World Health
Organization

Pre-review report:

Nitrous oxide

Expert Committee on Drug Dependence

Forty-sixth Meeting

Geneva, 16–20 October 2023

Contents

Executive summary

Nitrous oxide is an inert, non-irritating, colourless, slightly sweet-smelling gas widely used as an anaesthetic or analgesic agent in medicine and commercially in the food and beverage, electronics and motor fuel industries. The global market for both medical and industrial nitrous oxide was estimated at US$ 1.2 billion in 2022.

Nitrous oxide is listed on the 2023 WHO Model List of Essential Medicines and on the list of Essential Medicines for Children as an inhalational anaesthetic. It is approved as a medical gas and as a food additive by many national regulatory authorities. It is not currently under international control and has never been reviewed by the ECDD. Nitrous oxide is legal in all countries, although some countries limit the sale of nitrous oxide for direct human consumption or to minors.

Nitrous oxide is rapidly absorbed via inhalation. It is relatively insoluble in blood, resulting in rapid onset (15–30 s) and offset (10–15 min) of effects. Nitrous oxide is not metabolized and is eliminated unchanged in exhaled breath.

For non-medical use, nitrous oxide is usually inhaled through the mouth from small metal canisters or bulbs ("whippets"), from balloons filled with gas from other sources, such as cylinders ("smart whips"), or large tanks containing up to 10 kg nitrous oxide. Bulbs typically contain 10–50 mL of pure nitrous oxide.

The limited information on dosage and use patterns is from surveys of self-selected convenience samples by non-medical users. Many respondents used nitrous oxide several times a month, taking 3–10 "hits" each time. Heavier users may inhale 20 or more bulbs at each session.

Two neuropharmacological mechanisms have been proposed for the abuse potential and other behavioural effects of nitrous oxide: activation of opioid receptors and blockade of the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor. Evidence for a role of opioid receptors is suggestive but not consistent. In mice, pharmacological blockade of κ-opioid receptors consistently blocks nitrous oxide-induced analgesia, while results for pharmacological blockade of μ-opioid receptors are inconsistent. Deletion of the gene for the μ-opioid receptor does not influence the analgesic effect. One study in mice and one in rats found that direct injection of a μ-opioid receptor antagonist into the periaqueductal gray area of the brain reduced nitrous oxide-induced analgesia.

Studies of the influence of naloxone (an opioid receptor antagonist selective for the μ-opioid receptor at low doses) on nitrous oxide-induced analgesia, in a variety of models of pain, gave mixed results. Two show a decreased analgesic effect, four show no effect, and two (from the same research group) show a reduction or an enhancement of the analgesic effect, depending on the participant. One study found no significant effect of naloxone (0.1–10 mg/kg intravenously) on subjective effects or psychomotor impairment.

The second proposed major neuropharmacological mechanism is noncompetitive antagonism at the NMDA receptor, which has also been proposed as the mechanism of the rapid anti-depressant action of nitrous oxide, which resembles that of ketamine. There is no direct evidence that this mechanism occurs in humans.

Nitrous oxide, as an inert gas, has no acute biochemical or cellular toxicity. The rare deaths associated with nitrous oxide use are due to asphyxia or accidents. Chronic exposure causes irreversible oxidation of the cobalt ion in cobalamin (vitamin B12), which renders the vitamin functionless. This mechanism results in major neurological and haematological toxicity. Both chronic nitrous oxide use and cobalamin deficiency are associated with generalized demyelinating polyneuropathy and megaloblastic anaemia. Other possible mechanisms of neurotoxicity include elevated concentrations of homocysteine and methylmethionine, which are neurotoxic in rodents.

The true prevalence of adverse reactions associated with use of nitrous oxide is unknown, as no population-based or systematic unbiased surveys appear to have been conducted. The most common adverse effects reported by non-medical nitrous oxide users in anonymous online surveys include hallucinations (lifetime prevalence, 36.3%), confusion (31.5%), fainting (10.4%), nausea (9.7%), persistent numbness (5.2%) and accidents (3.2%).

The most common adverse reactions seen in chronic nitrous oxide users presenting for medical attention are neurological (usually related to demyelinization) and haematological. The most common symptoms include limb weakness or numbness (almost all patients), difficulty in walking, headache or dizziness, involuntary movements and constipation.

Nitrous oxide produced psychotic-like subjective effects similar to those of ketamine in some laboratory studies with humans. The effects included both positive and negative symptoms.

Nitrous oxide caused dose-dependent cognitive and psychomotor impairment in healthy adults at concentrations that had positive subjective effects. Impairments resolved within 5–20 min of cessation of nitrous oxide exposure.

Even brief exposure to nitrous oxide can impair cognition. Among 12 healthy young adults who took four deep inhalations of nitrous oxide, verbal recall and performance on the Digit-Symbol Substitution Test (DSST) were impaired by 10% nitrous oxide. Performance was normal 3 min after inhalation.

Nitrous oxide generates acute and chronic tolerance to many of its effects in rodents, although a few studies did not show tolerance. Rodents that were tolerant to nitrous oxide were partially cross-tolerant to ethanol but not to barbiturates or morphine.

Rodents exposed to nitrous oxide showed signs of withdrawal when exposure ended abruptly, most prominently tonic–clonic handling seizures.

Tolerance to the analgesic effects of nitrous oxide was observed in several laboratory studies with humans but usually not to its subjective or psychomotor effects. Tolerance can appear after 30 min of exposure. Of 111 cases of non-medical nitrous oxide use reported to the French national addictovigiliance system between 2012 and 2021, 27.9% showed tolerance and 11.7% showed withdrawal symptoms. Of 190 medical students at the University of Paris who met the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, fifth edition, revised (DSM-5) for nitrous oxide use disorder and who participated in an anonymous online survey, 45% reported tolerance.

Nitrous oxide had clear rewarding properties in two of the four widely used animal models of substance abuse (self-administration, intracranial self-stimulation [ICSS], drug discrimination) and mixed results in two others (ICSS, conditioned place preference), although the evidence is sparse. Some rats self-administered nitrous oxide, as did squirrel monkeys. Mice discriminated between 60% nitrous oxide and pure oxygen. Nitrous oxide has variable effects on the response rate of self-administration and ICSS and reduced the effort mice were willing to make. Nitrous oxide elicited dose-dependent conditioned place preference in rats at a low concentration (8%) and conditioned place aversion at higher concentrations (≥ 30%) but had no conditioning effect in mice.

The abuse potential of nitrous oxide was noted immediately after it was identified, in 1772. Inhalation of nitrous oxide produced a pleasurable euphoria and giddiness likened to alcohol intoxication, with pleasant thrilling sensations in the body and auditory and visual distortions. Modern laboratory studies with healthy young adults confirm that inhaled nitrous oxide has robust, dose-dependent pleasurable, rewarding subjective effects at sub-anaesthetic concentrations of inhaled gas (10–50%) but did not consistently generate a preference to oxygen in discrete choice procedures. Commonly reported subjective effects are: dreamy, detached reverie (e.g. "floating", "coasting", "spaced out"); happy, euphoric mood (e.g. "happy", "high", "elated", "stimulated") or psychedelic (e.g. "pleasant bodily sensations", changed body awareness and image, altered time perception, dissociative state). Subjective

effects resolved substantially by 15 min after cessation of nitrous oxide exposure and resolved completely within 1 h. Adults who took a single deep inhalation of 100% nitrous oxide or four consecutive deep inhalations of 40%, 60% or 80% nitrous oxide experienced positive subjective effects within 15–30 s, peaking at 2–3 min and subsiding within 15–20 min. Subjective effects were rated by participants as more similar to those of ketamine and alcohol than to those of cannabis or cocaine.

Individuals differed substantially in the quality and intensity of the subjective effects of nitrous oxide. A large proportion of individuals reported disliking or were neutral towards inhaling nitrous oxide rather than liking it.

The first medical use of nitrous oxide was as a dental anaesthetic, in 1844. It is now widely used as a supplementary agent in inhalational anesthesia, as it is not sufficiently potent to be used alone, for analgesia and sedation during childbirth and in painful short procedures in dentistry and emergency medicine.

Nitrous oxide has been proposed as a treatment for depression (especially that which is resistant to treatment) and alcohol withdrawal on the basis of several small controlled clinical trials, but it is not approved for these conditions by any national regulatory authority. Five controlled clinical trials found that nitrous was non-inferior to standard doses of oral benzodiazepines in reducing the signs and symptoms of acute alcohol withdrawal, while a later controlled clinical trial found no benefit.

Deaths directly related to the non-medical use of nitrous oxide appear to be rare. Coroners' inquests in England and Wales identified 62 deaths associated with nitrous oxide use between 2001 and 2021, corresponding to an average of three deaths per year. The Australian National Coronial Information System included 20 fatalities related to nitrous oxide misuse between 2000 and 2021.

The true prevalence of non-medical use of nitrous oxide or of nitrous oxide use disorder is unknown. The United Nations Office on Drugs and Crime annual World Drug Report does not include data on use of nitrous oxide. Few population-based surveys of psychoactive substance use have included nitrous oxide, and many of those that did grouped it with other inhalants (e.g. alkyl nitrites, volatile organic compounds).

The global prevalence of non-medical use of nitrous oxide appears to be low but may have increased during the past decade in some countries and population groups, such as in Lithuania and Netherlands (Kingdom of the). The lifetime prevalence was 0.6–7.6% in four countries that have conducted nationally representative, population-based cross-sectional surveys (Lithuania, Netherlands [Kingdom of the], New Zealand and the USA). The prevalence in the past year was 0.8–3.2% in England and Wales, Netherlands (Kingdom of the) and New Zealand.

The prevalence of non-medical use of nitrous oxide is relatively high in some population subgroups, including health profession students (perhaps because of easier access), high schools (secondary), colleges, attendees at dance clubs and music festivals and adolescents in psychiatric treatment settings.

1   Substance identification

A   *International nonproprietary name*

Not assigned

B   *Chemical Abstracts Service (CAS) registry number*

100024-97-2

C   *Other chemical names*

Canonical SMILES
N#N=O

InChI
InChI=1S/N2O/c1-2-3

InChI key
GQPLMRYTRLFLPF-UHFFFAOYSA-N

Other names
Dinitrogen monoxide
Dinitrogen oxide
Dinitrogen oxide (N_2O)
Hyponitrous acid anhydride
Nitrogen protoxide
Nitrous-oxide
2-Oxodiazen-2-ium-1-ide
1,2-Diazaethyne1-oxide
Diazyne 1-oxide
Nitrogen hypoxide
Nitrogen oxide
Nitrogenium oxydulatum
Oxodiazen-2-ium-1-ide
Oxidodinitrogen(N-N
Diazooxidane
Laughing gas
Factitious air
R 744A

D   Trade names

Nitrous oxide is used as a food additive (aerosol propellant to make whipped cream), a refrigerant, a leak detecting agent, an oxidizing agent, a chemical reagent and as an additive to fuels for racing cars under several trade names.

Medical nitrous oxide is sold as single ingredient in several countries under the trade name NIONTIX (Belgium, Czechia, Denmark, Finland, Germany, Netherlands [Kingdom of the], Norway and Sweden) or MAXICOOL (Israel). Medical nitrous oxide is also sold in multi-ingredient preparations under the trade names Entonox and Equanox in Australia; Livopan in Austria; Actynox, Kalinox and Relivopan in Belgium; Alnox, Entonax and Liquid-Med in Canada; Entonox in Czechia; Kalinox, Latox, Livopan and Medimix in Denmark; Livopan in Finland; Antasol, Entonax, Kalinox and Oxynox in France; Livopan in Germany; Livopan and Nitralgin in Hungary; Donopa and Entonox in Ireland; Protoxan in Italy; Actynox, Donopa, Kalinox, Protoxan and Relivopan in Netherlands (Kingdom of the); Livopan in Norway; Entonox in New Zealand; Entomix, Entonox and Kalinox in Poland; Kalinox, Livopan and Protoxan in Portugal; Entonox in South Africa; Actynox, Entonox, Kalinox, Nodolox and Protoxan in Spain; Livopan and Medimix in Sweden; Entonox and Kalinox in Switzerland; and Entonox and Equanox in the United Kingdom *(1)*.

E   Street names

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; Noss; Pan; Shoot the breeze; Tanks; Whippet; Whippets; and Wippets *(1)*. The name Nox is used when nitrous oxide

is combined with other substances, such as 3,4-methylenedioxymethamphetamione (MDMA) *(2)*.

F   Physical *appearance*

The European Pharmacopoeia describes nitrous oxide as a colourless gas *(3)* and the United States Pharmacopeia describes it as a colourless gas with no appreciable odour or taste *(4)*. It has also been reported as a colourless gas under ambient conditions, with a slightly sweet odour and taste *(5,6)*.

The British Pharmacopeia 2019 *(3)* states that nitrous oxide should be kept in approved metal cylinders painted blue and carry a label stating "nitrous oxide". Furthermore, "nitrous oxide" or "N_2O" should be stenciled in paint on the shoulder of the cylinder.

G   *WHO review history*

Nitrous oxide has not previously been reviewed by the WHO Expert Committee on Drug Dependence.

2   Chemistry

A   *Chemical name*

IUPAC Name: Nitrous oxide

Chemical Abstracts Index name: Nitrogen oxide (N_2O) (7CI, 8CI, 9CI, ACI)

B   *Chemical structure*

Free base:

$$\overset{-}{N}=\overset{+}{N}=O$$
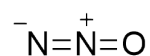
Molecular formula:

N_2O

Molecular weight:

44.013 g/mol

C   *Stereoisomers*

No stereoisomers of nitrous oxide have been described.

D   *Methods and ease of illicit manufacture*

Nitrous oxide was first isolated by Joseph Priestley in 1772 and was synthesized by Humphry Davy in the late 1790s. The synthesis of nitrous oxide has remained relatively unchanged, as it is widely used in industrial preparation of the gas *(7)*. The synthesis consists of thermal decomposition of a 92–94% hot solution of ammonium nitrate in reaction (1):

$$NH_4NO_3 \rightarrow N_2O + 2H_2O \text{ (1)}.$$
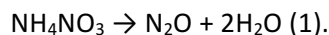
The ideal temperature of the reaction for the maximum yield is 265–278 °C. Higher and lower temperatures result in undesired side-products, such as nitrogen (N_2), nitrogen dioxide (NO_2), dinotrogen trioxide (N_2O_3), nitric oxide (NO), ammonia (NH_3) and nitric acid (HNO_3). The presence of impurities such as chlorides, carbonates, nitric acid, iron, organic substances and especially oil in a hot solution of ammonium nitrate can disturb the decomposition reaction. For

instance, in the presence of 0.1% chlorides (w/w) the formation of nitrous oxide is slower and the formation of impurities is faster. Additionally, the presence of oil traces leads to formation of carbon monoxide as a gaseous impurity, while the presence of iron traces increases the formation of impurities *(8)*. As the reaction is exothermal, a large increase in the reaction temperature can cause an explosion *(7)*. The gas obtained from the reaction undergoes a series of processes to achieve the desired purity.

Another synthesis reported in a US patent *(9)* involves reaction of urea in sulfuric acid with nitric acid and subsequent purification with an aqueous alkaline solution.

Clandestine and home production of nitrous oxide are possible but expensive and dangerous because of the risks of explosions and formation of noxious side products such as NO and NO_2 *(5)*. Other methods have been developed for producing nitrous oxide at home. For example, mixing sodium nitrate and ammonium sulfate according to reaction (2) minimizes the formation of side products; however, the procedure is unsafe *(10)*.

$$2NaNO_3 + (NH_4)_2SO_4 \rightarrow Na_2SO_4 + 2N_2O + 4H_2O \text{ (2)}.$$
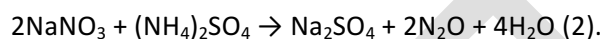
E   Chemical properties

Melting-point
−90.83 °C *(11)*

Boiling-point
88.57 °C *(11)*

Solubility

In water: 1 volume of nitrous oxide dissolves in about 1.5 volumes of water at 20 °C and at a pressure of 101 kPa *(3)*.

1 volume of nitrous oxide dissolves in about 1.4 volumes of water at 20 °C and at a pressure of 760 mmHg *(4)*.

It is also freely soluble in alcohol and soluble in ether and oils *(4)*.

F   Identification and analysis

Chromatographic, optical and amperometric techniques are currently used to measure nitrous oxide *(12)*. There are portable devices to measure nitrous oxide in gaseous media *(13)*, but they apparently have not been used to detect nitrous oxide in exhaled breath at the site of motor vehicle accidents or in cases of apparent intoxication. There is no portable device for detecting nitrous oxide in blood or other liquid matrices.

Chromatographic techniques

The most widely used analytical technique is gas chromatography (GC) coupled to an electron-capture detector with manual or automated sampling. The limit of detection is about 30 ppb, and the precision ranges from 0.18 to 0.4. The cost of this instrumental platform is low *(12)*, the methods are readily available, and the data are comparable to those previously collected *(14)*. As the presence of water vapour and carbon dioxide can result in inaccurate results, samples must be pre-treated *(12)*. High accuracy and precision can also be obtained with GC coupled to an isotope-ratio mass spectrometer for isotopic analysis of nitrous oxide *(15)*.

Optical techniques

The advantage of optical techniques is the short analysis time – fractions of a second – which makes them the techniques of choice for measuring trace gas fluxes *(12)*. Two optical techniques are used preferentially for measuring nitrous oxide: Fourier-transform infra-red spectroscopy

and laser-adsorption spectroscopy *(16)*. The limit of detection is about 1 ppb, and the precision may reach 0.1 ppb *(17)*. An indirect optical technique is photoacoustic spectroscopy, which measures the effect on the absorbing medium rather that direct light absorption. This technique is attractive because portable devices can be set up that work at atmospheric pressure, allowing measurements in situ *(18)*.

Amperometric techniques

The current produced by reduction of nitrous oxide at an electrode can be used to measure its concentration. Electrochemical amperometric sensors are sensitive, simple, easy to use and cheap *(19)*. Such instrumentation can reach a limit of detection of 22 ppb but cannot be used for prolonged monitoring *(20)*.

Nitrous oxide in biological specimens

Few methods have been reported for the determination of nitrous oxide in biological samples. Those reported are based on either GC-electron capture detection (ECD) or GC-mass spectrometry (MS) *(21–23)*. Brugnone et al. *(21)* determined nitrous oxide concentrations in plasma and urine of physicians exposed to the gas during anaesthesia of patients and reported concentrations far higher than in controls. Poli et al. *(22)* examined post-mortem samples from eight cases of intoxication due to erroneous replacement of the oxygen gas line with one containing nitrous oxide. Nitrous oxide levels were detected even 1 month after death by head-space GC-ECD. Abnormal amounts of nitrous oxide were found in all post-mortem tissues (blood, urine, liver, bile, kidney, fat and brain) by HS-GC-MS in toxicological studies of a death suspected to be due to nitrous oxide intoxication *(23)*.

Because of its short half-life, nitrous oxide is more difficult to detect in biological samples from living people than in post-mortem specimens. Levels can be measured in blood and urine immediately after exposure *(21)*, but routine analysis is more difficult. Mariller et al. *(24)* attempted to detect nitrous oxide in urine and exhaled air but found sampling and interpretation of the results to be difficult. They recommended early sampling, use of entirely filled airtight containers and freezing if immediate analysis is not possible.

3 Ease of conversion into controlled substances

Although nitrous oxide is used in a wide variety of chemical reactions, especially as an oxidizing agent, it is not known whether it can be converted into a controlled substance.

4 General pharmacology

A Routes of administration and dosage

Nitrous oxide is an inert, colourless, slightly sweet smelling, non-irritating gas that is administered via inhalation through the nose and/or mouth *(25)*. For non-medical use, nitrous oxide is typically inhaled from small metal canisters or bulbs used to generate whipped cream ("whippets", "nangs" [in Australia]) or from balloons filled with gas from other sources *(26)*. Bulbs typically contain 10–50 mL of pure nitrous oxide. Balloons may be filled from cylinders ("smart whips") or large tanks containing up to 10 kg of nitrous oxide that are sold legally for industrial or medical use. This is readily done with a "cracker", a device screwed onto the cylinder, and turning the cap to release nitrous oxide into the attached balloon *(5)*.

Cross-sectional, online, anonymous international surveys of convenience samples of self-selected individuals who use psychoactive substances (global drug surveys in 2014–2016)

identified 17 325 respondents who had used nitrous oxide for non-medical reasons in the past year *(27)*. The vast majority (82%) had inhaled nitrous oxide from balloons, which they had filled from bulbs (87.2%) or large tanks (6.8%). The remainder inhaled nitrous oxide from whipped cream dispensers (12.8%), plastic bags (0.7%) or gas bulbs (0.9%). Among 4883 respondents in the 2014 global drug survey (data collected in late 2013) who reported use of nitrous oxide in the past year, 80.6% had inhaled nitrous oxide from a balloon, 15.9% from a whipped cream dispenser, 0.8% from a plastic bag and 0.8% from a gas bulb *(28)*. The gas was almost always inhaled by mouth (98.3%) and rarely via the nose (0.7%) or both (1.0%).

Respondents in the global drug surveys in both 2014 (data collected in late 2013) and 2014–2016 reported taking a median (interquartile range, IQR) of 5 (3–10) "hits" each time they used nitrous oxide *(27,28)*. Respondents in the Australian Ecstasy and Related Drug Reporting System in Sydney, Australia (about 100 each year) (see section 13) reported using nitrous oxide on a median of 7 days (IQR 2–14), 5 days (2–10) and 5 days (2–10) over the previous 6 months in 2018, 2019 and 2020, respectively *(9,10)*. Respondents reported using a median of seven bulbs (IQR 4–10), five bulbs (3–10) and eight bulbs (4–20) at a typical session in 2018, 2019 and 2020, respectively. In 2018, 17% of respondents reporting using at least 15 bulbs at their most recent session *(29)*.

Among 158 lifetime "recreational" nitrous oxide users self-identified in a 2002 in-class survey of first-year students at the University of Auckland, New Zealand, the typical use pattern was two to five bulbs per session; 5% used more than 10 bulbs *(30)*.

B   *Pharmacokinetics*

Nitrous oxide is rapidly absorbed via inhalation. It is poorly soluble in blood and adipose tissue and does not bind to blood constituents, resulting in rapid onset (15–30 s) and offset (10–15 min) of effects *(31)*. Nitrous oxide is not metabolized and is eliminated unchanged in exhaled breath.

C   *Pharmacodynamics*

Two neuropharmacological mechanisms have been proposed for the abuse potential and other behavioural effects of nitrous oxide: activation of opioid receptors and blockade of the NMDA-type glutamate receptor *(26)*. Evidence for the role of opioid receptors is suggestive but not consistent. In mice, pharmacological blockage of κ-opioid receptors consistently blocks nitrous oxide-induced analgesia, while pharmacological blockage of μ-opioid receptors is inconsistent *(32–36)*. Administration of a κ-receptor agonist with the antagonist restored the analgesic effect *(35)*. In some studies, a μ-opioid receptor antagonist (usually naloxone) did not alter the analgesic effect of nitrous oxide *(32–36)*, while in others it reduced the analgesic effect *(37–39)*. The discrepancy may be due partly to differences in the type and dose of μ-opioid receptor antagonist used. Naloxone was not selective for the μ-opioid receptor at the higher doses used in some studies. In a study in which a wide range of naloxone doses were injected into the cerebral ventricles, higher doses blocked the analgesic effect, while lower doses potentiated the effect *(39)*. Deletion of the gene for the μ-opioid receptor did not influence the analgesic effect *(40)*. In one study in mice *(41)* and one in rats *(42)*, injection of a μ-opioid receptor antagonist directly into the periaqueductal gray area of the brain reduced nitrous oxide-induced analgesia. These studies suggest that the periaqueductal gray area, known to be an important site for pain perception, plays a role in the analgesic effect of nitrous oxide. In two studies, naloxone reduced nitrous oxide-induced hyperlocomotion *(43,44)*. Three studies in rats showed that μ-opioid receptor antagonists (naloxone, naltrexone) reduced nitrous-oxide induced analgesia *(45–47)*, while κ- and δ-opioid receptor antagonists had no effect *(46)*. In another study in rats, naloxone had no effect on nitrous oxide-induced analgesia *(48)*. A study

in cats also showed no effect of naloxone on nitrous oxide-induced analgesia *(49)*. A study in mice indicated that naloxone had no effect on nitrous oxide-induced anesthesia *(50)*. In rats, naltrexone (an opioid receptor antagonist selective for the μ-opioid receptor at lower doses) had no effect by itself but exacerbated nitrous oxide-induced impairment of performance in a visual vigilance task *(51)*.

Studies in humans on the influence of naloxone on nitrous oxide-induced analgesia, in a variety of pain models, had mixed results. Two showed a decreased analgesic effect, four showed no influence, and two (by the same research group) showed a reduction or increase in the analgesic effect depending on the participant (Table 1). Although a different experimental pain model was used in each study, there were no other methodological issues that would explain the different results. In one study, naloxone (0.1–10 mg/kg intravenously) had no significant effect on the subjective effects or psychomotor impairment induced by nitrous oxide (30% for 90 min) *(52)*.

Table 1. Studies on the influence of intravenous naloxone on the analgesic effect of nitrous oxide

Dose	No. of participants	Age (years)	Pain model	Effect	Reference
4, 8 mg	?	"Young"	Arm ischaemia	↓	*53*
0.4 mg/kg	12	18–32	Tooth pulp shock	↓	*54*
1.4 mg	7	27–41	Eyelid muscle stimulation	No change	*55*
10 mg	30	18–32	Tooth extraction	No change	*56*
0.01 mg	6	22–23	Skin heat	No change	*57*
30 mg/70 kg	14	21–39	Cold pressor test	No change	*58*
0.8–1.2 mg	15	21–30	Skin pressure	4 ↑	*59*
				7 ↓	
0.8 mg	6	28–40	Chronic musculo-skeletal pain	2 ↑	*60*
				4 ↓	

No change, no effect of naloxone on nitrous oxide-induced analgesia
↓ = reduced analgesia
↑ = increased analgesia

The second proposed neuropharmacological mechanism for the abuse liability of nitrous oxide is noncompetitive antagonism at the NMDA receptor *(26,61)*. This has also been proposed as the mechanism for the rapid anti-depressant action of nitrous oxide, which resembles that of ketamine *(62)*. There is no direct evidence for this mechanism in humans. Mice that lack the gene for the epsilon1 subunit of the NMDA receptor show reduced anaesthetic response to nitrous oxide *(63)*. Nitrous oxide, like ketamine, enhances glutamatergic neurotransmission in the rat hippocampus *(62)*.

Flumazenil (an antagonist at the benzodiazepine site of the γ-aminobutyric acid receptor) significantly reduced self-ratings of "high" during exposure to nitrous oxide (30% for 35 min), but only at the highest dose (5 mg/70 kg intravenously) *(64)*. Flumazenil had no effect on other subjective effects ("drunk", "high", "drug liking") nor on psychomotor impairment (Digit Symbol Substitution Test) due to nitrous oxide. In mice exposed to nitrous oxide (50% or 75%), pretreatment with flumazenil (10 mg/kg subcutaneously) attenuated the effect of nitrous oxide on reducing rearing (a putative model of anxiolytic action).

Nitrous oxide has other weak neuropharmacological actions in rodents, the relevance of which to its effects in humans is unknown *(65)*. They include activation of α-adrenergic receptors in

the brain stem and spinal cord, weak antagonism at 5-HT$_3$ receptors, and partial inhibition of certain nicotinic acetylcholine receptors.

5   Toxicology

Nitrous oxide, an inert gas, does not cause acute biochemical or cellular toxicity. Deaths associated with nitrous oxide use are due to asphyxiation when the inhaled substance prevents inhalation of sufficient oxygen (see section 14).

The main toxic action of nitrous oxide is irreversible oxidation of the cobalt ion in cobalamin (vitamin B12), which renders the vitamin functionless *(26)*. This is considered to be the mechanism by which chronic use of nitrous oxide causes major neurological and haematological toxicity. Cobalamin plays a major role in enzymatic formation of methionine and tetrahydrofolate, which are essential for formation of the myelin sheath surrounding neuronal axons and for formation of red blood cells. Thus, chronic nitrous oxide use and cobalamin deficiency are associated with several generalized demyelinating polyneuropathies, such as peripheral neuropathy, progressive spinal cord degeneration, subacute combined spinal cord degeneration and megaloblastic anaemia ("pernicious anemia"). A causal association is suggested by functional vitamin B12 deficiency in most chronic, heavy users of nitrous oxide and by recovery with abstinence from nitrous oxide use and/or treatment with cobalamin, although only about two thirds of patients have low blood concentrations of cobalamin and/or holotranscobalamin (the transporter for cobalamin) *(66)*. Another possible mechanism of nitrous oxide-induced neurotoxicity may be elevated levels of homocysteine and methylmalonic acid, which are found in about 75% of chronic users of nitrous oxide *(66)*. Both compounds are neurotoxic in rodents *(26)*.

6   Adverse reactions in humans

The true prevalence of adverse reactions due to use of nitrous oxide is unknown, as no population-based or systematic unbiased surveys appear to have been conducted. Among 4883 respondents with non-medical nitrous oxide use in the past year who participated in the anonymous, online global drug survey in 2014 (data collected in late 2013), the most common adverse effects associated with nitrous oxide use were hallucinations (lifetime prevalence, 36.3%; prevalence in the past year, 27.8%), confusion (31.5%/24.0%), fainting (10.4%/4.4%), nausea (9.7%/5.8%), persistent numbness (5.2%/4.3%) and accidents (3.2%/1.2%) *(28)*. Among 16 239 respondents to the global drug surveys in 2014–2016 who answered a question about paraesthesia (a proxy for peripheral neuropathy), a dose-dependent relation was found between the amount of nitrous oxide used per session and the prevalence of paraesthesia. The prevalence was 1.86% (95% CI 1.8 ; 2.5) for people who took one per session, 5.0% (4.4 ; 5.6) among those who took 20 doses per session, and 8.48% (6.6 ; 10.4) among those who took 100 doses per session *(67)*.

The adverse reactions seen most commonly in chronic nitrous oxide users presenting for medical attention are neurological (usually related to demyelinization) and haematological. In a case series of 110 patients (52% men; mean age, 21.4 years [range, 14–33 years]; mean duration of nitrous oxide use, 12.5 months [0.5–72 months]) who had used nitrous oxide non-medically and were treated in Shengjing Hospital of China Medical University between 2018 and 2020, the most common symptoms were limb weakness or numbness (97%), difficulty in walking (12%), headache or dizziness (8%), involuntary movements (6%) and constipation (5%) *(68)*. Among about 4000 cases reported to two toxicology units in the Sydney, Australia, area in 2017–2020, 22 involved individuals (50% women; 68% post-secondary students; median age, 22, IQR 20–28) who had used nitrous oxide daily for more than 1 week *(69)*. All were heavy users, with a median (IQR) peak use of nitrous

oxide 300 (200–370) bulbs daily for 6 (3–24) months. Only four (18%) reported use of illicit drugs, including ketamine, cannabis, MDMA ("ecstasy") and/or cocaine. The most common presenting symptoms were gait disturbance (68%) and psychiatric symptoms (23%), including hallucinations, paranoia and/or depression. Two patients each had urinary retention, urinary incontinence and confusion. Ten (45%) patients had low serum cobalamin concentrations, 8 of 10 patients (80%) had elevated homocysteine concentrations, 5 (23%) had anaemia, and 3 (14%) had neutropenia. Of the 18 patients who underwent magnetic resonance imaging, 10 (45%) had spinal cord abnormalities consistent with subacute combined degeneration due to cobalamin deficiency. All seven patients who underwent nerve conduction studies had abnormalities consistent with sensory peripheral neuropathy.

Nitrous oxide produced psychotic-like subjective effects similar to those of ketamine in some laboratory studies. They included both positive symptoms (e.g. perceptual distortion, paranoia, delusions) and negative symptoms (e.g. anhedonia, cognitive disorganization) *(70)*. At least 32 cases presenting for medical care with psychosis related to non-medical use of nitrous oxide have been reported in the published literature *(71,72)*. About half of the patients did not have concurrent neurological symptoms, suggesting that their psychosis was not due to neurological factors such as vitamin B12 deficiency. The most common psychotic symptoms were hallucinations, delusions and paranoia *(71)*.

Nitrous oxide causes dose-dependent cognitive and psychomotor impairment in healthy adults at concentrations that have positive subjective effects. Impairment resolves within 5–20 min of cessation of exposure *(73,74)*. For example, exposure to 15% and 30% nitrous oxide for 40 min impaired performance on the DSST by 10–20% and on a test of logical reasoning by 15% (only at 30%) *(75)*. No significant impairment was found in eye–hand coordination or auditory reaction time. Among 12 healthy adults exposed to nitrous oxide (at 0%, 3%, 5%, 7%, 10% or 15%) for 55 min, cognitive and psychomotor function was impaired only at 15% (DSST, continuous attention, choice reaction time, block pattern recognition, short-term memory recall), with one exception *(76)*. Long-term memory recall was impaired at all concentrations. Subjective effects (e.g., "drowsy", "feel well", "energetic", "drunk") were generated only at 15%, with one exception: "dizzy" was generated at 7% and higher concentrations.

Twelve healthy young adults exposed to nitrous oxide (0%, 5%, 10%, 20% and 40%) for 1 h experienced dose-dependent impairment of cognitive and psychomotor function *(77)*. At 5% nitrous oxide, no impairment was seen on any test. At 10%, DSST, finger-tapping rate and continuous attention were impaired. At 20%, choice reaction time, body sway, decision-making and visual vigilance were also impaired. At 40%, Gibson spiral maze (a test of visuo-motor coordination) and paired word learning were also impaired. Critical flicker fusion was not impaired at any concentration. In 12 healthy young adults exposed to nitrous oxide (0%, 10%, 20% and 40%) for 30 min, DSST performance was impaired in a dose-dependent manner: by 9% at 10%, 6% at 20% and by 20% at 40% *(73)*. No significant change was found in auditory reaction time at any concentration.

Even brief exposure to nitrous oxide can impair cognition. In 12 healthy young adults who took four deep inhalations of nitrous oxide (0%, 40%, 60% and 80%), verbal recall and DSST performance were significantly, albeit modestly (10%), impaired only at the highest concentration *(78)*. Performance was normal 3 min after inhalation.

Use of nitrous oxide has been associated with motor vehicle accidents, although the true prevalence is unknown due to lack of onsite tests for the substance. The number of driving incidents involving nitrous oxide in Netherlands (Kingdom of the) increased from 2652 in 2019 to 4860 in 2021 *(5)*. The incidents included both driving while intoxicated and while filling a balloon. Dutch police estimated that nitrous oxide use was a contributing factor in almost 1800 motor vehicle accidents between 2019 and 2021, with 362 injuries and 63 deaths *(79)*. Nitrous oxide (50%

or 70% for 5 min) significantly impaired performance in a driving simulator in 10 healthy young male dental students *(80)*.

Use of nitrous oxide from metal cannisters has occasionally been associated with frostbite injury, usually to the inner thighs (from holding a large tank between the legs), hands or lips and face. Frostbite can result from either direct skin contact with pressurized liquified nitrous oxide released from the container because of its boiling-point of – 55 °C to –88.5 °C or by touching the cold metal, which can be cooled to – 40 °C by release of the gas *(81–83)*.

The number of calls to poison centres related to nitrous oxide have increased substantially during the past decade in several European countries: from 16 (2015) to 73 (2021) in Denmark, 10 (2017) to 134 (2020) in France and 13 (2015) to 98 (2021) in Netherlands (Kingdom of the) *(5)*.

7   Dependence potential

A   *Studies in experimental animals*

Nitrous oxide generates acute and chronic tolerance to many, but not all, of its effects in rodents, including anaesthesia, analgesia, hypolocomotion, hypothermia and electrophysiological responses. Partial acute tolerance to the anaesthetic effect developed in mice after 60 min of continuous exposure *(84)*. Development of tolerance was blocked by concomitant treatment with nitrendipine, a calcium channel blocker. Tolerance to the analgesic effect occurred in rats within 45 min of > 16 h of exposure to 75% nitrous oxide *(78,85)*; however, development of tolerance was blocked by pretreatment with an enkephalinase inhibitor *(86)*, which presumably increased enkephalinergic activity in the brain. No tolerance developed to the anaesthetic effect over 3 h *(86)*, and there was no cross-tolerance with morphine-induced analgesia *(78)*. In mice exposed to 80% nitrous oxide for up to 30 h, naloxone did not elicit signs of withdrawal (as would have been seen in mice tolerant to morphine) *(87)*. In another study in rats, no tolerance to analgesia developed during 2 h of exposure to 75% nitrous oxide *(88)*. Mice became tolerant to analgesia after exposure to 75% nitrous oxide for 16 h *(78)*, to 50% or 70% for one week or to 40% for 3 weeks *(95)*, with partial cross-tolerance to ethanol *(89)* but no cross-tolerance to barbiturates *(90)*. Tolerance resolved within 6 days.

Tolerant mice had normal synaptic membrane fatty acid, phospholipid and cholesterol content *(85)*. Acute tolerance to analgesia occurred in mice within 41 min of continuous exposure *(91)*. Adolescent rats developed significant tolerance to nitrous oxide-induced hypothermia after three 90-min exposures to 60%, while adult rats developed minimal tolerance *(92)*. Mice developed partial tolerance to the hypolocomotor effects of 70% nitrous oxide during 48 h of exposure *(93)*, and rats developed partial tolerance to the suppression of cortical evoked potential amplitude during exposure to 70% nitrous oxide, within 2–10 min for somatosensory evoked potentials *(94)* and within 15 min for visual evoked potentials *(93)*. Exposure to nitrous oxide had no effect on spinal cord evoked potentials *(94)*. Rats developed partial tolerance to the aversive effect of 80% nitrous oxide (conditioned taste aversion) after four 60-min exposures *(95)*.

Rodents exposed to nitrous oxide experienced signs of withdrawal when exposure was ended abruptly. Mice exposed continuously to nitrous oxide (0.9–1.5 atmospheric pressure or 50–80% mixture) for 30–60 min at 34 or 68 h developed handling seizures (tonic–clonic) for 2 min to 6 h after the end of exposure *(84,87,96–99)*. The occurrence of handling seizures was significantly reduced in mice treated concomitantly with nitrendipine *(84)* or treated before nitrous oxide exposure with cholinesterase inhibitors (physostigmine or galantamine) *(98)*.

Withdrawal seizures were suppressed by nitrous oxide or ethanol treatment; however, nitrous oxide suppressed handling seizures that occurred during withdrawal from ethanol *(99)*. Tolerance did not occur to nitrous oxide-induced impairment of performance on a visual vigilance task over 7 days of exposure to 60% concentration for 7 h daily *(51)*.

μ-Opioid receptor ligands influence seizures in mice during withdrawal from nitrous oxide, the effects depending on when they are administered. Pretreatment with naloxone (μ-opioid receptor antagonist) just before exposure to nitrous oxide reduced withdrawal seizures *(98)*, while treatment 5 min before the end of exposure increased the occurrence of seizures *(87)*, and treatment with naloxone or naltrexone (another μ-opioid receptor antagonist) after exposure had no effect *(97,98)*. Conversely, treatment with morphine (μ-opioid receptor agonist) 5 min before the end of exposure decreased the occurrence of seizures *(87)*. Rats exposed for 48 h to 70% nitrous oxide had decreased levels of β-endorphin immunoreactivity in the brainstem and subcortex (but not the cortex) 30 min into withdrawal *(100)*. Brain β-endorphin immunoreactivity was unchanged during withdrawal from acute exposure (25 min) to 70% nitrous oxide or during exposure to 70% nitrous oxide.

B   *Studies in humans*

Tolerance to the analgesic effects of nitrous oxide was observed in several laboratory studies, but no tolerance was seen to its subjective or psychomotor effects. Three of seven healthy adult men exposed continuously to 33% nitrous oxide showed complete tolerance after 1 h *(101)*. In a study with healthy young adults, continuous exposure to 35% nitrous oxide led to about 33% tolerance after 30 min and about 50% tolerance after five 30-min exposures spaced at least one half-day apart *(102)*. In a study of eight healthy young adult men who were exposed continuously to 60–80% nitrous oxide for 3 h, tolerance was observed after 30 min, which was complete by 150 min *(103)*. In a study with 10 healthy young adults who were exposed continuously to 2–40% nitrous oxide for 2 h, partial tolerance developed to analgesia and to rewarding subjective effects (e.g. "like drug effect", "feel elated"); no tolerance developed to other subjective effects (e.g. "feel drug effect", "dizzy", "sedated") or to cognitive or psychomotor impairment *(104)*. In another study by the same research group, 11 healthy young adults who were exposed continuously to 20–40% nitrous oxide for 2 h showed no tolerance to rewarding subjective effects or to cognitive or psychomotor impairment *(105)*.

Among 190 medical students at the University of Paris, France, who met the DSM-5 diagnostic criteria for nitrous oxide use disorder and participated in an anonymous online survey in March–October 2021, 45% reported tolerance *(106)*.

For 525 cases of non-medical nitrous oxide use reported to the French national addictovigiliance system between 2012 and 2021 (mean age, 21.9 years, median 21 years, range 13–53 years; 38.3% women), there was sufficient clinical information to evaluate the individual DSM-5 diagnostic criteria for (nitrous oxide) use disorder in 111 cases. Of these, 27.9% reported tolerance *(107)*.

Among 10 individuals (median age, 23 years, range 18–26 years; 80% women) reported to the Dutch Poisons Information Centre between 16 January 2021 and 15 January 2022 who had nitrous oxide "intoxication" and neuropathy, eight reported tolerance to the effects of nitrous oxide and nine reported withdrawal effects, including insomnia, anxiety, restlessness, sweating or shaking *(108)*. Eight participants used nitrous oxide at least weekly; nine were "heavy" users (more than 50 balloons at a session).

Of 73 published cases of non-medical nitrous oxide use that resulted in "major side effects", identified in a recent systematic electronic literature search (in English and French), only one report in each language provided sufficient clinical information to evaluate tolerance to nitrous

oxide or withdrawal effects *(109)*. The case did meet DSM-5 diagnostic criteria for tolerance, but the other case did not meet the criteria for withdrawal effects.

8 Abuse potential

A Studies in experimental animals

In the four widely used animal models of substance abuse liability, nitrous oxide had clear rewarding properties in two (self-administration, drug discrimination) and mixed results in two (ICSS, conditioned place preference) *(26)*, although the evidence is sparse. Of four rats offered 60% nitrous oxide, two self-administered it, one avoided it, and one showed no preference *(110)*. Squirrel monkeys readily learnt to self-administer 60% nitrous oxide in 15-s bursts, even when 20 lever presses were required to receive one dose *(111)*. Mice discriminated between 60% nitrous oxide and pure oxygen, suggesting that nitrous oxide has distinctive stimulus effects *(112)*. Of other inhalants, toluene almost fully substituted for nitrous oxide in discrimination tests from pure oxygen, while 1,1,1-trichloroethane and ethanol only partially substituted and 2-butanol did not substitute.

Nitrous oxide alters the response rate in mice trained to self-administer ICSS, but with no clear dose–response pattern: 40% increased responding (at two of the electrical frequencies used), while 80% decreased responding (at a broader range of frequencies), 20% showed no effect, and 60% decreased responding only at the highest frequency *(113)*. Nitrous oxide at 40% and 60% reduced the effort (lever presses) mice made before stopping self-administration of ICSS. In comparison, cocaine (at 3, 10, 18 mg/kg intraperitoneally) increased the response at all doses and frequencies used and increased the effort made before stopping ICSS self-administration. Diazepam and toluene facilitated the ICSS response more strongly than nitrous oxide, but only at intermediate doses; higher doses suppressed the response.

Nitrous oxide generates a dose-dependent conditioned place preference in rats but not in mice. In rats, 8% nitrous oxide induced conditioned place preference, 15% had no effect, and 30% and 60% induced conditioned place avoidance, suggesting an aversive effect *(110)*. Nitrous oxide at 70% and 80% induced a conditioned taste aversion in rats, also suggesting an aversive effect *(95,114)*. In mice, 50% nitrous oxide for 20 min did not generate a conditioned place preference and it blocked development of morphine- or cocaine-elicited conditioned place preference *(115,116)*.

B Studies in humans

The abuse potential of nitrous oxide was noted immediately after its synthesis by Joseph Priestly in 1772. Humphrey Davy reported that inhalation of nitrous oxide produced a pleasurable euphoria and giddiness, which he likened to alcohol intoxication, with pleasant thrilling sensations in the body and auditory and visual distortions *(117)*. He promoted recreational use of nitrous oxide, including at "laughing gas" parties, which became popular in England.

Modern laboratory studies with healthy young adults have confirmed that inhaled nitrous oxide produces robust, dose-dependent, pleasurable, rewarding subjective effects at sub-anaesthetic concentrations (10–50%) but does not consistently generate preference to oxygen in discrete choice procedures.

Inhaling nitrous oxide at 10–50% for 2–30 min under blinded conditions (when participants did not know whether they were inhaling nitrous oxide or oxygen) generated a dose-dependent pleasurable subjective experience commonly described by participants as having one of three

main characteristics: dreamy, detached reverie (e.g. "floating", "coasting", "spaced out"); a happy, euphoric mood (e.g. "happy", "high", "elated", "stimulated") or psychedelic-like (e.g. "pleasant bodily sensations", changed body awareness and image, altered time perception, dissociative state) *(70,118–120)*. The subjective effects resolved substantially within 15 min of the end of exposure *(120)* and resolved completely within 1 h *(74)*. Adults who took a single deep inhalation of 100% nitrous oxide *(117,121)* or four consecutive deep inhalations of 40%, 60% or 80% nitrous oxide *(122)* experienced positive subjective effects within 15–30 s, peaking at 2–3 min and subsiding within 15–20 min. Subjective effects were rated by participants as more similar to those of ketamine and alcohol than to those of cannabis or cocaine *(70,120)*. In comparison with equipotent (strength of drug effect, psychomotor impairment) concentrations of other inhalational anaesthetics (e.g. isoflurane, sevoflurane), nitrous oxide (15% or 30% for 40 min) produced less sedation and more pleasant psychedelic-like subjective effects *(75)*.

Substantial individual differences have been found in the quality and intensity of the subjective effects of nitrous oxide *(119)*. Among 12 participants exposed to 30% or 40% nitrous oxide for 30 min, one "liked" the 30% concentration, while seven were neutral and four "disliked" it *(67)*. Four "liked" the 40% concentration, while three were neutral and five "disliked" it. Among 12 participants exposed to 30% nitrous oxide for 10 min in nine sessions daily on 5 days, the mean within-participant rating on a 100-mm visual analogue scale ranged from 1–84 mm for "high" *(123)*. Substantial inter-participant variation was also observed for self-ratings of "dreamy", "coasting" and "having pleasant thoughts". The ratings for placebo were consistently low (< 10 mm).

Considerable inter-individual variation was also seen among among healthy young adults in their preference for inhaling nitrous oxide rather than placebo (100% oxygen or room air), even when they reported positive subjective effects. In these studies, participants first sampled the two gases double-blinded and then chose which gas to inhale. Among 19 participants given the choice between 10–40% nitrous oxide for 17 min and 100% oxygen, 84% preferred nitrous oxide at 10%, 47% at 20%, 42% at 30% and 40% at 40% *(124)*. Among 12 participants given two or three single-blind discrete choices between 30% or 40% and placebo (100% oxygen), 41.6% chose nitrous oxide at 30% and 20% chose it at 40% *(67)*. A positive association was found between choice of nitrous oxide and degree of positive subjective effects. Among 16 participants given 18 discrete choices between 30% nitrous oxide for 5 min and 100% oxygen, seven chosen nitrous oxide at least two thirds of the time, three chose oxygen at least two thirds of the time, and six were neutral *(125)*. A significant positive association was found between choice of nitrous oxide and "liking" a drug (Spearman $r = 0.42$) or wanting to take the drug again ($r = 0.27$). Among 12 participants each given 45 discrete single-blind free choices (nine daily on 5 days) between 30% nitrous oxide and 100% oxygen or open-label "drug-free air" (100% oxygen) each for 10 min, 41% of the choices were for nitrous oxygen, 11% for placebo and 48% for "drug-free air" *(123)*. The choice of nitrous oxide was relatively consistent among participants during the 45 sessions. Five participants chose nitrous oxide more than two thirds of the time, five chose nitrous oxide less than 10% of the time, and two were neutral (40% and 51% of the time). The choice of nitrous oxide was significantly associated with several subjective effects, including "drunk" (Pearson $r = 0.60$), "dreamy" ($r = 062$) and "floating" ($r = 0.59$).

In a separate group of 20 participants given the same choice with four nitrous oxide concentrations, 25% preferred (chose more than half the time) placebo, 45% preferred 10% nitrous oxide, 65% preferred 20% nitrous oxide, and 65% preferred 30% nitrous oxide *(126)*. At all doses (including placebo), at least one participant never chose nitrous oxide and at least one always chose nitrous oxide. Significant associations were found between choosing nitrous oxide and subjective effects, although these did not occur at all nitrous oxide concentrations or

at all times. The choice of nitrous oxide was significantly associated with "liking" at the end of inhalation of all concentrations (Pearson r = +0.44 at 10%, +0.68 at 20%, +0.46 at 30% and +0.59 at 40%) and with "inhale again" at 20% (r = +0.71), 30% (+0.48) and 40% (+0.60). Among 14 participants each given 27 single-blind opportunities to choose among nitrous oxide (10%, 30% or 50%), placebo (100% oxygen) or neither, the cumulative choices were 27% for nitrous oxide, 16% for placebo and 57% for neither *(127)*. Eight participants showed a significant preference for nitrous oxide, but the preference was not always monotonically dependent on dose. Three participants showed an increasing preference with increasing dose, three showed decreased preference at the highest dose, and 2 showed no change with increasing dose.

Little is known about the reasons for individual differences in response to nitrous oxide. In a study of 80 psychologically healthy young adults exposed to 50% nitrous oxide for 30 min, participants with high trait impulsivity (assessed on an eight-item version of the Barratt Impulsiveness Scale-11) had a significantly greater liking for nitrous oxide than those with low impulsivity *(120)*. Depressed mood during the previous week was inconsistently associated with the nitrous oxide response, but a history of bipolar traits was not *(120)*. Gender did not appear to influence the abuse potential of nitrous oxide significantly. When 38 women and 72 men were exposed to 30% nitrous oxide for 15 min, no significant difference was seen between the two groups in the quality or intensity of positive subjective effects (e.g. "elated", "high", "pleasant thoughts") *(128)*.

Personal or family use of other psychoactive substances, such as alcohol or cannabis, influenced the positive subjective and rewarding effects of nitrous oxide in human laboratory studies. Among 32 participants exposed to 20%, 30% and 40% nitrous oxide for 10 min, only the 16 who were considered moderate drinkers (at least seven standard drinks weekly and drinking on at least 4 days per week) experienced positive subjective effects (e.g. "drug liking", "inhale again"), while light drinkers (no more than four drinks per month) did not *(129)*. Participants who were moderate drinkers were also more likely to choose nitrous oxide over placebo (100% oxygen), while light drinkers made equal choices. Among 19 healthy young adults exposed to 0–40% nitrous oxide for 30 min, those who drank alcohol moderately (mean [standard deviation] 11.4 [4.7] drinks per week; 40% were current cannabis smokers) chose nitrous oxide over placebo significantly more often than those who drank alcohol lightly (0.8 [0.2]; no current cannabis smokers) *(124)*. Th groups did not differ significantly in positive subjective effects or psychomotor impairment. Among 60 healthy adults who drank heavily (112 and 168 g/week for women and men, respectively) but did not have alcohol use disorder, those with a family history of alcohol use disorder experienced significant "stimulation" from exposure to 50% nitrous oxide for 30 min, while those without such a history did not *(130)*.

Among 18 healthy young adults exposed to 40% nitrous oxide or placebo for 30 min, those who used cannabis (mean [SD] 1.28 [1.02] joints per week), two thirds of whom also used hallucinogens), experienced stronger positive subjective effects than non-users of cannabis (none of whom used hallucinogens), but there was no difference in psychomotor impairment *(131)*. Of 24 healthy adults exposed to a single inhalation of 100% nitrous oxide, those who used cannabis and/or hallucinogens experienced less psychomotor impairment and were less likely to have an adverse experience than those who did not (8% and 58%, respectively) *(117)*. Users of cannabis and/or hallucinogens compared intoxication with nitrous oxide to a psychedelic experience, while those who did use these substances compared it to alcohol intoxication. Among 60 healthy young men exposed to 40% nitrous oxide for 20 min, those who had used cannabis in the previous 12 months experienced more positive mood and less anxiety or depression than those who had not *(132)*. Among 80 healthy young adults exposed to 40% nitrous oxide for 20 min, those who were experienced users of cannabis (at least 100 times; most had also used psychedelics) compared the experience to cannabis or LSD

intoxication; those who had used cannabis no more than 10 times (and never used psychedelics) compared the experience to alcohol intoxication *(133)*. These findings suggest that use of cannabis or psychedelics enhances the abuse potential of nitrous oxide. Thus, human laboratory studies in which only participants who have not used other substances are enrolled are likely to result in underestimates of the abuse potential of nitrous oxide.

Nitrous oxide is more rewarding than the potent inhalational anaesthetic sevoflurane. Among 14 participants given a choice between placebo (100% oxygen) and either 30% nitrous oxide or 0.2%, 0.4% or 0.6% sevoflurane, 71% chose nitrous oxide over placebo, while 50%, 57% and 50% chose the sevoflurane concentration over placebo, respectively *(134)*.

9   Therapeutic applications and extent of therapeutic use and epidemiology of medical use

The first medical use of nitrous oxide was as a dental anesthetic, in 1844 *(25)*. Nitrous oxide is now used as a supplement in inhalational anaesthesia (as it is not potent enough to be used alone) and for analgesia and sedation during childbirth and painful short procedures in dentistry, paediatrics and emergency medicine *(135,136)*. Use of nitrous oxide for inhalational analgesia appears to have decreased during the past few decades, in part because of concern about adverse effects (e.g. nausea and vomiting) after exposure for more than 1 h *(137)* and environmental damage due to its greenhouse gas properties *(136)*. The advantages of nitrous oxide over other inhalational anaesthetics include its analgesic and sedative effects (also present at subanaesthetic doses), its very rapid onset (within seconds) and the offset of effects (due to the very low blood:gas partition coefficient of 0.47), which allows more rapid induction and emergence from effects; it also has no adverse haemodynamic or pulmonary effects *(31,136)*. The advantages of nitrous oxide over other analgesics (e.g. opioids) and sedatives (e.g. benzodiazepines) include all of the above and also the fact that it is eliminated unchanged in exhaled breath, such that its pharmacokinetics is not affected by liver or kidney function. Furthermore, it has apparently less abuse potential than opioids and can be administered by inhalation, which allows intravenous catheter placement or avoids the need for such placement, both of which are advantages for infants and children *(138)*, and allows patient self-administration (self-titration). The advantages of its use during childbirth include lack of interference with labour and no significant retention by the neonate, as it is rapidly exhaled, or during breastfeeding *(139)*.

The number of patients who receive nitrous oxide for medical purposes is unknown. In an online survey conducted in November 2019–March 2020 of 171 paediatric emergency departments in 17 European countries plus Israel and Türkiye, which serve about 5 million children annually, 54% used nitrous oxide for sedation and/or analgesia *(140)*. It was estimated recently that more than 500 hospitals and birthing centres in the USA were using nitrous oxide for analgesia during labour in 2018 *(141)*.

Nitrous oxide has been proposed as a treatment for depression (especially treatment-resistant depression) on the basis of a limited number of favourable randomized, double-blind, placebo-controlled clinical trials, although it is not approved for this condition by any national regulatory authority. The interest is due partly to the rapid onset of anti-depressant effects seen in some studies (within 24 h, similar to the action of ketamine), whereas weeks are required with conventional antidepressants. At least eight clinical trials of treatment-resistant and other forms of depression are currently under way *(142)*. Three controlled clinical trials involving a total of 88 adult patients with major treatment-resistant depression found that a single treatment with nitrous oxide (50% for 1 h) significantly reduced depressive symptoms as compared with placebo (oxygen only) *(143–145)*. Significant anti-depressant effects appeared after 2 and 24 h in two studies (but not

after 1 and 2 weeks in the study in which those periods were evaluated) and after 1 and 2 weeks (but not after 2 or 24 h) in a third study *(144)*. Cognitive performance was improved throughout the 2 weeks of the study in which anti-depressive effects lasted only 24 h *(146)*. The anti-depressant effect was accompanied by increased brain cortical connectivity assessed by electroencephalogram *(147)*. A fourth controlled clinical trial involving 23 adults with major depression (not necessarily treatment-resistant) who remained on their prescribed anti-depressant medication found that nitrous oxide at 50% for 1 h twice weekly for 4 weeks significantly reduced depressive symptoms by 4 weeks *(148)*. Of the patients who received nitrous oxide, 91.7% showed a clinically significant response (at least 50% reduction in their score on the Hamilton Depression Rating Scale), and 75% achieved remission, as compared with 44.4% and 11.1%, respectively, in the placebo group. A controlled clinical trial involving 25 adults with bipolar disorder and current treatment-resistant depression found that nitrous oxide (25% for 20 min) resulted in a significantly larger proportion of patients with a clinically significant anti-depressant response (at least 50% reduction in scores on the Montgomery-Asberg Depression Rating Scale [MADRS]) 2 h after treatment (92% vs 38%) as compared with a group who received active placebo (medical air + 2 mg midazolam intravenously), but there was no significant difference 4 or 24 h after treatment *(149)*. There was no significant difference in the mean MADRS scores of the treatment groups at any time. The nitrous oxide-induced reduction in MADRS score at 24 h was significantly associated with a lower baseline cerebral blood flow (assessed by magnetic resonance imaging arterial spin labelling) in the frontal, ventral prefrontal and anterior cingulate cortical regions.

A systematic literature review conducted in 2005 identified five controlled clinical trials conducted by the same research group in South Africa, involving 212 participants, to evaluate the influence of nitrous oxide on acute alcohol withdrawal *(150)*. All five studies found that nitrous oxide (titrated to achieve mild sedation, "psychotropic analgesic nitrous oxide") was non-inferior to standard dosing with oral benzodiazepines in reducing the signs and symptoms of acute alcohol withdrawal. A controlled clinical trial conducted in Finland of 105 adults admitted for inpatient alcohol detoxification (not included in the systematic review) found that nitrous oxide (30–70% titrated to achieve an end-tidal concentration of 30%, duration not reported) had no significant effect on the signs and symptoms of acute alcohol withdrawal or dosing with benzodiazepines during 42 h after treatment *(151)*.

10   Listing on the WHO Model List of Essential Medicines

Nitrous oxide is listed on the 23rd WHO Model List of Essential Medicines *(152)* and on the 9th WHO Model List of Essential Medicines for Children as an inhalational anaesthetic *(153)*.

11   Marketing authorizations (as a medicinal product)

Nitrous oxide is approved by national regulatory authorities as a medical gas in the European Union, North America and many other countries. It is marketed as an equimolar mixture (50%/50%) of oxygen and nitrous oxide in France (known as EMONO) and the United Kingdom (Entonox). In other countries, nitrous oxide is marketed as the pure gas and mixed with the appropriate amount of oxygen at the site of clinical administration.

12   Industrial use

Nitrous oxide is widely used commercially in the food and beverage, electronics and motor fuel industries *(25,154)*. Nitrous oxide is used as a mixing, aerating and foaming agent in food and

beverage preparation and as a propellent for dispensing whipped cream and other toppings. It is preferred to other inert gases (e.g. nitrogen, carbon dioxide) for such culinary uses because it is tasteless, colourless, non-irritating, does not promote oxidation or bacterial growth, and is highly fat soluble *(155–157)*. It is approved for use as a food additive by most national regulatory authorities. Nitrous oxide is used as an oxidizing agent to increase the efficiency of fuels for racing cars and some rocket engines. It is preferred to other oxidizing agents because it is nonflammable and condenses under relatively low pressure at room temperature, making it easier to handle *(158)*. Nitrous oxide is used in the manufacture of semi-conductor chips *(135)*.

13   Non-medical use, abuse and dependence

The true prevalence of non-medical use of nitrous oxide or of nitrous oxide use disorder is unknown. Data on the use of nitrous oxide are not included in the United Nations Office on Drugs and Crime annual World Drug Report. As nitrous oxide is not a novel psychoactive substance, events are not reported to its ToxPortal, nor is its use monitored systematically in Europe in the European Monitoring Centre on Drugs and Drug Addiction Early Warning System. Few population-based surveys of psychoactive substance use include nitrous oxide, and those that do group it with other inhalants (e.g. alkyl nitrites, volatile organic compounds) *(159)*. Surveys on non-medical use of nitrous oxide do not include data on use disorder.

The global prevalence of non-medical use of nitrous oxide appears to be low but may be increasing in some countries and population groups (e.g. in Lithuania, Netherlands (Kingdom of the), see Table 1). The lifetime prevalence of use was 0.6–7.6% in four countries that have conducted nationally representative, population-based cross-sectional surveys (Lithuania, Netherlands (Kingdom of the), New Zealand, USA) (Table 2). The median age at first use was 19 years in New Zealand (2007–2008) *(160)*, and the prevalence in the past year was 0.8–3.2% in England and Wales, Netherlands (Kingdom of the) and New Zealand; the prevalence in the past month was 1.1% in Netherlands (Kingdom of the). The lifetime prevalence of use in the USA has been relatively stable in the past decade, ranging from 4.4% to 4.8% (see Table 1). The prevalence in Netherlands (Kingdom of the) was 3.2% in 2019 and decreased to 1.6% in 2021 (see Table 1).

Table 2. Prevalence of non-medical use of nitrous oxide in population-based surveys

Country or region	Year	Age range (years)	Population	Prevalence (%)		
				Lifetime	Past year	Past month
Denmark	2019	15–18	High-school students	15	7	
Denmark	2019	19–25	Vocational school students	12	6	
England and Wales	2022	16–59	National		1.3	
England and Wales	2020	16–59	National		2.4	
England and Wales	2013–2014	16–59	National		2	
England and Wales	2022	16–24	National		3.9	

Country	Year	Age	Population			
England and Wales	2020	16–24	National		8.7	
England and Wales	2013–2014	16–24	National		8	
England	2021	11–15	Students	3.0	1.6	0.9
Lithuania	2021		National	0.6		
Lithuania	2016		National	0.2		
Netherlands (Kingdom of the)	2021	≥ 18	National		1.6	
Netherlands (Kingdom of the)	2020	≥ 18	National	7.4	2.1	
Netherlands (Kingdom of the)	2019	≥ 18	National	7.6	3.2	1.1
Netherlands (Kingdom of the)	2018	≥ 18	National	6.9	2.7	
Netherlands (Kingdom of the)	2020	20–24			12.1	
Netherlands (Kingdom of the)	2018	20–24	National		14.6	
Netherlands (Kingdom of the)	2017	20–24	National		9.4	
Netherlands (Kingdom of the)	2015	20–24	National		7.8	
New Zealand	2007–2008	16–65	National	4.6	0.8	
USA	2021	≥ 12	National	4,4		
USA	2020	≥ 12	National	4.7		
USA	2019	≥ 12	National	4.6		
USA	2018	≥ 12	National	4.6		
USA	2017	≥ 12	National	4.7		
USA	2014	≥ 12	National	4.8		
USA	2013	≥ 12	National	4.7		

Sources: European Monitoring Centre for Drugs and Drug Addiction *(5)*; Office for National Statistics, United Kingdom *(161)*; van Amsterdam et al. *(162)*; van Laar et al. *(163,164)*; New Zealand Alcohol and Drug Use Survey *(160)*; Center for Behavioral Health Statistics and Quality *(165–168)*.

The prevalence of non-medical use of nitrous oxide was higher in men than in women in all three countries in which this variable was reported: 6.0% vs 3.3%, respectively, for lifetime use and 1.2% vs 0.6%, respectively, for use in the past year (2007–2008) in New Zealand *(160)*; 2.3% vs 1.9%, respectively, for use in the past year (2020) in Netherlands (Kingdom of the) *(164)* and 17% vs 10%, respectively, among high-school students (15–18 years) and 17% vs 8%, respectively, among vocational-school students (19–25 years) for lifetime use in Denmark *(158)*.

The prevalence of non-medical use of nitrous oxide varies by age. In most countries, the prevalence is greatest among adolescents and young adults *(160,164)*. For example, in Netherlands (Kingdom

of the), the highest prevalence in the past year (2020) was among those aged 18–19 years (14.5%), followed by those aged 20–24 years (12.1%), 25–29 years (4.0%), 30–39 years (1.8%), 40–49 years (0.3%), 50–64 years (0.2%) and ≥ 65 years (0%) *(174)*. The USA is an exception, as the highest lifetime prevalence (2021) was among people aged ≥ 26 years (5.1%) *(168)*.

Cross-sectional, online, anonymous international surveys of convenience samples of self-selected individuals who use psychoactive substances (global drug surveys) found a substantially higher prevalence of non-medical use of nitrous oxide than in population-based studies, presumably because of the bias inherent in their sampling method. In these biased samples, the prevalence of nitrous oxide use was also highest among adolescents and young adults. The 2020–2021 Global Drug Survey (with more than 147 000 respondents in 35 countries) found that the prevalence of use of nitrous oxide in the past year was 21% *(169)*, as compared with 7.2% in the 2014–2016 global drug surveys (241 566 unique respondents) *(8)*. The highest prevalence in both studies was among adolescents and young adults. A majority (57%) of nitrous oxide users reported "clubbing" at least weekly, and 18% reported clubbing at least five times a week. In the 2021 Global Drug Survey, 22.5% of respondents reported lifetime nitrous oxide use, which is similar to the 23.6% reported lifetime use in 2019 *(167)*. The majority (89.2%) of participants in these surveys also used other psychoactive substances. The prevalence of nitrous oxide use in the past year was higher among men than women (7.2% vs 4.9%, respectively) and among "clubbers" than non-"clubbers" (9.4% vs 3.0%, respectively). Of the six countries with the highest number of respondents, New Zealand, the United Kingdom and the USA had the highest prevalence of lifetime or past-year nitrous oxide use: 26.6% (25.5 ; 27.8), 38.6% (95% CI, 37.5 ; 39.7)/20.5% (19.6 ; 21.5) and 29.4% (28.3 ; 30.5)/8.2% (7.6/8.9), respectively. Australia, the United Kingdom and the USA had the highest prevalence of past-month nitrous oxide use: 2.0% (1.0 ; 2.4), 7.7% (7.1 ; 8.3) and 2.9% (2.5 ; 3.3), respectively. Germany and Switzerland had the lowest prevalence of lifetime or past-year/past-month nitrous oxide use: 11.2% (10.8 ; 11.6)/1.5% (3.3 ; 3.7)/0.9% (0.8 ; 1.0) and 13.4% (12.5 ; 14.4)/3.6% (3.1 ; 4.1)/1.0% (0.7 ; 1.3), respectively.

An anonymous online survey (on a website targeted at adolescents and young adults) of a self-selected convenience sample of 6070 Dutch residents conducted in May–October 2020 found that 40.7% had used nitrous oxide before onset of the COVID-19 pandemic, which decreased to 20.7% during the pandemic (when the Netherlands [Kingdom of the] was in lockdown) *(170)*. Among those who used nitrous oxide during the pandemic, 22.6% decreased their use from the pre-pandemic levels, 28.0% did not change their use, and 36.0% increased their use.

The prevalence of non-medical use of nitrous oxide is relatively high in some population subgroups, including health profession students (perhaps because of easier access), high school (secondary) and university students, attendees at dance clubs and music festivals and adolescents in psychiatric treatment *(162)*. A questionnaire administered in class to 1360 (61.2% of eligible students) first-year engineering, law and health science students at the University of Auckland, New Zealand, in 2002 indicated a 11.1% lifetime prevalence of "recreational" nitrous oxide use (56% men; median age, 20 years, range 17–48 years) *(30)*. Most (78%) used at least one other recreational drug, and 23% used another inhalational drug. An anonymous survey in 1976–1978 of a convenience sample of 351 medical students and 273 dental students at a university in the USA found that 10.3% and 17.9%, respectively, had ever used nitrous oxide in a social setting for recreational purposes *(171)*. In an anonymous online survey of a self-selected sample of 981 medical students at the University of Paris, France (29% of all eligible students), conducted in March–October 2021, 80.3% reported lifetime nitrous oxide use *(106)*. Users of nitrous oxide were significantly more likely than nonusers to be men (odds ratio [OR] = 1.63, P = 0.006), have an alcohol use disorder (1.83, P = 0.017) and be younger (0.90, P = 0.0005). Use of other psychoactive substances was significantly more common among nitrous oxide users than among nonusers: 96.6% (vs 58%) used alcohol, 62.8% (vs 2.6%) used alkyl nitrites ("poppers"), 47% (vs 9.3%) used cannabis, 43.9% (vs 9.3%) used tobacco, 11.8%

(vs 1%) used MDMA and 5.6% (vs 0%) used cocaine. An anonymous online survey in 2021 of a convenience sample of 593 health profession students (medicine, dentistry, midwifery, pharmacy) at a French university found a 76.6% lifetime prevalence of non-medical use of nitrous oxide, ranging from 66.5% among pharmacy students to 80.0% among medical students *(172)*. Only 2.6% had "given up" nitrous oxide use at the time of the survey. Nitrous oxide use was slightly more prevalent among men than among women (81.7% vs 74.2%,). In an anonymous, online, cross-sectional survey of a self-selected convenience sample of 10 066 French university students aged at least 18 years conducted in 2015–2017, the reported prevalence of nitrous oxide use was 26% for lifetime and 12% for past-year use *(173)*. Half (50.6%) of the past-year nitrous oxide users reported using only nitrous oxide; 23.7% also used cannabis, 20.2% also used MDMA, and 11.8% also used cocaine. A survey of a self-selected convenience sample of 140 young adults (18–25 years, 67% women, 95% university students) in southwest London, United Kingdom, in 2017 found that 77% had heard of nitrous oxide ("hippy crack") and 28% were past-year users *(174)*. The prevalence of use was not significantly associated with age or gender. More than three quarters (83%) of users had used nitrous oxide no more than 10 times in the past year, 31% had used it only once and 10% more than 20 times. An anonymous online survey of 555 Dutch secondary school students (14–18 years, 47% girls) indicated a 13.6% prevalence of lifetime use of nitrous oxide *(175)*. More than one third had used it only once (40.7%) or two or three times (34.9%), 16.3% had used it 4–10 times and 8.1% had used it more than 10 times. Most users (52.3%) reported that they would "definitely" use nitrous oxide again; only 7.0% said they would "never" use nitrous oxide again. An in-person interview survey in 2004 of 723 adolescents (97.7% of all those in treatment) in residential psychiatric treatment in Missouri, USA (mean age, 15.5 years, 87.0% male) found a 15.8% prevalence of lifetime nitrous oxide use and a 12.2% prevalence of past-year use *(176)*. Of the nitrous oxide users, 80.1% also had a history of lifetime inhalation of volatile organic solvents. Most (77.7%) of those who had used both nitrous oxide and volatile organic solvents had a psychiatric diagnosis, while 36% of those who used only nitrous oxide had such a diagnosis. Among 106 adolescents and young adults (mean [standard deviation] age 17.25 [1.33] years, 19.8% > 18 years, 71.7% men) court-ordered into outpatient treatment for "illicit drug use" in Taiwan (China) between September 2016 and September 2021, 22.6% had used nitrous oxide as their main drug *(177)*.

For the Australian Ecstasy and Related Drug Reporting System, interviews are conducted annually with a convenience sample of people in each state capital city who are at least 16 years of age, resident in the city for at least 12 months and had used stimulants, hallucinogens or novel psychoactive substances at least six times in the past 6 months *(178)*. Respondents are recruited by advertisements and peer referral. Among respondents interviewed in Sydney, New South Wales (about 100 annually), the prevalence of use of nitrous oxide in the past 6 months was relatively stable (8–12%) in 2003–2011, increased to 20% in 2012–2013 and steadily increased to 55% in 2016–2017, 75% in 2018, 72% in 2019 and 67% in 2020. Among the 69 respondents in 2020 who answered a question about changes in their use of nitrous oxide in the past 6 months, 25% reported decreased use, 15% increased use, 10% stopped use, and the remainder had not changed their use. A review of the Rapid Emergency Department Data for Surveillance for New South Wales, Australia, for 2012–2018 identified 118 patients for whom nitrous oxide use was the presenting problem or mentioned in their diagnosis, of whom 24% were chronic or heavy users *(179)*. More than half (56%) were men, 83% were aged 16–30 years, and 46% were "polydrug" users. The number of patients who used nitrous oxide increased gradually from 2 in 2012 to 10 in 2015–2016 and then dramatically to 61 in 2018.

A systematic review of four electronic databases conducted in January 2022 identified 91 case reports and 24 case series comprising 355 people with chronic nitrous oxide "misuse" or "abuse", of whom 55.5% were men with a mean age of 24 years (range, 14–60 years) *(180)*. The duration of

nitrous oxide use ranged from 1 month to 10 years, but most had used it for at least 1 year. All the patients used nitrous oxide at least weekly, using 15–900 bulbs per session. Most of the patients came to clinical attention because of medical problems (most commonly sensory changes, weakness or autonomic dysfunction) rather than psychiatric problems.

The prevalence of nitrous oxide use disorder, of heavy nitrous oxide use (suggesting use disorder) and the proportion of nitrous oxide users who develop a use disorder is unknown. This information has not been collected in large-scale, unbiased population-based surveys. Surveys of convenience samples suggest that only a minority of nitrous oxide users develop a use disorder, while a high proportion of cases that come to medical attention or are reported to monitoring systems have a use disorder.

Among 525 cases of non-medical nitrous oxide use reported to the French national addictovigilance system between 2012 and 2021 (mean age, 21.9 years; median, 21 years; range, 13–53 years; 38.3% women), 64.6% met the diagnostic criteria for nitrous oxide use disorder, and another 17.9% were considered "heavy" users (daily use or at least 20 cartridges per use occasion) *(107)*. The mean duration of nitrous oxide use was 16.5 months. About one quarter of cases also used alcohol (23.4%) or cannabis (23.8%). Only a few cases were reported annually. The number was stable in 2012—2018 but then increased from 40 in 2019 to 344 in 2021. The number of cases with use disorder more than tripled between 2020 (88) and 2021 (302), while the number of cases of "heavy" use only remained stable (37 and 42). For 111 cases, sufficient clinical information was available to evaluate the individual DSM-5 diagnostic criteria for (nitrous oxide) use disorder. Of these cases, 60.4% took nitrous oxide in larger amounts or for longer periods than initially intended, 40.1% had spent a great deal of time in obtaining nitrous oxide or recovering from its use, 35.1% continued use despite persistent health problems due to nitrous oxide, 30.1% had a persistent desire or had unsuccessfully attempted to reduce or stop their use, 27.9% showed tolerance, 23.4% had social, financial or vocational problems due to use, 15.3% had cravings to use, 11.7% had withdrawal symptoms, and 2.7% engaged in risky behavior while using nitrous oxide.

Among 788 medical students at the University of Paris, France, with self-identified lifetime nitrous oxide use who participated in an anonymous online survey in March–October 2021, 24.1% met the DSM-5 diagnostic criteria for nitrous oxide use disorder: 79% of cases were mild (meeting 2–3 of 11 possible criteria), 17% moderate (4–5 criteria) and 4% severe (≥ 6 criteria) *(106)*. The most common criteria were a persistent desire or unsuccessful attempts to reduce or stop use (46%), tolerance (45%), continued use despite persistent health problems due to nitrous oxide (44%), and use in larger amounts or for longer periods than intended (18%). Nitrous oxide users with a use disorder were significantly more likely than users without a use disorder to be men (36.8% vs 27.7%), to use nitrous oxide at least monthly (33.1% vs 12.9%) and to use it more than once a week (3.6% vs 0.8%). Use of other psychoactive substances was common in users with and without a use disorder, except that use of alkyl nitrites ("poppers") was more common in those with a use disorder (72.5% vs 59.7%).

In a systematic review of 73 published cases of non-medical nitrous oxide use that resulted in "major side effects", 62 provided sufficient clinical information to evaluate at least one of the DSM-5 diagnostic criteria for nitrous oxide use disorder *(109)*. Of the 73 cases, 55 (88.7%) met the criteria for taking nitrous oxide in larger amounts or over a longer period than intended and for spending time in obtaining nitrous oxide, using it or recovering from its effects (in all the cases for which these two criteria could be evaluated), qualifying as having mild use disorder. Eight cases met the criteria for use resulting in failure to fulfill major role obligations, interfering with important activities or continued use despite persistent social or interpersonal problems due to its use (88.9% of the cases for which these three criteria could be evaluated). Six cases met the criteria for

persistent use when it was physically hazardous or despite physical or psychological problems due to use, representing 66.7% of all the cases for which these two criteria could be evaluated.

In a retrospective case series of seven adults (21–33 years, four women) who sought treatment for "severe nitrous oxide use disorder" at a psychiatric centre in Taiwan (China) between 2017 and 2018. the duration of nitrous oxide use varied from 4 months to 20 years; five used nitrous oxide daily, one two or three times weekly and one weekly *(181)*. All had used other psychoactive substances before initiating nitrous oxide use; and all but one had a comorbid psychiatric disorder (five major depressive disorder, one bipolar disorder).

Little is known about the time course of nitrous oxide use disorder. Only two published studies were found. A 5-month follow-up of 430 patients (57% men, mean age 23.9 years, mean [SD] 2.2 [0.6] years of nitrous oxide use, mean daily dose 1800 [450] mL) who received 1 month of inpatient treatment (psychological therapy and vitamin B12) for nitrous oxide use disorder (diagnostic criteria not reported) at the Gaoxin Hospital in Beijing, China, found a non-linear relapse rate: 15.8% at 1 month, 38.6% at 2 months, 47% at 3 months, 51.2% at 5 months and 55.1% at 6 months *(182)*. Half the patients had relapsed by 108 days. Depressed or anxious mood at hospital discharge was associated with an increased risk of relapse.

A prospective, 3-month longitudinal study was conducted to follow 10 individuals (median age, 23 years, range 18–26 years; 80% women) who were reported to the Dutch Poisons Information Centre between 16 January 2021 and 15 January 2022 with nitrous oxide "intoxication" and neuropathy *(108)*. At baseline, all 10 participants met the DSM-5 diagnostic criteria for (nitrous oxide) use disorder: one mild, one moderate and eight severe. Eight participants used nitrous oxide at least weekly, and nine were "heavy" users (> 50 balloons at a session). At 1-month follow-up, six of the remaining seven participants still met the criteria for severe nitrous oxide use disorder, as did the one participant at the 3-month follow-up. The external validity of this study is low because of the high drop-out rate (90%). In addition, the 10 participants were drawn from a pool of 75 individuals who met the eligibility criteria but 65 of whom refused to participate.

14 Nature and magnitude of public health problems related to misuse, abuse and dependence

Deaths directly related to non-medical use of nitrous oxide appear to be rare. Coroners' inquests were conducted in England and Wales for 62 deaths associated with nitrous oxide use (both intentional and unintentional) between 2001 and 2021 *(183)*, for an average of three annually. The prevalence of nitrous oxide-associated deaths tripled, from 1.6 annually during the first decade to 4.5 annually during the last decade. A review of the Australian National Coronial Information System for 2000–2021 identified 20 fatalities related to nitrous oxide misuse, representing 12.2% of all fatalities related to inhalant misuse *(184)*. Nitrous oxide was detected in the blood in all five cases in which it was tested. More than 75% of all inhalant-related fatalities were considered to be unintended, about 10% were due to intentional self-harm and about 5% to unintended traumatic injury. A review of autopsies of cases of suicide in 2003–2017 in the files of Forensic Science South Australia identified only two cases associated with nitrous oxide use *(185)*.

The most common cause of death from non-medical use of nitrous oxide is asphyxia, either unintended or intended (in a suicide attempt). When nitrous oxide is inhaled in a confined space, it displaces the available oxygen *(186)*, and the resulting hypoxia may lead to death if nitrous oxide inhalation is continued. This may occur when nitrous oxide is inhaled with the person's head in a plastic bag *(185)*, through a tight-fitting face mask or in a car or a small unventilated room *(197)*.

15 Licit production, consumption and international trade

Nitrous oxide is legally manufactured for industrial and medical use. The leading manufacturers are based in France, Japan, Netherlands (Kingdom of the), Singapore and the USA. The global market for nitrous oxide was estimated in 2016 to be US$ 805 million for both the medical (about 85% of the market) and the industrial sector *(188)*, which increased to US$ 1.2 billion in 2022 *(2)*. North America accounts for almost half the market, followed by Europe and Asia with about one-quarter each. The electronics industry used an estimated 10 000 tonnes of nitrous oxide in 2022 *(154)*.

16 Illicit manufacture and traffic and related information

Nitrous oxide is not known to be manufactured illicitly. Non-medical use is with nitrous oxide manufactured legally for legitimate medical or industrial use but then purchased for non-medical use online or in person. Among 4883 self-selected respondents to an anonymous online survey in late 2013 (2014 Global Drug Survey) who reported use of nitrous oxide in the past year, nitrous oxide was most commonly obtained from friends (38.8%), a supermarket (34.4%), over the Internet (29.3%) or at a festival (28.7%) and rarely from "head shops" (9.9%), a dealer (7.3%) or an adult store (6.1%) *(28)*.

17 Current international controls and their impact

Nitrous oxide is not currently controlled under any international treaty.

18 Current and past national controls

Nitrous oxide is sold legally in all countries; however, many countries have imposed controls to limit non-medical use *(5)*. Such restrictions include bans on sale to minors, banning sales during certain hours (e.g. 22:00–05:00) and from certain locations (e.g. shops that sell alcohol or tobacco), restrictions on retail display and advertisement in shops, warning labels on packaging, prohibiting concurrent sale of products that enable non-medical use (e.g. balloons, "crackers"), and limits on importation and production. In the United Kingdom, production or supply (but not possession) of nitrous oxide for its psychoactive effects is illegal under the Psychoactive Substances Act of 2016 *(189)*. Netherlands (Kingdom of the) banned the production, sale and possession of nitrous oxide effective 1 January 2023, with exceptions for medical use and the food industry *(190)*. In South Australia, it is illegal to keep nitrous oxide visible in stores or to sell to minors or between 22:00 and 05:00 *(191)*.

19 Other medical and scientific matters relevant for a recommendation on scheduling of the substance

No other medical and scientific matters were identified.

References

1. Nitrous oxide. In: Reynolds JEF, editor. Martindale, The Extra Pharmacopoeia. 29th edition. London: Pharmaceutical Press; 1989. ISBN 0 85369 210 6 El 10.00.

2. Nitrous oxide overdose: How much is too much? Newport Beach (CA): WindwardWay; 2023 (https://windwardway.com/prescription-drug-abuse/nitrous-oxide/overdose/, accessed 28 June 2023).

3. Nitrous oxide. In: European Pharmacopoeia. 11th edition. Strasbourg: Council of Europe: European Directorate for the Quality of Medicines and Health Care; 2023 (https://www.edqm.eu/en/european-pharmacopoeia-ph.-eur.-11th-edition).

4. Nitrous oxide. In: United States Pharmacopoeia – The National Formulary (USP42-NF37). 42nd edition. Rockville (MD): United States Pharmacopeial Convention; 2019 (usp.org).

5. Recreational use of nitrous oxide: a growing concern for Europe. Lisbon: European Monitoring Centre for Drugs and Drug Addiction; 2022 (https://www.emcdda.europa.eu/publications/rapid-communication/recreational-use-nitrous-oxide-growing-concern-europe_en, accessed 9 July 2023).

6. Trogler WC. Physical properties and mechanisms of formation of nitrous oxide. Coordination Chem Rev. 1999;187:303–27. doi:10.1016/S0010-8545(98)00254-9.

7. Buslov A, Carroll M, Desai MS. 2018. Frozen in time: a history of the synthesis of nitrous oxide and how the process remained unchanged for over 2 centuries. Anesth Analg. 2018;127:65–70. doi:10.1213/ANE.0000000000003423.

8. Denisova KO, Ilyin AA, Rumyantsev RN, Ilyin AP, Volkova AV. Nitrous oxide: production, application, and protection of the environment. Russ J Gen Chem. 2019;89:1338–46. doi:10.1134/S107036321906032X.

9. Matuda H, Mishimura T, Tanaka T, Hatabu Y. 1981. Process for producing nitrous oxide. US patent US4376105A (https://patents.google.com/patent/US4376105A/en).

10. Helmenstine A. How to make nitrous oxide or laughing gas. Science Notes; 2020 (https://sciencenotes.org/how-to-make-nitrous-oxide-or-laughing-gas/, accessed 28 June 2023).

11. Blue RW, Giauque WF. The heat capacity and vapor pressure of solid and liquid nitrous oxide. The entropy from its band spectrum. J Am Chem Soc. 1935;57:991–7. doi:10.1021/ja01309a008.

12.. Rapson TD, Dacres H. Analytical techniques for measuring nitrous oxide. Trends Anal Chem. 2014;54:65–74. doi:10.1016/j.trac.2013.11.004.

13. Milam-Guerrero J, Yang B, To DT, Myung NV. Nitrous oxide is no laughing matter: a historical review of nitrous oxide gas-sensing capabilities highlighting the need for further exploration. ACS Sens. 2022;7(12):3598–610. doi:10.1021/acssensors.2c01275.

14. Kolb C, Wormhoudt JC, Zahniser MS. Recent advances in spectroscopic instrumentation for measuring stable gases in the natural environment. In: Matson PA, Harriss RC, editors. Biogenic trace gases: Measuring emissions from soil and water. Hoboken (NJ): Wiley-Blackwell; 1995:259–90 (https://www.wiley.com/en-sg/Biogenic+Trace+Gases:+Measuring+Emissions+from+Soil+and+Water-p-9780632036417).

15. Brenninkmeijer CAM, Janssen C, Kaiser J, Röckmann T, Rhee TS, Assonov SS. Isotope effects in the chemistry of atmospheric trace compounds. Chem Rev. 2003;103:5125–62. doi:10.1021/cr020644k.

16. Heard D, editor. Analytical techniques for atmospheric measurement. Hoboken (NJ): Wiley-Blackwell; 2008 (https://www.wiley.com/en-fr/Analytical+Techniques+for+Atmospheric+Measurement-p-9781405123570).

17. Griffith DWT, Deutscher NM, Caldow CGR, Kettlewell G, Riggenbach M, Hammer S. A Fourier transform infrared trace gas analyser for atmospheric applications. Atmos Meas Tech Discuss. 2012;5:3717–69. doi:10.5194/amtd-5-3717-2012.

18. Li J, Chen W, Yu B. Recent progress on infrared photoacoustic spectroscopy techniques. Appl Spectrosc Rev. 2011;46:440–71. doi:10.1080/05704928.2011.570835.

19. Compton RG, Northing RJ. Novel amperometric sensor for the detection of the anaesthetic gases isoflurane and nitrous oxide. J Chem Soc Faraday Trans. 1990;86:1077–81. doi:10.1039/FT9908601077

20. Andersen K, Kjær T, Revsbech NP. An oxygen insensitive microsensor for nitrous oxide. Sensors Actuators B Chem. 2001;81:42–8. doi:10.1016/S0925-4005(01)00924-8.

21. Brugnone F, Perbellini L, Cerpelloni M, Soave C, Cecco A, Giuliari C. Nitrous oxide in blood and urine of operating theatre personnel and the general population. Int Arch Occup Environ Health. 1995;68:22–6. doi:10.1007/BF01831629.

22. Poli D, Gagliano-Candela R, Strisciullo G, Colucci AP, Strada L, Laviola D et al. Nitrous oxide determination in postmortem biological aamples: a case of serial fatal poisoning in a public hospital. J Forensic Sci. 2010;55(1):258–64. doi:10.1111/j.1556-4029.01218.x.

23. Giuliani N, Beyer J, Augsberger M, Varlet V. Validation of an analytical method for nitrous oxide (N_2O) laughing gas by headspace gas chromatography coupled to mass spectrometry (HS-GC-MS): forensic application to a lethal intoxication. J Chromatogr B Analyt Technol Biomed Life Sci. 2015;983-984:90-3. doi:10.1016/j.jchromb.2014.12.034.

24. Marillier M, Karila L, Miguet-Alfonsi C. Quand le protoxyde d'azote ne fait plus rire: épidémiologie, aspects analytiques, incidences clinique et médicojudiciaire [When nitrous oxide is no longer a laughing matter: epidemiology and analytical, clinical incidents and medicolegal aspects]. Toxicol Analyt Clin. 2020;32:278–90. doi:10.1016/j.toxac.2020.07.002.

25. Xiang Y, Li L, Ma X, Li S, Xue Y, Yan P et al. Recreational nitrous oxide abuse: prevalence, neurotoxicity, and treatment. Neurotoxicity Res. 2021;39:975–85. doi:10.1007/s12640-021-00352-y.

26. Brunt TM, van den Brink W, van Amsterdam J. Mechanisms involved in the neurotoxicity and abuse liability of nitrous oxide: a narrative review. Int J Mol Sci. 2022;23:14747. doi:10.3390/ijms232314747.

27. Winstock AR & Ferris JA. Nitrous oxide causes peripheral neuropathy in a dose dependent manner among recreational users. Journal Psychopharmacol. 2020;34(2):229-236.

28. Kaar SJ, Ferris J, Waldron J, Devaney M, Ramsey J, Winstock AR. Up: The rise of nitrous oxide abuse. An international survey of contemporary nitrous oxide use. J Psychopharmacol. 2016;30(4):395–401. doi:10.1177/0269881116632375.

29. Gibbs D, Peacock A. New South Wales Drug Trends 2018: Key findings from the Ecstasy and Related Drugs Reporting System (EDRS) interviews. Sydney: National Drug and Alcohol Research Centre, University of New South Wales; 2018 (https://ndarc.med.unsw.edu.au/sites/default/files/NSW%20EDRS%20Interview%20Report%202018_2.pdf).

30. Ng J, O'Grady G, Pettit T, Frith R. Nitrous oxide use in first-year students at Auckland University. Lancet. 2003;361:1349–50. doi:10.1016/S0140-6736(03)13045-0.

31. Becker DE, Rosenberg M. Nitrous oxide and the inhalation anesthetics. Anesthesia Progress. 2008;55:124–31. doi:10.2344/0003-3006-55.4.124.

32. Quock RM, Graczik LM. Influence of narcotic antagonist drugs upon nitrous oxide analgesia in mice. Brain Res. 1988;440: 35–41. doi:10.1016/0006-8993(88)91156-0.

33. Chen DC, Quock RM. A study of central opioid receptor involvement in nitrous oxide analgesia in mice. Anesthesia Progress. 1990;7:181–5. PMID:1965769.

34. Quock RM, Walczak CK, Henry RJ, Chan DC. Effect of subtype-selective opioid receptor blockers on nitrous oxide antinociception in rats. Pharmacol Res. 1990;22(3):351–7. doi:10.1016/1043-6618(90)90733-t.

35. Quock RM, Mueller J. Protection by U-50,388H against β-chlornaltrexamine antagonisms of nitrous oxide antinociception in mice. Brain Res. 1991;549:162–4. doi:10.1016/0006-8993(91)90615-3.

36. Koyama T, Fukuda K. Involvement of the κ-opioid receptor in nitrous oxide-induced analgesia in mice. J Anesthesia. 2010;24:297–9. doi:10.1007/s00540-010-0886-5.

37. Smith EH, Rees JM. The effects of naloxone on the analgesic activities of general anaesthetics. Experientia. 1981;37(3):289–90. doi:10.1007/BF01991660.

38. Moody EJ, Mattson M, Newman AH, Rice KC, Skolnick P. Stereospecific reversal of nitrous oxide analgesia by naloxone. Life Sci. 1989;44(11):703–9. doi:10.1016/0024-3205(89)90381-0.

39. Quock RM, Curtis BA, Reynolds BJ, Mueller JL. Dose-dependent antagonism and potentiation of nitrous oxide antinociception by naloxone in mice. J Pharmacol Exp Ther. 1993.;267(1):117–22. PMID:8229738.

40. Koyama T, Mayahara T, Wakamatsu T, Sora I, Fukuda K. Deletion of mu-opioid receptor in mice does not affect the minimum alveolar concentration of volatile anaesthetics and nitrous oxide-induced analgesia. Br J Anaesthesia. 2009;103(5):744–9. doi:10.1093/bja/aep246.

41. Emmanouil DE, Dickens AS, Heckert RW, Ohgami Y, Chung E, Han S et al. Nitrous oxide-antinociception is mediated by opioid receptors and nitric oxide in the periaqueductal gray region of the midbrain. Eur Neuropsychopharmacol. 2007;18:194–9. doi:10.1016/j.euroneuro.2007.06.008.

42. Fang F, Guo TZ, Davies MF, Maze M. Opiate receptors in the periaqueductal gray mediate analgesic effect of nitrous oxide in rats. Eur J Pharmacol. 1997;336:137–41. doi:10.1016/s0014-2999(97)01219-3.

43. Hynes MD, Berkowitz BA. Nitrous oxide stimulation of locomotor activity: evidence for an opiate-like behavioral effect. J Pharmacol Exp Ther. 1979;209:304–8.

44. Hynes MD, Berkowitz BA. Lack of an opiate response to nitrous oxide in mice resistant to the activity-stimulating effects of morphine. J Pharmacol Exp Ther. 1982;220:499–503. PMID:6278126.

45. Tulunay FC, Tulunay M, Akkartal B, ayhan IH. A study of analgesic and dependence producing actions of nitrous oxide in mice. Pharm Res Commun. 1982;14(2):13–9. doi:10.1016/s0031-6989(82)80094-5.

46. Hodges BL, Gagnon MJ, Gillespie TR, Breneisen JR, O'Leary DF, Hara S et al. Antagonism of nitrous oxide antinociception in the rat hot plate test by site-specific mu and epsilon opioid receptor blockade. J Pharmacol Exp Ther. 1994;269(2):596–600. PMID:8182525.

47. Guo TZ, Poree L, Golden W, Stein J, Fujinaga M, Maze M. Antinociceptive response to nitrous oxide is mediated by supraspinal opiate and spinal adrenergic α_2 receptors in the rat. Anesthesiology. 1996;85:846–52. doi:10.1097/00000542-199610000-00020.

48. Fukihara N, Ishikawa T, Kinoshita H, Xiong L, Nakanishi O. Central noradrenergic mediation of nitrous oxide-induced analgesia in rats. Can J Anaesth. 1998;45:1123–9. doi:10.1007/BF03012404.

49. Shingu R, Osawa M, Omatsu Y, Komatsu T, Urabe N, Mori K. Naloxone does not antagonize the anesthetic-induced depression of nociceptor-driven spinal cord response in spinal cats. Acta anaesth scand. 1981;25:526–32. doi:10.1111/j.1399-6576.1981.tb01699.x.

50. Smith RA, Wilson M, Miller KW. Naloxone has no effect on nitrous oxide anesthesia. Anesthesiology. 1978;49:6–8. doi:10.1097/00000542-197807000-00003.

51. Courtière A, Hardouin J. Behavioural effects induced by nitrous oxide in rats performing a vigilance task. Behav Pharmacol. 1997;8:408–15. PMID:9832980.

52. Zacny JP, Coalson DW, Lichtor JL. Effects of naloxone on the subjective and psychomotor effects of nitrous oxide in humans. Pharmacol Biochem Behav. 1994;49(3):573–8. doi:10.1016/0091-3057(94)90071-x.

53. Yang JC, Clark WC, Ngai SH. Antagonism of nitrous oxide analgesia by naloxone in man. Anesthesiology. 1980;52:414-417.

54. Chapman CR, Benedetti C. Nitrous oxide effects on cerebral evoked potential to pain: Partial reversal with a narcotic antagonist. Anesthesiology. 1979;51:135–8. doi:10.1097/00000542-197908000-00009.

55. Willer JC, Bergeret S, Gaudy JH, Dauthier C. Failure of naloxone to reverse the nitrous oxide-induced depression of a brain stem reflex: an electrophysiologic and double-blind study in humans. Anesthesiology 1985;63(5):467-72.

56. Levine JD, Gordon NC, Fields HL. Naloxone fails to antagonize nitrous oxide analgesia for clinical pain. Pain. 1982;13:65-170. doi:10.1016/0304-3959(82)90026-4.

57. Yagi M, Mashimo T, Kawaguchi T, Yoshiya I. Analgesic and hypnotic effects of subanaesthetic concentrations of xenon in human volunteers: comparison with nitrous oxide. Br J Anaesth. 1995;74:670–3. doi:10.1093/bja/74.6.670.

58. Zacny JP, Conran A, Pardo H, Coalson DW, Black M, Klock AP et al. Effects of naloxone on nitrous oxide actions in healthy volunteers. Pain. 1999;83:411–8. doi:10.1016/S0304-3959(99)00127-X.

59. Gillman MA, Kok L, Lichtigfeld FJ. Paradoxical effect of naloxone on nitrous oxide analgesia in man. Eur J Pharmacol. 1980;61:175–7. doi:10.1016/0014-2999(80)90160-0.

60. Gillman MA, Lichtigfeld FJ. A comparison of the effects of morphine sulphate and nitrous oxide analgesia on chronic pain states in man. J Neurolog Sci. 1981;49:41–5. doi:10.1016/0022-510x(81)90186-6.

61. Jevtovic-Todorovic V, Todorovic SM, Mennerick S, Powell S, Dikranian K, Benshoff N et al. Nitrous oxide (laughing gas) is an NMDA antagonist, neuroprotectant and neurotoxin. Nat Med. 1998;4(4):460–3. doi:10.1038/nm0498-460.

62. Izumi Y, Hsu FF, Conway CR, Nagele P, Mennerick SJ, Zorumski CF. Nitrous oxide, a rapid antidepressant, has ketamine-like effects on excitatory transmission in the adult hippocampus. Biol Psychiatry. 2022;92:964–72. doi:10.1016/j.biopsych.2022.06.016.

63. Sato Y, Kobayashi E, Murayama T, Mishina M, Seo N. Effect of N-methyl-D-aspartate receptor epsilon1 subunit gene disruption of the action of general anesthetic drugs in mice. Anesthesiology. 2005;102:557–61. doi:10.1097/00000542-200503000-00013.

64. Zacny JP, Yajnik S, Coalson D, Lichtor JL, Apfelbaum JL, Rupani G et al. Flumazenil may attenuate some subjective effects of nitrous oxide in humans: a preliminary report. Pharmacol Biochem Behav. 1995;51(4):815–9. doi:10.1016/0091-3057(95)00039-y.

65. Kalmoe MC, Janski AM, Zorumski CF, Nagele P, Palanca BJ, Conway CR. Ketamine and nitrous oxide: the evolution of NMDA receptor antagonists as antidepressant agents. J Neurol Sci. 2020;412:116778. doi:10.1016/j.jns.2020.116778.

66. Menetrier T, Denimal D. Vitamin B12 status in recreational users of nitrous oxide: a systematic review focusing on the prevalence of laboratory abnormalities. Antioxidants. 2023;12:1191. doi:10.3390/antiox12061191.

67. Dohrn CS, Lichtor JL, Coalson DW, Uitvlugt A, de Wit H, Zacny JP. Reinforcing effects of extended inhalation of nitrous oxide in humans. Drug Alcohol Depend. 1993;31(3):265–80. doi:10.1016/0376-8716(93)90009-f.

68. Yu M, Qiao Y, Li W, Fang X, Gao H, Zheng D et al. Analysis of clinical characteristics and prognostic factors in 110 patients with nitrous oxide abuse. Brain Behav. 2021;12:e2533. doi:10.1002/brb3.2533.

69. Chiew AL, Raubenheimer JF, Berling I, Buckley I, Buckley NA, Becker T et al. Just "nanging" around – harmful nitrous oxide use: a retrospective case series and review of Internet searches, social media posts and the coroner's database. Intern Med J. 2022;52:1724–32. doi:10.1111/imj.15391.

70. Piazza GG, Iskandar G, Hennessy V, Zhao H, Walsh K, McDonnell J et al. Pharmacological modelling of dissociation and psychosis: an evaluation of the Clinician Administered Dissociative States Scale and Psychotomimetic States Inventory during nitrous oxide ("laughing gas")-induced anomalous states. Psychopharmacology. 2022;239:2317–29. doi:10.1007/s00213-022-06121-9.

71. Paulus MC, Wijnhoven AM, Maessen GC, Blankensteijn SR, van der Heyden MAG. Does vitamin B12 deficiency explain psychiatric symptoms in recreational nitrous oxide users? A narrative review. Clin Toxicol. 2021;59(11):947–55. doi:10.1080/15563650.2021.1938107.

72. Sood R, Parent T. Peripheral polyneuropathy and acute psychosis from chronic nitrous oxide poisoning. A case report with literature review. Medicine (Baltimore). 2022;101(31):e28611. doi:10.1097/MD.0000000000028611.

73. Dohrn CS, Lichtor JL, Finn RS, Uitvlugt A, Coalson DW, Rupani G et al. Subjective and psychomotor effects of nitrous oxide in healthy volunteers. Behav Pharmacol. 1992;3:19–30. doi:10.1097/00008877-199203010-00005.

74. Lichtor JL, Lane BS, Zimmerman MB. Residual sleepiness after N_2O sedation: a randomized control trial [ISRCTN88442975]. BMC Anesthesiology. 2004;4:5. doi:10.1186/1471-2253-4-5.

75. Beckman NJ, Zacny JP, Walker DJ. Within-subject comparison of the subjective and psychomotor effects of a gaseous anesthetic and two volatile anesthetics in healthy volunteers. Drug Alcohol Depend. 2006;81:89–95. doi:10.1016/j.drugalcdep.2005.06.002.

76. Armstrong PJ, Morton C, Sinclair W, Tiplady B. Effects of nitrous oxide on psychological performance. A dose-response study using inhalation of concentrations up to 15%. Psychopharmacology (Berl). 1995;117:486–90. doi:10.1007/BF02246223.

77. Fagan D, Paul DL, Tiplady B, Scott DB. A dose–response study of the effects of inhaled nitrous oxide on psychological performance and mood. Psychopharmacology (Berl). 1994;115:333–8. doi:10.1007/BF02245337.

78. Berkowitz BA, Finck AD, Hynes MD, Ngai SH. Tolerance to nitrous oxide in rats and mice. Anesthesiology. 1979;51:309–12. doi:10.1097/00000542-197910000-00006.

79. Laughing gas implicated in growing number of fatal accidents: NOS. Dutch News, 2 December 2021. https://www.dutchnews.nl/2021/12/laughing-gas-implicated-in-growing-number-of-fatal-accidents-nos/, accessed 27 August 2023).

80. Moyes D, Cleaton-Jones P, Lelliot J. Evaluation of driving skills after brief exposure to nitrous oxide. S Afr Med J. 1979;56:1000–2. PMID:550424.

81. Baran KC, van Munster IG, Meij-de Vries A, Gardien KLM, van Trier T, Pijpe A et al. Severe nitrous-oxide frostbite injuries on the rise in The Netherlands; let's raise awareness. Burns. 2020;46(6):1472–9. doi:10.1016/j.burns.2020.04.016.

82. Quax MLJ, Van Der Steenhoven TJ, Bronkhorst MWGA, Emmink BL. Frostbite injury: an unknown risk when using nitrous oxide as a party drug. Acta Chir Belg. 2020;122(2):140–3. doi:10.1080/00015458.2020.1782160.

83. Chan JHK, Eriksson S, Mohamed H, Bhatti S, Frew Q, Barnes D. Experiences of frostbite injury from recreational use of nitrous oxide canisters in a UK burns center: Not a laughing matter. J Plast Reconstr Aesthet Surg. 2023;83:282–8. doi:10.1016/j.bjps.2023.05.012.

84. Dolin SJ, Little HJ. Effects of "nitrendipine" on nitrous oxide anesthesia, tolerance, and physical dependence. Anesthesiology. 1989;70:91–7. PMC1917760.

85. Koblin DD, Dong DE, Eiger EI 2nd. Tolerance of mice to nitrous oxide. J Pharmacol Exp Ther. 1979;211:317–25. PMID:228023.

86. Rupreht J, Ukponmwan DE, Dworacek B, Admiraal PV, Dzoljic. Enkephalinase inhibition prevented tolerance to nitrous oxide analgesia in rats. Acta Anaesthesiol Scand. 1984:28:617–20. doi:10.1111/j.1399-6576.1984.tb02132.x.

87. Manson HJ, Dyke G, Melling J, Gough M. The effect of naloxone and morphine on convulsions in mice following withdrawal from nitrous oxide. Can Anaesth Soc J.1983;30(1):28–31. doi:10.1007/BF03007713.

88. Shingu K, Osawa M, Fukuda K, Mori K. Acute tolerance to the analgesic action of nitrous oxide does not develop in rats. Anesthesiology. 1985;62:502–4. doi:10.1097/00000542-198504000-00021.

89. Koblin DD, Deady JE, Dong DE, Eiger EI 2nd. Mice tolerant to nitrous oxide are also tolerant to alcohol. J Pharmacol Exp Ther. 1980;213:309–12. PMID:7365690.

90. Koblin DD, Deady JE, Nelson NT, Eiger EI 2nd, Bainton CR. Mice tolerant to nitrous oxide are not tolerant to barbiturates. Anesth Analg. 1981;60:138–41. PMID:7193990.

91. Matsushita Y, Ishikawa M, Abe K, Utsunomiya I, Chikuma T, Hojo H, Hoshi K, Quock RM, Taguchi K. Involvement of the protein kinase Cgamma isoform in development of tolerance to nitrous oxide-induced antinociception in mice. Neuroscience 2007;148(2):541-7

92. Kaiyala KJ, Butt S, Ramsay DS. Systems-level adaptations explain chronic tolerance development to nitrous oxide hypothermia in young and mature rats. Psychopharmacology (Berl) 2007b;191:233–242.

93. Dzoljic M, Rupreht J, Erdmann W, Stijnen TH, van Briemen LJ, Dzoljic MR. Behavioral and electrophysiological aspects of nitrous oxide dependence. Brain Res Bull. 1994;33:25–31. doi:10.1016/0361-9230(94)90046-9.

94. Rupreht J, Shimoji K, Fukuda S, Erdmann, Denda S. Tolerance to N$_2$O-induced alterations in somatosensory evoked potentials. J Neurosurg Anesthesiol. 1989;1(2):91–5. doi:10.1097/00008506-198906000-00002.

95. Fischer GJ, Gilman SC, Blank C. Corticosterone response underlying tolerance to stress induced by a gaseous (nitrous oxide) environment. Percept Mot Skills. 1987;64:799–808. doi:10.2466/pms.1987.64.3.799.

96. Harper MH, Winter PM, Johnson BH, Koblin DD, Eiger EI 2nd. Withdrawal convulsions in mice following nitrous oxide. Anesth Analg. 1980;59:19–21. PMID:7188661.

97. Milne B, Cervenko FW, Jhamandas KH. Physical dependence on nitrous oxide in mice: resemblance to alcohol but not to opiate withdrawal. Can Anaesth Soc J. 1981;28(1):46–50. doi:10.1007/BF03007289.

98. Rupreht J, Dworacek B, Ducardus R,Schmitz PI, Dzolijc MR. The involvement of the central cholinergic and endorphinergic systems in the nitrous oxide withdrawal syndrome in mice. Anesthesiology. 1983;58:524–6. doi:10.1097/00000542-198306000-00008.

99. Belknap JK, Laursen SE, Crabbe JE. Ethanol and nitrous oxide produce withdrawal-induced convulsions by similar mechanisms in mice. Life Sci. 1987;41:2033–40. doi:10.1016/0024-3205(87)90477-2.

100. Dzoljic MR, Haffman J, Rupreht J, Adolfs MJ, Dzolijic MM, Cappendijk SL. Decrease of β-endorphin in the brain of rats following nitrous oxide withdrawal. Drug Metab Drug Interact. 1991;9(2):139–48. doi:10.1515/dmdi.1991.9.2.139.

101. Whitwam, JG, Morgan M, Hall GM, Petrie A. Pain during continuous nitrous oxide administration. British Journal Anaesthesiology 1976 ;48(5):425-9

102. Ramsay DS, Leroux BG, Rothen M, Prall CW, Fiset LO, Woods SC. Nitrous oxide analgesia in humans: acute and chronic tolerance. Pain. 2005;114:19–28. doi:10.1016/j.pain.2004.12.011.

103. Rupreht J, Dworacek B, Bonke B, Dzoljic MR, van Eijndhoven JH, de Vlieger M. Tolerance to nitrous oxide in volunteers. Acta Anaesthesiol Scand. 1985 Aug;29(6):635-8. doi: 10.1111/j.1399-6576.1985.tb02271.x. PMID: 4061009.

104. Zacny JP, Cho AM, Coalson DW, Rupani G, Young CK, Klafta JM et al. Differential acute tolerance development to effects of nitrous oxide in humans. Neurosci Lett. 1996;209:73–6. doi:10.1016/0304-3940(96)12626-4.

105. Yajnik S, Zacny JP, Young CJ, Lichtor JL, Ruoani G, Klafta JM et al. Lack of acute tolerance development to the subjective, cognitive, and psychomotor effects of nitrous oxide in healthy volunteers. Pharmacol Biochem Behav. 1996;54(2):501–8. doi:10.1016/0091-3057(95)02278-3.

106. Cohen L, Duroy D, Perozziello A, Sasportes A, Lejoyeux M, Geoffroy PA. A cross-sectional study: nitrous oxide abuse in Parisian medical students. Am J Addict. 2023;32(1):60–5. doi:10.1111/ajad.13363.

107. Guerlais M, Aquizerate A, Lionnet A, Daveluy A, Duval M, Gérardin M et al. Nitrous oxide: a unique official French addictovigilance national survey. Front Public Health. 2023;11:1167746. doi:10.3389/fpubh.2023.1167746.

108. Nugteren-Van Lonkhuyzen JJ, van der Ben L, van den Hengel-Koot IS, de Lange D, van Riel AJHP, Hondebrink L. 2023. High incidence of signs of neuropathy and self-reported substance use disorder for nitrous oxide in patients intoxicated with nitrous oxide. Eur Addict Res. doi:10.1159/000530123.

109. Fidalgo, M., Prud'homme, T., Allio, A., Bronnec, M., Bulteau, S., Jolliet, P., & 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*, *40*(1), 33–42. https://doi.org/10.1080/08897077.2019.1573210

110. Ramsay DS, Watson CH, Leroux BG, Prall CW, Kaiyala KJ. Conditioned place aversion and self-administration of nitrous oxide in rats. Pharmacol Biochem Behav. 2003;74(3):623–33. doi:10.1016/s0091-3057(02)01048-1.

111. Wood, R. W., Grubman, J., & Weiss, B. (1977). Nitrous oxide self-administration by the squirrel monkey. *The Journal of Pharmacology and Experimental Therapeutics, 202*(3), 491–499.

112. Richardson KJ, Shelton KL. Discriminative stimulus effects of nitrous oxide in mice: comparison with volatile hydrocarbons and vapor anesthetics. Behav Pharmacol. 2014;25:2–11. doi:10.1097/FBP.0000000000000017.

113. Tracy ME, Slavova-Hernandez GG, Shelton KL. Assessment of reinforcement enhancing effects of toluene vapor and nitrous oxide in intracranial self-stimulation. Psychopharmacology. 2014;231:1339–50. doi:10.1007/s00213-013-3327-y.

114. Goudie AJ, Dickins DW. Nitrous oxide-induced conditioned taste aversions in rats: the role of duration of drug exposure and its relation to the taste aversion-self-administration "paradox". Pharmacol Biochem Behav. 1978;9(5):587–92. doi:10.1016/0091-3057(78)90207-1.

115. Benturquia B, Le Gruen S, Canestrelli C, Lagente V, Apiou G, Roques BP et al. Specific blockade of morphine- and cocaine-induced reinforcing effects in conditioned place preference by nitrous oxide in mice. Neuroscience. 2007;149(3):477–86. doi:10.1016/j.neuroscience.2007.08.003.

116. Benturquia B, Le Marec T, Scherrmann JM, Noble F. Effects of nitrous oxide on dopamine release in the rat nucleus accumbens and expectation of reward. Neuroscience. 2008;155(2):341–4. doi:10.1016/j.neuroscience.2008.05.015.

117. Lynn EJ, Walter RG, Harris LA, Dendy R, James M. Nitrous oxide: It's a gas. J Psychedelic Drugs. 1972;5:1–7. doi:10.1080/02791072.1972.10471462.

118. Block RI, Ghoneim MM, Kumar V, Pathak D. Psychedelic effects of a subanesthetic concentration of nitrous oxide. Anesth Prog. 1990;37(6):271–6. PMID:2097905.

119. Galinkin JL, Janeszewski D, Young CJ, Klafta JM, Klock PA, Coalson DW et al. Subjective, psychomotor, cognitive, and analgesic effects of subanesthetic concentrations of sevoflurane and nitrous oxide. Anesthesiology. 1997;87:1082–8. doi:10.1097/00000542-199711000-00012.

120. Komboj SK, Zhao H, Troebinger L, Piazza G, Cawley E, Hennessy V et al. Rewarding subjective effects of the NMDAR antagonist nitrous oxide (laughing gas) are moderated by impulsivity and depressive symptoms in healthy volunteers. Int J Neuropsychopharmacol. 2021;24(7):551–61. doi:10.1093/ijnp/pyab009.

121. Lynn EJ, James M, Dendy R, Harris LA, Walter RG. Non-medical use of nitrous oxide: a preliminary report. Mich Med. 1971;70(6):203–4. PMID:5547080.

122. Zacny JP, Lichtor JL, Coalson DW, Apfelbaum JL, Flemming D, Foster V. Time course of effects of brief inhalations of nitrous oxide in normal volunteers. Addiction. 1994;89:831–9. doi:10.1111/j.1360-0443.1994.tb00986.x.

123. Walker DJ, Zacny JP. Within- and between-subject variability in the reinforcing and subjective effects of nitrous oxide in healthy volunteers. Drug Alcohol Depend. 2001;64:85–96. doi:10.1016/s0376-8716(00)00234-9.

124. Cho AM, Coalson DW, Klock PA, Klafta JM, Marks S, Toledano AY et al. The effects of alcohol history on the reinforcing, subjective and psychomotor effects of nitrous oxide in healthy volunteers. Drug Alcohol Depend. 1997;45:63–70. doi:10.1016/s0376-8716(97)01346-x.

125. Walker DJ, Zacny JP. Lack of effects of ethanol pretreatment on the abuse liability of nitrous oxide in light and moderate drinkers. Addiction. 2001;96:1839–45. doi:10.1046/j.1360-0443.2001.9612183913.x.

126. Walker DJ, Zacny JP. Analysis of the reinforcing and subjective effects of different doses of nitrous oxide using a free-choice procedure. Drug Alcohol Depend. 2002;66(1):93–103. doi:10.1016/s0376-8716(01)00188-0.

127. Walker DJ. Zacny JP. Bitonic dose–response functions for reinforcing and self-reported effects of nitrous oxide in humans. Pharmacol Biochem Behav. 2003;74:851–7. doi:10.1016/s0091-3057(03)00015-7.

128. Zacny JP, Jun JM. Lack of sex differences to the subjective effects of nitrous oxide in healthy volunteers. Drug Alcohol Depend. 2010;112:251–4. doi:10.1016/j.drugalcdep.2010.06.008.

129. Zacny, J. P., Walker, D. J., & Derus, L. M. (2008). Choice of nitrous oxide and its subjective effects in light and moderate drinkers. *Drug and alcohol dependence*, *98*(1-2), 163–168. https://doi.org/10.1016/j.drugalcdep.2008.06.001

130. Walsh K, Das RK, Kamboj SK. The subjective response to nitrous oxide is a potential pharmaco-endophenotype for alcohol use disorder: a preliminary study with heavy drinkers. Int J Neuropsychopharmacol. 2017;20(4):346–50. doi:10.1093/ijnp/pyw063.

131. Yajnik S, Thapar P, Lichtor JL, Patterson T, Zacny JP. Effects of marijuana history on the subjective, psychomotor, and reinforcing effects of nitrous oxide in humans. Drug Alcohol Depend. 1994;36:227–36. doi:10.1016/0376-8716(94)90149-x.

132. Atkinson RM, Green JD. Personality, prior drug use, and introspective experience during nitrous oxide intoxication. Int J Addictions. 1983;18(5):712–38. doi:10.3109/10826088309027365.

133. Atkinson RM, Morozumi P, Green JD, Kramer JC. Nitrous oxide intoxication: subjective effects in healthy young men. J Psychedelic Drugs. 1977;9(4):317–28. doi:10.1080/02791072.1977.10472063.

134. Zacny JP, Janiszewski DJ, Sadeghi P, Black ML. Reinforcing, subjective, and psychomotor effects of sevoflurane and nitrous oxide in moderate-drinking healthy volunteers. Addiction. 1999;94(12):1817–28. doi:10.1046/j.1360-0443.1999.941218177.x.

135. Nagele P, Zorumski CF, Conway C. Exploring nitrous oxide as treatment of mood disorders. Basic concepts. J Clin Psychopharmacol. 2018;38:144–8. doi:10.1097/JCP.0000000000000837.

136. Dimic N, Djuric M, Nenadic I, Bojic S, Bobos M, Janicijevic A et al. Nitrous oxide – application in modern anesthesia. Curr Anesthesiol Rep. 2023;13:117–23. doi:10.1007/s40140-023-00554-4.

137. Hendrickx J, Peyton P, Carette R, De Wolf A. Inhaled anaesthetics and nitrous oxide. Complexities overlooked: things may not be what they seem. Eur J Anaesthesiol. 2016;33:611–9. doi:10.1097/EJA.0000000000000467.

138. Poonai N, Creene C, Dobrowlanski A, Geda R, Hartling L, Ali S et al. Inhaled nitrous oxide for painful procedures in children and youth: a systematic review and meta-analysis. Can J Emergency Med. 2023;25:508–28. doi:10.1007/s43678-023-00507-0.

139. Zuarez-Easton S, Erez O, Zafrar N, Carmeli J, Garmi G, Salim R. Pharmacologic and nonpharmacologic options for pain relief during labor: an expert review. Am J Obstet Gynecol. 2023;228(5S):S1246–59. doi:10.1016/j.ajog.2023.03.003.

140. Sahyoun C, Cantais A, Gervaix A, Bressan S, Löllgen R, Krauss B et al. Pediatric procedural sedation and analgesia in the emergency department: surveying the current European practice. Eur J Pediatr. 2021;180:1799–813. doi:10.1007/s00431-021-03930-6.

141. Broughton K, Clark AG, Ray AP. Nitrous oxide for labor analgesia: what we know to date. Ochsner J. 2020;20:419–21. doi:10.31486/toj.19.0102.

142. Liu H, Kerzman J, Demchenko I, Wijeysundera DN, Kennedy SH, Ladha KS et al. Nitrous oxide for the treatment of psychiatric disorders: a systematic review of the clinical trial landscape. Acta Psychiatrica Scandinavica. 2022;146:126–38. doi:10.1111/acps.13432.

143. Nagele P, Duma A, Kopec M, Gebara MA, Parsoei A, Walker M et al. Nitrous oxide for treatment-resistant major depression: a proof-of-concept trial. Biol Psychiatry. 2015;78:10–8. doi:10.1016/j.biopsych.2014.11.016.

144. Nagele P, Palanca BJ, Gott B, Brown F, Barnes L, Nguyen T et al. A phase 2 trial of inhaled nitrous oxide for treatment-resistant major depression. Sci Transl Med. 2021;13:eabe1376. doi:10.1126/scitranslmed.abe1376.

145. Yan D, Liu B, Wei X, Ou W, Liao M, Ji S et al. Efficacy and safety of nitrous oxide for patients with treatment-resistant depression, a randomized controlled trial. Psychiatry Res. 2022;317:114867. doi:10.1016/j.psychres.2022.114867.

146. Liu J, Zhao X, Wei X, Yan D, Ou W, Liao M et al. mpirical evidence for the neurocognitive effect of nitrous oxide as an adjunctive therapy in patients with treatment resistant depression: a randomized controlled study. Psychiatry Res. 2023;326:115326. doi:10.1016/j.psychres.2023.115326.

147. Shao X, Yan D, Kong W, Sun S, Liao M, Ou W et al. Brain function changes reveal rapid antidepressant effects of nitrous oxide for treatment-resistant depression: evidence from task-state EEG. Psychiatry Res. 32023;22:115072. doi:10.1016/j.psychres.2023.115072.

148.. Guimarães MC, Guimarães TM, Hallak JE, Abrão J, Macado-de-Sousa JP. Nitrous oxide as an adjunctive therapy in major depressive disorder: a randomized controlled double-blind pilot trial. Braz J Psychiatry. 2021;43(5):484–93. doi:10.1590/1516-4446-2020-1543.

149. Kim WSH, Dimick MK, Omrin D, Mitchell RHB, Riegert D, Levitt A et al. Proof-of-concept randomized controlled trial of single-session nitrous oxide treatment for refractory bipolar depression: focus on cerebrovascular target engagement. Bipolar Disord. 2023;25(3):221–32. doi:10.1111/bdi.13288.

150. Gillman MA, Lichtigfeld F, Young T. Psychotropic analgesic nitrous oxide for alcoholic withdrawal states. Cochrane Database Syst Rev. 2007;(2): CD005190. doi:10.1002/14651858.CD005190.pub2.

151. Alho H, Methuen T, Paloheimo M, Seppä K, Strid N, Apter-Kaseva N et al. Nitrous oxide has no effect in the treatment of alcohol withdrawal syndrome: a double-blind placebo-controlled randomized trial. J Clin Psychopharmacol. 2003;23(2):211–4. doi:10.1097/00004714-200304000-00019.

152. World Health Organization Model List of Essential Medicines – 23rd List. The selection and use of essential medicines 2023: Executive summary of the report of the 24th WHO Expert Committee on the Selection and Use of Essential Medicines, 24–28 April 2023. Geneva: World Health Organization; 2023 (https://www.who.int/publications/i/item/WHO-MHP-HPS-EML-2023.01).

153. World Health Organization Model List of Essential Medicines for Children – 9th List. The selection and use of essential medicines 2023: Executive summary of the report of the 24th WHO Expert Committee on the Selection and Use of Essential Medicines, 24–28 April 2023. Geneva: World Health Organization; 2023 (https://www.who.int/publications/i/item/WHO-MHP-HPS-EML-2023.01).

154. Nitrous oxide market share, size, trends, industry analysis report. New York (NY): Polaris Market Research; 2023 (https://www.polarismarketresearch.com/industry-analysis/nitrous-oxide-market, accessed 20 August 2023).

155. Adhikari BM, Truong T, Bansai N, Bhandar B. Use of gases in dairy manufacturing: a review. Crit Rev Food Sci Nutr. 2017;58(15):2557–69. doi:10.1080/10408398.2017.1333488.

156. Sikin AM, Walkling Rebeiro M, Rizvi SS. Synergistic processing of skim milk with high pressure nitrous oxide, heat, nisin, and lysozyme to inactivate vegetative and spore-forming bacteria. Food Bioprocessing Technol. 2017;10:2132–45. doi:10.1007/s11947-017-1982-4.

157. De Aguiar Saldanha Pinheiro AC, Urbaniti E, Toppi S, Picone G, Patrignani F, Lanciotti R et al. The impact of gas mixtures of argon and nitrous oxide (N_2O) on quality parameters of sardine (*Sardina pilchardus*) fillets during refrigerated storage. Food Res Int. 2019;115:268–75. doi:10.1016/j.foodres.2018.12.030.

158. Lemieux P. Nitrous oxide cooling in hybrid rocket nozzles. Prog Aerospace Sci. 2010;46:106–15. doi:10.1016/j.paerosci.2009.12.001.

159. Crossin R, Crossin, Whelan J, Ball J. Defining and measuring "inhalant" use in population-based surveys. Int J Drug Policy. 2023. doi:10.1016/j.drugpo.2023.103991.

160. The 2007/08 New Zealand Alcohol and Drug Use Survey: online data tables. Wellington: Ministry of Health; 2010 (https://www.health.govt.nz/publication/2007-08-new-zealand-alcohol-and-drug-use-survey-online-data-tables, accessed 13 July 2023).

161. Deaths related to volatile substances where nitrous oxide was mentioned on the death certificate, England and Wales, registered between 2001 and 2021. London: Office for National Statistics; 2022 (https://www.ons.gov.uk/file?uri=/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/adhocs/15557deathsrelatedtonitrousoxideenglandandwalesregisteredbetween2001and2021/nitrousoxide.xlsx).

162. van Amsterdam J, Nabben T, van den Brink W. Recreational nitrous oxide use: prevalence and risks. Regul Toxicol Pharmacol. 2015;73:790–6. doi:10.1016/j.yrtph.2015.10.017.

163. van Laar M, Cruts AAN, van Miltenburg CJA, Strada L, Ketelaars APM, Croes EA et al. Nationale Drug Monitor Jaarbericht 2019. Utrecht: Trimbos-Instituut; The Hague: Netherlands Institute of Mental Health and Addiction; 2020 (https://www.trimbos.nl/docs/2611d773-620a-45af-a9e5-c27a7e6688e4.pdf, accessed 17 September 2023).

164. van Laar MW, van Beek RJJ, Beenakkers EMT, Cruts AAN, Kuin MC, Meijer RF et al. Nationale Drug Monitor Jaarberich 2021. Utrecht: Trimbos-Instituut; The Hague: Netherlands Institute of Mental Health and Addiction; 2022 (https://www.nationaledrugmonitor.nl/wp-content/uploads/2022/07/Bevriezing-NDM-2021_DEF.pdf, accessed 17 September 2023).

165. Results from the 2014 National Survey on Drug Use and Health: detailed tables. Substance Abuse and Mental Health Services Administration, Rockville (MD): Center for Behavioral Health Statistics and Quality; 2015 (https://www.samhsa.gov/data/sites/default/files/NSDUH-DetTabs2014/NSDUH-DetTabs2014.pdf, accessed 23 July 2023).

166. Results from the 2018 National Survey on Drug Use and Health: detailed tables. Rockville (MD): Substance Abuse and Mental Health Services Administration (https://www.samhsa.gov/data/sites/default/files/cbhsq-reports/NSDUHDetailedTabs2018R2/NSDUHDetailedTabs2018.pdf, accessed 17 September 2023).

167. Results from the 2020 National Survey on Drug Use and Health: detailed tables. Rockville (MD): Substance Abuse and Mental Health Services Administration; 2021 (https://www.samhsa.gov/data/, accessed 23 July 2023).

168. Results from the 2021 National Survey on Drug Use and Health: detailed tables. Rockville (MD): Substance Abuse and Mental Health Services Administration; 2022 (https://www.samhsa.gov/data/report/2021-nsduh-detailed-tables, accessed 27 February 2023).

169. Winstock et al. (2022)

170. Benschop A, van Bakkum F, Noijem J. Changing patterns of substance use during the coronavirus pandemic: Self-reported use of tobacco, alcohol, cannabis, and other drugs. Front Psychiatry. 2021;12:633551. doi:10.3389/fpsyt.2021.633551.

171. Rosenberg H, Orkin FK, Springstead J. Abuse of nitrous oxide. Anesth Analg. 1979;58:104–6. PMID:571232.

172. Inquimbert C, Maitre Y, Moulise E, Gremillet V, Tramini P, Valcarcel J et al. Recreational nitrous oxide use and associated factors among health profession students in France. Int J Environ Res Public Health. 2022;19(9):5237. doi:10.3390/ijerph19095237.

173. Perino J, Tournier M, Mathieu C, Letiner L, Peyré A, Perret G, Pereira E et al. Psychoactive substance use among students: a cross-sectional analysis. Fundam Clin Pharmacol. 2022;36(5):908–14. doi:10.1111/fcp.12771.

174. Ehirim EM, Naughton DP, Petroczi A. No laughing matter: presence, consumption trends, drug awareness, and perceptions of "hippy crack" (nitrous oxide) among young adults in England. Front Psychiatry. 2018;8:312. doi:10.3389/fpsyt.2017.00312.

175. van den Toren SJ, van Grieken A, Raat H. Associations of socio-demographic characteristics, well-being, school absenteeism, and substance use with recreational nitrous oxide use among

adolescents: a cross-sectional study. PLoS One. 2021;16(2):e0247230. doi:10.1371/journal.pone.0247230.

176. Garland EL, Howard MO, Perron BE. Nitrous oxide inhalation among adolescents: prevalence, correlates, and co-occurrence with volatile solvent inhalation. J Psychoactive Drugs. 2009;41(4):337–47. doi:10.1080/02791072.2009.10399771.

177. Chen YH, Chen MH, Wei HT, Chen LY. Survey of substance use among adolescent drug offenders referred from juvenile courts in Taiwan: clinical epidemiology of single versus multiple illicit substance use. J Formosan Med Assoc. 2022;121:2257–64. doi:10.1016/j.jfma.2022.04.009.

178. Peacock A, Karlsson A, Uporova J, Price O, Chan R, Swanton R et al. Australian drug trends 2020: Key findings from the national Ecstasy and Related Drugs Reporting System (EDRS) interviews. Sydney: National Drug and Alcohol Research Centre, University of New South Wales; 2021 (https://ndarc.med.unsw.edu.au/resource/australian-drug-trends-2020-key-findings-national-ecstasy-and-related-drugs-reporting).

179. Bethmont A, Harper CE, Chan BSH, Dawson AH, McAnulty J. Increasing illicit use of nitrous oxide in presentations to NSW emergency departments. Med J Aust. 2019;211(9):429–429.e1. doi:10.5694/mja2.50377.

180. Marsden P, Sharma AA, Rotella JA. Clinical manifestations and outcomes of chronic nitrous oxide misuse: a systematic review. Emerg Med Australas. 2022;34(4):492–503. doi:10.1111/1742-6723.13997.

181. Chien WH, Huang MC, Chen LY. Psychiatric and other medical manifestations of nitrous oxide abuse: implications from case series. J Clin Psychopharmacol. 2020;40:80–3. doi:10.1097/jcp.0000000000001151.

182. Xu, J., Zhang, Y., Lei, C., Sun, P., Chen, R., & Yuan, T. (2023). Using machine learning to identify factors related to nitrous oxide (laughing gas) relapse among adolescents. *General psychiatry*, *36*(2), e101003. https://doi.org/10.1136/gpsych-2022-101003

183. Drug misuse in England and Wales: year ending June 2022. London: Office for National Statistics; 2022 (https://www.ons.gov.uk/peoplepopulationandcommunity/crimeandjustice/articles/drugmisuseinenglandandwales/latest, accessed 6 July 2023).

184. Darke S, Zahra E, Dufluo J, Peacock A, Farrell M, Lappin J. Characteristics and circumstances of volatile solvent misuse-related death in Australia, 2000–2021. Clin Toxicol. 2023;61(4):260–5. doi:10.1080/15563650.2023.2184243.

185. Byard R, Heath K. How common are suicides using nitrous oxide? Med Sci Law. 2019;59(3):160–3. doi:10.1177/0025802419852184.

186. Wagner SA, Clark MA, Wesche DL, Doesdens DJ, Lloyd AW. Asphyxial deaths from the recreational use of nitrous oxide. J Forensic Sci. 1992;37(4):1008–15. PMID:1506823.

187. Backstrom B, Johansson B, Eriksson A. Death from nitrous oxide. J Forensic Sci. 2015;60(6):1662–5. doi:10.1111/1556-4029.12879.

188. Nitrous oxide market size, share & trends analysis report. San Francisco (CA): Grand View Research; 2016 (https://www.grandviewresearch.com/industry-analysis/nitrous-oxide-market, accessed 20 August 2023).

189. Nitrous oxide: updated harms assessment. London: Advisory Council on the Misuse of Drugs; 2023 (https://www.gov.uk/government/publications/nitrous-oxide-updated-harms-assessment, accessed 17 September 2023).

190. Ministers press ahead with laughing bas ban in 2023. Dutch News, 15 November 2022 (https://www.dutchnews.nl/2022/11/ministers-press-ahead-with-laughing-gas-ban-in-2023/, accessed 27 August 2023).

191. Nitrous oxide regulations. Adelaide: Government of South Australia, Attorney-General's Department; 2022 (https://www.agd.sa.gov.au/law-and-justice/legislation/nitrous-oxide-regulations, accessed 27 August 2023).

Not cited:

Das RK, Tamman A, Nikolova V, et al. 2016. Nitrous oxide speeds the reduction of distressing intrusive memories in an experimental model of psychological trauma. *Psychological Medicine*. 46:1749–1759.

EU 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. *In:* Union European (ed.). Brussels (Belgium): Official Journal of the European Union.

Kingery WS, Sawamura S, Agashe GS, et al. 2001. Enkephalin release and opioid receptor activation does not mediate the antinociceptive or sedative/hypnotic effects of nitrous oxide. *European Journal of Pharmacology* 427: 27–35.

Quock RM, Emmanouil DE, Vaughn LK, et al. 1992. Benzodiazepine mediation of behavioral effects of nitrous oxide in mice. *Psychopharmacology*. 107:310-314.