



## Method developments and assessments of animal welfare in IVC-systems

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# **Method developments and assessments of animal welfare in IVC-systems**

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PhD.-thesis**

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## **Preface**

The different studies in this PhD.-project have been carried out from May 1999 until April 2002, and the project is the result of an industrial research project (Erhvervsforskeruddannelse) between Scanbur BK A/S, The Royal Veterinary and Agricultural University in Copenhagen, and the Academy of Technical Sciences (ATV).

The PhD.-project has been a worthwhile experience for me, and has provided me with many new skills, and improved some other skills considerably.

First of all I want to thank Axel Kornerup Hansen for your initial beliefs in me and my skills and for making a positive contact to the management of Scanbur BK A/S and the Academy of Technical Sciences. Also thanks for your always excellent supervising and fast and kind response to my questions or problems.

Then a thank-you to my other two supervisors, Henrik Møllegård and Bo Salling, for your supervising in technical and marketing problems, especially Bo for being very helpful in the development of the technical set-ups used in the study.

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At the Royal Veterinary and Agricultural University, Division of Laboratory Animal Science and Welfare, a thank-you to all, especially Vera Østvedt, Klaus Switon and Lennart Kurland for helping me with the animal and laboratory skills, and to all my fellow PhD. and master students for nice and pleasant company throughout the years.

And to all other people I have met and shared some time with during the last three years.

And finally a thank-you to my family, specially to my father, Christian, for introducing me to animal welfare and for giving me useful pieces of advice during the project, and to my wife, Pernille, for your love, patience and helpful attitude.

Thomas C. Krohn, Copenhagen, 1<sup>st</sup> of May 2002.

## Publications related to present project

- Article I** : Krohn TC, Hansen AK, Dragsted N (2003) Telemetry as a method for measuring impacts of housing conditions on rats. *Animal Welfare* **12** (1) **In Press**.
- Article II** : Krohn TC, Hansen AK (2001) The application of traditional behavioural and physiological methods for monitoring of the welfare impact of different flooring in rodents. *Scandinavian Journal of Laboratory Animal Science* **In Press**.
- Article III** : Krohn TC, Hansen AK (2001) Weighing as automatic registration of preferences when testing rats. *Scandinavian Journal of Laboratory Animal Science* **28**, 223-229.
- Article IV** : Krohn TC, Hansen AK, Dragsted N (2002) The impact of cage ventilation on rats housed in IVC-systems. *Laboratory Animals* **Submitted**
- Article V** : Krohn TC, Hansen AK, Dragsted N (2002) The impact of low levels of Carbon Dioxide on rats. *Laboratory Animals* **Submitted**
- Article VI** : Krohn TC, Hansen AK (2002) Carbon Dioxide Concentrations in Unventilated IVC Cages. *Laboratory Animals* **36**, 209-212.

All the articles are printed in the last part of the present thesis. **II**, **III** and **VI** are printed in the versions which have been published, whereas **I**, **IV** and **V** are printed in manuscript-version, and the journals are free to make minor editorial changes before the papers are published in the journals.



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## Introduction



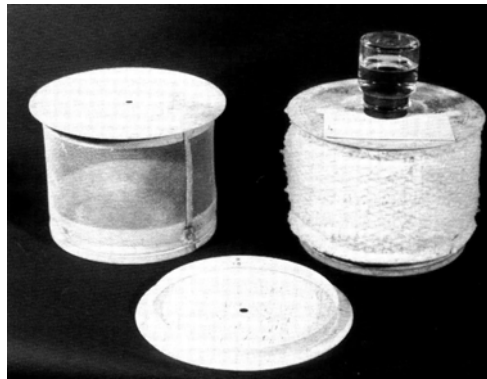


## The Individually Ventilated Cage Concept

The use of individually ventilated cage systems (IVC-systems) started thirty years ago, and today they are common in use, especially for housing transgenic rodents. In the following the history of IVC-systems will be described, starting with the development of the static filter top cage. Also the advantages and disadvantages using IVC-systems and how this will affect animal welfare will be discussed.

### *The history of the IVC-systems*

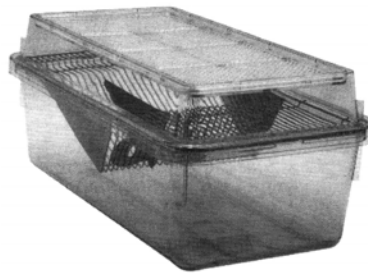
The IVC-era was founded in the late fifties with the development of the first static filter top cage. Dr. Lisbeth Kraft was doing research on rotavirus, the cause of epidemic diarrhoea in mice. To prevent the spread of the virus to the surroundings, she developed a metal cylinder with wire mesh walls wrapped in fibreglass insulation and with metal top and bottom (Figure 1). Inside the cylinder were bedding, water and food for the mice.



**Figure 1: The first filter top cage developed in 1958 by Dr. Lisbeth Kraft.** A metal cylinder with wire mesh walls wrapped with fibreglass insulation and a metal top and bottom.

This cage effectively protected the environment against the virus, as well as protected the mice inside the cage against other infections. In the sixties the filter top was further developed to fit a normal shoebox cage, but still fibreglass insulation was used as filter medium.

The filter top became a success in research, and disadvantages, if any, using the filter top were not considered. In the sixties most animal facilities installed various technological equipment for regulation of temperature, humidity and air quality, but the environment inside the filter top cage could not be controlled. In the late sixties and early seventies the first measurements on the microenvironment inside the filter top cages were conducted (Murakami 1971, Serrano 1971, Simmons et al 1968). They revealed that the filter top has great effects on intra-cage temperature, humidity and gasses such as CO<sub>2</sub> and NH<sub>3</sub>. The temperature inside the cage was 1-2 °C higher than the surrounding temperature, and the relative humidity 10-15% higher than that of the room. The CO<sub>2</sub>-level was 10 times higher in a cage with filter top compared to an open cage, and during the animals' active period CO<sub>2</sub> could reach 0.8 %. Also the NH<sub>3</sub> level was higher in a filter top cage compared to an open cage, as NH<sub>3</sub> levels up to 400 ppm were measured. In spite of these effects on the cage environment the static filter top is still in use today, but in a more practical design. In 1980 Robert Sedlacek invented a new type of static filter top fitting the shoebox cage with a more practical filter media than the fibreglass insulation (Figure 2). The filter top was placed on top of the cage with an overhang along the cage edge, like the principle used in the petri dish. Most static filter tops today are based on that design.



**Figure 2: The static filter top invented by Robert Sedlacek in 1980, which gave the principle for most modern filter tops.**

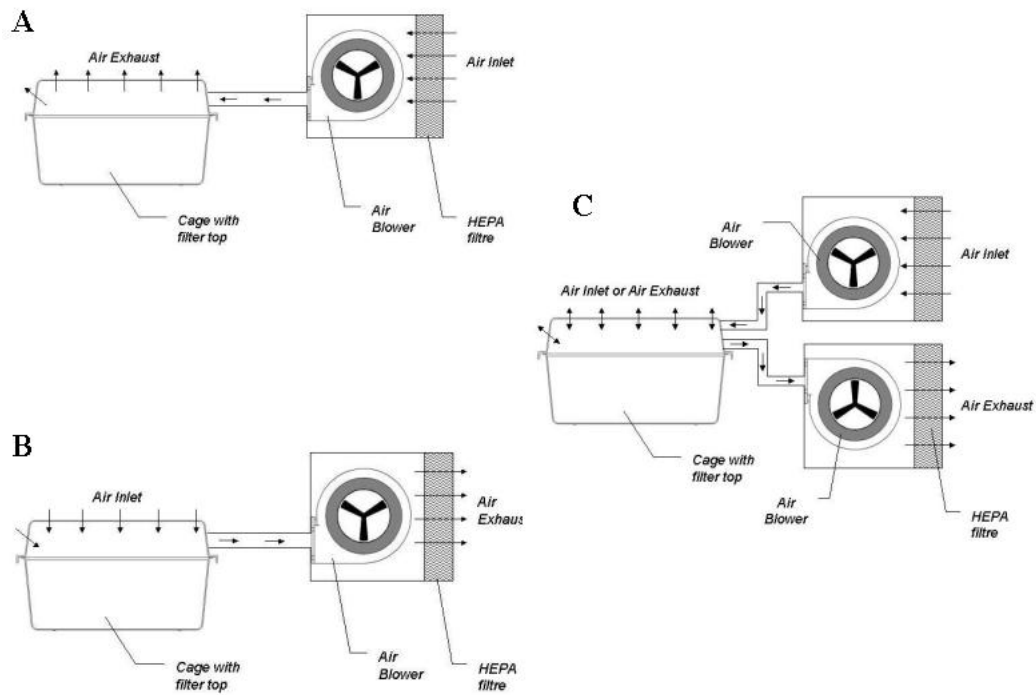
For improvement of the environment inside the filter top cage, each cage can be ventilated with clean fresh air. Although a spontaneous air change between the cage and the environment is taking place, it is not enough to secure the air quality inside the cage. In the early eighties the first system equipped with individually ventilated filter top cages became commercially available, and in 1985 the word “microisolator” was accepted as a common name for a filter top cage. Today, individually ventilated filter top cages are widely used for protection of animals and/or staff in the animal facility. In the following only IVC-systems using filter top cages will be discussed, although there

are other IVC-systems on the market using other barrier principles beside the filter top for protection of animals, e.g. MADU (mass air displacement unit), PIV (pressurized individually ventilated) cages, ventilated cabinets etc.

For the IVC-systems using filter top cages three different ventilation principles are used (Figure 3). The first type of ventilated filter top cage was equipped with one ventilator ventilating air into the cage diffusing it out through the filter top (Figure 3A). This type of cage has a positive pressure inside to that of the room, giving high protection to the animals inside the cage. Several experiments have shown that a cage running in positive mode is able to protect the animals against infections (Clough et al 1995, Lipman et al 1993, McGarrity & Coriell 1973, Mrozek et al 1994). When the cage is ventilated the microclimate is improved considerably as gasses ex. CO<sub>2</sub> and NH<sub>3</sub> are removed effectively and the bedding is dried out, reducing the growth of bacteria producing NH<sub>3</sub> (Corning & Lipman 1992, Huerkamp & Lehner 1994, Keller et al 1989, Lipman et al 1992, Perkins & Lipman 1996). In the nineties laboratory animal allergy came in focus and reduction of allergens in the room became an important issue. Setting the pressure in the cage on negative to that of the room the allergens can be kept inside the cage (Figure 3B). A ventilator ventilating air out of the cage and air diffusing in through the filter top giving a negative pressure. This keeps the allergens inside the cage and prevents infectious agents spreading from infected animals. With the cage kept on negative pressure the release of allergens is reduced significantly (Renström et al 2001, Sakaguchi et al 1990). Today most systems are using two ventilators, one ventilating air into the cage and one ventilating air out of the cage (Figure 3C).

Normally one of the ventilators is ventilating more air than the other, resulting in either a small positive pressure or a small negative pressure. This means that only a small amount of air is diffusing either in or out through the filter top. An example can be given: If ventilator A is ventilating 20 m<sup>3</sup> per hour and ventilator B 15 m<sup>3</sup> per hour an air amount of 5 m<sup>3</sup> will diffuse through the filter top. Using a system with two ventilators reduces the amount of air diffusing directly into or out of the cage by 70-80 percent depending on the settings of the ventilators.

For keeping the animals protected against infections, the inlet air must be ventilated through a HEPA-filter, which is able to clean the air from infectious agents with an efficiency of up to 99.97% (Mrozek et al 1994). Also, to prevent spread of allergens from the animals to the room, the exhaust air can be ventilated through a HEPA-filter or ventilated directly out of the room.



**Figure 3: The three different ventilation principles used for IVC systems with filter top cages.** A) One ventilator is ventilating air into the cage and diffusing it out through the filter top. Inside the cage is a positive pressure to that of the room. B) One ventilator is ventilating air out of the cage and air diffusing into the cage through the filter top. Inside the cage is a negative pressure to that of the room. C) One ventilator is ventilating air into the cage and one ventilator is ventilating air out of the cage. Depending on which of the ventilators ventilating most air, the pressure inside the cage can be either positive or negative to that of the room, as the air is diffusing either in or out of the cage through the filter top. See text for more details.

## ***The use of IVC-systems***

IVC-systems are common in many laboratories and animal facilities today, and maybe 10-20% of the European facilities house rodents in these systems today according to the companies selling these systems. Especially for transgenic rodents the IVC-systems are found useful as a supplementary protection of the animals, but in Scandinavia IVC-systems are also used to reduce release of allergens, and thereby preventing laboratory animal allergy among the staff. There may be some advantages (Table 1) and disadvantages (Table 2) using IVC-systems.

**Table 1: The advantages using IVC-systems**

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<b>Improved protection</b>	By using IVC-systems instead of conventional open cages, the protection of the animals improved considerably especially when running at positive pressure, and if the system is running in negative mode the protection of the staff against allergens will be improved.
<b>Protection at rack level</b>	As the inlet air, when the system is running in the positive mode, is HEPA filtrated, it is possible to have a protection at rack level compared to the conventional system where the protection is at room level.
<b>Improved micro climate</b>	Due to the high number of air changes in the cage, the microclimate is improved compared to a conventional cage. There are no build up of CO <sub>2</sub> and NH <sub>3</sub> , and temperature and humidity are kept on the same level as that of the room.
<b>Prolonged periods between cage changing</b>	As the cage is highly ventilated the period between each cage change can be longer. When there is no build up in humidity, the bacteria growth will be reduced and thereby the production of NH <sub>3</sub> limited. This means that the need for changing the cage is reduced.

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**Table 2: The disadvantages using IVC-systems**

<b>Health monitoring problems</b>	In IVC-systems it is more difficult to perform health monitoring, as each cage is protected against the environment. It is not logical to have a sentinel cage in the rack, as these animals are not in contact with the rest of the animals in the rack, neither physically nor through the air.
<b>Cage change problems</b>	To maintain a high level of protection cages need to be changed in special benches or in special areas of the room equipped with Laminar Air Flow (LAF). Even if the cage change does not take place in some kind of LAF-unit, it is more time-consuming to make cage changes in IVC-systems compared to open cages as each cage has a lid that must be opened before access to the animals.
<b>Requires constant ventilation</b>	The cage requires constant ventilation, as the spontaneous air change is reduced in the modern IVC-cage due to improved filter media and tighter sealing. The animals' respiration causes a rise in CO <sub>2</sub> concentration and will, if not removed quickly, reach harmful levels.

### ***The welfare aspects of using IVC-systems***

In the following some welfare consequences of the use of IVC-systems will be listed and discussed.

*Positive impact of IVC-systems on animal welfare:* Being protected from diseases is a welfare improvement compared to being exposed to diseases. When animals are housed in IVC-systems it is possible to keep them free from diseases (Clough et al 1995, Lipman et al 1993). Welfare is improved if the animal is protected from gasses such as NH<sub>3</sub>, which is toxic and CO<sub>2</sub>, which affects the physiology of the animals (Krohn & Hansen 2000). The ventilation secures fresh air inside the cage and thus prevents exposure to these harmful gasses. Ventilation also removes humidity in the bedding caused by urine or water and thereby keeping the bedding dry. Wet bedding promotes bacterial growth, which may lead to diseases or formation of NH<sub>3</sub>.

*Negative impacts of IVC-systems on animal welfare:* To ventilate each cage effectively a considerable amount of air must be ventilated into the cage. To ensure sufficient air change in each cage the air speed must be relatively high (between 0.2 and 0.5 m/sec) at the valve (Lipman et al 1993). This may cause draft inside the cage, affecting the animals in a negative way. In addition the high number of air changes may dry out the cage environment, and may cause more aggression -

especially when males are housed together (Gray & Hurst 1995). If the cage environment dries out it may affect the animals' pheromone marking used to signal the intra-cage hierarchy. Although there is a welfare improvement when the cage is ventilated, the welfare may be reduced when the cage is left as many of the IVC-cages are very tight and if not ventilated, an increase in the level of CO<sub>2</sub> and NH<sub>3</sub> may take place very quickly. This may affect the animals' welfare in a negative way as these gasses affect the animals' physiology.

## **Welfare**

The welfare term has become an important and well-established part of modern laboratory animal science and research. Today, animal welfare is considered before setting up an animal experiment. There are two reasons for this; Firstly, improved conditions for the animals are reflected in the experimental results: as an animal with good physiological and psychological state better deals with the experimental procedures. Secondly, it will give positive public relations for the company or research institute. With the increased public focus on laboratory animals this is very important, as there are many national and international organisations that invoke the right to protect the animals and abolish the use of these animals.

But to improve the animal welfare one must be able to define welfare and the factors that affect it, and set up methods for monitoring it.

### ***Definition of welfare***

The definition of welfare can be seen from three different points of view, the hedonism, the perfectionism, and the preference point of view.

*The hedonism point of view:* Welfare is the net sum of the good and the bad feelings experienced during an animal's life (Simonsen 1996). To consider welfare from this viewpoint, one must be able to assess whether the different things the animal experiences throughout its life are regarded as good or bad by the animal. Also, one must be able to give these experiences a weight in the animal's life. The difficulty is to determine how the animal regards and weights the situation; it may not be the same way as we do as human beings. Another problem with the hedonism point of view is using the net sum; the animal may have a good welfare even though it may suffer in its last part of life. The good part in the greater part of its life compensates for this suffering.

*The perfectionism point of view:* Welfare is the animal's attempts to cope with its environment and maintain homeostasis (Broom 1991a). The animal is challenged throughout its life, and it tries to cope physiologically and/or behaviourally with these challenges. As long as the animal is able to cope with the situation homeostasis is maintained, but if it fails to cope, homeostasis is disturbed and welfare may deteriorate (Broom 1991b). However, it is difficult to detect when the homeostasis

is disturbed, and thereby whether the welfare is reduced. It takes different measurements of physiological and behavioural parameters, e.g. hormone levels, weight gain, reproduction performance, and behavioural changes. Some of these parameters are difficult to measure and it may be a problem to detect small changes (Mason & Mendl 1993).

*The preference point of view:* Welfare is the animal's ability to make its own free choices in life and do whatever it wants to do (Jensen & Sandøe 1997). In reality, to give an animal a totally free choice is almost impossible to fulfil as options necessarily need to be limited. There are many factors deciding what an animal wants here and now, and these preferences may change very suddenly according to the animal's physical state, the surroundings, other animals etc.

The three different viewpoints presented above do not represent absolute beliefs or definitions in the welfare debate, but should be regarded as three different views each representing a way of looking at welfare. It is not representing a religious view, saying that one must adhere to strict hedonism or perfectionism, but rather should it be regarded as a draft giving different views of how welfare may be evaluated.

In the present thesis the welfare will be considered in principal lines from a perfectionism point of view, as I find this one the most appropriate in its explanation of how the animals deal with a stressor, but there may be some influence from the other two points of view, as none of these beliefs are absolute.

### ***What affects the welfare***

Factors such as housing conditions, the physical state of the animal, other animals, experimental procedures etc. may affect the welfare of the animals. To minimize this, the UK Ministry of Agriculture, Fisheries and Food in 1992 developed a guideline with five freedoms to use for farm animals, which, however, is so universal, that it may be used for laboratory animals as well (Table 3).

**Table 3: The five freedoms (Broom & Johnson 1993)**

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Freedom from hunger and thirst – by ready access to fresh water and a diet to maintain full health and vigour
Freedom from discomfort – by providing an appropriate environment including shelter and a comfortable resting area
Freedom from pain, injury or disease – by prevention or rapid diagnosis and treatment
Freedom to express normal behaviour – by providing sufficient space, proper facilities and company of the animal's own kind
Freedom from fear and distress – by ensuring conditions and treatment which prevent mental suffering

---

Obviously, although following the five freedoms solely does not entirely ensure welfare of the animal trying to fulfil them, much negative impact may be eliminated. The five freedoms deal mainly with parameters caused by housing conditions or basic conditions. Effects from the experimental procedures or special housing conditions are not controlled or eliminated by fulfilling the five freedoms.

The most common parameters affecting the welfare in addition to housing conditions are disease, social interaction, or the animal is placed in a situation it cannot cope with (Broom 1993). Some of these may affect the welfare due to change in the fitness and other due to change in the physiological parameters.

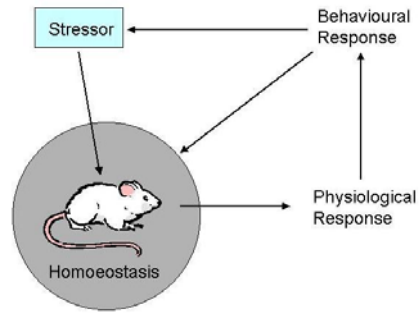
If an animal has a disease, e.g. such caused by infections, its welfare may be affected. An infection will have a major effect on the animal and many different physiological parameters are mobilised, including those used to maintain the animal's welfare. An infected animal is not very suitable for use in experimental procedures. Firstly, the animal may react unpredictably on the experimental procedure due to the infection circulating in the body. Also the infection may cause symptoms on the animal's physiology e.g. the circulation, the respiration or the digestive system. Secondly, an infected animal may have a reduced fitness and thereby a reduced welfare (Broom 1993). If the animal's fitness is reduced, the experimental procedures may challenge the animal more than expected and the animal may have more difficulty coping with the experimental procedures, and thereby reducing the animal's fitness additionally. Therefore, it is important to house the animal in an environment, which is as protective as possible. This is one of the reasons why the IVC-systems

have been so successful in the last decades, as these systems are able to protect the animals at rack or cage level against airborne infections.

Social contact may affect welfare both positively and negatively (Dantzer 1990). For a social animal being single housed may be very stressful. Rodents are by nature social animals and a considerable part of the natural behavioural repertoire is social behaviour. Therefore, if a rodent is single housed, the natural behavioural repertoire is reduced and consequently the welfare (Hurst et al 1997, Hurst et al 1998), as it is important for an animal to be able to perform its natural behaviour.

Social housing may also reduce welfare. Being a member of a group may be problematic and a challenge to the welfare. Being the weakest in the group, the one the others attack and chase is difficult, and this animal's welfare may be very poor, and in the worst case it may lead to death (Koolhaas et al 1997). This will especially be the case when housing non-social animals together e.g. male rabbits.

If the animal is placed in a situation it cannot cope with, the welfare is affected. The animal will get stressed and the fitness reduced. Stress might be defined as *an environmental effect on an individual, which overtaxes its control systems and reduces its fitness or appears likely to do so* (Broom & Johnson 1993), i.e. the more stressed the animal, and the poorer it's welfare. Irrespective of the stressor (e.g. housing conditions or pain), the animal is affected in a similar way. The stressor onsets different cascades of hormone pathways in the animal, affecting the physiology, e.g. heart rate, body temperature and immune system, leading to a behavioural response, which either eliminates the stressor or reduces the animal's fitness causing a change in the homeostasis. When measuring and evaluating the animal's welfare, these changes or effects are analysed. Figure 4 gives a schematic overview of the effects of a stressor.



**Figure 4: The stress-reaction used in present paper.** In an undisturbed animal the organism is in homoeostasis, which means all physiological and behavioural values are at a basal level. If a stressor affects the animal, the homoeostasis is disturbed leading to a physiological response. This response will lead to a behavioural response, e.g. the animal moves away from the stressor, and thereby eliminates the effects from the stressor. If the animal is not able to eliminate the stressor through a behavioural response, the animal's homoeostasis will be changed, causing a physiological stress response.

## The aim of present study

For evaluation of welfare of rodents housed in IVC-systems, different parameters must be analysed. These parameters are related to the ventilation of each cage. As the impact on animal welfare may be small, the methods used to evaluate the parameters must be very sensitive and able to reveal even small changes in welfare. The usual methods for welfare evaluation may not be that sensitive, so the methods must be evaluated and new methods for measuring animal welfare may have to be developed.

Therefore the thesis is divided in two sections. In the first, methods for evaluating animal welfare are analysed, and in the second, evaluation of different parameters in relation to IVC-systems and their effects on animal welfare are measured.

### *Methods for measuring animal welfare*

A number of different methods for evaluation of animal welfare have been developed over the years as reviewed in an earlier paper (Krohn et al 2001). Some of these methods are based on physiological analysis, while some are based on behavioural analysis. Combining the physiological and behavioural methods the welfare should reveal the most precise analysis of welfare.

The sensitivity of the following methods were tested and analysed in the first part of the thesis:

- Physiological methods
  - Telemetry, plasma corticosterone and barbiturate sleeping time
- Behavioural methods
  - Open field test and preference test

A physiological and a behavioural method were chosen for further use in the analysis for the effects of the IVC-system on animal welfare.



*Evaluation of different factors in relation to IVC-systems*

Some of the aspects from the use of IVC-systems have been analysed since the first systems were marketed in the seventies. The microclimate inside the cages has been analysed and evaluated (Baer et al 1997, Corning & Lipman 1991, Hasenau et al 1993, Iwarsson & Norén 1992, Lipman et al 1992, Perkins & Lipman 1996) in different types of systems, with different stocking density and with different types of bedding. Also the preferable number of air changes have been found (Reeb et al 1998, Reeb-Whitaker et al 2001) to ensure acceptably low levels of NH<sub>3</sub>.

In the thesis some of the factors that may cause welfare problems when the animals are housed in IVC-systems were analysed:

- Draught, measured as air speed is, in general, between 0.2 m/s up to more than 1.0 m/s, as each cage is ventilated and the air is entering the cage at animal level with different speed depending on the principle used. The air speed level considered draught for humans is given as 0.2 m/s (Lipman 1999), but it is unknown whether rodents react to higher speeds.
- Air changes, especially the high levels used in IVC-systems, may affect the animals.
- CO<sub>2</sub> may rapidly rise in an unventilated cage, and it may affect the animals.

It was analysed whether an air speed below 0.2 m/s and an air speed just above 0.5 m/s, air changes of 50, 80 and 120 times per hour, and CO<sub>2</sub> levels at 1, 3, and 5% affect the animal physiologically or ethologically. The time to reach unacceptable CO<sub>2</sub> levels in various and differently designed commercially available IVC-cages was also analysed.

## Development of methods for measuring welfare





## **How to monitor animal welfare – State of the art**

The welfare can be measured and evaluated in two different ways, either by physiological or behavioural methods. Neither of the ways is giving an exact measure of the welfare, but by combining two or more methods, it is possible to get reliable results, that provide information about the welfare.

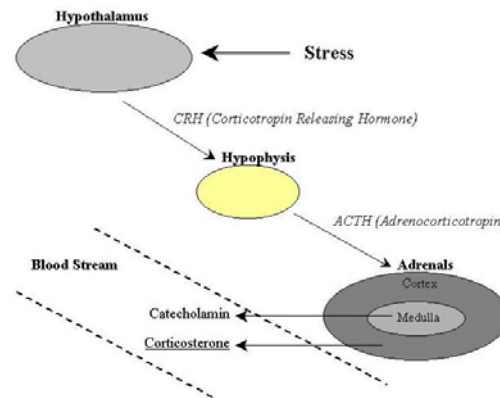
### ***Physiological ways of measuring welfare***

*Telemetry.* Telemetry means measuring from a distance, and that is exactly what is done. A small sensor is placed on or inside an animal and the sensor is via a receiver placed outside the animal's environment transmitting information about the animal's physiological state to a computer. The first implantable sensor was developed in 1986, and since then the technology has evolved, and today the sensors are small enough to be placed inside the abdomen of a laboratory mouse. An overview of the development in the telemetry sensors is given in a previous paper (Kramer et al 1998). Usually, the transmitter is placed in the abdomen of the animal and a small catheter is placed in the aorta. The surgical procedure is described in details in previous papers (Kramer et al 1993). Depending on the size and type of sensor, each sensor is able to measure one or more of the following physiological parameters: ECG, heart rate, blood pressure, body temperature, and activity. The results from these physiological investigations can be interpreted to give some information about the animal's reactions to certain factors. If the animal's welfare is affected, heart rate, blood pressure and temperature may change, and depending on the type of stressor and time of day these changes will be more or less distinct (Mason & Mendl 1993).

*Corticosterone.* When an animal is housed or treated unpleasantly, the animal's physiology will be affected. When stress is affecting an animal a cascade of hormones is being released, with a final release of corticosterone/cortisol (depending on the species) into the blood stream (Figure 5). The level of corticosterone varies over the day, and has a peak in the morning (Kilgour 1983). Corticosterone is very sensitive and even minor influences will lead to immediate release to the blood stream. Due to this high sensitivity, the use of corticosterone for measuring stress is very common and has been used for years for many different species and studies (Peng et al 1989, Sachser & Kaiser 1997, Tuli et al 1995, Verde & Piquer 1986, von Borrell & Ladewig 1992). Corticosterone is used as an indicator for acute stress, as the level in the blood stream rises within a

few minutes after exposure to the stressor. As a consequence, blood samples must be taken very quickly, within two minutes, as handling and restraint of the animal otherwise will affect the results. The high sensitivity of corticosterone means that most studies fail to prove stress by the use of corticosterone. It is only possible to measure the serum concentration of corticosterone, and it is difficult to distinguish between the natural level and the level caused by the stressor. The best way of measuring stress by the use of corticosterone would be measuring the immediately release from the adrenal glands when the animal is exposed to the stressor.

The use of corticosterone levels for measuring long-term stress is more controversial (Brown & Grunberg 1995), as stress-induced serum corticosterone increases will adapt and gradually return to normal level with time (Pitman et al 1988). If using corticosterone levels as indicator for long-term stress, repeated samples should be used and compared. Corticosterone levels from experimental animals must be compared to control animals, which has been identically treated beside the experimental factor.



**Figure 5: The hormone cascade caused by stress and leading to release of corticosterone into the blood stream.** A stressor is affecting the animal, thereby inducing the release of a releasing hormone (CRH) from the hypothalamus, which via the portal vessel is transported to the hypophysis, thereby inducing the release of adrenocorticotropic hormone (ACTH) from the hypophysis to the circulating blood. As the ACTH reaches the adrenals, corticosterone is released from the adrenal cortex to the blood stream.

*Barbiturate sleeping time.* The amount of liver cytochromes P-450 and other liver enzymes may indicate stress in the animal. Hormones (e.g. corticosterone and other stress hormones) are cleared from the blood by the liver and decomposed by the liver cytochrome P-450 complex, which also decomposes barbiturates and other foreign chemical components in the organism (Dairman &

Balazs 1970). The amount of liver cytochromes P-450 complex can be measured indirectly via the barbiturate sleeping time, which may be inversely correlated to stress (Dairman & Balazs 1970, Lovell 1986). Therefore the more hormones the liver has to remove from the blood, the higher amount of liver cytochromes P-450 complexes, and consequently the shorter barbiturate sleeping time. The method is not common for measuring stress and welfare in animals, although the method has been known and developed since the late sixties. Especially to evaluate the effects of being single housed compared to group housed, barbiturate sleeping time has been used, showing that the sleeping time was almost halved when rodents were housed singly compared with in groups (Dairman & Balazs 1970, Einon et al 1976).

### ***Behavioural ways of measuring welfare***

*Open field test.* An animal placed in an unfamiliar situation will react differently whether it is stressed or undisturbed. The open field test is one of the oldest behavioural tests developed in the thirties and has been used almost unchanged since (Walsh & Cummins 1976), although many variations over the traditional open field test is used today. The idea with the open field test is to analyse, how an animal reacts when placed in an unfamiliar situation. In general, an animal can only be tested once in the arena, although some prefer additional tests (Denenberg 1969). The parameters of interest are activity, indicated by the number of lines crossed in the arena, latency, indicated by time from start to first move, and then different behaviours, such as rearing, grooming, and defecation. An unstressed animal will react by performing high level of activity and exploration, whereas a stressed animal will show higher latency and less active behaviour (Quartermain et al 1996). Differences in species, strains, sexes etc may lead to major differences in the open field test (Dahlborn et al 1996, Prior & Sachser 1995, van-de-Weerd et al 1994), consequently complicating comparisons between different types of animals.

*The preference test* registers what the animal wants here and now. The preference test has been widely used for many different species to evaluate whether an animal prefers one set-up to another (Blom 1993, Held et al 1995, Hughes 1976, Manser et al 1995). Two or more cages are connected and in each cage is a different option. By registering in which cage the animal prefers to be, it is possible to decide, what option the animal wants here and now. Different preference test set-ups have been designed for evaluation of housing conditions for rodents (Baumans et al 1987, Chmiel & Noonan 1996, Manser et al 1995, Patterson-Kane et al 2001). Commonly, the activity is recorded

on video and analysed afterwards, or the set-up is equipped with micro switches detecting the animal entering and leaving each cage. The preference test is also one of the most controversial tests used in the animal welfare analysis, and its advantages and disadvantages have been discussed in several previous papers (Fraser et al 1993). (Dawkins 1976, Dawkins 1983, Duncan 1978, van Rooijen 1982)

**Table 4: The results from the discussion about the preference test**

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The animal cannot consider and evaluate the long-term consequences of the choice it makes. An animal will always make a here-and-now choice e.g. a rat may choose eating a very high-fat diet leading to obesity, although having the free choice of a low-fat diet.

The preferred option may simply be the lesser of two evils, as the animal must be present in either one of the cages, whether it prefers the option given or not.

The animal is only able to make a choice between those few options given, meaning that it is impossible to allow the animal a totally free choice.

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Keeping these reservations in mind (Table 4), the preference test may, however, be a very useful tool for evaluating different housing conditions, as long as overall conclusions are drawn in connection with other behavioural and physiological tests. Normally, only one animal at a time can be tested in the preference set-up. This is a very unnatural situation, especially for gregarious animals, such as mice and rats. Alternatively, groups of animals may be tested at the same time, but interpretation is difficult, as the dominant animal may influence decisions of the subordinates.

## **Methods for measuring welfare in the present study**

The methods for measuring animal welfare have been evolved and developed in the last century. Most of the methods have been made for evaluation of specific situations or set-ups, and then later on improved or changed for use in other situations.

Many of the methods are giving strong results in specific situations, while they in other situations are unable to reveal any effects in the experimental situation given. Corticosterone and barbiturate sleeping time show great effects when testing effects of social isolation in rodents (Brown & Grunberg 1995, Dairman & Balazs 1970, Einon et al 1976, Hurst et al 1997, Hurst et al 1998), whereas the open-field test is very useful when testing the impact from different kinds of enrichment (Eskola & Kaliste-Korhonen 1998, Kaliste-Korhonen et al 1995).

### ***Why evaluation and development of new methods***

In the present study different parameters related to ventilation of the cages will be tested and these parameters may not have that great impact on the animals compared to other parameters related to housing conditions. As the IVC-systems have been in use for the last thirty years without evidence of obvious symptoms of reduced welfare such as reduced breeding performance, pathological changes, increased mortality (Huerkamp et al 1994), this may indicate that welfare problems related to these systems are only minor. However, animals unable to cope satisfactorily with their environment may not necessarily show increased mortality or obvious pathological symptoms (Broom 1986). This challenges the methods used for analysing the welfare for the animals housed in IVC-systems, as the methods must be able to reveal even small impacts on the animals' physiology, behaviour and welfare.

So, starting from the established methods, each of the methods will be tested for their ability to reveal effects on the animals when they are exposed to a given stressor. Those methods that do not show any effects will not be used in the present study whereas those that reveal an effect will be used and eventually improved to be more suitable for measurements on the present parameters.

To add something new to the field of animal welfare measurements it can be necessary to look into other disciplines to see if there should be methods that could be improved and used for measuring different impacts on the welfare (ex. telemetry). It is important to try to develop new methods all



the time, as most of the established methods have their limitations and may not fit the present study completely, which may lead to use of less suitable methods giving weaker or misleading results.

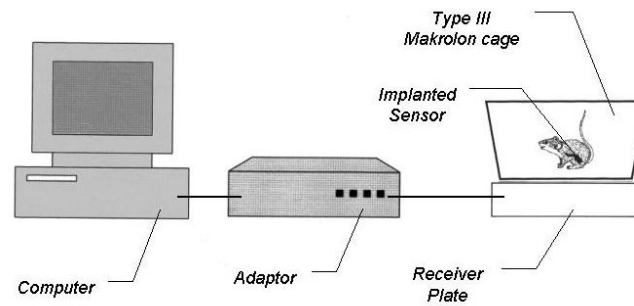
### ***Selection of stressor***

A stressor is defined as the environmental factors, which lead to stress (Broom 1984). To evaluate the methods in a standardised way a simple and standardized stressor related to housing conditions had to be selected, as animals react differently to a stressor depending on whether stress is caused by housing conditions, handling, pain or other stressful conditions (Ladewig et al 1993). Based upon known effects of different housing conditions housing on grid floor was the chosen as a suitable stressor. Preference tests have shown that given the opportunity rodents avoid sleeping on grid floors (Blom 1993, Manser et al 1995), i.e. grid floors may be stressful or at least unpleasant for the animal. Grid floors are very easy to use and the grid floor inlet for the cages is standardised, so the impact on the animals will be the same every time tested. A method suitable for registrations of reactions to housing conditions such as those caused by IVC-housing should also reveal some impact on animals housed on grid floors. If this is not the case, the chosen method may not be sensitive enough to register and reveal small impacts on the animal, which would be needed for studying the impact of less stressful parameters, such as ventilation and levels of different residuals in the environment.

### ***The set-ups used in present study***

*Telemetry.* In the telemetry set-up eight rats were used for testing the impact of different flooring, bedding, plastic or grid as schematically shown in

Figure 6. Data were collected both day and night and heart rate, systolic blood pressure, diastolic blood pressure, and body temperature were analysed. The animals were monitored for 72 hours and the data analysed after separated in day and night periods (**I**).

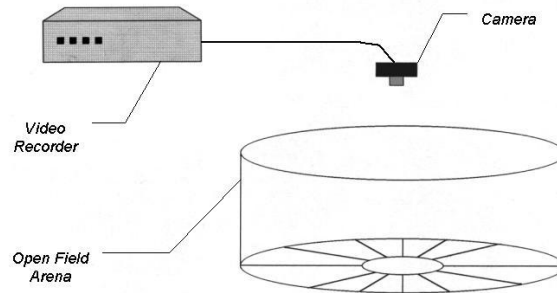


**Figure 6: The telemetric set-up used for present project.** A rat with an implanted sensor is housed in a cage placed on a receiver plate that receives the radio signals transmitted by the sensor. The receiver is sending the results through an adaptor and finally to a computer, where the data are stored and analysed. Depending on the sensor, information about the animal's heart rate, blood pressure, ECG and body temperature can be registered.

*Corticosterone.* Serum levels of corticosterone were tested in a set-up with 24 mice housed on bedding, plastic or grid for three weeks. During the study blood samples were collected from the animals every week. The blood samples were hereafter analysed and the amount of corticosterone determined by the use of a  $^{125}\text{I}$  RIA Kit (II).

*Barbiturate sleeping time.* This old method for indirect measurement of corticosterone and thereby stress was used for evaluating impact of different flooring. Thirty-two rats were housed on bedding or grid for a period of time. Then they were anaesthetised in a barbiturate, and the time from injection of barbiturate until return of interdigital reflexes were measured (II).

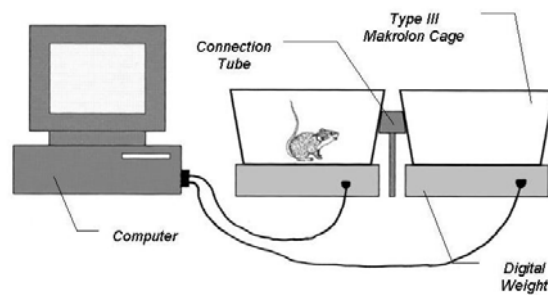
*Open field test.* In order to evaluate the sensitivity of the open field test for registering impacts of different housing conditions twenty-four mice were housed on bedding, plastic or grid for three weeks, and then tested in an open field arena (Figure 7) for five minutes and recorded on video for subsequent analysis (II).



**Figure 7: The open field set-up used in present study.** A circular arena divided into an inner circle and an outer circle divided into twelve subfields. Around the arena is a plastic wall. Above the arena a video camera recording all activities in the arena and sending the signal to a video recorder is placed. Afterwards the video can be analysed.

*The preference test.* The principals for the preference test were in the present study changed from registration by the use of small sensors to registration by the use of digital weights (

Figure 8). Thirty rats were used for evaluation of the preference test. The rats' preference for two identical cages was tested, as well as their preference for grid versus bedding (III).



**Figure 8: The preference study set-up used in present study.** Two cages are each placed on a digital weight and a computer is registering the weight of each cage as an indicator of in which cage the animal is present. The two cages are interconnected with a tube.

### ***The results from the validation of the different methods***

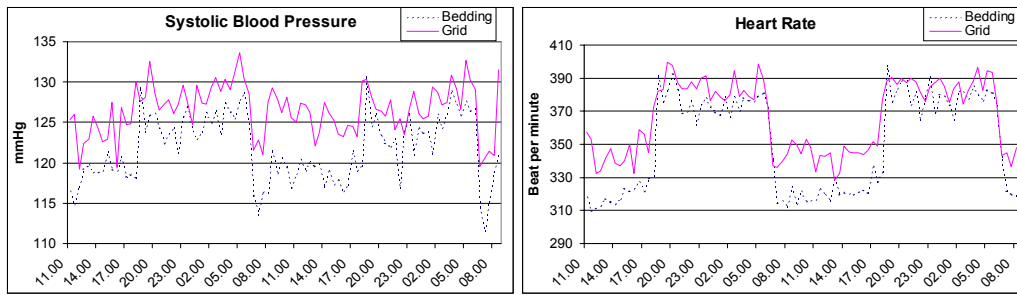
The results from validation of all the methods are summarized in the following table (Table 5).

**Table 5: Results of validation of methods for testing impacts of housing conditions equivalent to the difference between housing on grid and solid floors.**

<b>Method</b>	<b>Testing</b>	<b>Animal</b>	<b>Significance</b>	<b>Reference</b>
<i>Pulse and blood pressure registered by telemetry</i>	Bedding Grid Plastic	Rats	Significant difference	I
<i>Serum corticosterone</i>	Bedding Grid Plastic	Mice	Non-significant difference	II
<i>Barbiturate sleeping time</i>	Bedding Grid	Rats	Non-significant difference	II
<i>Open field test</i>	Bedding Grid Plastic	Mice	Non-significant difference	II
<i>Preference test</i>	Bedding Grid	Rats	Significant difference	III

*Barbiturate sleeping time, corticosterone and open field test.* None of these methods were found to be sensitive enough to reveal impacts on the animals caused by changes in housing conditions equivalent to the difference between solid and grid floors.

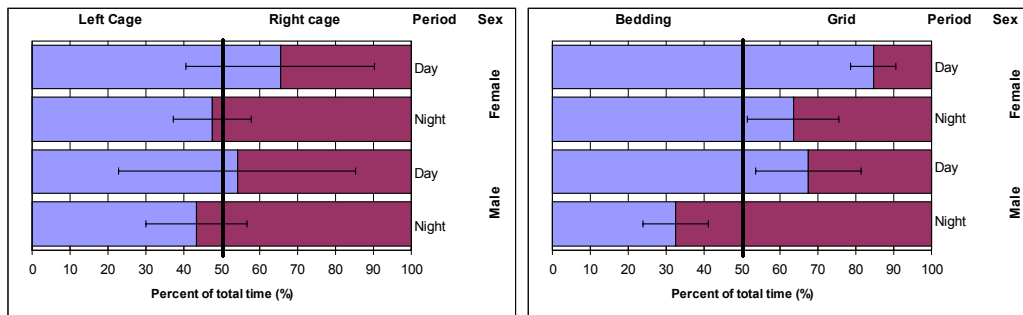
*Telemetry:* In the telemetric study it was possible to reveal an impact on the heart rate and systolic blood pressure when the rats were housed on either grid or plastic compared to bedding. An effect was shown both day and night, but the effects were most evident during daytime when the rats were resting. Daytime values of heart rate and systolic blood pressure were 6-7 % higher when housed on grid or plastic than values when housed on bedding (Figure 9).



**Figure 9: The results for the telemetric study showing the systolic blood pressure and the heart rate when the rats were housed on bedding or grid (I).**

*Preference test.* The preference test was further developed from the traditional test used in previous studies, so by placing the cages on digital weights connected to a computer, it was possible to use the weight of each cage as an indicator of the preference. For evaluation of the test the rats were given the free choice between a cage with bedding and a cage with grid. The result confirmed earlier studies showing that the rat would choose to rest on bedding, and if possible avoid grid floor (Figure 10). The results also confirmed the telemetric data showing a physiological impact when the rats were housed on grid.

When testing the preference, the standard deviation should turn out to be rather large, if the two options are equally preferable by the animal, as the animal must necessarily be inside one of the cages, and probably have a preference for one of the cages although they are identical. So, if one animal prefers the left cage and another animal prefers the right cage, the standard deviation will be very large when analysing data on group level. On the other hand, if the animal must choose between two different options, the standard deviation should turn out to be minor, as the animals in the same group should prefer the same cage.



**Figure 10: The results from the validation of the preference set-up.** The left figure showing the rats' preference when giving two identical options, and the right figure showing the rats' preference when giving the choice between bedding and grid floor (III).

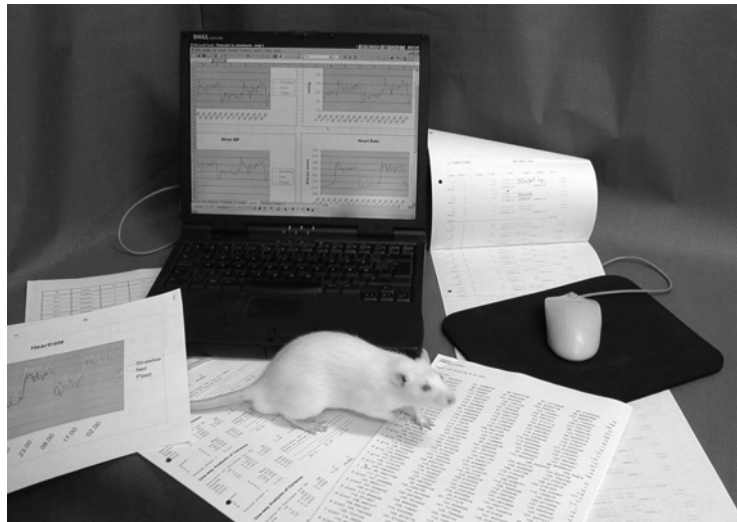
### ***The conclusion from the validation of the different methods***

As only two of the five methods tested revealed effects from the selected stressor, these two methods were used further to evaluate the effects of different factors in relation to individual ventilation of cages.

Although the three other methods were not found sensitive enough for evaluating the impact from different housing conditions, these methods may still be suitable for measuring stress and welfare under other conditions, as types of stress and the impacts of stressors differ.



## Evaluation of impacts on welfare in IVC-systems





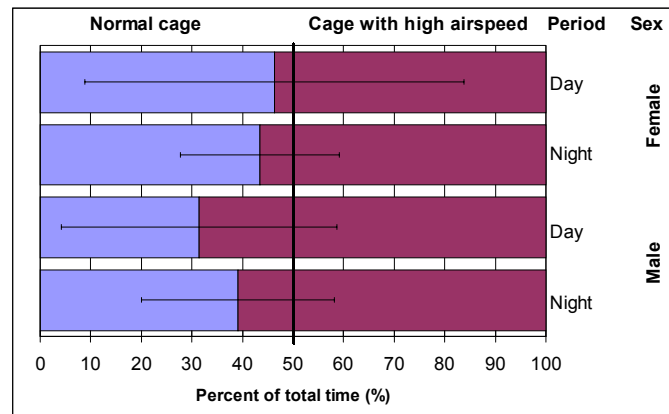


## Evaluation of impacts on welfare in IVC-systems

### *Impact of different air speeds*

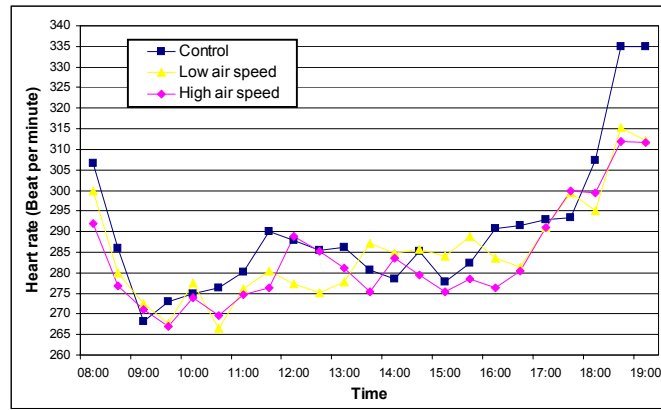
A study was designed to reveal the impacts of different air speeds at animal level. Air was blown into the cage at animal level with a speed of either 0.2 m/s or 0.5 m/s. The cage was designed to ensure uniform air speeds in the entire cage to minimize variation (IV).

In the preference study both male and female rats choose equally between the two cages both at day and night (Figure 11), and consequently no effects of air speed could be revealed.



**Figure 11: The results from the preference study on the effects of air speeds at 0.5 m/s.** The distribution of dwelling time for Sprague Dawley rats in the left and right cage is shown for day and night when the left cage is without any air speed and the right one with an air speed at 0.5 m/s. The 50% distribution is marked with a bold line, and for each result the standard deviation is marked (IV).

In the telemetric study the heart rate was significantly lower when the animals were exposed to either 0.2 m/s or 0.5 m/s air speed (Figure 12), whereas the systolic blood pressure was significantly higher for air speeds at 0.5 m/s.



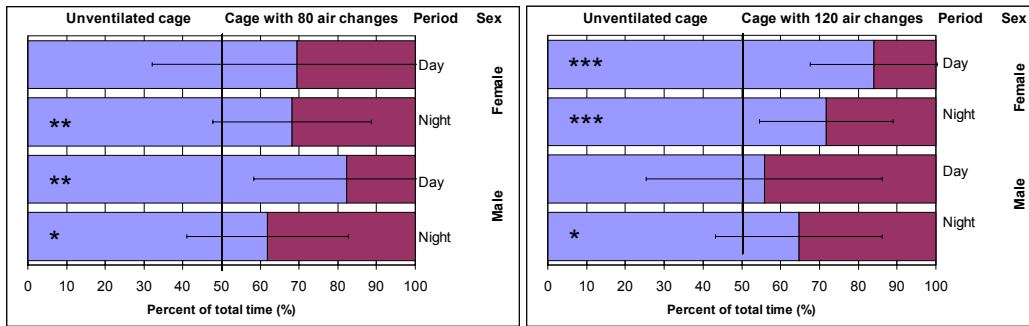
**Figure 12:** The results from the telemetric study on the effects of different air speeds on heart rate. The heart rate during daytime when the rats are exposed to a control period, a period with an air speed at 0.2 m/s, and a period with an air speed at 0.5 m/s is shown with the results for eight male rats with the same rats used for all three periods (IV).

The preference study was completed both at day and night, whereas the data from the telemetric study was only collected during daytime. In the preference study both male and females were used, while in the telemetric study only males were used.

### ***Impact of differences in air change***

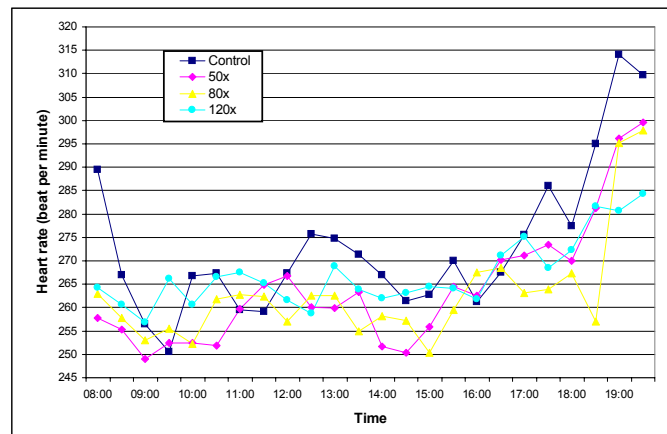
To evaluate the impact of number of air changes it is important that the air is entering the cage without causing draught, and therefore a special filter top was designed to give this opportunity. The number of air changes tested was 50, 80 and 120 times per hour to study whether this would affect the animals or not (IV).

In the preference test no effects were seen at 50 air changes per hour, whereas there was a significant preference for the unventilated cage at 80 changes per hour, except for the females at day, and at 120 changes per hour, except for males at day (Figure 13).



**Figure 13: The results from the preference study on the effects of different air changes.** The left figure shows the distribution of dwelling time for Sprague Dawley rats between left and right cage for day and night when the left cage is without any air changes and the right one with 80 air changes per hour. The right figure shows the same, but 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. Statistical significance is marked with \* ( $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ) (IV).

In the telemetric study there was a significant effect of all air changes on both systolic blood pressure and heart rate (Figure 14).



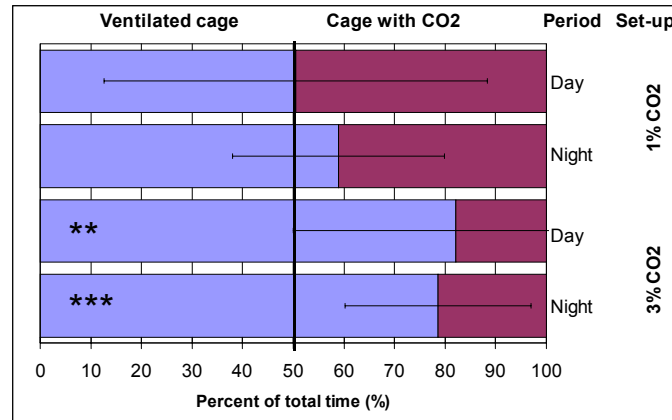
**Figure 14: The results from the telemetric study on the effects of different air changes.** Heart rate during daytime when the rats are exposed to a control period, a period with 50 air changes per hour, a period with 80, and a period with 120 air changes per hour is shown with the results for eight male rats with the same rats used for all three periods (IV).

The preference study was sampled both at day and night, whereas data in the telemetric study only were sampled during daytime. In the preference study both males and females were used, while in the telemetric study only males were used.

### Impact of different CO<sub>2</sub>-levels

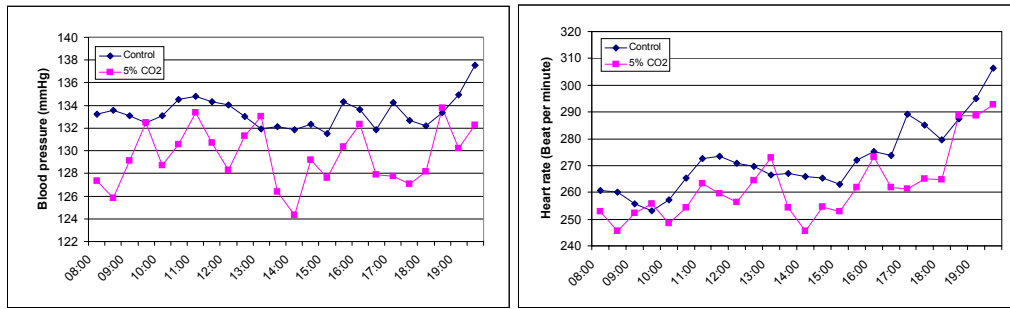
A study was designed to reveal the impact of different levels of CO<sub>2</sub> on the animals. CO<sub>2</sub> was added to the air used to ventilate the cage, and in the preference test 1% or 3% CO<sub>2</sub> was added, and in the telemetric study 3% or 5% CO<sub>2</sub> was added.

Evaluation of the impact of CO<sub>2</sub> revealed, that the animals avoid 3% CO<sub>2</sub> having the opportunity, whereas there are no preferences for either of the cages if the level is 1% (Figure 15).



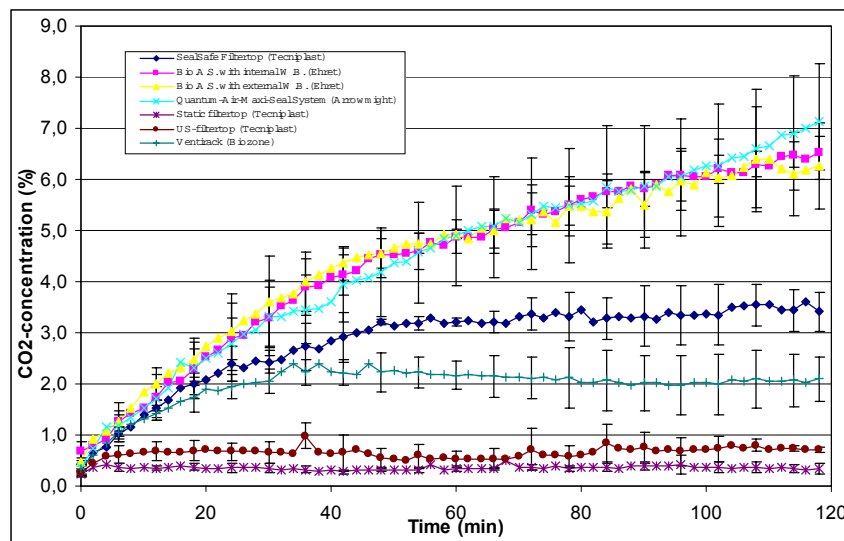
**Figure 15: The results from the preference study on the effects of CO<sub>2</sub>.** The distribution of dwelling time for male Sprague Dawley rats between left and right cage for day and night when the left cage is without any CO<sub>2</sub> and the right one with either 1% or 3% CO<sub>2</sub> is shown. The 50% distribution is marked with a bold line, and for each result the standard deviation is marked. Statistical significance is marked with \* (\* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001) (V).

In the telemetric study the heart rate was lower when the animals were exposed to either 3% or 5% CO<sub>2</sub>, compared with values for non-exposure. No effects of CO<sub>2</sub> were seen on the systolic blood pressure at 3% exposure, but at 5% there was a decrease in the blood pressure compared to the control value (Figure 16).



**Figure 16: The results from the telemetric study on the effects of 5% CO<sub>2</sub> on the systolic blood pressure and the heart rate.** The figures show the systolic blood pressure and the heart rate during daytime when the rats are exposed to a control period and a period with exposure to 5% CO<sub>2</sub>. Results for seven male rats with the same rats used for both periods are shown (V).

Major differences between commercially available IVC-cages in respect to concentrations of CO<sub>2</sub> when unventilated and filled with the maximum amount of mice were found. As cages commercially available today are of different sizes, the volume of the cages was measured and in each cage a number of 8-10 month old mice were placed, to achieve a standard of mouse per air volume of 20 g liter<sup>-1</sup> (VI). In the static filter top the CO<sub>2</sub> stabilizes on a level of 0.5%, while for other types of filter tops the level reaches values between 2 and 8% within 120 minutes (Figure 17).



**Figure 17: CO<sub>2</sub> concentration inside different types of sealed filter topped cages containing live mice (20 g liter<sup>-1</sup>) when the cages were unventilated for 120 min (VI).**

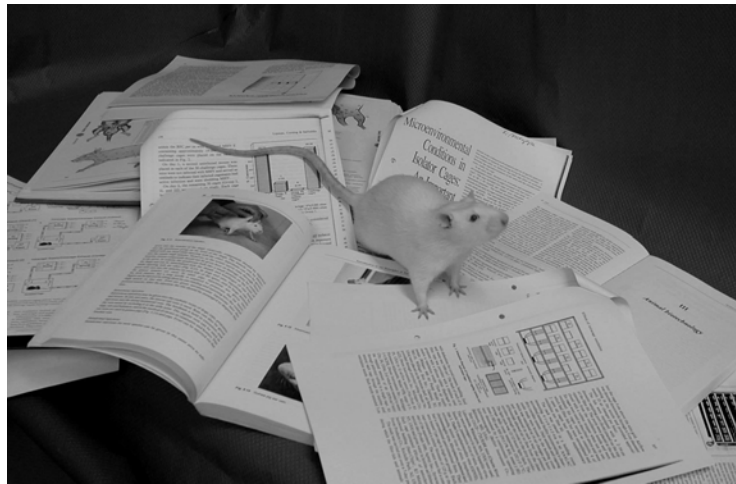
### **Summarizing the results for evaluation of impacts of ventilation**

The results found in the three studies on the ventilation parameters are summarised in the following table (Table 6).

**Table 6: The summarized results for the three studies investigating the effects from different ventilation parameters in relation to housing in IVC-systems.**

Study	Preference tests				Telemetric studies		Reference
	Male		Female		Heart rate	Systolic blood pressure	
	Day	Night	Day	Night			
Air Speed <b>0.2 m/s</b>	NS	NS	NS	NS	S	NS	<b>IV</b>
Air Speed <b>0.5 m/s</b>	NS	NS	NS	NS	S	S	
Air change <b>50 times per hour</b>	NS	NS	NS	NS	S	S	<b>IV</b>
Air change <b>80 times per hour</b>	S	S	NS	S	S	S	
Air change <b>120 times per hour</b>	NS	S	S	S	S	S	
CO <sub>2</sub> -level <b>1 %</b>	NS	NS	-	-	-	-	<b>V</b>
CO <sub>2</sub> -level <b>3 %</b>	S	S	-	-	S	NS	
CO <sub>2</sub> -level <b>5 %</b>	-	-	-	-	S	S	

## Discussion







## Discussion

### *The method evaluation*

In the present study a range of different methods were evaluated in order to find the most suitable methods for analysing impacts of small differences in housing conditions. Many existing methods have not been applied and evaluated in this work, but two methods were found efficient enough, i.e. telemetry and preference test. The telemetric study gives information about the physiological state of the animals, whereas the preference test gives information about the feelings or the will of the animals and their emotional state, and as can be seen, the two methods may be conflicting, as the animal may refuse one option to another although the condition does not affect the animal physiologically.

The grid floor was found to be well qualified as stressor used, and confirmed earlier studies, that being housed on grids is very uncomfortable and may be stressful (Blom 1993, Eskola & Kaliste-Korhonen 1998, Manser et al 1995) and, probably, it has a higher impact on the animals than the different ventilation factors.

The preference test is refined compared to earlier studies (Baumans et al 1987, Chmiel & Noonan 1996, Patterson-Kane et al 2001), and compared to video or micro switches the use of digital weights is much easier (III). The use of telemetry as a method for measuring impact from housing conditions is rather novel, as previously it has only been used in that sense in a few papers for studying the effects of group housing compared to single housing (Lawson et al 2000, Lemaire & Mormède 1995, Meerlo et al 1999). In the present study telemetry was found very suitable for evaluating the impact of housing conditions (I). It is advantageous that telemetry supplies information of the physiological state without disturbing the animals, which can be kept in their home cages during monitoring. It is known, that shortly after implantation, there are no effects on the animals caused by the sensor (Kramer et al 2000). In other studies telemetry has been used to investigate effects of different drugs on the animals' physiology (Clement et al 1989, Janssen et al 1991, Meehan et al 1995). The method may be refined further, as the mechanism behind the control of heart rate and especially blood pressure is rather complicated. Also the use of the telemetric registered activity may be considered for future studies as it may give some useful information about how a situation affects the animal comparable to information achieved by open field test (II).

The telemetric method is very sensitive. When using the animals as their own controls and having so many data as from the telemetric system the sensitivity is very high. The statistical model is able to show significant differences even at very small changes in either blood pressure or heart rate i.e. differences between control and experimental conditions of approximately 1% (two or three heart beats or 1 mmHg). In almost all the telemetric studies there were found statistical differences between the control and the experimental conditions (**I**, **IV**, **V**).

Revealing statistically significant differences in the telemetric studies indicates that there is an impact on the animals' physiology. An impact does not necessarily come from a stress effect or may be interpreted as reduced welfare. Stress will probably cause a change in pulse larger than two or three heartbeats per minute. In the evaluation of the method (**I**) the grid floor caused changes in the parameters in the size of 6-7% between the control and experimental conditions, which is also the size of stress impact found by others (Lemaire & Mormède 1995). Therefore, only a change in the size of what was found during the evaluation of the method will be considered as caused by stress in the conclusions of the present study. Smaller impacts on the animals' physiology may have been caused by other factors, and may therefore not be of major interest in the present study designed to investigate the welfare of rodents housed in ventilated cages.

### ***The ventilation evaluation***

Today, most commercially available systems ventilate cages with an air speed around 0.2 m/s at animal level (Lipman 1999). If the air speed becomes too low the air will not be homogeneously distributed in the entire cage (Tu et al 1997). The human draught factor is 0.2 m/s (Lipman 1999), but rodents seem to accept air speeds up to 0.5 m/s as shown in the preference test (**IV**) as well as in the telemetric studies (**IV**). The telemetric studies showed a statistical effect of the different air speeds and air changes, but this should not be interpreted as reduced welfare, as these changes are only minor. The preference study showed that the animals preferred cages with air changes below 80 times per hour.

Testing unventilated commercial IVC-cages showed that in some cage types the CO<sub>2</sub>-level could easily become a problem, which is also indicated by another paper (Höglund & Renström 2001). A level of 3%, which may be a critical level (Krohn & Hansen 2000) is reached within 20-45 minutes (**VI**). In the present study, the rats avoided 3% CO<sub>2</sub> if they were given the opportunity, and it

lowered the heart rate (V). The blood pressure was undisturbed at 3% exposure, but at 5% the systolic blood pressure decreased. When testing the impact of 1% CO<sub>2</sub> no effects were seen on the preference of the animals.

The reductions observed in systolic blood pressure and heart rate when exposed to either 3% or 5% CO<sub>2</sub> were probably not related to a stress reaction, as a previous study has shown that a stress reaction will cause an increase in both heart rate and systolic blood pressure (I). The effects seen are more likely to be related to the anaesthetic effect of CO<sub>2</sub> which may be the reason why the animals deselect cages with 3% CO<sub>2</sub>, as they may feel dizzy. The level at 3% CO<sub>2</sub> is corresponding with previous studies reviewed in an earlier paper (Krohn & Hansen 2000), in which the effects of 3% CO<sub>2</sub> were found to have impacts on the hormonal, respiratory parameters and blood parameters of the animals. As no effects were seen at 1% CO<sub>2</sub> this may indicate that the rats have CO<sub>2</sub>-tolerance different from humans, as humans are able to register CO<sub>2</sub>-levels from 1% and above (Glatte & Welch 1967). This difference between humans and rats may be explained by the differences in habitate. A study has revealed that CO<sub>2</sub> concentration may reach levels up to 1.4% in underground rat burrows (Studier & Bacce 1968), thereby giving an evolutionary explanation of the higher CO<sub>2</sub>-tolerance in rats.

### ***The IVC-system evaluation***

Most commercial systems today have the option of ventilating the cages from 25 up to 120 air changes per hour (Huerkamp & Lehner 1994, Perkins & Lipman 1996), so many systems are able to provide more than 80 air changes per hour. Previous studies have shown that an air changing frequency of at least 30-40 times per hour is required in order to keep the cages free from NH<sub>3</sub> (Reeb et al 1997, Reeb et al 1998). Therefore, around 50 air changes per hour in the cages may be recommendable, as this will keep the cages free of NH<sub>3</sub>, and as the rats deselected cages with higher air changes. By keeping the air change number around 50 times per hour it is easier to keep the air speed at animal level relatively low, as a higher number of air changes calls for more air to be ventilated into the cage, which may lead to higher air speed, if the inlet opening is not adjusted properly (Keller et al 1989).

In the same way the findings of the present study in regard to CO<sub>2</sub> will have some consequences for the use of IVC-systems. Air changes around 40-70 times per hour will easily remove all CO<sub>2</sub> from

the cages, even with the cages filled to a maximum with animals (Lipman et al 1992, Perkins & Lipman 1996). The problem occurs in cages unventilated due to cage changing or animal procedures. The very rapid raise in the concentration of CO<sub>2</sub> is not the only problem in unventilated cages (Corning & Lipman 1991), as studies have also shown a raise in humidity and NH<sub>3</sub> if such cages (Baer et al 1997), i.e. the rack and thereby the cages should only be without ventilation for the shortest possible time. In many facilities the racks are transported to a central place in the laboratory for cage changing. Therefore the rack may be left without ventilation for some time, and to prevent this, it may be recommendable to connect the rack to the ventilation from the LAF-cabin or the cage changing station to secure that the cages are ventilated during the period of cage changing. Also for the experimental purpose the cages may be taken out of the rack and placed in a small unit ventilating few cages in the laboratory. As reviewed in an earlier paper the animals become physiologically affected when exposed to CO<sub>2</sub> levels higher than 3% (Krohn & Hansen 2000), and the results of the present study seem to confirm this assumption even without effects on the systolic blood pressure (V).

Therefore, it may be recommendable that the rats are not exposed to CO<sub>2</sub>-levels higher than 3%, and consequently the IVC-cages should not be left without ventilation for periods longer than 15-30 minutes.

### ***Contributions and perspectives***

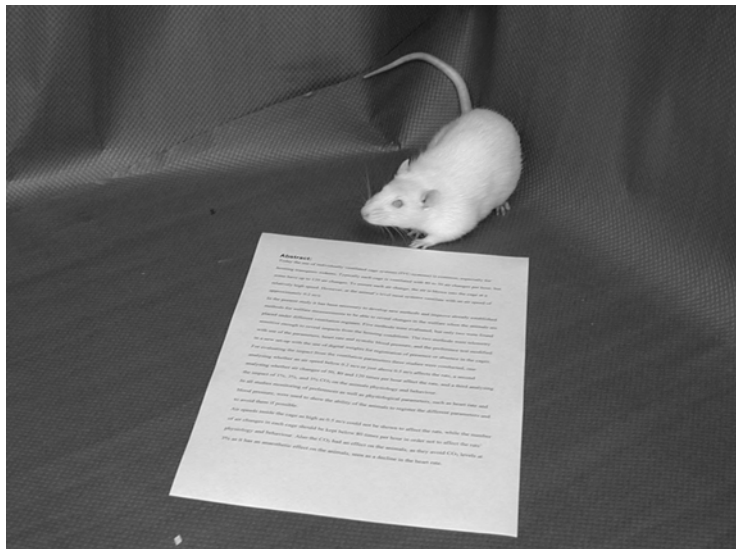
With the present thesis and the studies conducted during the present project some new information about animal welfare is added to our knowledge about laboratory animal science and to the discipline concerning animal welfare. The present study is of course only able to investigate a small part of the problem, as this seems to be the first study analysing any welfare consequences of housing rats in IVC-systems. Furthermore, the study has contributed to the field of animal welfare by analysing the applicability of different methods for measuring animal welfare and also by adding and refining methods for future use. In the present study the rat was used as the model, although mice is the most common rodent housed in IVC-systems. The choice of the rat as the model was primarily based upon the easier use of telemetry for the rats, but also upon easier registration on the weights used in the preference system. Although studying mice has a high chance of leading to the same conclusions, a study is needed to reveal whether the tolerances for air change and air speed is

the same for the mice as found for the rats. Also other aspects of welfare when housing rodents in IVC-systems need to be investigated, e.g. the impact of total isolation, light and transparency of the cages as well as long-term consequences of the air changes and air speed.

The present study has enlightened a small part of the problem, and far more studies are needed to find better methods for measuring animal welfare and reveal possible consequences from housing rodents in IVC-systems and improve the conditions as much as possible.



## Summary / Sammenfatning







## Summary (English)

Today the use of individually ventilated cage systems (IVC-systems) is common, especially for housing transgenic rodents. Typically each cage is ventilated with 40 to 50 air changes per hour, but some have up to 120 air changes. To ensure such air change, 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 study it has been necessary to develop new methods and improve already established methods for welfare measurements to be able to reveal changes in the welfare when the animals are placed under different ventilation regimes. Five methods were evaluated, but only two were found sensitive enough to reveal impacts from the housing conditions. The two methods were telemetry with use of the parameters, heart rate and systolic blood pressure, and the preference test modified in a new set-up with the use of digital weights for registration of presence or absence in the cages.

For evaluating the impact from the ventilation parameters three studies were conducted, one analysing whether an air speed below 0.2 m/s or just above 0.5 m/s affects the rats, a second analysing whether air changes of 50, 80 and 120 times per hour affect the rats, and a third analysing the impact of 1%, 3%, and 5% CO<sub>2</sub> on the animals physiology and behaviour.

In all studies monitoring of preferences as well as physiological parameters, such as heart rate and blood pressure, were used to show the ability of the animals to register the different parameters and to avoid them if possible.

Air speeds inside the cage 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 in order not to affect the rats' physiology and behaviour. Also the CO<sub>2</sub> had an effect on the animals, as they avoid CO<sub>2</sub> levels at 3% as it has an anaesthetic effect on the animals, seen as a decline in the heart rate.

## Sammenfatning (Dansk)

Individuelt ventilerede bursystemer er i dag almindelig brugt i dyrefaciliteter, og især til opstaldning af transgene gnavere. Typisk er hvert bur ventileret med et luftskifte på mellem 40 og 50 gange i timen, men nogle systemer har et luftskifte op til 120 gange i timen. For at sikre sådan et luftskifte må luften ventileres ind i buret med en relativ høj hastighed. Hos de fleste systemer er lufthastigheden på dyrenes niveau omkring 0,2 m/s.

I forbindelse med nærværende projekt har det været nødvendigt at udvikle nye metoder og forbedre allerede eksisterende metoder til brug for måling af velfærd for at være i stand til at påvise de ændringer i velfærden, der opstår, når dyrene opstaldes under forskellige ventilationsforhold. Fem forskellige metoder blev afprøvet, men kun to blev fundet fintfølende nok til at påvise en effekt fra opstaldningsforholdene. De to metoder var telemetri ved anvendelse af puls og systolisk blodtryk, og præference testen i en modificeret udgave, som bruger digitale vægte til at registrere i hvilket bur dyret er tilstede.

Til at vurdere effekten af ventilationen på dyrenes velfærd blev tre studier gennemført, et til at undersøge om lufthastigheder under 0,2 m/s og lige over 0,5 m/s påvirker rotter, et andet til at undersøge om luftskifte på 50, 80 og 120 gange i timen påvirker rotter, og et tredje til at undersøge hvordan 1, 3 og 5% CO<sub>2</sub> påvirker rotternes fysiologi og adfærd.

I alle forsøgene blev dyrenes præference målt, ligesom de fysiologiske parametre, som puls og systolisk blodtryk, blev brugt til at vurdere dyrenes evne til at registrere og undgå de forskellige parametre.

Lufthastigheder på op til 0,5 m/s i burene kunne ikke påvises at have en effekt på rotterne, mens antallet af luftskifte i hvert bur bør holdes under 80 gange i timen for ikke at påvirke rotternes fysiologi og adfærd. Også CO<sub>2</sub> viste sig at have en effekt på dyrene, da de undgik CO<sub>2</sub>-niveauer på 3%, ligesom et sådant niveau har en anæstetisk effekt på dyrene, der kan ses som et fald i puls.

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