# The impact of cage ventilation on rats housed in IVC systems

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## Summary

Today the use of individually ventilated cage systems (IVC systems) is common, especially for housing transgenic rodents. Typically, in each cage a ventilation rate of 40 to 50 air changes per hour is applied, but in some systems even up to 120 air changes per hour is applied. To reach this rate, the air is blown into the cage at a relatively high speed. However, at the animal's level most systems ventilate with an air speed of approximately 0.2 m/s. In the present paper, two studies were conducted, one analysing whether an air speed below 0.2 m/s or just above 0.5 m/s affects the rats, and another study analysing whether air changes of 50, 80 and 120 times per hour affect the rats. In both studies, monitoring of preferences as well as physiological parameters such as heart rate and blood pressure, was used to show the ability of the animals to register the different parameters and to avoid them if possible. Air speeds inside the cage of as high as 0.5 m/s could not be shown to affect the rats, while the number of air changes in each cage should be kept below 80 times per hour to avoid impacts on physiology (heart rate and systolic blood pressure). Also the rats prefer cages with air changes below 80 times per hour if they have the opportunity of choosing, as shown in the preference test.

**Keywords** IVC systems; ventilation; rats; housing; air change; air speed

The first individually ventilated cage systems (IVC systems) were marketed 30 years ago. Today they are in common use, especially for housing transgenic rodents. To keep a proper air quality with low levels of $CO_2$ and $NH_3$ inside the cage, at least 30–40 air changes per hour are needed (Reeb *et al.* 1998, Reeb-Whitaker *et al.* 2001), and a considerable amount of air must be blown into the cages. In unventilated IVC cages, significant rises in $CO_2$ and $NH_3$ levels occur within a very short time (Lipman *et al.* 1992, Krohn & Hansen 2002). Therefore, commercially available systems run at air changes ranging from 25 to 120 times per hour (Huerkamp & Lehner 1994, Perkins & Lipman 1996), and to ensure sufficient air exchange in each cage, the air speed must be relatively high, i.e. between 0.2 and 0.5 m/s at the valve (Lipman *et al.* 1993). The air speed declines with the distance from the valve, but when measured at 'animal level' the air speed is constantly approximately 0.2 m/s, which is normally regarded as draught level for human beings (Lipman 1999). Some systems may ventilate at even higher air speeds due to other principles of design (Wu *et al.* 1985, Corning & Lipman 1992).

So far, all studies on IVC systems have dealt with management, how to prolong the intervals between cage changes and how to

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achieve a high air quality for the animals. No studies have investigated whether the animals are affected by this high performance of ventilation.

Two methods for evaluating the effects of housing conditions have been established and evaluated, and found to be sensitive enough to reveal even small impacts on the animals' physiology and/or behaviour, when housed in conditions that may affect the animals' welfare negatively (Krohn & Hansen 2001, Krohn *et al.* 2003). In the first method the animals' heart rate and blood pressure are registered by the use of telemetry, placing animals with inserted transmitters under different housing conditions. The second method is based upon the traditional preference test, enabling the animal to show which housing conditions it prefers.

In the present paper, two studies were designed: one analysing whether air speeds below 0.2 m/s or just above 0.5 m/s affect the animals, and another study analysing whether air changes of 50, 80 and 120 times per hour affect them; and the impact on the animals was registered by both telemetry and in the preference test.

## Material and methods

### *Cage design*

A cage was specially designed for the evaluation of the effects of air speed (Fig 1). In a Makrolon type III cage (Tecniplast, Italy), 10 holes with a diameter of 0.8 cm were made in each side with a distance of 3.5 cm and 4.5 cm above the bottom. In front of the holes, on the outside of the cage, a PVC tube with a diameter of 4.0 cm opening into the holes was placed. One end of the tube was closed and the other end was connected to a silicone tube 2 m long with a diameter of 1.8 cm. The silicone tubes were connected to a ventilator supplying 50 air changes per hour. The air speed was measured using a Testo400 equipment (Testo GmbH, Germany). With all 20 holes open, the air speed at animal level was less than 0.2 m/s, and with every second hole open the air speed at animal level was 0.5 m/s.

**Fig 1 The specially designed type III cage for use in the air speed studies.** Ten holes were made in each side of the cage. In front of the holes, on the outside of the cage, a PVC tube was placed with an opening into the holes. One end of the tube was closed, and the other end was connected to a silicone tube. The silicone tubes were connected to a ventilator giving 50 air changes per hour. With all holes opened, the air speed at animal level was less than 0.2 m/s, and with every second hole opened, the air speed at animal level was 0.5 m/s

For evaluating the effects of air changes, a special filter top was designed (Fig 2) using a filter top (Tecniplast, Italy) for a type III cage. The filter was replaced with plastic parts, and a special chamber was designed in one half of the filter top. The front of the chamber was equipped with a 5 μm filter with an area of 160 $cm^2$ and the back of the chamber with two connections for silicone tubes 2 m long

**Fig 2 The type III cage with the special designed filter top for use in the air change studies**. The filter was replaced with plastic parts, and a special chamber was designed in one half of the filter top. The front of the chamber was equipped with filter and the back of the chamber with two connections for silicone tubes. The silicone tubes were connected to a ventilator giving 50, 80 or 120 air changes per hour

with a diameter of 1.8 cm. The silicone tubes were connected to a ventilator supplying 50, 80 or 120 air changes per hour.

For both cage designs, ultrasound measurements were conducted, and no ultrasound was registered from the ventilation.

### *Telemetry study*

Eight male 10–14 month Mol:SPRD rats (M&B Ltd, Ll. Skensved, Denmark) weighing 500–600 g were used for each study. The eight rats were pair-housed with eight social companions. One of the rats carried an inserted transmitter (TL11M2-C50-PXT, Data Sciences International, St Paul, Minnesota, USA), while the other one was a social companion without monitoring equipment and was not registered for the study. The transmitter was inserted 6 months prior to the present studies as previously described (Krohn *et al.* 2003). From arrival at the laboratory until the beginning of the study, the rats were pair-housed in type III cages (Tecniplast, Gazzada, Italy), with aspen bedding (Tapvei, Kortteinen, Finland) and fed Altromin 1324 (Brogaarden, Gentofte, Denmark) with water *ad libitum*. Cages were changed twice a week. Lights were on from 06.00–18.00 h with no twilight periods, and air was changed 8–15 times per hour. The room temperature was 21 ± 1°C and the humidity was 50 ± 5%. The set-ups were placed in a Scantainer (Scanbur BK A/S, Denmark) with four receiver plates placed opposite each other on the four shelves in the cabinet. The four plates were connected to a computer outside the cabinet. The ventilator for individual ventilation of the cages was placed on the top shelf inside the Scantainer, with the power supply placed outside. The cabinet was ventilated with external air, 50 times per hour, temperature was 21 ± 1°C and humidity was 50 ± 5%, and the Scantainer was illuminated from 08.00–20.00 h with half-an-hour twilight period. The animals were given one week for acclimatization in the Scantainer before each study. During both studies the cages were equipped with bedding and water/food *ad libitum*. During the studies, data for systolic blood pressure and heart rate were collected at daytime. The days were divided into 24 periods lasting 30 min each.

*Registering the impact of air speed* The rats were pair-housed in the specially designed cages. The number of air changes was 50 times per hour. Four pairs were monitored simultaneously. Data from the animals were recorded for 3 days in an unventilated cage, as this is their normal housing condition and thereby regarded as control values, whereas data from the animals were recorded for 4 days with an air speed of 0.2 m/s and 4 days with an air speed of 0.5 m/s. The study was conducted as a Latin square with two cages at low air speed and two cages at high speed simultaneously.

*Monitoring the impact of air change frequency* The rats were pair-housed in type III cages (Tecniplast, Italy) equipped with the special designed filter top. Four pairs were monitored simultaneously. The animals were recorded for 3 days in an unventilated cage, as a control value, and then for 4 days with 50 air changes per hour, 80 air changes per hour and then 120 air changes per hour. The study was conducted as a continuous study starting with the lowest number of air changes and ending with the highest.

### *The preference study*

Ten Mol:SPRD rats (M&B Ltd, Ll. Skensved, Denmark), i.e. five females weighing 200–300 g and five males weighing 250–400 g, were used for each study. Prior to testing, the rats were housed in four U1400 cages (Tecniplast, Italy) in groups of two or three for at least one week to allow them to acclimatize. Bedding (Tapvei, Finland), wood blocks (Tapvei, Finland) and wood wool (Tapvei, Finland) were used and the cages were changed twice a week. Food (Altromin 1324, Brogården, Denmark) and water were provided *ad libitum*.

The preference test was set up as previously described using two cages interconnected with a tube placed on a computer-logged digital weight (Krohn & Hansen 2001). Two set-ups were placed simultaneously in a Scantainer (Scanbur BK A/S, Denmark) in a separate room with no other animals present and with automatic day/night light shift

(06:00–18:00 h), room temperature at 23±1°C and relative humidity at 45±5%. The room was ventilated 10–15 times per hour and the Scantainer 70 times per hour. Three night-time (18:00–06:00 h) and two daytime (06:00–18:00 h) periods were analysed for each rat in each study.

*Monitoring the impact of air speed* The rats were placed in the preference set-up with a standard type III cage (Tecniplast, Italy) and one of the specially designed cages. First the animals were tested for preferences for low air speed (0.2 m/s) and then for high air speed (0.5 m/s). Between each test the animals were housed for 2 weeks in the normal cage with social companionship.

*Monitoring the impact of air change frequency* The rats were placed in the preference set-up in standard type III cages (Tecniplast, Italy) and with one of the cages in each set-up equipped with the special designed filter top. The preferences for 50, 80 and 120 number of air changes were registered. Between each test the animals were housed for 2 weeks in the standard cage with social companionship.

*Statistical analysis*

The data from the telemetric studies were tested independently by the use of a SAS procedure mixed with a Diggle model (The SAS System version 8, SAS Institute Inc., US). The parameters 'housing' (the housing conditions) were used. In addition, the effect of interaction was tested. As the same rats were tested for all three housing conditions they were tested as the subject in the model. Before using the model the data were tested by plotting the predicted values against the residuals. The null hypothesis was set as there being no effect of housing on the physiological parameters versus the alternative hypothesis, that an effect would be observable.

For the preference studies the results were statistically analysed by the use of a *t*-test (Minitab version 12.1, Minitab Inc., US) testing whether the distribution between the left and right cage was 50/50, as the data are normally distributed. The null hypothesis was set as there being no effect of housing on the preference (a 50/50 distribution between the two cages) versus the alternative hyphothesis, that an effect would be observable.

## Results

The results of the studies of different air speeds are summarized in Figs 3 and 4 and the results of the studies of different number of air changes are summarized in Figs 5 and 6. An overall summary of the results for the telemetric studies for both air speed and air change is given in Table 1.

*The impact of air speed*

For the telemetric study the heart rate was significantly lower when the animals were

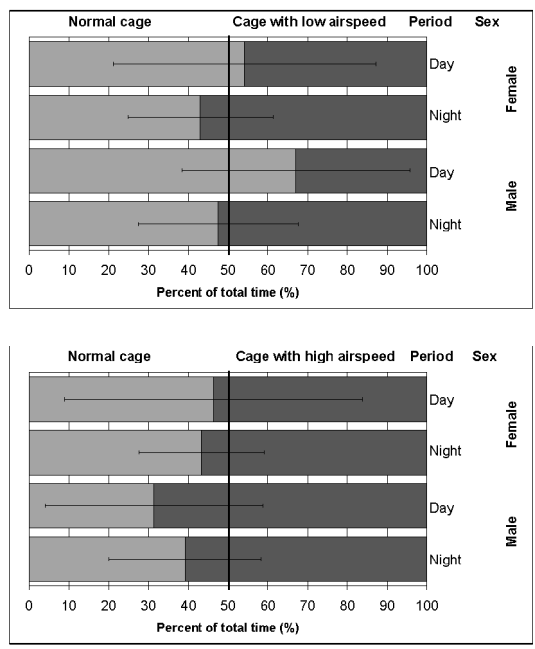
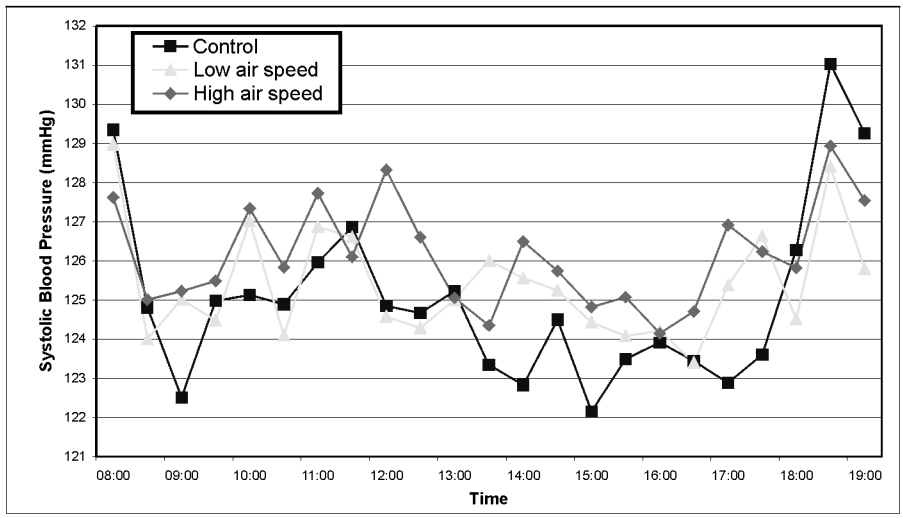



**Fig 3 The results from the preference study on the effects of different air speed**. The upper figure shows the distribution of dwelling time for Mol:SPRD rats between the left and right cages for day and night when the left cage is without any air speed and the right one with an air speed at 0.2 m/s. The lower figure shows the same, but with an air speed at 0.5 m/s in the right cage. Also results for both male and female rats are shown. The 50% distribution is marked with a bold line, and for each result the standard deviation is marked. The figure shows the results for 10 rats, five males and five females, and the same rats are used for both set-ups. No statistically significant differences were shown



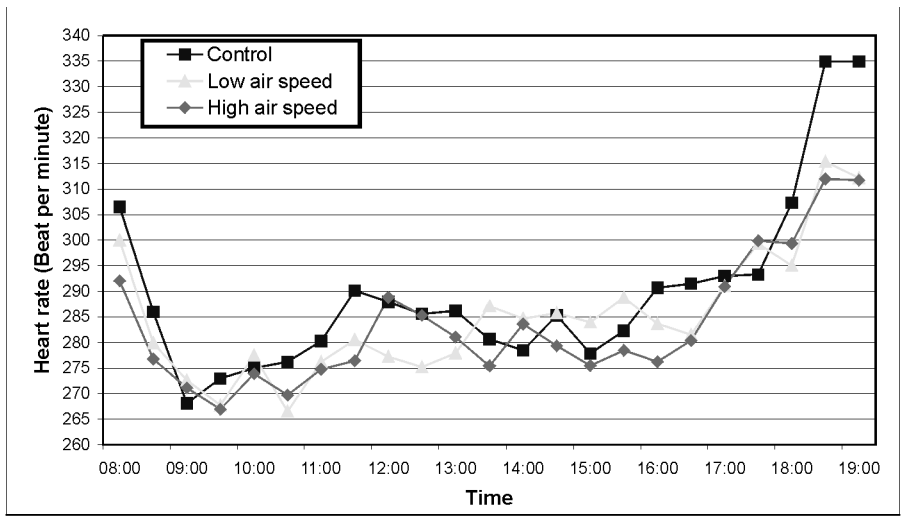




**Fig 4 The results from the telemetric study on the effects of different air speed**. The upper figure shows the systolic blood pressure during daytime when Mol:SPRD rats are exposed to a control period, a period with an air speed at 0.2 m/s, and a period with an air speed at 0.5 m/s. The lower figure shows the heart rate for the same periods. The result from the control period is a mean value for 3 day periods, and the two air speed periods show mean value for 4 day periods. The figure shows the results for eight rats with the same rats used for all three periods. The systolic blood pressure was significantly higher at high air speed compared to the control ($P < 0.0014$), and the heart rate was significantly lower for both air speeds compared to control ($P < 0.001$)

exposed to either 0.2 m/s or 0.5 m/s air speed (for both $P < 0.001$), whereas the systolic blood pressure was significantly higher for the set-up with air speed at 0.5 m/s ($P < 0.0014$). Irrespectively of sex and time of day, none of the rats showed any preferences in relation to different air speeds.

### *The impact of air change frequency*

In the telemetric study the heart rate was significantly lower when the animals were exposed to any kind of air change ($P < 0.0001$), whereas the systolic blood pressure compared with the control was significantly higher when air was changed

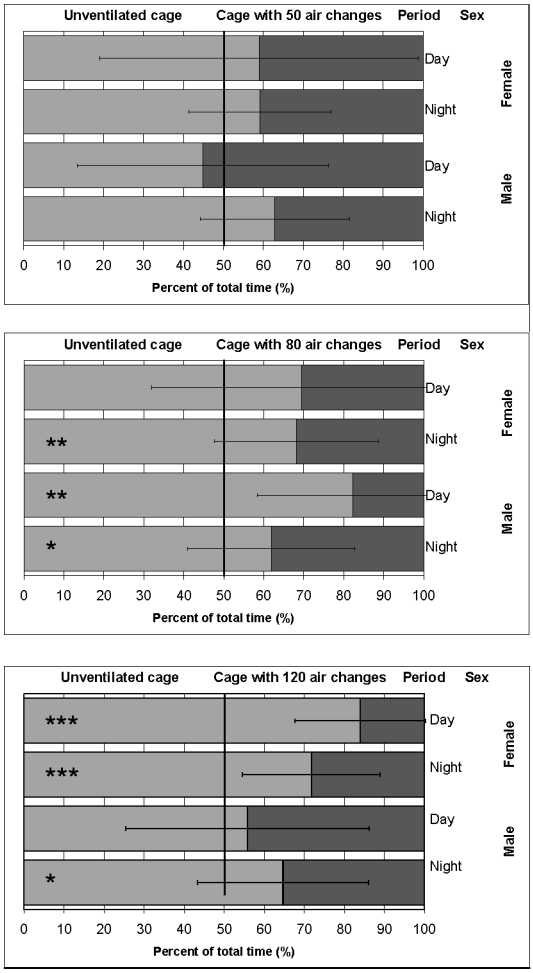



**Fig 5 The results from the preference study on the effects of different air changes**. The upper figure shows the distribution of dwelling time for Mol:SPRD rats between the left and right cages for day and night when the left cage is without any air changes and the right one with 50 air changes per hour. The middle figure shows the same, but with 80 air changes per hour, and the lower figure with 120 air changes per hour. Results for both males and females are shown. The 50% distribution is marked with a bold line, and for each result the standard deviation is marked. The figure shows the results for 10 rats, five males and five females, and the same rats are used for all set-ups. Any statistical significance is marked with * (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

80 times or 120 times ($P < 0.0001$) and lower when air was changed 50 times ($P < 0.0001$). There were no preferences for the cage with 50 air changes per hour compared to the unventilated cage, whereas there was a significant preference for the unventilated cage compared to the cage with 80 air changes per hour for the males at day ($P < 0.002$) and night ($P < 0.05$) and for the females at night ($P < 0.004$), but no preference for the females during daytime. For cages with 120 air changes per hour the males showed a significant preference for the unventilated cages at night ($P < 0.019$), while the females showed a significant preference at both day ($P < 0.0001$) and night ($P < 0.0002$).

The variation in the telemetric study did not differ significantly between the groups, i.e. differences in ventilation conditions neither increased nor decreased the variance observed when using heart rate or systolic blood pressure as a parameter.

## Discussion

In the present study it was possible to show that an air change frequency of more than 50 times per hour has an impact on the animals, while it was not possible to show any effects of the air speed on either the physiology or the animals' preference for the cages.

In another study on the impact of different housing conditions, the changes in the systolic blood pressure and the heart rate as a response to stressful housing conditions were found to be 6–7% (Krohn *et al.* 2003), whereas the changes in the present study are found to be 0.5–3.0%. Furthermore, the heart rate decreased in this study, while we previously found it to be increased by stressful conditions (Krohn *et al.* 2003). The changes in heart rate and systolic blood pressure might have been caused by the improvement in air quality in the cages. Systolic blood pressure and heart rate were both significantly influenced by the air change frequency as well as by the air speed, but we consider these too small to be biologically significant, although they are statistically significant.

The males will mark their territory with urine, which contains pheromones telling the male and other males that this area is occupied. The absent male preference for the unventilated cage during daytime compared

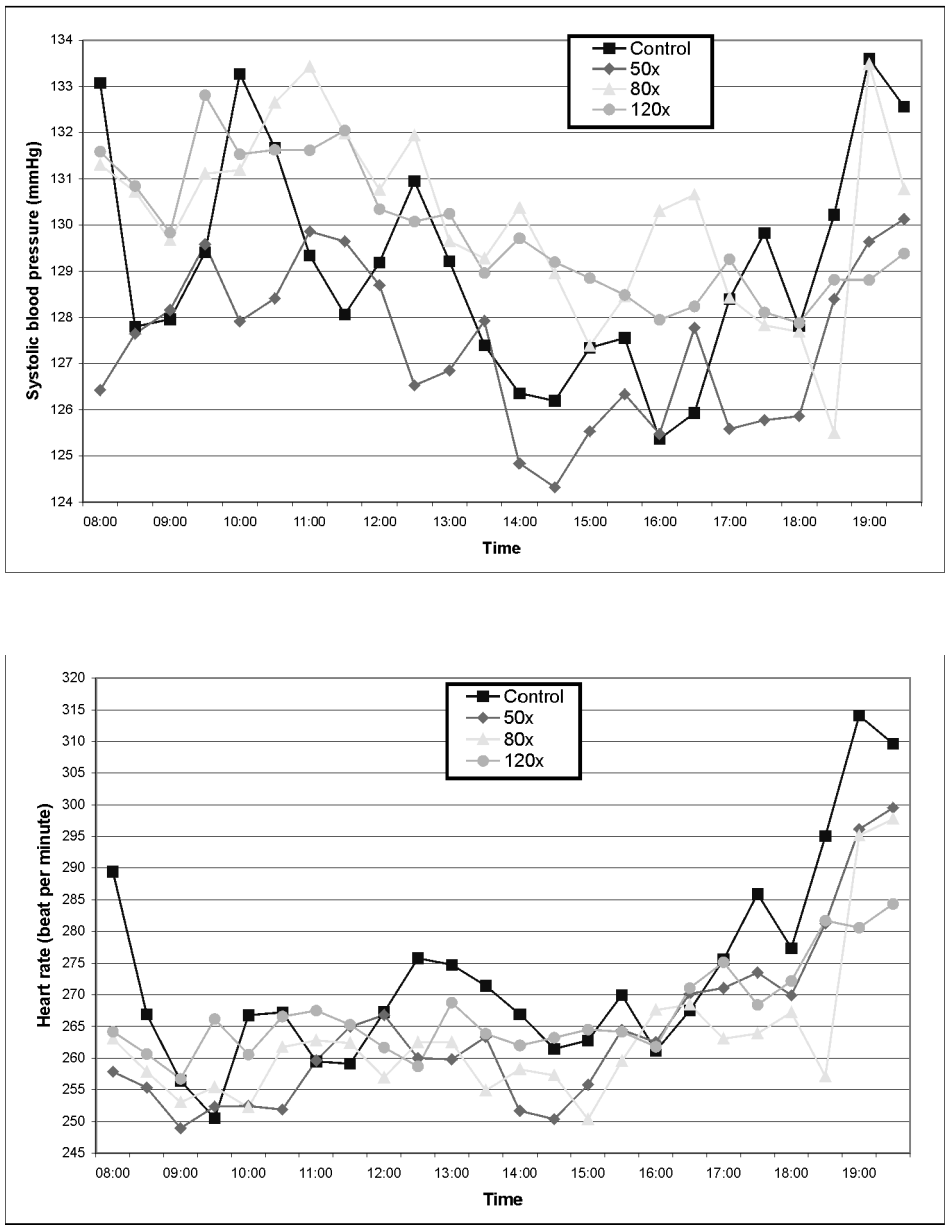



**Fig 6 The results from the telemetric study on the effects of different air changes**. The upper figure shows the systolic blood pressure during daytime when Mol:SPRD rats were exposed to a control period, a period with 50 air changes per hour, a period with 80 air changes per hour, and a period with 120 air changes per hour. The lower figure shows the heart rate for the same periods. The result from the control period is a mean value for the 3 day periods, and the three air change periods shown are the mean value for the 4 day periods. The figure shows the results for eight rats with the same rats used for all four periods. The systolic blood pressure was significantly lower at 50 air changes compared to the control ($P < 0.0001$), and significantly higher for 80 and 120 air changes compared to the control ($P < 0.0001$). The heart rate was significantly lower for all air changes compared to the control ($P < 0.0001$)

**Table 1 The overall results from the telemetric studies for both the air speed study and the air change study**

| | Mean values for air speed study | | | Mean values for air change study | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.2 m/s | 0.5 m/s | Control | 50 times | 80 times | 120 times |
| Systolic blood pressure (mmHg) | 125.0 ± 2.3 | 125.4 ± 1.4 | 126.1* ± 1.3 | 129.1 ± 2.4 | 127.4* ± 1.7 | 130.1* ± 2.0 | 129.8* ± 1.4 |
| Heart rate (beats/min) | 289.8 ± 17.1 | 283.5* ± 12.4 | 285.2* ± 12.5 | 273.0 ± 15.8 | 264.2* ± 13.2 | 263.0* ± 11.4 | 267.1* ± 7.2 |

The values are means ± SD for the whole period for the different set-ups. The systolic blood pressure is shown as mmHg and the heart rate as beats per minute. Any significant differences compared to the control values are marked with *. All significant differences are with $P < 0.001$

to the cage with 120 air changes per hour could eventually be due to removal of such marking from the ventilated cage. Although the rats are supposed to rest during the daytime, they may spend more time keeping the scent marking fresh in the ventilated cage, explaining the preference for presence in this cage.

For most commercial systems today, there are no problems keeping air speeds below 0.5 m/s, as most systems have air speeds at animal levels of around 0.2 m/s (Lipman 1999). One way to reduce the air speed at the animal level is to make the inflow area as large as possible, i.e. the larger area the lower the air speed. However, the air will be less uniformly distributed and inadequately turned-over in the cage if the air speed becomes too low.

Previous studies have shown that an air changing frequency of at least 30–40 times per hour is required to keep the cage free of $NH_3$ (Reeb *et al.* 1998, Reeb-Whitaker *et al.* 2001), and the present study shows that the air changing frequency must be kept below 80 times per hour to avoid any negative impact on the animal. So, to keep proper air quality inside the cages and to secure high standards of animal welfare the number of air changes should be kept at a level of around 50 times per hour.

The present study could not reveal if housing in IVC systems should increase or decrease the group variation on the selected physiological parameters. Housing in IVC systems may influence the variance on other physiological or behavioural parameters beside the ones measured in the present study, and any conclusion about that must be drawn when other studies dealing with the problem have been conducted.

In conclusion, a ventilation air speed in IVC cages of up to 0.5 m/s may be acceptable, while the air change frequency should be kept below 80 times per hour to avoid any negative impacts on rats. As mice are also frequently maintained in IVC systems, further studies should be made to reveal whether these assumptions are also valid for this species.

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## References

Corning BF, Lipman NS (1992) The effects of a mass air displacement unit on the microenvironmental parameters within isolator cages. *Laboratory Animal Science* 42, 91–3

Huerkamp MJ, Lehner NDM (1994) Comparative effects of forced-air, individual cage ventilation or an absorbent bedding additive on mouse isolator cage microenvironment. *Contemporary Topics in Laboratory Animal Science* 33, 58–61

Krohn TC, Hansen AK (2001) Weighing as automatic registration of preferences when testing rats. *Scandinavian Journal of Laboratory Animal Science* 28, 223–9

Krohn TC, Hansen AK (2002) Carbon dioxide concentrations in unventilated IVC cages. *Laboratory Animals* 36, 209–12

Krohn TC, Hansen AK, Dragsted N (2003) Telemetry as a method for measuring impacts of housing conditions on rats. *Animal Welfare* 12, 53–62

Lipman NS (1999) Isolator rodent caging systems (state of the art): a critical view. *Contemporary Topics in Laboratory Animal Science* 38, 9–17

Lipman NS, Corning BF, Coiro MA (1992) The effects of intracage ventilation on microenvironmental conditions in filter-top cages. *Laboratory Animals* 26, 206–10

Lipman NS, Corning BF, Saifuddin MD (1993) Evaluation of isolator caging systems for protection of mice against challenge with mouse hepatitis virus. *Laboratory Animals* 27, 134–40

Perkins SE, Lipman NS (1996) Evaluation of microenvironmental conditions and noise generation in three individually ventilated rodent caging systems and static isolator cages. *Contemporary Topics in Laboratory Animal Science* 35, 61–5

Reeb CK, Jones RB, Bearg DW, Bedigian H, Myers DD, Paigen B (1998) Microenvironment in ventilated animal cages with differing ventilation rates, mice populations, and frequency of bedding changes. *Contemporary Topics in Laboratory Animal Science* 37, 43–9

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 in ventilated cages. *Laboratory Animals* 35, 58–73

Wu D, Joiner GN, McFarland AR (1985) A forced-air ventilation system for rodent cages. *Laboratory Animal Science* 35, 499–504