Ammonia Measurement in the IVC Microenvironment

Robert B Morrow and Rhonda J Wiler*

In this review, we describe the methods and technology used to measure intracage ammonia levels; the data were derived from 38 articles published since 1970. Ammonia concentration is commonly used as a surrogate for assessing environmental quality inside rodent cages. Data generated from this group of publications have been used to support new husbandry practices, determine the effect of ammonia on health, and establish the effectiveness of caging systems. Consequently, the data generated from these studies have a direct effect on animal welfare and therefore should demonstrate a high level of reproducibility. Obtaining reproducible results requires a critical understanding of the methodology and the technology used to collect ammonia concentration data. This review highlights the need for consistent methodology for measuring ammonia that considers the technology used to capture the data as well as the environmental parameters that affect ammonia concentrations, to facilitate the design of future studies.

DOI: 10.30802/AALAS-JAALAS-18-000006

The *Guide for the Care and Use of Laboratory Animals*²¹ provides guidelines for the maintenance of a wide range of animals. Obtaining deviations from these guidelines requires data to support that the deviation does not diminish the welfare of the animals.³³ The introduction of IVC has revolutionized the way laboratory rodents are housed. The many benefits to using IVC include increased sanitation intervals. The *Guide*²¹ stipulates that an increased sanitation interval may be justified when the microenvironment is not compromised. The *Guide*²¹ also mentions ammonia as a microenvironmental pollutant. Therefore, many studies have measured intracage ammonia concentrations as an indicator of cage environmental health. In this review, we critically compare the technology and methodology used to measure ammonia within the microenvironment of mouse cages. We identified 38 publications that evaluated a variety of environmental parameters for their effects on the generation of ammonia, including cage bedding, husbandry methods, housing density, health, environmental parameters, ventilation flow rates, and ventilation design (Figure 1). Notably, we do not evaluate the validity of using ammonia levels as an indicator of intracage environmental quality.

Ammonia sampling technology. The availability of toxic gas detection technologies has evolved since the first application of a portable gas analyzer using colorimetric technology.¹⁰ The 3 types of toxic gas detection sensor technologies that have commonly been used to measure ammonia concentration in IVC are NO_x gas analysis, photoionization detection (PID), and colorimetric analysis (Figure 2). Electrochemical sensors are a fourth technology that is capable of measuring ammonia, but this technology has not been used to measure ammonia in IVC. Toxic gas devices are configured with sensors designed to meet the specific needs of the end user, including device portability, response time, cross-sensitivity to other toxic gases, and capital cost.¹⁴ Capital cost and limited device portability are

often barriers to more widespread use of highly accurate ammonia sensing technology in the vivarium setting. In contrast, colorimetric analyzers are designed to be portable, convenient, and easy to use to enable rapid assessment of an environment containing an unknown toxic gas. This technology allows the user to identify what gas is present, its concentration range, whether the gas concentration poses a danger to humans, and what personal protective equipment is needed to safely enter the unknown environment.⁸

Colorimetric analyzers. Colorimetric analyzers are designed to be portable, convenient, and easy to use, so that an environment containing an unknown toxic gas can be assessed quickly. Prior to 2005, this type of device was the only readily available portable instrument for measuring ammonia concentration. Of the 38 studies that we reviewed, 23 (61%) measured ammonia concentration by using a toxic gas detection device based on colorimetric analysis (Figure 2). The continued use of such instruments is likely due to the desire to repeat the methodology described in published studies. In addition, these instruments are readily available from institutional environmental health and safety groups, have a relatively low cost per sample, and are simple to use.

Colorimetric analyzers have a resolution of 1 ppm and an accuracy of 15% to 20%.⁸ These devices are available in a manual pump using a single-use tube design as well as the Dräger measurement chip, which has 10 capillaries and thus is able to make 10 measurements. Both models are designed to measure momentary concentrations and capture a spot measurement of the target environment⁸ and have no capability for real-time data logging to generate descriptive statistics, making it difficult to compare data between samples or studies. This instrument is not designed to provide quantitative or highly accurate ammonia concentration readings, and their use for these purposes should be discouraged.

NO_x analyzers. NO_x gas analyzers are designed to measure specific toxic gases according to the specific 'fingerprint' characteristics of the target gas. NO_x gas analyzers have a sensor range of 0 to 10,000 ppm, resolution of ±1% of range or 0.2 ppm,

Received: 11 Jan 2018. Revision requested: 22 Feb 2018. Accepted: 18 Jun 2018.
Transgenic Technology Department, Genentech, South San Francisco, California
*Corresponding author. Email: rwiler@gene.com

	No. of articles (<i>n</i> = 38)	References
Bedding material	11	6, 9, 18–20, 23, 32, 38, 50, 51, 58
Environmental parameters	3	37, 54, 55
Housing density	3	5, 56, 57
Husbandry and health	11	1–3, 10, 12, 13, 31, 52, 60, 62, 63
Ventilation design	5	28–30, 47, 49
Ventilation rates	5	17, 24, 44–46

Figure 1. Primary topics in reviewed publications.

	No. of articles (<i>n</i> = 38)	References
Detection technology		
Passive sensor plus colorimetric tube	2	6, 30
NO _x analyzer plus colorimetric tube	4	12, 13, 45, 46
Photoionization detection plus colorimetric tube	2	50, 51
Colorimetric tube	18	1–3, 10, 17–20, 23, 24, 29, 31, 32, 38, 47, 52, 59, 60
Dräger chip measurement system	5	5, 9, 54, 55, 62
Multipoint NO _x analyzer	4	44, 56–58
Passive diffusion	2	49, 63
Not reported	1	30

Figure 2. Device technology used to measure ammonia levels.

a response time of approximately less than 15 s (t_5 – t_{95}), and limited cross-sensitivity to other gases.^{16,43} This review lists 6 studies^{44–46,56–58} using the NO_x gas analyzer. The limited use of this instrument is due to its capital cost and minimal portability.

Photoionization detector (PID). Monitoring devices using a PID are designed to be portable and capable of measuring a wide concentration range (0 to 1000 ppm) of volatile organic compounds, with a resolution of 0.1 ppm and t_{90} response time of 3 to 5 s (sensor saturation time), making it easy to measure dynamic environmental conditions. However, PID are nonspecific to individual toxic gases and have negative interactions from cross-sensitivity with other gases.^{26,41,43} This review identified only 2 studies that used a PID device to measure ammonia.^{50,51}

Electrochemical sensors. Electrochemical sensors are designed to measure specific toxic gases. These devices have an ammonia sensor range of 0 to 100 ppm, resolution of 1 ppm, response time of approximately 150 s,⁴⁰ and limited cross-sensitivity to other gases.⁴³ Ammonia electrochemical sensors are limited by a fixed life expectancy of 1 to 3 y and a relatively low maximal over-range rating.⁴⁰ The maximal over-range rating corresponds to the highest concentration that an ammonia sensor can detect, typically within 200 to 300 ppm for ammonia electrochemical sensors; 300 ppm is the ammonia level considered to be immediately dangerous to human life or health.^{34,40} None of the publications reviewed used electrochemical sensors.

	No. of articles (<i>n</i> = 38)	Publication
Reported information		
Activity	4	6, 11–13
Bedding material	11	6, 9, 18–20, 22, 23, 32, 38, 50, 51
Ventilation rates	7	17, 24, 25, 44–46, 61
Ventilation design	6	28–30, 47–49
Animal density	8	4, 5, 10, 23, 27, 31, 56, 57
Sample tube length	12	5, 23, 24, 44–46, 50, 51, 54–57
Sample tube material	3	49, 54, 55
Sample duration	9	5, 6, 29, 47, 49–51, 54, 55
Sample location	31	1, 3, 5, 6, 9, 10, 12, 13, 18–20, 23, 28, 29, 31, 38, 44–47, 49–51, 54–58, 60, 62, 63
Pump rate	13	29, 31, 44–47, 50, 51, 54–57, 60
Relative humidity	37	1–3, 5, 6, 9, 12, 13, 17–20, 22–25, 28–30, 32, 37, 38, 44–47, 49–51, 54–58, 60, 61, 63
Temperature	31	1, 5, 6, 9, 10, 12, 17–20, 23, 24, 28–30, 37, 38, 44–47, 49, 51, 54–58, 60, 63
Macroenvironmental parameters	25	2, 3, 5, 6, 9, 10, 17–20, 23, 24, 28–30, 32, 37, 38, 44–47, 49, 51, 60
Microenvironmental parameters	37	2, 3, 5, 6, 9, 10, 12, 13, 17–20, 23, 24, 28–32, 37, 38, 44–47, 49–52, 54–58, 60, 62, 63

Figure 3. Intracage measurement parameters.

	No. of articles (<i>n</i> = 38)	Publication
Front bulkhead (external)	10	5, 38, 44–46, 54–58
Front bulkhead (internal)	10	2, 6, 9, 19, 20, 23, 31, 49–51
Exhaust port or plenum	5	12, 13, 30, 32, 47
Cage lid access (internal)	9	3, 9, 17, 18, 24, 29, 60, 62, 63
Not reported	4	1, 10, 52, 59

Figure 4. Reported sample locations. *, *n* = 38 because one publication (reference 9) measured ventilated and static cages by using different access point.

Combined measurement methods. Eight studies used 2 measurement methods, combining a colorimetric analyzer in conjunction with either a passive sensor, NO_x gas analyzer, or another technology. These devices did not provide simultaneous measurements (Figure 2). Toxic gas measurement technology has evolved rapidly through miniaturization to provide portable devices that measure gas concentrations in the ppm range with built-in user-defined functionality. PID and electrochemical sensors are 2 toxic gas sensor technologies that are often combined into multigas detectors and thus provide a simultaneous measurement of the environment by 2 different sensor types. The advantages of this technology are increased accuracy, sensitivity, and range. None of the studies reviewed reported using a multigas detector device.

Environmental parameters and sampling methodology. Sampling methodology influences ammonia measurements. Measurement parameters including instrument calibration, the material used to gather sample gas, spatial location, sample duration, sample pump rate, data analysis, ventilation

Measurement technology	Response time (t_r)	Range (NH ₃)	Precision and resolution (NH ₃)	Data logging	Application and selectivity	Calibration	Expense
Colorimetric chemical sensor*	40–120 s	Cartridge-specific 0.2–2000 ppm	1 ppm ±15% to 20%	Not available	Portable platform with selectivity limited to target toxic gas	Self-calibrating	Low
Colorimetric chip measurement	40–120 s	Cartridge-specific 0.2–2000 ppm	1 ppm ±15% to 20%	Not available	Portable platform with selectivity limited to target toxic gas	Self-calibrating	Moderate
Photoionization detector	150 s	0–1000 ppm volatile organic compounds	1 ppm	User-defined	Portable platform with selectivity limited to volatile organic compounds	Calibration gas by user	Low to moderate (configuration-dependent)
Diffusion sensor	150 s	0–50 ppm 0–100 ppm	1 ppm	User-defined	Versatile in portable and fixed platforms with specific sensor selectivity	Calibration gas by user	Low to moderate (configuration-dependent)
Electrochemical sensor	150 s	0–50 ppm 0–100 ppm	1 ppm	User-defined	Versatile in portable and fixed platforms with specific sensor selectivity	Calibration gas by user	Low to moderate (configuration-dependent)
Photoacoustic gas monitor	<30 s	Wide dynamic range of toxic gases	1 ppb	User-defined	Versatile in fixed platforms with specific selectivity	Specialist required	High
NO _x analyzer or monitor	30 s	Wide dynamic range of toxic gases	0.1 ppm (<2%)	User-defined	Versatile in fixed platforms with specific selectivity	Specialist required	High
Ultraviolet-visible	30 s	Wide dynamic range of toxic gases	1 ppb	User-defined	Versatile in fixed platforms with specific selectivity	Specialist required	High
Infrared spectrometer	60–300 s	Wide dynamic range of toxic gases: near (0.75–3.00 μ m), mid (3.00–6.00 μ m) and far (6.00–15.00 μ m)	3–4.00 $\times 10^{-3}$ nm	User-defined	Versatile in fixed platforms with specific selectivity	Specialist required	High

Figure 5. A comparison of toxic gas technologies. The top end of the range likely demonstrates increased variability due to erratic signal at saturation, which then becomes unreliable. *, The basis of direct reading of a colorimetric chemical sensor is the chemical reaction of the measured substance with the chemicals of the filling preparation. The substance conversion is proportional to the mass of the reacting gas, as indicated by the substance conversion as a length-of-stain indication with a response time dependent on target concentration.

design, temperature, relative humidity, animal activity, and cage ventilation rate all can contribute to sample variability (Figure 3).^{7,24,35,44–46,48,50,51,64}

Reliability of an ammonia sampling device depends on ensuring that the gas sensor is providing accurate measurement of the target gas concentration. Due to the stickiness of ammonia, this assurance is even more crucial, because the sensor becomes saturated and loses sensitivity over time. Manufacturer guidelines typically suggest calibration either weekly or according to the number of hours used, although device calibration may

vary depending on use.^{8,15,39–42} In addition, to ensure that the device sensors accurately measure the target environment, a daily calibration check using a known concentration of the target gas—commonly called a ‘bump test’—needs to be completed. A daily bump test demonstrates that the sensor is within the manufacturer’s prescribed tolerance; if it is not, then sensor calibration needs to be performed, thus ensuring the accuracy and reproducibility of those measurements. Two publications^{50,51} used a PID device that required frequent calibration, and 5 publications^{44,45,56–58} used a NO_x analyzer that

required maintenance calibration. Colorimetric analyzers do not require calibration. No articles in the review reported the frequency of sensor calibration or bump testing.

The pump rate and reactivity of the materials are important to ensure that an adequate air sample reaches the ammonia sensor. Ammonia readily absorbs onto most surfaces and can subsequently desorb if ammonia levels in sample air streams decrease.⁵⁹ Thus contact with absorptive surfaces can cause both positive and negative artifacts. The 'stickiness' and reactivity of ammonia require a high pump rate (that is, 300 to 1000 mL/min).^{33,59} Using nonreactive and nonabsorbent materials such as Teflon PFA or stainless steel further decreases the surface adhesion of ammonia and increases the accuracy of the reading.^{22,40,53,64} The pump rate was reported in 15 of the 38 studies in this review. Just 3 studies reported the sample tube material used.

Airflow dynamics and the activity of the cage occupants affect the distribution of ammonia within IVC.^{36,37} The collection point of an air sample, air flow dynamics, cage population, activity levels, temperature, and humidity levels are sources of variability in assessing ammonia concentrations and therefore should be described. This review revealed that air sampling has been conducted more frequently through a port at the front of the cage or outside the cage by using a vacuum pump than internally accessed through the cage wall or through the cage lid (Figure 4).

Although some of these variables are reported routinely, not all of them are. This inconsistency reduces the reproducibility of the results. Most of the studies reviewed reported the temperature and humidity or described the sample location as within the breathing zone. Fewer reports described the sample location as within the cage. Fewer still reported other parameters, such as the sample tube material, length, pump rate, and occupant activity—all of which influence the accuracy of intracage ammonia measures.

Discussion and Conclusion

In the *Guide*, ammonia is described as a microenvironmental pollutant. Therefore, quantification of ammonia concentration within the cage is often used as an indicator of microenvironmental health. Currently, there is no standardized methodology for assessing ammonia concentrations inside the cage. This review revealed marked variation across studies in the technology and sampling methodology used, thus confounding comparison. As a result, ammonia concentration data in these studies may not be reproducible at the study location or comparable across institutions.

The types of sensor used and their application are critical to obtaining reproducible ammonia concentration measurements. Data from one sensor technology cannot be directly compared with data from another sensor technology due to differences in accuracy, sensitivity, and analytical capabilities (Figure 5). One exception is a multigas instrument configured with a PID and electrochemical sensor, which simultaneously measures ammonia in an environment by using 2 sensor technologies. Even so, to yield reproducible results, these multigas devices require calibration and specific training in their use.

In addition to the type of sensor used, many other parameters introduce variation during sample acquisition—these also need to be considered and reported to enable comparison of results from one study to another. This variability is primarily due to ammonia chemistry in that ammonia is highly reactive and the amount generated depends on pH, temperature, and dissolved salts. In addition, ammonia readily adsorbs onto most surfaces

and is highly soluble in water. Ammonia is less dense than air; consequently airflow and animal activity introduce additional variation in ammonia concentration within the cage.

This review illustrates a need to develop reproducible experimental methodology for measuring ammonia levels within the IVC microenvironment, if ammonia levels are going to be used to indicate the quality of the environment within the cage. As laboratory animal scientists, one of our objectives is to support optimal animal care practices that ensure that animal welfare is not compromised. Many animal care practices have been based on historical use or professional opinion. In recent times, gaining a deviation from the *Guide* recommendations has required the development of performance-based standards.^{21,33} This process requires the generation of data, that are often published, to support that the deviation does not adversely affect animal welfare.

Taken together, results from these publications are often used to guide new norms or support regulatory change that can dramatically influence both animal welfare and research. Therefore, ensuring high-quality methodology is critical to generating reproducible and comparable data sets.

Acknowledgments

We thank those who have contributed to this effort: J Colin Cox, Sally Madden, and Erik Bierwagen for their detailed review of this manuscript and Chuck Murray (JAECO Fire and Safety) for his time and willingness to share his knowledge of measuring ammonia.

References

1. Broderston JR, Lindsey JR, Crawford JE. 1976. The role of environmental ammonia in respiratory mycoplasmosis of rats. *Am J Pathol* 85:115–130.
2. Burn CC, Peters A, Day MJ, Mason GJ. 2006. Long-term effects of cage-cleaning frequency and bedding type on laboratory rat health, welfare, and handleability: a cross-laboratory study. *Lab Anim* 40:353–370. <https://doi.org/10.1258/002367706778476460>.
3. Burn CC, Mason GJ. 2008. Effects of cage-cleaning frequency on laboratory rat reproduction, cannibalism, and welfare. *Appl Anim Behav Sci* 114:235–247. <https://doi.org/10.1016/j.applanim.2008.02.005>.
4. Davidson LP, Chedester AL, Cole MN. 2007. Effects of cage density on behavior in young adult mice. *Comp Med* 57:355–359.
5. DiVincenti L Jr, Moorman-White D, Bavlov N, Garner M, Wyatt J. 2012. Effects of housing density on nasal pathology of breeding mice housed in individually ventilated cages. *Lab Anim (NY)* 41:68–76. <https://doi.org/10.1038/labon0312-68>.
6. Domer DA, Erickson RL, Petty JM, Bergdall VK, Hickman-Davis JM. 2012. Processing and treatment of corncob bedding affects cage-change frequency for C57BL/6 mice. *J Am Assoc Lab Anim Sci* 51:162–169.
7. Dräger. [Internet]. 2008. Introduction to gas detection systems, ed. 1–36. [Cited 17 May 2016]. Available at: <https://www.draeger.com/Library/Content/infoplip-gas-detection-systems-9046421-en.pdf>.
8. Dräger. [Internet]. 2015. Drägersensor & portable instruments handbook ed. 280. [Cited 12 July 2016]. Available at: <https://www.draeger.com/Library/Content/sensors-ca-br-9046571-en.pdf>.
9. Ferrecchia CE, Jensen K, Van Andel R. 2014. Intracage ammonia levels in static and individually ventilated cages housing C57BL/6 mice on 4 bedding substrates. *J Am Assoc Lab Anim Sci* 53:146–151.
10. Gamble MR, Clough G. 1976. Ammonia build-up in animal boxes and its effect on rat tracheal epithelium. *Lab Anim* 10:93–104. <https://doi.org/10.1258/002367776781071477>.
11. Godbey T, Gray G, Jeffery D. 2011. Cage-change interval preference in mice. *Lab Anim (NY)* 40:225–230. <https://doi.org/10.1038/labon0711-225>.
12. Green AR, Wathes CM, Demmers TG, Clark JM, Xin H. 2006. Tolerance of atmospheric ammonia by laboratory mice. Conference proceeding. 2006 American Society of Agricultural and

- Biological Engineers (ASABE) Annual International Meeting, 9–12 July, Portland, Oregon. <https://doi.org/10.13031/2013.20904>.
13. **Green AR, Wathes CM, Demmers TG, Clark JM, Xin H.** 2008. Development and application of a novel environmental preference chamber for assessing responses of laboratory mice to atmospheric ammonia. *J Am Assoc Lab Anim Sci* **47**:49–56.
14. **Henderson B.** [Internet]. 2007. Choosing the best confined space gas detector ed. 9. [Cited 10 June 2016]. Available at: <https://ohsonline.com/Articles/2015/10/01/Choosing-the-Best-Confining-Space-Gas-Detector.aspx>
15. **Henderson RE.** [Internet]. 2008. Using electrochemical sensors for toxic gas measurement. [Cited 10 June 2016]. Available at: <https://www.thermofisher.com.au/Uploads/file/Electrochemical-Sensors-for-Toxic-Gas-Measurement.pdf>.
16. **Henderson RE.** [Internet]. 2013. Ap 1002: Understanding ammonia sensors and their applications 02/25/2013 ed. 6. [Cited 10 June 2016]. Available at: http://goodforgas.com/wpcontent/uploads/2013/12/AP1002_understanding_ammonia_sensors_and_their_applications_2_25_13.pdf
17. **Höglund AU, Renstrom A.** 2001. Evaluation of individually ventilated cage systems for laboratory rodents: cage environment and animal health aspects. *Lab Anim* **35**:51–57. <https://doi.org/10.1258/0023677011911372>.
18. **Horn MJ, Hudson SV, Bostrom LA, Cooper DM.** 2012. Effects of cage density, sanitation frequency, and bedding type on animal wellbeing and health and cage environment in mice and rats. *J Am Assoc Lab Anim Sci* **51**:781–788.
19. **Huerkamp MJ, Dillehay DL, Lehner ND.** 1994. Effect of intracage ventilation and automatic watering on outbred mouse reproductive performance and weanling growth. *Contemp Top Lab Anim Sci* **33**:58–62.
20. **Huerkamp MJ, Lehner ND.** 1994. Comparative effects of forced-air, individual cage ventilation or an absorbent bedding additive on mouse isolator cage microenvironment. *Contemp Top Lab Anim Sci* **33**:58–61.
21. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
22. **Kaliste E, Linnainmaa M, Meklin T, Torvinen E, Nevalainen A.** 2004. The bedding of laboratory animals as a source of airborne contaminants. *Lab Anim* **38**:25–37. <https://doi.org/10.1258/00236770460734362>.
23. **Koontz JM, Kumsher DM, Kelly R 3rd, Stallings JD.** 2016. Effect of 2 bedding materials on ammonia levels in individually ventilated cages. *J Am Assoc Lab Anim Sci* **55**:25–28.
24. **Lipman NS, Corning BF, Coiro MA Sr.** 1992. The effects of intracage ventilation on microenvironmental conditions in filter-top cages. *Lab Anim* **26**:206–210. <https://doi.org/10.1258/002367792780740503>.
25. **Lipman NS.** 1999. Isolator rodent caging systems (state of the art): a critical view. *Contemp Top Lab Anim Sci* **38**:9–17.
26. **Liu X, Cheng S, Liu H, Hu S, Zhang D, Ning H.** 2012. A survey on gas sensing technology. *Sensors (Basel)* **12**:9635–9665. <https://doi.org/10.3390/s120709635>.
27. **McGlone JJ, Anderson DL, Norman RL.** 2001. Floor space needs for laboratory mice: BALB/cJ males or females in solid-bottom cages with bedding. *Contemp Top Lab Anim Sci* **40**:21–25.
28. **Memarzadeh F.** 2000. Ventilation design in animal research facilities using static microisolators. *ASHRAE Trans* **106**:1–8.
29. **Memarzadeh F, Harrison PC, Riskowski GL, Henze T.** 2004. Comparison of environment and mice in static and mechanically ventilated isolator cages with different air velocities and ventilation designs. *Contemp Top Lab Anim Sci* **43**:14–20.
30. **Memarzadeh F, Manning A.** 2005. Control of ammonia production in animal research facilities through ventilation system design. *ASHRAE Trans* **111**:203–212.
31. **Mexas AM, Brice AK, Caro AC, Hillanbrand TS, Gaertner DJ.** 2015. Nasal histopathology and intracage ammonia levels in female groups and breeding mice housed in static isolation cages. *J Am Assoc Lab Anim Sci* **54**:478–486.
32. **Miyamoto T, Li Z, Kibushi T, Yamasaki N, Kasai N.** 2008. Use of soft hydrothermal processing to improve and recycle bedding for laboratory animals. *Lab Anim* **42**:442–452. <https://doi.org/10.1258/la.2007.007065>.
33. **National Academies of Sciences, Engineering, and Medicine.** 2015. Design, implementation, monitoring, and sharing of performance standards for laboratory animal use (a workshop summary). Washington (DC): National Academies Press.
34. **National Institute for Occupational Safety and Health.** [Internet]. 2005. NIOSH pocket guide to chemical hazards, 3rd ed. 454. [Cited 22 June 2016]. Available at: <http://www.cdc.gov/niosh/>.
35. **Ni JQ, Heber AJ.** 2008. Sampling and measurement of ammonia at animal facilities. *Adv Agronomy* **98**:201–269. [https://doi.org/10.1016/S0065-2113\(08\)00204-6](https://doi.org/10.1016/S0065-2113(08)00204-6).
36. **National Science and Technology Council (US), Air Quality Research Subcommittee.** [Internet]. 2000. Atmospheric ammonia: sources and fate. A review of ongoing federal research and future needs. [Cited 16 June 2016]. Available at: <https://digital.library.unt.edu/ark:/67531/metadc25995/>
37. **Pandy A.** [Internet]. 2005. Computational fluid dynamics study of ventilated cages for laboratory animals. [Cited 28 July 2016]. Available at: http://etd.fcla.edu/UF/UFE0010846/pandey_a.pdf
38. **Perkins SE, Lipman NS.** 1995. Characterization and quantification of microenvironmental contaminants in isolator cages with a variety of contact beddings. *Contemp Top Lab Anim Sci* **34**:93–98.
39. **RAE Systems.** [Internet]. 2001. Measuring ammonia (NH₃) with photoionization detectors (PIDs), AP-201 ed. 1-3. [Cited 3 August 2016]. Available at: https://www.raesystems.com/sites/default/files/content/resources/Application-Note-201_Measuring-Ammonia-%5BNH3%5D-With-Photoionization-Detectors_04-01.pdf
40. **RAE Systems.** [Internet]. 2001. Multirae IR multigas monitor: operation and maintenance manual, PGM-54. [Cited 3 August 2016]. Available at: <https://docplayer.net/21549638-Multirae-ir-multi-gas-monitor-pgm-54-operation-and-maintenance-manual-document-no-008-4028-rev-b.html>
41. **RAE Systems.** [Internet]. 2013. The PID handbook: theory and applications of direct-reading photoionization detectors (PIDs) 3 ed. 1-94. [Cited 3 August 2016]. Available at: https://www.raesystems.com/sites/default/files/content/resources/pid_handbook_1002-02.pdf
42. **RAE Systems.** [Internet]. 2014. Gas sequences for calibration and calibration check testing of the Multirae instrument family, TN-203. [Cited 4 August 2016]. Available at: https://www.raesystems.com/sites/default/files/content/resources/Technical-Note-203_Gas-sequences-for-calibration-and-calibration-check-testing-of-the-MultiRAE-instrument-family.pdf
43. **RAE Systems.** [Internet]. 2015. Sensor specifications and cross-sensitivities, TN-114 [Cited 4 August 2016]. Available at: <https://www.raesystems.com/customer-care/resource-center/tn-114-sensor-specifications-and-cross-sensitivities>
44. **Reeb-Whitaker CK, Paigen B, Beamer WG, Bronson RT, Churchill GA, Schweitzer IB, Myers DD.** 2001. The impact of reduced frequency of cage changes on the health of mice housed in ventilated cages. *Lab Anim* **35**:58–73. <https://doi.org/10.1258/0023677011911381>.
45. **Reeb CK, Jones RB, Bearg DW, Bedigian H, Paigen B.** 1997. Impact of room ventilation rates on mouse cage ventilation and microenvironment. *Contemp Top Lab Anim Sci* **36**:74–79.
46. **Reeb CK, Jones RB, Bearg D, Bedigian H, Myers D, Paigen B.** 1998. Microenvironment in ventilated animal cages with differing ventilation rates, mice populations, and frequency of bedding changes. *Contemp Top Lab Anim Sci* **37**:43–49.
47. **Riskowski GL, Harrison PC, Memarzadeh F.** 2006. Mass generation rates of ammonia, moisture, and heat production in mouse cages with 2 bedding types, 2 mouse strains, and 2 room relative humidities. *ASHRAE Trans* **112**:134–144.
48. **Riskowski GL, Memarzadeh F.** 2000. Investigation of static microisolators in wind tunnel tests and validation of CFD cage model. *ASHRAE Trans* **106**:1–10.
49. **Rivard GF, Neff DE, Cullen JF, Welch SW.** 2000. A novel vented microisolator container for caging animals: microenvironmental comfort in a closed-system filter cage. *Contemp Top Lab Anim Sci* **39**:22–27.

50. **Rosenbaum MD, VandeWoude S, Johnson TE.** 2009. Effects of cage-change frequency and bedding volume on mice and their microenvironment. *J Am Assoc Lab Anim Sci* **48**:763–773.
51. **Rosenbaum MD, VandeWoude S, Volckens J, Johnson T.** 2010. Disparities in ammonia, temperature, humidity, and airborne particulate matter between the micro- and macroenvironments of mice in individually ventilated caging. *J Am Assoc Lab Anim Sci* **49**:177–183.
52. **Schoeb TR, Davidson MK, Lindsey JR.** 1982. Intracage ammonia promotes growth of *Mycoplasma pulmonis* in the respiratory tract of rats. *Infect Immun* **38**:212–217.
53. **Schwab JJ, Li Y, Bae MS, Demerjian KL, Hou J, Zhou X, Jensen B, Pryor SC.** 2007. A laboratory intercomparison of real-time gaseous ammonia measurement methods. *Environ Sci Technol* **41**:8412–8419. <https://doi.org/10.1021/es070354r>.
54. **Silverman J, Bays DW, Cooper SF, Baker SP.** 2008. Ammonia and carbon dioxide concentrations in disposable and reusable ventilated mouse cages. *J Am Assoc Lab Anim Sci* **47**:57–62.
55. **Silverman J, Bays DW, Baker SP.** 2009. Ammonia and carbon dioxide concentrations in disposable and reusable static mouse cages. *Lab Anim (NY)* **38**:16–23. <https://doi.org/10.1038/labon0109-16>.
56. **Smith AL, Mabus SL, Stockwell JD, Muir C.** 2004. Effects of housing density and cage floor space on C57BL/6J mice. *Comp Med* **54**:656–663.
57. **Smith AL, Mabus SL, Muir C, Woo Y.** 2005. Effects of housing density and cage floor space on 3 strains of young adult inbred mice. *Comp Med* **55**:368–376.
58. **Smith E, Stockwell JD, Schweitzer I, Langley SH, Smith AL.** 2004. Evaluation of cage microenvironment of mice housed on various types of bedding materials. *Contemp Top Lab Anim* **43**:12–17.
59. **Targowski SP, Klucinski W, Babiker S, Nonnecke BJ.** 1984. Effect of ammonia on in vivo and in vitro immune responses. *Infect Immun* **43**:289–293.
60. **Teixeira MA, Chaguri LC, Carissimi AS, Souza NL, Mori CM, Saldiva PH, Lemos M, Macchione M, Guimaraes ET, King M, Merusse JL.** 2006. Effects of an individually ventilated cage system on the airway integrity of rats (*Rattus norvegicus*) in a laboratory in Brazil. *Lab Anim* **40**:419–431. <https://doi.org/10.1258/002367706778476398>.
61. **Tu H, Diberadinis LJ, Lipman NS.** 1997. Determination of air distribution, exchange, velocity, and leakage in 3 individually ventilated rodent caging systems. *Contemp Top Lab Anim Sci* **36**:69–73.
62. **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.
63. **Washington IM, Payton ME.** 2016. Ammonia levels and urine-spot characteristics as cage-change indicators for high-density individually ventilated mouse cages. *J Am Assoc Lab Anim Sci* **55**:260–267.
64. **Wrenn C.** [Internet]. 2014. Getting the most from toxic gas sensors, ap-109 ed. 18. [Cited 22 June 2016]. Available at: <https://www.environics.fi/wp-content/uploads/2014/06/ap-109-getting-the-most-from-toxic-gas-sensors-2014-02-05.pdf>