

Review of Nitrous Oxide Toxicity Data as it Applies to ASHRAE 110-1995 Procedure¹

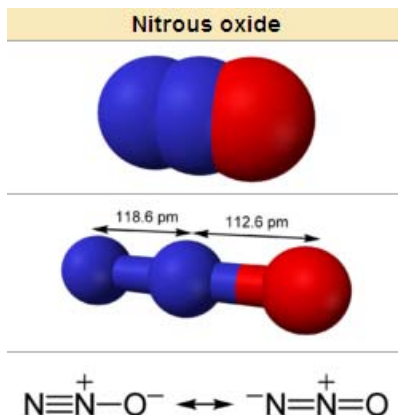
Authors

Russell Vernon, Ph.D., Environmental Health & Safety, University of California, Riverside, CA
 Shaun Larsen, MPH, Sr. EHS Engineer, Watson Pharmaceuticals, Inc, Corona, CA
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Abstract

Many people have considered using nitrous oxide as a tracer gas for conducting laboratory hood performance testing using the ANSI/ASHRAE Standard 110 (Method of Testing Performance of Laboratory Fume Hoods). The current tracer gas, sulfur hexafluoride (SF₆) has a highly undesirable global warming potential faces potential restrictions or outright banning as a tracer gas because of greenhouse gas concerns. Nitrous oxide is a potential replacement. However, the low NIOSH REL (25 ppm as an 8-hour time-weighted average) raises concerns about potential exposures to the affected stakeholders: technicians testing laboratory hoods, laboratory personnel in the room, maintenance personnel working on the roof or in a fan penthouse, and environmental exposures.

This paper reviews the toxicological literature, examines potential exposures, and generates a hazard assessment for testing laboratory hoods with nitrous oxide. With the proper handling methods (both work practices and administrative controls), testing laboratory hood using nitrous oxide as a tracer gas for Standard 110 does not present a health hazard potential.



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ANSI/ASHRAE Standard 110

ANSI/ASHRAE Standard 110 tests the performance of a laboratory hood by measuring the face velocity, conducting smoke visualization tests, and quantitatively testing the hood using tracer gas. This discussion focuses only on the tracer gas test. The test procedure releases tracer gas, usually Sulfur Hexafluoride (SF₆), inside a laboratory hood through an ejector and monitors the concentration of the tracer gas in the “breathing zone” of a mannequin at the face of the hood. Figure 1 shows the test arrangement.

The typical release rate of the tracer gas is 4 liters per minute, although some investigators have used different flow rates. The design of

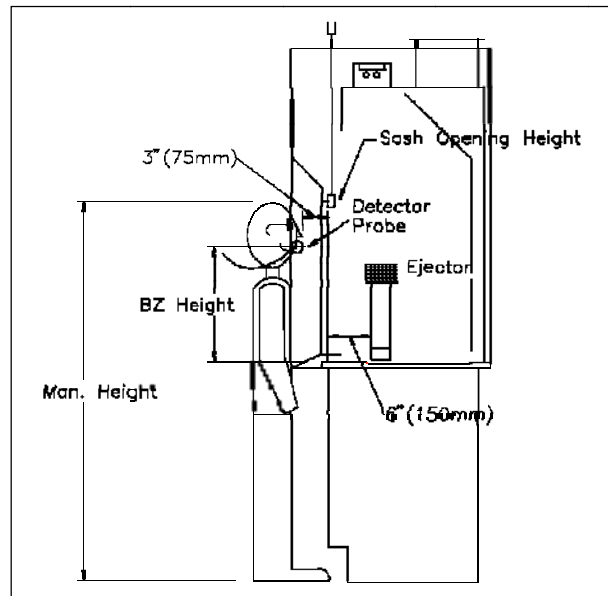


Figure 1: Test arrangement for ASHRAE Standard 110

the ejector causes air to aspirate through four ports near the base. The resulting air-tracer gas mixture discharges through the screen “bonnet” on top of the ejector. Typical acceptance levels, in the breathing zone of the mannequin are either 0.05 ppm for as installed or 0.10 ppm for as used when evaluated as a five-minute time weighted average.

Although potential exposures to personnel conducting the tests depend on the hood performance, the ventilation rate of the test hood, the room ventilation, operator work practices, and other similar conditions, exposure estimates can be made.

- 1) The maximum exposure, at 4 L/min of nitrous oxide flow rate, is approximately 16,000 ppm or 1.6%²; which would occur only if a tester would place his/her head in the plume at the ejector.
- 2) The tracer gas concentration inside the hood depends on the ventilation rate for the hood, the hood design, and the internal mixing. In all cases the concentration at any one point is less than the concentration in the plume and a significant exposure would only result from the tester would placing their head inside the hood for an extended time.
- 3) The tracer gas concentration in the exhaust duct, at the hood, would depend on ventilation rate for the hood. Using a small hood, 5-foot bench top hood with an 18-inch high sash height, and a nominal face velocity of 100 fpm, the volumetric flow rate would be about 600 acfm. With a release rate of 4 L/min (0.14 acfm), the concentration in the exhaust duct would be about 240 ppm.
- 4) The worst-case exposure in the breathing zone of the mannequin would be about 25 ppm (complete failure of the hood, which the investigator would normally be detected by the face velocity and smoke visualization tests).
- 5) The worst-case exposure in the room would be about 10 ppm.

Environmental Nitrous Oxide

Nitrous oxide is produced from a wide variety of natural and human sources. Nitrous oxide produced by nature is primarily by the bacterial decomposition of nitrogen in soils and the earth's oceans. Primary human-related sources are agricultural, animal manure, sewage treatment, mobile and stationary combustion of fossil fuel, and nitric acid production.

The atmospheric concentration of nitrous oxide in 1998³ was about 320 ppb or 0.32 ppm

Toxicological Background

Nitrous oxide has been in use as a general anesthetic gas for over 150 years with limited noted adverse effects beyond its association with asphyxiation. Recent studies have shown that nitrous oxide can cause spontaneous abortions, neuropathy, and embryotoxicity/fetotoxicity when anesthetic waste gas scavenging is not incorporated in occupational settings.

Toxicological concerns of nitrous oxide surround the key issues of;

- methionine synthetase production (and related neuropathy)
- spontaneous abortion
- embryotoxicity and fetotoxicity

N₂O Exposure standards:

Occupational Exposure Limits

Federal OSHA has not established a Permissible Exposure Limit (PEL) for nitrous oxide. California⁴ has established a PEL of 50 ppm (90 mg/m³) for nitrous oxide.

Non-Occupational Exposure Limits

The American Conference of Governmental Industrial Hygienists (ACGIH) has established a Threshold Limit value (TLV) of 50 ppm as an 8-hour Time-Weighted Average with a 4A notation (Not classified as a human carcinogen) ⁵

The National Institute for Occupational Safety and Health (NIOSH) has established a recommended exposure limit (REL) for nitrous oxide of 25 ppm (45 mg/m³) as a time-weighted average for exposure⁶ to waste anesthetic gases.

The National Institute of Occupational Safety and Health (NIOSH) based its Recommended Exposure Limit (REL) on a study by Bruce and Bach⁷, which focused on the neuronal implications of nitrous oxide exposure. The Bruce and Bach study's results however, have not been reproducible. This and other data lead the American Conference of Governmental Industrial Hygienists (ACGIH) to select 50 ppm for their Threshold Limit Value (TLV).

Exposure effects

There is no data on occupational exposure of nitrous oxide outside of medical settings where therapeutic exposures are many orders of magnitude greater than anything anticipated in an ASHRAE 110 test.

The low level (non-anesthetic gas levels) nitrous oxide studies were generated because of concern about anesthetic gas exposures in operating room to hospital personnel, especially to the anesthesiologists or anesthesiologist. The concerns were acute, intoxication of the anesthesiologists, as well as chronic, potential systemic exposures.

The vitamin B12 complex and related methionine synthetase enzyme have been shown to be affected by nitrous oxide. This is not the same as Arakawa's Syndrome which is a genetic disorder that causes a deficiency of a specific enzyme that affects Vitamin B12 metabolism.

At very high nitrous oxide levels (as in anesthetic gas use), the vitamin B12 complex and related methionine synthetase enzyme activity have been reduced. This is a potential concern for hepatic (liver) damage. However, Nunn et al⁸ found no effect in animals and humans repeatedly exposed to nitrous oxide at about 400 ppm. **This no effect level is much higher than any potential exposure associated with ASHRAE testing.**

A summary of the information available about spontaneous abortions, embryotoxicity, fetotoxicity, decreased litter size, and other animal effects is found in Table 3 "Reproductive/Developmental Nitrous Oxide Exposure". All the studies show effects only at or above 1000 ppm. The mechanism of action for the reported deleterious effects on the reproductive system does not appear to be fully understood at this time.

Data for acute exposure to nitrous oxide was not found; the most likely result to an acute exposure to nitrous oxide is asphyxiation.

Relevance of exposure studies to fume hood testing questioned

A majority of the toxicological studies regarding nitrous oxide have focused on concentrations based on the *maximum tolerated dose* where the anesthetic gas concentration of **80% N₂O** to 20% O₂ are coupled with a time surrogate for exposure in epidemiological models. These studies are not relevant to the ASHRAE 110-1995 method of fume hood testing as the concentrations of nitrous even at the diffuser outlet are significantly lower than 80%.

The data show minimal problems, except the anesthetic or asphyxiation effects. For tracer gas testing, the potential exposures are few parts per million rather than the high percentage used in the studies. Consequently, the lack of acute symptoms at anesthetic levels implies that there are no symptoms associated with very low, short-term exposures.

During a recent study at UCSD, Martin Burke of Technical Safety Services, Inc. reported data from a comparison test of SF₆ and N₂O on 30 laboratory hoods. They adjusted the hoods to leak a little (4 ppm & greater) by opening the sashes too far and introducing an intermittent cross currents (provided by a fan). The nitrous oxide measurements in the room were all less than 0.5 ppm during the hood testing at the University of California, San Diego⁹ while using Nitrous oxide.

Toxicological Literature Data:

Currently there is insufficient data relating acute exposure of nitrous oxide to adverse effects. The ACGIH recognizes the lack of data and does not indicate a short term exposure limit for nitrous oxide. The following data tables have been populated with information gathered from the ACGIH TLV documentation and subsequent articles cited therein. A recent review article supports the continued use of Nitrous Oxide as an anesthetic gas.¹⁰

Summary

The potential for human nitrous oxide exposure, above consensus standards, received during the ASHRAE 110 fume hood containment test is extremely low according to data gathered during a test run at the University of California. It is our position that substituting Nitrous Oxide sulfur hexafluoride poses negligible increased risk to test operators, room occupants, or maintenance personnel.

Tables

Table 1 Sub chronic Nitrous Oxide Exposure

Species	Dose	Effect	Citation
Swiss-Webster Mice	4hr/day, 5days/wk 14 weeks @ 0.5%, 5%, 50% (MTD)	No effect	Rice et al. ¹¹
Rats	2 or more days at 80% N ₂ O and 20% Oxygen	Bone marrow toxicity Is this a problem?	Green and Eastwood ¹²
Rats	1% nitrous oxide for 1week-6 months	No effect	Green and Eastwood ¹³
Rats	450ppm	No hepatic effect on methionine synthetase	Nunn et al. ¹⁴
Humans	400ppm	Normal serum methionine synthetase	

Table 2 Chronic/Carcinogen Nitrous Oxide Exposure

Species	Dose	Effect	Citation
None	Carcinogenic bioassay	No Effect	Eger et al. ¹⁷
Swiss ICR Mice	19% and 75% Nitrous Oxide in air	No increase in tumor incidence	Eger et al. ¹⁸

Rats	50ppm to 500ppm	No increase in tumor incidence	Coate et al. ¹⁹
Swiss Webster Mice	10%-40% Nitrous Oxide to air	No increase in tumor incidence	Baden et al. ²⁰
Human	50% nitrous oxide and 50% oxygen	Bone marrow depression. Bone marrow returned to normal within 4 days following exposure. Granulocytopenia.	Lassen et al. ²¹ Sando et al. ²² Stead et al. ²³
Human	Occupational exposure to anesthetic gas	Small increase in the incidence of cancer in women but as the tumor types and locations were inconsistent it do not indicate a common cause	Corbett et al. ²⁴ American Society of Anesthesiologists ²⁵ Cohen et al. ²⁶
Human	Occupational Exposure. Retrospective Study	Increased cancer rate of from 30%-100% higher than when compared to matched controls. However as there was not a significant indication of malignancy type or location. As a result there is significant doubt that occupational exposure to nitrous oxide is the cause.	American Society of Anesthesiologists ²⁷
Human (Dentists and Assistants)	Occupational Exposure	No effect of anesthetic exposure was observed in the dentists. Female assistants had a cancer rate of 1.06% compared to .72% (p=0.06) and a 2.4 fold increase in cervical cancer (p=0.04). This study did not find that exposure to nitrous oxide causes cervical.	Cohen et al. ²⁸

Table 3 Reproductive/Developmental Nitrous Oxide Exposure

Species	Dose	Effect	Citation
Sprague Dawley Rats	70-75% nitrous oxide in oxygen or room air for 24 hours on day 9 of pregnancy	Caused major soft tissue and skeletal anomalies. Embryotoxic and fetotoxic	Lane et al. ²⁹
Rats	5-9 days at 0, 100, 1000. or 15,000 ppm Nitrous Oxide	Fetal mortality increased. At 1000 and 15000ppm implantation was lessened.	Corbett et al. ³⁰
Wistar rats	0, 250, 500, 1000 ppm nitrous oxide	Decreased litter size at 1000ppm concentration, higher incidence of fetal resorptions and skeletal abnormalities	Vieira et al. ³¹
Swiss ICR Mice	4hours/day at 0.5, 5 or 50% nitrous oxide on days 5-15 of gestation	No increase in percentage of congenital anomalies	Mazze et al. ³²
Sprague Dawley Rats	1, 10, or 50% nitrous oxide for 8hrs/day throughout gestation	No increase in teratogenicity	Pope et al. ³³
Rats and Rabbits	1000ppm for 8hrs/day	No teratogenic effects	Hardin et al. ³⁴
Human	Occupational Exposure to waste anesthetic gas.	Increased incidence of spontaneous abortions. Only 2 of 5 studies showed increased spontaneous abortions in the wives of men exposed to nitrous oxide at work. It is very hard to see how exposure to	American Society of Anesthesiology ³⁵ Knill-Jones et al. ³⁶ Cohen et al. ³⁷

	a gas would affect the family..	
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Table 4 Neurotoxicity

Species	Dose	Effect	Citation
Human	Non specific (over 3000 hours of exposure during a decade. High exposure group)	Numbness, tingling, and weakness increased 3 to 4 fold.	Cohen et al. ³⁸
Human	50ppm for 4 hours. A battery of audiovisual tests followed	Results have not been confirmed in other studies No statistically significant results were shown in test performance.	Frankhuizen et al. ³⁹ Smith et al. ⁴⁰ Bruce and Bach ⁴¹

¹ This literature review and analysis was initiated by Shaun Larsen, MPH, MS and completed by Russell Vernon, Ph.D. at Environmental Health & Safety, University of California, Riverside in late 2008.

² Martin Burke's AIHce presentation 250 L/min induced flow with 4 L/min N₂O is 4/250*1000000 = 16,000 ppm = 1.6%

³ EPA, nitrous oxide, posted on <http://www.epa.gov/nitrousoxide/scientific.html>

⁴ http://www.dir.ca.gov/Title8/5155table_ac1.html

⁵ ACGIH, 2009 Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Limits, 2009

⁶ <http://www.cdc.gov/NIOSH/NPG/npgd0465.html> NIOSH pocket guide to Chemical Hazards, DHHS Publication No. 2005-149, 2005

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⁹ Private Communication from Martin Burke at TSS on March 25, 2009

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