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Nitrous oxide causes peripheral neuropathy in a dose dependent manner among recreational users. — Source link

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Abstract:	Background Nitrous oxide (N2O) has been used in clinical and recreational settings for over 150 years. Through inactivation of the Vitamin B12 -dependent enzyme methionine synthase N2O can lead to the development of peripheral neuropathy. This study sought to determine the relationship between exposure and risk of neurological symptoms in the largest ever sample of users. Design Data is drawn from the Global Drug Survey (GDS) over three consecutive years (2014-2016). The GDS is an online, cross sectional survey of substance use, translated into multiple languages. Participants Respondents to the GDS who indicated they had used N2O in the previous 12 months (n=16,239) Measurements Questions relating to N2O use, peripheral neuropathy, age and gender, were explored among last year users Findings Of 241,566 respondents 41,181 (17.0%) indicated ever using nitrous; of these 17,325 (42.1%) had used in the last 12 months. Overall 3.4% (n=561) reported persistent numbness / tinging (paraesthesia) in their hands or feet. Although the risk was very low among infrequent users, there was a strong dose-response relationship. For people indicating one or two doses per session the probability of reporting paraesthesia was approximately 0.018 by comparison, for people indicating 100 doses per session the probability was approximately 0.085. The association between dose and paraesthesia was influenced by gender and age. Conclusion While infrequent, episodic users are not at risk, a minority of heavy users are at dose dependent risk of developing serious neurological consequences. Better education and raised awareness of early symptoms

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TITLE PAGE

Manuscript title

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Key words nitrous oxide, neuropathy, vitamin B12, neurological deficits, laughing gas

Conflict of interest. ARW is the founder and CEO of Global Drug Survey Ltd, an independent, self-funded research organisation. JAF is part of the GDS core research team. Neither author received any funding to support the production of this manuscript.

Abstract (word count 283)

Background

Nitrous oxide (N2O) has been used in clinical and recreational settings for over 150 years. Through inactivation of the Vitamin B12 -dependent enzyme methionine synthase

N2O can lead to the development of peripheral neuropathy. This study sought to determine the relationship between exposure and risk of neurological symptoms in the largest ever sample of users.

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Data is drawn from the Global Drug Survey (GDS) over three consecutive years (2014-2016). The GDS is an online, cross sectional survey of substance use, translated into multiple languages.

Participants

Respondents to the GDS who indicated they had used N2O in the previous 12 months (n=16,239)

Measurements

Questions relating to N2O use, peripheral neuropathy, age and gender, were explored among last year users

Findings

Of 241,566 respondents 41,181 (17.0%) indicated ever using nitrous; of these 17,325 (42.1%) had used in the last 12 months. Overall 3.4% (n=561) reported persistent numbness / tinging (paraesthesia) in their hands or feet. Although the risk was very low among infrequent users, there was a strong dose-response relationship. For people indicating one or two doses per session the probability of reporting paraesthesia was approximately 0.018 by comparison, for people indicating 100 doses per session the probability was approximately 0.085. The association between dose and paraesthesia was influenced by gender and age.

Conclusion

While infrequent, episodic users are not at risk, a minority of heavy users are at dose dependent risk of developing serious neurological consequences. Better education and raised awareness of early symptoms is required.

Introduction

Nitrous oxide (N2O, laughing gas) is a colourless odourless gas that has a long history of use both in clinical settings as an anaesthetic and analgesic agent[1] and within recreational settings.[2+3] First popularised by Sir Humphry Davy in Victorian England[4] the inhalation of nitrous oxide produces an extremely short lived but intense euphoria with feelings of dissociation and mild changes in perception of body image, distinct from other inhalants and being described as more pleasant and psychedelic.[5] Often used in the context of poly-drug use it can enhance the effects of other drugs.

Nitrous oxide's psychoactive and anaesthetic effects are thought to be mediated through N-methyl-D-aspartate (NMDA) antagonism, decreasing excitatory neurotransmission throughout the CNS by non-competitive glutamate inhibition.[6] Nitrous oxide also acts as a partial mu, kappa and delta opioid receptor agonist modulating dopamine activity within the nucleus accumbens, that may in part be responsible for its analgesic effect.[7]

Although compared to other drugs nitrous oxide is remarkably safe, acute harms are seen following the recreational use of nitrous oxide, including falls, freezing of the lips, accidental injury, confusion, and hallucinations.[3] Fatalities are very rare; most fatalities are commonly reported following accidental asphyxiation due to nitrous oxide ability to displace oxygen in a closed space (in clinical practice nitrous oxide is combined with oxygen to minimise hypoxia).[8+9]. Underlying much of its clinical toxicity is nitrous oxide's interaction with vitamin B12 transforming the active monovalent from of B12, by irreversible oxidation into the inactive bivalent form.[10+11] Vitamin B12 is a co-factor for the conversion of L-methylmalonyl coenzyme A into succinyl coenzyme A[12] and for methionine synthase which produces methionine by methylation of homocysteine. The latter is required for DNA synthesis and the maintenance of the myelin sheath, with B12 deficiency causing demyelination within the central and peripheral nervous systems. Nitrous oxide-induced oxidation of vitamin B12 thus results in impairment of methylation reactions and DNA synthesis and an accumulation of homocysteine.[13] Other mechanisms for the observed neurological sequelae have been proposed including interference with the cytokine

system.[14] Complications attributable to interference with vitamin b12 metabolism[15] have been reported including megaloblastic anaemia,[16] myeloneuropathy,[17] subacute combined degeneration of the cord[18] and reversible psychosis.[19]

In previous work,[3] GDS had described current patterns of recreational nitrous oxide use and the 12-month incidence of acute adverse events. We reported that 4.2% of last year users reported persistent numbness in their limbs following use. In this current paper, we build on this research and use the biggest sample of nitrous oxide users ever recruited to better define neurological symptoms and explore any dose response relationship between exposure to nitrous oxide and symptoms consistent with peripheral neuropathy (persistent numbness). We hypothesize a positive significant relationship between 'per session' dose rate of nitrous oxide and frequency of use and reporting of symptoms consistent with B12 related peripheral neuropathy. Further, we will explore if differences exist that relate to a person's gender and age in the dose-response relationship of nitrous oxide and persistent numbness.

Study Design and Sample

The Global Drug Survey (GDS) runs the world's largest online drug survey. It is a cross sectional survey, self-completed, anonymous survey which has been conducted annually since 2012. GDS is translated into multiple languages and is promoted through partnerships with media organisations, harm reduction organisations and social media networks. Participants engage the survey on a self-nominating basis; as such, responses are not nationally representative, and data are drawn from a non-representative sample. For more information, see Barratt et al.'s paper [20] which provides extensive detail around the GDS methodology including the history of GDS, orientation towards specific group (i.e., drug users), survey design, and recruitment approaches. Ethical approval from the Kings College London Psychiatry, Nursing and Midwifery Research Ethics Subcommittee (PNM RESC: see PNM 141/02, PNM 14/15-18) and University of Queensland (Reference: 2017001452/11671/001).

For the current analysis, we combined identical questions exploring nitrous oxide use and associated health harms and socio-demographic variables collected as part of GDS2014, GDS2015 and GDS2016, conducted over November and December for each year 2013-2015 respectively. Data preparation and cleaning is described elsewhere.[20] Only cases where participants reported using nitrous oxide in the last twelve months are retained for analysis. As a conservative measure, to ensure data across the three years only contains unique cases, where participants reported that they had participated in the GDS in a previous year, these data were removed.

Measures

Demographic data used for analysis include gender (male and female only) and age (continuous). All participants were presented with a 'drug screen' module were participants indicated if they had ever used, used in the last 12 months or used in the last month over 150 drugs. Participants who indicated nitrous use during the last 12

months were also presented with questions about their context of use, source of substance, route of administration, frequency of use and quantity used.

To estimate the dose consumed on a day of use, we used the same approach adopted in our previous study[3] asking "how many hits (inhales) would you have on a day that you use?" (while not explicitly asked we determine a "hit" to represent one bulb of nitrous oxide). One bulb of nitrous oxide, pressurised at 7-9 bars, contains approximately 10ml of nitrous oxide in liquid form[2] and is sufficient to inflate one large-size 12-inch latex party balloon.[21] This is the most widely adopted method of administering nitrous oxide within recreational settings.

To assess the presence of peripheral neuropathy respondents answering the GDS 2014 survey were asked whether they had ever experienced "Persistent numbness/tingling in your arms/legs (lasting days and weeks after using)?" Response options were: yes (in last 12 months), yes (not in last 12 months), and no. In GDS 2015 and GDS 2016 to better capture peripheral neuropathy respondents were asked two separate questions: "Numbness / tingling around the face or mouth

that has persisted for at least 2 weeks following your last use of nitrous and that you had not experienced before you started using nitrous?" and "Numbness / tingling in hands or feet that has persisted for at least 2 weeks following your last use of nitrous and that you had not experienced before you started using nitrous?". Response options were: yes (within last 12 months), yes (not within last 12 months) and no. To maintain consistence across the three years of data, a binary response proxy (yes/no) for recent peripheral neuropathy was constructed from the GDS 2014 question and the GDS 2015 and GDS 2016 question relating to numbness / tingling in hands or feet. If the respondent indicated yes (within the last 12 months) this was coded as yes (experienced paraesthesia) or if the respondent indicated no this was coded as no (did not experience paraesthesia). Participants who indicated yes (not within the last 12 months) were excluding from analysis.

Analyses

Data were managed and analyzed using Stata 15.0.[22] When presenting descriptive statistics, to account for missing data, valid percentages are reported rather than absolute values. To examine gender differences in consumption practices and risks

of neurological harm we employ chi-squared and Fisher's exact tests. Finally, we use multivariable logistic regression models to examine whether specific demographics (age and gender) are associated with greater propensity for participants to report neurological harms. For models were age is included as a covariate both the linear form and the quadratic form of age is examined.

The following generalised logistic regression model (1) where $\pi_i = Pr(Y_{i=1}|X_i = x_i)$, Y_i=1 if respondent indicates tingles and 0 otherwise, X=(X_1, $X_{2,\ldots,}$ X_k) represents any type of covariate (e.g. continuous, dichotomous)

$$logit(\pi_i) = log\left\{\frac{\pi_i}{1-\pi_i}\right\} = \beta_0 + \beta_1 x_{i1} + \beta_k x_{ik} \quad (1)$$

Only the best fit model, based on AIC, BIC and likelihood ratio test statistics will be presented. As the method of recruitment was purposive, confidence intervals are provided to illustrate the range of values associated with the sample's standard error and to compare differences in point estimates. Both descriptive and inferential analyses were undertaken using Stata 15.0.[38] All statistical tests were two tailed and significance level was set at 0.05.

Results

Across the 3 years of data, there were 241,566 respondents; over 80,000 respondents in 2015 and 2016 (see table 1).

In total 17,325 respondents indicated using nitrous in the last 12 months; of these 812 respondents did not indicate how much nitrous oxide they used per session. Of the 16,513 respondents indicating a dose per session the median number of doses was around 5 (see Table 1) but this ranged from 1 dose (n=1,344; 8.1%) to 100 or more doses (n=130; 0.8%). Of these 16,513 respondents a further 274 did not provide a valid response to experiencing paraesthesia. The following analysis is based on the 16,239 respondents who indicated using nitrous oxide in the last 12 months, provided a per session dose response and provided a valid answer to experiencing paraesthesia.

Fitting the base model (see Table 2, Model 1), with the independent variable natural log of per session dose - ln(nitrous dose), suggests a significant relationship between dose and paraesthesia. That is, for every 10 percent increase in dose the there is a 3.5% increase in the likelihood of reporting paraesthesia. Figure 1 highlights the dose-response curve between number of doses per session and the predicted probability of reporting paraesthesia. For example, of the 1,313 (8.1%) respondents reporting only one nitrous oxide dose in a session, the probability of reporting paraesthesia was 1.86% (95% CI: 1.52--2.19). By contrast, for respondents reporting 20 nitrous oxide doses in a session, the probability of reporting paraesthesia was 5.04% (95% CI: 4.44—5.65) and for respondents reporting 100 nitrous oxide doses in a session, the probability of reporting paraesthesia was 8.48% (95% CI: 6.58—10.4).

Of the 16,124 respondents 11,184 (69.3%) were men. Including gender as a covariate in the fitted model (see Table 2, Model 2) suggests that the dose-response curve relating to nitrous use and reporting paraesthesia significantly differs for men

and women. As depicted in Figure 2, at low doses, 8 doses per session or less, the predicted probability for reporting paraesthesia is significantly greater for men than for women. By 16 doses per session the two curves intersect and following this the predicted probability of reporting paraesthesia is greater for women than for men. However, due to relatively small numbers of respondents using 16 doses or more (men = 1,311 (11.7%); women = 457 (9.3%) there is insufficient power for this difference to be significant. By 100 doses of nitrous oxide, the predicted probability for women reporting paraesthesia was 12.1% (95% CI: 7.16—17.0%) compared to men 7.2% (95% CI: 5.3—9.2%); the Wald test for statistical differences was (χ^2_1=3.22; p=0.073).

Of the 16,239 respondents the median age was 22 years (25th percentile: 19 years and 75th percentile: 25 years). Including age as a covariate in the fitted model (see Table 2, Model 3) the best model was including age only as a main effect. This suggests that the main effects of age and dose per session significantly influence respondents reporting paraesthesia but, per there is no statistically significant interaction between the covariates. As depicted in Figure 3, the younger the

respondent the more likely the respondent would report paraesthesia; similarly, the greater the dose per session the greater the predicted probability of reporting paraesthesia. A respondent who was 40 years of age reporting two doses per session had a predicted probability of approximately 1 to 2 percent; a 16-year-old, by comparison, having the same dose would have a predicted probability of reporting tingle between 3-4 percent.

The Full Model (see Table 2) includes the significant covariates gender (and the interaction term with nitrous dose) and age. Overall, for men, compared to women, the predicted probabilities for reporting paraesthesia, is substantially less as the nitrous dose per session increases. Moreover, at younger ages, compared to women, men are more likely to report paraesthesia, however the increased probability of reporting paraesthesia is less for men compared to women as the nitrous oxide dose increases. Finally, for young men indicating up to 100 doses per session, the predicted probability of reporting paraesthesia is less than 10% by comparison, for young women using up to 100 doses per session the predicted probability of reporting paraesthesia is almost 16%.

Limitations

Consumption of nitrous oxide is based upon self-report and is to recall bias. None of the neurological symptoms or functional disturbances reported by users were confirmed by clinical examination and there is no supportive biochemistry to determine levels of vitamin B12. However, our choice of clinical symptoms reflects the most common neurological presentations of those with B12 deficiency and would be easily recognisable by the person themselves. For example, in a study of 143 patients with B12 deficiency assessed by Healton,[22] almost three quarters presented with neurologic symptoms, with isolated numbness or paraesthesia being present in a third and gait abnormalities in 12%. Our choice of symptoms is also supported by those first reported by Layzer[17] and more recently seen as presenting symptoms in cases associated with recreational nitrous oxide abuse including uncomfortable tingling sensations in the feet and poor balance,[23] hand numbness and difficulty with fine motor movements and gait ataxia[15+24] The limitations and utility of non-probability samples in exploring drug related harms are addressed in previous work published by the group.[20]

Discussion

This study represents the largest investigation of neurological symptoms among nitrous oxide users ever conducted with over 16,000 last year users recruited for the study over 3 years. Our findings support our hypothesis that nitrous oxide causes peripheral neuropathy in a dose dependent manner among recreational users. We present a clear dose response relationship that is both statistically significant and clinically relevant to a large of number of users of this easily accessible drug around the world. While our findings also suggest that for the vast majority of consumers who use infrequently and at low levels, its use poses little or no risk, the potential neurological consequences for a minority of users warrants effective health promotion and raised awareness among users and clinicians. The greater vulnerability among women reflects the higher incidence of Vitamin B12 in the women of reproductive age25+26 and perhaps related to diet. For example, those adhering to vegetarian and vegan diets were more susceptible to nitrous induced neurological symptoms is consistent with the high rates of B12 deficiency in these

population due to suboptimal intake, with rates of 21-40% noted among adolescents.[27]

Nitrous oxide induced neuropathy was first reported nearly 40 years ago, among 3 habitual users who presented with a mainly sensory but disabling peripheral neuropathy with numbness that was 'radicular rather than purely distal'.[15] Initial case reports of nitrous oxide-induced myeloneuropathy typically involved health care professionals with presenting clinical features comprising of patchy tingling and paraesthesia in the hands and feet, weakness, loss of balance, difficulty with walking impaired manual dexterity, poor balance and leg weakness.[28-29] The first case report of polyneuropathy associated with the use of whippets was made in 1978.[30] Since that time case series involving recreational users have been reported internationally, typically in the context of heavy chronic exposure have appeared.[15+16, 23+24+ 31]

Rarely presenting with the classic triad of weakness, sore tongue, and paraesthesia, the varied array of non–specific symptoms (including depression, irritability and personality change) can often the lead to the diagnosis being overlooked or

attributed to other conditions.[32+33] In otherwise healthy well-nourished individuals, a presentation of a peripheral neuropathy and functional loss should prompt direct enquiry about recreational use of nitrous oxide. Imaging may be useful assess for the presence of Subacute Degeneration of the cord (SACD) typically showing abnormal hyper intensity of the dorsal columns.[15] The neurological presentation may be diverse however with sensorimotor peripheral neuropathy occurring in the absence of clinical or imaging evidence of myelopathy.[24]

Early diagnosis and prompt treatment are required to avoid disease progression and to increase the chance of full recovery, with response to treatment strongly related to the severity and duration of the condition before treatment.[22+34]

High dose oral vitamin B12 (1-2mg/day) may be effective in reversing anaemia and neurological symptoms, although high dose injection with loading doses and weekly supplements leads to swifter resolution of symptoms and should be considered in those presenting with more significant deficits. [35] Full neurological recovery is seen in about half of those with Vitamin B12 deficiency following treatment.[22] However, in

those presenting late even high dose therapy may leave individuals with persisting disability, including those associated with recreational abuse.[24] Biochemical diagnosis relies upon a combination of tests including serum B12, holotranscobalamin (holoTC) (both reduced) and methylmalonic acid levels (raised) [36+37]. Response and compliance should be determined with both clinical and biochemical (vitamin B12, homocysteine, and methylmalonic acid levels) two to three months after initiating treatment.[33+36]

Nitrous oxide use is unlikely to diminish as result of change in regulation or enforcement. From a public health perspective, smart education not blunt regulation is required. The most effective response is likely to be targeted health promotion campaigns raising awareness of early neurological symptoms among heavy consumers and signposting the need for cessation of use and urgent self-referral should index symptoms be experienced. Clinicians across the field require a high index of suspicion and direct enquiry about the use of nitrous oxide to allow the early diagnosis and treatment of nitrous oxide induced B12 deficiency.

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Tables and figures

Table 1: Demographic and nitrous use characteristics of respondents from GDS 2014, 2015, and 2016

	2014	2015	2016	All years
Count	72,765	86,616	82,185	241,566
Gender: male (%)	48,591 (67.1)	51,914 (60.6)	52,681 (64.9)	153,186 (64.0)
Missing†	375	950	963	2,288
Age: $\bar{x}, sd, p50$	29.6, 10.9, 26	28.8, 10.6, 25	28.3, 10.6, 25	28.9, 10.7, 25
Nitrous use				
Ever (%)	13,714 (18.9)	13,367 (15.4)	14,100 (17.2)	41,181 (17.1)
Last 12 months (%) of ever used	4,741 (34.6)	5,624 (42.1)	6,960 (49.4)	17,325 (42.1)
Last month (%) of last 12-month use	1,603 (33.8)	1,940 (34.5)	2,574 (37.0)	6,117 (35.3)
Nitrous dose (per session): *p50, p25-p75*	5, 3-10	4, 3-8	5, 3-10	5, 3-10

Missing	271	258	283	812
Paraesthesia (proxy for peripheral neuropathy) of those using in last 12 months (%)¥	198 (4.4)	160 (3.0)	203 (3.0)	561 (3.4)
Missing	192	263	274	729

†Missing include transgender (n=848)

¥ Percentage excludes count of missing

Table 2: Bivariable and multivariable logit models: DV – experiencing persistent numbness/paraesthesia in the hands or feet in the last 12 months. IV – log(nitrous oxide dose per session). Additional covariates include gender (male/female) and age, and interaction terms with IV

	Model 1: Dose (N=16,239)	Model 2: Model 1 + Gender (N=16,124)	Model 3: Model 1 + Age¥ (N=16,239)	Full model: Models 1, 2 + 3 (N=16,124)
Ln(nitrous dose)	0.35 (0.26 to 0.43) p<0.001	0.56 (0.39 to 0.73) p<0.001	0.36 (0.27 to 0.44) p<0.001	0.57 (0.40 to 0.74) p<0.001

Gender: Male		0.82 (0.39 to 1.26) p<0.001		0.85 (0.41 to 1.28) p<0.001
Gender × Ln(nitrous dose)		-0.30 (-0.50 to -0.11) p=0.002		-0.30 (-0.49 to -0.10) p=0.003
Age			-0.04 (-0.06 to -0.02) p<0.001	-0.04 (-0.06 to -0.02) p<0.001
Constant	-3.97	-4.56	-3.06	-3.64
LR χ^2; df	60.78; 1	72.88; 3	84.78; 2	98.41; 4
Pseudo R^2	0.0128	0.0155	0.0178	0.0209
Linktest: _hat (β;p-value)	3.71; p=0.024	2.28; p=0.028	2.21; p=0.038	1.80; p=0.040
Linktest: _ hat^2: (β;p-value)	0.42; p=0.100	0.19; p=0.213	0.18; p=0.252	0.120; p=0.357
Goodness-of-fit (df; χ^2; p-value) (10 groups)	6; 8.12; p=0.230	8; 6.72; 0.567	8; 7.14; 0.5221	8; 16.99; 0.030

¥ LR test with (age^2, age and interaction terms) vs (age and interaction term) = LR $\chi^2_{(2)} = 2.45$, p=0.293; (age and interaction term) vs (age and no interaction) = LR $\chi^2_{(1)} = 1.25$; p=0.264. Therefore, on parsimonious grounds (age and no interaction) is best model.

Figure 1: Predicted probability of reporting paraesthesia given number of doses per session.

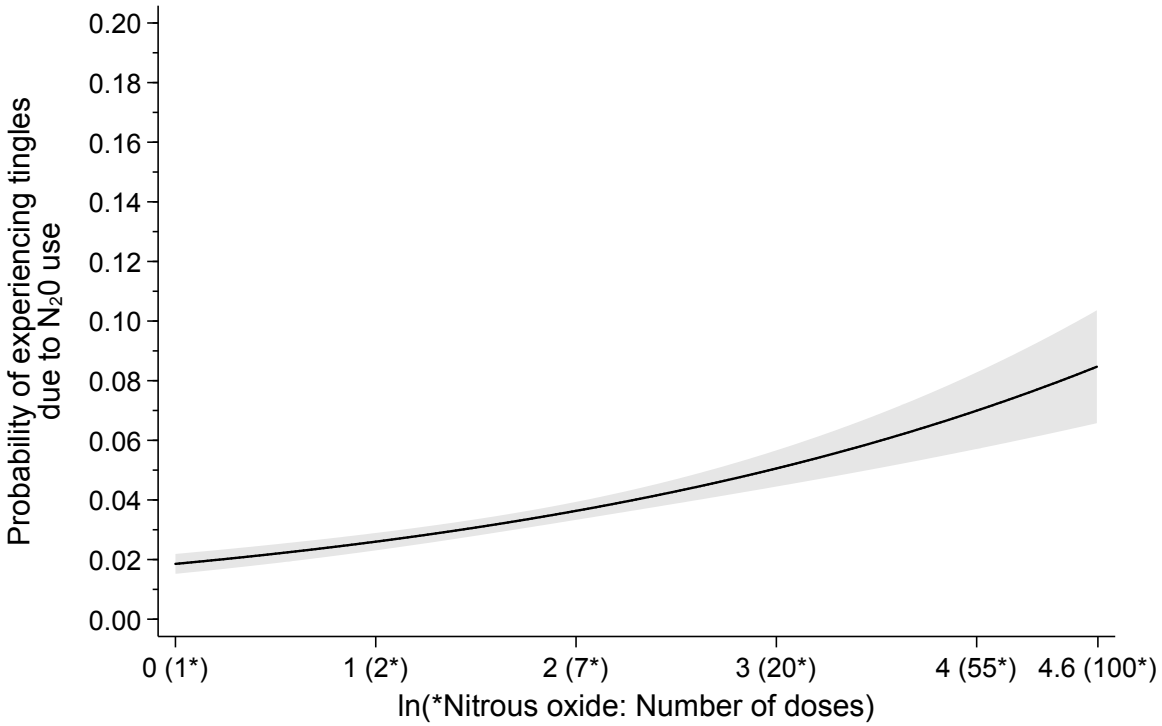


Figure 2: Stratified by gender, predicted probability of reporting paraesthesia given number of doses per session.

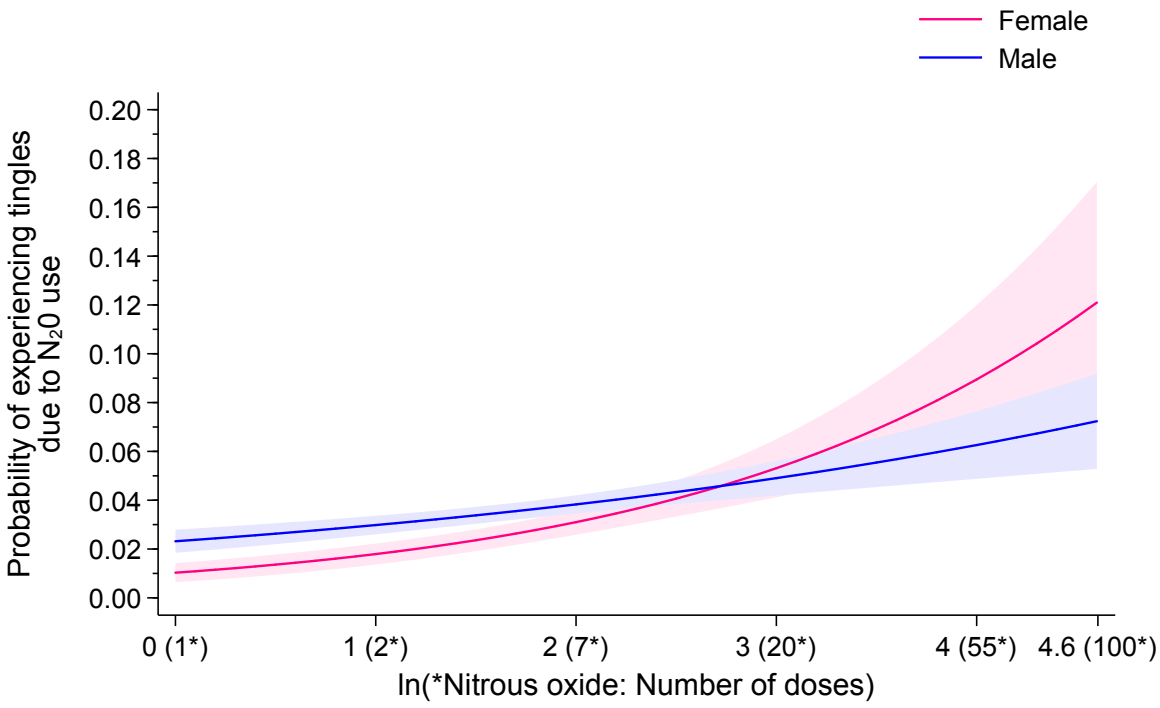
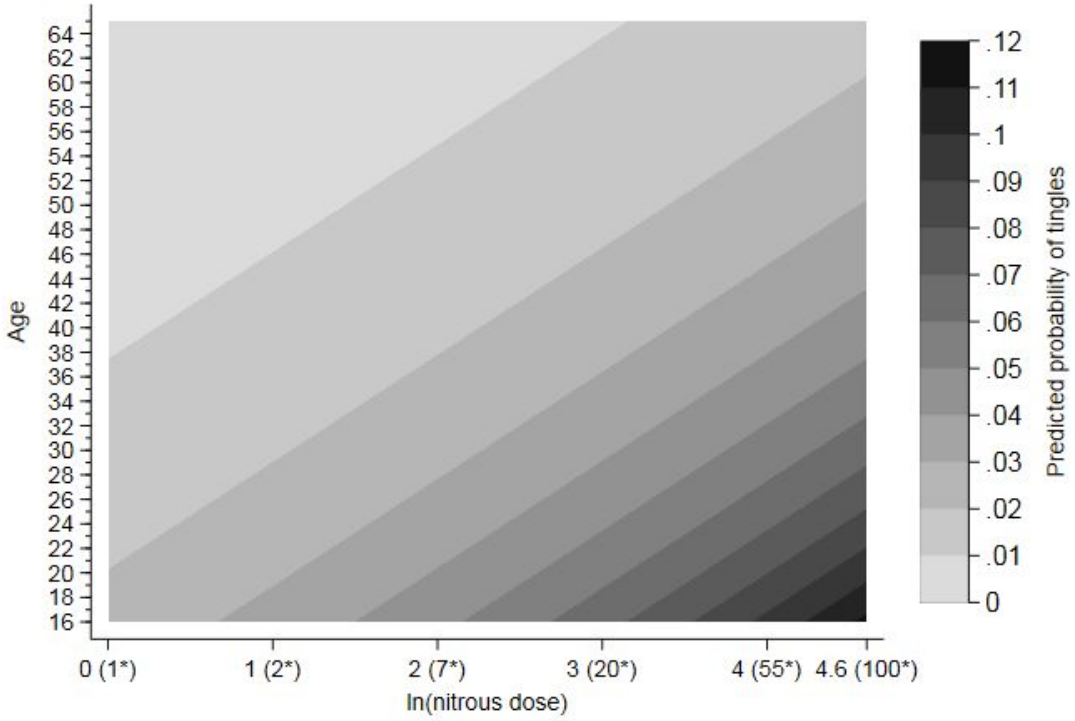


Figure 3: Predicted probability of paraesthesia by age (in years) and ln (nitrous dose).



References

1. Brown SM, Sneyd JR. Nitrous oxide in modern anaesthetic practice. *BJA Education* 2016; **16**(3): 87-91.

2. van Amsterdam J, Nabben T, van den Brink W. Recreational nitrous oxide use: Prevalence and risks. *Regulatory Toxicology and Pharmacology* 2015; **73**(3): 790-6.

3. Kaar S, Ferris J, Waldron J, Devaney M, Ramsey J, Winstock A. Up: the rise of nitrous oxide abuse. An international survey of contemporary nitrous oxide use. *Journal of Psychopharmacology* 2016; **30**(4): 1-7.

4. Davy H. Researches, chemical and philosophical: Chiefly concerning nitrous oxide or dephlogisticated nitrous air, and its respiration. London, United Kingdom: Biggs and Cottle; 1800.

5. 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 and Alcohol Dependence* 2006; **81**(1): 89-95.

6. Jevtovic-Todorovic V, Todorovic SM, Mennerick S, et al. Nitrous oxide (laughing gas) is an NMDA antagonist, neuroprotectant and neurotoxin. *Nat Med* 1998; **4**(4): 460-3.

7. Gillman MA, Lichtigfeld FJ. Clinical role and mechanisms of action of analgesic nitrous oxide. *Int J Neurosci* 1998; **93**(1-2): 55-62.

8. Ghodse AH, Corkery J, Ahmed K, Schifano F. Trends in UK deaths associated with abuse of volatile substances, 1971-2009. St George's, University of London, UK: Volatile Substance Abuse (VSA) Mortality Project International Centre for Drug Policy, 2012.

9. Wagner SA, Clark MA, Wesche DL, Doedens DJ, Lloyd AW. Asphyxial Deaths from the Recreational Use of Nitrous-Oxide. *J Forensic Sci* 1992; **37**(4): 1008-15.

10. Banks RGS, Henderson RJ, Pratt JM. Reactions of Gases in Solution .3. Some Reactions of Nitrous Oxide with Transition-Metal Complexes. *J Chem Soc A* 1968; (12): 2886-+.

11. Chanarin I. The Effects of Nitrous-Oxide on Cobalamins, Folates, and on Related Events. *Crc Cr Rev Toxicol* 1982; **10**(3): 179-213.

12. Krautler B. Biochemistry of B12-cofactors in human metabolism. *Subcell Biochem* 2012; **56**: 323-46.

13. Flippo TS, Holder WD. Neurologic Degeneration Associated With Nitrous Oxide Anesthesia in Patients With Vitamin B12 Deficiency. *Archives of Surgery* 1993; **128**(12): 1391-5.

14. Hathout L, El-Saden S. Nitrous oxide-induced B-12 deficiency myelopathy: Perspectives on the clinical biochemistry of vitamin B12. *J Neurol Sci* 2011; **301**(1-2): 1-8.

15. Stockton L, Simonsen C, Seago S. Nitrous oxide-induced vitamin B12 deficiency. *Proc (Bayl Univ Med Cent)* 2017; **30**(2): 171-2.

16. Chiang TT, Hung CT, Wang WM, Lee JT, Yang FC. Recreational nitrous oxide abuse-induced vitamin B12 deficiency in a patient presenting with hyperpigmentation of the skin. *Case Rep Dermatol* 2013; **5**(2): 186-91.

17. Layzer RB, Fishman RA, Schafer JA. Neuropathy Following Abuse of Nitrous-Oxide. *Neurology* 1978; **28**(5): 504-6.

18. Chaugny C, Simon J, Collin-Masson H, et al. Vitamin B12 deficiency due to nitrous oxide use: Unrecognized cause of combined spinal cord degeneration. *Rev Med Interne* 2014; **35**(5): 328-32.

19. Sethi NK, Mullin P, Torgovnick J, Capasso G. Nitrous oxide "whippit" abuse presenting with cobalamin responsive psychosis. *J Med Toxicol* 2006; **2**(2): 71-4.

20. Barratt MJ, Ferris JA, Zahnow R, Palamar JJ, Maier LJ, Winstock AR. Moving on from representativeness: testing the utility of the Global Drug Survey. *Substance Abuse: Research and Treatment* 2017; **11**: 1-17

21. Butzkueven H, King JO. Nitrous oxide myelopathy in an abuser of whipped cream bulbs. *J Clin Neurosci* 2000; **7**(1): 73-5.

22. StataCorp. Stata: Release 15. Statistical Software. College Station, Texas: StataCorp LP; 2017.

22. Healton EB, Savage DG, Brust JCM, Garrett TJ, Lindenbaum J. Neurologic Aspects of Cobalamin Deficiency. *Medicine* 1991; **70**(4): 229-45.

23. Cheng HM, Park JH, Hernstadt D. Subacute combined degeneration of the spinal cord following recreational nitrous oxide use. *BMJ case reports* 2013; **2013**.

24. Thompson AG, Leite MI, Lunn MP, Bennett DL. Whippits, nitrous oxide and the dangers of legal highs. *Pract Neurol* 2015; **15**(3): 207-9.

25. Green R, Allen LH, Bjorke-Monsen AL, et al. Vitamin B12 deficiency. *Nat Rev Dis Primers* 2017; **3**: 17040.

26. Pawlak R, Parrott SJ, Raj S, Cullum-Dugan D, Lucus D. How prevalent is vitamin B(12) deficiency among vegetarians? *Nutr Rev* 2013; **71**(2): 110-7.

27. Allen LH. How common is vitamin B-12 deficiency? *Am J Clin Nutr* 2009; **89**(2): 693S-6S.

28. Brodsky JB, Cohen EN, Brown BW, Wu ML, Whitcher CE. Exposure to Nitrous-Oxide and Neurologic Disease among Dental Professionals. *Anesth Analg* 1981; **60**(5): 297-301.

29. Jameson M, Roberts S, Anderson NE, Thompson P. Nitrous oxide-induced vitamin B12 deficiency. *J Clin Neurosci* 1999; **6**(2): 164-6.

30. Sahenk Z, Mendell JR, Couri D, Nachtman J. Polyneuropathy from Inhalation of N2o Cartridges through a Whipped-Cream Dispenser. *Neurology* 1978; **28**(5): 485-7.

31. Nevins MA. Neuropathy after Nitrous-Oxide Abuse. *Jama-J Am Med Assoc* 1980; 244(20): 2264-.

32. Lindenbaum J, Healton EB, Savage DG, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. *N Engl J Med* 1988; **318**(26): 1720-8.

33. Oh R, Brown DL. Vitamin B12 deficiency. *Am Fam Physician* 2003; **67**(5): 979-86.

34. Ralapanawa DM, Jayawickreme KP, Ekanayake EM, Jayalath WA. B12 deficiency with neurological manifestations in the absence of anaemia. *BMC research notes* 2015; **8**: 458.

35. Langan RC, Goodbred AJ. Vitamin B12 Deficiency: Recognition and Management. *Am Fam Physician* 2017; **96**(6): 384-9.

36. Stabler PS Vitamin B12 deficiency. Clinical Practice. N Engl J Med 2013; 368:149-160

37. Green R Vitamin B12 deficiency from the perspective of a practicing hematologist Blood 2017 129:2603-2611