
Isoflurane leakage from non-rebreathing rodent anaesthesia circuits: comparison of emissions from conventional and modified ports

J C Smith¹ and B Bolon^{2*}

¹Department of Laboratory Animal Resources and ²Department of Pathology, Amgen Inc, Thousand Oaks, California, USA

Summary

Chronic exposure to low levels of fluorocarbon-based waste anaesthetic gas (WAG) has been linked to a multitude of human health problems. We have shown that isoflurane exhaust from passive gas-scavenging canisters is often quite high when using conventional rodent anaesthesia protocols and equipment. Another likely source of WAG build-up in rodent procedure rooms is leakage at the interface between the breathing circuit and the animal's face. We evaluated this possibility using three non-rebreathing circuits: traditional Bain, modified Bain, and Mapleson (type E). For the Mapleson E circuit, a conical rodent facemask was attached and used in one of two configurations: normal aperture, or aperture modified with a latex diaphragm (cut from an unpowdered surgical glove) to reduce the orifice diameter and tighten the seal. Adult female Sprague-Dawley rats were anaesthetized with isoflurane (5% for induction, 2% or 3.5% for maintenance) in oxygen (2 L/min for induction, 1 L/min for maintenance). Isoflurane leakage was assessed by real-time spectrophotometry. In 94% of the trials, three configurations – traditional Bain, modified Bain, and Mapleson E with unmodified mask – permitted isoflurane leakage approaching or exceeding 100 ppm at the face/port interface. In contrast, the Mapleson circuit with diaphragm-modified mask emitted significantly ($P \leq 0.0003$) less isoflurane (peak of 9.5 ± 1.7 ppm [mean \pm standard error]). These data indicate that (1) WAG leakage from standard rodent non-rebreathing circuits is substantial, and that (2) a simple, rapid, and economical modification to a conventional rodent facemask can significantly reduce WAG exposure to workers performing many rodent anaesthesia procedures in one session.

Keywords Isoflurane; waste anaesthetic gas; rodent; emission

Chronic exposure to trace levels of fluorocarbon-based waste anaesthetic gas (WAG) ('anaesthetic pollution'), such as halothane and methoxyflurane, is an occupational hazard experienced by 200,000 or more health-care professionals in the United States – including an estimated

50,000 veterinarians and veterinary technicians (National Institute for Occupational Safety and Health [NIOSH] 1977). Many epidemiological and experimental data suggest that chronic exposure to WAG is causally related to neurological and reproductive dysfunction, hepatic and renal toxicity, and neoplasia (for reviews, see Manley & McDonell 1980, Paddleford 1986, Smith 1993, Dorsch & Dorsch 1999a,b). Co-workers and family members who mingle with exposed

*Present address: GEMpath Inc, 2540 N 400 W, Cedar City, UT 84720, USA

Correspondence: Jennifer Smith, Amgen, One Amgen Center Drive, M/S 15-1-A, Thousand Oaks, CA 91320-1789, USA. Email: smithj@amgen.com

individuals may also be at risk (Cohen *et al.* 1980, Guirguis *et al.* 1990) as metabolites of halogenated inhalation agents (e.g. halothane, methoxyflurane) are amassed by the body (Pfaffli *et al.* 1972, Corbett 1973) and exhaled in appreciable quantities (Corbett & Ball 1971, 1973, Whitcher *et al.* 1971). The evidence for WAG-induced health hazards is inconclusive (Rosenberg & Vanttinnen 1978, Axelsson & Rylander 1982, Buring *et al.* 1985, Tannenbaum & Goldberg 1985, McGregor 2000), and the impact of long-term exposure to trace isoflurane levels has not been defined. Nevertheless, the US NIOSH composed a criteria document in 1977 to ameliorate occupational exposure to waste halogenated anaesthetics based on effects attributed to halothane and methoxyflurane (NIOSH 1977). While the US Occupational Safety and Health Administration (OSHA 2002) has not instituted a formal standard based on this criteria document, the NIOSH recommendation (2 ppm) has been applied to newer halogenated agents – including isoflurane, an anaesthetic agent that is commonly used in conventional laboratory animal facilities.

Anaesthetic pollution in the occupational setting has been substantially reduced during the past three decades by improved control practices. Nonetheless, previous work in our facility reveals that isoflurane will be present at trace levels in the room atmosphere of conventionally equipped rodent procedure rooms even when a well-maintained anaesthesia system is used (Smith & Bolon 2002, 2003). In these studies, the primary WAG sources that we identified were emissions from the exhaust ports of passive gas-scavenging canisters and bench-top induction boxes. We predict that another significant source of WAG during large-scale rodent experiments will be leakage of isoflurane from poorly fitted facemasks. Furthermore, we anticipate that the atmospheric waste gas levels resulting from standard rodent anaesthesia protocols will expose research personnel to isoflurane at concentrations approaching or exceeding the NIOSH recommendation of 2 ppm. In the current experiment, we tested these

suppositions using a standard rodent procedure room, routine rodent inhalation anaesthesia protocol, and commercially available equipment.

Materials and methods

Experimental animals

This study was conducted in accordance with federal animal care guidelines, in an AAALAC accredited facility, and was pre-approved by the Amgen Institutional Animal Care and Use Committee. Adult, female, Sprague-Dawley rats (CrI:CD (SD)IGS BR; Charles River Laboratories, Wilmington, MA, USA; $n = 15$) weighing 212–337 g (i.e. retired breeders slated for removal) were acclimatized for at least one week prior to use. Animals were housed two per cage in filter-capped polycarbonate cages (Allentown Caging and Equipment, Inc, Allentown, NJ, USA), containing sterilized wood shavings (Sani-chip, Harlan-Teklad, Madison, WI, USA); cages were placed on a ventilated rack (Allentown Caging and Equipment, Inc) and maintained under constant environmental conditions ($22 \pm 1^\circ\text{C}$, relative humidity 50%). Rats were kept on a 12 h:12 h light-dark cycle (lights on at 06:00 h) and given bottled drinking water (purified by a reverse osmosis system; Edstrom Industries, Waterford, WI, USA) and pelleted chow (#8640; Harlan-Teklad) *ad libitum*.

Choice of anaesthetic agent

Isoflurane is commonly used for rat inhalation anaesthesia (Waynforth & Flecknell 1992), and is the agent of choice in many laboratory animal facilities. Its suitability is attributed to both its desirable physical properties and its limited toxicity (briefly reviewed in Smith & Bolon 2002). In the present study, we employed IsoFlo (Abbott Laboratories, North Chicago, IL, USA).

Configuration of anaesthesia machines

Isoflurane was administered using Laboratory Animal Anaesthesia Systems (VetEquip, Pleasanton, CA, USA). Delivery

was controlled using precision isoflurane vaporizers (Isotec3, Cyprane, West Yorkshire, UK), with an adjustable dial to regulate the output of isoflurane (concentration range, 0–5%) coupled with a separate oxygen flow meter (range, 0.2–4 L/min). Each unit was configured with two circuits, one directed to a 2 L acrylic induction chamber, and the other to a non-rebreathing circuit suitable for anaesthesia maintenance in rats; this arrangement represents a typical conformation for anaesthesia units in rodent procedure rooms (Short & Harvey 1983). Both circuits were connected to the vaporizer by silicone tubing (length, 1.5 m; inner diameter [i.d.], 6.35 mm; McMaster-Carr, Santa Fe Springs, CA, USA) equipped with composite plastic stopcocks (Delryn, VetEquip) to control access of the anaesthetic mixture. In a like manner, exhaust ports of both devices were attached to passive gas-scavenging canisters (EnviroPure, SurgiVet Inc, Waukesha, WI, USA) by 1.2 m of corrugated evacuation tubing (i.d., 19 mm; Global Medical, Trenton, Ontario, Canada). All components (seals and tubes) were tested for leaks prior to initiation of the study by passing isoflurane (5%, carried in 4 L per minute of oxygen) through the system after all circuits, but the one to the induction chamber had been tightly sealed with two or more layers of plastic wrap and duct tape. The extent of isoflurane leakage was quantified using an infrared spectrophotometer (described in detail below); isoflurane release was not detected either before or during the investigation (data not shown). The vendor had recently serviced all units.

Breathing circuit and face mask variants

Three non-rebreathing (semi-open) circuits employing four configurations (Figure 1) for the interface between the snout and the mask/breathing circuit (hereafter referred to as the 'port') were evaluated for waste isoflurane emissions. The three circuits were traditional Bain (22 mm outer diameter [o.d.] aperture with 6.4 mm i.d. inlet tube; Hudson RCI, Temecula, CA, USA), modified Bain (9 mm o.d. aperture with 3.2 mm i.d.

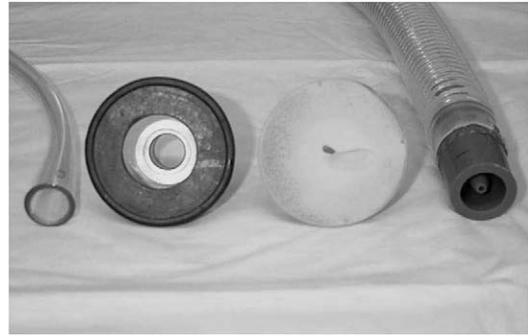


Figure 1 Rodent circuit and mask configurations assessed for isoflurane emissions at the face/port interface. Variants (from left to right) are modified Bain circuit, Mapleson E circuit with unmodified conical facemask*, Mapleson E circuit with diaphragm-modified conical facemask*, and traditional Bain circuit

*Only the mask which was attached to the system is shown

inlet tube; VetEquip), and Mapleson type E (22 mm o.d. aperture with 6.4 mm i.d. inlet tube; Hudson RCI [as described in Dorsch & Dorsch 1999b]). Due to the confusion in terminology of breathing systems and different definitions of terms, the following explanation is added for clarification: a traditional Bain circuit is also classified as a Mapleson D system. A modified Bain system can also be termed the Bain modification of the Mapleson D system (for detailed information, refer to Dorsch & Dorsch 1999b). The traditional Bain device is characterized by the fresh gas supply tube running within the exhaust tubing. The modified Bain apparatus uses the same configuration, but on a smaller scale. Additionally, the modified Bain apparatus has been altered to include a bell-shaped extension of the exhaust tubing to serve as a mask. The two Bain circuits were used as supplied by the vendor (i.e. without an additional facemask or diaphragm covering the snout), while a conical facemask (24.1 mm i.d. aperture with 15 mm i.d. inlet port; Matrix Medical, Orchard Park, NY, USA) was attached to the Mapleson E system. We tested the facemask both with and without a simple homemade latex diaphragm manufactured from the thumb portion (Figure 2) of a commercially

available, unpowdered surgical glove (small size; Evolution One[®], Microflex Corporation, Reno, NV, USA).

Analysis of atmospheric isoflurane concentrations

Realtime monitoring of waste isoflurane emissions was performed using a commercially available, portable, ambient air analyzer containing a single-beam infrared spectrophotometer (MIRAN SaphiRe, Series 205A, Foxboro Co, Foxboro, MA, USA). Room air was sampled for all four mask variants at three sites: (1) the face/mask interface (probe ≤ 2 cm from the interface, and held perpendicular to the mask margin); (2) the operator's breathing zone (probe held 25 cm from the face/mask interface), and (3) the background (probe at 150 cm [1.5 m] from the face/mask interface). Isoflurane leakage was assessed at 2.5 and 5 min after application of the mask. Values above the upper limit of the linear analysis range (100 ppm) were recorded as '>100'. A

designation of 'fast' (reading exceeded 100 ppm in 15 s or less; i.e. very high emissions) or 'slow' (a gradual increase, typically ranging from 30–60 s) was used as a qualitative index of waste gas levels for '>100' readings.

Anaesthetic regimen

One rat was anaesthetized at a time. Anaesthesia was induced in a standard 2 L, airtight induction chamber (placed in a non-recirculating fume hood) using isoflurane in oxygen (2 L/min) delivered via a precision vaporizer (set at 5%). Each animal was then transferred to a bench-top station and placed in right lateral recumbency. The snout was restrained using a surgical tape, and all four facemask variants were applied in succession to the rat's face. Anaesthesia was maintained using isoflurane (2% [all circuits] or 3.5% vaporizer setting [only for Mapleson system with added latex diaphragm]) in oxygen (1 L/min). The low isoflurane level was selected as it represents

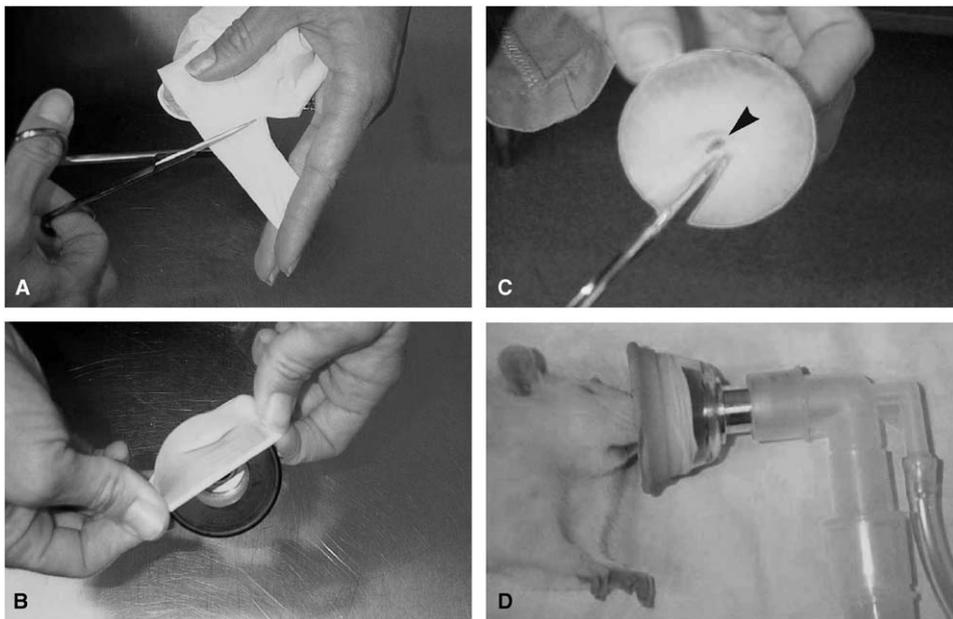


Figure 2 The simple homemade diaphragm is manufactured from the thumb portion of a small unpowdered latex surgical glove. The thumb is removed using ordinary scissors (A) and then stretched over the orifice of a conventional conical rodent facemask (B). The resulting latex diaphragm is nicked with scissors (C) to create a small (0.5 to 0.8 cm long) aperture (arrow head) just large enough to contain the animal's nose (Panel D)

a standard setting for rodent anaesthesia procedures that utilize non-rebreathing circuits (Flecknell 1996), while the high gas level was chosen to test the integrity of the seal provided by the diaphragm-modified facemask. Flow of the anaesthetic mixture was stopped when circuits were switched. Changing circuits during multi-animal rodent procedures that employ inhalation anaesthesia is not a common practice; we employed this design merely to ensure that all circuits and port configurations were tested using an identical system on the same day. Isoflurane was administered for 25–40 min to each rat (at least 5 min per mask variant), after which animals were euthanized with carbon dioxide while still anaesthetized with isoflurane. A period of 5 min between each rat anaesthesia study was used to allow atmospheric levels of isoflurane at the bench top to return to the basal level of 0 ppm (period established in pilot studies [unpublished]).

All anaesthetic procedures were conducted in a room (74 m³) in which the air turnover rate was 26 non-recirculating changes per hour. Two replicate experiments were performed to minimize interference with the spectrophotometric measurements resulting from waste isoflurane accumulation in the room atmosphere. In each replicate, all four port configurations were tested on the first four rats, while only the diaphragm-modified facemask was

assessed on the remaining animals. The anaesthetist did not wear a respirator during the experiments.

Statistical analysis

Results (expressed as mean \pm standard error [SEM]) were compared using the conservative, non-parametric Wilcoxon rank sum test. A *P* value of 0.05 was used to delineate significant differences between groups. Readings of '>100 ppm' were converted to '100' for statistical purposes.

Results

The traditional Bain circuit, modified Bain circuit, and Mapleson type E circuit with unmodified conical facemask were evaluated in eight rats (16 measurements per configuration) using 2% isoflurane carried in oxygen (1 L/min). For all three units, isoflurane leakage through the face/port interface exceeded 100 ppm in 94% (45/48) of measurements (Table 1).

The rate at which emissions surpassed the 100 ppm boundary was uniformly rapid (less than 15 s) for the traditional Bain circuit and the Mapleson E circuit with unmodified conical mask. Low levels of isoflurane were detected in the operator's breathing zone (measured 25 cm from the face/mask interface) for both these configurations (Table 1). In addition, both of these units

Table 1 Comparative isoflurane emissions of four rodent circuit/mask variants

Equipment configurations		Emissions (ppm)*					
Circuit	Facemask	Rats (n)	Trials* (n)	Face/port interface [†]	Breathing zone [‡]	Background [§]	Isoflurane odour in breathing zone
Traditional Bain	None	8	16	>100	2.4 \pm 0.3	1.0 \pm 0.2	12/16 (75%)
Modified Bain	None	8	16	92.9 \pm 5.6	1.0 \pm 0.1	0.6 \pm 0.2	2/16 (13%)
Mapleson E	Unmodified conical	8	16	>100	1.7 \pm 0.2	0.8 \pm 0.2	12/16 (75%)
Mapleson E	Diaphragm-modified conical	15	30	7.9 \pm 0.9 [¶]	0.2 \pm 0 [¶]	1.3 \pm 0.2	0/30 (0%)

*Readings were taken 2.5 and 5 min after the rat's nose was positioned in the port. Anaesthesia conditions: 2% isoflurane carried in oxygen (1 L/min). Probe position relative to the face/mask interface:

[†]Distance away=2 cm

[‡]Distance away=25 cm

[§]Distance away=150 cm

[¶]Denotes significant difference relative to the other three facemask types, *P* \leq 0.05, by the Wilcoxon rank sum test

were often associated with a transient odour of isoflurane in the operator's breathing zone (positive in 12/16 trials [75%] for both types for two different anaesthetists). This odour was not detected when the circuits were being switched, but only after the units had been properly attached and the carrier gas flow was restored; in all cases, the vapours were detected for only 1–2 s. The high emissions leading to fleeting but regular contamination of the breathing zone resulted in a mid-experiment decision not to test these two configurations on the remaining rats.

The modified Bain circuit delivered three values below 100 ppm, as well as four readings in which the 100 ppm limit was crossed only after 30–90 s. The modified Bain circuit also greatly reduced the gas odour in the operator's breathing zone (Table 1); isoflurane could be smelled transiently in only 2/16 readings (13%). Again, the occasional detection of an isoflurane odour in the breathing zone resulted in a mid-experiment decision not to test this configuration on the remaining rats.

The Mapleson E circuit equipped with diaphragm-modified conical facemask leaked significantly less isoflurane ($P \leq 0.0003$) using the same anaesthesia protocol than did the other circuit/port variants (Table 1). Atmospheric isoflurane levels in the operator breathing zone detected by realtime spectrophotometry were significantly lower by at least five-fold ($P \leq 0.0007$) for the diaphragm-modified port relative to the other configurations (Table 1). For all 15 rats, concentrations at the face/port interface were equivalent for isoflurane vaporizer settings of 2% or 3.5% (Table 2). Isoflurane was not detected by smell in the operator's breathing zone using this device.

Isoflurane was not detected as a baseline atmospheric contaminant (measured 1.5 m from the face/port interface). The background isoflurane level increased gradually during the two 4 h continuous anaesthesia experiments. The peak mean concentration was measured near the end of the experiment while using the Mapleson type E circuit and diaphragm-modified

Table 2 Impact of isoflurane concentration on emissions at the face/port interface

Isoflurane flow* (%)	Emissions (ppm) [†]		
	Face/port interface [‡]	Breathing zone [§]	Background [¶]
2.0	7.9 ± 0.9	0.2 ± 0	1.3 ± 0.2
3.5	10.9 ± 2.1	0.2 ± 0	1.1 ± 0.2

*Anaesthetic conditions: delivery with oxygen (rate, 1 L/min) using a Mapleson E non-rebreathing circuit equipped with a diaphragm-modified conical facemask

[†]Readings were taken 2.5 and 5 min after the rat's nose was positioned in the port. Probe position relative to the face/mask interface:

[‡]Distance away=2 cm

[§]Distance away=25 cm

[¶]Distance away=150 cm

conical rodent mask, while the lowest was determined early during the study with the modified Bain unit (Table 1).

Discussion

Occupational exposures to fluorocarbon-based WAG (e.g. halothane, methoxyflurane) at or below 10 ppm are well below the concentrations at which any significant adverse effects occur in animals, and represent levels at which there is no evidence to imply that human health will be affected (reviewed in ASA 1999). Given appropriate control procedures, atmospheric waste anaesthetic levels can readily be limited to this degree.

At present, the most significant contributing factor to fluorocarbon-based anaesthetic pollution in medical and veterinary operating theatres is inadequate scavenging of unused gases (Cohen *et al.* 1971, Ward & Byland 1982, Pothmann *et al.* 1991, Stimpfel & Gershey 1991, Smith 1993, Imberti *et al.* 1995). The overriding importance of this source results from the routine use of endotracheal tubes to maintain anaesthesia, as properly fitted tubes with inflated cuffs prevent passage of the anaesthetic mixture into the room atmosphere. However, rodent anaesthesia protocols routinely use non-rebreathing (semi-open) circuits with loosely fitting facemasks to maintain anaesthesia due to their ease of use and the brevity of many common procedures. Such circuits are

designed to allow expired gases to exhaust through the face/port interface to prevent re-uptake of exhaled vapours (Flecknell 1996). However, industrial hygiene studies of veterinary surgical suites have shown that anaesthetic delivery via non-rebreathing (semi-open) circuits equipped with facemasks results in more anaesthetic pollution than does intubation, apparently due to the requirement of non-rebreathing systems for a more rapid carrier gas flow rate and leaks at the animal/facemask interface (Wingfield *et al.* 1981, Short & Harvey 1983, Hoerauf *et al.* 1998). We performed the present study to explore the potential for isoflurane exposure afforded by several standard port configurations used in rodent procedures. Our data show that conventional variants will likely result in substantial occupational exposure when used according to routine rodent anaesthesia protocols. Fortunately, our work also identified a simple and inexpensive means of greatly decreasing anaesthetic pollution associated with administration of inhalation anaesthetic agents to rodents.

Our present data indicate that the flow of unused anaesthetic mixture through the face/port interface was plentiful for the three commercially available rodent non-rebreathing circuits (traditional Bain, modified Bain, and Mapleson E equipped with an unmodified conical facemask) that we utilized in this study. Atmospheric isoflurane levels detected at this boundary exceeded 100 ppm (Table 1) in 94% of measurements obtained when the anaesthesia system was operating at a standard flow rate (2% isoflurane carried in 1 L/min of oxygen). In fact, for the traditional Bain circuit and Mapleson E circuit with unmodified facemask, the rapid rate at which isoflurane levels surpassed the 100 ppm boundary (uniformly less than 15 s) implies that emissions at the face/port interface could have been several hundred ppm. Such anaesthetic discharge from these two variants resulted in detection of isoflurane in the operator's breathing zone at levels approaching (1.7 ppm, Mapleson E circuit with unmodified mask) or exceeding

(2.4 ppm, traditional Bain circuit) the American 1 h ceiling concentration (2 ppm, recommended by NIOSH 1977) (Table 1). Indeed, we predict that such outflow will present workers with intermittent, short-term exposure to isoflurane levels surpassing the most common European 8 h time-weighted average (10 ppm, established by regulatory agencies in Germany, Sweden, and Switzerland; reported in Hoerauf *et al.* 1999) defined for stand-alone use of isoflurane. This inference was supported by our ability to smell isoflurane – albeit transiently – in the operator's breathing zone (measured 25 cm from the face/port interface) during 75% of trials with the traditional Bain circuit and Mapleson E circuit with the unmodified mask. We estimate that the atmospheric isoflurane concentration for these instants was over 50 ppm (the olfactory threshold of halothane, a closely related anaesthetic; Lecky 1977, Short & Harvey 1983). However, the transitory nature of these odours coupled with the low isoflurane levels detected in the operator's breathing zone by realtime spectrophotometry indicates that personnel are unlikely to be exposed to anaesthetic concentrations of more than a few ppm for any substantial period. Relative to the traditional Bain circuit and Mapleson E circuit with unmodified mask, the modified Bain circuit passed less isoflurane through the face/port interface (Table 1). This conclusion was warranted because the modified Bain unit yielded three isoflurane readings less than 100 ppm (range, 29–83 ppm), four values for which the 100 ppm limit was reached only after a long delay (at least 30 s), and was infrequently associated with an isoflurane odour in the operator's breathing zone (13% of trials). Thus, the modified Bain device provided less exposure to waste isoflurane than did the traditional Bain circuit and the Mapleson E circuit equipped with an unmodified facemask. Regardless, our data show that all three of these commercially available configurations are likely to yield intermittent occupational contact with isoflurane emissions, including sporadic exposures at levels well above those

recommended by health-monitoring agencies.

Using the same anaesthesia protocol, addition of a latex diaphragm to the conical mask attached to the Mapleson E circuit significantly reduced isoflurane emissions at the face/port interface (Table 1). Leakage was reduced by at least 12-fold for the diaphragm-modified version (7.9 ± 0.9 ppm; $n = 30$ trials), relative to the unmodified mask (>100 ppm; $n = 16$ trials). Under conditions of normal use, the diaphragm-modified variant was equally efficient in preventing isoflurane leakage at isoflurane vaporizer settings of 2% or 3.5% (Table 2). In particular, the diaphragm-modified facemask prevented the perception of isoflurane odour that was experienced when the other three units were used. These data suggest that inclusion of a diaphragm-modified facemask was the only configuration that adequately limited occupational exposure to waste isoflurane. This finding is important as the latex diaphragm we developed is simple and quick to make from a latex surgical glove (Figure 2), and thus might be adopted readily throughout an entire facility for minimal cost in terms of both equipment and training time. Furthermore, each researcher will be able to tailor the atmospheric isoflurane level to suit their own preference, as the amount of waste isoflurane leakage will vary directly with the size of the opening made in the diaphragm and inversely with the tightness of the seal between the diaphragm and the animal's snout. Interestingly, the highest background isoflurane readings were obtained for the diaphragm-modified conical mask. This increase was measured near the end of a 4 h experiment that also included the three circuit configurations in which the port was not modified, and thus likely reflects redistribution of gas emitted by them into the room atmosphere rather than from substantial leakage through the latex diaphragm occurring at the bench-top. The main evidence supporting this conclusion was that isoflurane levels present in the operator's breathing zone were significantly higher during use of the unmodified devices, which were deployed during the first half of

the 4 h replicate experiments, relative to those detected when the diaphragm-modified conical unit was in use.

Taken together, these data indicate that addition of a latex diaphragm with an appropriately sized opening will significantly reduce but not eliminate occupational exposure to WAG, especially during lengthy studies in which multiple anaesthesia procedures are performed.

In conclusion, our data suggest that researchers working with standard rodent non-rebreathing circuits equipped with conventional conical facemasks will be regularly exposed to waste isoflurane emissions flowing through the face/port interface. Transient contact will likely occur at levels exceeding 50 ppm even if typical delivery systems, routine protocols, and standard work practices are used. Our current findings indicate that isoflurane waste will be significantly reduced by a simple adjustment that decreases the aperture diameter of the standard rodent facemask, thereby improving the face-to-mask seal. Two alternative practices to achieve the same end would be to move all rodent procedures requiring anaesthesia into chemical fume hoods and/or to only employ circuits equipped with evacuation lines (Henry & Casto 1989); the special equipment necessitated for the latter option would be more costly than the simple latex diaphragm that we devised. Additional procedures to reduce occupational exposure of laboratory animal personnel to waste isoflurane emissions include improving the efficiency of gas-scavenging systems, increasing the air turnover rate in animal procedure rooms, and avoiding bench-top use of induction chambers (Smith & Bolon 2002, 2003). Finally, our data clearly demand that regular and quantitative environmental monitoring programmes be initiated to detect and eliminate sources of WAG in laboratory animal facilities. We anticipate that adoption of such techniques coupled with the brevity of most rat anaesthesia procedures will mitigate any risk of adverse health effects for most, if not all, laboratory animal professionals.

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