

# Ammonia Levels

## in Animal Care Systems Cages

Animal Care Systems' revolutionary approach employs a unique one-pass low-velocity linear air technology without the introduction of air turbulence and high air velocity, while ensuring maximum cage air quality at the lowest airflow. Cage ammonia and other environmental parameters were measured in all three Animal Care Systems rack types (Optimice®, Optirat® Plus and Optirat® GenII) at standard recommended airflow rates, to determine values for routine monitoring of cage ammonia between cage changes over time.

### Materials and Methods

Ammonia data shown was taken at day 14 for mice and day 7 for rats after initial cage change. Several different categories of bedding types were tested to represent the most commonly used substrates (Table 1). Equal volumes of bedding were used per cage type except Alpha Pads [fixed stamped size] for mice.

**Ammonia measurements.** Ammonia was measured with either a BW Honeywell GasAlert ammonia sensor (lowest detection threshold > 3 ppm; Honeywell Analytics, Lincolnshire, IL) or a Vivaristat ammonia sensor (lowest detection threshold 0.5 ppm; InPoint Systems, Inc., Los Altos, CA) and an ammonia strip sensor (low 0-1 ppm, medium 1-25 ppm, high 25-50 ppm, danger 50+ ppm; Pacific Sentry LLC Small Animal Ammonia Sensor, Redmond, WA), all placed directly inside the cage for the duration of the tests.

**Rack and cage airflow measurements.** Rack airflow was monitored via the Animal Care Systems rack status monitor and cage level airflow with a handheld anemometer (Fieldpiece STA2 In-duct hot wire anemometer, Fieldpiece Instruments, Inc., Orange, CA).

**Animals and housing.** Animals were sourced from Charles River Laboratories, USA. Rats were either Sprague Dawley, Wistar or Long Evans females, up to 15 months of age. A combination of both CD1(ICR) male and female mice, ages 16 weeks to 15 months of age, were used. Rat cages had a shelter (Rat Retreat™, BioServ, Flemington, NJ) and mice were given one Bed-r'Nest® puck (all mice; Andersons Lab Bedding, Maumee, OH) and one DomicISLE shelter (females only; Animal Care Systems, Centennial, CO).

### Results

Data were collected between May 2017 and April 2019. Ammonia levels in mice and both types of rat cages remained at very low to undetectable levels for the duration of 14 days in mice and 7 days in rats. NH<sub>3</sub> strip color changes ranged from low to medium (low 0-1 ppm, medium 1-25 ppm); otherwise all other ammonia levels remained between 0-1 ppm. Room humidity levels ranged between 9.6 and 66% (Table 2).

Table 1. Types and sources of bedding used per species

Bedding Name	Bedding Material	Source	Species
Teklad sani-chips	Aspen chip	Envigo, Somerset, NJ	Mice/Rats
Teklad corn cob	Corn cob	Envigo, Somerset, NJ	Mice/Rats
Clean Comfort™	Paper	Kaytee Products, Inc., Clinton, WI	Rats
Alpha Pads	100% cotton	Shepherd Specialty Papers, Waterton, TN	Mice
Cell Sorb Plus	Recycled newspaper pellets	Fangman Specialties, Inc., Cincinnati, OH	Mice/Rats

**Table 2.** Summary of ammonia levels in all cage types, number of animals, number of cages, and room temperature and humidity

Cage Type	No. animals per cage	Average weight (g)	Average NH <sub>3</sub> (ppm)	Average room temp (°C)	Average room humidity (%)	Total no. cages	Bedding Material			
							Aspen	Corn cob	Cotton/paper	Pellets
Optimice	4.5	51	0.6 (day 14)	21.5	15.8	n=15	n=5	n=3	n=4	n=3
Optirat Plus	3	386	0.5 (day 7)	21.9	15.8	n=19	n=6	n=5	n=4	n=4
Optirat GenII	3	339	0.2 (day 7)	22.1	29.9	n=17	n=5	n=4	n=4	n=4

## Discussion

Currently there are no upper level ammonia exposure guidelines for mice; for humans, the 8-h time-weighted average exposure limit is 25 ppm or 50 ppm maximal exposure<sup>1</sup>. Numerous studies have described detrimental health effects of increased ammonia levels on laboratory rodents<sup>2,3</sup>. Most of these effects are subclinical but often include histologic and immunologic changes<sup>4</sup> that may impact animal welfare and variably alter research results. Intracage ammonia levels are influenced by several factors: sex, strain, age of animals housed; number of animals in the cage; temperature; humidity; number of hourly air changes; and amount and type of bedding substrate used. In the current conditions tested, and over 2 year seasonal changes, ammonia levels in mice and both types of rat cages remained at very low to undetectable levels for the duration of 14 days in mice and 7 days in rats, when cages were stocked at or near capacity for non-breeding animals per allowable floor space according to the “Guide for the Care and Use of Laboratory Animals.”<sup>5</sup> These values are also consistent with other available published data using Optimice cages<sup>6,7</sup>. Other published studies using the same strain of mice have shown much higher (in some instances greater than 150 ppm) levels of ammonia in other individually ventilated cages (IVC) cages<sup>8,9</sup>. The majority of the data in this study were collected at naturally low room humidity levels in Colorado (average range 15.8 – 29.9%; room temperature 21.5 – 22.1°C), as humidity was not controllable at the room level. Since ammonia levels are closely influenced by humidity levels, studies are being undertaken to look at ammonia levels at higher room/cage humidity levels, as might be representative in other areas of the country.

## References

1. **American Conference of Governmental Industrial Hygienists (ACGIH).** 2007. Threshold limit values (TLV) and biological exposure indices (BEI). Cincinnati (OH): ACGIH.
2. **Broderson JR, Lindsey JR, Crawford JE.** 1976. The role of environmental ammonia in respiratory mycoplasmosis of rats. *Am J Pathol* **85**:115–130.
3. **Vogelweid CM, Zapien KA, Honigford MJ, Li L, Li H, Marshall H.** 2011. Effects of a 28-day cage-change interval on intracage ammonia levels, nasal histology, and perceived welfare of CD1 mice. *J Am Assoc Lab Anim Sci* **50**:868–878.
4. **Sanford AN, Clark SE, Talham G, Sidelsky MG, Coffin SE.** 2002. Influence of bedding type on mucosal immune responses. *Comp Med* **52**:429–432.
5. **Institute for Laboratory Animal Research.** Guide for the Care and Use of Laboratory Animals 8th ed., 2011.
6. **Carbone E, Kass P, Evans K.** Feasibility of Using Rice Hulls as Bedding for Laboratory Mice. 2016. *JAALAS* **55**(3):268–276.5.
7. **Kostomitsopoulos N, Alexakos P, Eleni K, Doulou A, Paschidis K, et al.** 2012. The effects of different types of individually ventilated caging systems on growing male mice. *Lab Animal; New York*, **41**(7):192-197.
8. **Levy, DRM, Flores R, Garcia GE, Craig SL, Jensen VB.** 2018. Effects of Extruded Compared with Pelleted Diets on Laboratory Mice Housed in Individually Ventilated Cages and the Cage Environment. *JAALAS* **57**(6):686-694.
9. **Silverman J, Bays DW, Cooper SF, Baker SP.** 2008. Ammonia and Carbon Dioxide Concentrations in Disposable and Reusable Ventilated Mouse Cages. *JAALAS* **47**(2):57-62.