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A Quick Method to Assess Rodent Cage Ammonia Levels

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Introduction

Ammonia toxicity causes biochemical [1,2] and mid-respiratory [3] and nasal epithelial damage [4,5]. In rodent housing situations, ammonia is often used as a bio-indicator to assess rodent well-being, and directly applicable to a wide array of housing and management regimes [6]. OSHA standards are often cross-applied to rodents in lieu of established regulatory standards for them. However, unlike humans in the work place, rodents generally do not leave their cage environment for every long. Modern rodent caging may be static or ventilated, but newer caging in general is self-contained and limits outside air exchanges. Here we attempt to adapt aquarium ammonia test strips to see as a quick assessment of cage level ammonia concentrations by comparing values obtained with these strips to independently measured values. We then evaluated them under real-life conditions through time in statically caged mice as a practical demonstration of their potential utility.

Keywords: Rodent; Ammonia toxicity; Cage; Mice

Materials and Methods

Milli-pore® water was used throughout. A hermetically sealed rat cage (BCU®, Allentown, NJ) maintained at room temperature was used to produce a standard curve relating independently measured decreasing ammonia concentrations to time to maximum color change of water pre-moistened ammonia strips (Tetra EasyStrips® Ammonia Aquarium Test Strips; Cat. #19542-00). Independently, ammonia concentrations were measured with a RKI Eagle II Gas Monitor (Union City, CA; sensitive to 0.1 ppm ammonia) that was pre-calibrated with a known ammonia gas standard according to the manufacturer's instructions prior to use. Increasing ammonia (Ammonia hydroxide; Sigma # 05002) concentrations were measured and tested (time to maximum color change as shown on the container) in triplicate by first placing 10 µl of each Ammonia concentration in a petri dish inside at the center of the cage. Each sample was allowed to equilibrate 10 min, so that stable gas monitor measurements could be made. Twenty-six different ammonia solutions of varying concentrations were measured by analyzer and with test strips.

As a demonstration of their utility, 5 static cages (Allentown NextGen® mouse cages; Allentown, NJ) bedded to ½" depth using 3/8" diameter corncob bedding, were populated with 3 GRHL2-floxxed black mice; all cages were set adjacent to each other on the same shelf. Animals were supplied with water and food (Envigo 2018) ad lib. Cage tops were of the molded polysulfone type fitted with Remay® filters and external water bottles so the water bottle port could be used for sampling. Both analyzer and test strip (in replicate) measurements were taken at the same time daily until a threshold value of 20 ppm was exceeded and a cage change was needed. Room humidity and temperature, and water bottle weight were recorded daily to determine consumption.

Results

A standard curve was created by comparing the time for full aquarium test strip change (X=sec) to analyzer ammonia levels (Y=ppm). When plotted, the data fit a power curve with the following equation: $Y=115.24X^{-0.834}$ with an $R^2=0.95$ (Figure 1).

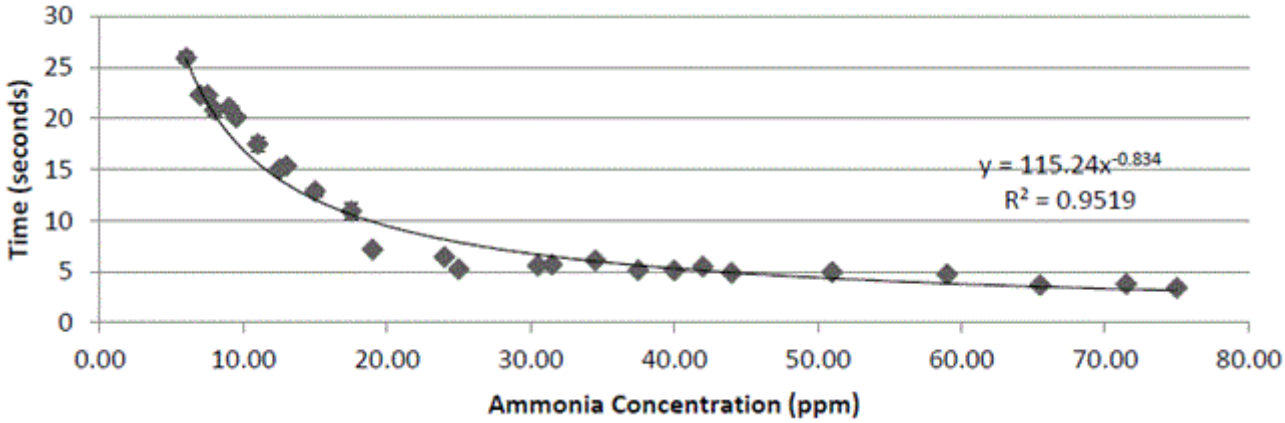

Figure 1: Standard Curve.

Table 1 summarizes useful milestones that were developed for monitoring rodent cages based on published work. Daily ammonia levels from static cages increased gradually until there was a precipitous increase. Ammonia levels precipitously exceeded 20 ppm in 1 cage on the 9th day, in 3 cages on the 10th day, and in 1 cage on the 11th day. Strong agreement of the monitor readings and time required for indicator strips to reach the full green were noted when sampling through the water bottle port without removing the outer molded cage top was used. During the study period, room temperature and humidity levels did not undergo wild excursions; temperature ranged 70.7°F-75.2°F and relative humidity ranged 44%-56%.

Criterion Used	Concentration/Time	Test Strip Color Change time	Literature adapted Rodent cut-offs (ppm)	Rodent Literature Reference
PEL	50 ppm/8 h	~ 6 sec	<100 ≤ 7 d	[7]
REL	25 ppm/10 h	~ 8 sec	100/6 h	[8]
WVU Standard	>20 ppm	>7 sec	~ 25 for 4 m	[9]

Table 1: Common cut-offs based on human OSHA standards and conservative threshold values derived from the rodent literature are shown. The OSHA standards for ammonia exposure are highlighted in dark grey [10]. WVU's adopted standard is highlighted in light grey. Since the test strips only provide a point estimate, more conservative values were selected for our use. Other convenient test strip metrics include: 10-15 sec corresponds to 10-20 ppm; <10 sec corresponds to ≥ 20 ppm; <5 sec corresponds to ≥ 40 ppm (+). REL=recommended exposure limit; PEL=permissible exposure limit.

Table 2 summarizes ammonia levels as determined independently in five static cages with the Eagle II unit, and as determined separately using moistened ammonia test strips and the standard curve in Figure 1. Cages were not opened during the complete testing period and access was gained for testing through the water bottle port. Water consumption is reported. Cage level room relative humidity and temperature during the study period were relatively stable Temperature and humidity (mean ± s.e.) were 52.3 ± 1.2°F and 72.3 ± 0.4% respectively during the study period.

Day	1	2	3	4	5	6	7	8	9	10	11	12
Reference*	0.0	0.0	0.3	0.0	0.0	0.3	0.5	0.6	15.4	1.7	43.3	52.0
Reference se	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.1	14.9	0.8	14.6	-
EasyStrip®	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.7	15.5	1.3	40.9	40.9
EasyStrip® se	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	14.9	0.5	13.8	-
Water Consumed	12.0	12.0	12.0	13.2	12.8	14.4	14.4	15.2	14.0	15.2	13.0	18.0
Water se	0.5	0.4	0.6	0.8	1.2	2.4	0.7	0.5	0.6	1.0	0.9	-
No. of Cages	5	5	5	5	5	5	5	5	5	4	4	1

Table 2: Summary of daily ammonia (ppm) measured independently after reference gas pre-calibration using an Eagle II® gas monitor*, and as estimated using EasyStrips®. Cages remained closed during the complete study period. Ammonia values were obtained by insertion of the monitor probe or distilled water remoistened strips the same distance into each static cage through the water bottle port. Daily water consumption was measured. Each cage was only monitored until ammonia methods indicated ammonia exceeded 20 ppm. Ammonia is reported in ppm, and water in mL consumed. During the 12 day study period, room temperatures at the cage level averaged 72.3 ± 0.4 °F, and room relative humidity was 52.3 ± 1.2%. Values are expressed as mean ± s. e.

Discussion

For short exposure periods in rats, ammonia causes minimal systemic effects except at extremely high levels (e.g., >100 ppm) [7]. Even when not clinically recognized, elevated ammonia may significantly affect drug studies and liver function [11] and may contribute to elevated corticosterone values [9]. Excessive ammonia accumulation emerges with reduced cage changing frequency [10], in the presence of inadequate ventilation [9,12], under conditions of higher humidity, and with increased animal densities or nitrogen load [13]. Certain bedding types accentuate ammonia production [14,15] while excessive ventilation rates may affect breeding success (Wimsatt, unpublished data). Test strip detection of ammonia levels allows a more refined approach, and may reduce costs, human labor and occupational risks; testing by this means has advantages in that it is simple, rapid, and relatively cost effective (~ $1 retail per test strip). Frequent cage changing may have adverse effects on body condition [16] and behavioral measures [17]. Likewise, in biocontainment settings, minimizing cage changes reduces worker exposure risk. Threshold ammonia screening is easily implemented by any animal care worker during routine animal care (e.g., >7 sec for the strip to turns fully green indicates >20 ppm has been reached in that cage). Initial rodent cage testing suggests ammonia levels can change rapidly and the importance of measuring levels often in high risk situations.

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