Refinement of Isoflurane Anesthesia Scavenging Method by Using Modified Prototypes and Personnel Monitoring Exposure Assessment in Vivarium

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Abstract

Anesthesia is an important procedure performed in laboratory animals as part of the experimental activity. A calibrated vaporizer is recommended for precision delivery and efficient scavenging is an integral part of avoiding possible health hazards for personnel involved in gas anesthesia. The vivarium facility is equipped with scavenging devices such as activated charcoal canisters, spot extractors, fume hoods, and biosafety cabinets those are engineering controls used during isoflurane anesthesia. A modified prototype was necessitated especially when rodent procedures were conducted on the table due to a higher turnover in a barrier-maintained facility. Hence, a well-designed prototype with different shapes was developed to improve the scavenging methods during long-term tabletop anesthesia procedures where numerous animals are handled to complete the activities within the timeframe. The concentration of waste anesthetic gas was captured systematically from 13 laboratory personnel at different intervals and analyzed for quantitative exposure assessment for isoflurane. The results revealed that the waste anesthetic concentration captured for laboratory personnel was between 0.08 to 1.45 ppm. This result is below the reported recommended laboratory concentration (0.23 - 3.40 ppm). Moreover, the Time Weighted Average (TWA) personal exposure to isoflurane was found to be below the Health and Safety Executive (HSE) established Workplace Exposure Limit (WEL)-TWA (8 hours) of 50 ppm. The National Institute for Occupational Safety and Health (NIOSH) has a non-regulatory recommended exposure limit (REL) for halogenated agents (e.g., isoflurane) of 2 ppm or 15 mg/m^3 as a ceiling limit (over a sampling period not to exceed one hour) during anesthetic administration. The Occupational Safety and Health Administration (OSHA) has not established a regulatory permissible exposure limit (PEL) for anesthetic gases; therefore, 2 ppm is used as the exposure threshold. Since each laboratory research and environmental conditions vary, it is important that exposure monitoring is performed at least one time to ensure isoflurane levels are less than 2 ppm. Overall, the implementation of a newly developed prototype along with canisters and an intensive exposure assessment was found to efficiently capture the waste anesthetic gas from induction chambers and surgical areas with nose-cone where isoflurane is constantly delivered during anesthesia. The portable setup with active suction arms and flexible hose connectors can be placed on a table and/or enclosure to reduce the occupational exposure of Isoflurane to laboratory personnel and demonstrated that the prototype had improved the scavenging practices in the vivarium.

Keywords: Isoflurane anesthesia, Laboratory Animals, Waste anesthetic gas, Scavenging Methods, Occupational Health and Safety, Exposure Assessment.

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Introduction

The laboratory animal facility has a variety of functions to carry out the research activities and cross-functional teams such as engineering and maintenance, environmental health safety and sustainability also play a pivotal role in the successful operation of the vivarium by ensuring employees' safety as a priority. Anesthesia is primarily administered through either inhalational or injectable methods and isoflurane has been used as an inhalant agent predominantly in rodents at our facility apart from other available agents. It is recommended to perform anesthesia under the approved research protocol using pharmaceutical-grade compounds (Navarro *et al* 2021) with appropriate dose levels for the species whenever possible.

The isoflurane is volatile with a pungent odor and the time to loss of consciousness may be prolonged due to breath-holding capacity and their anaerobic metabolism (AVMA 2020) this agent is also used for euthanasia of animals. However, inhalational anesthesia is commonly used in laboratory animals (Cesarovic *et al.,* 2010; Li *et al.,* 2001 and Fay 2017), and establishing a system to monitor the waste anesthetic gas requires an effective scavenging method to reduce personal exposure (Todd *et al.,* 2013). The relatively easy and quick method is inhalation anesthesia and a precision vaporizer is required including a regulator with oxygen as a carrier to deliver recommended concentrations for smooth induction and maintenance of animals. Moreover, the guide (NRC 2011) reiterate that waste anesthetic gas scavenging and adequate monitoring of animal is important. Isoflurane emissions may originate from anesthesia induction procedure or gas delivery through the nose cone (Nesbitt *et al.,* 2013) and individuals who perform the activity may be accidentally exposed which becomes hazardous over the period. Generally, an activated charcoal canister is used as an absorbent for an effective scavenging method of choice and several others are also available that can be used based on the purpose and duration of activities.

Health and Safety Executive (HSE 2011) from the UK has established a Workplace Exposure Limit (WEL) -Time Weighted Average (TWA) of 50 ppm (8 hours) for Isoflurane. However, the country-specific Occupational Exposure Limits (OELs), such as the Permissible Limit of Exposure (PLE) prescribed by The Indian Factories Act and global employee exposure guideline values (TLV) established by the American Conference of Governmental Industrial Hygienists have not been established for isoflurane (Achutan *et al.,* 2016). The WELs, TLVs, and PLEs are intended for use in industrial hygiene as guidelines and/or recommendations in the control of potential workplace health hazards. In addition, low levels of chronic exposure to waste anesthetic gases is likely to be a potential health hazard such as reproductive, renal, and hepatic toxicity including neoplasia to professionals (Smith and Bolon, 2002) and there may be chances that the emission of isoflurane can also occur even well-maintained precision vaporizers and canisters used depending upon the scavenging systems employed at the facility. The exposure of dose rates and risks of isoflurane are reviewed through open literature (1995 to 2020) and summarized the potential exposure scenarios and adverse effects of isoflurane among anesthetists, operating room personnel, investigators and veterinarians (Pokhrel and Grady, 2021) and further reported that the isoflurane target on the central nervous system, dose-dependent effects of cardiac hemodynamics, impair the pulmonary systems and cross the placental barrier leading to potential congenital malformation in fetus. A systematic review was performed using PubMed, EMBASE, and Web of Science to assess all the available evidence of the effects of isoflurane from studies of controlled exposure in laboratory animals. The systematic review outcomes revealed that reproductive health effects are reported (Struijs *et al* 2023). Even though the isoflurane was introduced for clinical use in 1979 the scavenging method was not so prevalent until 2000. Hence, these exposure limits are considered as a relative index of toxicity depending on the concentrations with time. In general, the average exposure to a contaminant in any form that the workers may be exposed to without any adverse effect for 8 hours/day (40h/week an average work shift) (ACGIH, 2007) and this refers to the time-weighted average (TWA). Recently, the NIOSH Recommended Exposure Limit (REL) for isoflurane was 2.0 ppm to protect personnel in occupational settings (NIH, 2016). Hence, it is the employer's responsibility to comply with safety standards and to ensure that their employees' work environment is free from hazards (OSHA, 2000). Considering the above factors and the limited availability of published data on personal exposure to isoflurane, the project was undertaken at our facility with the objectives of developing a prototype and its validation for the efficiency of the scavenging system, quantitative assessment of isoflurane waste anesthetic gas capturing while routine animal procedures to limit the personnel exposure, to refine the existing practices to improve workplace standards by ensuring occupational exposure levels within limits in vivarium.

Materials and Methods

Case Report

The isoflurane exposure monitoring for laboratory personnel was performed as a Quantitative Exposure Assessment (QNEA) by the Certified Industrial Hygienist (CIH) and supervised the activities as well as provided technical guidance for successful evaluation by adhering to the policies and recommendations. The OSHA has recommended that air sampling and analytical methods be used for the QNEA. However, an American Industrial Hygiene Association (AIHA) accredited laboratory (Galson Laboratories, NY, USA / Bureau Veritas North America Inc. MI, USA) analyzed all the samples. An employee's exposure during air monitoring was representative of any typical work exposure assessment. Hence, the representative samples were captured randomly from laboratories (Surgical suite and Immunology section) with higher experimental turnovers during the period of occupational exposure assessment on routine days. The samples were collected from 13 laboratory personnel (6 Investigators and 7Animal care staff) involved in various animal procedures.

Vivarium Overview

Syngene Laboratory Animal Research (SLAR) facility at Syngene International Limited (AAALAC accredited and GLP certified since 2009) offers discovery and development support for various therapeutic areas of pre-clinical research. The barrier facility is built by using clean room panels with epoxy floor and provision of heating ventilation and air conditioning (HVAC) with 15-20 air changes per hour (100% exhaust) controlled by Building Automation System (BAS). The facility has 12 laboratories wherein inhalant anesthesia is used for at least 25 procedure areas identified and all the anesthesia vaporizer system was calibrated annually by the

certified agencies. The exposure monitoring was conducted after the necessary approval from the health safety committee and obtaining consent from employees and two laboratories were selected with ongoing experiments in different phases. The Institutional Animal Ethics Committee (IAEC) approved animals under different protocols (Form B) and a list of investigators for the ongoing experiments was monitored for exposure assessment. The facility has approved Standard Operation Procedure (SOP)/guidelines for all the animal procedures by veterinary sciences and institutional policy for the safe use of chemicals including workplace safety also monitored by the Environmental Health Safety and Sustainability (EHSS) within the premises.

Prototype Development and Scavenging Methods

The in-house engineering team developed a custom device as an additional support system for the existing scavenging methods which are under practice. The Local Exhaust Ventilation (LEV) (spot extractor) of different capacities (L shape, E shape) was developed and connected with an independent exhaust blower and operated by an ON/OFF switch controller. The LEV was made up of stainless steel for easy sanitation and provision of perforated holes at the horizontal arms of the medial plenum for active suction during its operation from the surroundings. The prototype (L shape) was used where the surgical platform was positioned in between LEV arms for surgery purposes. Similarly, E E-shaped prototype was used for routine anesthesia where anesthetic chambers were kept in-between the arms for capturing waste anesthetic gas released due to the opening of the top lids of twin anesthetic chambers. The prototype LEV was validated by performing air velocity measurements by the engineering and maintenance team using a calibrated air velocity meter (LUTRON digital anemometer). The functional activity of prototype LEV was monitored during the assessment period thereafter samples were collected from the laboratories for analysis.

Inhalant Anesthesia Systems

A mixture of isoflurane vapors and oxygen gas (3-5 % isoflurane at a 2-4 lpm flow rate of oxygen) was supplied either from SurgiVet system / Drager Vapor 2000 system / VetEquip anesthesia system through a manifold (with flow regulator valves) for the anesthetic induction chambers to rodents. At the surgery suite, anesthesia was supplied through a nose cone as a maintenance dose to rats during the evaluation where jugular vein cannulation and bile duct cannulation surgery were performed at different anesthesia stations. However, the induction chambers were used for anesthesia at the immunology laboratory for planned procedures including bleeding of mice.

Evaluation Criteria and Documentation

The degree of exposure was assessed by evaluation criteria to determine personnel exposure to identify if the exposure limit is within the stipulated recommendation by an approved agency. All the exposed personnel did not use respiratory protection except a face mask and other regular Personnel Protective Equipment (PPE's) was used such as a facility apron, lab shoes, shoe covers, head cap, goggles and gloves while performing the activities and evaluated as like routine days. The analytical reference method (Modified OSHA 103) was used and sampling media (coconut shell charcoal tubes - SKC 226-09 was attached with user's apron collar while on sampling) for exposure absorption of isoflurane and Limit of Quantitation (LOQ - 10 µg) was adopted by the laboratory for isoflurane detection. However, there is no information available for isoflurane (ACGIH, 2013 TLV: NA) and the Indian Factories Act PLE (TWA: NA). Hence, the recommendation of HSE established WEL-TWA (50 ppm) was adopted as a reference to compare the isoflurane concentrations of our laboratory personnel and expressed in units (ppm).

Result

The individual employee samples (n=13) analysis report revealed that the concentrations of isoflurane were detected below 50 ppm (Table 1). However, the total duration of exposure measurement was between 64 to 394 min and the average flow rate was maintained (0.05 L/min) during the entire assessment period. The laboratory-reported concentration (0.23-3.4ppm) was compared with TWA concentrations (8 hours/day) and both measurements were monitored by two different CIHs. The concentrations captured by the investigators and animal care staff were 0.08 - 0.96 and 0.12 - 1.45 ppm, respectively. All thirteen laboratory personnel were given feedback that no differences were observed in their general health conditions during the assessment period. The recovery percentage of isoflurane (98.3 ± 12.44) from all the samples were within acceptable limit and met the analysis criteria. The LEV (L shape and E shape) was found to be quiet and less noisy while functioning where the surgery platform as well as induction chambers were also used for animal procedures and waste anesthetic gases were scavenged correspondingly by the custom prototype (Figure 1-3).

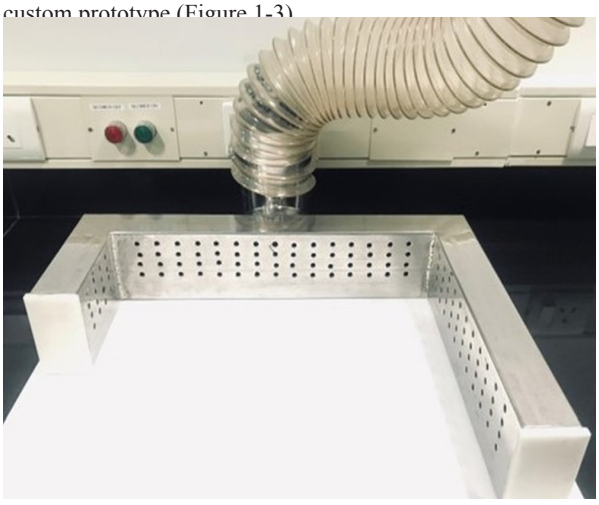

Figure 1: The Prototype - Local Exhaust Ventilation (L Shape) with perforated holes for active scavenging of waste anesthetic gas.

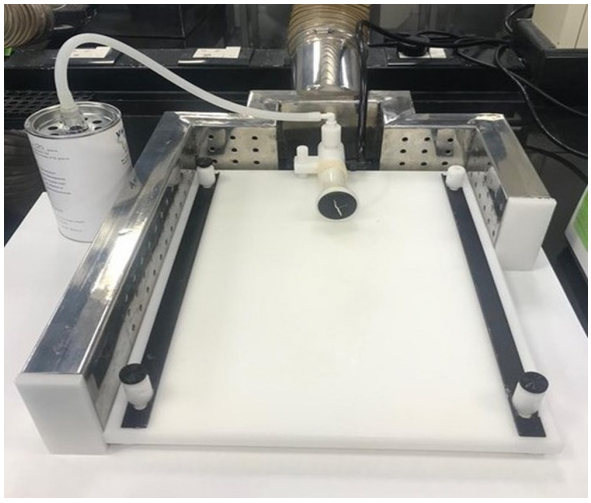

Figure 2: The Prototype - Local Exhaust Ventilation (L Shape) with surgical platform and activated carbon scavenging canister used for long term surgery.

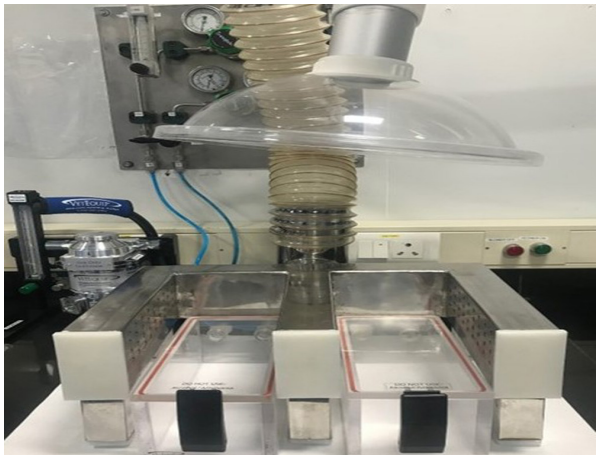

Figure 3: The Prototype - Local Exhaust Ventilation (E Shape) aligned over the acrylic induction chambers for effective scavenging of anesthetic gas during opening of the top lids while placing/removing of rodents.

Discussion

The exposure concentration of each employee was carefully assessed and their personal exposure levels and compared with laboratory-reported concentration. At the surgery suite, the TWA exposures to isoflurane for the five samples collected for investigators/surgeons during the QNEA ranged from 0.08 ppm - to 0.96 ppm. The investigators performed surgeries i.e., jugular vein cannulation and bile duct cannulation that required an approximate duration of 10-15 min and 30-45 min, respectively. During the monitoring period, investigators performed 4-5 jugular vein surgeries for three of the five employee exposure monitoring samples collected. Similarly, in the remaining two samples, the investigators performed 3 bile duct cannulation surgeries including jugular vein cannulation for the same rats. However, the number of surgeries performed during the shift may vary based on the research requirement, but the surgery activity was well distributed between surgeons in case of higher workload. During the above scenario, prototype LEV (L shape) was installed for capturing the waste anesthetic gas while on surgeries. On the other hand, TWA employee exposures to isoflurane for the three samples collected for animal care staff during the QNEA were 0.65 ppm, 1.42 ppm, and 1.45 ppm and hence both investigators and animal care staff concentrations were below the HSE-established WEL-TWA of 50 ppm at surgery suite. A flexible hose was connected to induction chambers for carrying waste anesthetic gases to an activated carbon canister (used for induction of anesthesia) and another hose was fitted with a nose cone over the thermo-controlled surgical platform (maintenance of surgical anesthesia) that was scavenged by prototype LEV. The carbon canister was periodically monitored for its weight increase and recorded on the canister then disposed of once it reached 50g of its initial weight (as per the manufacturer's recommendation). In addition, animal care staff also assisted investigators while performing surgery by handling surgical instruments/tools needed for surgery, as and when required, and occasionally stood near the investigator during surgery as a helping hand. The prototype LEV was aligned above the induction chamber to capture gases being released from the acrylic induction chamber whenever placing and removing the rats. In addition, the prototype LEV was validated by performing air velocity measurements and the average actual volumetric airflow rate of the LEV was greater than the designed airflow volumetric rate of 100 and/or 110 cfm.

A study showed that the isoflurane exposures were significantly higher significant among the procedures conducted on rodents as compared with the large body mass patients such as human non-human primates and swine ($P < 0.05$) while using the charcoal canister scavenging system when compared with the central vacuum exhaust system (Newcomer and Chopra, 2019). Furthermore, elevated occupational exposures were experienced while performing surgeries by the individuals with both direct-reading instruments and passive diffusion monitors where the exposure was measured significantly higher in the breathing zone as compared with any area within the room ($P < 0.05$). Similarly, another study demonstrated that the residual isoflurane concentrations were measured by using an infrared analyzer, especially the corner of the opposite side of the anesthesia machine, surgeons' breathing zones, anesthesiologist, and the animals subjected to anesthesia including the anesthesia machine at three-time points (5, 30 and 120 minutes intervals). The residual concentrations were gradually increased opposite to the anesthesia machine the surgeon's breathing zone and the anesthesiologist ($P < 0.05$). Further, the high residual isoflurane concentrations in the operating room where there was no exhaust system and suggested implementing exhaust systems to reduce the waste anesthetic gas to minimize occupational exposure (Drielle et al, 2020).

A study reported that waste anesthetic gas (WAG) levels measured by active scavenging had improved when a relief intake provision was made in the induction chamber while opening, the vacuum line draw waste anesthetic gas after the induction and vacuum scavenging draw also balanced

Sample ID	Employee ID	Total Duration (Min)	Average Flow Rate (L/Min)	Volume (Liter)	Laboratory reported Concentration (ppm)	TWA Without RPE (ppm)
Laboratory 1: Surgery Suite (Pharmaceutical Candidate Optimization)						
SLAR 101	Investigator 1	245	0.04884	11.96	1.8	0.92
SLAR 102	Investigator 2	64	0.05201	3.33	4	0.53
SLAR 103	Investigator 3	46	0.04862	2.24	0.85	0.08
SLAR 104	Investigator 4	46	0.05106	2.35	10	0.96
SLAR 105	Animal Care Staff 1	347	0.05179	17.97	2	1.45
SLAR 106	Animal Care Staff 2	234	0.05151	12.05	3.4	NA*
SLAR 107	Animal Care Staff 3	310	0.05120	15.87	2.2	1.42
SLAR 108	Animal Care Staff 4	368	0.05120	18.84	0.85	0.65
Laboratory 2: Immunology Section						
SLAR 109	Investigator 5	274	0.04830	13.23	0.23	0.13
SLAR 110	Investigator 6	339	0.04820	16.34	0.73	0.52
SLAR 111	Animal Care Staff 5	394	0.05113	20.15	0.43	0.35
SLAR 112	Animal Care Staff 6	350	0.05029	17.60	0.57	0.42
SLAR 113	Animal Care Staff 7	130	0.05022	6.53	0.45	0.12
SLAR 114	Blank Sample 1	0	NA	0	< 10 µg	NA
SLAR 115	Blank Sample 2	0	NA	0	< 10 µg	NA

Table 1: Summary of personal exposure monitoring results of isoflurane in vivarium

NA - Not Applicable; RPE-Respiratory Protective Equipment

*NOTE - Potential for employee exposure to isoflurane existed for the animal care staff before the monitoring and has not been captured during employee exposure monitoring, hence TWA for that employee exposure was not calculated for the particular animal care staff.

during maintenance by wearing diaphragm-sealed facemask that separated the scavenging zone from breathing space (Taylor and Mook, 2009). However, another finding revealed that extraneous emissions of isoflurane were determined and originated from the pre-procedural induction process as well as gas delivery nose cone that was decreased below the recommended occupational levels by the implementation of local exhaust ventilation controls in the operating room including surgeons breathing zone (Nesbitt *et al*, 2013).

The immunology laboratory TWA employee exposures to isoflurane for the two samples collected from investigators during the QNEA were 0.13 ppm and 0.52 ppm. The investigator injected test article (32 mice) for the first employee exposure monitoring sample, thereafter, euthanasia was performed by deep isoflurane (100 mice) as part of the terminal procedure (death ensured by the investigator) during the second employee exposure monitoring sample collected. The animal care staff involved in anesthesia and transferring the mice from the home cage to induction chambers then removed them before handling them to the investigator for further procedures (anesthesia monitored by investigator/veterinarian). Similarly, the investigator placed mice in between the suction arms of the prototype (L shape) for immunization of mice. On the other hand, TWA employee exposure to isoflurane was collected for three animal care staff involved and supported investigators during anesthesia in mice at the immunology laboratory during QNEA were 0.12 ppm, 0.35 ppm, and 0.42 ppm hence both the investigators as well as animal care staff concentrations were below the HSE established WEL-TWA of 50 ppm at immunology laboratory. The animal care staff was involved as part of anesthesia (32 mice) during the first employee exposure monitoring sample collected and 100 mice during the second employee exposure monitoring samples collected. However, the animal care staff performed shaving of 50 mice after anesthetizing them during the third employee exposure monitoring the samples collected. A prototype LEV was provided (suction arms of LEV aligned semi-peripherally around the top lid of twin induction chambers) for capturing the waste anesthetic gas released due to the opening of the top lid of the chamber for replacing mice during anesthesia. The prototype LEV was validated by performing air velocity measurements and averaged the actual volumetric airflow rate of LEV greater than the designed airflow volumetric rate of 201 cfm. A previous study reported that the ventilation system installed by the employer in the procedure room had reduced approximately 86% of isoflurane concentration after

the installation from 12 full shift sampling periods (Achutan *et al*, 2016). Similarly, isoflurane exposure was investigated from a horizontal laminar flow with the semi-open anesthetic chamber (1-4 liters) either partially or tightly fitted condition and without any scavenging system shown that measured concentrations of all three chambers and nose cone had elevated levels of anesthetic gas in the breathing zone as well as exhaust level when compared with the baseline (Cooper *et al*, 1996). Moreover, the atmospheric concentrations of isoflurane were assessed (active and passive) for the period ranging from 135-268 min, and TWA (8 hours) adjusted levels showed that 1.76 (0.73 ± 9.13) ppm of isoflurane (Johnstone *et al*, 2017) which is comparable with our laboratory findings and analyzed levels were well within the acceptable limits. In addition, a report suggested the use of a "T" shape connector showed that a single charcoal scavenging canister was sufficient to scavenge from multiple ports instead of 3 waste anesthetic gas canisters used while inhalational anesthesia such as induction box, operative table, and gas monitoring purposes and opined that "T" connector was acceptable for their practice and safe to use in laboratory (Palmisano and Deininger, 2015). Overall, the custom-made prototype LEV efficiently scavenged isoflurane and corroborated with other published information about the waste anesthetic gases scavenging process, hence further study is needed to set the recommendation of isoflurane within the context of various procedures performed in laboratory animal facilities.

Considering the above, it is concluded that the refinement of anesthesia procedure by adopting prototype LEV systems demonstrated through efficient scavenging of isoflurane during rodent procedures including survival surgeries. The results demonstrated that all the measured concentrations were below the HSE-established WEL-TWA (8 hours) of 50 ppm and performed over a period of 3 days from two different laboratories where 13 employees were involved in various animal procedures. Collectively, the refined method of the newly developed prototype LEV scavenging system along with other engineering controls had reduced the potential hazards of volatile waste anesthetic gases thereby ensuring the laboratory personnel's safety and improving the scavenging practices in the vivarium.

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Conflicts of interest

The authors declare No conflicts of Interest

Author Contributions:

The authors contributed at various phases such as designing of experiment, execution, sample analysis and data interpretation.

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