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Murine cage density: cage ammonia levels during the reproductive performance of an inbred strain and two outbred stocks of monogamous breeding pairs of mice

J. R. EVELEIGH

AFRC Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Woking, Surrey GU24 0NF, UK

Summary

The Laboratory Animal Breeders Association guidelines recommend a minimum floor area of 300 cm^2 for a monogamous pair of inbred/outbred mice or a trio of inbreds. The mean level of ammonia produced during lactation from BALB/c, TO and CD-1 breeding pairs housed in M2 cages with a floor area of 300 cm^2 on Day 4 after cleaning was 30 ppm, 87 ppm and 92 ppm, respectively. All 3 strains of mice, particularly the outbred strains, were subjected to high levels of ammonia as compared with human long-term health and safety occupational exposure limits (25 ppm). However, there is a gradient of ammonia within an M2 breeding cage from the nest (19 ppm), to the food hopper, 77 ppm. By housing CD-1 pairs of mice in RM2 cages which have more than double the floor area of M2 cages (676 cm^2), the mean level of ammonia during lactation on Day 4 after cleaning was reduced to 26 ppm. The reproductive performance on inbred/outbred strains of mice has to be equated with cage size (floor area) to maintain acceptable levels of ammonia. It is suggested that the recommended minimum floor areas for breeding mice be reviewed.

Keywords: Murine; Cage density; Ammonia; Reproduction

Eveleigh and Williams (1992) compared reproductive performance and cage density in relation to weight, between monogamous breeding pairs of outbred CD-1 and inbred BALB/c mice housed in M2 polypropylene cages with a floor area of 330 cm^2. They found that outbred CD-1 mice weaned up to 8·0 more young per litter than the BALB/c and had a greater biomass of up to 97 g.

Having obtained these results, Eveleigh and Williams proposed that further investigation was warranted into whether the minimum floor area of 300 cm^2 as recommended by the Laboratory Animal Breeders Association (1991) was optimal for outbred monogamous breeding pairs of mice and possibly inbred trios.

One of the problems associated with increased cage density is the greater production of ammonia (NH3). Gamble and Clough (1976) found that at 7 days 5 mice per M2 cage were exposed to ammonia in excess of 150 ppm and that rats exposed to 200 ± 50 ppm of ammonia developed histopathological changes in the tracheal epithelium. The Health and Safety Executive (1989) for humans list the upper limit for exposure to ammonia as 25 ppm for an 8-h exposure period and 35 ppm of ammonia for a 10-min exposure period.

Eveleigh (1991) in a brief paper showed that:

1. Monogamous pairs of inbred BALB/c and outbred CD-1 mice housed in M2 cages produced a mean cage ammonia level of 26 ppm and 154 ppm, respectively, over a 4-day period prior to weaning their litters.
2. CD-1 monogamous pairs housed in M2 cages produced a significantly lower level of ammonia over a 4-day period prior to weaning their litters than when they were housed in M2 cages, 42 ppm against 154 ppm.

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3. A gradient of ammonia exists in M2 cages from the nest to the food hopper when housing CD-1 monogamous pairs, 30 ppm to 99 ppm.

The purpose of this paper is to provide more detailed information on these findings and to measure and compare M2 cage ammonia levels between an inbred and two outbred stocks of mice of different fecundity potentials during stages of the reproductive cycle.

Materials and methods

Animals

Inbred BALB/c mice (Harlan OLAC Ltd, Oxfordshire, UK), outbred TO mice (Bantin and Kingman Ltd, Hull, UK) and outbred CD-1 mice (Charles River UK Ltd, Kent, UK) were used in this investigation.

Cageing

M2 polypropylene solid floor cages 33 cm × 15 cm × 13 cm with a floor area of 330 cm^2 and RM2 polypropylene solid floor cages 38 cm × 25 cm × 18 cm with a floor area of 676 cm^2 were used (North Kent Plastic Cages Ltd, Kent, UK).

Food, bedding and environmental controls were described by Eveleigh and Williams (1992). The volume of dust-free contact bedding used (softwood fibres) per M2 and RM2 cage was 20 and 50 g, respectively.

Method and frequency of measuring ammonia (NH3)

The presence of ammonia was detected and measured by a gas detector bellows pump with short-term direct indicating diffusion tubes which have a relative standard deviation of ± 10 to 15% (Draeger Ltd, Northumberland, UK). Up to 10 × 100 cc of air was evaluated to formulate a single reading.

All the mouse cages were cleaned out once a week with cage and room levels of ammonia being measured on Day 4 and/or Day 7 prior to cleaning. Air samples were taken approximately 1 cm above the soiled bedding. All the mouse cages were removed individually from their rack to gain access for air sampling.

Experiment 1

Ten monogamous pairs of virgin BALB/c, TO and CD-1 mice 8–10 weeks of age were mated in M2 cages. The first 5 breeding pairs, of each strain with litters born within 2 days of one another were used in this experiment. Air samples were taken throughout each cage to ascertain a single mean reading and recorded with litter size during the following stages of parity 1:

Stage 1 Day 7 after pairing.
2 Day 7 after cage cleaning with litters 1–2 days old.
3 Day 4 after cage cleaning with litters 5–7 days old.
4 Day 7 after cage cleaning with litters 8–10 days old.
5 Day 4 after cage cleaning with litters 12–14 days old.
6 Day 7 after cage cleaning with litters 15–17 days old.
7 Day 4 after cage cleaning with litters 19–21 days old.

Experiment 2

The 5 parity 2 CD-1 monogamous pairs from experiment 1 were placed in RM2 cages. These littered within 3 days of each other. As for experiment 1, air samples were taken from each cage and recorded with the litter size and noted during the following stages and of reproduction:

Stage 1 Day 4 after cage cleaning with litters 4–6 days old.
2 Day 7 after cage cleaning with litters 7–9 days old.
3 Day 4 after cage cleaning with litters 11–13 days old.
4 Day 7 after cage cleaning with litters 14–17 days old.
5 Day 4 after cage cleaning with litters 18–21 days old.

Table 1. M2 cage levels of ammonia (NH_3) recorded from 5 monogamous pairs and their litters of inbred BALB/c and outbred TO and CD-1 mice Day 4 and 7 after cleaning during reproductive stages 1–7 (mean ± standard deviation)

	Level of ammonia (ppm)					
	BALB/c		*TO*		*CD-1*	
Stage	*Day 4*	*Day 7*	*Day 4*	*Day 7*	*Day 4*	*Day 7*
1		0·4 ± 0·6		5·4 ± 3·2		2·0 ± 2·1
2		2·0 ± 0·0		90·0 ± 26·5		30·2 ± 22·0
3	10·6 ± 16·5		78·0 ± 37·7		47·6 ± 25·9	
4		72·0 ± 30·3		132·0 ± 41·5		68·6 ± 19·0
5	52·0 ± 19·2		88·0 ± 11·0		74·0 ± 19·5	
6		66·0 ± 13·4		116·0 ± 49·8		104·0 ± 16·7
7	26·0 ± 23·0		94·0 ± 16·7		154·0 ± 45·6	

Experiment 3

Ten monogamous pairs of virgin CD-1 mice 8–10 weeks of age were mated in M2 cages. The first 5 breeding pairs with litters born within 2 days of each other were used. Air samples were taken and recorded separately from the nest, the centre of the cage and beneath the food hopper during the following stages of parity 1 and litter sizes noted:

Day 4 after cage cleaning with litters 5–6 days old.
Day 4 after cage cleaning with litters 12–13 days old.
Day 4 after cage cleaning with litters 19–20 days old.

Observations and air samples were taken and recorded between 0900 and 1100 h during experiments 1, 2 and 3.

Results

Experiment 1. M2 cage ammonia levels

At Stage 1 very low levels of ammonia were associated with all 3 strains of mice. Within 48 h of parturition (Stage 2), high mean cage ammonia levels to 90 ppm were recorded in the outbred cages, particularly the TO cages. The mean BALB/c litter size at birth was 6·40 ± 1·8, and for the CD-1 13·2 ± 2·8 and the TO 13·2 ± 2·2. Cage ammonia levels increased with the growth of all the litters during Stages 3–7. Ammonia levels rose to 154 ppm in the CD-1 cages, 6 times the level of the BALB/c cages. The mean litter size weaned remained at 6·4 ± 1·8 for the BALB/c but was reduced through preweaning losses to 12·0 ± 1·9 for the TO and 13·0 ± 2·4 for the CD-1. Ammonia levels measured at Stages 1–7 and at the cleaning out intervals are shown in Table 1.

Experiment 2. RM2 cage ammonia levels

Ammonia levels increased with the growth of the litters from 12·8 ± 15·4 ppm–42·0 ± 20·5 ppm throughout the experiment. The CD-1 litter size at birth was 14·6 ± 3·5 and 13·0 ± 3·4 at weaning. The mean ammonia levels measured at the 4 and 7 day intervals after are shown in Table 2.

Experiment 3. M2 ammonia levels within the nest, centre of cage and beneath the food hopper

At all stages of the litters' development the level of ammonia was lowest in the nesting area (16–25 ppm) gradually building up towards the

Table 2. RM2 cage levels of ammonia (NH_3) recorded from 5 monogamous pairs of outbred CD-1 mice at 4 and 7 day intervals after cleaning when the young were between 4–21 days of age (mean ± standard deviation)

	Level of ammonia (ppm)				
	Interval (days)				
	4	*7*	*4*	*7*	*4*
Age of litter (days)	4–6	7–9	11–13	14–17	18–21
Mean ammonia level	12·8 ± 15·4	21·6 ± 11·5	22·4 ± 7·7	42·0 ± 8·4	42·0 ± 20·5
Mean litter size	14·6 ± 3·5	13·8 ± 3·7	13·6 ± 3·4	13·4 ± 3·5	13·0 ± 3·4

Table 3. M2 cage levels of ammonia (NH_3) recorded from the nesting area, centre of cage and beneath the food hopper from 5 monogamous pairs of outbred CD-1 mice 4 days after cleaning when the young were 5–6, 12–13 and 19–20 days old (mean ± standard deviation)

Age of litter (days)	*Level of ammonia (ppm)* *Nesting area*	*Centre of cage*	*Beneath food hopper*	*Mean litter size*
5–6	17·2 ± 8·6	36·0 ± 11·4	72·0 ± 16·4	11·0 ± 3·0
12–13	16·0 ± 5·5	31·0 ± 11·4	72·0 ± 19·2	11·0 ± 3·0
19–20	25·0 ± 5·0	32·0 ± 4·5	88·0 ± 11·0	11·0 ± 3·0

food hopper to 72–88 ppm 4 days after cleaning (Table 3). The nest had dispersed by weaning and the ammonia level, although low, had increased by approximately 47%. During lactation the mean level of ammonia in the nesting area was 19 ppm, 33 ppm at the centre of the cage and 77 ppm beneath the food hopper. The mean litter size at birth and weaning was 11·0 ± 3·0.

Throughout the experiment room ammonia levels were negligible measuring only 1–2 ppm on 3 occasions.

Discussion

Gamble and Clough (1976) showed that the most rapid build-up of ammonia occurred during conditions of high humidity, as when a water bottle leaked. This did not occur during this study. Good husbandry such as cleaning the cages twice weekly significantly reduced cage ammonia levels during lactation (Table 1). However, ammonia levels recorded on Day 4 after cleaning when the litters were 4–21 days of age showed that individual cage levels of up to 80 ppm, 120 ppm and 200 ppm could still be associated with BALB/c, TO and CD-1 mice in cages with a floor area of 320 cm^2. The mean level of ammonia present during this period of neonatal growth was 29·5 ± 9·0 ppm for BALB/c, 86·7 ± 8·0 ppm for TO and 91·7 ± 55·6 ppm for CD-1 mice. All 3 strains of mice, particularly the 2 outbred strains TO and CD-1, were therefore subjected to high levels of ammonia when compared to the 25 ppm limit identified for long-term human health and safety occupational exposure limits.

Cage ammonia levels can be reduced by additional cage cleaning but this frequent interference to the mouse's environment is stressful and can cause preweaning losses (Donnally, 1989). It is shown that lower levels of ammonia can be achieved by housing CD-1 monogamous pairs in larger cages, i.e. 676 cm^2/42 ± 20·5 ppm compared with 330 cm^2/154 ± 45·6 ppm.

Within the length of an M2 cage (33 cm), there was a gradient of ammonia from the nest to the food hopper. It is acknowledged that mice frequently nest under the food hopper, but it is not known how much time the litters and their parents spend in the low and high ammonia zones. What is the health and safety exposure limit to ammonia for mice and other animals confined in a restricted environment? These and other questions require further investigation.

CD-1 M2 cage ammonia levels on Day 4 in experiments 1 and 3 are at variance. The higher levels of ammonia in experiment 1 were probably due to the greater litter size born and weaned 2·2 and 2·0, respectively. However, there was little increase in variance in cage ammonia levels with time in experiment 3. This may be attributed to the different location of air sampling points for a single reading, e.g. experiment 1 air samples were taken throughout the cage and experiment 3 air samples were taken from a specific region of the cage.

As shown cage cleaning, litter size, growth and cage floor area, i.e. cage and biomass density, are factors which influence the level of ammonia produced during reproduction. Hence, the ammonia production level is another reason why the minimum floor area for the housing of inbred/outbred monogamous pairs and inbred trios should be reviewed by all laboratory animal users.

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