Comparison of Three Commercially Available Activated Charcoal Canisters for Passive Scavenging of Waste Isoflurane during Conventional Rodent Anesthesia

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Chronic, low-level exposure to waste anesthetic gases has been linked to increased incidences of neurologic and reproductive dysfunction, hepatic and renal toxicity, and neoplasia in humans. We have shown previously that one brand of activated charcoal canister (F/Air) used for passive scavenging of halogenated gases does not completely remove isoflurane during anesthetic protocols used in conventional laboratory animal facilities. For the present study, we compared the scavenging capacities of three commercially available canister brands (Breath Fresh, EnviroPure, F/Air) using the same protocol. Well-maintained precision isoflurane vaporizers were equipped with two circuits (a nonrebreathing one hooked to a modified Bain facemask and the other to an induction box), each of which was attached to a canister. Isoflurane concentration and oxygen flow rate were set at 2% and 1 liter/min, respectively. Real-time atmospheric isoflurane emissions from canister exhaust ports were assessed using a portable infrared spectrophotometer. In a random survey of canisters that had not reached their maximal use life (specified by the manufacturers as a weight gain of 50 g), the percentage of canisters emitting ≥ 5 ppm but < 100 ppm of waste isoflurane was 46% for Breath Fresh (n = 24), 8% for EnviroPure (n = 39), and 27% for F/Air (n = 37). Failure (defined as an isoflurane efflux of ≥ 100 ppm) occurred in 42% of Breath Fresh units but 0% for the other brands. In a subsequent experiment (n = 6/brand), all Breath Fresh and F/Air but no EnviroPure canisters had at least one reading of ≥ 5 ppm by the time they gained 30 g. These data indicate that marked variability in gas-scavenging capacity exists between different brands of commercially available activated charcoal canisters and suggest that trace levels of waste isoflurane may occur in high-throughput laboratory animal anesthesia rooms unless canister exhaust also is captured.

A common hazard of inhalation anesthesia is the release of waste anesthetic gases ("anesthetic pollution") into the environment. More than 200,000 health care professionals—including an estimated 50,000 veterinarians and veterinary technicians—are exposed routinely to trace levels of waste anesthetic gases (1). Chronic occupational exposure to waste anesthetic gases has been linked to increased incidences of neurologic and reproductive dysfunction, hepatic and renal toxicity, and neoplasia (for reviews, see refs. 2-7). People who interact with exposed health care workers may be at risk as well (8, 9), because older inhalation agents (e.g., halothane, methoxyflurane, nitrous oxide) are metabolized and retained by the body for extended periods (10, 11) and exhaled in appreciable quantities (12-14). Based on effects produced by halothane and methoxyflurane, the U.S. National Institute for Occupational Safety and Health (NIOSH) prepared a criteria document in 1977 to alleviate occupational exposure to emitted halogenated anesthetics (1). To date, the U.S. Occupational Safety and Health Administration (OSHA) has not promulgated a formal standard based on this criteria document, although it has assembled an array of information on waste anesthetic gases in general (15). Nevertheless, in recent years the NIOSH recommendation (2 ppm) also has been applied to newer halogenated agents—including isoflurane, an inhalation anesthetic commonly used in conventional laboratory animal facilities.

Although anesthetic pollution has been reduced substantially during the last 3 decades, it cannot be completely eliminated in high-throughput operating theaters even when well-maintained anesthesia systems equipped with gas-scavenging units are used in well-ventilated rooms (16, 17). In the laboratory animal setting, we recently have shown that passive scavenging of waste isoflurane using a commercially available activated charcoal canister (Omnicon F/Air) in conjunction with conventional rodent anesthesia equipment and protocols does not completely remove waste isoflurane from the work environment (18). The level of halothane emission is known to vary greatly among different canister brands (19-21), and it seems reasonable that isoflurane would respond in a similar manner. Therefore, we postulated that this apparent breakdown in scavenging capacity resulted not from design or manufacturing flaws in F/Air canisters but rather reflected the inability of passive systems in general to completely remove anesthetic pollution. We tested this hypothesis in the present study by comparing the isoflurane scavenging capacities of three different commercially available, activated charcoal canisters (Breath Fresh, EnviroPure, F/Air) that are commonly used for inhalation anesthesia in conventional laboratory animal facilities. Our findings demonstrate that isoflurane scavenging by different canister brands, and between individual canisters, varies greatly in this setting.

Materials and Methods

IACUC approval. This study was conducted in accordance with federal animal care guidelines and was preapproved by the Amgen Institutional Animal Care and Use Committee.

Choice of anesthetic agent. Isoflurane is a standard agent for rodent inhalation anesthesia (22) and is the inhalation anesthetic of choice in most laboratory animal facilities. Its suitability is attributed to both its desirable physical properties and its limited

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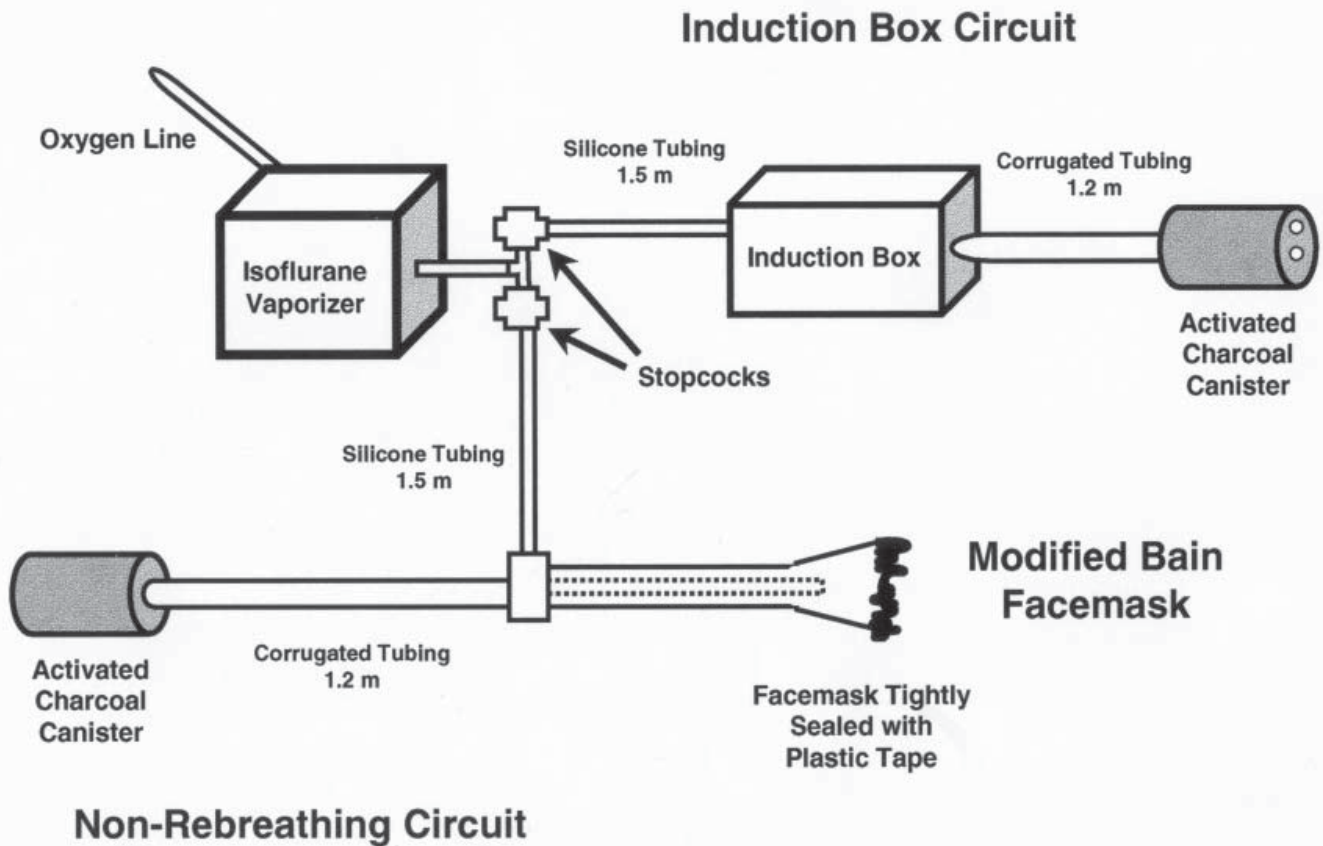
Induction Box Circuit

Figure 1. Schematic diagram (not to scale) showing the configuration of the anesthesia system used in this study. Predominant sources of waste isoflurane emission (facemask, induction box, and exhaust ports of activated charcoal gas-scavenging canisters) were widely separated.

toxicity (for review, see ref. 18). In the present study, we used IsoFlo (Abbott Laboratories, North Chicago, Ill.).

Configuration of anesthesia machines. Isoflurane was administered using Laboratory Animal Anesthesia Systems (VetEquip, Pleasanton, Calif.). Delivery was controlled using precision isoflurane vaporizers (Isotec3, Cyprane, Keighley, West Yorkshire, England) with an adjustable dial to regulate the output of isoflurane (concentration range, 0% to 5%) coupled with a separate oxygen flow meter (range, 0.2 to 4 liters/min). Each unit was configured with two circuits, one directed to a 2-L acrylic induction box and the other to a modified Bain, nonrebreathing facemask suitable for maintaining anesthesia in rodents (Fig. 1). The facemask was occluded with several layers of plastic wrap and duct tape to achieve a gas-tight seal. Both circuits were connected to the vaporizer by silicone tubing (length, 1.5 m; inner diameter, 0.25 in.; McMaster-Carr, Santa Fe Springs, Calif.) equipped with composite plastic stopcocks (Delryn; VetEquip) to control access of the anesthetic mixture. In like manner, the exhaust ports of both devices were attached to passive gas-scavenging canisters by 1.2 m of corrugated evacuation tubing (inner diameter, 19 mm; Global Medical, Trenton, Ontario, Canada) (Fig. 1). The vendor (VetEquip) recently had maintained and calibrated all machines.

Analysis of atmospheric isoflurane concentrations. Real-time monitoring of waste isoflurane emissions in the work environment was undertaken with a commercially available, portable, ambient air analyzer containing a single-beam infrared spectophotometer (Miran SapphiRe, Series 205A, Foxboro Co., Foxboro, Mass.). For each experiment, the air was sampled at the canister exhaust port (probe ~2 cm from the exit holes). Each canister was placed in the horizontal position for measurements. 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 sec or less; i.e., very high emissions)

or "slow" (a gradual increase) was used as a qualitative index of waste gas levels for "> 100" readings. For the present work, 5 ppm was selected arbitrarily as the lower boundary for statistical analysis, as this value approximates the midpoint between the 1-h ceiling concentration (2 ppm) recommended by NIOSH (1) and the most common European 8-h time-weighted average (10 ppm) established by European regulatory agencies (compiled in ref. 23) for exposure to isoflurane.

Experimental design. The efficacy of three different commercially available active charcoal scavenging canisters (Breath Fresh, Jorgensen Laboratories, Loveland, Colo.; EnviroPure, SurgiVet Inc., Waukesha, Wis.; Omnicon F/Air, A.M. Bickford, Wales Center, N.Y.) was explored using a common rodent anesthesia protocol (22). Two experiments were performed as defined below. All circuit components (seals and tubes) were tested for leaks prior to initiation of the experiments. Isoflurane release was not detected from seals and tubes either before or during the experiments.

(i) Experiment 1: effectiveness of different canisters brands under normal use. Randomly selected canisters (Breath Fresh, n = 24; EnviroPure, n = 39; F/Air, n = 37) were weighed prior to use and placed into service in our facility for various lengths of time (ranging from 1 week to 6 months). This range encompassed the span required within our animal facility for canisters to near or reach the end of their recommended use life (per the manufacturer's specifications, canisters should be retired once they have gained 50 g, which represents 12 to 15 h of use). The breadth of this time range resulted from differences in the experimental protocols employed by the facility's users (performed under their own preapproved IACUC protocols) and physiology of the animals to be anesthetized (generally adult mice [*Mus musculus*] and adult rats [*Rattus norvegicus*]). On each apparatus, both the facemask and induction box circuits were equipped with previously unused gas scavenging canisters (at

least 24 of each brand). All canisters of each brand were from the same manufacturing lot. Each canister was weighed weekly. Canisters were not alternated between circuits during the course of this experiment, as this practice is not a standard procedure in our facility. At the end of the study, each canister was attached to the facemask circuit of a bench-top anesthesia unit. With the induction box circuit closed, measurements were acquired using a uniform isoflurane concentration (2%) and oxygen flow rate (1 liter/min). These conditions were chosen because this regimen is a standard means of maintaining anesthesia for an individual rodent when performing simple bench-top procedures. Samples were taken in an animal procedure room (74 m^3) in which the air turnover rate was 26 nonrecirculating changes per hour.

(ii) Experiment 2: assessment of emissions from different canister brands during continuous use. Nine VetEquip units were configured as described (Fig. 1). Machines were distributed between two procedure rooms, one of 74 m^3 with an air-turnover rate of 26 nonrecirculating changes per hour (housing six machines) and the other of 50 m^3 with an air-turnover rate of 46 nonrecirculating changes per hour (housing three machines). Measurements of canister emissions were not impacted by the difference in air turnover rates between these two rooms, likely because the spectrophotometric probe is held in close proximity to the exhaust ports when assessing the waste isoflurane throughput (data not shown). On each apparatus, both circuits were equipped with previously unused gas scavenging canisters (n = 18, i.e., 6 per brand). For each brand, all canisters were from the same lot. With the induction box circuit open, all machines were run at a uniform isoflurane concentration (2%) and oxygen flow rate (1 liter/min) continuously until they neared or surpassed their rated use life (a weight gain of 50 g). Canister weight gain and isoflurane emissions from canister exhaust ports were measured hourly. All canisters were assessed while attached to the facemask loop and with the induction box circuit open by using a uniform isoflurane concentration (2%) and oxygen flow rate (1 liter/min). Flow through the induction box loop (inner diameter, 0.25 in.) far exceeded that through the nonrebreathing loop due to heightened resistance through the modified Bain facemask resulting from the small bore of the gas-delivery tube (inner diameter, 0.0625 in.). Therefore, canisters were alternated between the two circuits every hour to ensure that all canisters adsorbed isoflurane (i.e., gained weight) at approximately similar rates.

Statistical analysis. Results (expressed as mean ± standard error of the mean [SEM]) were compared using the nonparametric Wilcoxon rank sum test provided in commercially available software (JMP, v. 4.0; SAS Institute, Cary, N.C.). 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, thereby providing a conservative estimate of waste anesthetic gas concentrations.

Results

Experiment 1: effectiveness of different canisters brands under normal use. Isoflurane emissions differed substantially among the three canister brands (Fig. 2). For all three brands, isoflurane levels ≥ 5 ppm but ≤ 100 ppm were detected from the exhaust ports of canisters that had not reached their maximum use life as defined by the manufacturer's specifications—from 46% (11 of 24) of the Breath Fresh canisters, 8% (3 of 39) of the EnviroPure devices, and 27% (10 of 37) of the F/Air units. Isoflurane levels exuded by another 42% (10 of 24) of the Breath Fresh canisters exceeded 100 ppm, indicating an essentially complete absence of gas-scavenging capacity ("failure"; [20]). We verified the validity of this interpretation by testing the scrub-
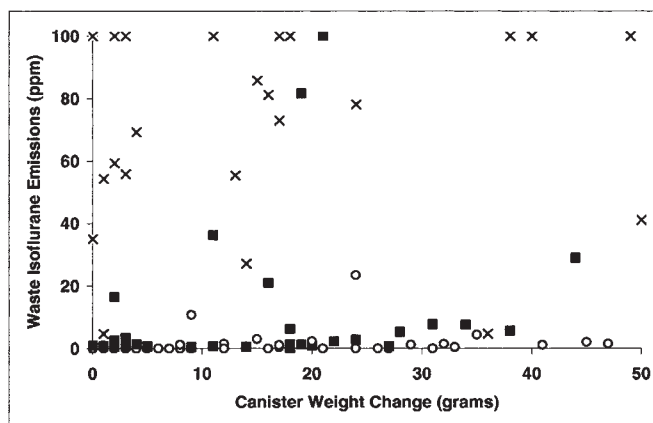

Figure 2. Waste isoflurane emissions measured from the exhaust ports of randomly chosen activated charcoal canisters of three different brands (Breath Fresh, n = 24, crosses; EnviroPure, n = 39, open circles; F/Air, n = 37, black squares). Emissions were measured prior to saturation (defined as a canister weight gain over baseline of 50 g).

bing efficiency of eight "retired" canisters that had gained from 68 to 86 g: all rapidly exuded isoflurane at levels > 100 ppm. Four of the 29 (14%) previously unused Breath Fresh units failed according to this criterion. In contrast, only 1 of 24 (4%) F/Air canister and no EnviroPure canisters passed >100 ppm of isoflurane. As a group, emissions (mean ± SEM) from EnviroPure canisters were 3.9 ± 2.4 ppm and were significantly (*P* < 0.001) lower than those emanating from the two other canister brands. In like manner, levels emitted by F/Air canisters (16.3 ± 4.9 ppm) were significantly (*P* < 0.001) lower than those given off by the Breath Fresh units (67.2 ± 6.2 ppm).

Experiment 2: assessment of emissions from different canister brands during continuous use. The scavenging efficiency of new canisters varied substantially across brands as well as among individual canisters from a given brand (Table 1, Fig. 3). At some point during their lives, all canisters from all three brands, except for a single EnviroPure unit, emitted at least 1.5 ppm isoflurane on at least one occasion. However, during the course of the entire experiment, isoflurane was exuded at > 5 ppm from Breath Fresh and F/Air canisters but not from EnviroPure canisters. For all six Breath Fresh canisters, the first reading exceeding 5 ppm was obtained before 50% of the rated use life had been reached. Only four of the six F/Air canisters emitted > 5 ppm before reaching 50% of their life span, while the other two F/Air canisters passed > 5 ppm by the time they had reached 60% of the rated use life. During their rated use life, waste isoflurane emissions from Breath Fresh and F/Air canisters varied up and down, whereas EnviroPure canisters emanated essentially no isoflurane (Fig. 3). Only one canister (an F/Air unit) gave off more than 100 ppm, and this value was recorded after a weight gain of 46 g (92% of the rated life span).

Discussion

The most important contributing factor to anesthetic pollution in operating theaters is inadequate scavenging of unused gases (6, 24-28). Improved scavenging equipment removes 90% or more (14, 29) of waste anesthetic gases, but atmospheric contamination cannot be entirely avoided (16, 17). We recently demonstrated that this scenario also applies to laboratory animal facilities in which activated charcoal canisters are used to scavenge waste isoflurane (18). Different canister brands are known to emit waste halogenated anesthetics at different rates (19-21), so we compared the isoflurane scavenging capacities of three different commercially available, activated charcoal canisters (Breath Fresh, EnviroPure, and F/Air) that are commonly

Table 1. Breakthrough of waste isoflurane emissions varies among canister brands and between canisters

Canister type	Charcoal canister no.[1]	Canister weight change (g) at breakthrough[2]	% of maximal use life[3]	Isoflurane level (ppm) at breakthrough	Canister weight change (g) at 1st reading ≥ 5 ppm	% of maximal use life[3]	Isoflurane level (ppm) at 1st reading ≥ 5 ppm	Peak emissions (ppm)[4]	Canister weight change (g) at peak emission	Overall change in canister weight (g)life[1]	% of maximal use (ppm)	Terminal isoflurane emissions
Breathe Fresh	1	4	4	2.0	20	40	5.1	11.5	39	52	104	78.2
Breathe Fresh	2	17	34	4.1	23	46	10.9	33.4	44	44	88	33.4
Breathe Fresh	3	9	18	31.4	9	18	31.4	52.8	28	50	100	19.7
Breathe Fresh	4	5	10	4.1	13	26	29.0	29.0	13	51	102	22.6
Breathe Fresh	5	7	14	3.6	12	24	12.0	17.5	32	49	98	1.5
Breathe Fresh	6	11	22	15.3	11	22	15.3	66.2	49	49	98	66.2
EnviroPure	1	54	108	2.1	N/A	–	–	0.6	31	66	132	0.8
EnviroPure	2	21	42	1.7	N/A	–	–	1.7	7	56	112	0.8
EnviroPure	3	N/A	–	–	N/A	–	–	1.0	44	44	88	1.0
EnviroPure	4	24	48	1.7	N/A	–	–	1.7	24	41	82	0.4
EnviroPure	5	2	4	4.8	N/A	–	–	4.8	2	51	102	0.5
EnviroPure	6	2	4	1.9	N/A	–	–	3.2	48	48	96	3.2
F/Air	1	9	18	2.9	23	46	18.7	33.0	48	48	96	33
F/Air	2	5	10	6.0	5	10	6.0	>100	46	46	92	>100
F/Air	3	7	14	1.6	30	60	7.3	12.6	37	50	100	10.1
F/Air	4	7	14	4.0	26	52	15.2	37.4	50	50	100	37.4
F/Air	5	8	16	25.9	8	16	25.9	62.8	34	47	94	17.2
F/Air	6	10	20	5.6	10	20	5.6	54.4	39	47	94	7.3

Canisters were attached to the nonrebreathing circuit with the stopcock to the induction box closed during spectrophotometric testing.

[1]The canister numbers in this table match those shown in Fig. 3.

[2]Initial breakthrough denotes emission of isoflurane levels of at least 2 ppm.

[3]Maximal use life (as defined by the manufacturer's specifications) is a weight change of 50 g from the baseline canister weight.

[4]Peak emissions denotes the highest reading during the course of each canister's rated use life (i.e., weight change of 50 g).

used for inhalation anesthesia in conventional laboratory animal facilities. The results of our experiments demonstrate that isoflurane scavenging capacity among different canister brands, and between individual canisters, indeed is quite dissimilar.

Our current data support several important conclusions. First, the number of used canisters emitting at least 5 ppm of isoflurane varied across a 6-fold range (from 8% to 46%) among the three canister brands (Fig. 2). Second, in a head-to-head comparison among previously unused units, all canisters of two brands (Breath Fresh and F/Air) but none of the third (EnviroPure) exhibited this trait after only 60% of their rated use-life had passed (Table 1). Third, a substantial percentage of canisters from one brand (Breath Fresh) failed to scavenge isoflurane (indicated by emissions exceeding 100 ppm [19]), even when previously unused units were tested (Fig. 2). Fourth, almost all working canisters from all three brands exuded 1.5 ppm or more of isoflurane at some point during prolonged anesthesia sessions (Table 1). Finally, the emission signatures of individual Breath Fresh and F/Air canisters varied up and down over time, whereas those of the EnviroPure units were low and essentially constant (Fig. 3).

These data are provocative. The emission levels from some canisters of all three brands exceeded the American 1-h ceiling concentration (2 ppm as recommended by NIOSH [1]) and the most common European 8-h time-weighted average (10 ppm as established by regulatory agencies in Germany, Sweden, and Switzerland; reported in [23]) defined for stand-alone use of isoflurane. These concentrations reflect occupational exposures well below the levels at which any significant adverse effects occur in animals and represent levels at which there is no evidence to imply human health will be affected (reviewed in ref. 30), suggesting that transient isoflurane emissions in the range of 10 ppm—or even modestly higher—likely pose no risk to laboratory animal researchers. However, under challenge conditions (anesthetic concentration of 1.5%, carrier gas flow of 5 liters/min), canister emissions should not exceed 10 ppm (20). In light of these criteria, our current anesthesia protocol (2% isoflurane, oxygen flow rate of 1 liter/min) did not seem to represent a substantial challenge, yet many canisters did not pass this test. The implications of these data are that the efficacy of individual canisters cannot be predicted with reliability, even in brands (e.g., EnviroPure) that

exhibited the best scavenging efficiency as a group.

We have no data that explain the differences in scavenging capacity among activated charcoal canisters of the same brand. One reasonable prospect is that redistribution of charcoal granules during shipping and handling led to the formation of low-resistance channels through some units. The channeling phenomenon has been described as a cause of reduced scavenging efficiency in soda lime-filled canisters used to remove carbon dioxide from circle anesthesia systems (31, 32). In the present study, shifting of the contents clearly happened in some canisters as substantial quantities of charcoal dust surrounded the exhaust ports (chiefly for the Breath Fresh units). Other potential sources of variation that might have affected canister efficiency include the degree of charcoal saturation (33) as well as differences in the physical properties (especially particle size) of the charcoal used by the different vendors. We controlled for charcoal saturation in the present study by including previously unused canisters as well as unsaturated units (i.e., weight gain < 50 g), but we did not analyze the contents to explore the potential impact of charcoal structure.

In summary, our data confirm that significant variability in isoflurane scavenging capacity exists among different brands of commercially available activated charcoal canisters. Therefore, personnel working with any of the three canister brands tested likely will experience at least occasional exposure to elevated waste isoflurane concentrations. Taken together, these findings strongly support the use of alternative work practices to prevent exposure of research personnel. A simple option available in most facilities is to combine passive gas-scavenging canisters with an active exhaust system. We institute this practice in our facility by placing either the gas-scavenging canister or the entire anesthesia unit in a nonrecirculating fume hood—arrangements that showed no evidence of anesthetic build-up during our preliminary studies. Other possibilities include implementing active gas scavenging systems (known to be more effective than passive systems [25, 34]), using facemasks equipped with evacuation lines (35), and increasing the air turnover rate in animal procedure rooms (18). Finally, our data also warrant more aggressive environmental monitoring of waste anesthetic gases in laboratory animal facilities.
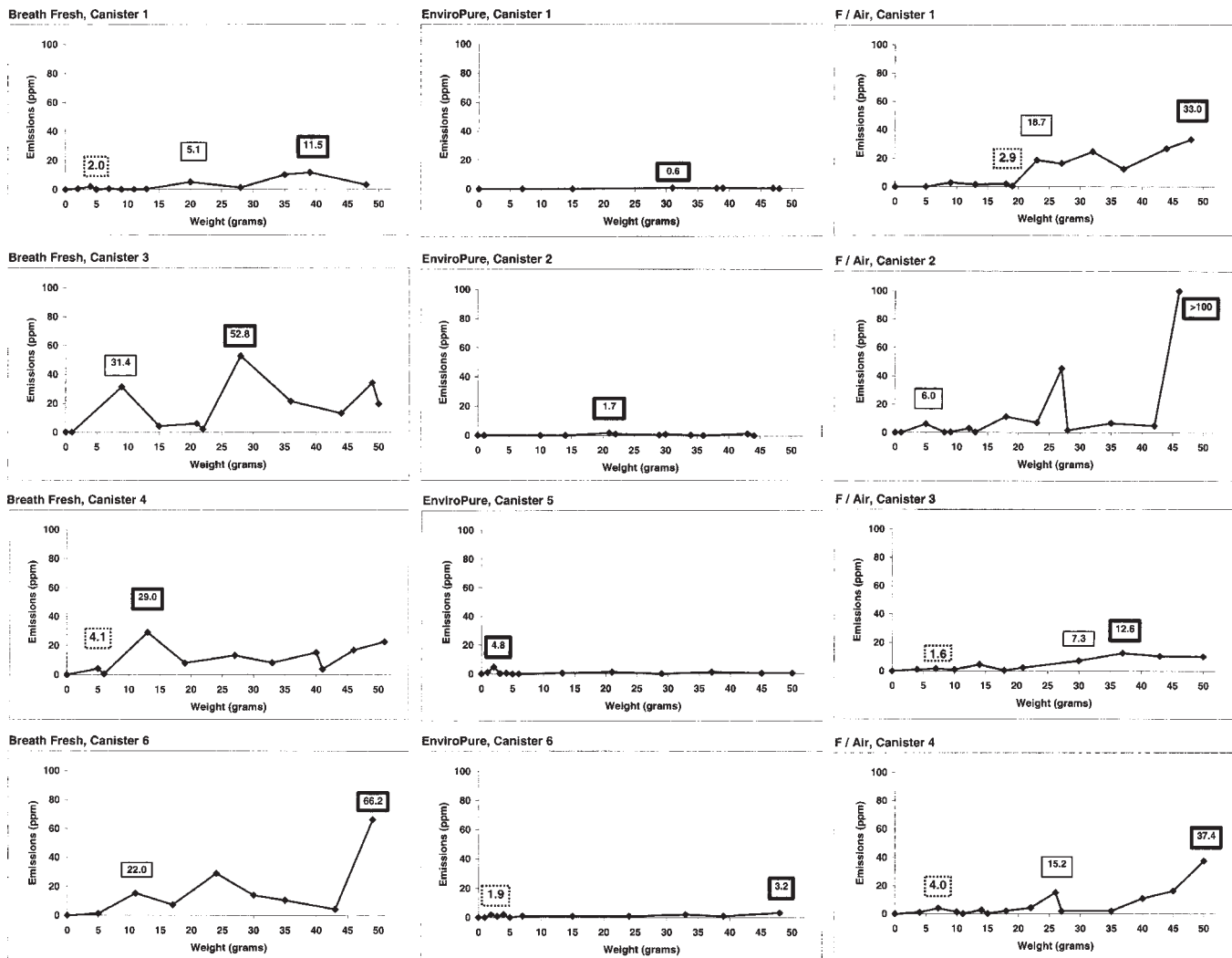
Figure 3. Emission signatures of individual activated charcoal canisters during their rated use life (a weight gain of up to 50 g), showing that the scavenging efficiency varies among both canister brands and individual canisters. The large values are positioned over the times at which isoflurane emissions escaping through the exhaust port were first detected at ≥ 1.5 ppm (dotted box) and 5 ppm (thin-walled box) as well as the highest level detected (thick-walled box). The canisters chosen (whose numbers match those listed in Table 1) had approached or reached their rated use life. Canisters were weighed hourly, so the interval between two points represents 60 min. Small gains in weight (short intervals) transpired when the canister was attached to the facemask circuit, whereas larger gains (long intervals) occurred when attached to the induction box circuit.

Acknowledgment

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