.7434002).
- Langton, J. A. and Hutton, A. (2009), 'Respiratory gas analysis', *Continuing Education in Anaesthesia, Critical Care & Pain*, Vol. 9, No 1, pp. 19-23 (doi:10.1093/bjaceaccp/mkn048).
- Largeau, B., Karam, A., Potey, C., Caous, A. S., Tard, C., Carton, L., Kuchcinski, G. et al. (2022), 'Myeloneuropathy induced by recreational nitrous oxide use with variable exposure levels', *European Journal of Neurology,* 10. 1111/ene.15370 (doi:10.1111/ene.15370).
- Lassen, H. C., Henriksen, E., Neukirch, F. and Kristensen, H. S. (1956), 'Treatment of tetanus; severe bone-marrow depression after prolonged nitrous-oxide anaesthesia', *Lancet*, Vol. 270, No 6922, pp. 527-530 (doi:10.1016/s0140-6736(56)90593-1).
- Leslie, K., Myles, P. S., Chan, M. T., Forbes, A., Paech, M. J., Peyton, P., Silbert, B. S. and Williamson, E. (2011), 'Nitrous oxide and long-term morbidity and mortality in the ENIGMA trial', *Anesthesia and Analgesia*, Vol. 112, No 2, pp. 387-393 (doi:10.1213/ANE.0b013e3181f7e2c4).
- Leslie, K., Myles, P. S., Kasza, J., Forbes, A., Peyton, P. J., Chan, M. T., Paech, M. J. et al. (2015), 'Nitrous oxide and serious long-term morbidity and mortality in the evaluation of nitrous oxide in the gas mixture for anaesthesia (ENIGMA)-II trial', *Anesthesiology,* Vol. 123, No 6, pp. 1267-1280 (doi:10.1097/ALN.0000000000000908).
- Lewińska, D., Stepnik, M., Krajewski, W., Arkusz, J., Stańczyk, M. and Wrońska-Nofer, T. (2005), 'Increased incidence of micronuclei assessed with the micronucleus assay and the fluorescence in situ hybridization (FISH) technique in peripheral blood lymphocytes of nurses exposed to nitrous oxide', *Mutation Research*, Vol. 581, No 1-2, pp. 1-9 (doi:10.1016/j.mrgentox.2004.10.018).
- Lewis, B., Nelson, G., Vu, T. and Judge, B. (2021), 'No laughing matter - Myeloneuropathy due to heavy chronic nitrous oxide abuse', *The American Journal of Emergency Medicine*, Vol. 46, pp. 799.e1-799.e2 (doi:10.1016/j.ajem.2021.01.079).
- Li, S., Chung, E. and Quock, R. M. (2004), 'Role of cyclic GMP in nitrous-oxide-induced anxiolytic-like behavior in the mouse light-dark exploration test', *Behavioral Neuroscience*, Vol. 118, No 3, pp. 648-652 (doi:10.1037/0735-7044.118.3.648).
- Liu, M., Zhang, J. and Bu, B. (2020), 'Isolated cortical vein thrombosis after nitrous oxide use in a young woman: a case report', *BMC Neurology*, Vol. 20, No 1, p. 378 (doi:10.1186/s12883-020-01961-4).
- Liu, Y., Lin, D., Wu, B. and Zhou, W. (2016), 'Ketamine abuse potential and use disorder', *Brain Research Bulletin*, Vol. 126, No 1, pp. 68-73 (doi:10.1016/j.brainresbull.2016.05.016).
- Long, H. (2019), 'Inhalants', in Nelson, L. S. et al. (eds.), *Goldfrank's toxicologic emergencies,* 11th edition, McGraw-Hill Education, New York (http://accesspharmacy.mhmedical.com/content.aspx?aid=1163018693).
- López-Valverde, A., Montero, J., Albaladejo, A. and Gómez de Diego, R. (2011), 'The discovery of surgical anesthesia: discrepancies regarding its authorship', *Journal of Dental Research*, Vol. 90, No 1, pp. 31-34 (doi:10.1177/0022034510385239).
- Mancke, F., Kaklauskaitė, G., Kollmer, J. and Weiler, M. (2016), 'Psychiatric comorbidities in a young man with subacute myelopathy induced by abusive nitrous oxide consumption: a case report', *Substance Abuse and Rehabilitation*, Vol. 7, pp. 155-159 (doi:10.2147/SAR.S114404).
- Manoli, I., Sloan, J. L. and Venditti, C. P. (2005), 'Isolated methylmalonic acidemia', in Adams, M. P. et. al. (eds.), *GeneReviews®*, University of Washington, Seattle.
- Marcus, D. J. and Bruchas, M. R. (2021), 'Where ketamine and dopamine collide', *eLife*, Vol. 10, e70148 (doi:10.7554/eLife.70148).

Marillier, M., Karila, L. and Miguet-Alfonsi, C. (2020), 'Quand le protoxyde d'azote ne fait plus rire : épidémiologie, aspects analytiques, incidences clinique et médicojudiciaire', *Toxicologie Analytique et Clinique*, Vol. 32, No 4, pp. 278-290 (doi:10.1016/j.toxac.2020.07.002).

Marotta, D. A. and Kesserwani, H. (2020), 'Nitrous oxide induced posterior cord myelopathy: beware of the methyl folate trap', *Cureus*, Vol. 12, No 7, e9319 (doi:10.7759/cureus.9319).

Marsden, P., Sharma, A. A. and Rotella, J.- A. (2022), 'Review article: clinical manifestations and outcomes of chronic nitrous oxide misuse: a systematic review', *Emergency Medicine Australasia*, Vol. 34, No 4, pp. 492-503 (doi:10.1111/1742-6723.13997).

Maslekar, S., Gardiner, A., Hughes, M., Culbert, B. and Duthie, G. S. (2009), 'Randomized clinical trial of Entonox versus midazolam-fentanyl sedation for colonoscopy', *The British Journal of Surgery*, Vol. 96, No 4, pp. 361-368 (doi:10.1002/bjs.6467).

Mazze, R. I., Fujinaga, M. and Baden, J. M. (1987), 'Reproductive and teratogenic effects of nitrous oxide, fentanyl and their combination in Sprague-Dawley rats', *British Journal of Anaesthesia*, Vol. 59, No 10, pp. 1291-1297 (doi:10.1093/bja/59.10.1291).

Mazze, R. I. and Lecky, J. H. (1985), 'The health of operating room personnel', *Anesthesiology*, Vol. 62, No 3, pp. 226-228.

Mazze, R. I., Rice, S. A., Wyrobek, A. J., Felton, J. S., Brodsky, J. B. and Baden, J. M. (1983), 'Germ cell studies in mice after prolonged exposure to nitrous oxide', *Toxicology and Applied Pharmacology*, Vol. 67, No 3, pp. 370-375 (doi:10.1016/0041-008x(83)90320-4).

Mazze, R. I., Wilson, A. I., Rice, S. A. and Baden, J. M. (1982), 'Reproduction and fetal development in mice chronically exposed to nitrous oxide', *Teratology*, Vol. 26, No 1, pp. 11-16 (doi:10.1002/tera.1420260103).

Mazze, R. I., Wilson, A. I., Rice, S. A., and Baden, J. M. (1984), 'Reproduction and fetal development in rats exposed to nitrous oxide', *Teratology*, Vol. 30, No 2, pp. 259-265 (doi:10.1002/tera.1420300213).

McCormick, J. P., Sharpe, S., Crowley, K., Dudley, A., O'Laoi, R., Barry, M., Owens, L. et al. (2022), 'Nitrous oxide-induced myeloneuropathy: an emerging public health issue', *Irish Journal of Medical Science* (doi:10.1007/s11845-022-02945-8).

McDermott, R., Tsang, K., Hamilton, N. and Belton, M. (2015), 'Recreational nitrous oxide inhalation as a rare cause of spontaneous pneumomediastinum', *BMJ Case Reports*, Vol. 2015, bcr2015209750 (doi:10.1136/bcr-2015-209750).

McGee, H. (2004), *On food and cooking. The science and lore of the kitchen*, 1st revise, Scribner, New York.

McNeely, J. K., Buczulinski, B. and Rosner, D. R. (2000), 'Severe neurological impairment in an infant after nitrous oxide anesthesia', *Anesthesiology*, Vol. 93, No 6, pp. 1549-1550 (doi:10.1097/00000542-200012000-00036).

Mennerick, S., Jevtovic-Todorovic, V., Todorovic, S. M., Shen, W., Olney, J. W. and Zorumski, C. F. (1998), 'Effect of nitrous oxide on excitatory and inhibitory synaptic transmission in hippocampal cultures', *The Journal of Neuroscience*, Vol. 18, No 23, pp. 9716-9726 (doi:10.1523/JNEUROSCI.18-23-09716.1998).

Merriam-Webster (2022a), 'Antinociception', in *Merriam-Webster.com medical dictionary* (https://www.merriam-webster.com/medical/antinociception).

Merriam-Webster (2022b), 'Analgesia', in *Merriam-Webster.com medical dictionary* (https://www.merriam-webster.com/medical/analgesia).

Messer (2017), *Summary of Product Characteristics Nitrous Oxide Messer 100%* (https://mri.cts-mrp.eu/Human/Downloads/NL_H_2606_001_FinalSPC.pdf).

Messina, F. V. and Wynne, J. W. (1982), 'Homemade nitrous oxide: no laughing matter', *Annals of Internal Medicine*, Vol. 96, No 3, pp. 333-334 (doi:10.7326/0003-4819-96-3-333).

Metz, J. (1992), 'Cobalamin deficiency and the pathogenesis of nervous system disease', *Annual Review of Nutrition*, Vol. 12, pp. 59-79 (doi:10.1146/annurev.nu.12.070192.000423).

Monshouwer, K., van Miltenburg, C. J. A., van Beek, R. J. J., den Hollander, W., Schouten, F., van Goor, M., Spronk, D., Blankers, M. and van Laar, M. (2021), *Het grote uitgaansonderzoek 2020*, Utrecht (https://www.trimbos.nl/aanbod/webwinkel/product/af1861-het-grote-uitgaansonderzoek-2020).

Morales, M. and Margolis, E. B. (2017), 'Ventral tegmental area: cellular heterogeneity, connectivity and behaviour', *Nature Reviews. Neuroscience*, Vol. 18, No 2, pp. 73-85 (doi:10.1038/nrn.2016.165).

Moudgil, G. C., Gordon, J. and Forrest, J. B. (1984), 'Comparative effects of volatile anaesthetic agents and nitrous oxide on human leucocyte chemotaxis in vitro', *Canadian Anaesthetists' Society Journal*, Vol. 31, No 6, pp. 631-637 (doi:10.1007/BF03008758).

van Munster, I. G., Baran, K. C., Gardien, K., van Trier, T. and Meij-de Vries, A. (2020), 'Bevriezingswonden door recreatief lachgasgebruik [Frostbite due to recreational nitrous oxide use]', *Nederlands Tijdschrift voor Geneeskunde*, Vol. 164, p. D4794.

Myles, P. S., Leslie, K., Chan, M. T., Forbes, A., Paech, M. J., Peyton, P., Silbert, B. S., Pascoe, E. and ENIGMA Trial Group (2007), 'Avoidance of nitrous oxide for patients undergoing major surgery: a randomized controlled trial', *Anesthesiology*, Vol. 107, No 2, pp. 221-231 (doi:10.1097/01.anes.0000270723.30772.da).

Myles, P. S., Leslie, K., Chan, M. T., Forbes, A., Peyton, P. J., Paech, M. J., Beattie, W. S. et al. (2014), 'The safety of addition of nitrous oxide to general anaesthesia in at-risk patients having major non-cardiac surgery (ENIGMA-II): a randomised, single-blind trial', *Lancet*, Vol. 384, No 9952, pp. 1446-1454 (doi:10.1016/S0140-6736(14)60893-X).

Nabben, T. (2010), 'High Amsterdam. Ritme, roes en regels in het uitgaansleven. [High Amsterdam. Rhythm, highs and rules in night life], PhD thesis, University of Amsterdam (https://dare.uva.nl/search?identifier=bbf4e68a-82cc-423d-9223-159f9a353a43).

Nabben, T. (2020), *Antenne Nederland. Regiomonitor drugs en risicojongeren 2019. [Antenne Netherlands. Regional monitor drugs and young people at risk 2019]*, Amsterdam (https://www.hva.nl/akmi/gedeelde-content/publicaties/publicaties-algemeen/coordinatie-grootstedelijke-vraagstukken/2020/antenne-nederland.html).

Nabben, T. and Bahara, K. (2020), *Als de tank je beste vriend wordt… Problematisch lachgasgebruik bij jonge marokkaanse Amsterdammers. Adviezen en aanbevelingen voor een interventie en preventiecampagne [When the tank becomes your best friend…. Problematic use of nitrous oxide amongst young Moroccans in Amsterdam]*, Amsterdam (https://www.hva.nl/akmi/gedeelde-content/publicaties/publicaties-algemeen/coordinatie-grootstedelijke-vraagstukken/2020/als-de-tank-je-beste-vriend-wordt.html).

Nabben, T., Benschop, A. and Korf, D. J. (2014), *Antenne 2013, trends in alcohol, tabak en drugs bij jonge Amsterdammers [Antenne 2013, trends in the use of alcohol, tobacco and drugs amongst young people in Amsterdam]*, Amsterdam.

Nabben, T., Benschop, A. and Korf, D. J. (2016), *Antenne 2015 trends in alcohol tabak en drugs bij jonge Amsterdammers [Antenne 2015. Trends in alcohol, tobacco and drug use amongst young people in Amsterdam]*, Amsterdam.

Nabben, T., van der Pol, P. and Korf, D. J. (2017), *Roes met een luchtje. Gebruik, gebruikers en markt van lachgas (Inebriating air. Nitrous oxide use, users and market]*, Amsterdam (https://www.trimbos.nl/docs/e3f9a3a4-d25c-4fa7-a981-ccba3d937997.pdf).

Nabben, T., Quaak, L. and Korf, D. J. (2005), *NL.Trend-Watch. Gebruikersmarkt uitgaansdrugs in Nederland 2004-2005 [NL. Trend-Watch. Recreational drug market in the Netherlands 2004-2005]*, Amsterdam.

Nabben, T., Weijs, J. and van Amsterdam, J. (2021), 'Problematic use of nitrous oxide by young Moroccan-Dutch adults', *International Journal of Environmental Research and Public Health*, Vol. 18, No 11, p. 5574 (doi:10.3390/ijerph18115574).

Nagele, P., Brown, F., Bass, V., and Yohanna, D. (2020), 'Prolonged remission of major depressive disorder after single nitrous oxide inhalation treatment', *Frontiers in Psychiatry*, Vol. 11, p. 692 (doi:10.3389/fpsyt.2020.00692).

Nagele, P., Duma, A., Kopec, M., Gebara, M. A., Parsoei, A., Walker, M., Janski, A. et al. (2015), 'Nitrous oxide for treatment-resistant major depression: a proof-of-concept trial', *Biological Psychiatry*, Vol. 78, No 1, pp. 10-18 (doi:10.1016/j.biopsych.2014.11.016).

Nagele, P., Metz, L. B. and Crowder, C. M. (2004), 'Nitrous oxide (N(2)O) requires the N-methyl-D-aspartate receptor for its action in Caenorhabditis elegans', *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 101, No 23, pp. 8791-8796 (doi:10.1073/pnas.0402825101).

Nagele, P., Zorumski, C. F. and Conway, C. (2018), 'Exploring nitrous oxide as treatment of mood disorders: basic concepts', *Journal of Clinical Psychopharmacology*, Vol. 38, No 2, pp. 144-148 (doi:10.1097/JCP.0000000000000837).

Navas, M. J., Jiménez, A. M. and Asuero, A. G. (2012), 'Human biomarkers in breath by photoacoustic spectroscopy', *Clinica Chimica Acta; International Journal of Clinical Chemistry*, Vol. 413, No 15-16, pp. 1171-1178 (doi:10.1016/j.cca.2012.04.008).

Neghab, M., Kargar-Shouroki, F., Mozdarani, H., Yousefinejad, S., Alipour, H. and Fardid, R. (2020), 'Association between genotoxic properties of inhalation anesthetics and oxidative stress biomarkers', *Toxicology and Industrial Health*, Vol. 36, No 6, pp. 454-466 (doi:10.1177/0748233720935696).

Nijkamp, L. (2020), *Lachgas: van zorgen naar acties [Nitrous oxide: from concerns to actions] update 2020*, Utrecht, https://www.trimbos.nl/aanbod/webwinkel/product/af1839-lachgas-van-zorgen-naar-acties, accessed on 5 May 2021.

NileRed (2017), 'Making laughing gas', *YouTube* (https://www.youtube.com/watch?v=uzSe3BDCkF8).

NIOSH (The National Institute for Occupational Safety and Health) (2019), *Nitrous oxide* (https://www.cdc.gov/niosh/npg/npgd0465.html).

NOS (2021), *Helft gemeenten wacht niet op kabinet en voert zelf lachgasverbod in [Half of the municipalities do not wait for the cabinet and implement nitrous oxide prohibitions]* (https://nos.nl/artikel/2374225-helft-gemeenten-wacht-niet-op-kabinet-en-voert-zelf-lachgasverbod-in).

Nugteren-van Lonkhuyzen, J.J., van Velzen, A. G., Mulder-Spijkerboer, H. N., Visser, C. C., Dijkman, M. A., Kan, A. A., de Lange, D. W., and van Riel, A. J. H. P. (2021), *Acute vergiftigingen bij mens en dier. NVIC jaaroverzicht 2020 [Acute poisonings in humans and animals. Dutch Poisoning Information Centre yearly overview 2020]*, Utrecht (https://nvic.umcutrecht.nl/nl/jaaroverzichten).

Nunn, J. F. and O'Moráin, C. (1982), 'Nitrous oxide decreases motility of human neutrophils *in vitro*', *Anesthesiology*, Vol. 56, No 1, pp. 45-48 (doi:10.1097/00000542-198201000-00010).

NVIC (Nationaal Vergiftigingen Informatie Centrum) (2022), *Acute vergiftigingen bij mens en dier. NVIC Jaaroverzicht 2021* (https://nvic.umcutrecht.nl/nl/jaaroverzichten).

O'Donovan, M. R. and Hammond, T. G. (2015), 'Is nitrous oxide a genotoxic carcinogen?', *Mutagenesis*, Vol. 30, No 4, pp. 459-462 (doi:10.1093/mutage/gev024).

OECD (2020), *Bacterial reverse mutation test* (https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1622137922&id=id&accname=guest&checksum=943C1FD16674FF6CA4FF1E36C7AED359).

OFDT (Observatoire français des drogues et des tendances addictives) (2019), *Usagers, marchés et substances : évolution en France en 2018-2019. Tendances*, No 136 (https://www.ofdt.fr/publications/collections/tendances/usagers-marches-et-substances-evolution-recentes-2018-2019-tendances-136-decembre-2019/).

OFDT (2020), *Substances psychoactives, usagers et marchés - Tendances récentes (2019-2020).Tendances*, No 141 (https://www.ofdt.fr/publications/collections/tendances/substances-

psychoactives-usagers-marches-tendances-recentes-2019-2020-tendances-141-decembre-2020/).

OFDT (2021), *Usages d'alcool, de tabac et de cannabis chez les élèves de 3e en 2021* (https://www.ofdt.fr/BDD/publications/docs/eftxss2bc.pdf).

OFDT (2022), *Les usages psychoactifs du protoxyde d'azote* (https://www.ofdt.fr/publications/collections/tendances/tendances-151/).

Office for National Statistics (2018), *Drug-related deaths involving nitrous oxide in England and Wales, 1993 to 2017* (https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/adhocs/009096drugrelateddeathsinvolvingnitrousoxideinenglandandwales1993to2017).

Office for National Statistics (2022), *Deaths related to volatile substances, helium and nitrogen in England and Wales: 2001 to 2020 registrations* (https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/articles/deathsrelatedtovolatilesubstancesheliumandnitrogeninenglandandwales/2001to2020registrations).

Olney, J. W. (2002), 'New insights and new issues in developmental neurotoxicology', *Neurotoxicology*, Vol. 23, No 6, pp. 659-668 (doi:10.1016/S0161-813X(01)00092-4).

O'Neil, M. J. (ed.) (2013), *The Merck index - an encyclopedia of chemicals, drugs, and biologicals*, Royal Society of Chemistry, Cambridge, p. 1236.

Oomens, T., Fokkema, T., van den Bogaard, B., de Metz, J., van Nieuwenhuizen, R. C., Riezebos, R. K., and Kuipers, R. S. (2021a), 'Trombo-embolieën door recreatief lachgasgebruik [Thromboembolisms due to recreational use of nitrous oxide]', *Nederlands Tijdschrift voor Geneeskunde*, Vol. 165, p. D5607.

Oomens, T., Riezebos, R. K., Amoroso, G. and Kuipers, R. S. (2021b), 'Case report of an acute myocardial infarction after high-dose recreational nitrous oxide use: a consequence of hyperhomocysteinaemia?', *European Heart Journal Case Reports*, Vol. 5, No 2, ytaa557 (doi:10.1093/ehjcr/ytaa557).

Oussalah, A., Julien, M., Levy, J., Hajjar, O., Franczak, C., Stephan, C., Laugel et al. (2019), 'Global burden related to nitrous oxide exposure in medical and recreational settings: a systematic review and individual patient data meta-analysis', *Journal of Clinical Medicine*, Vol. 8, No 4, p. 551 (doi:10.3390/jcm8040551).

Pacak, K. and McCarty, R. (2007), 'Acute stress response: experimental', in Fink, G. (ed.), *Encyclopedia of stress*, 2nd edition, Academic Press, pp. 7-14 (doi:10.1016/B978-012373947-6.00003-9).

Park, J., Jung, S., Kim, S. M., Park, I. Y., Bui, N. A., Hwang, G. S. and Han, I. O. (2021), 'Repeated hypoxia exposure induces cognitive dysfunction, brain inflammation, and amyloidβ/p-Tau accumulation through reduced brain O-GlcNAcylation in zebrafish', *Journal of Cerebral Blood Flow and Metabolism*, Vol. 41, No 11, pp. 3111-3126 (doi:10.1177/0271678X211027381).

Pascale, R., Caivano, M., Buchicchio, A., Mancini, I. M., Bianco, G. and Caniani, D. (2017), 'Validation of an analytical method for simultaneous high-precision measurements of greenhouse gas emissions from wastewater treatment plants using a gas chromatography-barrier discharge detector system', *Journal of Chromatography A*, Vol. 1480, pp. 62-69 (doi:10.1016/j.chroma.2016.11.024).

Patel, A. J. and Honoré, E. (2001), 'Properties and modulation of mammalian 2P domain K+ channels', *Trends in Neurosciences*, Vol. 24, No 6, pp. 339-346 (doi:10.1016/s0166-2236(00)01810-5).

Perino, J., Tournier, M., Mathieu, C., Letinier, L., Peyré, A., Perret, G., Pereira, E. et al. (2022), 'Psychoactive substance use among students: a cross-sectional analysis', *Fundamental & Clinical Pharmacology*, 10.1111/fcp.12771 (doi:10.1111/fcp.12771).

Pfeiffer, C. M., Sternberg, M. R., Fazili, Z., Lacher, D. A., Zhang, M., Johnson, C. L., Hamner et al. (2015), 'Folate status and concentrations of serum folate forms in the US population: national health and nutrition examination survey 2011-2', *The British Journal of Nutrition*, Vol. 113, No 12, pp. 1965-1977 (doi:10.1017/S0007114515001142).

Poli, D., Gagliano-Candela, R., Strisciullo, G., Colucci, A. P., Strada, L., Laviola, D., Goldoni, M. and Mutti, A. (2010), 'Nitrous oxide determination in postmortem biological samples: a case of serial fatal poisoning in a public hospital', *Journal of Forensic Sciences*, Vol. 55, No 1, pp. 258-264 (doi:10.1111/j.1556-4029.2009.01218.x).

Pope, W. D., Halsey, M. J., Lansdown, A. B., Simmonds, A., and Bateman, P. E. (1978), 'Fetotoxicity in rats following chronic exposure to halothane, nitrous oxide, or methoxyflurane', *Anesthesiology*, Vol. 48, No 1, pp. 11-16 (doi:10.1097/00000542-197801000-00003).

PRAC (Pharmacovigilance Risk Assessment Committee) (2018a), *Minutes of the Meeting on 03-06 September 2018. EMA/PRAC/675727/2018* (https://www.ema.europa.eu/en/documents/minutes/minutes-prac-meeting-3-6-september-2018_en.pdf).

PRAC (2018b), *Minutes of the Meeting on 08-11 January 2018. EMA/PRAC/71458/2018* (https://www.ema.europa.eu/en/documents/minutes/minutes-prac-meeting-8-11-january-2018_en.pdf).

Pratt, D. N., Patterson, K. C. and Quin, K. (2020), 'Venous thrombosis after nitrous oxide abuse, a case report', *Journal of Thrombosis and Thrombolysis*, Vol. 49, No 3, pp. 501-503 (doi:10.1007/s11239-019-02010-9).

Quax, M., Van Der Steenhoven, T. J., Antonius Bronkhorst, M. and Emmink, B. L. (2022), 'Frostbite injury: an unknown risk when using nitrous oxide as a party drug', *Acta Chirurgica Belgica*, Vol. 122, No 2, pp. 140-143 (doi:10.1080/00015458.2020.1782160).

Ramsay, D. S., Al-Noori, S., Shao, J., Leroux, B. G., Woods, S. C. and Kaiyala, K. J. (2015), 'Predicting addictive vulnerability: individual differences in initial responding to a drug's pharmacological effects', *PloS one*, Vol. 10, No 4, e0124740 (doi:10.1371/journal.pone.0124740).

Ramsay, D. S., Watson, C. H., Leroux, B. G., Prall, C. W. and Kaiyala, K. J. (2003), 'Conditioned place aversion and self-

administration of nitrous oxide in rats', *Pharmacology, Biochemistry, and Behavior*, Vol. 74, No 3, pp. 623-633 (doi:10.1016/s0091-3057(02)01048-1).

Rantamäki T. (2019), 'TrkB neurotrophin receptor at the core of antidepressant effects, but how?', *Cell and Tissue Research*, Vol. 377, No 1, pp. 115-124 (doi:10.1007/s00441-018-02985-6).

Rantamäki, T. and Yalcin, I. (2019), 'Depression and antidepressant action-from molecules to networks', *Cell and Tissue Research*, Vol. 377, No 1, pp. 1-4 (doi:10.1007/s00441-019-03042-6).

Redmond, J., Cruse, B. and Kiers, L. (2022), 'Nitrous oxide-induced neurological disorders: an increasing public health concern', *Internal Medicine Journal*, Vol. 52, No 5, pp. 740-744 (doi:10.1111/imj.15544).

Reinelt, H., Marx, T., Schirmer, U., Luederwald, S., Topalidis, P. and Schmidt, M. (2002), 'Diffusion of xenon and nitrous oxide into the bowel during mechanical ileus', *Anesthesiology*, Vol. 96, No 2, pp. 512–513 (doi:10.1097/00000542-200202000-00043).

Reitz, M., Antonini-Rumpf, E. and Lanz, E. (1993), 'DNA single strand breaks in peripheral human lymphocytes after anesthesia with isoflurane-nitrous oxide-oxygen', *Arzneimittel-Forschung*, Vol. 43, No 12, pp. 1258-1261.

Reynolds E. (2006), 'Vitamin B12, folic acid, and the nervous system', *The Lancet. Neurology*, Vol. 5, No 11, pp. 949-960 (doi:10.1016/S1474-4422(06)70598-1).

Richardson, K. J. and Shelton, K. L. (2015), 'N-methyl-D-aspartate receptor channel blocker-like discriminative stimulus effects of nitrous oxide gas', *The Journal of Pharmacology and Experimental Therapeutics*, Vol. 352, No 1, pp. 156-165 (doi:10.1124/jpet.114.218057).

Riedel, B., Fiskerstrand, T., Refsum, H. and Ueland, P. M. (1999), 'Co-ordinate variations in methylmalonyl-CoA mutase and methionine synthase, and the cobalamin cofactors in human glioma cells during nitrous oxide exposure and the subsequent recovery phase', *The Biochemical Journal*, Vol. 341, No 1, pp. 133-138.

van Riel, A., Hunault, C. C., van den Hengel-Koot, I. S., Nugteren-van Lonkhuyzen, J. J., de Lange, D. W. and Hondebrink, L. (2022), 'Alarming increase in poisonings from recreational nitrous oxide use after a change in EU-legislation, inquiries to the Dutch Poisons Information Center', *The International Journal on Drug Policy*, Vol. 100, p. 103519 (doi:10.1016/j.drugpo.2021.103519).

RIVM (Rijksinstituut voor Volksgezondheid en Milieu) (2016), *Beoordeling gezondheidsrisico's lachgas (N2O) [Assessment health risks nitrous oxide (N2O)]* (https://www.nvwa.nl/documenten/consument/consumentenartikelen/non-food/overige-non-food/rapport-rivm-–-beoordeling-gezondheidsrisico's-lachgas-n2o).

Roberts, D., Farahmand, P. and Wolkin, A. (2020), 'Nitrous oxide inhalant use disorder preceding symptoms concerning for primary psychotic illness', *The American Journal on Addictions*, Vol. 29, No 6, pp. 525-527 (doi:10.1111/ajad.13048).

Roizen, M. F., Plummer, G. O. and Lichtor, J. L. (1987), 'Nitrous oxide and dysrhythmias', *Anesthesiology*, Vol. 66, No 3, pp. 427-431 (doi:10.1097/00000542-198703000-00033).

Rombouts, M., van Dorsselaer, S., Scheffers - van Schayck, T., Tuithof, M., Kleinjan, M. and Monshouwer, K. (2020), *Jeugd en riskant gedrag 2019 [Youth and risky behaviour 2019]*, Utrecht (https://www.trimbos.nl/aanbod/webwinkel/product/af1767-jeugd-en-riskant-gedrag-2019).

Rooks, J. P. (2011), 'Safety and risks of nitrous oxide labor analgesia: a review', *Journal of Midwifery & Women's Health*, Vol. 56, No 6, pp. 557-565 (doi:10.1111/j.1542-2011.2011.00122.x).

Rosen, M. A. (2002), 'Nitrous oxide for relief of labor pain: a systematic review', *American Journal of Obstetrics and Gynecology*, Vol. 186, No 5 Suppl Nature, pp. S110-S126 (doi:10.1067/mob.2002.121259).

Rosenberg, P. (1974), 'The effect of N2O-oxygen inhalation on subjective experiences of healthy young adults', *Annales Chirurgiae et Gynaecologiae Fenniae*, Vol. 63, No 6, pp. 500-504.

Rosiers, J. (2019), *Uitgaansonderzoek 2018*, Brussels (https://www.vad.be/assets/uitgaansonderzoek_2018).

Rough, E. and Brown, J. (2020), 'Nitrous oxide: no laughing matter?', *UK Parliament House of Commons Library* (https://commonslibrary.parliament.uk/nitrous-oxide-no-laughing-matter/).

Rowland, A. S., Baird, D. D., Weinberg, C. R., Shore, D. L., Shy, C. M. and Wilcox, A. J. (1992), 'Reduced fertility among women employed as dental assistants exposed to high levels of nitrous oxide', *The New England Journal of Medicine*, Vol. 327, No 14, pp. 993-997 (doi:10.1056/NEJM199210013271405).

Royston, B. D., Nunn, J. F., Weinbren, H. K., Royston, D. and Cormack, R. S. (1988), 'Rate of inactivation of human and rodent hepatic methionine synthase by nitrous oxide', *Anesthesiology*, Vol. 68, No 2, pp. 213-216 (doi:10.1097/00000542-198802000-00006).

Rupreht, J., Dworacek, B., Bonke, B., Dzoljic, M. R., van Eijndhoven, J. H., and de Vlieger, M. (1985), 'Tolerance to nitrous oxide in volunteers', *Acta Anaesthesiologica Scandinavica*, Vol. 29, No 6, pp. 63-–638 (doi:10.1111/j.1399-6576.1985.tb02271.x).

Sakamoto, S., Nakao, S., Masuzawa, M., Inada, T., Maze, M., Franks, N. P. and Shingu, K. (2006), 'The differential effects of nitrous oxide and xenon on extracellular dopamine levels in the rat nucleus accumbens: a microdialysis study', *Anesthesia and Analgesia*, Vol. 103, No 6, pp. 1459–1463 (doi:10.1213/01.ane.0000247792.03959.f1).

Samia, A. M., Nenow, J., and Price, D. (2020), 'Subacute combined degeneration secondary to nitrous oxide abuse: quantification of use with patient follow-up', *Cureus*, Vol. 12, No 10, e11041 (doi:10.7759/cureus.11041).

Sanders, R. D., Weimann, J. and Maze, M. (2008), 'Biologic effects of nitrous oxide: a mechanistic and toxicologic review', *Anesthesiology*, Vol. 109, No 4, pp. 707-722 (doi:10.1097/ALN.0b013e3181870a17).

Sangle, P., Sandhu, O., Aftab, Z., Anthony, A. T. and Khan, S. (2020), 'Vitamin B12 supplementation: preventing onset and improving prognosis of depression', *Cureus*, Vol. 12, No 10, e11169 (doi:10.7759/cureus.11169).

Sato, Y., Kobayashi, E., Murayama, T., Mishina, M. and Seo, N. (2005), 'Effect of N-methyl-D-aspartate receptor epsilon1 subunit gene disruption of the action of general anesthetic drugs in mice', *Anesthesiology*, Vol. 102, No 3, pp. 557-561 (doi:10.1097/00000542-200503000-00013).

Savage, S. and Ma, D. (2014), 'The neurotoxicity of nitrous oxide: the facts and "putative" mechanisms', *Brain Sciences*, Vol. 4, No 1, pp. 73-90 (doi:10.3390/brainsci4010073).

Scalabrino, G., Carpo, M., Bamonti, F., Pizzinelli, S., D'Avino, C., Bresolin, N., Meucci, G., Martinelli, V., Comi, G. C. and Peracchi, M. (2004), 'High tumor necrosis factor-alpha [corrected] levels in cerebrospinal fluid of cobalamin-deficient patients', *Annals of Neurology*, Vol. 56, No 6, pp. 886-890 (doi:10.1002/ana.20325).

Scalabrino, G., Mutti, E., Veber, D., Aloe, L., Corsi, M. M., Galbiati, S. and Tredici, G. (2006), 'Increased spinal cord NGF levels in rats with cobalamin (vitamin B12) deficiency', *Neuroscience Letters*, Vol. 396, No 2, pp. 153-158 (doi:10.1016/j.neulet.2005.11.029).

Scalabrino, G., Nicolini, G., Buccellato, F. R., Peracchi, M., Tredici, G., Manfridi, A. and Pravettoni, G. (1999), 'Epidermal growth factor as a local mediator of the neurotrophic action of vitamin B(12) (cobalamin) in the rat central nervous system', *FASEB journal: Official Publication of the Federation of American Societies for Experimental Biology*, Vol. 13, No 14, pp. 2083-2090 (doi:10.1096/fasebj.13.14.2083).

Scalabrino, G., Tredici, G., Buccellato, F. R. and Manfridi, A. (2000), 'Further evidence for the involvement of epidermal growth factor in the signaling pathway of vitamin B12 (cobalamin) in the rat central nervous system', *Journal of Neuropathology and Experimental Neurology*, Vol. 59, No 9, pp. 808-814 (doi:10.1093/jnen/59.9.808).

Scalabrino, G., Veber, D. and Mutti, E. (2007), 'New pathogenesis of the cobalamin-deficient neuropathy', *Medicina nei Secoli*, Vol. 19, No 1, pp. 9-18.

Schifilliti, D., Mondello, S., D'Arrigo, M. G., Chillè, G. and Fodale, V. (2011), 'Genotoxic effects of anesthetic agents: an update', *Expert Opinion on Drug Safety*, Vol. 10, No 6, pp. 891-899 (doi:10.1517/14740338.2011.586627).

Schneemilch, C. E., Hachenberg, T., Ansorge, S., Ittenson, A. and Bank, U. (2005), 'Effects of different anaesthetic agents on immune cell function in vitro', *European Journal of Anaesthesiology*, Vol. 22, No 8, pp. 616-623 (doi:10.1017/s0265021505001031).

Schwark, T., Schaul, M., Schneider, S. and Yegles, M. (2022), 'Two cases of fatal inhalation of easily available "recreational" substances', *The American Journal of Forensic Medicine and Pathology*, Vol. 43, No 2, pp. 186-190 (doi:10.1097/PAF.0000000000000740).

Scofield, M. D., Heinsbroek, J. A., Gipson, C. D., Kupchik, Y. M., Spencer, S., Smith, A. C., Roberts-Wolfe, D. and Kalivas, P. W. (2016), 'The nucleus accumbens: mechanisms of addiction across drug classes reflect the importance of glutamate homeostasis', *Pharmacological Reviews*, Vol. 68, No 3, 816-871 (doi:10.1124/pr.116.012484).

Selvaraj, A. and Wong, K.E., (2017), 'An unusual case of 'laughing gas' addiction in Singapore', *European Psychiatry,* Vol. 41, p. S878 (doi:10.1016/j.eurpsy.2017.01.1772).

Selzer, R. R., Rosenblatt, D. S., Laxova, R. and Hogan, K. (2003), 'Adverse effect of nitrous oxide in a child with 5,10-methylenetetrahydrofolate reductase deficiency', *The New England Journal of Medicine*, Vol. 349, No 1, pp. 45-50 (doi:10.1056/NEJMoa021867).

Shapiro, J., Jersky, J., Katzav, S., Feldman, M. and Segal, S. (1981), 'Anesthetic drugs accelerate the progression of postoperative metastases of mouse tumors', *The Journal of Clinical Investigation*, Vol. 68, No 3, pp. 678-685 (doi:10.1172/jci110303).

Shen, Q., Lu, H., Wang, H. and Xu, Y. (2021), 'Acute cognitive disorder as the initial manifestation of nitrous oxide abusing: a case report', *Neurological Sciences*, Vol. 42, No 2, pp. 755-756 (doi:10.1007/s10072-019-04183-w).

Shouroki, K. F., Neghab, M., Mozdarani, H., Alipour, H., Yousefinejad, S. and Fardid, R. (2019), 'Genotoxicity of inhalational anesthetics and its relationship with the polymorphisms of GSTT1, GSTM1, and GSTP1 genes', *Environmental Science and Pollution Research International*, Vol. 26, No 4, pp. 3530-3541 (doi:10.1007/s11356-018-3859-0).

Singer, M. A., Lazaridis, C., Nations, S. P. and Wolfe, G. I. (2008), 'Reversible nitrous oxide-induced myeloneuropathy with pernicious anemia: case report and literature review', *Muscle & Nerve*, Vol. 37, No 1, pp. 125-129 (doi:10.1002/mus.20840).

Smith, A. D. and Refsum, H. (2016), 'Homocysteine, B vitamins, and cognitive impairment', *Annual Review of Nutrition*, Vol. 36, pp. 211-239 (doi:10.1146/annurev-nutr-071715-050947).

Smith, G. and Shirley, A. W. (1978), 'A review of the effects of trace concentrations of anaesthetics of performance', *British Journal of Anaesthesia*, Vol. 50, No 7, pp. 701-712 (doi:10.1093/bja/50.7.701).

Smith, W. D. (1965a), 'A history of nitrous oxide and oxygen anaesthesia. I. Joseph Priestley to Humphry Davy', *British Journal of Anaesthesia*, Vol. 37, No 10, pp. 790-798 (doi:10.1093/bja/37.10.790).

Smith, W. D. (1965b), 'A history of nitrous oxide and oxygen anaesthesia. II. Davy's researches in relation to inhalation anaesthesia', *British Journal of Anaesthesia*, Vol. 37, No 11, pp. 871-882 (doi:10.1093/bja/37.11.871).

Smith, W. D. (1972), 'A history of nitrous oxide and oxygen anaesthesia. IA. The discovery of nitrous oxide and of oxygen', *British Journal of Anaesthesia*, Vol. 44, No 3, pp. 297-304 (doi:10.1093/bja/44.3.297).

Smulders, Y. M. and Blom, H. J. (2011), 'The homocysteine controversy', *Journal of Inherited Metabolic Disease*, Vol. 34, No 1, pp. 93-99 (doi:10.1007/s10545-010-9151-1).

Solt, K. and Forman, S. A. (2007), 'Correlating the clinical actions and molecular mechanisms of general anesthetics', *Current Opinion in Anaesthesiology*, Vol. 20, No 4, pp. 300-306 (doi:10.1097/ACO.0b013e32816678a5).

Spronk, D., Nijkamp, L., and de Jonge, M. (2020a), *Lachgasgebruik bij jongeren met een niet-westerse migratieachtergrond [Use of nitrous oxide by young people with a non-Western migration background] info sheet*, Utrecht (https://www.trimbos.nl/aanbod/webwinkel/product/af1829-lachgasgebruik-bij-jongeren-met-een-niet-westerse-migratieachtergrond).

Spronk, D., Nijkamp, L., Nabben, T. and de Jonge, M. (2020b), *Lachgasgebruik bij jongeren met een niet-westerse migratieachtergrond. Een verkennend onderzoek [Use of nitrous oxide by young people with a non-Western migration background. An explorative study]*, Utrecht (https://www.trimbos.nl/aanbod/webwinkel/product/af1828-lachgasgebruik-bij-jongeren-met-een-niet-westerse-migratieachtergrond).

Steinberg, H. (1956), 'Abnormal behaviour induced by nitrous oxide', *British Journal of Psychology*, Vol. 47, No 3, pp. 183-194 (doi:10.1111/j.2044-8295.1956.tb00581.x).

Sturrock, J. (1977), 'Lack of mutagenic effect of halothane or chloroform on cultured cells using the azaguanine test system', *British Journal of Anaesthesia*, Vol. 49, No 3, pp. 207-210 (doi:10.1093/bja/49.3.207).

Sun, W., Liao, J. P., Hu, Y., Zhang, W., Ma, J. and Wang, G. F. (2019), 'Pulmonary embolism and deep vein thrombosis caused by nitrous oxide abuse: A case report', *World Journal of Clinical Cases*, Vol. 7, No 23, pp. 4057-4062 (doi:10.12998/wjcc.v7.i23.4057).

Tani, J., Weng, H. Y., Chen, H. J., Chang, T. S., Sung, J. Y. and Lin, C. S. (2019), 'Elucidating unique axonal dysfunction between nitrous oxide abuse and vitamin B12 deficiency', *Frontiers in Neurology*, Vol. 10, p. 704 (doi:10.3389/fneur.2019.00704).

Tavare, A. N., Li, D., Hare, S. S. and Creer, D. D. (2018), 'Pneumomediastinum and pneumorrhachis from recreational nitrous oxide inhalation: no laughing matter', *Thorax*, Vol. 73, No 2, pp. 195-196 (doi:10.1136/thoraxjnl-2017-210291).

Team Alert (2020), *Word geen clown in het verkeer. Rij ook ballonvrij [Don't be a clown in traffic. Drive balloon free]*, Ministry of Transport, Public Works and Water Management (https://rijballonvrij.nl/).

Tian, H., Xu, R., Canadell, J. G., Thompson, R. L., Winiwarter, W., Suntharalingam, P., Davidson, E. A., Ciais, P. et al. (2020), 'A comprehensive quantification of global nitrous oxide sources and sinks', *Nature*, Vol. 586, No 7828, pp. 248-256 (doi:10.1038/s41586-020-2780-0).

Tracy, M. E., Slavova-Hernandez, G. G. and Shelton, K. L. (2014), 'Assessment of reinforcement enhancing effects of toluene vapor and nitrous oxide in intracranial self-stimulation', *Psychopharmacology*, Vol. 231, No 7, pp. 1339-1350 (doi:10.1007/s00213-013-3327-y).

Trimbos-instituut (2022), *Nationale drug monitor, editie 2022. Lachgas 13.0 laatste feiten en trends - nationale drug monitor*, Trimbos-instituut, Utrecht & WODC, Den Haag (https://www.nationaledrugmonitor.nl/lachgas-laatste-feiten-en-trends/).

Trojan, J., Saunders, B. P., Woloshynowych, M., Debinsky, H. S. and Williams, C. B. (1997), 'Immediate recovery of psychomotor function after patient-administered nitrous oxide/oxygen inhalation for colonoscopy', *Endoscopy*, Vol. 29, No 1, pp. 17-22 (doi:10.1055/s-2007-1004055).

den Uil, S. H., Vermeulen, E., Metz, R., Rijbroek, A. and de Vries, M. (2018), 'Aortic arch thrombus caused by nitrous oxide abuse', *Journal of Vascular Surgery Cases and Innovative Techniques*, Vol. 4, No 2, pp. 80-82 (doi:10.1016/j.jvscit.2018.01.001).

UK Home Office (2013), *Drugs misuse: findings from the 2012/13 Crime Survey for England and Wales*, London (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/225122/Drugs_Misuse201213.pdf).

UK Home Office (2014a), *Drugs misuse: findings from the 2013/14 Crime Survey for England and Wales*, London (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/335989/drug_misuse_201314.pdf).

UK Home Office (2014b), *Guidance on restricting the supply of nitrous oxide for recreational use* (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/903285/RestrictingSupplyNitrousOxide.pdf).

UK Home Office (2017), *Drugs misuse: findings from the 2016/17 Crime Survey for England and Wales*, London (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/642738/drug-misuse-2017-hosb1117.pdf).

UK Home Office (2018a), *Drugs misuse: findings from the 2017/18 crime survey for England and Wales*, London (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/729249/drug-misuse-2018-hosb1418.pdf).

UK Home Office (2018b), *Review of the Psychoactive Substances Act 2016* (https://www.gov.uk/government/publications/review-of-the-psychoactive-substances-act-2016).

UK Home Office (2019), *Drugs misuse: findings from the 2018/19 Crime Survey for England and Wales* (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/832533/drug-misuse-2019-hosb2119.pdf).

UK Home Office (2020), *Drug misuse in England and Wales: year ending March 2020* (https://www.ons.gov.uk/peoplepopulationandcommunity/crimeandjustice/articles/drugmisuseinenglandandwales/yearendingmarch2020).

UK Home Secretary (2021), *Nitrous oxide: Home Secretary's letter to the ACMD* (https://www.gov.uk/government/publications/nitrous-oxide-home-secretarys-letter-to-the-acmd).

Uyanik, A. (1997), 'Gas chromatography in anaesthesia. I. A brief review of analytical methods and gas chromatographic detector

and column systems', *Journal of Chromatography. B, Biomedical Sciences and Applications*, Vol. 693, No 1, pp. 1-9.

VAD (Vlaams expertisecentrum Alcohol en andere Drugs) (2021), *Lachgas. Een nuchtere kijk op de aanpak van een terugkerend fenomeen [Nitrous oxide. a sober view on a recurrent phenomenon]. VAD-Visietekst: 5* (https://www.vad.be/assets/visietekst_lachgas).

Vallejo, M. C. and Zakowski, M. I. (2019), 'Pro-con debate: nitrous oxide for labor analgesia', *BioMed Research International*, Vol. 2019, 4618798 (doi:10.1155/2019/4618798).

Veber, D., Mutti, E., Galmozzi, E., Cedrola, S., Galbiati, S., Morabito, A., Tredici, G., La Porta, C. A. and Scalabrino, G. (2006), 'Increased levels of the CD40:CD40 ligand dyad in the cerebrospinal fluid of rats with vitamin B12(cobalamin)-deficient central neuropathy', *Journal of Neuroimmunology*, Vol. 176, No 1-2, pp. 24-33 (doi:10.1016/j.jneuroim.2006.04.002).

Verdejo-García, A., Lawrence, A. J. and Clark, L. (2008), 'Impulsivity as a vulnerability marker for substance-use disorders: review of findings from high-risk research, problem gamblers and genetic association studies', *Neuroscience and Biobehavioral Reviews*, Vol. 32, No 4, pp. 777-810 (doi:10.1016/j.neubiorev.2007.11.003).

VNG (Vereniging van Nederlandse Gemeenten) (2020), *Ledenbrief lachgas [Letter to the members on nitrous oxide]* (https://vng.nl/sites/default/files/2020-05/20200514_ledenbrief_lachgas.pdf).

VIAS (2021), *Nationale verkeersonveiligheidsenquête 2021 [National survey on unsafe driving 2021]* (https://www.enquetevias.be/nl/nationale-verkeersonveiligheidsenquete-2021/zelfgerapporteerd-gedrag/).

Vieira, E., Cleaton-Jones, P., Austin, J. C., Moyes, D. G. and Shaw, R. (1980), 'Effects of low concentrations of nitrous oxide on rat fetuses', *Anesthesia and Analgesia*, Vol. 59, No 3, pp. 175-177.

Vieira, E., Cleaton-Jones, P. and Moyes, D. (1983), 'Effects of intermittent 0.5% nitrous oxide/air (v/v) on the fertility of male rats and the post-natal growth of their offspring', *Anaesthesia*, Vol. 38, No 4, pp. 319-323 (doi:10.1111/j.1365-2044.1983.tb10452.x).

Walker, D. J. and Zacny, J. P. (2001), 'Within- and between-subject variability in the reinforcing and subjective effects of nitrous oxide in healthy volunteers', *Drug and Alcohol Dependence*, Vol. 64, No 1, pp. 85-96 (doi:10.1016/s0376-8716(00)00234-9).

Walker, D. J. and Zacny, J. P. (2002), 'Analysis of the reinforcing and subjective effects of different doses of nitrous oxide using a free-choice procedure', *Drug and Alcohol Dependence*, Vol. 66, No 1, pp. 93-103 (doi:10.1016/s0376-8716(01)00188-0).

Walker, D. J. and Zacny, J. P. (2003), 'Bitonic dose-response functions for reinforcing and self-reported effects of nitrous oxide in humans', *Pharmacology, Biochemistry, and Behavior*, Vol. 74, No 4, pp. 851-857 (doi:10.1016/s0091-3057(03)00015-7).

Walsh, K., Das, R. K. and Kamboj, S. K. (2017), 'The subjective response to nitrous oxide is a potential pharmaco-endophenotype for alcohol use disorder: a preliminary study with heavy drinkers', *The International Journal of Neuropsychopharmacology*, Vol. 20, No 4, pp. 346-350 (doi:10.1093/ijnp/pyw063).

Weimann, J. (2003), 'Toxicity of nitrous oxide', *Best Practice & Research. Clinical Anaesthesiology*, Vol. 17, No 1, pp. 47-61 (doi:10.1053/bean.2002.0264).

White, A. E., Takehisa, S., Eger, E. I., 2nd, Wolff, S. and Stevens, W. C. (1979), 'Sister chromatid exchanges induced by inhaled anesthetics', *Anesthesiology*, Vol. 50, No 5, pp. 426-430 (doi:10.1097/00000542-197905000-00010).

WHO (World Health Organization) (2021a), *WHO model list of essential medicines - 22nd list* (https://www.who.int/publications/i/item/WHO-MHP-HPS-EML-2021.02).

WHO (2021b), *WHO Model List of Essential Medicines for Children - 8th list* (https://www.who.int/publications/i/item/WHO-MHP-HPS-EML-2021.03).

Wiesner, G., Harth, M., Szulc, R., Jurczyk, W., Sobczynski, P., Hoerauf, K. H., Hobbhahn, J. and Taeger, K. (2001), 'A follow-up study on occupational exposure to inhaled anaesthetics in Eastern European surgeons and circulating nurses', *International Archives of Occupational and Environmental Health*, Vol. 74, No 1, pp. 16-20 (doi:10.1007/s004200000189).

van de Wijngaart, G., Braam, R., de Bruin, D., Fris, M., Maalsté, N. and Verbraeck, H. (1997), *Ecstasy in het uitgaanscircuit [Ecstasy on the night life scene]*, Utrecht

Winstock, A. R., Barratt, M. J., Maier, L. J., Aldridge, A., Zhuparris, A., Davies, E., Hughes et al. (2019), *Global Drug Survey (GDS) 2019 key findings report* (https://issuu.com/globaldrugsurvey/docs/gds2019_key_findings_report_may_16_).

Winstock, A. R. and Ferris, J. A. (2020), 'Nitrous oxide causes peripheral neuropathy in a dose dependent manner among recreational users', *Journal of Psychopharmacology*, Vol. 34, No 2, pp. 229-236 (doi:10.1177/0269881119882532).

Wood, R. W., Grubman, J., and Weiss, B. (1977), 'Nitrous oxide self-administration by the squirrel monkey', *The Journal of Pharmacology and Experimental Therapeutics*, Vol. 202, No 3, pp. 491–499.

Wrońska-Nofer, T., Nofer, J. R., Jajte, J., Dziubałtowska, E., Szymczak, W., Krajewski, W., Wąsowicz, W. and Rydzyński, K. (2012), 'Oxidative DNA damage and oxidative stress in subjects occupationally exposed to nitrous oxide (N(2)O)', *Mutation Research*, Vol. 731, No 1-2, pp. 58-63 (doi:10.1016/j.mrfmmm.2011.10.010).

Wrońska-Nofer, T., Palus, J., Krajewski, W., Jajte, J., Kucharska, M., Stetkiewicz, J., Wasowicz, W. and Rydzyński, K. (2009), 'DNA damage induced by nitrous oxide: study in medical personnel of operating rooms', *Mutation Research*, Vol. 666, No 1-2, pp. 39-43 (doi:10.1016/j.mrfmmm.2009.03.012).

Wu, M., Minkowicz, S., Dumrongprechachan, V., Hamilton, P., Xiao, L. and Kozorovitskiy, Y. (2021), 'Attenuated dopamine

signaling after aversive learning is restored by ketamine to rescue escape actions', *eLife*, Vol. 10, e64041 (doi:10.7554/eLife.64041).

Wyse, A., Bobermin, L. D., Dos Santos, T. M. and Quincozes-Santos, A. (2021), 'Homocysteine and gliotoxicity', *Neurotoxicity Research*, Vol. 39, No 3, pp. 966-974 (doi:10.1007/s12640-021-00359-5).

Xiang, Y., Li, L., Ma, X., Li, S., Xue, Y., Yan, P., Chen, M. and Wu, J. (2021), 'Recreational nitrous oxide abuse: prevalence, neurotoxicity, and treatment', *Neurotoxicity Research*, Vol. 39, No 3, pp. 975-985 (doi:10.1007/s12640-021-00352-y).

Yacoub, O., Doell, D., Kryger, M. H. and Anthonisen, N. R. (1976), 'Depression of hypoxic ventilatory response by nitrous oxide', *Anesthesiology*, Vol. 45, No 4, pp. 385-389 (doi:10.1097/00000542-197610000-00006).

Yagiela, J. A. (1991), 'Health hazards and nitrous oxide: a time for reappraisal', *Anesthesia Progress*, Vol. 38, No 1, pp. 1-11.

Yamakura, T. and Harris, R. A. (2000), 'Effects of gaseous anesthetics nitrous oxide and xenon on ligand-gated ion channels. Comparison with isoflurane and ethanol', *Anesthesiology*, Vol. 93, No 4, pp. 1095-1101 (doi:10.1097/00000542-200010000-00034).

Yu, J. T., Xu, W., Tan, C. C., Andrieu, S., Suckling, J., Evangelou, E., Pan, A. et al. (2020), 'Evidence-based prevention of Alzheimer's disease: systematic review and meta-analysis of 243 observational prospective studies and 153 randomised controlled trials', *Journal of Neurology, Neurosurgery, and Psychiatry*, Vol. 91, No 11, pp. 1201-1209 (doi:10.1136/jnnp-2019-321913).

Zacny, J. P. and Jun, J. M. (2010), 'Lack of sex differences to the subjective effects of nitrous oxide in healthy volunteers', *Drug and Alcohol Dependence*, Vol. 112, No 3, pp. 251-254 (doi:10.1016/j.drugalcdep.2010.06.008).

Zacny, J. P., Lichtor, J. L., Coalson, D. W., Apfelbaum, J. L., Flemming, D. and Foster, V. (1994a), 'Time course of effects of brief inhalations of nitrous oxide in normal volunteers', *Addiction*, Vol. 89, No 7, pp. 831-839 (doi:10.1111/j.1360-0443.1994.tb00986.x).

Zacny, J. P., Sparacino, G., Hoffmann, P., Martin, R., and Lichtor, J. L. (1994b), 'The subjective, behavioral and cognitive effects of subanesthetic concentrations of isoflurane and nitrous oxide in healthy volunteers', *Psychopharmacology*, Vol. 114, No 3, pp. 409-416 (doi:10.1007/BF02249330).

Zacny, J. P., Walker, D. J. and Derus, L. M. (2008), 'Choice of nitrous oxide and its subjective effects in light and moderate drinkers', *Drug and Alcohol Dependence*, Vol. 98, No 1-2, pp. 163-168 (doi:10.1016/j.drugalcdep.2008.06.001).

Zacny, J. P., Yajnik, S., Coalson, D., Lichtor, J. L., Apfelbaum, J. L., Rupani, G., Young, C., Thapar, P. and Klafta, J. (1995), 'Flumazenil may attenuate some subjective effects of nitrous oxide in humans: a preliminary report', *Pharmacology, biochemistry, and behavior*, Vol. 51, No 4, pp. 815-819 (doi:10.1016/0091-3057(95)00039-y).

Zarate, C. A., Jr and Machado-Vieira, R. (2015), 'Potential pathways involved in the rapid antidepressant effects of nitrous oxide', *Biological Psychiatry*, Vol. 78, No 1, pp. 2-4 (doi:10.1016/j.biopsych.2015.04.007).

Zhang, C., Davies, M. F., Guo, T. Z. and Maze, M. (1999), 'The analgesic action of nitrous oxide is dependent on the release of norepinephrine in the dorsal horn of the spinal cord', *Anesthesiology*, Vol. 91, No 5, pp. 1401-1407 (doi:10.1097/00000542-199911000-00033).

Zhang, S., Zhou, Z., Wu, W., Yu, Q., and Hong, M. (2021), 'Methylprednisolone combined vitamin supplementation reversed rapidly subacute combined degeneration of the spinal cord induced by the abuse of nitrous oxide', *Acta Neurologica Belgica*, 10.1007/s13760-020-01589-8 (doi:10.1007/s13760-020-01589-8).

Zheng, R., Wang, Q., Li, M., Liu, F., Zhang, Y., Zhao, B., Sun, Y. et al. (2020), 'Reversible neuropsychiatric disturbances caused by nitrous oxide toxicity: clinical, imaging and electrophysiological profiles of 21 patients with 6-12 months follow-up', *Neuropsychiatric Disease and Treatment*, Vol. 16, pp. 2817-2825 (doi:10.2147/NDT.S270179).

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About this publication

The purpose of this report is to examine the current situation, risks and responses to the recreational use of nitrous oxide in Europe. To support this, the report also provides a state-of-the-art review of the chemistry, pharmacology and toxicology of the gas. It is intended for policymakers and practitioners.

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