AMMONIA

PARAMETERS	EVIDENCE/REFERENCES
PEOPLE	
<25 ppm	• People notice the presence of ammonia at 1 ppm. (reference: Smyth, 1956 as cited in Memarzadeh F. 2005. Control of ammonia production in animal research facilities through ventilation system design. *American Society of Heating, Refrigeration and Air-Conditioning Engineers* (AHSHRAE).) • Ammonia odour threshold for human detection is at 0.04 ppm. (reference: Haz-Map: Information on hazardous chemicals and occupational diseases. Bethesda (MD): NIH; http://hazmap.nlm.nih.gov/index.html.) • The level of ammonia is based on detection by odours—humans can smell ammonia at more than 25 ppm. (participant's comment) • should be no odour outside animal rooms (participant's comment)
25 ppm for 8 hours	Supported by other standards: • the American Conference of Governmental Industrial Hygienists (ACGIH) • the US National Institute for Occupational Safety and Health (NIOSH) • the European Occupational Exposure Limit • Worksafe BC
35 ppm	• The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a short-term (15-minute) exposure limit of 35 ppm. (reference: Gay S.W. & Knowlton K.F. 2009. Ammonia emissions and animal agriculture; http://pubs.ext.vt.edu/442/442-110/442-110.html.)
50 ppm	• The US Occupational Safety and Health Administration (OSHA) standard is 50 ppm over an 8-hour period. (reference: US Department of Labor, http://www.osha.gov/dts/chemicalsampling/data/CH_218300.html.) • Ammonia odour detectable at 50 ppm is used as a baseline for quality air exchange and cage change rates. We try not to let animals go too long without a cage change if we detect much odour at the cage levels. Certainly if it is an irritant, levels are too high. (participant's comment)
RATS	
<25 ppm	• "Young adult pathogen-free rats … were inoculated intranasally with … *M pulmonis* and housed for 4 to 6 weeks in environments with ammonia maintained at specific concentrations from 25 to 250 ppm. All levels of NH_3 −whether produced naturally from soiled bedding or derived from a purified source− significantly increased the severity of the rhinitis, otitis media, trachelitis, and pneumonia … characteristic of murine respiratory

	mycoplasmosis (MRM) …. It was concluded that environmental NH_3, at concentrations commonly encountered in present day cage environments for rats, plays an important role in pathogenesis of MRM." (reference: Broderson et al. 1976. The role of environmental ammonia in respiratory mycoplasmosis of rats. *Am J Pathol* 85(1):115–130.)
25 ppm	• "Currently, upper-level ammonia exposure guidelines are not available for rodents; for humans, the 8-h time-weighted average exposure limit is 25 ppm, with a maximal exposure of 50 ppm, and it is generally accepted these human limits should not be exceeded for laboratory-housed rodents." (reference: Rosenbaum M.D. et al. 2009. Effects of cage-change frequency and bedding volume on mice and their microenvironment. *J Am Assoc Lab Anim Sci* 48(6):763–777; http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2786931/.)
50 ppm	• Ammonia odour detectable at 50 ppm is used as a baseline for quality air exchange and cage change rates. We try not to let animals go too long without a cage change if we detect much odour at the cage levels. Certainly if it is an irritant, levels are too high. (participant's comment)
<100 ppm	• "Long-Evans rats and Swiss mice, individually housed in running wheels, were exposed to … ammonia (100 or 300 ppm) for 6 hr. … Running in both species decreased in a concentration-related manner… Concentrations of ammonia that eliminated running during exposure led to an increase in activity following exposure. At comparable concentrations of both compounds, activity in rats decreased more than in mice." (reference: Tepper J.S. et al. 1985. Alterations in behavior produced by inhaled ozone of ammonia. *Fundam. Appl. Toxicol*. 5(6 Part 1):1110–1118.)
>100 ppm	• "Low environmental ammonia concentrations (less than 100 ppm) produced extremely small changes in blood ammonia concentration, and they had no measurable effects on other parameters examined in the study. These findings suggest that environmental ammonia concentrations found in animal holding rooms may cause minimal adverse effects in healthy rats." (reference: Schaerdel A.D., White W.J., Lang C.M., Dvorchik B.H. & Bohner K. 1983. Localized and systemic effects of environmental ammonia in rats. *Lab Anim Sci*. 33(1):40–45.)
Other evidence	• Growth of infective agents in the respiratory tract of rats was much greater for rats exposed to ammonia [rats inoculated intranasally with *Mycoplasma pulmonis* and exposed to $\leq$2 or 100 ppm ammonia] (reference: Schoeb T.R., Davidson M.K. & Lindsey J.R. 1982. Intracage ammonia promotes growth of Mycoplasma pulmonis in the respiratory tract of rats. *Infect Immun* 38(1):212–217.) • "The purpose of this study was to determine if environmental ammonia is absorbed through the lungs of rats into the blood and, in turn, exerts an effect on blood pH, blood gases, and hepatic drug metabolizing enzyme activity …. These findings suggest that environmental ammonia

	concentrations found in animal holding rooms may cause minimal adverse effects in healthy rats." (reference: Schaerdel A.D., White W.J., Lang C.M., Dvorchik B.H. & Bohner K. 1983. Localized and systemic effects of environmental ammonia in rats. *Lab Anim Sci*. 33(1):40–45.) • "From the results it can be seen that even in closely controlled environmental conditions a commonly observed change in the amount of ammonia vapour present can radically alter the 'normal' histological picture of the trachea of apparently healthy animals." (reference: Gamble M.R. & Clough G. 1976. Ammonia build-up in animal boxes and its effect on rat tracheal epithelium. *Lab Anim*. 10(2):93–104.) • "Studies described in the literature are not based on the exposure of healthy rodents to naturally developing ammonia concentrations and do not reflect the typical laboratory environment." (reference: Reeb C.K., Jones R.B., Bearg D.W., Bedigian H., Meyer D.D. & Paigen B. 1998. Microenvironment in ventilated animal cages with differing ventilation rates, mice populations, and frequency of bedding changes. *Contemp Top in Lab Anim Sci* 37(2):43–49.)
MICE	
<25 ppm	• 25 ppm is seen to be problematic to mice (reference: Memarzadeh F. 2005. Control of ammonia production in animal research facilities through ventilation system design. *American Society of Heating, Refrigeration and Air-Conditioning Engineers* (AHSHRAE).) • "Our data do not support absolute numbers regarding safe levels for ammonia exposure in mice, but we can make some inferences from our histology data. We saw rhinitis when the exposure level was 181 ppm for 18 d, necrosis of the olfactory epithelium when exposure was 93 ppm for 16 d, and degeneration of epithelium when exposure was 52 ppm for 13 d. We did not observe lesions in the nasal passages when the mean ammonia level was 32 ppm for only 7 d or 10 ppm or less for 28 d. Therefore, our data suggest that for mice housed in IVC, which have the potential for 24-h exposure to ammonia for days or weeks at a time, intracage ammonia levels should remain below 25 ppm." (reference: Vogelweid C.M. et al. 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(6):868–878 (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3228923/) • "An experimental study demonstrated that twice weekly cage cleaning would reduce the incidence of corneal opacities to a very low level. A bacterial product, such as ammonia, is proposed as a significant factor in the pathogenesis of spontaneous corneal opacities in laboratory mice." (reference: Van Winkle T.J. & Balk M.W. 1986. Spontaneous corneal opacities in laboratory mice. *Lab Anim Sci* 36(3):248–255.)
50 ppm	• "Without unambiguous documentation of the potential adverse health effects of given concentrations of ammonia and carbon dioxide on

	laboratory animals, and without a consensus on maximum desirable intracage concentrations of these gasses, the recommended frequency of cage change can only be estimated. We suggest, on the basis of guidelines for human exposure and a liberal reading of the available veterinary literature, that intracage concentrations of 50 ppm of ammonia should lead to cage changing." (reference: Silverman J., Bays D.W. & Baker SP. 2009. Ammonia and carbon dioxide concentrations in disposable and reusable static mouse cages. *Lab Anim* 38(1):16–23.)
<100 ppm	• "Long-Evans rats and Swiss mice, individually housed in running wheels, were exposed to … ammonia (100 or 300 ppm) for 6 hr. … Running in both species decreased in a concentration-related manner… Concentrations of ammonia that eliminated running during exposure led to an increase in activity following exposure. At comparable concentrations of both compounds [ammonia and ozone], activity in rats decreased more than in mice." (reference: Tepper J.S. et al. 1985. Alterations in behavior produced by inhaled ozone of ammonia. *Fundam. Appl. Toxicol*. 5(6 Part 1):1110–1118.)
>100 ppm	• "… mice exhibited no clear preference for, or aversion to, any of the experimental concentrations of ammonia" [concentrations of 4, 30, 56 & 110 ppm] (reference: Green A.R., Wathes C.M., Demmers T.G., Clark J.M. & 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(2):49–56.) • "Mean intracage NH_3 levels in reusable IVCs were occasionally greater than 25 ppm and, in some instances, were greater than 150 ppm. Animals in the cages with high NH_3 concentrations were closely monitored, but no overt problems (that is sneezing, rubbing of eyes or nose, erythema, changes in behavior patterns) were noticed…." (reference: Silverman J., Bays D.W., Cooper S.F. & Baker S.P. 2008. Ammonia and carbon dioxide concentrations in disposable and reusable ventilated mouse cages. *J Am Assoc Lab Anim Sci* 47(2):57–62.) • "Ammonia concentrations of more than 500 ppm were recorded toward the end of the initial study period [7 days], and concentrations of more than 700 ppm were recorded toward the end of the crossover study period [a 2nd 7-day period]. Continuous observations of the mice during the gas collection periods did not indicate an untoward behaviors or overt health problems" (reference: Silverman J., Bays D.W. & Baker SP. 2009. Ammonia and carbon dioxide concentrations in disposable and reusable static mouse cages. *Lab Anim* 38(1):16–23.) • "We found no pathological changes in the nasal passages of mice exposed to high concentrations of ammonia [ranging from 2 to358 ppm]. Thus, our results are at variance with somewhat similar studies in rats (Broderson et al. 1976) in which mild lesions consisting of epithelial hyperplasia accompanied by cell death and submucosal oedema, congestion, and fibrosis were reported in association with exposure to

	150±250 ppm ammonia….These differences may be due to: the exposure period being at least 35 days compared with about 10 days for our mice, that the rats were continuously exposed, or due to species differences. Similarly, Buckley et al. (1984) found mild lesions in mice exposed to 303 ppm of ammonia, but of a more inflammatory nature. Again, differences in the experimental design, such as the type of exposure and strain of mouse, could account for the differences." (reference: Reeb-Whitaker C.K., Paigen B., Beamer W.G., Bronson R.T., Churchill G.A., Schweitzer I.B. & Myers D.D. 2001. The impact of reduced frequency of cage changes on the health of mice housed in ventilated cages. *Lab Anim* 35(1):58–73.)
Other evidence	• Human's odour sensitivity would make working in conditions difficult yet mice can and have happily existed in very elevated NH_3 conditions for long periods of time. (participant's comment) • "There are no specific guidelines for exposure limits of mice to ammonia, probably because the literature is not clear on what levels are harmful … species differ widely in their ability to tolerate ammonia. … humans cannot safely tolerate concentrations of 100 ppm for more than an hour (Studier et al. 1967). Mice fall into an intermediate range, surviving one hour at 3440 ppm (Kapeghian e t al. 1982) and 16 h at 1000 ppm (Studier et al. 1967). …. Strains of mice also differ in their response to ammonia (Schaper 1993). For example, the level of ammonia that results in a 50% reduction of the respiratory rate (RD50) is 303 ppm in Swiss Webster mice (Barrow et al. 1978) and 790 ppm in BALB/c (Tomas et al. 1985)." (reference: Reeb-Whitaker C.K., Paigen B., Beamer W.G., Bronson R.T., Churchill G.A., Schweitzer I.B. & Myers D.D. 2001. The impact of reduced frequency of cage changes on the health of mice housed in ventilated cages. *Lab Anim* 35(1):58–73.) • "A second study was undertaken housing C57BL/6J mice with as little as 3.2 in^2/mouse (ca. 20.6 cm^2). The major effect was elevated ammonia concentrations that exceeded limits acceptable in the workplace at increased housing densities; however, the nasal passages and eyeballs of the mice remained microscopically normal." (reference: Smith A.L. et al. 2004. Effects of housing density and cage floor space on C57BL/6J mice. *Comp. Med*. 54(6):656–663.) • "Studies described in the literature are not based on the exposure of healthy rodents to naturally developing ammonia concentrations and do not reflect the typical laboratory environment." (Reference: Reeb C.K., Jones R.B., Bearg D.W., Bedigian H., MeyerD.D. & Paigen B. (1998) Microenvironment in ventilated animal cages with differing ventilation rates, mice populations, and frequency of bedding changes. *Contemp Top in Lab Anim Sci* 37(2):43–49.)
GUINEA PIGS	
<50 ppm	• "Exposure of animals to ammonia significantly reduced the cell-mediated

	response (i.e., dermal lesion) [experimental guinea pigs vaccinated with *Mycobacterium bovis* BCG were exposed to 50 ppm ammonia or 90 ppm ammonia]…. The results of this study indicate that the responsiveness of blood or bronchial lymphocytes to PPD was reduced in ammonia-exposed animals. Therefore, it can be suggested that in vivo ammonia inhibits the release of lymphokines and the mediation of the specific inflammatory reaction, consequently compromising the ability of the host to eliminate infection." (reference: Targowski S.P., Klucinski W., Babiker S. & Nonnecke B.J. 1984. Effect of ammonia on in vivo and in vitro immune responses. *Infect Immun.* 43(1):289–293.)
POULTRY	
<25 ppm	• "Elevated levels of ammonia can have a negative impact on animal health and production. For example, reduced final body weights have been observed in poultry produced in houses with indoor ammonia levels of approximately 25 [ppm] or higher during brooding." (reference: Reece et al, 1980 cited in Gay S.W. & Knowlton K.F. 2009. Ammonia emissions and animal agriculture (http://pubs.ext.vt.edu/442/442-110/442-110.html.) • "There was a significant difference between the responses in 0 and 25 ppm (p<0.05) but not between 25 and 45 ppm (p>0.05). This suggests that ammonia may be aversive to hens with a threshold for this aversion between 0 and 25 ppm." (reference: Kristensen H.H., Burgess L.R., Demmers T.G. & Wathes C.M. 2000.The preferences of laying hens for different concentrations of atmospheric ammonia. *Appl. Anim. Behav. Sci*. 68(4):307–318.)