SPECIAL ARTICLE

Occupational hazards, DNA damage, and oxidative stress on exposure to waste anesthetic gases

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Abstract

Background and objectives: The waste anesthetic gases (WAGs) present in the ambient air of operating rooms (OR), are associated with various occupational hazards. This paper intends to discuss occupational exposure to WAGs and its impact on exposed professionals, with emphasis on genetic damage and oxidative stress.

Content: Despite the emergence of safer inhaled anesthetics, occupational exposure to WAGs remains a current concern. Factors related to anesthetic techniques and anesthesia workstations, in addition to the absence of a scavenging system in the OR, contribute to anesthetic pollution. In order to minimize the health risks of exposed professionals, several countries have recommended legislation with maximum exposure limits. However, developing countries still require measurement of WAGs and regulation for occupational exposure to WAGs. WAGs are capable of inducing damage to the genetic material, such as DNA damage assessed using the comet assay and increased frequency of micronucleus in professionals with long-term exposure. Oxidative stress is also associated with WAGs exposure, as it induces lipid peroxidation, oxidative damage in DNA, and impairment of the antioxidant defense system in exposed professionals.

Conclusions: The occupational hazards related to WAGs including genotoxicity, mutagenicity and oxidative stress, stand as a public health issue and must be acknowledged by exposed personnel and responsible authorities, especially in developing countries. Thus, it is urgent to establish maximum safe limits of concentration of WAGs in ORs and educational practices and protocols for exposed professionals.

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Introduction

Waste anesthetic gases (WAGs) are small amounts of inhaled anesthetics present mainly in the operating room (OR) and post-anesthesia care unit (PACU) ambient air. Halogenated anesthetics, including halothane, isoflurane, sevoflurane, desflurane, and nitrous oxide (N\textsubscript{2}O) are the main constituents of WAGs, as they are the most frequently used anesthetics.

According to estimates by the American Occupational Safety and Health Administration (OSHA), more than 200,000 health professionals are at risk of occupational diseases due to chronic exposure to WAGs. Because it is a public health issue, knowledge of these risks and adoption of formal practices and regulations to reduce ambient air pollution in ORs to safe minimum levels of exposure are critical. The aim of this article is to show the impacts of occupational exposure to WAGs on exposed professionals’ health, with emphasis on topics more recently explored in the literature, as well as the definition of genotoxicity, mutagenicity, and oxidative stress applied to anesthesiology.

Background

Inhaled anesthetics are drugs widely and routinely used in general anesthesia. The unprecedented public demonstration of diethyl ether as an inhalation anesthetic by William Morton in 1846 at the Massachusetts General Hospital in Boston in the United States enabled to perform a pain-free surgical procedure and gave rise to one of the most significant scientific discoveries in medicine.

Since then, the practice of anesthesiology has witnessed the profound evolution in this field, as other anesthetics emerged, such as N\textsubscript{2}O, chloroform, and trichloroethylene. However, the high toxicity and risk of explosion within the surgical environment related to these agents discontinued its use and encouraged the search for safer anesthetics. In the 1950s, the first compound derived from fluoride ion (fluoroxene) was tested clinically, but was soon ruled out as extremely toxic. Halothane is a halogenated hydrocarbon synthesized in 1957, whose reduced flammability compared to agents available at that time consolidated it as the main inhaled anesthetic of the time, which lasts until today. In 1960, it was followed by methoxyflurane, which had limited use due to its high nephrotoxicity. At the same time, reports of rare cases of halothane-related fatal hepatitis led to the search for newer and safer volatile anesthetics synthesized in the 1960s, such as enflurane in 1963 and its structural isomer isoflurane in 1965, in addition to sevoflurane and desflurane (popularized in the mid-1990s). Xenon, recognized as an inert, odorless gas, has rapid absorption and elimination through the lungs, no hepatic and renal metabolism, and minimal cardiovascular effects. However, its use is still restricted due to its high cost and limited availability. Thus, the optimal inhaled anesthetic is still missing, being an important research topic.
Waste anesthetic gases (WAGs)

The surgical environment pollution with WAGs is essentially due to three causes: anesthetic techniques, anesthesia workstation, and OR with or without a scavenging system. Regarding anesthetic techniques, two main factors may be enumerated: (1) induction and/or maintenance of general anesthesia with inhaled anesthetics, particularly in pediatric patients via face mask; (2) failure to turn off both the valve that controls the flow (gas flowmeter) and vaporizer when OR is without patient; (3) leakage of anesthetic when filling the vaporizer; (4) performing flushing at the end of surgical procedure to accelerate recovery from inhalational anesthesia (common and extremely harmful practice); (5) problems with facial mask coupling, either by material that is inappropriate for use, inadequate size or even by difficulties related to the patient’s airway; (6) leakage of gas after inadequate endotracheal tube (ETT) cuff or laryngeal mask inflation, or by the use of uncuffed ETT; (7) use of intermediate fresh gas flow (2–4 L.min⁻¹) and particularly high flow (>4 L.min⁻¹) 10; (8) use of sidestream type capnograph with no gas return to the anesthesia machine; (9) use of Mapleson respiratory system, particularly in pediatric anesthesia. 10,11

Regarding anesthesia workstation, numerous components may be the reason for anesthetic leakage into the ambient air. Possible leaks may come from valves and respiratory circuit connections, defects in parts and reservoir bags. 9,12 ORs may or may not have a scavenging system. When there is a scavenging system, it may be global (when there is central suction that draws air from the OR through negative pressure, venting all the air with waste gases outside the room, without air recirculation) or partial (when there is central suction that draws air from the OR through negative pressure, partially venting the air with anesthetic gas to the outside, with air recirculation). In OR with no scavenging system, there is only the natural circulation of airflow from non-central air conditioners. 12

WAG environmental risks

WAGs eliminated from ORs to the external environment reach the atmosphere unchanged and cause environmental impact. The environmental damage caused by anesthetic gases depends on its molecular weight, proportion of halogen atoms, and half-life in the atmosphere. The approximate atmospheric half-life of anesthetic gases are: N₂O: 114 years; desflurane: 10 years; halothane: 7 years; sevoflurane: 5 years; and isoflurane: 3 years. 13 All inhaled anesthetics being used contain halogenated compounds that resemble chlorofluorocarbons and thus have deleterious effects on the ozone layer. Besides being one of the depleting gases in the ozone layer, N₂O seizes the thermal radiation emanated from the Earth’s surface and contributes to the phenomenon of global warming, known as the “greenhouse effect”. 13

Occupational health and WAG exposure

The possibility of health damage related to the inhaled anesthetic exposure has been the subject of debates in the last decades. 14 Several professionals (anesthesiologists, veterinarians and surgeons, nurses and related health professionals, as well as students) active in ORs and/or PACUs are the people most exposed to WAGs. 1

The first study that drew the scientific community attention to the risks associated with exposure to WAGs was conducted by Vaisman in the Soviet Union in 1967. It involved 198 men and 110 women anesthesiologists exposed primarily to diethyl ether, N₂O, and halothane and found not only symptoms such as fatigue, headache and irritability, but also showed, for the first time, an adverse effect on the reproductive system. There were 18 cases of spontaneous abortion in 31 pregnancies in the group of female anesthesiologists exposed to WAGs. 15 This finding raised great concern about the safety of exposed professionals. In 1974, the American Society of Anesthesiologists (ASA) published in the United States the study Occupational disease among operating room personnel: a national study. Report of an ad hoc committee on the effect of anesthetics on the health of operating room personnel. 16 Through the use of a questionnaire, a group of 49,585 professionals exposed to WAGs were compared with a group of 23,911 subjects without exposure. In exposed women, an increased risk of spontaneous abortion, congenital anomalies, cancer, and liver and kidney disease were seen. Male anesthesiologists, however, had an increased risk of liver disease and of having children with congenital abnormalities. 16

Subsequently, these studies were reviewed by other authors, who found numerous methodological errors and biases (for example, respondent bias in the analysis of questionnaires and confounding factors, such as psychological stress and long working hours). This mainly weakens the evidence of the causal association between exposure to inhaled anesthetics and negative reproductive outcomes (spontaneous abortion and congenital abnormalities). 14

Limits of occupational exposure to WAGs

In view of the foregoing, there was a need for formal recommendations to reduce occupational exposure to WAGs, especially the National Institute for Occupational Safety and Health (NIOSH) in 1977 17 that suggested the adoption of exposure limits to WAGs in any susceptible environment using these agents. Occupational Exposure Limits were defined as: 2 parts per million (ppm) – ceiling – to halogenated agents and 25 ppm – time-weighted average (TWA) – to N₂O during its administration time. Furthermore, it was recommended the implementation of effective scavenging systems that allow an efficient air renewal in ORs. 17 Thus, protocols and technical procedures have been instituted in the United States to prevent anesthetic gas leakage in OR, such as careful handling of face mask, vaporizers, and flowmeters and tests to identify leaks in high and low pressure systems. Surveillance of exposed physicians’ health status with physical and laboratory examinations, as needed, was also addressed, as well as the need to ambient air monitoring to determine WAG concentrations, with documentation through reports and serial inspections. 17

Following the regulation of these safety measures concerning occupational exposure to WAGs, other countries have also implemented their own legislation. The British Government Health Services Advisory Committee, for example, established limit-values of 8 h of 100 ppm (TWA) for
N\textsubscript{2}O, 50 ppm for enflurane and isoflurane, and 10 ppm for halothane, because these values are much lower than those that cause adverse effects reported in experimental studies.\textsuperscript{18} Other examples of nations with their own legislation are France, Switzerland, Germany, Austria, the Netherlands, Italy, Sweden, Norway, Denmark, and Poland.\textsuperscript{14}

In Brazil, the occupational exposure to WAGs is still a subject rarely explored and lacks regulation by labor legislation. The maximum limits of anesthetic gases that are safe for the worker are absent, as well as recommendations on monitoring and inspection. The Regulatory Standard NR 15 (on unhealthy activities and operations) refers to N\textsubscript{2}O, limited only to “asphyxiating doses”. In turn, NR 32 (health and safety standard at work in health care establishments), although addressing the issue more directly mentioning the rights of the pregnant worker exposed to WAGs, it does so in an unclear and insufficient way.\textsuperscript{11}

A national study conducted in the 1980s compared halothane inhaled anesthetics in the air and blood of animals exposed to experimental room pollution with and without the Venturi system.\textsuperscript{19} The authors have shown the effectiveness of this anti-pollution system in exhausting WAGs. Most anesthesiologists in Brazilian surgical centers use the most varied types of inhaled anesthetics (from halothane to desflurane) without protocols for reducing leakage and pollution in ORs, which have no scavenging system to eliminate WAGs. It is worth noting the work conducted in the Department of Anesthesiology of the Botucatu Medical School (Unesp), which measured, for the first time, the environmental concentration of anesthetics in ORs of Brazilian surgical theaters, with half of the ORs with partial scavenging system, with a 6–8 air exchange ar/h and half of the ORs without a scavenging system, with the latter reflecting the reality of many hospitals in developing countries.\textsuperscript{20}

The mean concentration of halogenated isoflurane, sevoflurane, and desflurane were above 5 ppm and for N\textsubscript{2}O it was higher than 170 ppm (TWA). According to the international standards advocated by the American Institute of Architects (1993),\textsuperscript{21} at least 15 air changes per hour are recommended to ensure that the air circulating in ORs is completely filled with fresh air. Moreover, the ideal is to use a unidirectional or laminar air flow system, which allows all the contamination generated in the environment to be taken out of it as soon as possible.\textsuperscript{12}

Thus, a quality standard is required, followed by routine inspections and regular measurement of WAG concentrations in OR to ascertain their proper functioning. It is also worth noting that there is a small number of studies addressing occupational exposure to WAGs and its possible deleterious effects in developing countries, such as Brazil, which makes it difficult to perceive this impact in the population and health personnel.\textsuperscript{20,23-26}

The concern with occupational exposure, regarding the limitation of WAG concentrations, is a relevant issue due to the potential health risks of exposed professionals. It is well documented that such exposure, even for a short time, can be reflected in signs and symptoms, such as headache, irritability, fatigue, nausea, dizziness, difficulty judgment and coordination.\textsuperscript{1} More serious changes in exposed individuals, including kidney and liver damage and neurodegenerative conditions, such as Parkinson’s disease and proprioceptive changes, have also been reported.\textsuperscript{27,28}

Genotoxic and mutagenic potential of WAGs

One of the important focuses of several studies is the potential of inhaled anesthetics to induce damage to genetic material (genotoxicity and mutagenicity) evaluated in animals,\textsuperscript{29,30} patients,\textsuperscript{31-33} and occupationally exposed professionals.\textsuperscript{20,34,35} In fact, genetic biomarkers have been widely used to monitor human exposure to genotoxic and/or mutagenic agents with potential carcinogenic effect.\textsuperscript{36} Among the major markers of genotoxicity and mutagenicity are the comet and micronucleus (MN) tests.

The comet test is a sensitive and cost-effective method to measure DNA damage, which has been established as an important tool to evaluate genotoxicity in occupational risk studies.\textsuperscript{37} Such methodology consists of immersion of eukaryotic cells in agarose gel, cell membrane lysis and subsequent electrophoresis. Under alkaline conditions of electrophoresis (pH >13), nucleoids with DNA damage (which have negative charge) migrate to the positive pole, mimicking the appearance of a comet (head and tail). Thus, the fragments resulting from single- and/or double-strand breaks of DNA, in addition to alkali-labile sites, migrate toward the anode of the electrophoresis trough.\textsuperscript{37} The greater the presence of damaged genetic material, the greater the migration of these DNA fragments. Thus, the tail extension proportionally reflects the amount of DNA damage (Fig. 1).\textsuperscript{37}

Although the genotoxicity and mutagenicity mechanisms of halogenated anesthetics are not fully elucidated, possible explanations include oxidative metabolism capable of generating reactive oxygen species (ROS) and the induction of direct damage to the genome at any stage of the cell cycle.\textsuperscript{33} On the other hand, N\textsubscript{2}O oxidizes the cobalt ion present in cobalamin (vitamin B12), leading to the inhibition of methionine synthetase with reduced production of methionine and tetrahydrofolate and its byproducts thymidine and nucleic acids (including DNA).\textsuperscript{38} Such changes are related
to megaloblastic anemia, agranulocytosis, spinal cord subacute combined degeneration, and neurobehavioral disorders in individuals under chronic exposure and/or elevated concentrations of N₂O.¹⁸

In a pioneering study conducted in the northern region of Brazil,²⁵ the effects of occupational exposure to WAGs on genetic material were seen during medical residency. The authors found a significant increase of primary lesions in the DNA of resident physicians at eight, 16, and 22 months exposure to isoflurane, sevoflurane, and N₂O compared to a control group, in ORs with no scavenging system. On the other hand, there was no increased basal damage in lymphocytes evaluated in anesthesiologists chronically exposed to isoflurane, sevoflurane, desflurane, and N₂O in a surgical center with partial scavenging system of a teaching hospital in southeastern Brazil.²⁰

The basal DNA damage, detected using the comet test, has been evaluated in the population chronically exposed to WAGs, but the results are controversial.²⁵,³⁹,⁴⁰ In Turkey, for example, there was a significant increase in lymphocyte DNA damage of 66 professionals (anesthesiologists, nurses, and technicians) exposed to halothane, isoflurane, and N₂O compared to a control group.³⁹ In contrast, a Polish study showed no difference in DNA damage in 100 professionals exposed to N₂O, isoflurane, sevoflurane, and halothane compared to control group or interference from exposure time in the outcomes.³⁹

There is evidence of interaction between free radicals derived from oxygen or nitrogen with DNA bases, which results in damages that produce oxidized bases, abasic sites and/or DNA strand breaks. The comet test, traditionally used to assess basal DNA damage, can also be modified with the use of specific enzymes to assess oxidation at DNA bases (pyrimidic and purine). This approach was found in only one study in the literature, which evaluated oxidative DNA damage in nurses chronically exposed to WAGs, and showed an increase in oxidized purines.⁴¹

MN are extranuclear corpuscles formed from fragments of chromosomes or whole chromosomes that were excluded from the main nucleus of the daughter cell during cell division (Fig. 2). Its occurrence represents genetic instability and impairment in cellular viability caused by genetic defects or exogenous exposure to genotoxic/mutagenic agents.⁴² The association between MN detected in peripheral lymphocytes and cancer has theoretical support. A cohort study conducted by the international Human MicroNucleus (HUMN) project from 1980 to 2002 involving 10 countries and 6718 individuals related the frequency of MN in peripheral lymphocytes to increased cancer risk in a population considered healthy.⁴³

A study comparing ORs in Germany with concentrations below the recommended limits of WAGs (without scavenging system) with other ORs with high concentrations of WAGs (without scavenging system) in an Eastern European country, found a significant increase of MN in lymphocytes only in professionals exposed to WAGs in ORs from an Eastern European hospital.⁴⁴ In Slovenia, a study showed that female professionals exposed to isoflurane, halothane, and N₂O (of which only isoflurane was above the recommended concentration limits in OR) had a significantly higher frequency of MN and other chromosome changes in lymphocytes than female radiology technologists and controls.⁴⁵

The use of MN in oral cells (evaluated by the Buccal Micronucleus Cytome Assay) is well established and internationally validated and it has been widely disseminated in the last decade by human biomonitoring studies to evaluate exposure to genotoxic and/or carcinogenic agents, as well as neoplastic or degenerative diseases. Its advantages include: (1) minimally invasive collection of oral mucosal cells; (2) high sensitivity; (3) specificity in detecting the effects of exposure to inhaled or ingested genotoxic agents; (4) ease of storage of samples at room temperature without the need for cell culture; and (5) low cost.⁴⁶ The buccal MN assay also allows the evaluation of nuclear changes and different stages of cell differentiation and death.⁴⁷ Fig. 3 shows the oral mucosal layers and the different cell types that can be detected in the micronucleus buccal test.⁴² The frequency of MN in the exfoliated oral cells has a positive correlation with that found in lymphocytes, showing that the genotoxic and/or mutagenic effects seen in bloodstream, as well as their potential risks (such as the association with cancer), are detected in buccal mucosa.⁴⁸ In addition, exfoliated cells of buccal mucosa represent the first biological barrier of contact with inhaled anesthetics. In the literature, there are only two reports on the use of buccal MN test in professionals chronically exposed to WAGs. The first study was conducted in India and a significant increase in MN was seen in several health professionals (surgeons, anesthesiologists, nurses, and technicians) exposed to halothane, enflurane, isoflurane, sevoflurane, and N₂O.⁴⁹ The second study was performed in Botucatu, SP, Brazil, and showed that anesthesiologists exposed for 16 years, on average, to the most modern WAGs have increased MN and cytotoxic alterations, as well as changes in cell proliferation of oral mucosa.²⁰

Oxidative stress and WAGs

By definition, oxidative stress is the imbalance between ROS production and antioxidant defenses (Fig. 4). Free
radicals are unstable molecules with unpaired electrons, which are extremely reactive. When these free radicals and other molecules arise as a result of oxidative reactions in biological systems, they are referred to as ROS, and can onset a cascade of reactions with biological molecules.\textsuperscript{49} Important examples of these reactions are lipoperoxidation or lipid peroxidation, protein damage, and oxidative damage to nucleic acids. The first involves free radical/ROS attack on membranes and lipoproteins and is implicated in the development of numerous diseases, such as atherosclerosis, cancer, and degenerative and inflammatory diseases.\textsuperscript{10} Protein damage occurs by the formation of protein groups called carbonyls, which can induce proteolysis in DNA bases (oxidative DNA damage), as well as single and double strands breaks in genetic material (such as guanidine conversion into 8-hydroxyguanidine). Ultimately, free radicals can be toxic to tissues or organs, with consequent cell damage, necrosis, and apoptosis.\textsuperscript{51} In fact, there is a relationship between genotoxicity and oxidative stress.\textsuperscript{52} Oxidative stress can mainly induce damage to macromolecules, including nucleic acids, lipids, and proteins, resulting in cellular damage, as well as a variety of diseases.\textsuperscript{53}

Oxidative stress has been studied using several biomarkers (Fig. 5). The use of protein oxidation byproducts (carboxylated proteins, S-glutathionation, and nitrotyrosine), DNA oxidation (e.g.: 8-hydroxy-2-deoxyguanosine or 8-OH-dG, phosphorylation of histone residues and increased DNA migration using the comet test) and lipid peroxidation (malonaldehyde or MDA and 4-hydroxynonenal or 4-HNE, among others) is well known to determine the evaluation of oxidative stress.\textsuperscript{52} From another perspective, oxidative stress may be evaluated by reducing antioxidant defenses, either by measuring enzymatic (e.g.: superoxide dismutase or SOD, glutathione peroxidase or GPX, catalase or CAT) or non-enzymatic antioxidant agents (e.g.: ascorbic acid or vitamin C, α-tocopherol or vitamin E, albumin, uric acid) or by tests that quantify the antioxidant capacity.
A possible relationship between occupational exposure to WAGs and oxidative stress has been studied since last decade, but it is still a relatively unexplored field. A study conducted in ORs with no scavenging system showed increased lipid peroxidation by thiobarbituric acid-reactive-substances and reduced antioxidant thiol groups in personnel exposed for nine years, on average, to halothane and N₂O, but without change in the antioxidant capacity test. Nurses working in ORs with no scavenging system, exposed to an average of 14.5 years mainly to isoflurane, sevoflurane, desflurane, and N₂O, had increased breaks in genetic material and reduced enzyme and antioxidant capacity compared to the non-exposed group. On the other hand, a study carried out with Turkish personnel exposed to enflurane, halothane, isoflurane, sevoflurane, and desflurane in ORs with partial scavenging system showed reduced plasma GPX and SOD antioxidant enzymes and copper and selenium microelements, but with increased zinc compared to controls. In personnel exposed to halothane, isoflurane, sevoflurane, desflurane, and N₂O, working for 3–11 years in surgical theater with scavenging system, there was a negative correlation between genetic material damage and antioxidant capacity. In another study, when comparing nurse exposed (5–27 years) to isoflurane and sevoflurane (low concentration) and N₂O (high concentration) with a control group, it was detected an increase in DNA bases oxidative damage and lipoperoxidation markers and reduced GPX antioxidant enzyme, but without changes in vitamin E concentration in exposed personnel. Thus, most studies show that chronic exposure to WAGs induces both oxidative damage and decreased antioxidant defense markers. In a research performed with physicians during medical residency in anesthesiology and surgery (therefore, with shorter exposure time) exposed to WAGs in ORs with no scavenging system, there was increase in basal level of DNA damage with changes in CAT and GPX enzymes, with negative correlation between DNA damage and GPX antioxidant enzyme compared to a control group.

**Conclusion**

Evidence has shown that prolonged/chronic occupational exposure to WAGs may induce damage to genome and lead to oxidative stress. Thus, it is urgent to implement appropriate legislation in our country, as well as in developing countries, regarding the limit of occupational exposure to inhaled anesthetics. Knowledge of anesthetic measurements in OR and SRPA is also fundamental. It is also worth mentioning the need for further biomonitoring studies to detect early changes caused by WAGs in exposed personnel, favoring environment intervention by implanting effective scavenging systems in ORs and individual intervention by education and protocols that ensure the use of anesthetic techniques to reduce ambient air pollution.

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**Conflicts of interest**

The authors declare no conflicts of interest.

**References**


17. NIOSH. Criteria for a recommended standard: occupational exposure to anesthetic gases and vapors; 1977.


