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Heating, ventilation, and air conditioning:

Addendum to the CCAC guidelines on laboratory animal facilities – characteristics, design and development

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PREFACE

The Canadian Council on Animal Care (CCAC) is the national peer review organization responsible for setting and maintaining standards for the ethics and care of animals in science throughout Canada. This Addendum provides general guidance for laboratory animal facilities. It has been developed based on expert peer advice and current interpretation of scientific evidence.

This Addendum has been developed to address Guideline 96 of the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003) by providing additional guidance for assuring clean air is available to animals and personnel at all times. In most cases, 15-20 air changes per hour of 100% clean, fresh air, evenly distributed within the room, meets this requirement. However, in some cases 15-20 air changes per hour may not be adequate and hence the air changes per hour may have to be increased, the number of animals held within the room reduced, or the types of animal enclosures changed. In other cases, such as where animal numbers are low, 15-20 air changes per hour in a room may be higher than required. A reduction in the air changes per hour in those rooms where 15-20 is not needed may result in significant energy and cost savings.

Institutions that choose to deviate from 15-20 air changes per hour (CCAC, 2003) must provide the infrastructure, monitoring, control mechanism, and documentation necessary to ensure appropriate air quality at all times for animals and personnel, as specified in this document. While monitoring is required when facilities operate at less than 15-20 air changes per hour, it is also encouraged as good practice for rooms operating at 15-20 air changes per hour, as there is still the potential for problems with air quality.

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Institutions must ensure clean air is available to all animals and personnel at all times.

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Guideline 2:

Institutions that operate at less than 15-20 air changes per hour, as specified in the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development*, must monitor and record air quality contaminants and ensure a mechanism is in place to correct deviations from the performance standards outlined in this document.

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1 INTRODUCTION

Throughout this document, the term 'should' is used to indicate an obligation for which any exceptions must be justified to, and approved by, an animal care committee. The term 'must' is used for mandatory requirements.

The provision of clean air is critical in a laboratory animal facility to maintain the health and well-being of both the animals and personnel, and for consistency of research outcomes. The heating, ventilation and air conditioning (HVAC) system plays a major role in providing stable, non-variable, clean air within the facility. The HVAC system should be capable of replacing potentially degraded room air with clean air; removing potentially harmful contaminants; heating or cooling the air to the appropriate temperature; and adding or removing humidity as required. The HVAC system is also used to maintain pressure gradients and directional air flow between rooms or parts of a facility as necessary to minimize cross-contamination.

Guideline 1:

Institutions must ensure clean air is available to all animals and personnel at all times.

In the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003), Section C.12.3.5, "Air Exchange", Guideline 96 states:

> The rate of air exchange within a room must be such that clean, fresh air is available to all animals and personnel at all times. For conventional animal holding rooms, the HVAC system should be capable of supplying and exhausting 15 to 20 air exchanges per hour.

The discussion that follows Guideline 96 indicates that "ideally HVAC systems should be designed so that the number of air exchanges can be altered according to how the room is being used; however, increased flexibility must be weighed against the potential for air balancing problems."

This Addendum has been developed to address Guideline 96 by detailing air quality performance-based standards and monitoring requirements for those institutions that choose to deviate from providing 15-20 air changes per hour to a room. Diversity of room use within an animal facility may make it possible to supply clean air without requiring 15-20 air changes per hour in all rooms; however, in all cases, clean air must be maintained.

Guideline 2:

Institutions that operate at less than 15-20 air changes per hour, as specified in the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development*, must monitor and record air quality contaminants and ensure a mechanism is in place to correct deviations from the performance standards outlined in this document.

Monitoring air quality is encouraged for all facilities to ensure clean air is available at all times; however, it is critical for any facilities operating at less than 15-20 air changes per hour. Regardless of the air changes per hour, temperature and humidity should be monitored, as stated in the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003).

1.1 AIR RECIRCULATION

The main incentive for facilities considering air recirculation is energy savings; however, recirculated air still requires conditioning and filtering, along with increased fan power associated with filtering. Therefore, the energy savings may not be as great as anticipated. The approach of reducing air change rates through monitoring, as described in this document, significantly addresses the issue of energy savings (Sharp, 2010).

The main concern with air recirculation is cross-contamination between rooms, since filters are seldom 100% efficient indefinitely, and the risk of introducing variability in the research environment, as filters are not able to capture pheromones and various gases that are not typically monitored.

While air recirculation is rare in laboratories in general, there is insufficient evidence of this approach being used in laboratory animal facilities and the risk of cross-contamination precludes air recirculation in laboratory animal facilities at this time.

2 FACTORS TO CONSIDER

2.1 SYSTEMIC AND OPERATIONAL ISSUES

Systemic sources of room air contamination are related to the design and maintenance of the facility, the HVAC system and the equipment being used, while operational sources stem from ineffective application of standard operating procedures for tasks conducted within the room that affect indoor air quality. A number of systemic and operational factors that affect air quality in laboratory animal facilities are discussed in the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003). These include the type of animal enclosure (e.g., open pens, static cages, and individually ventilated cages), exhaust configuration, air distribution, requirements for pressurization and air flow to maintain barriers, the species and density of animals, equipment in the room (such as change stations), and husbandry and research activities.

As noted in the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003), "the impact of the overall HVAC system must be evaluated at the cage level". This is further addressed in CCAC guidelines on particular types of animals.

2.2 EPISODIC ISSUES

Episodic issues result from events that cannot be predicted and are not related to the use of good systemic and operational approaches and controls (e.g., events that occur due to improper training of an individual, human error, or broken or failing equipment). Due to unexpected problems, one or more contaminants (see Section 3, "Air Quality Performance-Based Standards") can fall outside of acceptable limits for long periods of time, unless quickly detected. Episodic events can also be very short in duration and may not be detected if air quality is monitored on a weekly to monthly basis, rather than continually. For systems that only monitor air quality periodically, this may lead to an erroneous assumption that air quality is consistently good, while there may be fluctuations that could affect the health of the animals or personnel, or the consistency of the research environment. Even a weekly analysis of air quality may not permit a fast enough response to episodic events if short-term studies are underway or the elevation in contaminant levels is great.

Minimizing the occurrence of episodic events is important for all ventilation systems; however, it is critical for systems where there cannot be a timely increase in ventilation when required (see Section 2.3, "HVAC Systems"). The use of ventilated cages and cage change stations or biosafety cabinets and adherence to animal care committee-approved standard operating procedures for all functions occurring in a room that could affect the air quality can reduce, but not eliminate, the occurrence of episodic events.

The most effective solution to an episodic event is to increase the ventilation rate to 'flush' the room, and implement short-term measures to protect the health of the animals and personnel (e.g., personal protective equipment), as appropriate. In the case of HVAC malfunction, there should be a contingency and a crisis

management plan in place to address possible animal and personnel health and welfare issues if the HVAC system cannot be brought back online within a short period of time.

Responding to episodic events through increased ventilation is most efficiently achieved through a demand-based HVAC system, in which air quality is continually monitored and the ventilation rate is automatically increased when set criteria are detected, while maintaining air pressure differentials. For systems that cannot increase ventilation rates fast enough to flush the room air following an episodic event and maintain air pressure differentials, it is safer to operate at 15-20 air changes per hour. Maintaining 15-20 air changes per hour will allow a return to acceptable air quality standards faster when episodic events occur, than if these systems operate at a lower air change rate.

For facilities that cannot promptly increase ventilation rates in response to episodic events, any consideration of reducing ventilation rates for energy conservation must be thoroughly analyzed, based on: 1) an evaluation of the risks to the animals, people and research projects; 2) assurance that procedures are in place and adhered to that will minimize the occurrence of episodic events and minimize their impact when they occur; and 3) implementation of an air quality monitoring program (see Section 4, "Air Quality Monitoring"). If consideration of these factors and the air quality monitoring of a room indicate that a reduction in air changes per hour is acceptable, a lower limit of 12 air changes per hour should be maintained in that room, given that there cannot be an automatic response to episodic events.

2.3 HVAC SYSTEMS

The operation of the HVAC system depends on how the system is controlled, the diversity of ventilation requirements throughout the facility, and the ability to minimize the occurrence of episodic events that affect air quality, as well as the capacity to promptly respond when they occur. Any changes made to air flow rates must be accompanied by verification of air quality and air pressure differentials through documented monitoring.

2.3.1 Types of Systems

The types of HVAC systems in current use differ in the capacity to change the rate of air supplied to and exhausted from a room. Some HVAC systems have automatic air valves for supply and exhaust that adjust to maintain appropriate differential pressures. When these systems are combined with an air quality monitoring system, the ventilation rate in each room or space is adjusted automatically according to the air quality in the space, while maintaining appropriate air pressure differentials. These systems are referred to as air quality demand-based systems.

For other systems, such as constant air volume systems or demand-based systems that are not linked to air quality monitoring, changing the rate of air supply and exhaust is done manually. This is time consuming and often requires the expertise of a mechanical technician in order to maintain the required air pressure differentials of all rooms possibly affected by that adjustment. Therefore, the ventilation rate for these systems cannot be changed promptly in response to air quality monitoring or even with routine changes in the use or number of animals in a room. For these systems, the ventilation rate in each room should be set to accommodate the largest number of animals to be held at one time and a variety of species if that is the intended use of the room. However, the ventilation rate may need to be adjusted if the use of the room changes significantly. When air change rates are altered manually, there must be assurance that clean air and appropriate air differentials are maintained through monitoring (see Section 4, "Air Quality Monitoring").

2.3.2 Diversity Factor

Diverse air exchange rates may be used within a facility provided the air quality in each room is maintained within acceptable limits (outlined in Section 3, "Air Quality Performance-Based Standards"), and the required air pressure differentials are maintained. The ability to take advantage of the diversity factor is very limited in systems other than an air quality demand-based system.

Each situation will be unique, based on facility design, species and animal density in the room, type and location in the room of caging or pens, air flow patterns, whether ventilated cage change stations or biosafety cabinets are used, and, in the case of ventilated racks, how the rack ventilation system is set up.

This diversity factor may also be used by designers to determine the HVAC capacity required. The diversity factor can incorporate factors based on the intended operation of the facility, experience on typically required air flow levels to maintain desired air quality, air flow requirements from exhaust devices such as biosafety cabinets and rack ventilation systems, and animal and equipment thermal loads.

3 AIR QUALITY PERFORMANCE-BASED STANDARDS

The quality of the air in a room deteriorates as contaminants are generated within the room, based on room usage and activity.

Guideline 3:

The key contaminants that should be monitored and documented to assure acceptable air quality are ammonia, carbon dioxide, particulate matter and total volatile organic compounds.

Table 1 summarizes the recommendations for these key contaminants at the room level, with the aim of providing clean air that is close in composition to fresh air. Further explanation of each contaminant is provided in Sections 3.1-3.4.

In Table 1, supply air target values refer to the air being delivered to the room. These are important targets when a ventilation system is being set up, and considerations for ensuring clean air is entering the facility are described in the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development*, Section 12.3.3, "Fresh Air" (CCAC, 2003). During operations, if the supply air exceeds these target values, an investigation of the source and corresponding steps to mitigate the situation (e.g., appropriate filters) are required.

For indoor air, the target values in Table 1 indicate the acceptable operating range. If any of the contaminants rise above this level, facilities should investigate the source of the increase of that particular contaminant and take action to reduce the level to the target value. Note that these levels assume that the indicated supply air levels are being met, and they represent the increase in levels above that supply air level. As a result, it is important to identify the source of the problem in order to take appropriate action. For example, if the increase is due to an increase in the concentration of that component in the supply air, increasing the air changes per hour will not bring the room air quality within the target value, and steps should be taken to address the quality of the supply air. However, if the source of the problem is the generation of contaminants within the room, then increasing the air changes per hour may be necessary. Therefore, consideration of the difference in contaminant levels between room air and supply air should always be part of the investigation and any control of room air ventilation rates should only be done based on measurement of the difference between the supply air levels and the room air levels.

The maximum limit value for each component in Table 1 indicates a serious problem and these values should not be reached. Whenever the target values are exceeded, there should be an investigation into the source of the increase and action should be taken to reduce contaminant levels to the target values.

TABLE 1 TARGET VALUES FOR AIR QUALITY

CONTAMINANT	SUPPLY AIR TARGET VALUE	INDOOR AIR		METHOD OF MEASUREMENT [A]
		TARGET VALUE	MAXIMUM VALUE	
Ammonia	0 ppm	<5 ppm	<25 ppm [B]	Photoionization detector
Carbon dioxide	350 – 600 ppm	<500 ppm [C]	<5,000 ppm [D]	Infrared
Particulates (PM 2.5)	<28.2 million/m³ (<0.8 million/ft³) <10 µg/m³	<35.3 million/m³ [E] (<1 million/ft³) <12 µg/m³	<176.5 million/m³ (<5 million/ft³) <60 µg/m³	Laser particle counter Optical particle mass [F]
Total volatile organic compounds	0 ppb 0 µg/m³	<200 ppb [E] <500 µg/m³	<1 ppm [G] <2500 µg/m³	Photoionization detector Gas chromatography [H]

A. The values in the table relate to the method of measurement indicated. If another method is used, the values will need to be converted for that method.

B. This corresponds to the safety standards for humans for continuous 8-hour exposure (United States Department of Labor, 2012a; WorkSafeBC, 2017).

C. This is a differential value compared to the supply air provided to the room. Relatively high levels of carbon dioxide are not necessarily a health concern (as indicated by the maximum limit of 5,000 ppm (United States Department of Labor, 2012b; WorkSafeBC, 2017; Deutsche Forschungsgemeinschaft, 2012)); however, an increase in carbon dioxide in the room serves as a proxy for a ventilation problem.

D. It is recognized that 5,000 ppm is the 8-hour exposure limit determined by American Society of Heating, Refrigeration and Air Conditioning Engineers (ASHRAE) and Occupational Safety and Health Administration (OSHA) (ASHRAE, 2016; United States Department of Labor, 2012b). Since the animals are exposed to their environment continually, 24 hours/day, the maximum value should be lower than this; however, there is no specification in the literature as to what this value should be.

E. This is a differential level compared to the supply air provided to the room.

F. This is a weight-based measure, which is supported in the literature; however, the laser particle counter is a more accessible method for use in laboratory animal facilities, and therefore the value provided for a laser particle counter was derived from the value for the optical particle mass method (Sharp, 2010). The supply and weight-based differential target value is based conservatively on the recommended design indoor air quality value for PM 2.5 of 15 ug/m³ from Appendix C of S*tandard 62.1: Ventilation for Acceptable Indoor Air Quality* (ASHRAE, 2016), which is itself based on the *National Ambient Air Quality Standard (NAAQS)* (US EPA, 2012). There is no established maximum PM 2.5 value relating to potential health hazards, so the value listed here is meant to be a reasonable maximum value based on trying to maintain PM 2.5 levels to less than 12 ug/m³.

G. There is no established maximum total volatile organic compounds value relating to potential health hazards, so the value listed here is meant to be a reasonable maximum value based on trying to maintain the total volatile organic compounds levels to less than 500 µg/m³ or <200 ppb which is a widely recommended target value for total volatile organic compounds (Appendix C of ASHRAE, 2016; Sharp, 2010).

H. This method requires laboratory analysis and the value provided is supported in the literature (USGBC, 2007; Washington State Department of Health, 2003); however, the photoionization method is more accessible for use in laboratory animal facilities, and therefore the value provided for the photoionization method was derived from the value for the gas chromatography method.

3.1 AMMONIA

Guideline 4:

Ammonia levels must be maintained below 25 parts per million (ppm) and, in general, should not exceed 5 ppm.

Further limits on ammonia levels may need to be applied to address particular requirements of any research studies being undertaken.

An ammonia level of 25 ppm corresponds to the safety standards established for humans for continuous 8-hour exposure (e.g., American Conference of Governmental Industrial Hygienists; the US National Institute for Occupational Safety and Health (United States Department of Labor, 2012a); and WorkSafeBC Exposure Limits (WorkSafeBC, 2017)). While experience has shown that exposure to this level for this period of time does not result in significant health problems in humans, it does not mean that it is pleasant for humans or animals. People can detect the presence of ammonia at concentrations of 1 ppm (Smyth, 1956), and the smell becomes obvious at 2-3 ppm.

The production of ammonia will vary according to the species, strain, sex and density of animals, as well as some disease conditions. The concentration of ammonia in the room will also be affected by the research protocol (e.g., how frequently the cages are opened, the caging system, and whether procedures are conducted within an exhausted biosafety cabinet or ventilated cage change station).

Ammonia is produced by the action of urease-positive bacteria on urea present in urine and feces. The activity of the bacteria, and therefore the level of ammonia, is influenced by temperature and humidity (Gamble and Clough, 1976). Ammonia levels can rise exponentially (Gamble and Clough, 1976); hence, the facilities, type of enclosure and husbandry practices (e.g., type of bedding and frequency of bedding changes and cage/pen cleaning (Ferrecchia et al., 2014; Perkins and Lipman, 1995)) should be designed to ensure levels of ammonia remain within safe limits.

Ammonia can be measured with dedicated ammonia sensors or a photoionization detector type total volatile organic compounds sensor, since these devices react to ammonia as well as measure total volatile organic compounds in the room. This measurement should be done differentially with respect to the supply air since dilution ventilation can only help with the increase in ammonia levels in the room. As noted in Table 1, the

supply air should be free of ammonia, and therefore the ventilation should be overridden and begin to be increased if the differential ammonia level exceeds 5 ppm. Maximum ventilation or purge levels of 15-20 air changes per hour should be provided if the differential ammonia level approaches 20 ppm.

3.2 CARBON DIOXIDE

Guideline 5:

Increases in room carbon dioxide levels should be kept below 500 ppm.

An upper limit of a 500 ppm increase in carbon dioxide in a space as compared to outdoor levels (defined in Table 1 as clean air) is in keeping with the ASHRAE Standards (McLeod, 2011), and other international standards (a summary is provided by the Government of the Hong Kong Indoor Air Quality Management Group, 2003). Ideally, the increase in carbon dioxide levels in the room should be similar to that of an office building, which is typically 250-500 ppm (HC, 1995).

Outdoor air contains approximately 350-600 ppm carbon dioxide; however, this can fluctuate significantly with environmental factors. In office buildings, normal human activity can result in a carbon dioxide concentration increase of 500 ppm (HC, 1995).

Monitoring carbon dioxide is particularly important in rooms where carbon dioxide euthanasia is being performed, since carbon dioxide levels approaching or possibly exceeding the maximum health-related value of 5,000 ppm could occur without proper ventilation levels.

3.3 PARTICULATE MATTER

Guideline 6:

The increase in particulate levels above supply air levels should be kept below 12.0 micrograms per cubic meter (µg/m^3) or 35.3 million particles per cubic metre (1 million particles per cubic foot) as measured with an optical particle counter's 0.3 micrometre channel.

Particulates in indoor air originate from a variety of sources and can cause irritation to the eyes, skin and respiratory tract. In a laboratory animal facility, the additional presence of animal allergen particulates can be of considerable concern due to the potential for repeated exposure and sensitization of animal facility personnel and researchers. Allergens typically are attached to particles and are released into the air in the presence of other particulates, such as from cage bedding materials. The presence of particulates can be used then as both a proxy for the potential presence of allergens as well as an indication of high levels of particulates that should be removed.

Concerning the relationship between allergens and particulates, the absence of particulates in the environment would indicate that there are most likely no allergen particulates in the air as well. However, the presence of particulates in the air, due perhaps to the dropping of a cage or changing of a cage without the proper

use of a cage change station, could indicate the potential presence (although not the certainty) of allergen particles in the air, and therefore to be safe in all cases, extra or purge levels of ventilation should be provided until the particulates return to an acceptable level.

In terms of determining what should be measured, particle size affects how far particles can penetrate into the respiratory tract and determines the sites of possible health effects. Because respirable suspended particles less than 2.5 micrometres in aerodynamic diameter can reach the gas exchange region, they may present long-term health concerns. Additionally, when particles are released into the air, it is the fine particles less than 2.5 micrometres (also known as PM 2.5) that will be suspended for the longest periods of time, versus the larger particles that will fall onto surfaces and the floor much more rapidly.

For these reasons, PM 2.5 should be continuously monitored. Accurate real time measurements of PM 2.5 can be done for example with a laser based particle counter able to measure particulates down to 0.3 micrometre in the range of 353,000 to 176,500,000 particles per cubic metre (10,000 to 5,000,000 particles per cubic foot). This measurement, which is for ventilation control purposes, should represent the increase in particulates in the room, not the absolute particle level in the space. This is because dilution ventilation can only impact the contaminants generated in the room and will have no impact on the source concentration in the supply air which is used for the dilution ventilation.

Differential levels of fine particles (0.3 to 2.5 micrometres) in the room as compared to the supply air entering the room should be less than 12.0 micrograms per cubic meter (μg/m^3) (US EPA, 2012). For a laser based particle counter measuring particles greater than or equal to 0.3 micrometre, this corresponds to a level of approximately 35,300,000 particles per cubic metre (1,000,000 particles per cubic foot). It is important to also note that this differential threshold level can be measured by taking the difference between individual measurements of both the supply air in the supply duct before it enters the room and the air in the room itself, or preferably after it leaves the room in an exhaust duct. When appropriate filtration is used on the supply air, the particulate count should be less than 28.2 million/m^3 (0.8 million/ft^3), using a laser particle counter.

Ventilation levels should begin to be increased when the differential particle levels approach 35.3 million particles per cubic metre (1 million particles per cubic foot). Maximum ventilation or purge levels of 15-20 air changes per hour should be provided if the differential particle level approaches 176.5 million particles per cubic metre (5 million particles per cubic foot) or less.

3.4 TOTAL VOLATILE ORGANIC COMPOUNDS

Guideline 7:

The increase in room total volatile organic compounds levels above supply air levels should be kept below 500 micrograms per cubic meter (μg/m^3) or 200 parts per billion (ppb), as measured with a photoionization detector based total volatile organic compounds instrument.

Total volatile organic compounds of concern in animal facilities include vapors from chemicals and solvents that can often be used in procedure rooms, support rooms and even in the animal holding rooms. Other

contaminants of concern in laboratory animal facilities include beta- and alpha-pinene given off from certain bedding materials, and hydrogen sulfide given off from animal waste. Total volatile organic compounds are also of particular concern in new or renovated facilities, where the building materials and new furnishing may cause increased levels, and this should be checked prior to occupancy. As with particulates, dilution ventilation can only help with those total volatile organic compounds contaminants generated internally and not the level of contaminants coming from outdoors. Therefore, the measurement of total volatile organic compounds needs to be done differentially between the level of total volatile organic compounds entering the room in the supply air versus the actual room level, or preferably, the level of contaminant in the exhaust duct after it leaves the room.

The best instrument for accurate real time detection of differential total volatile organic compounds levels is a photoionization detector based total volatile organic compounds instrument. As noted in Section 3.1, "Ammonia", this instrument is also capable of measuring ammonia. The best location for measuring the room total volatile organic compounds levels, if at all possible, is in the room's dedicated exhaust duct.

Ventilation should begin to be increased if differential levels exceed 200 ppb as measured by a photoionization detector based total volatile organic compounds instrument. Maximum ventilation or purge levels of 15-20 air changes per hour should be provided if this differential total volatile organic compounds level approaches approximately 1 ppm or less.

An additional instrument that can be used for supplemental measurements is a metal oxide semiconductor based total volatile organic compounds sensor. This instrument is less accurate than a photoionization detector based total volatile organic compounds instrument, but can be used specifically for measurements of methane-based compounds, especially in large animal facilities, and methyl alcohol, if those compounds are of interest.

4 AIR QUALITY MONITORING

Air quality monitoring must be performed where rooms operate at less than 15-20 air changes per hour; however, it is also encouraged in rooms operating at 15-20 air changes per hour, as there can still be problems with air quality. Acceptable deviation from the recommended room ventilation rate of 15-20 air changes per hour specified in the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003) depends on the level of air quality monitoring being conducted and the ability of the HVAC system to respond to changes in air quality as follows:

1) For rooms where there is continual monitoring (defined as once every 15 minutes or less) and the HVAC system is a demand-based system that provides an automatic response to changes in the levels of contaminants identified in Section 3, "Air Quality Performance-Based Standards", there is no prescriptive lower limit for air changes per hour. Enough room airflow needs to be provided to achieve the performance based target values defined in Table 1.

2) For rooms where there is not continuous monitoring and the capacity for an automatic response to changes in levels of contaminants, a reduction in air changes per hour to a minimum lower limit of 12 air changes per hour may be acceptable if the level of monitoring specified in Section 4.1, "Minimum Monitoring Requirements for Reducing Air Changes Per Hour" is conducted and monitored contaminant levels are within the target ranges specified in Section 3, "Air Quality Performance-Based Standards".

3) For rooms where the monitoring requirements noted in the previous two scenarios are not met, the recommended level of 15-20 air changes per hour, as stated in the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003), applies. However, these facilities are encouraged to incorporate as much of the monitoring required for systems operating at <15-20 air changes per hour as possible, in order to gain a better understanding of the air quality of their rooms.

Monitoring room air quality must take into account the influence of room design and air distribution, so that monitors are positioned to ensure an accurate assessment of the air quality supplied to animals and humans. If at all possible, the monitoring devices should be positioned to directly monitor in the exhaust air duct from the room for best accuracy.

Sensors used for these measurements should be checked and calibrated approximately every six months or more frequently as needed to maintain sensor accuracy (see Section 4.4, "Sensor Calibration Requirements").

4.1 MINIMUM MONITORING REQUIREMENTS FOR REDUCING AIR CHANGES PER HOUR

For facilities that are unable to continuously monitor air quality and respond automatically to any changes, the HVAC system may operate below 15-20 air changes per hour to a minimum of 12 air changes per hour if the following level of monitoring is implemented and there is a record of all activities (e.g., cage changing, research activities, etc.) and conditions (e.g., humidity) that could affect the air quality of the room. The time of

year that monitoring takes place should be recognized as a factor, since humidity levels fluctuate annually and influence ammonia levels. The record of activity for the room should be correlated to the data from air quality monitoring, so that the source of any air quality problems can be investigated and appropriate action taken.

4.1.1 Initial Monitoring Period

The period of monitoring must encompass every activity that takes place in the room, and should cover each activity at least twice (e.g., two cage changes). The minimum time for the initial monitoring period is one month. In order to maintain an air change rate below 15-20 air changes per hour, recordings of contaminant levels must be consistently below the target levels specified in Section 3, "Air Quality Performance-Based Standards". If episodic increases above these levels are detected, the source must be investigated, the problem rectified, and the monitoring period extended.

If activities in a room fluctuate so much that all events cannot realistically be captured over a reasonable period of time, the air changes per hour should not be reduced. In other words, if room activities are highly variable, it may not be meaningful to monitor air quality with the goal of reducing the air change rate, and 15-20 air changes per hour should be maintained.

4.1.2 Follow-up Monitoring

Follow-up monitoring should occur every six months, with each monitoring period lasting for a period that covers all activities in the room at least once, with a two-week minimum period of monitoring.

4.2 ADDITIONAL CONSIDERATIONS

There are potential risks associated with monitoring air quality intermittently, instead of continuously. Favourable readings can lead to the conclusion that the air is consistently within the target values, but there may actually be periods between readings when these values are exceeded.

The type of research should be a consideration, and constant monitoring may be needed (or alternatively a high air changes per hour with verification that the source air is within the target value) for some research where consistent air quality is critical.

4.3 SENSOR ACCURACY REQUIREMENTS

For both continual monitoring (every 15 minutes) and the periodic monitoring of Section 4.1, "Minimum Monitoring Requirements for Reducing Air Changes Per Hour", the sensors need to meet the following accuracy and calibration requirements.

4.3.1 Ammonia

If a dedicated ammonia sensor is used, the following specifications apply. If a photoionization detector type of total volatile organic compounds sensor is used to detect ammonia, then the specifications in Section 4.3.3, "Particulates", for the photoionization detector type total volatile organic compounds sensor apply.

- Accuracy: ± 2 ppm or 2.5% of reading (whichever is greater)
- Resolution: 0.25 ppm

4.3.2 Carbon Dioxide

- Accuracy: ± 75 ppm up to 1000 ppm
- Resolution: 3 ppm
- Repeatability: 10 ppm

4.3.3 Particulates

A laser-based optical particle counter should be used for this application with a range or channel of 0.3 to 2.5 µm (PM 2.5) with the following minimum specifications:

- Accuracy: ± 25% of reading
- Resolution: <353,000 particles per cubic meter (10,000 particles per cubic foot)
- Concentration Range: >176,500,000 particles per cubic meter (5,000,000 particles per cubic foot)

4.3.4 Total Volatile Organic Compounds

A photoionization detector type total volatile organic compounds sensor with a 10.6 eV lamp should be used for this application with the following minimum specifications:

- Accuracy: ± 0.2 ppm (as isobutylene) or 2.5% of reading (whichever is greater)
- Resolution: 0.025 ppm
- Drift Stability: ± 2 ppm/6 months @ 5 ppm

4.4 SENSOR CALIBRATION REQUIREMENTS

Whether spaces in laboratory animal facilities are being monitored on a permanent, installed basis, or periodically on a temporary basis for two to four weeks or more, high accuracy and stability of measurement are needed due to the differential measurements that are required for this application. As noted above, measurements need to be taken of both the supply air feeding the room as well as the room air or exhaust air leaving the room to determine the generation and impact on air quality of contaminant sources in the room. The types of sensors used for this application have readings that will drift over time for various reasons. Laboratory animal facilities in particular, due to the potential presence of ammonia, particles, hair, organic material and other contaminants generated by the animals, can quickly foul air quality sensors and are especially challenging to the accurate use of sensors. Therefore, to ensure accurate and meaningful results, a frequent calibration regimen must be followed that is appropriate to the selected method of utilizing the sensors. Two recommended approaches are provided below.

The best approach to provide high accuracy and stability of these differential measurements is to use the same sensor for both supply and room/exhaust air measurements via an air sampling approach, so that normal offset drift of the sensor is cancelled out. If this air sampling or centralized sensor approach is followed, calibration is still needed since sensors can have other types of drift, but the frequency can be significantly reduced. A full factory or certified calibration every six months is appropriate for this method.

An alternative approach can also be followed that uses individual or separate sensors for the measurement of the supply air feeding a room as well as the room/exhaust air itself. When this approach is followed,

calibration becomes much more critical since sensor drift is not being canceled out through an inherent differential measurement approach. Each sensor can drift up or down so large signal errors can quickly appear from using the supply reference and room/exhaust air sensors. To keep the measurements accurate, the sensors must be calibrated more frequently as a practical means to eliminate the errors from sensor drift. As a result, facilities that use this approach of individual, separate sensors should follow the standard scientific approach of doing a full factory or certified sensor calibration before every new set of measurements. In other words, for this application, sensors that are being used for the two-week follow up monitoring should be calibrated every two weeks at the beginning of every new monitoring session. For the four-week initial monitoring, the sensors would then be calibrated every four weeks at the start of each initial monitoring session. Note that in no situation should these individual or separate sensors be used for over one month without recalibrating the sensors. For facilities where a measurement time of over one month is required for either the initial or follow-up monitoring of a room or space, each sensor must be replaced by a new calibrated sensor after one month of use to maintain measurement accuracy.

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GLOSSARY

Air changes per hour (also known as **air exchange per hour**) – the number of times the volume of air in a room is supplied to and exhausted from the room in one hour.

Allergen – a substance that causes an allergic reaction.

Biosafety cabinet – A primary containment device that provides protection for personnel, the environment, and the product (depending on biosafety cabinet class), when working with biological material.[1]

Clean air – air that meets the supply air target values of 0 ppm ammonia, 350-600 ppm carbon dioxide, <28.2 million particulates (PM 2.5)/m^3, and 0 ppb total volatile organic compounds.

Cross-contamination – the unintentional transfer of contaminants from one location to another.

Dilution ventilation – the replacement of a portion of the contaminated air with clean air to reduce the concentration of contaminants.

Directional air flow – the movement of air from an area of high pressure to an area of low pressure (see also pressure gradients).

Diversity factor (also known as **diverse air exchange rates**) – the operation of different rooms at different ventilation rates within a facility, taking into account the air quality of the room and the required pressure differences with respect to adjacent rooms and corridors.

Episodic events – situations that cannot be predicted and are not related to the use of good systemic and operational approaches and controls (e.g., human error, or broken or failing equipment).

Particulates – very small particles of a substance; in terms of monitoring air quality, particles less than 2.5 micrometres in aerodynamic diameter are of concern, as they can remain suspended in the air for a long time and may present health concerns.

Pressure gradients – different air pressures that are maintained between rooms and corridors to control the movement of air and eliminate a potential source of cross-contamination; for more information, see Section 12.3.6, "Differential Pressure", in the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003).

Thermal load – the amount of heat generated from all sources present such as equipment, lighting, animals and people.

Total Volatile Organic Compounds – a grouping of various organic compounds that are present in gaseous state.

1 From the *Canadian Biosafety Standard* (CBS), 2nd ed., (2015).

HEATING, VENTILATION, AND AIR CONDITIONING

DATE OF PUBLICATION: February 2019

The following is a selection of frequently asked questions (and their respective answers) concerning *Heating, ventilation, and air conditioning: Addendum to the CCAC guidelines on laboratory animal facilities – characteristics, design and development* (CCAC, 2019).

1. Why was the addendum developed?

CCAC program participants requested that the CCAC consider revision of guidance concerning heating, ventilation, and air conditioning systems to address the operation of newer systems aimed at maximizing energy efficiency and lowering operating costs. The addendum focuses on performance standards, rather than a specified air change rate, to help institutions utilize technological advancements while ensuring clean air is provided for the animals and personnel.

2. Are all institutions expected to implement an air quality monitoring system in accordance with the addendum?

Institutions are expected to follow either the requirement specified in the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003) (i.e. 15-20 air changes per hour) or implement the infrastructure and monitoring and generate the documentation necessary to ensure clean air is provided for animals and personnel at all times, as described in the addendum. As noted in the addendum, monitoring is required when facilities operate at less than 15-20 air changes per hour, but it is also encouraged as good practice for facilities operating at 15-20 air changes per hour as there is still the potential for air quality problems.

3. What is "clean air"?

Clean air in a laboratory environment refers to air that supports the health and well-being of the animals maintained within the facility and the staff who work there. The composition of clean air meets the supply air target values noted in the addendum: 0 ppm ammonia, 350-600 ppm carbon dioxide, <28.2 million particulates (PM 2.5)/m^3, and 0 ppb total volatile organic compounds.

4. What is the basis for the performance standards defined in the addendum?

The performance standards for air quality specified in the addendum are the result of the work of the expert volunteer subcommittee to assimilate evidence gathered from the published literature, an online survey, and the extensive feedback received from three external reviews of the draft addendum.

5. Why do the performance standards include total volatile organic compounds when there have been articles showing it does not reliably predict air quality in buildings designed for human occupancy? Wouldn't a single volatile organic compound (e.g., formaldehyde, toluene, etc.) be a better indicator?

The guidelines are intended to not only protect animals and personnel, but also the integrity and repeatability of the research. It is therefore important to ensure a stable environment as well as a safe environment. Monitoring total volatile organic compounds is appropriate to indicate any change or trend that requires further investigation.

Measurement of total volatile organic compounds is supported by green building standards such as LEED and has also been used in the WELL standard. The total volatile organic compounds limit noted in the addendum is widely accepted as indicative of good indoor environmental quality. Although it is not perfect and not usable directly as a health measure since different volatile organic compounds have different

threshold limits, total volatile organic compounds is easily measured and the best general, comprehensive measure available, particularly as a diagnostic tool.

It is possible to get a high total volatile organic compounds reading that is not a health problem due to the volatile organic compounds involved, but it does indicate an abnormal change that is worth investigating. By only measuring some individual volatile organic compounds, other compounds of interest could be missed, as there are a large numbers of volatile organic compounds and it is difficult to measure more than a few compounds continuously.

6. What are episodic events and why is it important to consider them in determining appropriate ventilation rates?

Episodic events are unexpected problems that influence air quality. Examples of such events include the following:

- improper training of an individual or human error – e.g., dropping a cage while changing cages, or opening a cage outside of a biosafety cabinet or change station;
- failing to plan for research procedures that cannot be conducted in a biosafety cabinet or change station, such as stereotaxic surgery; or chemicals that are used in conjunction with research procedures;
- procedures that do not follow best practice – e.g., using different chemical products to clean the room; bottom-only cage changes that stack dirty cage bottoms outside the biosafety cabinet or change station without cage tops; and
- broken or failing equipment – e.g., malfunction of individually ventilated cages; improper cage connections and failure to maintain cage top filters that may be worn or torn.

In each of these examples, contaminants would be released into the air of the room. Depending on the type and amount of contaminants released, this could affect the health and well-being of the animals and personnel, and/or the scientific studies being conducted.

When an episodic event occurs in a room with a ventilation rate of 15-20 air changes per hour, the air in the room is diluted with the source air, and the air quality components return to acceptable levels within a reasonable amount of time. However, when an episodic event occurs in a room where the ventilation rate has been lowered, dilution of the air in the room with the source air will be slower, relative to the air changes per hour of the room. In such situations, the ventilation rate needs to be increased to the maximum rate to efficiently remove the contaminants.

7. Why is there a lower limit of 12 air changes per hour for facilities that do not have a demand-based system?

Responding to episodic events through increased ventilation is most efficiently achieved through a demand-based heating, ventilation and air conditioning (HVAC) system, in which air quality is continually monitored and the ventilation rate is automatically increased when set criteria are detected, while maintaining air pressure differentials. For these systems there is no prescribed minimum air changes per hour.

For systems that do not have an automatic response to changes in air quality, it will take longer to dilute contaminants if the air changes per hour is lowered. For these systems, if air quality monitoring of a room indicates that a reduction in air changes per hour is acceptable, maintaining a minimum operating level of 12 air changes per hour will allow for some energy savings while still maintaining the ability to flush contaminants from the room at a reasonable rate.

8. Do the same air quality standards apply to rooms where animals are housed in ventilated racks?

Yes, the air quality standards for the room are the same, regardless of the equipment in the room. Although individually ventilated cages have a much higher air change rate (i.e. 30-70 air changes per hour) than the room, the air supply for ventilated cages is often drawn from the room. In these cases, the animals are susceptible to the air quality conditions of the room. Even if the air entering the cages is high efficiency particulate air (HEPA) filtered, there is no protection for the animals against the organic and inorganic gases present in the room air, or from the heat load created from equipment and people working in the room.

Individually ventilated cages connected directly to the outside do exist; however, unless they are completely sealed (which is rare) there is still some air exchange with the room at the top of the individually ventilated cages, which is used to maintain pressure differentials.

9. Does the addendum apply to situations where animals are taken from animal facilities to labs for procedures, surgery, imaging, etc. or where animals are housed in an investigator's lab?

The intent of the addendum is to ensure that the health and safety of the animals and personnel are protected at all times. The CCAC expects institutions to adhere to the principle of the addendum, with the critical requirement being that clean air is available to all animals and personnel at all times, with minimal or no potential for cross-contamination; however, for short-term holding and for areas external to the laboratory animal facility where procedures are carried out, it is recognized that the air changes may not meet the standards described in the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003) and addendum. Animals may only be held in these conditions for up to 12 hours, provided that their health and safety are not affected. It is also important to recognize that there may be some impact on research results if animals are moved from a controlled environment into an uncontrolled one. If there is any question about the negative impact of such a move, it should not be carried out.

10. Do the same air quality standards apply to institutions that hold animals for teaching purposes?

The intent of the addendum is to ensure that the health and safety of the animals and personnel are protected at all times. However, for small teaching programs where the animals are held for a maximum period of 12 hours, it is recognized that the principle stated in the addendum may be difficult to achieve. In these cases, the air changes may be lower than the required 15-20 per hour, provided that the health and safety of the animals are not affected. This does not apply to institutions where only some of the animals held in the general animal facility are included on teaching protocols.

11. Does the addendum apply to aquatic rooms?

The conditions in aquatic rooms differ significantly from rooms that house terrestrial animals, and therefore the air quality of aquatic rooms is addressed in separate guidelines for fish and other aquatic species.

12. Are there requirements for air quality within cages?

The limits for each of the components (ammonia, carbon dioxide, particulates, and total volatile organic compounds) within enclosures depend on the species. Therefore, these will be described in each of the guidelines on particular types of animals based on the available evidence.

13. When air quality monitoring is required, how long should records be retained?

Records for air quality monitoring should be retained for the same period as required for other records that can have an impact on the animals, personnel or research. Section 12.1, "Access to Records and Record Retention" in the *CCAC guidelines: Husbandry of animals in science* (CCAC, 2017) states "Health records and records for food, water and bedding should be retained for a period of time suited to the type of research and institutional requirements; a minimum of one year is required, or as long as necessary to meet the requirements of the government, relevant professional associations and the research." In keeping with this, air quality records should be retained for as long as necessary to meet the needs of the research and other requirements, with a one year minimum. However, retaining records for up to five years can be useful for gaining an understanding of the overall system performance.

14. The *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003) includes Appendix C excerpts from the CCAC *Guide to the Care and Use of Experimental Animals* (vol. 1, 2nd ed., 1993) listing recommendations for air changes per hour for different types of animals. Do these still apply?

Heating, ventilation, and air conditioning: Addendum to the CCAC guidelines on laboratory animal facilities (CCAC, 2019) applies to all types of animals within a laboratory setting and supersedes the recommendations currently listed in Appendix C of the CCAC guidelines on laboratory animal facilities. The *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003) is currently undergoing review, and Appendix C will be removed once the document is updated on the CCAC website. Where recommendations related to air quality for different types of animals are necessary, these will be included within the individual animal-type guidelines, as they are developed.