TECHNICAL BULLETIN
GENERAL INFORMATION

2100 Fernbrook Lane
Plymouth, MN 55447-4722
U.S.A.

Phone: 763.553.1270
Fax: 763.553.0459
Toll Free: 1.800.328.3352

Use of the Anesthetic Gas Isoflurane within a BSC

Background

The use of anesthetic gas, Isoflurane is common in vivariums today. There are several manufactures of anesthetic gas systems to both deliver and scavenge the gas for routine surgical procedures. However, if the surgical procedure requires a sterile or contained environment, the procedure will most likely be performed within a Biological Safety Cabinet (BSC).

The use of a BSC for surgical procedures offers HEPA filtered air (99.99%efficient @ 0.3 microns) for sterility. The BSC also offers containment from particulate hazards (bacteria, viruses) and may also offer containment from the anesthetic gas itself, depending upon the BSC type and configuration. If gas containment is to be achieved, the BSC must be exhausted either through a hard or a canopy connection.

To review the BSC types and configurations with respect to exhausting, please refer to table 1.

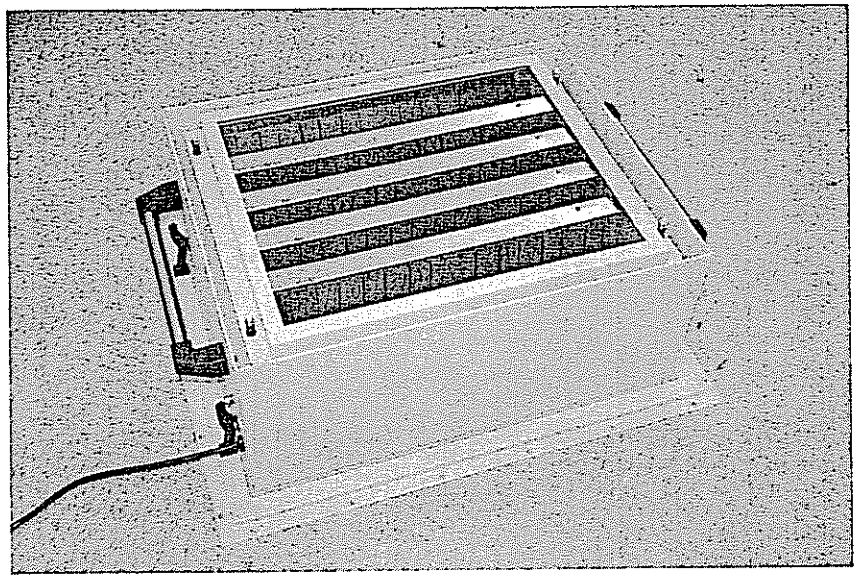
Table 1

BSC Type and Configuration	Recirculation/Exhaust Percentage (%)	Exhaust Type and Connection	Anesthetic Gas Protection
Class II, Type A1	70/30	Room Circulation	No
Class II, Type A2	70/30	Room Circulation	No
Class II, Type A2	70/30	Canopy or Thimble	Yes
Class II, Type B1	30/70	Hard	Yes
Class II, Type B2	0/100	Hard	Yes

As you can see, if the BSC is room re-circulated, no gas protection is achieved. This can be a critical issue if the vivarium only has room re-circulated Class II, Type A2 BSC's. What are the possible alternatives to still perform the required surgical procedures? One solution might be to install the anesthetic gas delivery and scavenging system within the BSC. As with the use on an open laboratory bench, some small amounts of anesthetic gas will be released, but performing these functions within a BSC will not increase or decrease this small negligible release level. A BSC may be configured to aid the delivery and scavenging system by additional service ports through the BSC's workzone wall, if necessary. If a scavenging system is not available, another choice would be to scavenge the room re-circulation portion or exhaust airflow of the BSC. To accomplish this, charcoal filter with sufficient efficiency must be fitted over the exhaust airflow of the BSC. In order to provide such a charcoal filter and not affect the BSC containment performance, a supplemental blower must be used to assure the rate of exhaust volume remains the same with the charcoal filter in place. In addition, the charcoal filter blower must be interlocked to the BSC's blower. This assures the charcoal filter blower will turn on and off with the cabinet's blower switch.

The design of this charcoal filter/blower must be relatively light weight and modular to be easily removed for room to room BSC movement and charcoal filter changes. We designed such a Charcoal Filter Blower (CFB) module that includes the above features (see photo 1) that is attached over the exhaust HEPA filter of the BSC. To test the effectiveness of the CFB module, we ran a series of tests with the help of a Certified Industrial Hygienist, Mr. Gerhard Knutson of Knutson Ventilation Consulting, Inc. I have attached his report summary of the testing along with some of his application guidance to perform a risk assessment.

Photo 1



Testing methods and results

Our thought pattern for the testing was to vaporize a high Isoflurane dose rate, 1.5 lpm at a 5% Isoflurane mixture into a room re-circulated Class II, Type A2 (Nuaire model NU-602-600) and measure the concentration above the exhaust airflow. The first filter test would measure the concentration above the exhaust HEPA filter running normally. The second test would measure the concentration with the charcoal module in place. The result difference would indicate the charcoal module efficiency in reducing the Isoflurane concentration.

The results of the testing are shown in table 2. At a 5% Isoflurane mixture, the CFB module reduced the concentration measured above the BSC's exhaust airstream from 3.5 ppm to .98 ppm. The reduction equals a 70% collection efficiency of Isoflurane gas from the exhaust airstream. In addition to the 5% Isoflurane mixture testing, we calculated lower mixture percentages assuming a linear reduction as additional information.

Table 2

Isoflurane Mixture Percentage	Concentration Measured without Charcoal (ppm)	Concentration Measured with Charcoal (ppm)
5%	3.5	.98
4%	2.8	.78
3%	2.1	.59
2%	1.4	.39
1%	0.7	.20

In addition to testing the collection efficiency of the CFB module, I asked Mr. Knutson to apply the results into a typical vivarium scenario. The results of his evaluation and calculation are shown in table 3. At a 5% Isoflurane mixture, he calculated the steady state gas room concentration level based on the following; a continuous supply of 5% Isoflurane mixture within the BSC, a room size of 12' x 20' x 8', 30 air changes per hour (960 cfm) and a mixing factor of 3 producing 320 cfm effective ventilation rate. Based on the above, the CFB module reduced the steady state gas room concentration from 8.3 ppm to 2.5 ppm applying the 70% collection efficiency as tested above. Again, we calculated lower Isoflurane mixture percentages as additional information.

Table 3

Isoflurane Mixture Percentage	Steady State Gas Room Concentration without Charcoal (ppm)	Steady State Gas Room Concentration with Charcoal (ppm)
5%	8.3	2.5
4%	6.6	2.0
3%	5.0	1.5
2%	3.4	1.0
1%	1.6	0.5

Discussion and Conclusion

The use of a CFB module on the exhaust airstream of a room re-circulated Class II, Type A2 does provide a 70% collection efficiency of Isoflurane gas. However, from a risk assessment perspective, several questions must be evaluated as mentioned in Mr. Knutson's summary. Selection of the Occupational Exposure Limit (OEL) is required, since there is no defined limit for Isoflurane. Using existing information on similar halogenated anesthetic gases can aid in the determination.

Other considerations to make in the risk assessment as discussed include the room design, size and number of air changes. All contribute to the potential room gas concentration level exposure. In addition to the room, the Isoflurane delivery process also has an impact. The Isoflurane mixture percentage used, duration of the used and the process itself all contribute to the release of Isoflurane into the BSC and subsequently into the room.

The test results in this paper represent the worst case scenario directly vaporizing a 5% Isoflurane mixture into the BSC. Most likely the delivery process will include a 5% mixture chamber for the initial anesthetizing, then a 1% to 3% continuous delivery rate during the surgical procedure. The amount of gas delivered will be both consumed by the lab animal and released back into the surgical environment. All have to be considered in the risk assessment.

In conclusion, the use of Isoflurane anesthetic gas within a vented Class II BSC should be utilized at all possible. The use of a room re-circulated Class II, Type A2 might be possible through the use of a CFB module. A thorough risk assessment should be performed using the information provided within this study. Please contact NuAire for any additional questions or concerns.

Knutson Ventilation Consulting, Inc.
3404 West 60th Street, Edina, MN 55410
gerhard.knutson@knutsonventilation.com
Phone (952) 928-0195
Fax (952) 920-1510

E-MAIL TRANSMITTAL

August 18, 2006

Bill Peters
NuAire
2100 Fernbrook Lane
Plymouth, MN 55447

BPeters@NuAire.com

Re: Project 326-013
Isoflurane Risk Assessment

Dear Mr. Peters:

At your request, I have investigated the potential risk associates with using isoflurane in a Type A2 biological Safety Cabinet (BSC). Since the Type A2 BSC discharges a portion of the exhaust into the laboratory environment, the risk clearly depends on the ventilation for the room in which the unit is installed.

DISCUSSION

Isoflurane

Neither the Occupational Safety and Health Agency (OSHA) nor the American Conference of Governmental industrial Hygienists (ACGIH) has established an exposure standard or guideline for Isoflurane. In an OSHA Guideline on Anesthetic Gases[1], OSHA states:

> NIOSH also recommended that no worker should be exposed at ceiling concentrations greater than 2 ppm of any halogenated anesthetic agent [halothane and enflurane] over a sampling period not to exceed one hour. In 1989, the American Conference of Governmental Industrial Hygienists (ACGIH) assigned a threshold limit value-time-weighted average (TLV-TWA) for nitrous oxide of 50 ppm for a normal 8-hour workday. ACGIH TLV-TWAs also exist for halothane and enflurane, and are 50 ppm and 75 ppm, respectively. No NIOSH REL's exist for the three most currently used anesthetics (isoflurane, desflurane, and sevoflurane).

[1] U. S. Department of Labor, "Anesthetic Gases: Guidelines for Worker Exposures", posted on the internet at http://www.osha.gov/dts/osta/anestheticgases/index.html, on August 17, 2006.

Since isoflurane does not have a TLV, PEL or REL, we reviewed material safety data sheets for isoflurane for an in-house occupational exposure level (OEL). Halocarbon Laboratories (a division of Halocarbon Products Corp) listed an internal guideline of 50 ppm. A copy of the MSDS is attached.

Reviewing the toxicological data included in the MSDS for isoflurane, an OEL of 50 ppm appears to be reasonable.

No Ventilation

If the room has no ventilation, or inadequate ventilation, and isoflurane is discharged through a Type A2 BSC, the isoflurane concentration will be unacceptable.

Description of Typical Scenario

Consider a small room where animals are anesthetized with isoflurane. Assume the room is 12 foot by 20 foot by 8 foot high and that the ventilation rate is 30 air changes per hour. In this room, the ventilation rate is equivalent to 960 cfm. In all ventilation systems, the ventilation system has some inefficiency. The measure of the inefficiency is called a mixing factor.[2] The mixing factor usually ranges from 1 to 10. For an animal room, a mixing factor of 3 is reasonable. Thus the effective ventilation is 320 cfm = 960/3 cfm.

If the isoflurane were released directly into the room, the potential exposure to the operator would depend on the geometry of the room, the positioning of the operator and the specific design of the ventilation system. However, the procedure usually is conducted in a biological safety cabinet for sterility reasons. If the cabinet discharges outside, the potential exposure would be caused by leakage from the BSC. With good work practices, an adequately designed BSC and a well designed room, the exposure due to leakage from the BSC would be well below 1 ppm and more likely below 0.1 ppm.[3] This would be well below the proposed OEL (50 ppm) and even below the REL of similar anesthetic gases (2 ppm).

With a Type A2 BSC, the anesthetic gas would not be removed by the HEPA filter. The gas would be discharged through the top of the BSC into the room. The discharge gases would then mix with the supply air and eventually most of the gases would be exhausted through the room ventilation system. Since the room ventilation system will mix with and substantially dilute the gases discharged from the BSC, the anesthetic gasses would be diluted and distributed around the room.

---

[2] Mixing factors are discussed in Chapter 2 of Industrial Ventilation: A Manual of Recommended Practices, 25th edition, 2004.

[3] Based on testing of several total exhaust biological safety cabinets, using a slightly modified procedure following the ANSI/ASHRAE Standard 110, the BSC performance level is often below 0.1 ppm.

Assume a high dose rate of 1.5 lpm of a 5% isoflurane mixture.[4] At a release of 1.5 lpm of 5% isoflurane, the isoflurane release rate would be 0.0027 cfm. Using an exhaust rate of 960 cfm (nominal 30 air changes per hour) and a mixing factor of 3, the steady state concentration in the room would be 8.3 ppm[5].

Operator Exposures

An operator at a Type A2 BSC could have exposures from leakage from the BSC and from any anesthetic gas discharged into the room air. The exposure caused by discharging the BSC into the room would be less than the steady state concentration, approximately 8.3 ppm.

Carbon Adsorption

If the discharge from the Type A2 BSC were to pass through a carbon filter before discharging into the room, the steady state concentrations would be lowered by the efficiency of the carbon adsorption.

In a rough preliminary test, we investigated the efficiency of a carbon filter. We released 5 percent mixture of isoflurane at a rate of 1.5 lpm within a Type A2 BSC. To eliminate the potential of recirculating isoflurane, we attached a hood to the BSC discharge and exhausted the discharge air from the building. Without the carbon filter, we measured an average of 3.5 ppm in the exhaust. We placed a carbon filter in the exhaust from the cabinet, adjusted the volumetric flow to match the preliminary test (575 cfm exhaust), and measured the average concentration in the outlet after the carbon filter. The average concentration was about 0.98 ppm and the removal efficiency about 70 percent. Using the steady state number level described above and assuming a 70 percent efficiency on the activated carbon filter, the new stead state level would be about 2.5 ppm.

Assumptions on the effect of the activated carbon collection must be tempered with the possibility of overloading the filter, channeling of the activated carbon and striping of the carbon bed as "clean" air passes the bed. A complete evaluation of the risk with an activated carbon bed requires further analysis. However, the activated carbon appears to have a beneficial effect.

---

[4] If oxygen is the mixing gas, the potential for fire increases due the elevated oxygen concentrations. This analysis ignores that possibility and focuses on the potential exposure to isoflurane.

[5] Steady state concentration would be 0.0027/320*1,000,000 = 8.3 ppm.

SUMMARY

1) The evaluation of the risk of using a Type A2 BSC, in the above scenario, depends on the OEL used.
   a. Assuming an OEL for isoflurane of 50 ppm, the steady state exposure would be below the OEL in a small laboratory with typical ventilation rates.
   b. Assuming an OEL of 2 ppm, steady state exposures would be marginal or high, depending on release rate, duration of the use of the anesthetic gas, and whether carbon filtration (including the depth of the bed) is used.
2) Use of activated carbon on the exhaust appears to reduce the potential exposure at the anesthetic operation. The degree of benefit will depend on the room set up and ventilation rates, release rate of the anesthetic gas, and maintenance of the activated carbon filter.

If you have any questions concerning this letter, please contact us.

Sincerely,

Gerhard W. Knutson, Ph.D., CIH
President

MATERIAL SAFETY DATA SHEET

IDENTITY: ISOFLURANE (1-CHLORO-2,2,2-TRIFLUOROETHYL DIFLUOROMETHYL ETHER)

SECTION I: MANUFACTURER

HALOCARBON LABORATORIES
(Div. of Halocarbon Products Corp.)
P.O. Box 661
River Edge, N.J. 07661

Emergency Number: (803) 278-3504
Customer Service & Sales: (201) 262-8899
Prepared by: Dr. Neville P. Pavri

SECTION II: CHEMICAL IDENTITY

Components	CAS No.	OSHA PEL	ACGIH TLV	Other Internal Guide
1-Chloro-2,2,2-Trifluoroethyl Difluoromethyl Ether	26675-46-7	None	None	50 ppm (8 hour TWA)

OSHA HAZARD RATING:

This product contains the following toxic chemical(s) subject to Section 313 Title III reporting requirements (40 CFR Part 372).

None

SECTION III - PHYSICAL/CHEMICAL CHARACTERISTICS

Boiling Point : 48.5 C
Melting Point : Not known
Specific Gravity(H2O=1): 1.50
Vapor Pressure: 330mmHg @ 20 C
Vapor Density(Air=1): >1
Solubility in Water : Negligible

Appearance and Odor: Clear, colorless liquid with slight pungent odor

SECTION IV - FIRE AND EXPLOSION HAZARD DATA

Flash Point/Method: None
Autoignition Temp: Not determined
Flammability Limits in Air - LEL: N/A UEL: N/A

Extinguishing Media: Non-flammable. Use methods appropriate for surroundings.

Special Fire Fighting Procedures: Wear self-contained breathing

apparatus if there is danger of leakage.

Unusual Fire and Explosion Hazards: Emits toxic and corrosive fumes under fire conditions.

SECTION V - REACTIVITY DATA

Unstable [ ] Conditions to Avoid: N/A
Stable [X]

Incompatibility (Materials to Avoid): Reactive metals such as sodium, potassium, or finely divided zinc, aluminum or magnesium, especially at high temperature.

Hazardous Decomposition or By-products: Halogen acids and carbonyl halides formed by thermal or oxidative decomposition.

Hazardous Polymerization [ ] May Occur [X] Will Not Occur

Conditions To Avoid: N/A

SECTION VI - HEALTH HAZARD DATA

RTECS Number KN6799000
Rat: oral LD50 4770 mg/kg (KSRNAM 21,3031,87)
Rat: inhalation LC50 15,300 ppm/3 hours
Rat: intraperitoneal LD50 4280 mg/kg
Mouse: oral LD50 5080 mg/kg
Mouse: inhalation LC50 16,800 ppm/3 hours
Mouse: intraperitoneal LD50 3030 mg/kg
Reproductive effects (RTECS)

Inhalation of isoflurane at a concentration of 0.5-3.0% can induce general anesthesia in 7 to 10 minutes, with analgesia, muscle relaxation, and loss of consciousness. Isoflurane is mildly pungent and may cause coughing, laryngospasm and breath holding in an unconscious individual; secretions may be slightly stimulated and pharyngeal and laryngeal reflexes may be obtunded. Isoflurane is a severe respiratory depressant, causing a decreased tidal volume that may produce hypercapnia. Blood pressure is depressed with an initial decrease in systemic vascular resistance, heart rate and cardiac output, although rate and output may increase due to compensatory mechanisms. Arrhythmias can occur, and the myocardium may be slightly sensitized to epinephrine. Renal blood flow, glomerular filtration and urine flow are decreased without residual renal depression or renal injury following isoflurane anesthesia. Isoflurane does not appear to produce liver injury when given for prolonged periods. Inhalation of higher concentrations may lead to death by medullary paralysis. Those recovering from exposure may exhibit shivering, nausea, vomiting, ileus, or excitation,

and there may be a transient white blood count increase. A slight decrease in intellectual function may persist for 2-3 days, with small mood changes or symptoms possible for 6 days. Induction of general anesthesia may cause malignant hyperthermia from hypermetabolism of skeletal muscles in susceptible individuals.

Target organs are respiratory, cardiovascular and central nervous system.

Primary routes of entry: [X] Inhalation [X] Skin [ ] Eyes [ ] Oral

Acute Effects of Overexposure: Anesthesia, respiratory depression, coughing

Chronic Effects of Overexposure: No present evidence demonstrates that isoflurane is a mutagen, teratogen or carcinogen.

In a study by Corbett, male Swiss ICR mice (but not females) exposed to isoflurane were found to have a higher incidence of liver tumors than control mice. The study was found to be flawed. When the flaws were corrected the results were negative.

May cause sterility or other reproductive effects.

Carcinogenicity listing: [NO] NTP [NO] IARC [NO] OSHA
[NO] Other:

IARC Cancer Review: Group-3, Human Inadequate Evidence, Animal Inadequate Evidence.

Exposure Limits/Toxicity: See also Section II
NIOSH: 2ppm/1 hr. ceiling limit is the recommended exposure limit to waste anesthetic gas
Internal: 50 ppm TWA (same TWA recommended by the ACGIH for Halothane, a similar inhalation anesthetic)

First Aid

Inhalation: Remove to fresh air. If necessary give artificial respiration and seek medical help.
Skin: Wash immediately with soap and water.
Eye: Flush eyes out for at least 15 minutes with water. Seek medical help.
Oral: Induce vomiting if concious. Seek medical help.

Medical Conditions Generally Aggravated by Exposure: Myocardial sensitization to epinephrine.

Other Health Hazards: None known

SECTION VII - PROTECTION INFORMATION

Respiratory: Self-contained breathing apparatus for emergency use

Ventilation: Adequate general and local ventilation

Eye and Face: Safety glasses or goggles and/or face shield

Gloves: Impervious gloves

Other equipment: Provide safety shower and eye wash facilities

SECTION VIII - SPILL, LEAK AND DISPOSAL PROCEDURES

Spill, Leak, or Release: Allow small spills to dissipate with good ventilation. For large spills wear self-contained breathing apparatus and absorb on vermiculite and place in closed container.

Waste Disposal: This material may be incinerated by licensed waste disposal company. Observe all federal, state & local regulations.

SECTION IX - OTHER INFORMATION

1. Hazardous Materials/Dangerous Goods Shipping Regulations

Anesthetics are classified as Dangerous Goods/Hazardous Materials when shipped by air. U.S. and international shipping regulations require that any person(s) shipping Dangerous Goods be properly trained and certified. Shipping Dangerous Goods without meeting these requirements is a violation of U.S. law and the shipper could be subject to fines and/or imprisonment. Anesthetics cannot be shipped by U.S. Mail.

U.S.
(49 CFR): N/A (Regulated by Air Only)

IATA: Proper Shipping Name: Aviation Regulated Liquid, N.O.S.
(1-Chloro-2,2,2-Trifluoroethyl Difluoromethyl Ether)
Hazard Class: 9; ID No.: UN 3334
Packaging Group: NA

IMDG: N/A (Regulated by Air Only)

2. Other Information: HMIS Labeling: H1; F 0; R0, PB

REVISED: JULY 7, 2003