Monitoring and mitigating isoflurane emissions during inhalational anesthesia of mice

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Establishing a program to monitor waste anesthetic gas (WAG) in order to limit personnel exposure requires measuring the levels of WAG emitted and determining the effectiveness of scavenging methods to reduce such levels. In this study, the authors used infrared spectroscopy to measure levels of WAG emitted while anesthetizing mice with isoflurane for 15 min. They evaluated four different WAG scavenging conditions during induction and maintenance anesthesia: two conditions that used passive techniques and two that used active techniques. Isoflurane concentrations were measured at three different locations: in the operator's vicinity, at the mouse-facemask interface and in the room environment. Passive scavenging of WAG improved when chambers were purged with oxygen after induction and when a diaphragm-sealed facemask delivered a reduced anesthetic flow rate during maintenance anesthesia. Active scavenging of WAG improved when a relief intake opening was provided in the induction chamber's vacuum line, vacuum draw after induction was regulated and the anesthetic flow rate and vacuum scavenging draw were balanced during maintenance anesthesia using a facemask that separated the breathing space from the scavenging zone. Additionally, time-weighted average isoflurane WAG levels detected by personal dosimeters correlated with real-time measurements made using infrared spectroscopy. These observations contribute to the development of a substantiated program for monitoring WAG air quality.

Continuous exposure to high levels of waste anesthetic gas (WAG) poses risks to human health[1,2], leading the Occupational Safety and Health Administration (OSHA) to advise that WAG be scavenged to ensure that levels are kept as low as is practical in the anesthetic environment[3]. Institutional animal care and use programs are similarly encouraged to require that WAG be scavenged during inhalational anesthesia of animals[4] and that personnel exposures to WAG be monitored[5]. Mice are the most prevalent model species in the laboratory animal setting, yet neither levels of WAG during mouse inhalational anesthesia nor the effectiveness of scavenging methods to modulate such levels have been measured. Consequently, there are no peer-reviewed recommendations or guidelines regarding scavenging methods or implementation of WAG monitoring practices during anesthetic induction or maintenance of mice, including how to respond to detectable levels of WAG[1].

Anesthetic and scavenging practices proposed to limit WAG exposures in the laboratory animal environment are often extrapolated from practices implemented in human operating suites and post-anesthetic care units[1]. Such extrapolations ignore the many

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logistical, physical and practical differences between human and mouse anesthetic environments. Inhalational anesthesia of mice rarely involves intubation of a single mouse using a closed re-breathing anesthetic system in an actively scavenged environment. Instead, owing to the small size of the mouse and the need for large experimental group sizes to ensure statistical strength of data, mice are frequently anesthetized using induction boxes and non-rebreathing circuits.

Batch, high-throughput anesthesia methodologies, often used to simultaneously anesthetize multiple mice, have been shown to result in the steady accumulation of WAG in the room environment[6,7]. Open non-rebreathing circuits with variable facemask interfaces are often used. Using conventional, non-rebreathing facemasks without a diaphragm when anesthetizing rats results in elevated WAG levels[8]. Activated charcoal canisters typically used in passive scavenging methods with rodents were once prone to breakthrough emissions and varied effectiveness and capacity[7]. These practices when used during inhalational anesthesia of mice may similarly contribute to detectable WAG levels, although this has not been documented.

Modern scavenging techniques in human anesthetizing environments effectively maintain WAG at trace levels[2], defined as below concentrations needed for anesthesia or detectable by smell[9]. Trace WAG exposure levels have not been associated with occupational diseases or adverse health affects[1,2,9]. Consequently, the American Society of Anesthesiologists concludes that there is no justification for routine monitoring of trace WAG levels in human anesthetizing areas or medical surveillance for effects relating to trace WAG exposure, provided that effective scavenging techniques are used[2].

In contrast, it is not known whether scavenging techniques during mouse inhalational anesthesia effectively maintain WAG levels below trace levels. Active and passive scavenging methods during rodent anesthesia have been shown to mitigate WAG in a few studies. Purging rodent induction chambers prior to opening reduces WAG levels emitted upon opening[10]. Active scavenging equipment designs and techniques can limit WAG exposure during inhalational anesthesia of rats[11,12]. In addition, charcoal filters installed on the blower module of a biological safety cabinet exhausted to the room reduces WAG emissions from the cabinet[13]. Measurable levels of WAG were emitted when administering isoflurane by the open-drop method[14], but it is recommended that this technique be conducted in a fume hood[15]. The relative efficacy of various WAG scavenging practices during mouse inhalational anesthesia has not been reported.

Due in part to the variety and range of anesthetic and scavenging practices used in the multi-species laboratory animal environment, there is no consensus regarding acceptable limits of isoflurane WAG exposure[2]. A National Institute for Occupational Safety and Health (NIOSH) criteria document for federal standards regarding WAG, never officially adopted nor enforced by OSHA, proposed a recommended exposure level (REL) of $\leq$2 ppm/h for halogenated agents, but isoflurane was not among them[16]. The American Conference of Government Industrial Hygienists has assigned threshold limit value time-weighted averages for some halogenated agents, but isoflurane is not among them. It has been suggested that trace isoflurane levels may be similar to the threshold concentration of halothane that is detected by smell ($<$50 ppm)[8,10]. Although exposure limits for isoflurane have not been established by OSHA[3], some isoflurane manufacturers' Material Safety Data Sheets indicate an employee exposure limit of 60 ppm time-weighted average[17]. Whether passive or active scavenging methods used during mouse inhalational anesthesia can maintain isoflurane WAG exposure levels below these exposure limits has not been determined.

Some authors[1] have recommended biannual monitoring of WAG emissions and proposed components of a laboratory animal WAG monitoring program. WAG levels in the laboratory animal setting can be monitored using infrared spectroscopy and continuous air sampling or using personal dosimeters. It has not been reported whether isoflurane levels detected using personal dosimeters and using infrared spectroscopy during inhalational anesthesia of mice are comparable. Personal dosimeters do not provide real-time data and have a greater potential for error in use[1,9]. When we implemented a WAG monitoring program that biannually assessed isoflurane WAG during inhalational anesthesia of mice using personal dosimeters alone, we obtained highly variable and unpredictable findings. Many variables, including differences in the type and quality of mouse anesthetic and scavenging practices and equipment, appeared to contribute to the variation in isoflurane WAG levels detected using personal dosimeters. These initial efforts to monitor WAG using only personal dosimeters contributed to the design of the experiments presented in the current report.

We designed experiments to determine whether passive scavenging or active scavenging could effectively reduce isoflurane WAG concentrations during mouse inhalational anesthesia to below trace concentrations. We also sought to determine whether WAG levels measured using personal dosimeters and using infrared spectroscopy were similar. We did not intend to isolate or assess a specific scavenging device or technique for its contribution to WAG containment, nor did we compare the relative efficiencies of passive and active WAG scavenging. Our observations should

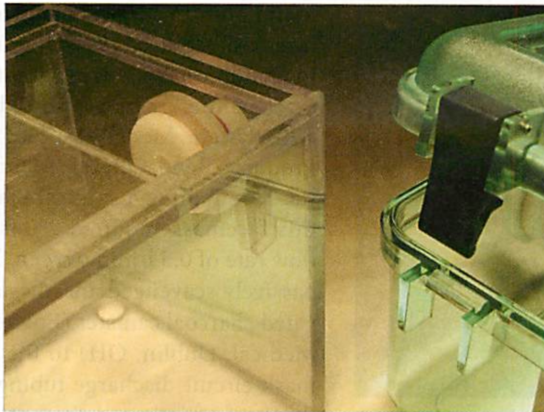

FIGURE 1 | Induction chambers. An induction chamber with a hinged top, locking latches and a silicone seal (right) was used for anesthetic induction during passive scavenging in Condition 1 and Condition 2. A sliding-top induction chamber without a capture hood (left) was used during active scavenging in Condition 3 and Condition 4.

form the basis for the development of a substantiated program for monitoring WAG.

METHODS

Animals

All work involving animals was done in accordance with international, national and institutional guidelines for humane treatment of animals, including those set forth in the *Guide for the Care and Use of Laboratory Animals*[4], the Public Health Service *Policy on Humane Care and Use of Laboratory Animals*[18], the US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training[19] and the IACUC Principles and Procedures of Animal Care and Use of the University of South Florida, an institution accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All studies described were approved by the University of South Florida IACUC.

Twenty-eight C57BL/6NHsd female mice (Harlan Laboratories, Indianapolis, IN), 8–12 weeks of age, were housed four per cage in individually ventilated microisolator cages (Tecniplast, Inc., Philadelphia, PA) on sterilized bedding (Harlan Teklad Tek-Fresh paper bedding #7099, Harlan Laboratories, Indianapolis, IN) and provided with autoclaved water and food (Harlan Teklad Sterilizable Diet #7012, Harlan Laboratories, Indianapolis, IN) *ad libitum*. The room in which they were housed was maintained at 20–22 °C and 40–60% relative humidity, on a 12-h:12-h light:dark cycle and under specific pathogen–free and viral antibody–free conditions. Quarantine and sentinel health evaluations excluded a comprehensive list of potential pathogens, including murine norovirus; parainfluenza virus Type I; mouse hepatitis virus; *Mycoplasma pulmonis*; paramyxovirus; mouse parvoviruses strains 1, 2 and 3; mouse minute virus; Theiler's murine encephalomyelitis virus GDVII; reovirus Type 3; lymphocytic choriomeningitis virus; adenovirus strains 1 and 2; ectromelia virus; rotavirus; papovavirus; and *Helicobacter* spp.

Anesthesia equipment

We carried out all studies in a common procedural room (6.3 m × 6.9 m × 3.1 m) with an air change rate of 16 complete air changes per h. The room was subject to standard pre-procedural and post-procedural decontamination and sanitization procedures. Both induction and maintenance anesthesia were provided side-by-side to all mice on a stainless steel bench top. The anesthetic circuits and scavenging equipment that we used, as well as the chamber purge rate and anesthetic rates, were the same as those commonly used by IACUC-certified faculty in our institution's animal care and use program. Anesthetic circuits were subject to standard operating procedures, including annual recalibration of vaporizers, key filling of vaporizers and testing of anesthetic circuits for high- and low-pressure leaks prior to each use.

Anesthesia and scavenging techniques

We randomly assigned each mouse to one of four experimental conditions, with seven mice in each condition. We used passive scavenging methods in Condition 1 and Condition 2, and we used active scavenging methods in Condition 3 and Condition 4.

In the passive scavenging conditions, we induced anesthesia by placing each mouse in an induction

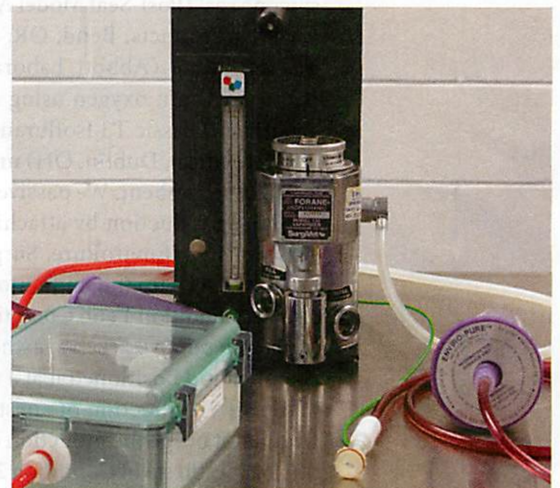

FIGURE 2 | Passive scavenging equipment. Activated charcoal canisters connected to the induction chamber (left) and to the discharge tubing of the non-rebreathing facemask (right) provided passive scavenging of WAG during anesthesia induction and maintenance, respectively, in Condition 1 and Condition 2.

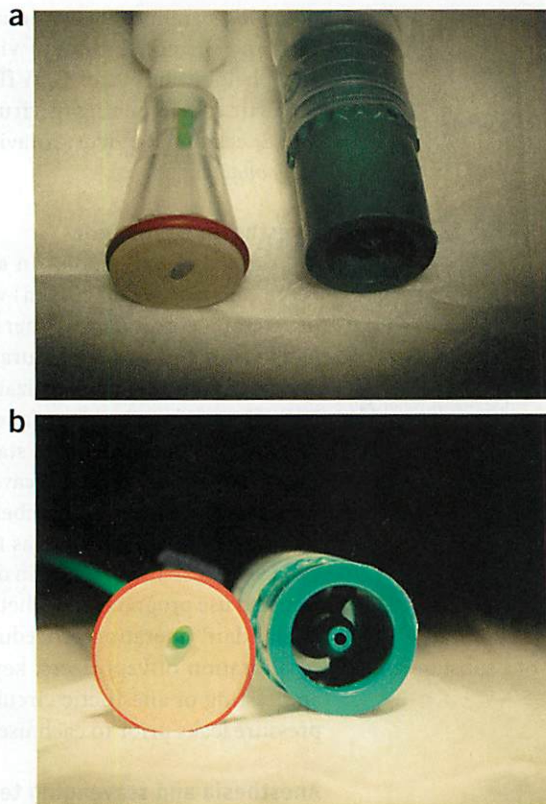

FIGURE 3 | Facemasks used during passive scavenging. (a) Top view. (b) Side view. A loose-fitting facemask (right), which provided no diaphragm seal, was used during maintenance anesthesia in Condition 1. A tight-fitting facemask with diaphragm seal (left) was used during maintenance anesthesia in Condition 2.

chamber (10.2 cm × 24.7 cm × 19.7 cm, 4.9 l interior volume) with a hinged top, locking latches and a silicone seal (Posi-Seal Model AS-01-0532, Molecular Imaging Products, Bend, OR; **Fig. 1**). We delivered 3% isoflurane (Abbott Laboratories, Abbott Park, IL) in 1.5 l/min oxygen using a calibrated precision vaporizer (Classic T3 Isoflurane Vaporizer, SurgiVet, Smith Medical, Dublin, OH) until the mouse became laterally recumbent. We passively scavenged anesthesia during induction by attaching an activated charcoal canister (EnviroPure, SurgiVet, Smith Medical, Dublin, OH) to the induction chamber (**Fig. 2**). In both Condition 1 and Condition 2, we turned off the vaporizer, removed each anesthetized mouse from the chamber and positioned it at a non-rebreathing facemask. After induction of anesthesia in Condition 1, we did not purge the induction chamber. After induction of anesthesia in Condition 2, we purged the chamber with 1.5 l/min oxygen for 5 s prior to opening the chamber and removing the anesthetized mouse. In Condition 1, we used a loose-fitting facemask with a coaxial design and without a diaphragm seal (Rodent Circuit Set model V7103, SurgiVet, Smith Medical, Dublin, OH; **Fig. 3**) for maintenance anesthesia. Maintaining a surgical plane of anesthesia using this facemask required 2% isoflurane in 1.5 l/min oxygen. In Condition 2, we used a tight-fitting facemask with a coaxial design and with a diaphragm seal (URN-NRB System, Model AS-01-0525, Molecular Imaging Products, Bend, OR; **Fig. 3**) for maintenance anesthesia. Maintaining a surgical plane of anesthesia using this facemask required 2% isoflurane at a reduced flow rate of 0.5 l/min oxygen. In both conditions, we passively scavenged anesthesia by attaching an activated charcoal canister (EnviroPure, SurgiVet, Smith Medical, Dublin, OH) to the non-rebreathing facemask circuit discharge tubing (**Fig. 2**). We selected this type of activated charcoal canister in part for its documented integrity and relative low tendency for failure and early-breakthrough WAG emissions[7]. We monitored the canisters weekly to assure that there was no WAG breakthrough.

In the active scavenging conditions, we induced anesthesia by placing each mouse in a sliding-top chamber without capture hood (10.8 cm × 11.6 cm × 23.5 cm, interior volume of 3 l; Model AS-01-0530-SM, Molecular Imaging Products, Bend, OR; **Fig. 1**). We delivered 3% isoflurane (Abbott Laboratories, Abbott Park, IL) in 1.5 l/min oxygen using a calibrated precision vaporizer (Classic T3 Isoflurane Vaporizer, SurgiVet, Smith Medical, Dublin, OH). After anesthesia induction, we turned off the vaporizer and then actively scavenged WAG using a building-wide house vacuum system that provided a default draw of 65 mmHg, as determined by a low-vacuum regulator (Vacutron, Model 22-19-1205-0802, Allied Healthcare Products, St. Louis, MO). We activated the house vacuum at the induction chamber by placing an on/off stopcock in the 'on' position for 5 s.

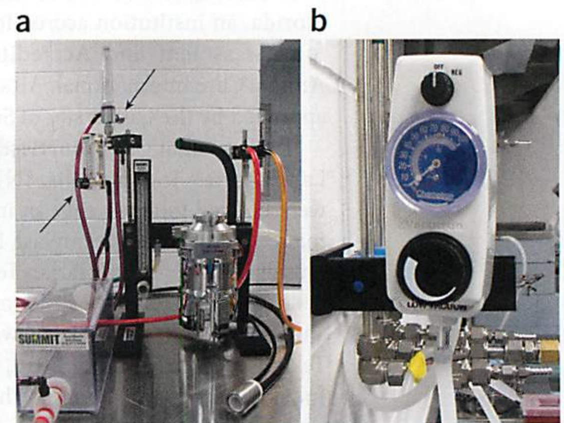

FIGURE 4 | Active scavenging equipment. (a) In Condition 4, one flow meter (left arrow) regulated vacuum WAG evacuation of chambers after each induction. Another vacuum flow meter (right arrow) regulated vacuum draw at the mouse-facemask interface during maintenance anesthesia. (b) In Condition 3, a low vacuum regulator regulated vacuum draw at the mouse-facemask interface during maintenance anesthesia.

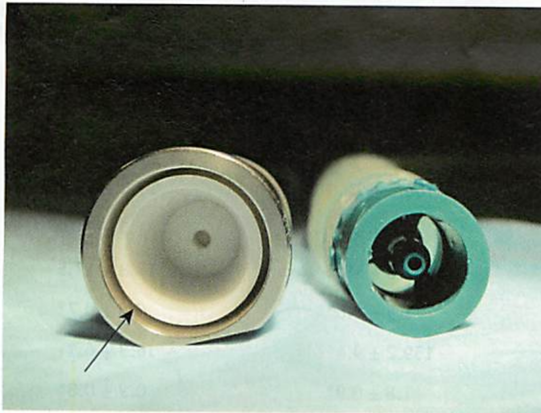

FIGURE 5 | Facemasks used during active scavenging. A loose-fitting facemask (right) that did not separate the mouse's breathing space from the WAG scavenging zone was used during maintenance anesthesia in Condition 3. A machined facemask (left), which has a slit-like circumferential opening at its periphery for WAG scavenging (arrow) separate from the facemask's central breathing zone, was used during maintenance anesthesia in Condition 4.

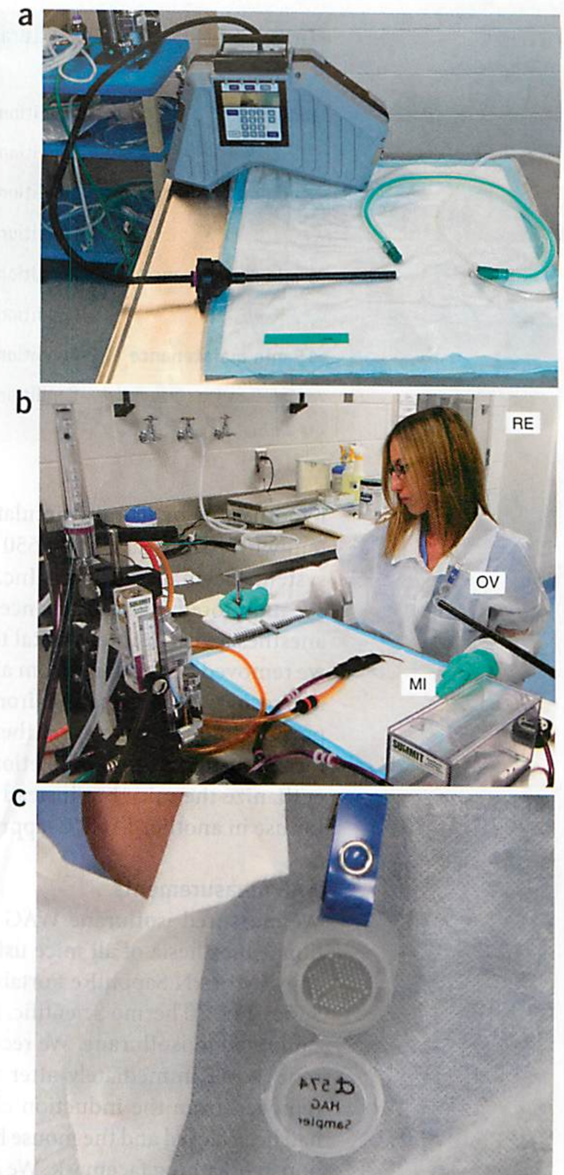

FIGURE 6 | Measurement of isoflurane WAG levels. Infrared spectroscopy equipment (a) was used to take measurements at the mouse-facemask interface, within the operator's vicinity and in the room environment (b) after induction and during maintenance of anesthesia. Personal dosimeters (c) were worn by operators. MI, mouse-facemask interface. OV, operator's vicinity. RE, room environment.

In Condition 3, the vacuum was unregulated, resulting in a 65-mmHg draw, and the connection between the house vacuum and the chamber was direct. In Condition 4, the vacuum was regulated by a flow meter (Vapor-Vac Complete, Model AS-01-0542, Molecular Imaging Products, Bend, OR; **Fig. 4**) set at 8 l/min, resulting in a 32-mmHg draw, and the connection between the house vacuum and the chamber had a relief intake opening, comprised of a fork in the vacuum tubing line, that drew in room air. In both conditions, we removed each anesthetized mouse from the chamber and positioned it at a non-rebreathing facemask. In Condition 3, we positioned mice at the same type of mask used in Condition 1 (a loose-fitting facemask with a coaxial design and without a diaphragm seal; **Fig. 5**). Maintaining a surgical plane of anesthesia using this facemask required 2% isoflurane in 1.5–2.0 l/min oxygen. In Condition 4, we positioned the mice at a machined facemask with a slit-like circumferential opening at its periphery (Posi-Vac Mouse, Model AS-01-0502, Molecular Imaging Products, Bend, OR; **Fig. 5**). Maintaining a surgical plane of anesthesia using this facemask required 2% isoflurane in 0.5–1.0 l/min oxygen. We maintained adequate anesthesia in both conditions by balancing vacuum scavenging draw with anesthetic oxygen flow. In Condition 3, we adjusted the scavenging of WAG at the facemask-mouse interface using a low-vacuum regulator (Vacutron, Model 22-19-1205-0802, Allied Healthcare Products, St. Louis, MO; **Fig. 4b**) set at 35–50 mmHg. It was difficult to balance anesthetic delivery and vacuum draw, however, because of the facemask used in this condition. Some individual mice momentarily became responsive to an inter-digital pinch, and we frequently needed to adjust the anesthetic oxygen flow rate and vacuum regulator settings. In Condition 4, we adjusted the scavenging of WAG at the facemask-mouse interface using a vacuum flow meter (Vapor-Vac Complete, 2-station, Model AS-01-0542; **Fig. 4a**) set at 12 l/min, resulting in a 48-mmHg draw.

In all conditions, we monitored the respiratory rate, body temperature and withdrawal and palpebral reflexes of the mice while under anesthesia. We prevented

TABLE 1 | Mean (± s.d.) isoflurane WAG levels (ppm) measured during passive scavenging of anesthesia.

		Mouse-facemask interface	Operator's vicinity	Room environment
Induction	Condition 1	67.8 ± 44.6	19.9 ± 16.6	1.0 ± 0.7
	Condition 2	52.8 ± 38.5	7.1 ± 5.4	0.8 ± 0.7
2 min maintenance	Condition 1	166.3 ± 12.9	13.3 ± 11.8	1.2 ± 0.3
	Condition 2	2.1 ± 0.9*	1.0 ± 0.7*	0.9 ± 0.6
5 min maintenance	Condition 1	167.8 ± 19.6	17.8 ± 17.5	2.9 ± 1.2
	Condition 2	1.5 ± 0.6*	0.8 ± 0.7*	0.7 ± 0.6
15 min maintenance	Condition 1	159.2 ± 9.4	16.3 ± 6.1	3.7 ± 1.6
	Condition 2	1.8 ± 0.9*	0.9 ± 0.8*	0.7 ± 0.5

*$P < 0.01$.

hypothermia by using a circulating warm-water system with pads (Gaymar TP650 temperature therapy system, Gaymar Industries, Inc., Orchard Park, NY). We monitored the maintenance of a surgical plane of anesthesia via the inter-digital toe pinch reflex. After we removed each mouse from anesthesia, we allowed it to recover in a warmed environment until capable of purposeful movements and then returned it to long-term housing. At the completion of study, we did not euthanize the mice but instead made them available for use in another IACUC-approved protocol.

WAG measurements

We measured isoflurane WAG levels during inhalational anesthesia of all mice using infrared spectroscopy (MIRAN SapphIRe Portable Ambient Analyzer, Series 205A, Thermo Scientific, Foxboro, MA; **Fig. 6a**) calibrated to isoflurane. We recorded levels of isoflurane WAG immediately after the mouse had been removed from the induction chamber, the chamber had been closed and the mouse had been placed on the non-rebreathing facemask. We also recorded levels of WAG after 2 min, 5 min and 15 min of maintenance anesthesia. At each time point, we took measurements in three locations (**Fig. 6b**): at the mouse-facemask interface (2 cm from the mouse's nostrils); within the operator's vicinity (25 cm from the mouse-facemask interface, at the level of the personal dosimeter worn on the lapel of the operator's laboratory coat); and in the room environment (150 cm from the mouse-facemask interface). Between measurements of isoflurane levels, personnel ensured that the infrared spectroscopy analyzer registered undetectable isoflurane levels.

In order to determine whether personal dosimeters provide similar measurements of WAG levels to real-time measurements using infrared spectroscopy, we also measured WAG levels within the operator's vicinity using an unexposed personal dosimeter for halogenated agents (Dosimeter 574, Assay Technology, Inc., Boardman, OH; **Fig. 6c**). The operator wore the dosimeter during induction and maintenance of anesthesia for each mouse of each of the four conditions, so that 28 dosimeters were exposed for the time it took to induce anesthesia plus 15 min of maintenance anesthesia.

Statistical analysis

We carried out analysis of variance (ANOVA) tests for all statistical comparisons. We compared all

TABLE 2 | Mean (± s.d.) isoflurane WAG levels (ppm) measured during active scavenging of anesthesia.

		Mouse-facemask interface	Operator's vicinity	Room environment
Induction	Condition 3	26.4 ± 26.6	13.4 ± 18.3	0.2 ± 0.1
	Condition 4	3.9 ± 3.1	0.3 ± 0.4*	0.2 ± 0.2
2 min maintenance	Condition 3	16.8 ± 22.9	8.0 ± 11.1	0.4 ± 0.4
	Condition 4	0.5 ± 0.4*	0.1 ± 0.3*	0.1 ± 0.1
5 min maintenance	Condition 3	8.2 ± 9.7	5.6 ± 3.7	0.3 ± 0.1
	Condition 4	0.4 ± 0.2*	0.1 ± 0.2*	0.1 ± 0.1
15 min maintenance	Condition 3	7.8 ± 8.6	6.8 ± 4.4	0.7 ± 0.4
	Condition 4	0.3 ± 0.2*	0.1 ± 0.2*	0.1 ± 0.2

*$P < 0.01$.

TABLE 3 | Mean (± s.d.) isoflurane WAG levels (ppm) measured in the operator's vicinity using infrared spectroscopy or personal dosimeter.

		Condition 1	Condition 2	Condition 3	Condition 4
Infrared spectroscopy	**Induction**	19.9 ± 16.6	7.1 ± 5.4	13.4 ± 18.3	0.3 ± 0.4
	2 min maintenance	13.3 ± 11.8	1.0 ± 0.7	8.0 ± 11.1	0.1 ± 0.3
	5 min maintenance	17.8 ± 17.5	0.8 ± 0.7	5.6 ± 3.7	0.1 ± 0.2
	15 min maintenance	16.3 ± 6.1	0.9 ± 0.8	6.8 ± 4.4	0.1 ± 0.2
Personal dosimeter		15.8 ± 7.3	0.5 ± 1.4	11.7 ± 9.8	0

mean ± standard deviation (s.d.) isoflurane WAG levels that were detected in Condition 1 with those that were detected in Condition 2. We compared all mean ± s.d. isoflurane WAG levels that were detected in Condition 3 with those that were detected in Condition 4. We also compared mean ± s.d. time-weighted average levels of isoflurane WAG detected in the operator's vicinity by personal dosimeters during induction and 15 min of maintenance anesthesia with those detected using infrared spectroscopy after 15 min of anesthesia maintenance in each of the four conditions. *P* values <0.01 were considered significant.

RESULTS

Passive scavenging

WAG levels measured at the mouse-facemask interface after anesthesia induction were only modestly lower in Condition 2 than in Condition 1 (**Table 1**), but WAG levels measured at the mouse-facemask interface during maintenance anesthesia after 2 min, 5 min and 15 min were significantly lower in Condition 2 than in Condition 1 ($P < 0.01$). During maintenance anesthesia, WAG levels measured within the operator's vicinity after 2 min, 5 min and 15 min were significantly lower in Condition 2 than in Condition 1 ($P < 0.01$). WAG levels in the room environment did not significantly differ between the two conditions.

Active scavenging

WAG levels measured at the mouse-facemask interface after anesthesia induction were lower in Condition 4 (3.9 ± 3.1 ppm) than in Condition 3 (26.4 ± 26.6 ppm; **Table 2**). WAG levels measured at the mouse-facemask interface during maintenance of anesthesia after 2 min, 5 min and 15 min were significantly lower in Condition 4 than in Condition 3 ($P < 0.01$). Isoflurane WAG levels measured in the operator's vicinity after induction and during maintenance of anesthesia were significantly lower in Condition 4 than in Condition 3 ($P < 0.01$). Isoflurane WAG levels measured in the room environment did not differ significantly between the two conditions.

Infrared spectroscopy versus personal dosimeter

Personal dosimeter readings registered during anesthesia induction and 15 min of maintenance anesthesia correlated significantly with the infrared spectroscopy measurements taken in the operator's vicinity after 15 min of maintenance anesthesia ($P > 0.01$; **Table 3**). No isoflurane WAG was detected by personal dosimeters in the operator's vicinity in Condition 4.

DISCUSSION

Effective scavenging practices to limit personnel exposure to isoflurane WAG during inhalational anesthesia of mice, the most commonly utilized laboratory animal species, must be established in order to implement an institutional program to monitor and mitigate such exposure. Although guidelines encourage scavenging and monitoring of WAG[4,5], exposure limits for isoflurane have not been established[3]. For the present report, we sought to determine whether WAG scavenging practices during inhalational anesthesia of mice could keep isoflurane levels below trace concentrations detectable by smell for halothane (< 50 ppm) or below the NIOSH REL of ≤2 ppm for halogenated agents. The present report documents for the first time the levels of WAG emitted during inhalational anesthesia of mice, the reduction of WAG levels to below trace concentrations by careful implementation of scavenging methods and the similarity of WAG levels detected using personal dosimeters to those measured in real-time using infrared spectroscopy.

In our laboratories, passive scavenging techniques frequently have been used during bench-top procedures involving mice due to their low cost, ease of use and portability[1]. Previous reports have demonstrated that purging induction chambers before opening them reduces WAG levels emitted[1,2]. In this report, mean isoflurane WAG levels in the operator's vicinity after induction were lower in Condition 2 (which included purging of the chamber after induction) than in Condition 1 (which did not include purging of the chamber). WAG levels in both conditions were below the 50-ppm threshold but well above the 2-ppm threshold. Whether implementing a longer purge time

or higher oxygen flow purge rate would result in a further reduction in WAG after anesthesia induction, while still maintaining mice at an appropriate depth of anesthesia amenable to transfer to the facemask, requires future investigation.

Effective passive scavenging during maintenance anesthesia of rats requires the use of an effectively sealed facemask interface[8]. An effective seal retains the pressurized anesthetic gas flow and permits discharge of the gas from the non-rebreathing circuit without leakage to the work environment. Coaxial facemasks are designed to provide such a discharge route for WAG, pushed by the pressure of gas flow. In the present report, WAG levels in the operator's vicinity during maintenance anesthesia were lower in Condition 2, which included a tight-fitting facemask with diaphragm and a reduced flow rate of 0.5 l/min oxygen, than in Condition 1, which included a facemask without a diaphragm and a flow rate of 1.5 l/min oxygen. In Condition 1, residual WAG accumulated not only in the operator's vicinity but also in the room environment, resulting in mean WAG levels >2 ppm after 15 min of anesthesia. Our data indicate that activated charcoal canisters connected to the induction chamber and facemask outflow do not necessarily ensure effective passive WAG scavenging.

In the present report, we also measured WAG levels when active scavenging methods were used during inhalational anesthesia of mice. When the house vacuum setting, which provided a steady draw of 65 mmHg, was used in the absence of a relief intake opening during induction in Condition 3, an audible sealed static vacuum developed within the induction circuit, resulting in inadequate evacuation of isoflurane vapors. Consequently, mean WAG levels in the operator's vicinity after induction were higher in Condition 3 than in Condition 4, when the vacuum draw was reduced from 65 mmHg to 32 mmHg and the vacuum line was provided with a relief opening to draw in room air during induction. Our data indicate that the presence of a house vacuum connection leading from the induction chamber does not necessarily ensure effective active WAG scavenging.

In Condition 3, maintenance anesthesia was delivered using a loose-fitting facemask that did not separate the mouse's breathing space from the WAG scavenging zone. This lack of separation between the breathing space and scavenging zone complicated efforts to balance anesthetic delivery and vacuum WAG scavenging, causing both anesthetic depth and WAG levels emitted to be highly variable. As a result, WAG levels during maintenance anesthesia in Condition 3 were consistently above the 2-ppm threshold in the operator's vicinity. In contrast, use of a facemask that separated the breathing space and WAG scavenging zones in Condition 4 permitted a reliable balance between anesthetic depth and vacuum scavenging draw and resulted in lower mean isoflurane WAG levels that were nearly undetectable. These results indicate that the presence of a house vacuum connection to the non-rebreathing facemask alone did not ensure effective WAG scavenging.

Our results suggest that with careful anesthetic and passive or active scavenging techniques, it is possible to maintain WAG levels below trace levels in the operator's vicinity during inhalational anesthesia of mice. Occupational WAG exposure in the mouse anesthetic environment can be reduced by establishing a substantiated WAG air quality program to document, train and monitor personnel in the use of best anesthetic and scavenging practices. Recommendations regarding WAG monitoring in the laboratory animal setting include conducting biannual assessments for emitted WAG levels and using multiple WAG monitoring modalities during WAG monitoring program development and implementation[1]. In the present report, we describe the use of continuous air sampling and infrared spectroscopy to monitor WAG levels during inhalational anesthesia of mice. These data allow us to document detectable WAG levels and to determine whether scavenging devices and methods contribute to reduced WAG emissions and improved methodologies.

In order for a WAG air-monitoring program to become routine, the monitoring process must be cost-effective, efficient and verifiable. Correlation of infrared spectroscopy findings with *personal dosimeter* readings ensures that routine checks using personal dosimeters appropriately reflect WAG levels in the operator's vicinity. Personal dosimeter readings using time-weighted-average sampling correlated with the infrared spectroscopy measurements taken in the operator's vicinity after 15 min of maintenance anesthesia, confirming that the real-time WAG levels detected were not transient. Our results indicate that WAG scavenging practices can be reliably assessed using time-weighted-average personal dosimeters.

This study contributes towards a substantiated WAG air-monitoring program for the laboratory animal setting. Many variables contribute to WAG emissions. Additional studies are required to establish best anesthetic and scavenging practices applicable to various experimental settings involving mice and to ensure that monitoring programs actually lead to reductions in WAG emissions in the mouse anesthetic environment.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Producing timed-pregnant Mongolian gerbils for developmental studies

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The developing fetus is differentially susceptible to chemicals and pathogens depending on the stage of its development. In developmental studies, embryos or fetuses therefore must be exposed to experimental interventions at the same gestational stage. Acquiring sufficient numbers of embryos of the same developmental stage requires the use of timed-pregnant animals. Timed-pregnant Mongolian gerbils currently are not available for purchase. The authors developed a novel method for timed mating of virgin female gerbils. Female gerbils were housed in the same cage as males, but physically separated by a partition, for 3 d in order to expose the females to the males' pheromones before they were allowed to interact. Females were monitored for lordosis to determine sexual receptivity. Lordosis was observed in 10 of 15 females (67%), and 9 of these females (90%) became pregnant. When lordosis was not observed, none of the females became pregnant. These results demonstrate that the timed mating method produces a high rate of mating success and indicate that lordosis is a reliable predictor of sexual receptivity and subsequent successful mating in the Mongolian gerbil.

Adverse outcomes resulting from fetal exposure to certain pathogens, chemicals or pharmacological agents often vary depending on the stage of development at which the exposure occurs. Because of these variations in susceptibility of the embryo or fetus, studies of developmental exposure to potentially harmful substances or organisms require accurate tracking of the gestational age so that such substances are administered to the pregnant female at the correct time. This is necessary for both the reliability and reproducibility of a study, and it is especially important for rodents with short gestation periods because relatively large advances in development can occur in a single day.

The developmental stage cannot easily be determined in utero and often requires restraint or anesthesia, which produce stress during pregnancy and can confound experimental design. For these reasons, developmental exposure studies use timed-pregnant animals, which are actively monitored during mating and then removed from their partners, allowing researchers to pinpoint the time of conception to within a few hours. Gestational day (GD), as measured from the date that mating occurred, is used as a surrogate for the developmental stage of the fetus.

Our laboratory carries out dose-response research to investigate the adverse effects of exposure to chemicals and pathogens during prenatal development. We have consistently used timed-pregnant mice[1], guinea pigs[2] and primates[3] as animal models in our studies. In some rodent species, pregnancies can be timed by exploiting the female's post-partum estrus period and mating sows or dams immediately after the births of their litters[4]. The feasibility of using this technique to consistently produce adequate numbers of timed-pregnant animals relies on multiple dams giving birth at the same time, and this method precludes the use of virgin females in the experimental design. In large breeding colonies, probability and density dictate that several animals will give birth on the same day and, thus, all can be bred again at the same time, but in smaller breeding colonies,

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