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1.0 INTRODUCTION

1.1 Objectives

This document describes a research program undertaken by the National Institutes of Health, Office of Research Services, Division of Engineering Services. The work was carried out in collaboration with the University of Illinois Bioenvironmental Engineering Research Laboratory (BERL) and Flomerics Inc.

The hypothesis of this research was that the room ventilation parameters, which include room ventilation rate, diffuser type, diffuser location, number of exhausts, exhausts location, cage density, changing station location, changing station status (on/off), room size, cage rack arrangement, and room pressurization, affect both room (macro) and cage (micro) environment for laboratory animal research facilities.

In order to develop relationships between micro- and macroenvironmental conditions and to better determine ventilation system designs that provide appropriate micro- and macroenvironments, the following objectives were set:

1. Conduct a study to determine typical mass generation rates of CO₂, H₂O, and NH₃, and consumption of O₂ with groups of mice in shoebox cages with bedding at different room air relative humidities using open-system calorimeters with precisely controlled fresh air exchange rates.

2. Create and measure various airflows within a known mouse cage in such a manner as to lay the groundwork for determining the boundary conditions for the computational fluid dynamics (CFD) analysis of the cage. Cage boundary conditions include resistance and coefficient of loss created by both the cage top and the surrounding edge on which the cage top sits. The tracer gas method using 1 L/min, and 100 mL/min injection rate for measuring the airflows within the cage was used. Comparison were conducted between tracer gas types CO₂ vs. SF₆, as well as changing airflow measurement techniques using a constant injection rate and decay methods.

3. Obtain data in an empty room as well as a room with racks, cages, and simulated animals to verify the accuracy of CFD. Room air velocity, temperature, and CO₂ concentration patterns were used throughout the room for verification of CFD model predictions.

4. Utilize over 500 CFD simulations to establish a relationship between micro- and macroenvironments by changing room ventilation rate, diffuser type, diffuser location, number of exhausts, exhausts location, cage density, changing station location, changing
station status (on/off), room size, cage rack arrangement, and room pressurization. The simulations’ variations would determine the affect on both room (macro) and cage (micro) environments for laboratory animal research facilities.

1.2 Background to Project

Air quality within macro (room) and micro (cage) environments of laboratory animal facilities is essential for the health and welfare of humans and animals, and the integrity of the studies being conducted. It is well-known that biological responses are influenced by both genetic heritage and the environment. Information on the influence of the physical environment on the animals’ biological responses is needed to improve laboratory animal facility design and management. At the optimum environmental condition, not only does the laboratory animal experience a state of well-being, the researcher obtains reliable and repeatable experimental results from the animal.

While many thousands of square feet of animal research facilities are designed and constructed each year, inadequate information is available regarding ventilation rates and patterns required to maintain acceptable micro- and macroenvironments. A scientific basis is needed for selecting the ventilation rates of the macroenvironment and microenvironment and for designing effective ventilation systems for laboratory animal facilities. Design information is also needed for engineers to improve design, ensure air quality, and minimize energy cost.

Current ventilation guidelines are based largely on anthropomorphic views as opposed to scientifically defined animal needs. Limited research has been conducted to determine macro- and microenvironment relationships in animal research facilities in regard to ventilation rates, room air distribution, supply relative humidity and temperature, and other factors required to maintain acceptable and uniform cage environments. Most research has focused only on room conditions.

Laboratory animal ventilation should balance air quality, animal comfort, and energy efficiency to provide cage environments that optimize animal welfare and research efficiency. Conditions that optimize animal welfare automatically tend to improve research efficiency because good conditions minimize unintended stress factors on the animals. Additionally, the laboratory animal ventilation system should provide a healthy and pleasant environment for researchers and animal caretakers.

On the subject of ventilation, the most commonly accepted sources of performance criteria for research animal facility ventilation systems are the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources (1996) and the American Society of Heating Refrigerating and Air Conditioning HVAC Systems and Application Handbook.

Ventilation recommendations are based on room air exchanges, but cage ventilation rates may be inadequate in some cages and excessive in other cages depending on cage and facility design.
Recommendations for room ventilation rates of 10 to 15 air changes per hour (ILAR, 1996) are an attempt to provide adequate ventilation for the room and the cages. This recommendation is based on the assumption that adequate ventilation in the macroenvironment provided sufficient ventilation to the microenvironment. This may be a reasonable assumption when cages have a top of wire rods or mesh. However, several studies have shown that covering cages with filter tops provide a protective barrier for rodents and reduced airborne infections/diseases, especially neonatal diarrhea, but can result in a significant differences in microenvironmental conditions due to changes in air movement caused by the cover.

1.3 Literature Review

The following is the conclusion of pertinent research studies and publications on room air distribution and the relationship between macro- and microenvironments, ambient temperature on growth, behavioral thermoregulatory responses in rats, and moisture production of rats and mice.

This section also includes a review of ammonia and carbon dioxide physiochemical properties and their relevance to the current study.

1.3.1 Literature Search on Macro and Micro Environmental Relationships


To assess the impact of room ventilation on animal cage microenvironments, intracage ventilation rates, temperature, humidity, and concentrations of carbon dioxide and ammonia were monitored in non-pressurized, bonnet-topped mouse cages housing four C57BL/6J male mice that weighed 26.5 ± 0.4 g. with autoclaved pine shavings as bedding. The top, middle, and bottom rows of a mouse rack were monitored at room ventilation rates of 0, 5, 10, and 20 air changes per hour (ACH).

Ventilation inside the animal cage increased somewhat from 12.8 to 18.9 ACH as the room ventilation rate increased from 0 to 20 ACH, but the differences were not statistically significant. Most of the increases occurred in cages in the top row nearest to the fresh air supply. Cages containing mice had ventilation rates between 10 and 15 ACH even when room ventilation was reduced to 0 ACH. This ventilation is a result of the thermal heat load of the mice.

After six days of soiled bedding, intracage ammonia concentration was < 3 ppm at all room ventilation rates and was not affected by increasing room ventilation. Although
earlier reports, such as M.R. Gamble and G. Clough (1976), indicated ammonia values > 200 ppm, recent studies testing cage densities of four mice/microisolator cage, such as G.C. Choi, J.S. McQuinn, B.L. Jennings (1994), and J.J. Hasenau, R.B. Baggs, and A.L. Kraus (1993), are consistent with the observed data that ammonia concentration can be low. Two possibilities may explain why the ammonia concentrations were low. The first is that improved cage washing procedures and animal room cleanliness may reduce concentrations of ammonia-producing bacteria, resulting in lower ammonia concentration. In this facility, the cages are washed with water at 82 ± 6 °C followed by a dry heat cycle at 118 °C. The second possibility is that formation of ammonia may be dependent on the strain of mice.

Temperature inside the cages did not change with increasing ventilation. The temperature inside the cages was 1 to 3 °C higher than the room temperature, an observation consistent with an earlier study that proved the use of a filter top results in a temperature increase of about 2 °C in the cage.

Humidity inside cages significantly decreased with increasing ventilation, from 55 percent relative humidity at 5 ACH to 36 percent relative humidity at 20 ACH. Humidity inside the cage increased compared with humidity in the room, and the average difference between room and cage RH was about 10 percent, similar to the increased cage humidity previously observed by M.L. Simmons, D.M. Robie, J.B. Jones, and L.J. Serrano (1968).

Carbon dioxide concentration decreased from 2,500 ppm to 1,900 ppm when ventilation rate increased from 5 ACH to 10 ACH, but no further significant decrease was observed at 20 ACH. In conclusion, increasing the room ventilation rate higher than 5 ACH did not result in significant improvements in the cage microenvironment.


Microenvironment and macroenvironment: The microenvironment of an animal is the physical environment immediately surrounding it, the primary enclosure with its own temperature, humidity, and gaseous and particulate air composition. The physical environment of the secondary enclosure, such as a room, a barn, or an outdoor habitat constitutes the macroenvironment. Although the microenvironment and the macroenvironment are linked by ventilation between the primary and secondary enclosures, the environment in the primary enclosure can be quite different from the environment in the secondary enclosure. It is affected by the design of both enclosures. Measurement of the characteristics of the microenvironment can be difficult in small primary enclosures. Available data indicate that temperature, humidity, and concentrations of gases and particulate matter are often higher in an animal's
microenvironment than in the macroenvironment (Besch 1980; Gamble and Clough 1976; Murakami 1971; Serrano 1971; Flynn 1959). Microenvironmental conditions can induce changes in metabolic and physiologic processes and/or alterations in disease susceptibility (Schoeb and others 1982; Broderson and others 1976; Vesell and others 1976).

Temperature and Humidity: Regulation of body temperature within normal variation is necessary for the well-being of homeotherms. Generally, exposure of unadapted animals to temperatures above 85°F (29.4°C) or below 40°F (4.4°C) without access to shelter or other protective mechanisms might produce clinical effects (Gordon 1990) that could be life-threatening. Animals can adapt to extremes by behavioral, physiologic, and morphologic mechanisms, but such adaptation takes time and might alter protocol outcomes or otherwise affect performance (Gordon 1993; Garrard and others 1974; Pennycuik 1967). Environmental temperature and relative humidity can depend on husbandry and housing design and can differ considerably between primary and secondary enclosures. Factors that contribute to variation in temperature and humidity include housing material and construction, use of filter tops, number of animals per cage, forced ventilation of the enclosures, frequency of bedding changes, and bedding type.

Recommended dry-bulb temperatures for mice, rats, hamsters, gerbils, and guinea pigs are 18 to 26°C (64 to 79°F). However, some conditions might require increased environmental temperatures. These conditions include postoperative recovery, maintenance of chicks for the first few days after hatching, housing of some hairless rodents, and housing of neonates that have been separated from their mothers.

Ventilation: The purposes of ventilation are to supply adequate oxygen; remove thermal loads from animals, lights, and equipment; dilute gaseous and particulate contaminants; adjust the moisture content of room air; and, where appropriate, create static-pressure differentials between adjoining spaces. Establishing a room ventilation rate, however, does not ensure the adequacy of the ventilation of an animal’s primary enclosure and hence does not guarantee the quality of the microenvironment. The degree to which air movement (drafts) causes discomfort or biologic consequences has not been established for most species. The volume and physical characteristics of the air supplied to a room and its diffusion pattern influence the ventilation of an animal’s primary enclosure and so are important determinants of its microenvironment. The relationship of the type and location of supply air diffusers and exhaust vents to the number, arrangement, location, and type of primary enclosures in a room or other secondary enclosure affects how well the primary enclosures are ventilated and should therefore be considered. The use of computer modeling for assessing those factors in relation to heat loading and air diffusion patterns can be helpful in optimizing ventilation of primary and secondary enclosures. The guideline of 10 to 15 fresh-air changes per hour has been used for secondary enclosures for many years and is considered an acceptable general standard. Although it is effective in many animal housing settings, the guideline does not take into account the range of possible heat loads; the species, size, and number of animals involved; the type
of bedding or frequency of cage changing; the room dimensions; or the efficiency of air distribution from the secondary to the primary enclosure. In some situations, the use of such a broad guideline might pose a problem. A secondary enclosure that contains few animals could be over-ventilated and would waste energy. Under-ventilating a secondary enclosure that contains many animals would allowing heat and odor accumulation. To determine more accurately the ventilation required, the minimal ventilation rate (commonly in cubic feet per minute) required to accommodate heat loads generated by animals can be calculated with the assistance of engineers. The heat generated by animals can be calculated with the average-total-heat-gain formula as published by the American Society of Heating, Refrigeration, and Air-Conditioning Engineers ASHRAE (1997). The formula is species-independent, so it is applicable to any heat-generating animal. Minimal required ventilation is determined by calculating the amount of cooling required (total cooling load) to control the heat load expected to be generated by the largest number of animals to be housed in the enclosure, any heat expected to be produced by non-animal sources, and heat transfer through room surfaces. The total-cooling-load calculation method can also be used for an animal space that has a fixed ventilation rate to determine the maximal number of animals, based on total animal mass, that can be housed in the space. Even though that calculation can be used to determine the minimal ventilation needed to prevent heat buildup, other factors, such as odor control, allergen control, particle generation, and control of metabolically generated gases, might necessitate ventilation beyond the calculated minimum. When the calculated minimal required ventilation is substantially less than 10 air changes per hour, lower ventilation rates might be appropriate in the secondary enclosure, provided that they do not result in harmful or unacceptable concentrations of toxic gases, odors, or particles in the primary enclosure. Similarly, when the calculated minimal required ventilation exceeds 15 air changes per hour, provisions should be made for additional ventilation to address the other factors. In some cases, fixed ventilation in the secondary enclosure might necessitate adjustment of sanitation schedules or a limit on animal mass to maintain appropriate environmental conditions.


Eight ventilation parameters for laboratory animal facilities were studied in a full-scale room ventilation simulator. They were room air exchange rate, diffuser neck diameter, diffuser type, number of returns, return location, cage type, room size and cage rack arrangement. Air exchange rates, velocities, and temperatures within the room and the cages were studied as well. Room airflow pattern and cage airflow patterns were determined using smoke tests. Cage conditions varied widely among cages within the same room. Cage type was the most important factor influencing cage conditions. Room air exchange rate, air velocity approaching the cage, number of returns, return location, and diffuser type did not significantly influence cage conditions in the ranges studied.
Room air velocities approaching the cage were not high enough to have a major influence on shoebox cage ventilation rates. Air diffusion performance index (ADPI) did not show a difference between the diffuser type, the number of returns, or return location, but ADPI did show a difference with the room ventilation rate and diffuser neck size.

The air velocity, air temperature, and ventilation rate were measured in the room and within cages at selected locations in the room. The following conclusions were drawn:

Cage conditions varied widely with cage location in a room.

Cage type was the most important factor that influenced cage conditions. Wire cages had ventilation rates three times higher than shoebox cages even though the sensible heat production in the wire cage was only one-fourth that of the shoebox cage.

In general, room air exchange rates, velocity approaching the cage, number of returns, location of return, and diffuser type did not significantly influence cage conditions for a range of factors studied in this project. Room air velocities approaching the cage were not high enough to have a major influence on shoebox cage ventilation rate.

The thermal comfort conditions in the human-occupied region of laboratory animal rooms were affected by room ventilation rate. In general, ADPI had lower values at lower ACH.

ADPI did not show a difference between the diffuser types, the number of returns, or the return location, however, the diffuser neck size (neck velocity) did affect the ADPI values.

For the range of conditions tested in this study, the number of air returns and the location of air returns did not have a measurable effect on cage or room conditions. The perforated ceiling and laminar flow diffuser performed equally well, but the neck size needs to be carefully matched to the overall design. Room ACH values from 5 to 15 had the same effect on cage conditions, so the higher room air exchange rates did not provide better conditions for the animals.

This study did not address the concern of odors and contaminants in the room or the cages. This needs further study. However, higher ventilation rates in the cages were found to reduce contaminant levels in the cages. Another area that needs more study is cross-contamination between cages due to air movement from cage to cage. More study is needed to explain the wide variation of cage conditions at different locations in the same room. The magnitude of air velocity approaching the cage did not have a large effect on cage ventilation rate at the low room velocities encountered in this study. However, the direction of airflow approaching the cage could have a major effect and could explain
some of the differences. More precise measurements are needed on effects of air direction and magnitude on cage ventilation rates.


Microenvironmental contaminants were measured within isolator-type cages housing DBA/1J mice on eight contact beddings. Each cage contained 850 cm$^3$ of bedding and five mice randomized by body weight. Seven cages with and two to four cages without mice were evaluated per bedding. Macroenvironmental conditions were defined and controlled. Macro- and microenvironmental temperatures, relative humidity, and carbon dioxide and ammonia concentrations were determined daily during each of three 7-day test periods. An air sampling pump and detector tubes were used to measure hydrogen gas, 2-butanol, acetone, ethanol, carbon monoxide, acetic acid, hydrogen sulfide, sulfur dioxide, and formaldehyde on the final day of each test period. In addition, gas chromatographic analysis was used on the seventh day to detect additional volatile alcohols and ketones. Ammonia concentrations ranged from 0 to 410 ppm, depending on the bedding type and day of measurement. On the basis of the mean microenvironmental ammonia concentration in the cages with mice, the beddings were ranked from highest to lowest in ammonia generated, as follows: aspen shavings, pine shavings, reclaimed wood pulp bedding, virgin pulp loose bedding, hardwood chip bedding, recycled paper bedding, virgin cellulose pelleted bedding, and corn cob bedding. The temperature, relative humidity, and carbon dioxide concentration were similar among beddings. No other contaminants were detected except acetic acid (mean = 0.86 ppm) in the cages with and without mice containing corncob bedding. Sulfur dioxide (mean = 0.42 ppm) was only detected in cages with mice and corncob bedding. In summary, the concentration of ammonia generated varied significantly in cages containing mice and different contact bedding.

Temperature: There were no significant differences in temperature detected among cages with mice and various bedding throughout the 7-day observation periods. Temperature rise in the cages with hardwood chip bedding was approximately 4 °F.

Relative humidity: Intracage relative humidities were not significantly different among cages with different bedding through the 7-day observation periods. Relative humidity rise in the cages with hardwood chip bedding was approximately 25 percent.

Carbon dioxide: There were no significant variations detected in carbon dioxide concentration among cages with various bedding throughout the 7-day observation
periods. Carbon dioxide concentration in the cages with hardwood chip bedding was approximately 4,500 ppm with background concentration of 370 ppm.

Ammonia: Microenvironmental ammonia concentration ranged from 0 to 410 ppm, depending on the bedding type and day of measurement. The cages containing aspen shavings had a significantly higher 7-day mean ammonia concentration compared with other beddings. Additionally, the cages containing reclaimed wood pulp and pine shavings had a significantly higher 7-day mean ammonia concentration compared with other beddings, with the exception of aspen shavings. Cages containing virgin pulp loose bedding had a significantly higher 7-day mean ammonia concentration compared with the cages that had virgin cellulose and corncob bedding. Ammonia was first detected in cages containing aspen and pine shavings on day 2. On day 3, ammonia was first detected in cages containing reclaimed wood pulp. Cages with virgin loose pulp and hardwood chip bedding had detectable ammonia beginning on day 4, followed by the cages with recycled paper bedding on day 6. The cages containing virgin cellulose bedding had no detectable ammonia production until day 7, whereas the cages with corncob bedding never had detectable ammonia concentration. There was a significant difference in ammonia concentration in cages with different bedding and the day of evaluation, indicating that ammonia production differed among the beddings over time.


A survey was conducted of 46 laboratory animal rooms (33 rat rooms, 8 mouse rooms, 2 rabbit rooms, 2 primate rooms, and 1 dog room) at three commercial use sites, five universities, and a research organization. Air exchange rates, air velocities, temperature, humidity, ammonia concentrations, noise levels, and light levels were measured throughout the room and within selected cages in the room. Cage conditions varied widely among cages within the same room and among similar cages in different rooms. Cage type was the most important factor that influenced cage conditions and uniformity in cage conditions. Room air exchange rate had little influence on cage conditions. Velocity approaching the cage, number of returns and diffusers, and diffuser type did not significantly influence cage conditions and uniformity in cage conditions.

The following conclusions were drawn:

Cage conditions varied widely among cages within the same room and among similar cages in different rooms.

Cage type was the most important factor that influenced cage conditions and uniformity in cage conditions.
Room air exchange rate, air velocity approaching the cage, number of returns and diffusers, and diffuser type did not significantly influence cage conditions and uniformity in cage conditions.


A study was undertaken to determine the effect of population size on the buildup of ammonia and humidity in individually ventilated microisolation cages over time as compared with static microisolation cages. Ammonia and relative humidity measurements were taken daily from sterilized, pressurized, individually ventilated microisolation cages and sterilized, static microisolation cages containing one to four, 24 to 26 gram female Crl: CF-1 BR mice per cage. The effect of population size was evaluated over a 32-day period in the ventilated cages and over a 10-day period in the static cages. The bedding in each cage was 140 grams (360 cm³) of combination 1/4-in and 1/8-in. No detectable levels of ammonia were present in any of the ventilated cages throughout the duration of the study. Ammonia was detected after eight days in the static microisolation cages containing three or four mice. Relative humidity levels in ventilated cages were not significantly different than room relative humidity levels indicating that the ventilation provided to the microisolation cages was effective in controlling intracage humidity. Relative humidity levels in static microisolation cages were significantly higher than room humidity levels, and a significant increase in relative humidity with increasing population density was observed. The complete lack of detectable ammonia levels in the ventilated cages and the low levels of ammonia detected in the static cages may have been influenced by the stock of animal studied, the autoclaving of the bedding, and the differences in ventilation rates of the two caging types.

The intracage relative humidity was approximately 23 percent to 36 percent higher than the room relative humidity depending on cage population density.

No ammonia was detected during the study period in cages containing one or two mice. Ammonia was detected after eight days in cages containing three or four mice. On days 9 and 10, the contrast of ammonia production between low population density (one or two mice/cage) and high population density (three or four mice per cage) was statistically significant.

Ammonia production by bacteria can be influenced by the strain or stock of animal as well as by population density, relative humidity, temperature, and type of cage bedding. Strain, stock and sex differences in ammonia production have been shown with BALB/c mice producing less ammonia than CD1 mice. For mice housed in microisolator cages, ammonia concentrations were > 100 ppm for strains DBA and CD-1 and approximately 21 ppm for strain BALB/c on the 7th day of soiled bedding.


Recognizing the research needs for laboratory animal facility design and management is important for both cost-effective design of laboratory research animal facilities and the reliability of laboratory animal experiments. The Laboratory Animal Consortium (LAC), which consists of representatives from various industries and organizations involved with laboratory animals, has identified the following five major research priorities: the effects of microenvironmental conditions on the response of laboratory rats, the means for achieving appropriate ranges of environmental conditions, the environmental preferences of rats, the relationships between cage and room environmental conditions in typical laboratory animal facilities and caging systems, and the practical methods for measuring cage environmental conditions.

Recognizing the research needs for laboratory animal facility design and management is important for both cost-effective design of animal facilities and reliability of experimental procedures. The LAC, consisting of representatives from various organizations involved with laboratory animals, has identified five major research priorities:

- Effects of micro environmental conditions on the heat, moisture, gas, and particulate production rates of rats;
- Practical means for achieving appropriate ranges of environmental conditions;
- Environmental preferences of rats;
- Relationship between cage and room environmental conditions in typical laboratory animal facilities and caging systems; and
Practical methods for measuring cage environmental conditions.

This research agenda will ultimately provide scientifically based criteria for laboratory animal facility design and management, improve the scientific validity of research using laboratory animals, enable managers to adjust the animal facility systems and practices to conform to animal welfare guidelines, and develop a basis for reducing animal facility design and operational costs. It should be noted that meeting this challenge requires the following three other activities:

Adequate funding and support from both public and private organizations,

Conduct of research by multidisciplinary teams, and

Evaluation and utilization of research results.

S. Frei: *Facilities Approaches To Reducing Operating Costs*, 1993, Affiliated Engineers, Inc.

Frei noted that scientific justification for recommended ventilation rates is rather weak. The 1985 ILAR (Institute of Laboratory Animal Resources) Committee on Care and Use of Laboratory Animals recommendations can be traced back to Munkelt’s (1938) work, in which he proposed a ventilation rate of 1.5 cfm/lb for rats or guinea pigs. A historical summary of the development of ventilation rates follows:

Munkelt (1938) developed a standard of 1.5 cfm/lb of animal or 11 air changes per hour while working with rats and guinea pigs.

Runkle (1964) extrapolated Munkelt’s (1938) rates for mice through dogs.

In 1964, a laboratory extrapolated Runkle’s (1964) work to imply 10 to 20 air changes per hour (Frei 1993).

ILAR (1978) set the first standards at 10 to 15 air changes per hour.


Four different mouse caging systems were evaluated for microenvironmental temperature, humidity, and ammonia levels. All had polycarbonate bases and lids holding Reemay 2024 filter material in three different designs or a control without filter. Comparisons were made of BALB/c and CRL: CD-1 (ICR) BRV mice at four and two per cage under different macroenvironmental (room) conditions. All studies were conducted in an animal
room measuring 11 ft. x 8.58 ft. x 8.0 ft. in an AAALAC accredited animal facility. Ventilation of 20 air changes per hour was balanced so that pressure differences allowed air to flow from the corridor and ceiling supply vent (130 CFM) over the entry door through the room into the far wall exhaust ceiling vent (270 CFM). A 12/12-hour light/dark cycle with no twilight was used. Room temperatures averaged 22.8 ± 1.7 °C (range 18.4 to 26.9 °C) in the trials where nationally accepted standard macroenvironmental RHs were used and averaged 24.1 ± 0.7 °C (range 22.2 to 25.2 °C) during below normal macroenvironmental RH trials. The average range in room temperature per trial was ± 1.35 °C. Room humidity in the standard RH group was 51.5 ± 8.2 percent (range 38.5 to 66.5 percent RH). Room humidity in the below normal RH group was 22.7 ± 7.7 percent (range 11.4 to 35.1 percent RH). The range in room humidity per trial averaged 11.7 percent RH. At macroenvironmental room humidities of 40 to 70 percent, the relative humidity (RH) levels in the microisolation cages with four animals per cage averaged 27.1 percent RH above room RH levels. With two animals per cage, the RH levels in the microisolation cages averaged 16.1 percent RH above the room RH levels. At macroenvironmental room humidities of <40 percent, the RH levels in microisolation cages with four animals averaged 35.4 percent RH above the room RH levels. At two animals per cage the RH levels in the microisolation cages averaged 22.6 percent above room levels.

Study I (BALB/c mice): Population density of four animals per cage (Trials 1 and 2):

Temperature: There was no significant temperature variation between microisolator caging systems.

Relative humidity: Microenvironmental RH in the type microisolator cages averaged 28 percent higher than the room RH levels.

Ammonia: All cages accumulated less than 5 ppm ammonia at day 9.

Study I (BALB/c mice): Population density of two animals per cage (Trials 3 and 4):

Relative humidity: Micro environmental RH in microisolator cages averaged 9 percent higher than the room RH levels.

Ammonia: All cages contained less than 3 ppm ammonia at day 9.

Study II (CD-1 mice): Population density of four animals per cage (Trials 1 and 2):

Relative humidity: Micro environmental RH in microisolator cages averaged 36 percent higher than the room RH levels.

Ammonia: In trial 1, 8-day trial averages were, 50.6 ppm, in microisolator cages. In
trial 2, 8-day trial averages were 8.7 ppm, in microisolator.

Study II (CD-1 mice): Population density of two animals per cage (Trials 3 and 4):

Relative humidity: Micro environmental RH in microisolator cages averaged 13 percent higher than the room RH levels.

Ammonia: All cages accumulated less than 5 ppm at day 8. There were no significant sex differences or significant differences in cages with replaced damaged filters in any of the parameters measured in either of the two studies. Air velocity across each cage and shelf of the rack was 0 to 0.65 m/second. The outermost cages were subject to the highest air velocity.

Study 3 (Strain/stock comparison):

Temperature: The CD-1 cages, DBA cages, and BALB/c cages measured 23.5 to 23.6 °C, which were not significantly different.

Relative humidity: With average humidities measured at CD-1 cage (82.9 percent), DBA cage (86.3 percent), and BALB/c cage (79.0 percent), only the BALB/c cages had significantly lower humidities than the DBA cages. For four mice housed in microisolator cages Ammonia time period averages (day 7) were CD-1 = 149.5 ppm, DBA = 214.2 ppm, and BALB/c = 21.1 ppm.


It was shown that ammonia is produced in greater amounts under conditions of high humidity. Desiccation was shown to be helpful in the prevention of ammonia and humidity accumulation as urease-producing bacteria grow less efficiently in the presence of reduced humidity. Lowering the macroenvironmental relative humidity levels and providing dehumidified air have been suggested as methods for preventing the elevation of humidity observed in barrier caging systems.

C.M. Collins and S.E.F. D’Orazio: Bacterial Ureases-Structure, Regulation Of Expression and
Ammonia is produced by enteric bacteria through two possible mechanisms of enzymatic activity. Bacterial ureases can catalyze the hydrolysis of urea to ammonia and carbamate. A second series of enzymes, D-and L-amino acid oxidases can deaminate amino acids to their corresponding keto acid and ammonia.


A survey of laboratory animal environmental conditions in seven laboratory rat rooms was conducted. These rooms were designed and operated according to the ILAR (1985) guidelines, which recommended at least 10 ACH. Room air changes varied from 11 to 24 ACH, room air ammonia levels were under 0.5 ppm, and macroenvironmental conditions were considered satisfactory. However, cage air quality conditions and ventilation rates in typical rat cages may be “inadequate, which” or “inadequate and could” compromise the rat's respiratory systems. For those same rooms, ammonia levels ranged from negligible to 60 ppm in the cages. The authors noted that designs based only on room air changes may not be adequate to achieve desired conditions in the animal cages.


Four different mouse caging systems were evaluated for micro environmental temperature, carbon dioxide, relative humidity (RH) and ammonia levels during a 7-day testing period. All caging systems evaluated had polycarbonate bases and consisted of either a molded polyester (MP) filter lid, one of two different polycarbonate filter lids, or no filter lid which served as a control. At 50 percent macro environmental RH (study I), all systems maintained an intracage temperature of 75.5 ± 0.5°F. Both polycarbonate systems averaged > 2,200 ppm of carbon dioxide, more than the MP system and the controls. When compared with RH in the control cages, RH levels averaged over 20 percent and five to eight percent RH greater in the polycarbonate filter lid systems and the MP system, respectively. There were no appreciable ammonia levels in either the MP or control systems. In the polycarbonate filter lid systems, ammonia levels were undetectable on day 4 and were > 200 ppm by day 6. At 20 percent macro environmental RH (Study II), there was a proportional 15 to 30 percent RH decrease from study I levels. Ammonia levels were undetectable in any system until day 7 and averaged only 17 ppm in one of the polycarbonate systems. Minimal differences were observed in studies III, IV and V when pine shavings were used instead of hardwood chips, a CD-1 stock instead of a DBA/2J strain, and different grades of filter inserts in the polycarbonate systems, respectively.
Study I:

The temperature range for all caging systems was 75.5 ± 0.5 F.

Carbon dioxide: The Microisolator and Micro-Barrier systems averaged greater than 2,200 ppm more CO₂ than those in the molded polyester system which was 2,900 ppm greater than the control. The control cage CO₂ levels of 316 ppm approximated the 279 ppm in the macro environment.

Relative humidity: Microenvironmental humidity levels in the Microisolator and Micro-Barrier systems averaged greater than 20 percent RH higher than in the control cages. RH in one cage reached over 85 percent.

Ammonia: Weekly NH₃ levels in the control cages were 1.6 ppm and the molded polyester systems were 0.9 ppm. Weekly levels for the Microisolator and Micro-Barrier systems were 139.1 ppm and 162.8 ppm of NH₃ respectively. By day 4, one of the Micro-Barrier cages was already at 260 ppm NH₃, the maximum level attainable for the sampling tube. By day 6, seven of eight cages from the Microisolator and Micro-Barrier systems contained greater than 200 ppm of NH₃.

Study II:

There were no significant temperature variations among caging systems at 20 percent macro environmental humidity.

Carbon dioxide: Similar trends, as in study I, were observed for CO₂ levels among caging systems at the lower room humidity. No significant differences were observed when compared to study I.

Relative humidity: There was a proportional 15 to 30 percent RH decrease in microenvironmental RH levels in all caging systems when macroenvironmental humidity was lowered to 20 percent RH. Similar trends were observed among caging systems when compared with humidity levels in study I.

Ammonia: Weekly intracage NH, levels, which averaged 139.1 and 162.8 ppm for the Microisolator and Micro-Barrier caging systems respectively, when macro environmental humidity was 50 percent RH, were reduced to four and 17 ppm of NH₃ for these systems when room humidity was reduced to 20 percent. Ammonia detection at the lower humidity was not evident until day 7. There were no detectable NH₃ levels in any of the molded polyester or control cages at 20 percent macroenvironmental humidity.
Study III:

Temperature: No significant temperature differences were observed when pine shavings were used instead of hardwood chips.

Carbon dioxide: There were statistically significant (P < 0.05) elevations in CO₂ levels in all caging systems when pine shavings were used. The molded polyester system averaged 1,157.8 ppm versus 967.9 ppm with hardwood chips. The Microisolator and Micro-Barrier systems averaged 3,485.9 ppm and 3,651.6 ppm with pine shavings versus 3,187.5 ppm and 3,616.1 ppm with hardwood chips. Trends among caging systems were similar to those observed when hardwood chips were used.

Relative humidity: There were similar trends to studies I and II in RH and no significant differences were observed when shavings were used.

Ammonia: There were similar trends to studies I and II and no significant differences in NH₃ levels were observed when pine shavings were used.

Study IV:

Temperature: No significant differences in temperature were detected when the CD-1 stock was used instead of the DBA/2J strain.

Carbon dioxide: There were statistically significant (P < 0.05) elevations in CO₂ levels when the CD-1 stock was used in the Microisolator and Micro-Barrier caging systems. The Microisolator system averaged 4351.8 ppm versus 3187.5 ppm with the DBA/2J strain. The Micro-Barrier system averaged 4376.8 ppm versus 3616.1 ppm with the DBA/2J strain. Trends among caging systems were similar to those noted when the DBA/2J strain was used.

Relative humidity: There were significantly higher humidity levels (P < 0.05) within the Microisolator cage type with the CD-1 stock (79.5 percent RH vs. 69.7 percent RH). Trends among caging systems were similar to those noted when the DBA/2J strain was used.

Ammonia: Trends in ammonia levels among caging systems were similar to those noted when the DBA/2J strain was used. No significant differences were observed.

Study V:
No significant differences were observed between polycarbonate systems having either a Reemay filter insert or when the two different frames were compared for temperature, CO₂, relative humidity, or NH₃. Daily NH₃ levels detected in study V were as high as 450 ppm with humidities over 90 percent RH in individual cages from the Microisolator and Micro-Barrier systems. Detector tubes with the broader range of 50 to 900 ppm of NH₃ were used for this study.


Although temperature and relative humidity have been quantified and their effects on research data studied, few studies have measured the air turnover rates at cage level. We evaluated the air distribution and air turnover rates in unoccupied shoebox mouse cages, filter-top covered cages, and shoebox mouse cages housed in a flexible film isolator by using discontinuous gas chromatography/mass spectrometry and smoke. Results showed that air turnover was most rapid in the unoccupied shoebox mouse cage and slowest in the filter-top covered cage. Placing mice in the filter-top covered cage did not significantly improve the air turnover rate. Although filter-top covered cages reduce cage-to-cage transmission of disease, the poor airflow observed within these cages could lead to a buildup of gaseous pollutants that may adversely affect the animal’s health.


Carbon dioxide and ammonia are the major gaseous pollutants generated by animals and animal waste within the cage. Ammonia levels in an animal’s environment are dependent on fecal bacteria flora, population density, frequency of bedding changes, type of bedding material, cage design, temperature, humidity, cage sanitation, ventilation, and the animal’s level of activity.
To determine the affect of rod, wire-mesh, and fibrous filter types of covers on diffusion or convection of gases produced in the cage, groups of 4, 8, or 16 mice were placed in cages. Samples of air from each cage were analyzed daily. Filter and mesh covers had a major influence on the composition of air in the cages. They allowed the accumulation of CO$_2$, NH$_3$, and probably other gases to rise to levels considerably higher than in cages with open covers. When the mice were active, or when the number of mice in the cage was doubled, the CO$_2$ level was increased 50 to 100 percent above the original level of each cage. NH$_3$ was not detected until the 3rd to 6th day (3rd day for 16 mice per cage and to 6th day for 4 mice per cage), depending on the number of mice per cage. By the 7th day air reached noxious levels under some covers. Limiting the number of mice per cage and frequently removing soiled bedding could prevent excessive levels of CO$_2$ and NH$_3$ from accumulating in protectively covered cages.

Significance of ammonia levels: Ammonia was not detected until the 5th or 6th day in a protectively covered cages holding no more mice than the number recommended for its size.

Carbon dioxide is a metabolic by product of respiration and its accumulation is influenced by activity level of the animals, population density, and airflow within the cage. CO$_2$ levels were significantly higher in all cages than in the room. The levels in protective cover cages were in the range of 1217 parts per million for 4 mice per cage to 3155 parts per million for 16 mice per cage.


Ammonia concentrations >25 ppm promote the growth of infective agents in the respiratory tracts of rats.


To determine how a filter cap affects the heat and moisture buildup in a mouse cage and how that buildup is affected by ambient conditions of temperature and relative humidity, 50 adult female mice were housed 10 per cage in polycarbonate cages that were covered with a fibrous filter and sealed with a neoprene gasket and hold-down rod. The cages were placed in a chamber that controlled ambient temperature and humidity. Observations were made at 1 F intervals of temperature 68 to 74 F and at three different
relative humidities (40, 55, and 70 percent). Every 24 hours the chamber conditions were changed, and cage, bedding, water, filter caps, and food were replaced. Three sensors suspended just under the filter cover measured temperature and humidity and transmitted the data to a recorder. The first reading was taken after a 3-hour equilibration period, then every three hours until the next day’s change. By measuring the average temperature and humidity, it was possible to study the differences between cage and ambient conditions. At 68 °F and 40 percent relative humidity, the mean conditions in the cage were 72 °F and 50 percent relative humidity; at the upper limit of 74 °F, and 70 percent relative humidity, the mean cage conditions were 78 °F and 75 percent relative humidity.


Most of the current recommended guidelines for heating, ventilation, and air conditioning systems for the animal research facilities appear to be based on the ventilation criteria described by Runkle, which were in turn based on studies done by Munkelt.

1.3.2 **Literature Search on Effects of Ambient Temperature on Growth and Behavioral Thermoregulatory Responses in Mice**


The ambient temperature (T<sub>a</sub>) to house and study laboratory mice is critical for nearly all biomedical studies. The ideal T<sub>a</sub> for housing mice should be based on their thermoregulatory requirements. The T<sub>a</sub> for housing mice should approximate their zone of thermoneutrality where neither autonomic nor behavioral thermoeffectors are activated. In other words, the T<sub>a</sub> for housing should not stress the animal’s thermoregulatory system. Although laboratory mice are usually housed in groups of five or more, most of the information on thermoregulation in mice has been collected in individual animals. Overall, individual mice have a thermoneutral zone of approximately 30 to 32 °C, which is considerably warmer than the standard housing T<sub>a</sub> of 22 to 24 °C. Thus, it is expected that standard housing conditions impart varying amounts of cold stress on mice. However, because they frequently huddle and thereby reduce heat loss it can be assumed that a standard housing T<sub>a</sub> of 22 to 24 °C is probably not thermally stressful for groups of mice. If huddling behavior alters the thermoneutral zone, then groups of mice should prefer markedly cooler T<sub>a</sub>s compared to individual mice. To address this issue, it is necessary to measure the thermoregulatory behavior of individual
and groups of mice. In one study, female CD-1 mice were housed in groups of five or individually in a temperature gradient while selected $T_a$ and motor activity were monitored. Single- and group-housed mice displayed a circadian oscillation of selected $T_a$ with relatively warm $T_a$ of ~29°C selected during the daytime and cooler $T_a$ of ~25°C during the night. Motor activity was low during the day and high at night. Selected $T_a$ of aged (11 month old) mice was ~1.0°C warmer for single mice as compared to groups. Thermal preference of younger mice (two months old) was similar for individual- and group-housed animals. In another study, autonomic thermoregulatory requirements were measured in groups of female CD-1 mice that were placed inside a direct calorimeter. Groups of five mice displayed a minimal metabolic rate at a $T_a$ of 30°C. As $T_a$ decreased below 30°C, metabolic rate increased in a near linear fashion. Thus, groups of five mice display a metabolic profile that is qualitatively similar to individual mice. Their zone of metabolic thermoneutrality is approximately 30°C. Daytime metabolic rate has not been determined in groups of mice. The operative $T_a$ of mice housed in standard facilities was estimated by measuring the cooling rate of "phantom" mice modeled from aluminum cylinders. The results show that the typical housing conditions for single- and group-housed mice are much cooler than their $T_a$ for ideal thermal comfort. Wood shaving bedding material provides the best type of insulation that allows mice to maintain a relatively warm microenvironment. Wire-screen floors and wood chip bedding has minimal thermal insulative value.

To summarize, groups of five mice display a behavioral and autonomic thermoneutral zone that is surprisingly similar to individual mice. The standard housing $T_a$ of 22 to 24°C is significantly below the thermoneutral zone of groups of mice suggesting that they are subjected to varying degrees of cold stress under standard housing conditions.


Two consecutive generations of mice were raised at graded room temperatures ranging from 12 to 32°C at intervals of 2°C. The delivery rate decreased at 30 and 32°C, and the litter size and weaning rate decreased above 28°C. No significant difference was demonstrated within the 12 to 26°C range for any reproduction parameters observed. The body weights of the first-generation mice born at 22°C did not significantly differ after being transferred to rooms in the 14 to 28°C range after eight and 16 weeks of exposure. The second-generation mice born and reared at various temperature levels did not show significant intergroup differences in weight within the 20 to 26°C range at any age in the growth period. No significant intergroup difference was observed within the 20 to 26°C range with respect to food and water intakes in the second-generation mice. Hematological values and organ weights in the first- and second-generation mice of both
sexes did not significantly differ in any parameter within the 20 to 26 °C range. The results suggest the temperature range of 20 to 26 °C to be optimal for laboratory mouse rooms.


Two generations of rats were raised at animal room temperature ranging from 12 to 32 °C at steps of 2 °C. The body weight of rats born in 22 °C environment and exposed to each temperature did not significantly differ within the range of 16 to 28 °C. The delivery rate, litter size, and weaning rate decreased at 30 °C and 32 °C, and only the weaning rate had a tendency to decrease at 12 °C. No significant difference was demonstrated within the 14 to 28 °C range for any reproduction parameter observed. The body weight of sucklings did not differ at birth within the 12 to 32 °C range; and at three weeks of age, there were no differences within the range of 18 to 28 °C. The body weight gain in both sexes after weaning was generally small when the temperature was below 18 °C or above 30 °C. There were no significant differences in food intake within the 20 to 26 °C range, in water intake within the 12 to 26 °C range, in hematological and serum biochemical values within the 20 to 26 °C range, and in organ weights within the 18 to 28 °C range. Therefore, the range of 20 to 26 °C (68 to 78 °F) was the optimum temperature range in rat rooms.


The zone of minimal metabolic rate for wistar rats was between 28 and 32 °C. At this range of ambient temperatures the rats exhibited low motor activity. The rats were most active at temperatures between 18 and 28 °C.

1.3.3 Literature Search on Rats and Mice Moisture Production

Animals produce heat and moisture from their metabolic processes in consistent, predictable quantities. Moisture is vaporized from (1) the passive loss of the water by diffusion through the skin and through respiration and (2) the active loss of water via sweating, panting, and application of saliva, urine, and other forms of moisture to the fur and skin.

Chongyang Liu: *Indirect Calorimetry Study on Laboratory Rat Metabolism at Various Air Temperatures and Velocities*, University of Illinois at Urbana-Champaign, unpublished thesis 1995, 1996.
The study reported evaporative water loss of 4.82 g H₂O/kg/hr at 20 °C.


The study reported a mean evaporative water loss of 5.03 gH₂O/kg/hr at 22 °C.


The most prominent effects on evaporative water loss occurred when temperature was elevated above 30 °C for rats. The temperature ranges studied ranged from 14 to 34 °C. He measured values of evaporative water loss of 1.2, 1.5, and 1.8 mg H₂O/mLO₂ at 16, 22, and 30 °C, respectively.

**Note:**

The difference in the values in the above studies reflects the importance of how the body fluids were handled. Gordon (1987) had a pan filled with oil below the rats to collect all the waste and prevent any of that moisture from getting into the air. The oil in the Liu (1996) study formed only a thin layer over water and may not have been sufficient to stop moisture evaporation from both drinking water spills and body fluids voided into the air.

Schmidt-Nielsen gives a value for the albino mouse at “laboratory temperature” of 0.85 mg water per ml oxygen consumed. This is equivalent to 3.06 g water/kg/hr. Schmidt-Nielsen, B. and Schmidt-Nielsen, K. *Pulmonary Water Loss in Desert Rodents*. Am. J. Physiol. 162,31-36, 1950.


In studies of wind effects on deer mice, evaporative water loss at 30 and 35 °C indicated that evaporative water loss increased with increasing temperature, and wind speed from 0.05 to 3.75 m/s had little effect at 30 °C while an increase in air velocity increased moisture production at 35 °C.

Studies of rats and other rodents have found that approximately 50 percent of the passive water loss occurs via diffusion across the skin. Respiratory moisture production is highly affected by changes in body temperature and ambient temperature. An increase in breathing rate was found to elevate evaporative water loss in restrained rats, accounting for as much as 21 percent of the total heat loss at an ambient temperature of 31 °C.

1.3.4 Ammonia and Carbon Dioxide Physiochemical Properties

Ammonia and carbon dioxide are the major gaseous pollutants derived from animals and animal waste within the cage. Below are the physiochemical properties as well as current standards set by American Conference of Governmental Industrial Hygienists for ammonia and carbon dioxide.

Ammonia (NH₃):

Ammonia is a colorless gas with a sharp, intensely irritating odor. It is lighter than air and easily liquefied by pressure, and it is very soluble in water, alcohol, and ether. Physiochemical properties include the following:

- Molecular weight: 17.03
- Specific gravity: 0.77 at 0 °F; 0.6819 at boiling point
- Boiling point: -3.5 °C
- Freezing point: -77.7 °C
- Vapor pressure: liquid, 8.5 atmospheres at 20 °C

Henderson and Haggard (1927) record temporary blindness and intolerable irritation from high concentrations. Osmond et al. (1968) describes severe eye damage and irritation of the glottis from exposure at high concentration. Schenker (1967) reports that toxic doses of ammonia acutely affect cerebral energy metabolism and this effect is localized at the base of the brain. Smyth (1956) found 1 ppm detected and identified by 10 subjects. Analysis of data obtained in plant surveys conducted by the Bureau of Industrial Hygiene, Detroit Department of Health, during 1965 to 1970 and National Institute For Occupational Safety and Health: Criteria For A Recommended Standard Occupational Exposure to Ammonia, 1974, identifies the limit of detection to be below 5 ppm and the complaint level to be 20 to 25 ppm. Patty’s Industrial Hygiene and Toxicology (1996) states that the odor threshold is between 5 to 53 ppm. As these last three references show, the actual level of initial odor detection varies significantly.

The American Conference of Governmental Industrial Hygienists recommended a time-weighted average (TWA), threshold limit values (TLV) of 25 ppm (approximately 18 mg/m³) to protect against irritation to eyes and the respiratory tract and minimize discomfort among workers. It also
recommended that the short term exposure limit (STEL), however, be set at 35 ppm (approximately 27 mg/m$^3$).

Other countries’ standards are as follows:

West Germany (1974), Japan, Yugoslavia, 50 ppm; East Germany (1973) 33 ppm; USSR (1976), Sweden (1975), Australia, Belgium, Finland, Hungary, Italy, Netherlands, Poland, Romania, Switzerland, all 25 ppm.

**Carbon Dioxide (CO$_2$):**

Carbon dioxide is a colorless, odorless, noncombustible gas. Chemical and physical properties include:

- Molecular weight: 44.01
- Density: 1.527 (air = 1.0)
- Melting point: sublimes at -78.33 °C at 76 torr
- Vapor pressure: > 1 atm at 20 °C
- Solubility: soluble in water to the extent of 171 ml/100 ml at 0 °C and 76 torr, 88 ml/100 ml at 20 °C, and 36 ml/100 ml at 60 °C. Under higher pressure carbon dioxide is more soluble.

Carbon dioxide when inhaled in elevated concentrations may act to produce mild narcotic effects, stimulation of the respiratory center, and asphyxiation, depending on the concentration present and the duration of exposure. The literature contains a variety of reports on human response at varying concentrations. Deaths have been reported from asphyxiation in workers exposed at high concentrations of carbon dioxide. Winter (1937) found that stimulation of the respiratory center is produced at 50,000 ppm (five percent). Schaefer (1951) reported that submarine personnel exposed continuously at 30,000 ppm were only slightly affected as long as the oxygen content of the air was maintained at normal concentrations (minimal content 18 percent by volume). When the oxygen content was reduced 15 percent to 17 percent, the crew complained of ill effects. The gas is weakly narcotic at 30,000 ppm, giving rise to reduced acuity of hearing and increasing blood pressure and pulse. Above this concentration subjective symptoms occur. Signs of intoxication are produced by a 30-minute exposure at 50,000 ppm. Aero Medical Association; Committee on Aviation Toxicology, Blakston, New York, (1953) concluded that 7 to 10 percent produces unconsciousness within a few minutes. Flury and Zernik (1931) quote Lehman-Hess as stating that exposure at 5,500 ppm of carbon dioxide for six hours caused no noticeable symptoms.

Based on the long-term exposure studies, even though the majority of references are concerned with studies on physically fit males in confined spaces, the American Conference of Governmental Industrial Hygienists (ACGIH) recommended a TLV-TWA of 5,000 ppm (9,000
mg/m³) for carbon dioxide. In light of the short-term exposure studies of Sinclair and associates (1969), ACGIH also recommended a STEL of 30,000 ppm (54,000 mg/m³).

Other countries’ standards are as follows:

Australia: 5,000 ppm, STEL 30,000 ppm (1990); Federal Republic of Germany: 5,000 ppm, short-term level 10,000 ppm for 60 minutes, three times per shift (1989); Sweden: 5,000 ppm, 15-minute short-term level 10,000 ppm (1984); United Kingdom: 5,000 ppm, 10-minute STEL 15,000 ppm (1987).

1.4 Project Summary

At project conception, NIH recognized that a comprehensive study of air movement, heat transfer, and contamination dispersal in the macro- and microenvironment in animal facilities could only be undertaken using Computational Fluid Dynamics (CFD). CFD is an advanced three-dimensional mathematical technique that can be used to compute the motion of air, water, or any other gas or liquid. If you were learning fluid dynamics as recently as, say 1960, you would be operating in the “two-approach world” of theory and experiment. However, the advent of the high-speed digital computer combined with the development of accurate numerical algorithms for solving physical problems on these computers has revolutionized the way we study and practice fluid dynamics today. The CFD approach provides a tool with which you can carry out numerical experiments and in this way undertake the comprehensive study envisaged.

It was clear from the beginning that the disadvantage of this approach would be the vast amount of data generated for each and every simulation, compounded by undertaking many simulations. This project presents summaries of these data, in terms of mean cage values or mean values in the scientists’ breathing zones, to allow a designer or specifier to identify a satisfactory animal facility configuration.

One of the major advantages of using CFD for such research is the confidence to simulate different configurations knowing that all conditions, except those being varied, remain constant. This makes comparison of CFD simulations much more reliable than comparison of experimental studies, where there is always uncertainty that all conditions are kept the same.

However, it is important that all conditions are understood and correctly specified in the CFD simulation so the results it produces are as accurate as possible. In this study, inputs for the CFD, such as heat dissipation and surface temperature of the mice; in addition to moisture, CO₂, and NH₃ generation rates for mice; needed to be defined. Also the characteristics of the cages under study needed to be understood so an accurate mathematical model of a Microisolator cage could be built. As most of these data were not available from literature, an unprecedented set of experimental measurements was undertaken.
Experimental work was undertaken with an instrumented shoebox cage and Microisolator placed in a wind tunnel. Using different approach velocities, the following were measured:

- Cage air velocities
- Cage ventilation rate
- Cage temperature rise

The measurements were taken for:

- Different cage orientations
- With and without simulated mice
- Different cage configurations and designs
- L/min CO₂ injection rate using infrared analyzers
- 100 mL/min CO₂ injection rate using infrared analyzers
- 100 mL/min SF₆ injection rate using gas chromatography

All combinations of approach velocity and cage configuration were simulated in several hundred CFD simulations with appropriate boundary conditions specified to ensure the CFD model agreed with the measured data. This work is documented in section 4.

A further set of extremely careful experiments was undertaken with outbred female mice of HSD-ICR strain, five per cage on hardwood (Beta chip) shavings as bedding. Three indirect, convective calorimeters were used for this phase of project. Air temperature, velocity, and relative humidity were controlled in each calorimeter. The calorimeters were used to measure mice heat, and CO₂ and NH₃ mass production for different cage humidity conditions. This gave constant CO₂ generation rates but showed that NH₃ generation rose over the 10-day period of the experiments and depended on the relative humidity level. This meant the CFD data needed to be post-processed to reflect a room’s performance over a 10-day period.

A third series of experiments were carried out to measure the following factors at several thousand points in an “empty” room using a sampling frequency at each point of 40 Hz.:

- Average velocity
- Minimum velocity
- Maximum velocity
- Turbulence intensity

Further measurement of boundary conditions was carried out in an “occupied” room with racks, cages, and simulated animals. At each point the following were measured:

- Mean air velocity
Median air velocity
Turbulence intensity
Air velocity fluctuation range
Temperature
Concentration of CO₂

Using the experimental data to provide boundary conditions, various room and ventilation conditions were simulated by CFD. These included:

Supply type
Exhaust number and location
Supply flow rate
Use of change station and its location
Number cages on the racks

In total, 100 variations were simulated and these are detailed in section 2. The results are presented in section 3.

Over 18,000 super computer hours (CRAY equivalent) and 10,000 man-hours were needed to complete the CFD and experimental parts of this project.

It should be noted that these reports use Systems International (S.I.) units, that is kilogram, meter, second, Pascal (1 Newton per square meter), watt (1 joule per second) and degrees Celsius, with approximate conversions to Imperial units displayed for reference only. One exception to this rule is the use of cfm (cubic feet per minute) which is in common use for flow rates.