Comparison of microenvironmental conditions in standard versus forced-air ventilated rodent filter-top cages

by *K. Iwarsson*[1] & *L. Norén*[2]

[1] Laboratory Animal Unit, Karolinska Institute, Box 60 400, S-104 01 Stockholm, Sweden,
[2] Barriärteknik, St. Topphem, Fågeltofta, S-270 44 Brösarp, Sweden.

Introduction

Rodent caging systems employing filter-top covered cages are commonly used and found useful for containment and preserving microbial integrity at the cage level (*Dillehay et al.* 1990, *Lipman et al.* 1991).

Among the problems encountered with filter-top cages are reduced ventilation through the filter with increased intracage levels of carbon dioxide, humidity and ammonia (*Keller et al.* 1989, *Corning & Lipman* 1991, *Keller et al.* 1983).

The purpose of this study was primarily to evaluate the levels of relative humidity (RH), carbon dioxide (CO_2) and ammonia (NH_3) in commercial filter-top cages provided with a novel developed forced-air ventilated system versus those without, with standard unfiltered cages used as controls. The forced-air individually ventilated caging system was constructed in the initial phase of a developmental undertaking conducted to investigate and improve the microenvironmental conditions of rodent caging systems (*Iwarsson & Norén* 1991).

Materials and methods

Animals: Seventy female SPF-derived outbred NMRI mice (National Veterinary Institute (SVA), Uppsala, Sweden), aged about 12 weeks, were used. The animals were fed a commercial, non-autoclaved pelleted diet (R36, Ewos AB, Södertälje, Sweden) and tap water *ad libitum*. The animals were housed in an animal room measuring 2.5 × 5.5 × 3.1 m, with a photoperiod of 12 h light/12 h dark and maintained at 21–22°C, 50–55 % RH and 12–14 changes per hour of 100 % fresh air during the study.

Caging systems: Shoebox-type polycarbonate cages with dimensions of 42 × 26 × 15 cm, Macrolon® type III with Micro-Isolator® filter lids and Reemay® (polyester) Filter # 2024 (UNO, bv, Holland, supplied by B & K, Sollentuna, Sweden), placed in a rack, were used.

One filter cage was provided with a forced-air distribution system (Vent A Cage, Barriärteknik, Brösarp, Sweden) constructed using standard polyvinyl chloride tubing for direct mounting on standard rack units of variable sizes. For the present studies the system utilized a speed-adjustable centrifugal fan unit for sterile filtered air (Munktell Sterile Air Flow Mod. # 20, Stora Kopparberg, Grycksbo, Sweden) delivering pressurized air via a horizontal probe into each cage. The probe entered the cage above the wire bar lid through a 40 mm drilled hole at the rear end of the filter top frame. In the present study the system provided 1.2 m/s at the horizontal probe orifice with approximately 150 air changes per hour within the cage. Each cage contained 800 cm^3 of hot-air dried, non-autoclaved, hardwood chip bedding (Finn Tapvei Ky, Kaavi, Finland).

All cages were modified with a 40 mm-diameter port for sampling, placed 30 mm from the bottom of the front of the cage and covered with a metal lid supported by plastic tape.

Measurements: Twenty-four-hour room temperature and humidity measurements were recorded with a continously operating

thermohygrograph (Lambrecht 252, KEBO Lab AB, Spånga, Sweden) with a whirling psychrometer for calibration. All cage-sampling procedures were done at 8:00 am each day and performed through the modified ports in the cage front. Cage temperature and humidity were measured with a portable instrument (Vaisala Humidity & Temperature Indicator HMI 31, Vaisala OY, Helsinki, Finland) and air-flow with a thermal anemometer (Lambrecht 641 S 15 KEBO Lab AB, Spånga, Sweden). Carbon dioxide and ammonia levels were obtained with an air-sampling pump (Dräger Multi Gas Detector Mod. 21/31, Dräger Svenska AB, Nacka, Sweden), with Dräger Tubes Carbon Dioxide 0,1 %/a and 0,01 %/a respectively, and CH 20501 Ammonia 5/a (Bicapa AB, Stockholm, Sweden). The tube accuracy according to the manufacturers' specification corresponds to a standard deviation of ± 10 % depending on the concentration of the gas.

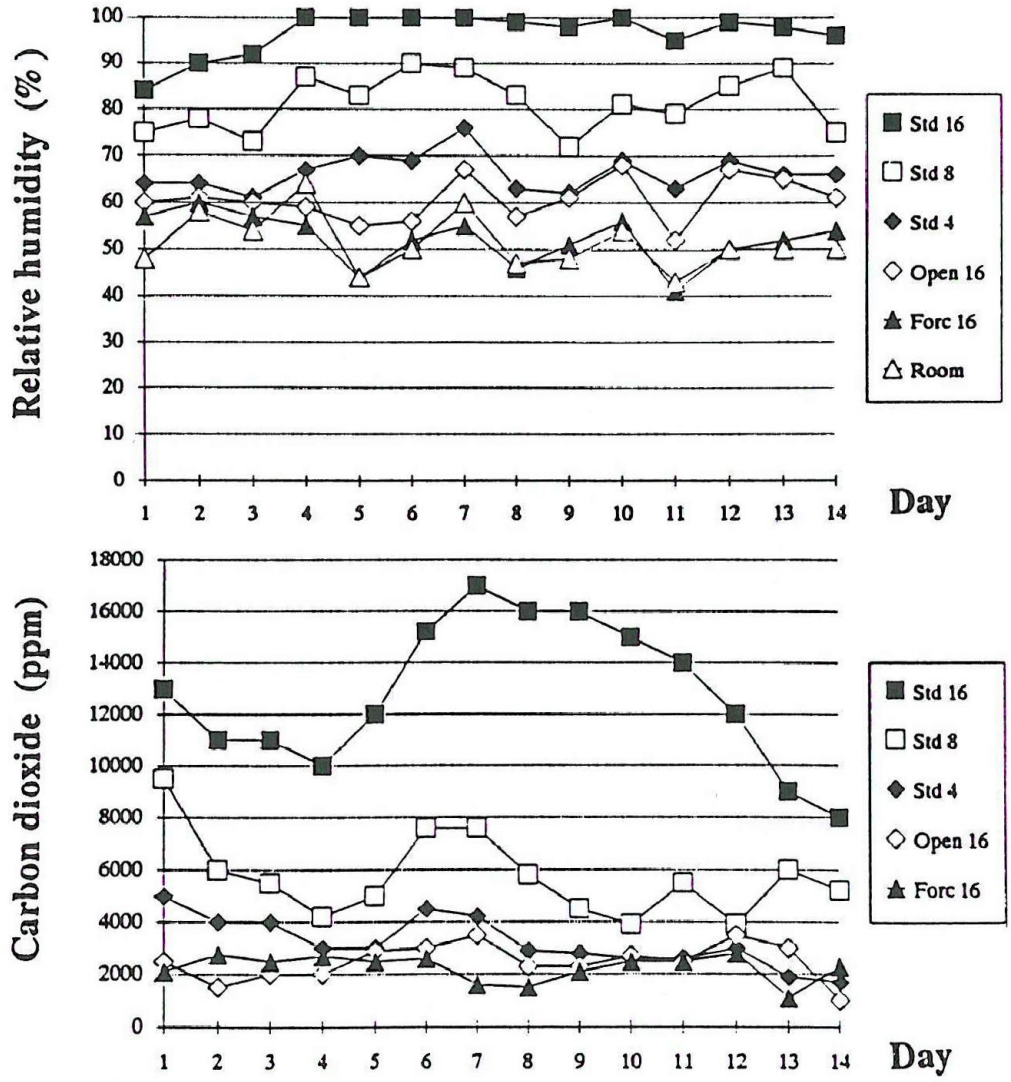

Figure 1. Microenvironmental relative humidity and carbon dioxide levels measured once a day in different caging systems: Standard Micro-Isolator filter top cages (*Std*), open cage without filter top (*Open*), forced-air ventilated cage (*Forc*) and the animal room (*Room*). Numbers after the symbols in legends denote the number of mice housed in the actual cage.

Experimental settings: The following experimental settings were evaluated: Micro-Isolator cages without forced air (Standard), housing 4, 8 and 16 female mice with body weights ranging between 23–28 g, were compared to two cages housing 16 mice each: a Micro-Isolator provided with an individual Vent A Cage system (Forced-air) and a cage without filter lid (Open).
Intracage RH, CO_2 and NH_3 levels were evaluated once a day in each caging system during a 2-week period. Bedding and feed were not removed during the testing period, but water bottles were changed on day 6 and 12.

Results
Control measurements of intracage temperatures showed only small deviations from actual room temperature for all caging systems throughout the study (data not shown).
Air velocity measurements conducted at start and on days 3 and 10 in the forced-air ventilated cage housing 16 mice varied between 0.1–0.3 m/s at bedding level with low values in the periphery and rear part of the cage floor.
The results of RH measurements are shown in Figure 1. Micro-Isolator cages housing 8 and 16 mice increased from 75–85 % RH on day 1 to an average of 80–100 % from day 4 and onwards, while the cage with 4 mice varied between 65–75 % during the study. The forced-air ventilated Micro-Isolator cage averaged 40–60 % RH with daily levels close to those of the ambient room air and on an average 10 % lower RH than the open control cage.
Carbon dioxide levels ranged from 5,000–17,000 ppm in standard Micro-Isolator cages with 8 and 16 mice, and from 2,000–5,000 ppm in the standard cage with 4 mice (Figure 1). A CO_2 concentration of 5,000 ppm, detected after 24 hours basal housing of 4 mice in a standard Micro-Isolator cage, is more than double as high as the simultaneous concentration of the unfiltered control cage housing 16 mice. Similarly, cages housing 8 and 16 mice showed CO_2 concentrations 5–6 times higher than the open cage. From day 4 the CO_2 levels gradually increased with a maximum of 17,000 ppm noted in the 16 mouse cage on day 7, after which the levels gradually decreased.
The forced-air cage housing 16 mice ranged from 1,000–2,700 ppm CO_2 during the whole observation period with daily levels close to or below the open control cage.
No appreciable ammonia levels were detected in either system until day 14 (Figure 2 a). Concentrations close to the detection limit of the system used were noted with start on day 10 (Std 16), 11 (Open 16) and 12 (Std 8), respectively, as indicated in Figure 2 b. In the standard filter cage housing 16 mice the ammonia levels increased from about 7 ppm (traces) on day 13 to 650 and 700 ppm on days 14 and 15, respectively. The investigations were stopped immediately after the sudden increase in ammonia concentration.

Discussion
It seems obvious that with use of Micro-Isolator filter top cages the number of animals (the total biomass) in the cage must be limited as compared to open cages to prevent a build up of RH and CO_2 at basal housing of mice. It should be noted that the biomass per floor area of 16 mice as used in the present study corresponds to about 60 % of the allowed total biomass of mice housed in a cage according to current Swedish regulations.
Already after 24 hours housing CO_2 levels in excess of those acceptable in humans were noted. Cages with 8 and 16 mice showed values twice the current hygienic limit value of 5,000 ppm for CO_2 in the human workplace according to the Swedish National Board of Occupational Safety and Health. The highest concentrations observed in the present study, with a maximum concentration of 17,000 ppm in the Micro-Isolator housing 16 mice, exceed on an average 3–5

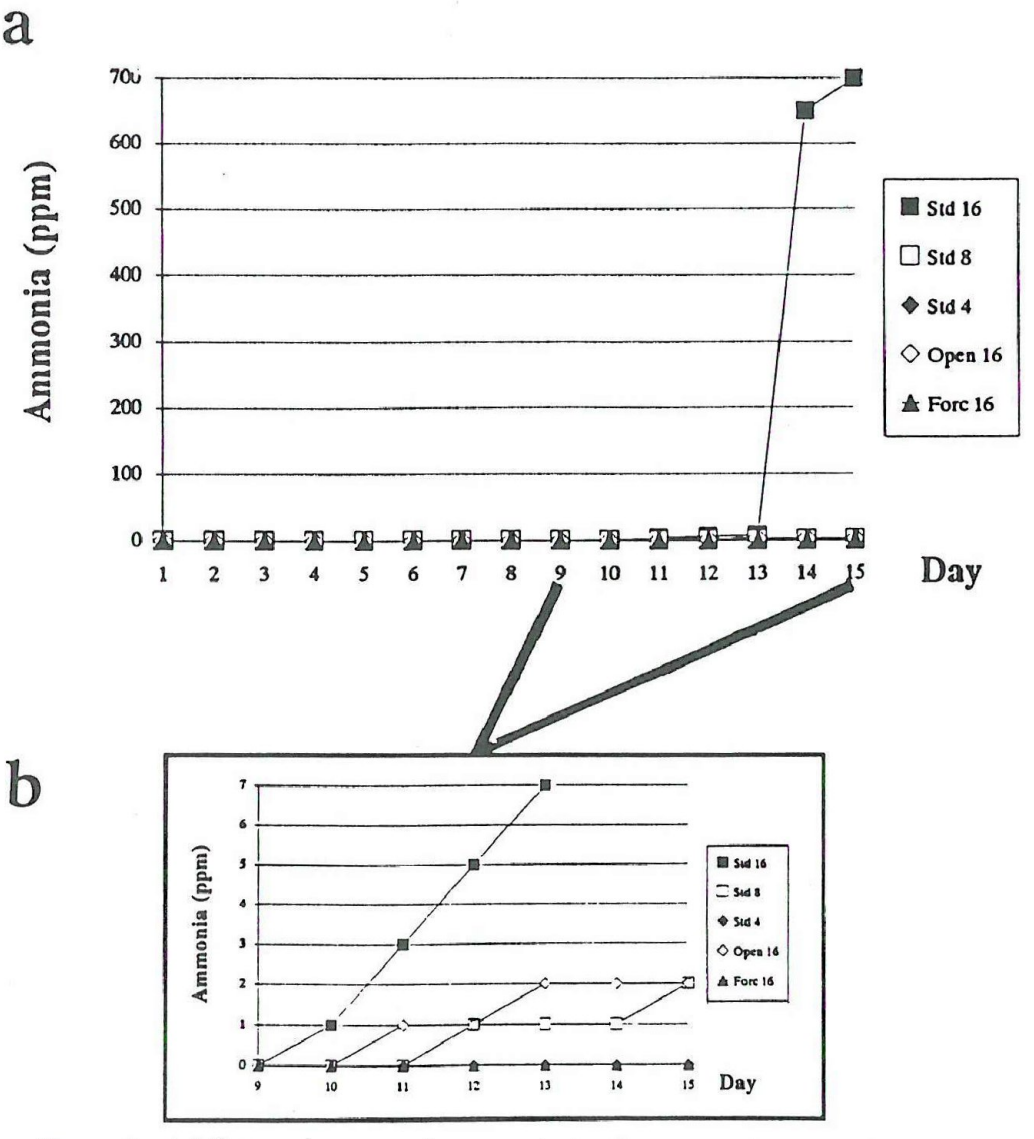

Figure 2. a) Microenvironmental ammonia levels measured once a day during a 15-day period in different caging systems: Standard Micro-Isolator filter top cages (*Std*), open cage without filter top (*Open*), forced-air ventilated cage (*Forc*). Numbers after the symbols in legends denote the number of mice housed in the actual cage. *b)* Ammonia concentrations in the range 0–7 ppm on day 9–15 (expansion of Figure 2 a).

times the recommended upper limit for livestock (3,000 ppm) laid down by the Swedish Board of Agriculture in 1989.

In addition relative humidity levels far in excess of those normally recommended for mice were noted already on day one in the standard filter top cages. *Clough* (1988) stated a RH range of 55 ± 10 % for rat and mouse rooms in a review of published recommendations for environmental requirements of laboratory rodents with special emphasis on housing and animal welfare.

The results of RH and CO_2 measurements of filter top-cages without a forced air system are in accordance with recently published studies by *Corning & Lipman* (1991, 1992) on different filter-top systems, which included the Micro-Isolator filter-top cage system. The same authors compared another commonly used filter cage system provided

with individual fresh air (Micro-FLO, Allentown Caging Equipment Co., Inc., NJ, USA) versus those without (*Corning & Lipman* 1989). Micro-FLO cages housing 5 mice supplied with individual fresh air providing 17–25 changes per hour within the cage, corresponding to about twice as much as registered for the room, still showed higher RH and CO_2 levels than the open control cage and the animal room.

The Vent A Cage System used in the present study utilized about 150 air changes per hour and showed values close to the open control cage throughout a 2-week study without changing the bedding. Comparing Micro-Isolator cages housing 16 animals with and without a forced-air system, the ventilated cage showed values on an average 10,000 ppm lower than the standard filter cage. A possible explanation for those differences in efficiency may be that the Vent A Cage System is based on a concept assuming a much higher air turnover rate at cage level in open standard rodent cages of shoebox type. Based on extensive studies, *Allander & Abel* (1973) reported about 60 airchanges per hour within a cage housing 8 mice at 15 changes per hour in the room. This air turnover rate is higher than those reported by e.g. *Clough* (1988) or *Keller et al.* (1983).

The comparatively late occurring ammonia levels detected in the present study as compared to other studies on the microenvironmental effects of using filter top caging systems (*Lipman et al.* 1991, *Corning & Lipman* 1991, 1992, *Keller et al.* 1983) may be partially explained by the type and quality of bedding used. In a separate study on the effect of different bedding materials and autoclaving procedures of bedding on intracage NH_3 levels, we found ammonia levels starting on day 6 from 50–300 ppm with 10 mice kept on "ordinary" sawdust in an open cage. Furthermore, detectable ammonia appeared earlier on autoclaved hardwood chip bedding compared to non-autoclaved (*Iwarsson & Norén* unpublished results).

Acknowledgements

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The study was performed after approval by the Stockholm Northern Ethical Committee for Animal Experimentation (Application N 293/90).

Summary

The microenvironmental conditions of a commercial rodent filter top caging system was evaluated when housing mice for a 2 week testing period, with an open cage with no filter used as a control. The results were compared to the effect of utilizing a novel developed forced-air ventilated system (Vent A Cage, Barriärteknik, Brösarp, Sweden) for filter cages, delivering filtered air directly to the cage and providing approximately 150 air changes per hour.

In an animal room maintained at 21–22°C, 50–55 % RH and 12–14 airchanges per hour the following experimental settings were evaluated: Micro-Isolator® cages of Macrolon® type III without forced air (Standard), housing 4, 8 or 16 female mice with body weights ranging between 23–28 g were compared to two cages housing 16 mice each: a Micro-Isolator provided with an individual Vent A Cage system (Forced-air) and a cage without a filter lid (Control).

Intracage RH, CO_2 and NH_3 levels were evaluated once a day in each caging system during a 2 week period. Bedding and feed were not removed during the testing period, but water bottles were changed on day 6 and 12. Control measurements of intracage temperature of filter cages showed only small deviations from actual room temperature throughout the study (data not shown).

RH and carbon dioxide levels ranged between 70–100 % and 4,000–17,000 ppm respectively in standard cages with 8 and 16 mice, and between 60–75 % and 2,000–5,000 ppm respectively in the standard cage with 4 mice. The open control and the forced-air cages housing 16 mice showed levels of RH and CO_2 similar to the macroenvironment (animal room); the forced-air system averaged 40–60 % and 1,000–2,700 ppm, respectively.

No appreciable ammonia levels were detected in either system until day 14. In the standard filter cage housing 16 mice the ammonia levels increased from about 7 ppm (traces) on day 13 to 650 and 700 ppm on day 14 and 15, respectively.

The results indicate that housing of $\geq$ 8 adult mice in a Macrolon III Micro-Isolator cage within a day leads to a buildup of humidity and CO_2 levels in excess of recommended levels for mice. The comparatively late occurring ammonia levels detected in the present study as compared to other studies of filter top cages may be explained by the type and quality of bedding used. The forced-air system evaluated provided acceptable intracage

air conditions with 16 mice for two weeks without cage changing and resulted in a remarkable improvement of the microenvironmental conditions as compared to a standard filter top cage.

Sammanfattning
Ett kommersiellt filterbursystem för gnagare undersöktes med avseende på klimatförhållanden i buren vid förvaring av möss under en 2-veckors period, med en standardbur utan filter som kontroll. Resultaten jämfördes med ett nyutvecklat separatventilerat system (Vent A Cage, Barriärteknik, Brösarp, Sverige) för filterburar som genererar filtrerad luft direkt till buren motsvarande ca 150 luftomsättningar per timme.
I ett djurrum med standardiserat klimat (21–22°C, 50–55 % RH och 12–14 luftomsättningar per timme) undersöktes följande bursystem experimentellt: Makrolon® typ III med Micro-Isolator® filter lock av standardmodell med 4, 8 eller 16 honmöss med kroppsvikt varierande mellan 23–28 g, jämfördes med dels en Micro-Isolator®-bur försedd med individuellt Vent A Cage system och dels en konventionell bur utan filterlock (kontroll), vardera med 16 möss.
Halten relativ fuktighet (RH), CO_2 och NH_3 mättes i samtliga burar en gång dagligen under en 2-veckors period. Bäddmaterial och foder avlägsnades inte under testperioden medan filterlocken öppnades för byte av vattenflaskor dag 6 och 12. Kontrollmätning av temperaturen i filterburarna visade endast små avvikelser från aktuell rumstemperatur under studien.
RH- och koldioxidnivåerna varierade mellan 70–100 % respektive 4.000–17.000 ppm i standardfilterburarna med 8 och 16 möss och mellan 60–75 % respektiva 2.000–5.000 ppm i filterburen med 4 möss. Den öppna kontrollburen och den separatventilerade buren, vardera med 16 möss, uppvisade fukt- och koldioxidhalter överensstämmande med djurrummet; i den separatventilerade buren uppmättes i genomsnitt 40–60 % RH och 1.000–2.700 ppm CO_2.
Inga anmärkningsvärda ammoniakhalter detekterades i något bursystem förrän dag 14. I standardfilterburen med 16 möss ökade ammoniakhalterna från ca 7 ppm (spår) på dag 13 till 650 respektive 700 ppm på dag 14 och 15.
Resultaten talar för att förvaring av $\geq$ 8 möss i en Makrolon III Mikro-Isolator®-bur inom ett dygn leder till RH- och koldioxidhalter överstigande rekommenderade värden för möss. De jämförelsevis sent uppträdande ammoniakhalterna i föreliggande studie jämfört med andra studier av filterburar kan eventuellt förklaras av skillnader i typ och kvalité av bäddmaterial. Det separatventilerade system som prövats resulterade i en acceptabel luftmässig burmiljö vid förvaring av 16 möss i 2 veckor utan burbyte och innebar en påtagligt förbättrad burmiljö jämfört med en filterbur av standardtyp utan separat ventilation.

Yhteenveto / K. Pelkonen
Artikkelissa verralaan kaupallisesti saatavissa olevan suodatinkannen, avoimen häkin ja uudelaisen pakkoilmastoidun (150 ilmanvaintoa tunissa) häkkäarjestelmän vaikuluksia makrolon III – häkin mikroilmastoon, kun sinä pidettiin 4, 8 tai 16 naarashärtä. Seuranta-aika oli 2 vikkoa, jonka aikana ei vaihdettu kuivikskeita rai lisätty nuokaa. Havaittiin, etlä 8 tai 16 hiiren suodatinkansihäkeissä kohosivat sekä ilmankosteus etlä hiilidioksidimäärä ylt hiirille suasiteltujen arvojen jo ensimmäisenä päivänä. Neljäntenätoista päivänä alkoi ammoniakkimäärässä lapahtua nousua suodatinkansilaatikossa nän, että sen pitoisuus ilmassa oli 15. päivänä 700 ppm. Nään hidas ammoniakkitason nousu saattoi johtua käytelyn kuivikkeen ominaisuuksista.
Loppupäätekmä tutkitusta uudesta ilmastointijärjestelmästä oli, että se pystyy ylläpitämään hyväksyttävät ympäristöolosuhteet 16 naarashiinelle makrolon III:ssa jopa kaksi viikkoa, vaikka ei kuivikkella vaihdettaisi. Ero lavanomaiseen suodatinkanteen oli kirjoittalien mielestä merkitlävä.

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