Assessment of Ventilated Rodent Cage Air Exchange Using an Optical Detection Method

BACKGROUND/PROBLEM

Rodents housed for biomedical research require isolation from neighboring cages and lab personnel in order to reduce environmental variables on experiments. Researchers often prefer individually filtered housing systems over static or non-ventilated cages because the rodents conditions are more controlled. For instance the air exchange into the cages is not a function of the building's HVAC system or the stacking density of the cages. In addition, Individual Vented Cages (IVC) extend the cage change interval due to the superior cage air exchange that expels gas buildup. IVC's are also safer for rodents and lab personnel because the filtration is a biological barrier.

One negative consequence associated with IVC's are the resultant air drafts in the cage that the rodents experience. While it is known that humans can feel air drafts of 2 m/s [394 fpm], the threshold for rodents is unknown. Some researchers believe that air drafts cause stress to the rodents and negatively affect their skin. The objective of this paper is to discuss the development of an IVC system that effectively operates with less air drafts to the rodents. Mechanical and in vivo tests were also developed to validate the systems benefits to rodents and researchers.

AIR FLOW THEORY

Before the air drafts can be arbitrarily reduced careful consideration must be given to how the air moves through the cage. Individually vented rodent cage systems reduce the build-up of gases and particulate by flowing filtered air into and out of the cage. Two distinct modes of cage air exchange are possible; air mixing and air purging. Both models assume an air intake and exhaust in the cage with identical flow rates as governed by conservation of mass.

The *air-mixing model* assumes that the incoming air mixes with the existing contaminated air perfectly. This assumption means that anywhere in the cage the concentration of contaminates is uniform. The *air purging model* does not assume that the contaminate concentration is uniform. Instead, the air-purging model assumes that a curtain of air sweeps the contaminated air toward the exhaust like a piston. For the air purging model to be most effective the air intake and exhaust should be on opposite sides of the cage. The time required to remove all the cage contaminates is simply related to the incoming flow rate and the volume of the cage by the following relation:

$$z = z_i - \frac{w}{V} t$$

Where z is the concentration of contaminate, z_i is the initial concentration, V is the cage air volume in [ft^3], w is the cage flow in [CFM], and t is time in [min].

The evacuation rate can be described as w/V in [min^{-1}]. The industry often uses the term ACH (air exchanges per hour), defined as:

$$ACH = \frac{60w}{V}$$

Therefore, the evacuation rate for the purging model can be expressed as:

$$R_{purge} = \frac{ACH}{60} = \frac{w}{V}$$

The air purging method is the best case performance target that any ventilated cage can attempt to achieve. However, it is extremely difficult to flow a curtain of air from a small intake port all the way across the cage to the exhaust vent.

The air mixing model is more typical in existing IVC systems where the intake air flows into the cage in a swirling fashion. The resulting turbulence in the cage mixes contaminates with fresh air. Assuming perfect mixing (isotropic concentration), then the following differential equation can be written governed by conservation of mass:

$$g - wz = V \frac{dz}{dt}$$

Where g is the rate of contaminant generation, z is the concentration of contaminate, dz/dt is the time rate of change of concentration.

The solution to the above equation is:

$$z = \frac{g}{w}\left(1 - e^{-\frac{w}{V}t}\right) + z_i e^{-\frac{w}{V}t}$$

Where z_i is the initial concentration at time t=0

A comparison to the purging model can be made by assuming that $g = 0$. The time required to remove 98% of contaminates is defined as four time constants. This can be inferred from the graph and equation in Figure 1.

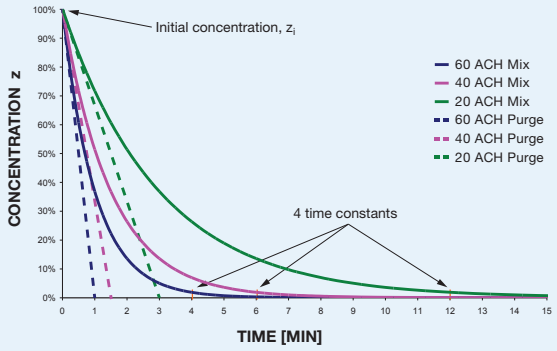

FIGURE 1. CONTAMINATE CONCENTRATION VS. TIME

$$\frac{z}{z_i} = e^{-\frac{w}{V}t}$$

$$\tau_{mixing} = \frac{V}{w}$$

The mixing time constant is equal to the inverse of the purging rate as seen in the above equations, however the mixing model requires 4-5 time constants to remove most of the contaminates. Remember the purging model removed all contaminates in one V/w [min]. Five time constants will remove 99% of contaminates, but a very large time would be required to remove all contaminates due to the exponential behavior of mixing theory.

The *mixing method* requires at least four times more time than the purging method to reduce cage contaminates assuming perfect mixing. In practice, perfect mixing cannot be achieved because some areas of the cage have very poor airflow and stagnation and/or stratification occurs. Theory sets the maximum performance that can be obtained based on the purging and mixing models. In practice a complex combination of both methods exist.

Complex numerical analysis and 3D simulations can be performed to study air flow paths and local particle concentrations. While these numerical methods are useful for complex geometries and high-speed air flows, they are probably overkill for an effective rodent cage design. Software and analysis time may cost over $20,000 per design iteration. Basic understanding of the theoretical best performance of both methods and the assumptions required to achieve the most effective cage flow is typically enough to design an optimized system.

TEST METHODS

A novel optical measurement technique was developed to quantify the effectiveness of several ventilated cages available on the market. This optical technique allows an engineer to quantify the time required to remove at least 98% of cage contaminates at various positions in the cage.

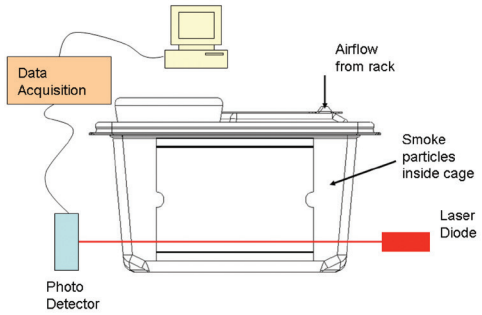

FIGURE 2. OPTICAL MEASUREMENT PROCESS

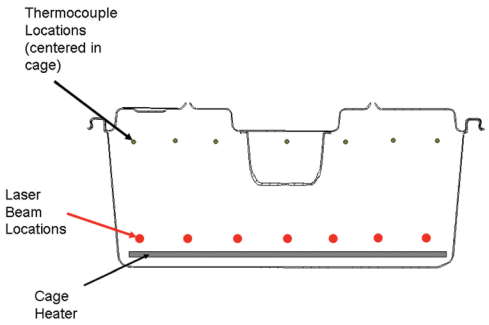

FIGURE 3. OPTICAL MEASUREMENT TECHNIQUE

Smoke emitted from incense sticks was used as the source of contaminates. (Approximately 30 sec burn time) Smoke particle sizes emitted from incense range from .05 to 10 micron in size. For comparison airborne bacteria are typically about .5 micron in size.

Once the cage was visibly filled with smoke the incense stick was extinguished and removed from the cage. One to two minutes without forced cage airflow is required to allow the smoke particles to cool and reach concentration equilibrium. Next the laser, photo detector and data logger are turned on. Laser light intensity is recorded every .5 seconds for a period of at least 10 minutes. As the smoke is evacuated by the IVC air flow system, the scattering of light is reduced and the photo detector measures a larger signal. This signal is then normalized and subtracted by one to yield the smoke concentration versus time plots shown in the following section.

The initial amount of smoke is not critical because the experiment is only interested in the characteristic time to

evacuate the cage. This can be seen from the equation below. At time t=0, $z = z_i$, therefore no matter how much smoke is present initially the left side of the equation is always one. As time increase concentration z exponentially diminishes to zero at a rate only dependent on the cage flow rate and the volume of the cage.

$$\frac{z}{z_i} = e^{-\frac{W}{V}t}$$

The laser and photodetector measure the average concentration across the width of the cage through a beam size of 2 mm. Four or more locations across the depth of the cage were measured to study local concentration effects. All measurements were taken 1 inch from the bottom of the cage at rodent level.

A heater and array of thermocouples were used to simulate the metabolic heat release of the animals. The purpose of this test was to determine if the heated air emanating from the animals caused an increase in air evacuation performance. The heater was set to 4 Watts to simulate five active mice. Temperatures were recorded along the center of the cage and as illustrated in Figure 3. Smoke concentration measurements were performed with and without the heater powered on to determine if heat rise contributed to cage evacuation performance. Buoyancy or chimney effects typically are negligible in forced ventilated applications, especially when the heat release is low. However, in static cages buoyancy effects are certainly important.

Gas detectors are sometimes used to measure concentrations at various points within the cage. One advantage of an optical measurement versus a gas detection method is the smoke particles path can be visualized using a laser-generated plane of light. This allows an engineer to quickly determine if the isotropic smoke distribution assumption required in the mathematical mixing model is valid. Gas detectors are well suited for steady-state experiments because the time constant of the gas detector is typically longer than the time constant of the system.

TEST RESULTS / SOLUTION

Using the laser-generated plane of light, internal cage flow can be visualized and used to quickly determine whether the mixing model or purging model is dominant. The first step is to fill the cage with smoke and allow the smoke to reach its thermal and concentration equilibrium. This equilibrium condition is satisfied when the smoke particles are stationary or very low velocity. The next step is to turn on the cage airflow and witness the smoke particles path. If the purging model is dominant the particles generally travel in one direction towards the exhaust. Areas of high and low velocity can easily be visualized. If the mixing model is dominant then the particles appear to go in circles with no particular direction. In some cases stagnant zones can be seen near the cage corners. As the smoke eventually clears, these cage corners are the last to clear due to the poor flow in these areas. When some areas of the cage, such as the cage corners, evacuate slower then other areas the assumption of isotropic concentration used in the mixing model is not valid. This leads to performance reductions from the theoretical best according to the mixing model. This can be seen in the smoke concentration results below.

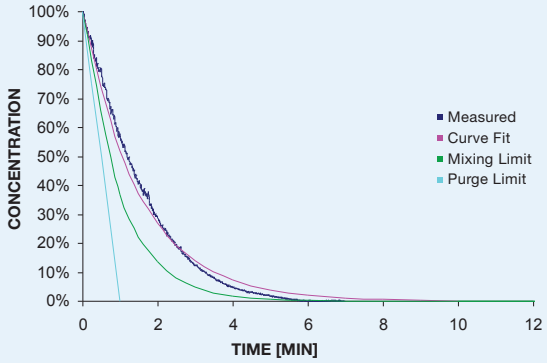

FIGURE 4. COMPETITOR A SYSTEM PERFORMANCE AT 60 ACH
The green line is the mathematical limit for the mixing method. The light blue line is the mathematical limit of the purging method. The pink line is a curve fit of the measured data in blue.

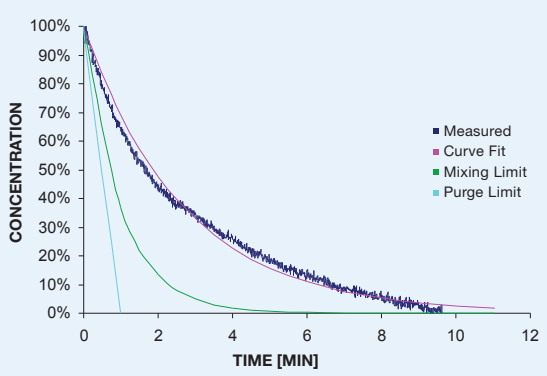

FIGURE 5. COMPETITOR B SYSTEM PERFORMANCE AT 60 ACH

The Competitor A system behaves closer to the mathematical limit of mixing, but still has some areas that are stagnate or exhibit poor mixing. Since the entire top of the Competitor A cage is a filtered exhaust, there is no deliberate airflow path to the front of the cage. High air velocity forces some mixing in the front of the cage, but this technique is not optimized and may cause unwanted stress to the animals.

One factor that is difficult to quantify in the Competitor B system is how much of the incoming air actually fills the cage. Competitor B relies on a connection that is located on the top rear of their cage, which seems to primarily affect the air at the ceiling of the cage. Competitor A also uses a direct connection into the rear of the cage.

A system can be designed to more closely follow the purging model. As mentioned earlier the intake and exhaust should be on opposite ends of the cage.

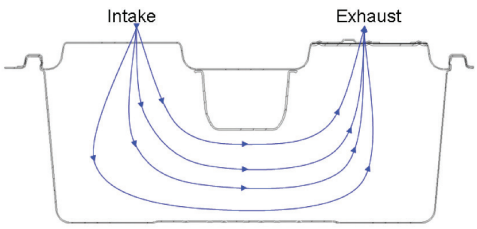

FIGURE 6. OPPOSITE SIDE INTAKE AND EXHAUST SYSTEM

The food tray located in the center of the cage separates the cage into two compartments; intake and exhaust. All the air entering the intake compartment must flow to the exhaust compartment via the reduced area underneath the food tray. This technique creates a bulk flow of particles and gases underneath the food tray in a front to rear manner. The diagram above does not show mixing, but it does occur in each of the compartments. As air enters the intake compartment it diverges and swirls into the front wall of the cage. From there air is pulled into the exhaust compartment and evenly pulled into the exhaust filter area. Results of this improved design are below:

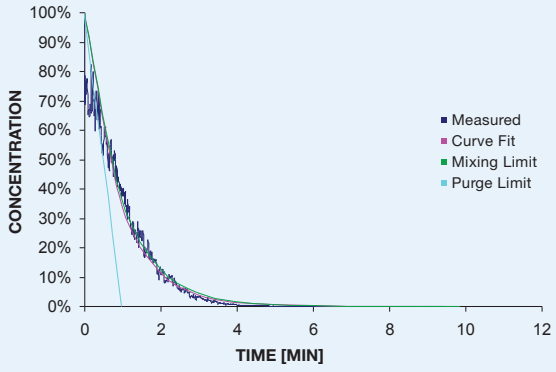

FIGURE 7. INNOVIVE SYSTEM PERFORMANCE AT 60 ACH

As expected, the performance of the Innovive system beats the mathematical mixing limit because it more closely approaches the purging model. The light blue line represents the purging limit.

When the cage heater was turned on to 4W no measurable difference was apparent in the smoke evacuation time constant.

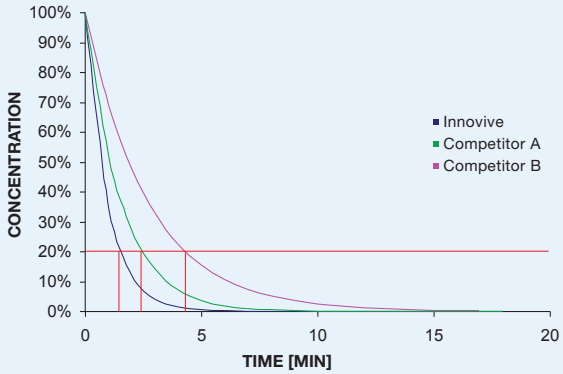

FIGURE 8. SYSTEM PERFORMANCE COMPARISON AT 60 ACH

Particulate concentration	Time - Innovive	Time - Competitor A	Innovive advantage over Competitor A	Time - Competitor B	Innovive advantage over Competitor A
20%	1.47	2.47	168.03%	4.48	304.76%
10%	1.57	3.15	200.64%	6.41	408.28%

FIGURE 9. RESULTS TABLE

As can easily be seen in the above graphs and table, when all three systems are evaluated using this assay, the Innovive transversal airflow system achieves substantially higher airflow efficiency than the traditional systems at a selected airflow rate of 60ACH.

CONCLUSION

Two simple mathematical theories, purging and mixing, are crucial to understanding intra-cage airflow and how to design an improved rodent housing system. A laser-generated plane of light facilitates the understanding of particulate flow within various cage locations. The same plane of light can also be used to quantify the evacuation time constants with the addition of a photodetector and data acquisition system. Knowledge of the performance and limitations of existing systems yielded an improved design that resulted in improved performance with less airflow.