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BRIEF REVIEW

Health Hazards and Nitrous Oxide: A Time for Reappraisal

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Recent adoption by the American Conference of Governmental Industrial Hygienists of a Threshold Limit Value of 50 ppm for an 8-hour average exposure to nitrous oxide (N_2O) increases the likelihood for its regulation by state and federal occupational health agencies. This review outlines current information on the health risks of N_2O inhalation to provide a basis from which safe and reasonably attainable exposure limits can be proposed. Although N_2O was for many years believed to have no toxicity other than that associated with its anesthetic action, bone marrow depression in patients administered N_2O for extended periods of time and neurological abnormalities in health care workers who inhaled N_2O recreationally have disproved this notion. Retrospective surveys of dental and medical personnel have also linked occupational exposure to N_2O with a number of health problems and reproductive derangements. Nitrous oxide reacts with the reduced form of vitamin B_{12}, thereby inhibiting the action of methionine synthase, an enzyme that indirectly supports methylation reactions and nucleic acid synthesis. Many, if not all, of the nonanesthetic-related adverse effects of N_2O may be ascribed to this action. Animal and human studies indicate that the toxic effects of N_2O are concentration- and time-dependent. It is suggested that a time-weighted average of 100 ppm for an 8-hour workday and/or a time-weighted average of 400 ppm per anesthetic administration would provide adequate protection of dental personnel and be achievable with existing pollution control methods.

Over a decade has passed since a battery of reports alerted the dental profession to the potential health hazards of nitrous oxide (N_2O) in dentistry. In May 1977, the National Institute for Occupational Safety and Health (NIOSH) released its criteria document, *Occupational Exposure to Waste Anesthetic Gases and Vapors,* which recommended a maximum permissible time-weighted average exposure of 25 ppm N_2O per anesthetic administration for all health care workers.[1] In October of that year, in an issue of the *Journal of the American Dental Association* largely devoted to the topic of trace inhalation anesthetics in the dental office, the ADA Ad Hoc Committee on Trace Anesthetics as a Potential Health Hazard in Dentistry published its position paper.[2] Although the Committee had serious reservations regarding the research available then, it recognized the potential for a health hazard and strongly urged that, "every effort should be made to reduce, by presently existing technology, the trace concentration of anesthetic/sedative agents in the dental environment." In 1978, publication of two seminal papers would broaden concerns and lead the way to a possible understanding of the basis for adverse reponses to N_2O exposure. In one, Layzer et al reviewed three health workers who developed disabling peripheral neuropathy following habitual abuse of N_2O.[3] In the other, Amess et al found that in patients undergoing open heart surgery N_2O could cause hematopoietic changes indicative of pernicious anemia.[4] Subsequent important landmarks included the large-scale epidemiologic study of occupational disease in the dental profession and chronic exposure to N_2O by Cohen et al[5] and elucidation of the effects of N_2O on vitamin B_{12} biochemistry.[6,7]

The year 1978 also witnessed publication of two comprehensive and critical reviews of the scientific evidence that led to the NIOSH report.[8,9] These reviews served two purposes: (1) they presented the findings of the various studies in a form that facilitated comparison and evaluation; and (2) they pointed out methodological limitations that should caution the reader from making decisions or judgments based on insufficient or erroneous information. Perhaps in recognition of these shortcomings, the Occupational Safety and Health Administration (OSHA) de-

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Table 1. Changes in Peripheral Blood in Relation to Drugs Used in 13 Patients with Severe Tetanus. (From Lassen et al[11])

Treatment	*Patients With Complications**	*Patients Without Complications*
Chloral hydrate	3	7
Pentobarbital	5	5
Meperidine	0	1
Tubocurarine	3	0
Bromethol	0	1
Continuous N_2O	6	0

* Granulocytopenia and/or thrombocytopenia.
NOTE: All patients also received antibiotics, but these were not tabulated.

ferred establishing an official maximal exposure for N_2O. There are indications, however, that this restraint is about to end.

Recently, the American Conference of Governmental Industrial Hygienists (ACGIH) set a Threshold Limit Value of 50 ppm for an 8-hour average exposure to N_2O.[10] Although this organization has no regulatory power, OSHA historically has followed its recommendations. Under the rubric of the General Duty Clause of the 1970 Occupational Health and Safety Act, which requires employers to protect employees from recognized hazards in the workplace, OHSA inspectors are empowered to cite dentists for failure to equip N_2O delivery systems with scavenging circuits. It appears only a matter of time before a Permissible Exposure Limit of N_2O will be proposed.

Given this regulatory climate, a balanced understanding of the salient facts concerning health hazards of N_2O and recommendations and procedures for limiting occupational exposure becomes critically important.

BIOCHEMICAL BASIS FOR INJURY

Historically, N_2O was thought to be chemically inert in the human body. Adverse reactions to N_2O were attributed to hypoxia (from failure to administer sufficient oxygen with the anesthetic) or to known risks of general anesthesia (eg, respiratory or cardiac complications). The first hint the N_2O had toxic potential unrelated to anesthesia or hypoxia per se was offered by Lassen et al, who noted in a Lancet article in 1956[11] that severe bone marrow depression occurred after prolonged N_2O anesthesia in patients being treated for tetanus. In fact, two patients died as a consequence of septicemia that developed after severe leukopenia. A study of 13 patients indicated that the only consistent correlate between tetanus patients who exhibited hematologic complications and those who did not was the administration of N_2O (Table 1). Subsequent reports published shortly thereafter confirmed this association, but no explanation for the effect was given.

Nitrous Oxide and Vitamin B_{12}

Banks and co-workers reported in 1968[12] that N_2O could oxidize nonenzymatically the cobalt atom in reduced vitamin B_{12} (cob[I]alamin) as follows:

$$\text{Cob[I]alamin} + N_2O + H_2O \rightarrow \text{Cob[III]alamin} + N_2 + 2OH^- \quad (1)$$

$$\text{Cob[I]alamin} + \text{Cob[III]alamin} \rightarrow 2\ \text{Cob[II]alamin}$$

This discovery had biological implications because only the monovalent (reduced) form of vitamin B_{12} is active in vivo. However, buried among a number of similar observations of gases and transitional metal complexes, the reaction was not recognized as important clinically until Amess and associates made the connection between N_2O and pernicious anemia.[4]

Amess et al found that patients exposed to 50% N_2O for 6 to 24 hours developed megaloblastic changes in bone marrow cells and that these changes were accompanied by increases in the deoxyuridine suppression test, which measures in vitro the ability of deoxyuridine (dU) to compete with ^{3}H-thymidine for incorporation into bone marrow DNA:

$$\text{dU suppression} = \frac{^3\text{H-DNA in presence of dU}}{^3\text{H-DNA in absence of dU}} \times 100(\%) \quad (2)$$

A high dU suppression score indicates impaired conversion of dU monophosphate to thymidine monophosphate, a process ultimately dependent on active vitamin B_{12}. In these patients, as in patients with pernicious anemia, dU suppression scores were increased. A variety of studies quickly followed substantiating the fact that N_2O can inactivate vitamin B_{12} and therefore cause biochemical derangements similar to those seen in pernicious anemia.

Vitamin B_{12} is essential for the function of three enzymes in mammals: (1) methionine synthase (methionine synthetase), which catalyzes the synthesis of methionine and tetrahydrofolate from homocysteine and 5-methyltetrahydrofolate; (2) methylmalonyl-CoA mutase, which supports the transformation of methylmalonyl-CoA to succinyl-CoA; and (3) leucine 2,3-aminomutase, which permits the interconversion of leucine and β-leucine. The synthase and mutase enzymes differ in the form of the vitamin B_{12} cofactor used and in their susceptibility to inactivation by N_2O. Methionine synthase uses methylcobalamin and is rapidly affected by N_2O. The mutase enzyme relies on 5-deoxy-adenosylcobalamin and is essentially immune to direct inactivation. Since the Co^+ in 5-deoxyadenosylcobalmin is believed to be subject to oxidation just as it is in methylcobalamin, it is postulated that the mutase may work equally well with oxidized cofactor.[13] Because the vitamin B_{12}-dependent aminomutase enzyme is a fairly recent discovery,[14] it has not been investigated concerning

N_2O. Its reliance on 5-deoxyadenosylcobalamin as the cofactor implies, however, that it may be resistant to N_2O.

Exactly how N_2O inactivates methionine synthase is unknown. Recent biochemical studies suggest that a variant of the reaction described by Banks et al[12] might lead to both vitamin B_{12} oxidation and enzyme damage. In this reaction:

$$\text{Cob[I]alamin} + N_2O + H^+ \rightarrow \text{Cob[II]alamin} + N_2 + {}^{\cdot}OH \quad (3)$$

a hydroxyl radical is generated, which is highly reactive and could proceed to alter chemically the adjacent synthase enzyme. In support of this proposal is the finding in mice that administration of a hydroxyl radical scavenger impedes destruction of methionine synthase by N_2O.[15]

Metabolic Derangements

A variety of metabolic disturbances are caused by a decline in methionine synthase activity. As can be deduced from Figure 1, the biosynthesis of methionine, tetrahydrofolate, and thymidine monophosphate is diminished.[16] Derivatives of tetrahydrofolate (ie, 10-formyl- and 5,10-methenyltetrahydrofolate) essential to the formation of purine nucleotides are also affected, and DNA synthesis is impaired. A number of additional single-carbon transfer reactions dependent on S-adenosylmethionine and tetrahydrofolate analogues is likewise affected.

None of the substrates illustrated is totally dependent on methionine synthase activity for its existence, at least in the short term. Methionine is a natural constituent of the diet, and it can also be synthesized by way of the so-called betaine pathway; thymidine and purines are also dietary constituents and can be recycled through salvage pathways for reincorporation into nucleic acids; interconversion of folate analogues (with the notable exception of 5-methyltetrahydrofolate) can ameliorate temporarily the loss of tetrahydrofolate.

Clinically evident (but nonhypoxic or anesthesia-related) changes in response to N_2O inhalation depend on a variety of factors, many of which are co-dependent. These factors include:

(1) the intensity, duration, and pattern of exposure to N_2O;
(2) the degree of inactivation of methionine synthase and the time course of recovery;
(3) the extent to which body stores and dietary intake (including vitamin and/or amino acid supplements) offset the biochemical block caused by N_2O;
(4) the sensitivity of important biochemical pathways to substrate decrements; and
(5) the sensitivity of tissues to alterations by the affected biochemical pathways.

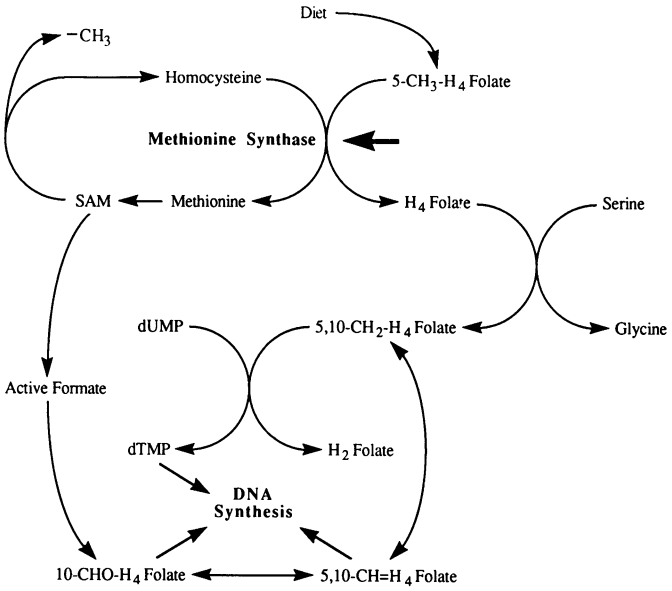


Figure 1. Biochemistry of methionine synthase-associated reactions (large arrow indicates block by N_2O). Major pathways involving folate analogues that support DNA synthesis are included. Abbreviations: 5-CH_3-H_4 Folate, 5-methyltetrahydrofolate; H_4 Folate, tetrahydrofolate; 5,10-CH_2-H_4 Folate, 5,10-methylenetetrahydrofolate; H_2 Folate, dihydrofolate; 5,10-CH=H_4 Folate, 5,10-methenyltetrahydrofolate; 10-CHO-H_4 Folate, 5,10-formyltetrahydrofolate; SAM, S-adenosylmethionine; $-CH_3$, methylation reactions; Active Formate, methylthioribose phosphate; dUMP, deoxyridine monophosphate; dTMP, thymidine monophosphate.

Factor 1 is under the control of the clinician, and the rest of this article will concentrate on how and to what extent exposure should be controlled. Factor 3 is also potentially modifiable, and the use of agents to prevent "biochemical" toxicity from N_2O will be mentioned. One caveat should be raised at this time. Although the biochemical basis for toxicity is strongly supported, it remains possible that other aspects of N_2O pharmacology may contribute to health hazards associated with exposure to the gas.[17] These include the formation of toxic substances during the metabolism of N_2O by enteric bacteria[18] and interaction with the endogenous opioid system leading to such physiological disturbances as decreased release of luteinizing hormone releasing hormone.[19]

TOXICITY AND CLINICAL EXPOSURE

Evidence is overwhelming that prolonged exposure to clinical concentrations of N_2O inhibits cellular proliferation of the formed elements of blood and can lead to megaloblastic anemia, leukopenia, and thrombocytopenia.[4,7,13] As shown in Figure 2, these hematopoietic changes are preceded by disturbances in thymidylate metabolism as measured by the dU suppression test.[7] Inhibition of

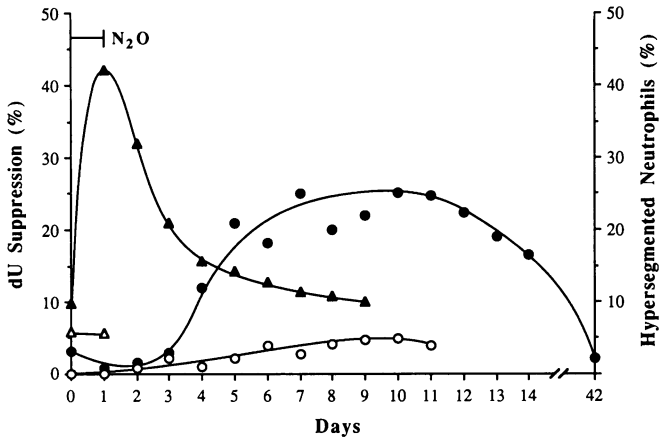


Figure 2. Hematopoietic toxicity of N_2O in humans. Mean dU suppression scores in 5 surgery patients receiving 50% N_2O for 24 hours (▲) and two patients receiving etomidate (△) are shown along with the mean percentage of hypersegmented neutrophils in peripheral blood (less 1 N_2O patient; ● = N_2O, O = etomidate). Abnormal thymidylate synthesis in the N_2O group presages an altered hematopoiesis. Data from Skacel et al.[7]

methionine synthase is an expected prerequisite for depression of thymidylate synthesis by N_2O. Whereas rats and mice are not susceptible to megaloblastic anemia during vitamin B_{12} deficiency, they are even more responsive[20,21] than humans[22] with respect to inhibition of methionine synthesis (Figure 3).

Biochemical recovery from exposure to clinical concentrations of N_2O does not begin until inhalation of the gas is terminated. Afterward, the induced derangements subside in association with the formation of active holoenzyme. Data (eg, as in Figure 2) suggest that several days may be required for full return of methionine synthase activity.[6,7] Slow reversal of N_2O toxicity raises the possibility that repeated exposures may lead to a cumulative effect. Indeed, Nunn et al have reported that a patient receiving 50% N_2O for 15 min 3 times a day for 24 days developed megaloblastic changes and increased dU suppression values.[23] Recovery rate may also depend on the health of the individual. Patients who can return immediately to a normal diet might hasten recovery be replacing some of the lost essential metabolites. At the other extreme, gravely ill patients are likely to be more seriously affected by the same degree of exposure to N_2O and take longer to recover.[24]

Several investigators have shown that the hematopoietic changes induced by N_2O can be reversed or even prevented by administration of appropriate agents. Vitamin B_{12} given after N_2O can at least partially offset the effect of N_2O as measured by the dU suppression test.[4,7] Folinic acid (formyltetrahydrofolate) and several analogues (eg, tetrahydrofolate) but not 5-methyltetrahydofolate can reverse or even prevent disturbances in nucleic acid synthesis and thus the hematologic sequelae to N_2O.[7,25,26] However, as in the neuromyopathy of pernicious anemia, folate derivatives have little influence on reactions dependent on methionine synthesis.

The clinical implications of the interaction between N_2O and vitamin B_{12} have been reviewed by Nunn and Chanarin.[13] Even though inactivation of methionine synthase must occur routinely, disturbances in cellular proliferation are generally quite minor. Nevertheless, continuous administration beyond 24 hours, or repeated administration more frequently than once every 3 to 4 days, will result in leukopenia and megaloblastic changes. Patients who have serious pre-existing hematologic disturbances or who are morbidly ill may have unique sensitivity to N_2O, but data are too scanty to make any particular recommendation for these patients.

Spontaneous abortion and fetotoxicity are important concerns regarding chronic exposure to N_2O in the workplace. It is not known if these concerns should also apply to the clinical use of N_2O. Crawford and Lewis[27] reviewed 433 operations in which N_2O was administered to women in the first two trimesters of pregnancy and failed to uncover a single instance in which N_2O caused any deleterious effect. Most of these anesthetics involved exposures under 30 min, however, and until this issue is explored more fully, the potential of N_2O for inhibiting DNA synthesis must be considered in any decision to use N_2O on a pregnant patient, especially when prolonged or multiple administrations are planned. Similarly, the influence of N_2O on leukocyte proliferation raises some concern over

Figure 3. Inactivation of hepatic methionine synthase in rats given 50% N_2O,[20] mice exposed to 80% N_2O,[21] and humans administered 50% to 70% N_2O.[22] Exposure to N_2O is calculated as the product of the concentration and the time.[13]

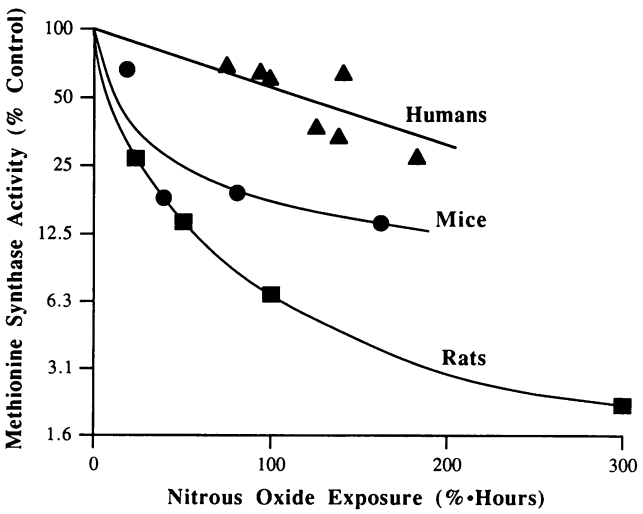


Table 2. Neurological Symptoms in 15 Patients with N_2O Myeloneuropathy. (From Layzer[33])

Symptom	*Patients With Symptoms Initially*	*Patients With Symptoms Later*
Lhermitte's sign	0	12
Numbness, paresthesia, or clumsiness of hands	7	13
Numbness or paresthesia in legs	7	15
Numbness of trunk	0	10
Impairment of equilibrium or gait	3	12
Inability to walk unassisted	0	7
Impotence	0	7
Impairment of sphincter control	0	4
Altered mood or difficulty in thinking	0	7
Relapsing course	—	6
Inability to work	—	10

its use in patients with severe infections. Although short-term (less than 6 hours) exposure probably is without potential danger insofar as affecting white cell counts is concerned,[25] Nunn and O'Morain have found that N_2O impairs mobilization of neutrophils from peripheral storage sites.[28] Nitrous oxide also inhibits neutrophil chemotaxis, but this effect is shared by other inhalation anesthetics and seems unrelated to a specific toxic effect of N_2O. Together, these data suggest that N_2O would be a suboptimal choice as a sedative/analgesic for infected patients requiring protracted or repeated anesthesia.

TOXICITY AND DRUG ABUSE

By modern definition, Humphrey Davy was the first abuser of N_2O. In his treatise on N_2O,[29] Davy described both the euphoric and the addicting potential of laughing gas:

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

He also described symptoms of chronic toxicity from overindulgence:

> *"During the last week in which I breathed it uniformly, I imagined that I had increased sensibility of touch: my fingers were pained by anything rough . . . My bodily strength was rather diminished than increased."*

N_2O began as a "social drug," and it is still used in that manner today. Occasional reports[30,31] of individuals being asphyxiated by improper self-administration have not dampened enthusiasm for N_2O as a safe drug available in pure form in medical gas cylinders or as a propellant for whipping cream.[32]

The clinical report by Layzer et al[3] describing the delayed development of a distinctive neurologic syndrome in 3 health care workers who used N_2O recreationally engendered communications of similar reactions, and Layzer was able to compile data quickly on 15 individuals with the same syndrome.[33] Only two of this group denied N_2O abuse; both of these were oral surgeons who were heavily exposed to the gas in their professional practice. Table 2 lists the major symptoms reported by the 15 patients. Initial symptoms consisted of numbness or parasthesia of the limbs. With continued abuse, sensory symptoms would worsen and motor impairment become manifest. Concomitant neurologic signs (Table 3) included evidence of muscle weakness, impaired sensation, and altered reflexes. Cessation of N_2O inhalation led to improvement of the myeloneuropathy, but residual deficits indicative of potentially permanent neural injury were noted in approximately half of the patients.

Although patients with N_2O myeloneuropathy generally have had normal serum folate and vitamin B_{12} concentrations at time of testing, the similarity of signs and symptoms with those of vitamin B_{12} deficiency naturally led to investigations of a linkage between the two conditions. The monkey has proved especially useful as a model. Monkeys self-administer N_2O when the gas is the sole reinforcer of behavior.[34] Moreover, exposure to 15% N_2O for 2 weeks causes ataxia, uncoordination, and degeneration of the spinal cord, effects that can be largely prevented by providing methionine supplementation in the diet.[35] Methionine has also been shown to reverse the neuropathy caused by vitamin B_{12} deprivation or N_2O administration in the pig[36] and fruit bat.[37] Interference with methionine metabolism may impair transmethylation reactions[36] and/or nucleic acid synthesis[38] vital to neuronal health.

The easy access to N_2O and a general lack of strict control of its use makes N_2O a simple substance to abuse for health professionals, particularly those in the dental profession. A questionnaire distributed to dental and medical students at an eastern university revealed that 16% of the 524 respondents had used N_2O in a social setting.[39]

Table 3. Neurologic Signs in 15 Patients with N_2O Myeloneuropathy During Active Stage of Illness and Recovery. (From Layzer[3])

Sign	*Patients During Active Stage*	*Patient With Residual Deficits*
Romberg sign or ataxic gait	11	4
Muscle weakness in distal legs	7	5
Muscle weakness in proximal legs	3	1
Muscle weakness in arms	4	0
Impaired sense of touch, vibration, or position	15	8
Diminished or absent knee jerks	8	3
Increased knee jerks	6	3
Diminished or absent ankle jerks	12	5
Increased ankle jerks	3	1
Extensor plantar reflexes	8	3

Using a questionnaire sent to licensed dentists in West Virginia who graduated from the West Virginia School of Dentistry between 1969 and 1977, Gutmann and Johnsen[40] found that 51% of the 143 respondents used N_2O regularly in practice. Eight user-dentists reported one or more neurologic symptoms in themselves or their assistants. Three of these respondents identified themselves and were interviewed. In two instances, recreational drug use was uncovered, one involving the dentist, the other involving two assistants. In the third case, the dentist used 50% N_2O on patients routinely (6 to 7 hours daily) without the benefit of scavenging equipment.

Taken together, the findings described in this section reasonably support several conclusions: (1) N_2O is a potential drug of abuse especially targeted to the dental profession; (2) A small percentage of dentists (possibly 1% to 5% of practitioners using N_2O in their offices), and some auxiliary personnel as well, self-administer N_2O, or at least did so during the late 1970s; (3) Repeated exposures can, probably by oxidation of vitamin B_{12} and inhibition of methionine synthesis, lead to myeloneuropathic disturbances; (4) Although the dangers of N_2O abuse should caution the reader against self-administration, it seems clear that such abuse is not a major public health concern, being overshadowed by more ubiquitous habituations, such as alcoholism and cigarette smoking. As will be mentioned in the next section, a final conclusion that can be drawn is that potential abusers of N_2O should be considered in evaluating survey results of health hazards to trace contamination of inhalation agents.

TOXICITY AND OCCUPATIONAL EXPOSURE

Unquestionably, the primary health concern regarding the use of N_2O in dentistry is the possibility of occupational disease resulting from chronic exposure to trace amounts of gas. The question was first raised by a report in the Russian literature describing a variety of ailments and an unusual number of miscarriages occurring in anesthesiologists.[41] Since 1967, numerous epidemiologic surveys, animal studies, and other investigations have been conducted in attempts to determine what the true dangers of chronically inhaling low concentrations of anesthetic agents are. Studies that relate directly to N_2O will be reviewed according to the research methodology used.

Retrospective Surveys

The large-scale epidemiologic study by Cohen and coworkers,[5,42] carried out in conjunction with the American Dental Association, remains the definitive epidemiologic survey of N_2O exposure and related health problems. It was an impressive effort and seemed to avoid major pitfalls (eg, low response rate, multiple anesthetic gases, inequality of control and at-risk groups) that have bedeviled previous studies of this kind.[8,9] Figures 4 and 5 summarize the statistically significant findings of this investigation of the relationship between N_2O exposure and health and reproductive outcome. Chief among these were a higher incidence of hepatic, renal, and neurologic disorders among exposed personnel, increased spontaneous abortions in chairside assistants and wives of male dentists, and congenital abnormalities in children born to chairside assistants.

Because of the important medical and legal implications of this work, and the possibility that a study of similar magnitude may never be attempted in the future, it deserves careful scrutiny. As with most surveys, the Cohen study was subject to possible respondent bias. The simple fact that inhalation agents were linked to a survey of health problems might have had some effect on respondents. Using recall as the sole source of evidence of adverse events occurring in the previous decade is a serious limitation to this study. Another question regarding the study derives from the fact that 18.7% of the dentists who were

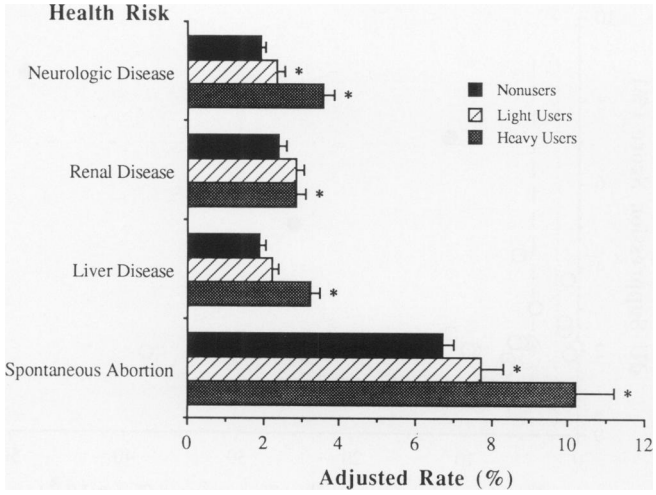
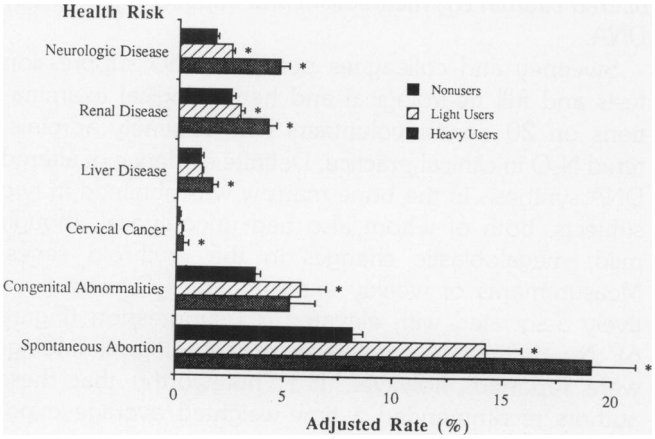


Figure 4. Incidence of health problems in male dentists, and spontaneous abortion in their wives, grouped by exposure to N_2O. Rates were adjusted for age, smoking, and pregnancy history. Light users were dentists with 1 to 2999 hours of exposure the previous decade; heavy users were exposed to 3000+ hours. For spontaneous abortion, light exposure was defined as 1 to 8 hours of exposure per week for the year beginning 6 months before conception. Brackets indicate the standard errors; asterisks reflect significant differences from nonusers ($P \leq 0.05$). Data from Cohen et al.[5]

exposed to N_2O were also exposed to halogenated anesthetics. Although subsequent analysis excluding these individuals from the incidence rates shown in Figures 4 and 5 did not significantly affect outcomes,[42,43] the simple fact that so many dentists were exposed occupationally to general anesthetics indicates that a high number of oral surgeons and/or hospital dentists were disproportionately included in the user category. Thus, the cohort groups might not have been as similar as one would otherwise have expected. A final concern involves the failure to exclude dentists and chairside assistants who abused N_2O. In Figure 4, for instance, heavy exposure to inhalation anesthetics was associated in male dentists with an 86% increase in neurologic disease. Yet, the total percentage of respondents reporting these disorders rose only 1.66%. This number could easily reflect dentists who inhaled high amounts of N_2O habitually and therefore be spurious with respect to occupational disease and trace gas exposure. Because abusers of N_2O may represent a special subset of respondents, it is possible that other problems, such as liver and kidney derangements, are also linked to this group.

Of all the findings of the Cohen group, the strongest evidence of a health hazard is spontaneous abortion in female chairside assistants. The absolute increase in miscarriage rate is much too high to be ascribed to recreational N_2O inhalation. It is also the most consistent finding in other surveys of this nature.[44] However, to quote from Tannenbaum and Goldberg, "The consistency of the results may be explained in part by consistency of the methodologic problems, specifically lack of criteria for exposure or outcome, poor survey response rates, selection bias, lack of validation of outcome, recall bias, and lack of control of potentially confounding variables.[45] It is interesting that two recently published restrospective studies that used examination of public health registries in Sweden[46] and in Finland[47] could find no link between working in an operating room and/or being exposed to anesthetic gases and miscarriage or congenital malformation.

Figure 5. Incidence of health problems and reproductive abnormalities in female chairside assistants grouped by exposure to N_2O. Rates were adjusted for age, smoking, and pregnancy history. Light users were assistants with 1 to 2999 hours of exposure the previous decade; heavy users were exposed to 3000+ hours. For spontaneous abortion, light exposure was defined as 1 to 8 hours of exposure per week for the year beginning 6 months before conception. Brackets indicate the standard errors; asterisks reflect significant differences from nonusers ($P \leq 0.05$). Data from Cohen et al.[5]



Animal Studies

An impressive number of animal investigations have been conducted to provide data on health and reproductive effects of N_2O administered in a wide array of concentrations and exposure times. Prolonged exposure to clinical concentrations has resulted in a variety of toxicologic effects in some but not all studies. In addition to the neurologic deficits described previously, these effects have included changes in growth,[48] hematopoiesis,[49] and testicular weight.[50] Similar concentrations (10% to 75% N_2O) have also been shown to cause fetal death, teratogenesis, and retarded development.[51,52] As a recent example, Fujinaga and Baden demonstrated that 50% to 75% N_2O increased the malformation rate of embryos grown in tissue culture 11-fold (from a control value of 6%) when administered for 24 hours on a day 9 of gestation.[53] Fetal toxicity can be separated from the anesthetic effect per se, as equipotent concentrations of xenon do not cause such disturbances.

Of special concern to health care workers is the minimal concentration or exposure at which toxic effects can develop. Alteration in methionine synthase activity is a sensitive indicator of a biological response to N_2O. The threshold for inhibition with continuous exposure is between 500 and 1000 ppm in the rat.[55] Studies on the lowest exposure that causes toxicity have yielded mixed results. Whereas Mazze and colleagues found no adverse reproductive responses to intermittent doses of N_2O (24 hours at day 9 of gestation or 4 hours per day on days 6 through 15) in subclinical concentrations (<25%),[56,57] Vieira et al determined that continuous exposure throughout pregnancy to 1000 ppm was associated with reduced litter size, skeletal anomalies, and prenatal growth retardation[58] and that intermittent exposure (6 hours per day 5 days per week) to 5000 ppm caused decreased litter size and a reduction in body length by day 19 of gestation.[59] Healy and colleagues reported recently that exposure to as little as 50 ppm for 5 days per week for 13 weeks resulted in decreased liver weight and leukocyte count in mice.[60] However, there was no dose-response relationship evident in these effects, and it is possible they reflected random variations. A similar study by Rice et al (4 hours exposure per day, 5 days per week for 14 weeks) found no such effects at concentrations of N_2O up to 500,000 ppm.[61]

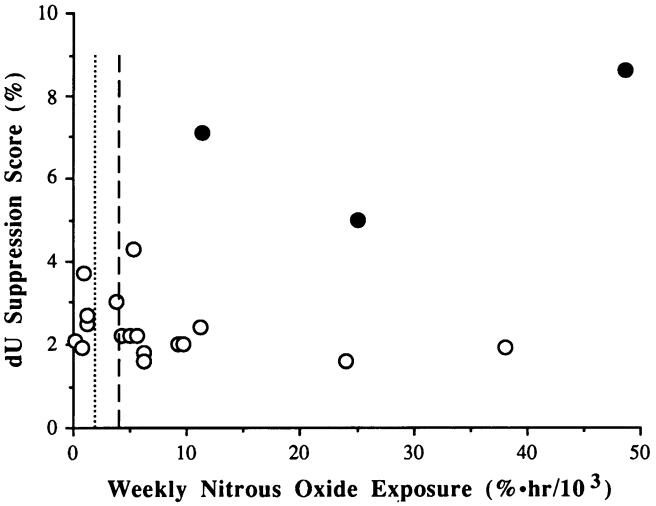


Figure 6. Altered dU suppression tests scores as a function of the weekly total exposure to N_2O measured by passive dosimetry. Each circle represents a single dentist subject. Shaded circles denote two subjects with altered dU suppression scores and megaloblastic changes in the bone marrow. The dotted line depicts the ACGIH recommendation of 50 ppm; the dashed line indicates 100 ppm. Data from Sweeney et al.[73]

Human Studies

Experimental investigations involving humans exposed to trace amounts of N_2O have concentrated on two separate issues: psychomotor performance and biochemical disturbances. Bruce et al first raised the possibility that low concentrations of N_2O could alter perceptual, cognitive, and motor skills of health care workers.[62] They reported that inhaling N_2O at 500 ppm for 4 hours significantly impaired performance on the digit span test. Subsequently, Bruce and Bach found that 50 ppm inhaled for over 2 hours caused impairment in audiovisual performance tasks.[63] This last finding was instrumental in causing NIOSH to recommend 25 ppm as the standard for maximum exposure in the workplace. These findings have not been reproduced by subsequent workers.[64,65] Cook et al[66] in particular tried to reproduce the methodology of Bruce but were still unable to detect any performance deficit with administration of trace concentrations of N_2O. In fact, concentrations over 5% were required before significant impairment was uncovered. It seems clear from the preponderance of evidence that psychomotor deficits are probably not a concern with concentrations to which a dentist could conceivably be exposed as a result of clinical practice.[67]

Several investigations of health care personnel have attempted to discover biochemical alterations caused by exposure to trace amounts of N_2O. Results have been largely negative. For instance, methionine concentrations were not altered in anesthetists chronically exposed to N_2O concentrations of 150 to 400 ppm as a consequence of their normal daily activities.[68] Similarly, analyses of neurologic function in dentists,[69] sister chromatid exchange in dental personnel,[70] sperm indices in anesthesiologists,[71] and peripheral blood studies in operating room personnel[72] revealed no significant effects of low-dose N_2O exposure. These investigations were admittedly small in scope and perhaps limited by low ambient concentrations of N_2O and insensitive methods used to detect physiological disturbances. They are collectively eclipsed by the work of Sweeney et al,[73] who provided the *first direct evidence that occupational exposure to N_2O can result in altered vitamin B_{12} metabolism and impaired synthesis of DNA.*

Sweeney and colleagues performed dU suppression tests and full neurological and hematological examinations on 20 dentist volunteers who routinely administered N_2O in clinical practice. Definite evidence of altered DNA synthesis in the bone marrow was obtained in two subjects, both of whom also had unequivocal, though mild, megaloblastic changes in the erythroid series. Measurements of weekly exposure to N_2O were positively associated with elevated dU suppression (Figure 6). No definite hematological or neurological findings were reported, however. It is noteworthy that these authors recommended a time-weighted average expo-

sure (TWA) of 400 ppm per anesthetic administration as being safe and reasonably obtainable with existing technology.

RECOMMENDATIONS FOR CONTROL OF OCCUPATIONAL EXPOSURE

Existing evidence indicates that a potential danger exists for adverse health effects occurring as a consequence of exposure to N_2O. There is a known mechanism by which N_2O could induce deleterious effects, and various biochemical and toxicological responses have been described in animals and humans. The evidence for danger associated with inhalation of trace amounts of N_2O is much less compelling. It would appear that some dentists and auxiliary personnel exposed to ambient N_2O *may* be at risk of developing biochemical disturbances that *could* have deleterious consequences, such as neurological deficits and miscarriage of offspring. Assuming that the toxicity of N_2O is dose-dependent and that sensitivity to N_2O is normally distributed, the preponderance of findings outlined in this review suggest that the minimum threshold for biologic effects in humans lies above both a continuous TWA of 100 ppm N_2O for an 8-hour day and a TWA of 400 ppm per anesthetic administration in the dental setting. These values are, respectively, the 100 ppm continuous exposure limit adopted in Sweden[74] and the 400 ppm limit per anesthetic administration suggested by Sweeney et al.[73] Clearly, the 1977 NIOSH guideline for a TWA of 25 ppm per anesthetic use is much too restrictive according to current information.[55]

If 400 ppm is a reasonable TWA standard per N_2O administration in the dental office, it becomes of interest to determine what steps would be necessary to ensure compliance with that standard. Numerous investigations have shown that dental personnel are often exposed to concentrations an order of magnitude higher than 400 ppm when no attempts are made to limit N_2O pollution and/or hasten its elimination from the operatory environment.[75,76] In the Sweeney study, all but 3 of the dentists were above 400 ppm (Figure 7). With respect to an 8-hour TWA of 100 ppm, all but 6 would exceed the limit (Figure 6), assuming a 40-hour work week and a balanced daily distribution of exposure.

Under tightly controlled circumstances, the use of scavenging gas delivery systems can reduce the ambient N_2O to under 50 ppm.[77,78] Recent surveys of occupational exposure to N_2O in dental operatories suggest, however, that practical difficulties often limit the efficacy of scavenging equipment.[79,80] Some of these problems reflect leaks in the system or poor adminstration technique; others involve poor mask fit or the patient talking or exhaling orally; and still others may be inherent in the scavenging apparatus itself.[74] Periodic assessments of N_2O are essential to establishing and maintaining an effective scavenging system.[77]

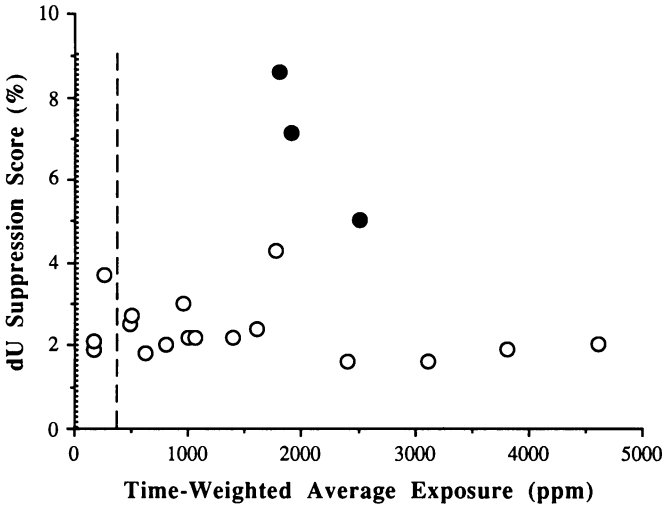


Figure 7. Altered dU suppression test scores as a function of the time-weighted average exposure to N_2O per anesthetic administration. Each circle represents a single dentist subject. Shaded circles denote two subjects with altered dU suppression scores and megaloblastic changes in the bone marrow. The dotted line depicts the NIOSH recommendation of 25 ppm; the dashed line indicates 400 ppm. Data from Sweeney et al.[73]

Should the NIOSH guideline or even the less stringent ACGIH recommendation of 50 ppm be adopted by OSHA, a scavenging system removing 90% to 95% of the administered N_2O might not be sufficient to maintain occupational exposure within maximum permissible limits. Local exhaust ventilation systems hold promise for reducing N_2O pollution, but such equipment is expensive (approximately $1000 per installation) and must encroach on the dentist's work area to be truly effective.[81] An even more costly option would be to revamp the ventilation system to improve air turnover in the operatory. By contrast, adoption of the standards suggested here would protect the health of exposed dental personnel while making it possible for private practitioners to achieve compliance using existing and relatively inexpensive methodologies.

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