**Vivarium Design And Management For The Laboratory Animal Veterinarian.**

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The Physical Plant And Operational Procedures Need To Be Able To Safeguard The Animals’ Environment

ENVIRONMENT = Physical components of the animal’s surroundings that may impact animal well being or have research effects

The practices used to modify it for the purposes of controlling the introduction or spread of disease

**Primary Enclosure Environment**

**Physical Factors**

- Mass
- Energy
- Gaseous
- Particulate
- Heat
- Sound
- Light
- Vapors
- Gases
- Radiation
- Conduction
- Convection

Viable
Non-Viable

**Temperature**

- The most critical environmental parameter to control
- Elevated temperatures more problematic than low temps.
  - Limited animal adaptive mechanisms
  - Substantial departures from recommended ranges and large daily fluctuations are the principle problem
  - Control within a very narrow range is not essential for animal health but may reduce variation caused by animal adaptive processes

**Heating, Ventilation and Air Conditioning Systems**

**Thermoregulatory Effector Responses**

(Rats and Mice)
Factors Influencing Temperature

Cage design and location
- Heat transferred by conduction, convection and radiation

- Cage design affects cage/room coupling (Woods and Besch); location in room affects heat transfer as well as other coupling:
  - Also affects air and gaseous contaminant transfers, particulate dilution, water vapor transfer, light incursion, and noise attenuation
  - Addition of a filter top alters internal cage temperature as a function of ventilation

- Cage materials also affect internal cage temperature
  - Heat sinks versus insulators

- Number of animals in cage also affects temperature

Recommended Temperature (°C) and Relative Humidity (%)

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Relative Humidity

- HVAC system should maintain between 30 – 70%
  - Tight control not required but large daily fluctuations should be avoided if possible

- Influences total heat load on animal since affects insensible heat loss

- Influences NH3 concentration and other gases that are water soluble

- Some association presumed with “ringtail” in rodents – never established scientifically.

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Veilation

- What are we trying to accomplish with the ventilation system?
  - Control the thermal environment of the animal
  - Refresh the microenvironment
  - Provide a mechanical solution to a husbandry issue (delay bedding change)
  - Provide a mechanical solution to disease or allergy control (dilute out agents)
Ventilation

**Definition:** The process using air to affect the temperature, humidity, gaseous and particulate concentration of an animal's environment.

- Traditionally, the quality of air in animal rooms has been monitored in the room, not the animal cages.
  - Sampling methodologies, sampling volumes and assay methods difficult to adapt to individual cages.
  - Relates better to caged large animals than small animals in rack mounted cages.

Ventilation Rate - Percentage of outside air introduced into the supply air quantity.

Air Exchange Rate - Number of times in one hour the air volume of animal room is removed and exchanged with conditioned air.

\[ \text{ACH} = \frac{\text{Supply CFM} \times 60}{\text{Room Volume}} \]

Cooling Load Calculation

- Sets ventilation requirements for each space.
  - Cooling capacity ventilation requirements exceed heating season requirements.
- Need to set animal type, size and number for each room.
- Need to specify the temperature range the space is to be maintained within.
- Need to set maximum outside ambient conditions that system will operate under.
  - Usually 95-97% degree days.

Space Heat Loads

- Solar
- Transmission
- Infiltration
- Ventilation

Animal Heat Gain Formula

\[ \text{Q Total} = 0.167 \times \text{[# of Animals (avg. wt. in Grams)]}^{3/4} \text{ BTU/Hr} \]
\[ \text{Q Latent} = \text{Q Total} \times 0.33 \]
\[ \text{Q Sensible} = \text{Q Total} \times 0.66 \]

**Note:** Calculation based on standard metabolic rate for laboratory animals given in “ASHRAE Fundamentals Handbook”.

Ventilation

- Where did 10-15 AC/hr come from?
  - “Rule of thumb” – many cooling load calculations will result in AC rates in this range but not all.
  - High and low animal mass outside this range.
- What about 100% Fresh air exchange?
  - Maximum particulate and gaseous dilution but energy intensive!
  - Tends to counteract suboptimal husbandry program.
  - Allergen and odor dilution but not proportional to the risk or cost.
Recycled Air

- Two sources:
  - 1) Air from animal Areas
  - 2) Air from non animal areas
- Must be:
  - Conditioned – temp and rh
  - Particulate removal in proportion to contaminant risk
  - Removal or dilution of gaseous contaminants
- Inappropriate for biocontainment areas

- Provides energy savings
- Unlikely to maintain other air quality parameters if recycle more than 50%
  - Animal population dependent
  - If using microisolators or isolators with independent HVAC then secondary enclosure can be ventilated only to maintain temp and rh

- Draftiness/point velocity (ADPI): measured or calculated values used to describe the abstract concept of discomfort perceived by humans when sensing air movement
  - No data to suggest any biological consequences in animals
  - No general agreement even in humans as to what conditions of air movement are objectionable

HEPA Filter Considerations

- Only addresses particulates – not gases or vapors
- Only as good as housing and seal
- Duct work design should allow for routine testing (mass or scan DOP)
- Air Flow Restriction
  - Cost (operational & Capital)

Air Filtration

- ASHRE rated pre-filter (15,30,65,85,90%)
  - Use prefilters to take large particle load of large particles
- Hospital or Industrial Grade (90-95% DOP or non certified) – not HEPA
  - Fine particle removal
- HEPA filter (High Efficiency Particulate Arrestance)
  - Absolute or Pharmaceutical Grade (99.97% DOP or better) pretested
  - Tested to arrest 0.3 micron size particle (112 million 0.3 micron particles is size of pencil tip); medical grade air

- Use 1 or 2 sets of prefilters to take out large particles; should replace frequently
  - Slows loading of downstream small particle filters
- Terminal filters can be 90-99% depending on risk evaluation
  - Mount in gel seals to prevent particulate blow by
  - Should Mass or scan test regularly or replace at preset frequency

- No filter is 100% efficient at all particle sizes and will also get bleed through as filter loads with particulates
- Exhaust filtration seldom required
  - Coarse washable filters in room exhaust grates
  - Exception is biocontainment – may require prefilters, HEPA's and "bag out" change system with bypass
CFD Technology

- Computer modeling using animal, equipment and structural heat gains
  - Properly locate diffuser, exhaust grilles, racking, work station, etc.
  - Can look at particulate and gaseous movement in rooms as well as thermal gradients
  - Candle effect of racking shown as vectors on plot
- Can model the whole site as well
  - Look at particulate dispersion and mass-less particle diffusion around and between buildings

Room Air Volume And Distribution

- Supply high and return high versus supply high and return low
  - No simple or universal answers
- Powered versus passive exhaust
- Variable volume air supply
  - Adjusts air supply based on monitored room conditions (e.g. temp, rh, NH3)

Air Pressure Differentials

- Static pressure difference (inches of water) between 2 spaces
  - (+) pressure to keep things out
  - (-) pressure to keep things in
- When door opened between areas static pressure differential lost
  - No directional control of air movement
  - Can't depend on SP Diff for disease control

Air Pressure Differentials

- Cascading pressures in entry locks to barrier facilities
  - Ventilate each lock component separately
  - No grills in doors: particulate transfer
- Pressures above ½ inch of water makes opening doors difficult
- Install static pressure gauges/indicators across filters and critical housing spaces

ANIMAL HOUSING

Room Design

- Rectangular shape most efficient if caging is to be located parallel to wall
  - 12 x 20 feet for rodent caging gives most wall space for the least center room space
    - Based on two corridor concept
  - Caging perpendicular to wall requires wider room
- Pens/ runs require larger rooms
  - 16x20 feet or larger
Rooms

- Designed for frequent cleaning and disinfection with aqueous agents
  - Smooth surfaces
  - Durable surfaces (masonry best; gypsum board worse)
  - Chemically resistant finishes on walls
  - Chemically resistant floor (e.g., troweled on epoxy)
  - Non-rusting fixed equipment
  - Water resistant outlet covers and lighting

- Small rooms
  - Investigators usually prefer
    - "Their own territory that they can control"
  - Very inefficient especially for microisolation caging
  - No room for change station
  - Large room better for microisolation housing (barrier at cage level)
    - 16X20 or larger
    - Gives room for multiple change stations and room to work on individual racks
  - Remember barrier at cage level not room

- Anterooms
  - Used where extensive PPE protection is required
  - Used for housing monitoring equipment
  - Can be used for procedure area
  - Commonly just accumulate clutter
  - Usually get little use

Corridors

- Wide enough to accommodate bidirectional equipment movement
  - Recess wall mounted equipment
  - 6 to 8 feet wide
  - Wall protection critical
- Utilities accessible through ceiling panels
  - Plan for frequent access
- Use sound absorbing strategies
  - E.g., air locks, doors, ceiling tiles, etc.

FLOW PATTERNS
**Operating Philosophies for Animal Housing**

- **Perimeter/ Facility Bioexclusion** – Conventional Facility; open cages
  - Treat the entire facility as one microbiological unit
    - Try to detect and exclude microorganisms at perimeter
      - Vendor qualification
      - Quarantine
  - Assume door to animal room will limit spread of infection
  - Open cages are used for animal housing
    - Airborne and fomite cross contamination
  - Control of spread of infection relies almost exclusively on disinfection, dilution and culling

- **Room Level Bioexclusion** – Barrier Room; open cages most common

- **Group Level Bioexclusion** – Isolators; open cages

- **Cage Level Bioexclusion** – Microisolation Caging

**Housing Systems**

- **Open caging**: Still common in most facilities
  - Pens/runs, also suspended wire and solid bottom rodent cages
  - Used in toxicologic research extensively
  - Cage to cage transmission of infectious agents not prevented
    - Dilution, disinfection and culling are a principle tools for controlling infection

- **Open Cages**
  - Cage wash is principal means of disinfection
    - No guarantees
  - Room/surface sanitation used to reduce (but not eliminate) contaminant pressure
  - Animal manipulation not done aseptically
Large Animals (e.g., Dogs, NHP, Rabbits, Swine, etc.)

- Considerations
  - Consume a large amount of space
    - Need to be relevantly certain of level of usage
  - Mechanically intensive and large waste production
  - Need to address exercise and enrichment requirements
    - Also social housing

- Tight microbiological control, not really achievable with large animals
  - Microisolators and isolators not available
  - Barrier possible but expensive if done correctly.
    - Even then, there are no guarantees
  - Single or dual housing in cages requires greater mechanical support and labor
    - Can only do in some species (e.g. dogs, NHP)

- Cleaning in place often the only option (especially for pens and runs)
  - Wet floor injuries

- Pens / Runs
  - Collapsible / convertible versus fixed pens
  - NHP: safe handling and capture is critical
    - Need to build into design: also enrichment strategies
    - Large pens for group housing need means of separating animals
    - 2-door rule
    - Need provisions for logical clothing change and use of PPE
    - Possibility of changing space requirements
  - Waste removal and processing
    - Environmental issues: high biological oxidation load
    - On site pretreatment may be necessary
  - Flushing has its own problems
    - Mixture of solid waste, liquid waste and often bedding
    - Large drains (6”), troughs, rim flush
    - May need in line macerator

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Barrier Room/ Facility

- No such thing as a partial barrier
- Personnel entry Lock
  - 4 compartment (complete separation), clothing change, individually ventilated
  - Size to anticipated traffic
- All materials equipment and waste enter and exit through autoclave or spray port
- Independent mechanical services
- Provide necessary support services within barrier.
  - Minimize passage of animals and equipment across barrier

- Expensive to construct and operate

Microisolation Cages

- Static
- Ventilated

The same infection control practices regardless of which type is used!
Microisolation caging

- Static versus Ventilated
- Ventilated
  - These choices influence architectural and mechanical design
    - Supply and exhaust from and into the room
    - Captured exhaust
    - Dedicated supply
    - How much of cage will be disinfected / sterilized
    - What level of aseptic technique will be used

Housing Density and Cleaning Frequency

<table>
<thead>
<tr>
<th>Microisolation Cages</th>
<th>Reliable Microbiological Control</th>
<th>Better Environment</th>
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ISOLATORS

- Used to divide the population into microbiologically independent groups
- Infection control is almost technique independent
- No direct contact between animals and personnel
  - People and clothing not much of a risk
- Ideally, everything is done within the isolator
  - If animals must be removed and returned:
    - Must be maintained in a microbiologically controlled environment at all times
- HVAC for isolators
  - Central versus individual air supply
  - Captured exhaust
- Can be used in less sophisticated spaces, but still need an area for aseptic experimental manipulation
  - To be efficient, need a ceiling height of at least 10.5 feet
  - Chemical resistant flooring needed due to high disinfectant use

- Cubicles, bio-bubbles and ventilated cabinets
  - Space intensive secondary enclosures
  - Physical separation, not microbiological separation
    - Assumes all microbial transmission is airborne – Bad Assumption
  - Utilizes other caging systems, therefore need to accommodate their requirements

Cage Wash
Cage Wash Location

**Central**
- High equipment utilization—little redundancy
- Increased transit time
- Greater staff efficiency
- Lower equipment cost

**Decentralized**
- Reduced transit time
- Redundancy
- Less efficient use of staff
- High equipment cost

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**The Separation Between “Clean” and “Dirty” is Often Not Complete**

*Open Door Between Sides Of Cage Wash*

OPEN DOOR  
BETWEEN SIDES  
OF CAGE WASH

SAME TECHNICIAN 1

DIRTY SIDE  
CLEAN SIDE

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**Cage Wash**

- Need to consider how cage wash associated supplies will be stored and locations of those stores (e.g., bedding, feeding, detergents, etc.)
- Waste removal from dirty side of cage wash
  - How will waste be contained and transported
  - Where will it be stored
  - Need to treat it like it was infectious
  - Bedding dump stations
  - Not a fail proof substitution for PPE

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**Cage Wash**

- If using microisolation cages and autoclaving
  - How will you maintain disinfection status?
  - Autoclaving assembled versus disassembled caging
  - Point of assembly—cage wash/clean room versus in the animal room
  - What ancillary functions will be located in cage wash?
    - Floor cleaning equipment storage
    - Storage of general cleaning supplies and utensils
    - Disinfectant preparation
    - Euthanasia equipment
    - Carcass storage

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**Cage Wash**

- Need to allow for a 15-20 percent cage turn around for conventional cages
  - Add 5 to 10 percent more if autoclaving microisolation cages
  - Provide point source capture of heat and moisture from washing and autoclave equipment
  - Complete separation between clean and dirty
    - Especially important if assuming the cage washer is supplying necessary disinfection

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**Capacity calculations for equipment**

- Cycle time
- Load volume / configuration
- Hours of operation
- Subsequent processing
  - Slowest component of operations drives process
- Downtime

**Automation versus manual feed of equipment**

- Automation: $1.5 to $2 million (minimum)
  - $70 to $90 K per year maintenance contract
  - Need specialized cage washing equipment to interface with robot
  - Need adequate cage volume to make economic sense—at least 10 to 20K cages in use

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**Cage Wash**

- Bulky supplies will often dictate a cage wash location near loading docks
- Clearance for door openings and work areas around equipment
  - Need to establish realistic boundaries for cage / rack holding
**Cage Sanitation - Process Flow Open Cage**

- Animal Room → Cage Change → Soiled Cage → Wash → Clean Cage → Storage
- Bedding → Feed → Contaminant Movement

**Cage Sanitation - Process Flow Microisolation Cage**

- Animal Room → Cage Change → Soiled Cage → Wash → Clean Cage → Disinfection → Storage
- Bedding → Feed → Contaminant Movement

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**WATER**

- Treatment used to alter chemical content and to remove microbial contamination
  - **Bacteria**
    - Filtration used for reducing load
    - Physical or chemical disinfection used to kill off organisms
    - Condition dependant
    - Capacity of disinfectant and spectrum of action
    - Need to set realistic goals

- **Viruses**
  - Rodent specific viruses unlikely to be an issue:
    - Filtration will do little to affect load due to usable pore size
    - Chemical disinfection is a practical way to address
  - Mixed bed deionization and reverse osmosis used to alter ion composition

**Water Treatment**

- Water treatment methods
  - Acidification
  - Halogenation (Chlorination)
  - Reverse Osmosis
  - Ozonation
  - Filtration (Particulate) and Activated Charcoal
  - Deionization
- Need to use a combination of methods and treat all water used in animal rooms

**Physical Means of Disinfection/Sterilization**

- **Heat**
  - Moist (autoclave)
  - Dry (oven, hot auger)
  - Ohmic
  - Microwave
- **Irradiation**
  - Gamma ray
  - β-electron (E-beam)
  - X-Ray
  - UV
  - Gas plasma
- **Chemical**
  - Liquid
  - Vapor
  - Gas
- **Filtration**
  - Membrane filter
  - Depth filter
  - Reverse osmosis (?)

**Autoclaves: Key Operational Considerations**

- Load configuration by load type
- Regular Vacuum hold testing
  - Monthly
- Multiple probe temperature calibration
  - Twice a year
  - At least 12 to 16 probes around and in the load
  - F value of 24 for "sterilization"
- Multiple point temperature and biological indicator validation
  - Once a year
  - At least a four to six log reduction in spores
### Gravity Displacement Steam Sterilizer

![Gravity Displacement Steam Sterilizer Diagram](image)

### Food And Bedding Using Gamma Irradiation

![Food And Bedding Using Gamma Irradiation](image)

### Units and Conversion Factors for Radiation

- 1 roentgen (r) = $2.58 \times 10^{-4}$ ?/kg
- 1 rad = 100 erg/g
  - = $10^{-2}$ Joules/kg
  - = $6.29 \times 10^{13}$ eV/g
  - = $2.4 \times 10^{-6}$ cal/g
- 1 gray (Gy) = 100 rad
- 1 megarad (Mrad) = $10^{6}$ rad

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### Irradiation: Operational Issues Associated With “Sterilization”/Disinfection

- Bio-burden reduction is dose dependant
  - Dose variables: Intensity, time, target size, type of radiation, radio-absorbency of substrate as well as concentration and radiosensitivity of microorganisms in the substrate
- Various areas of the load get different doses of radiation
  - Need to specify dosage in terms of maximum or minimum dosage
    - Minimum dose is the minimum amount of irradiation that is applied to all areas of the load even though some areas will experience greater amounts of radiation.

### Irradiation: Operational Issues Associated With “Sterilization”/Disinfection

- Radiation is considered a food additive
  - Limits set on maximum dose that can be applied and still be transported and sold commercially across state lines
    - 50 kGy (5 Mrad) maximum dose – minimum dose is much lower
  - No limits if it is irradiated as an experimental diet
- For pelleted rodent feed 30-35 Mrad is needed under usual bioburden levels to achieve sufficient disinfection to support axenic animals
- Bedding requires 15 Mrads minimum dose to achieve necessary disinfection to support axenic animals
Lighting

- Control of photoperiod critical for rodents
  - Less critical in some larger species
  - Timer with manual override and indicator
  - Optimum photoperiod for rodents is 14hr.(L):10hr.(D)
- Fluorescent – ceiling mounted – most common
  - Rapid change out fixtures for barrier rooms
- Moisture resistant covers
  - Waterproof generally not needed

- Intensity
  - Decreases with the square of the distance from the source
  - 30 ft candles one meter off floor (Guide)
    - High intensity can cause photoretinal injury in albinos
    - No recommendations for non-albinos
    - Recommended for adequate husbandry
    - Effective intensity within cage lower than room
- Full spectrum unnecessary
  - Mixed bulbs (same type of bulbs from different mfgs.) will achieve near full spectrum

Continuity of Critical Services

- Emergency electrical power
  - Sufficient generator capacity to run essential environmental controls
    - Also mission critical research equipment (e.g. sample freezers, tissue culture incubators, etc.)
  - Sufficient fuel for at least 72 hours
  - Auto transfer and regular testing under load
  - Consider dividing load between several units with provision for cross-tying in case of single unit failure

- Consider provisions for water back up for:
  - Animal drinking water
  - Cooling tower operation
  - Storage for a minimum of 14 days of working supplies of food and bedding
  - Also need to maintain supply of essential mechanical system spare parts

Floors

- Epoxy resin floors most common
  - Surface preparation is everything
  - Chemical resistance
  - Finish is important:
    - Smooth versus rough
    - Balancing ease of cleaning with safety
  - Can be a safety issue with pen housed animals such as swine and ruminants
- Sloping – only in areas of high water use
  - Important for pens, runs and certain cages that will be cleaned in place
  - Use where stables are employed
    - Minimum of 1/8 inch per running foot
- Vapor transmission
  - Water vapor accumulates under concrete slab as a result of high alkalinity of concrete
  - Moisture collection affects bond to concrete—blisters, formation and delamination
  - Water vapor moves through the concrete and the flooring causing destruction in localized areas
  - Broadcast floors block water vapor passage above 3 pounds / 24 hours / 1000 square feet
  - Minimize impact by using high density concrete as sub-flooring
- Concrete slab joints
  - Use expansion or control joints and elastomeric caulk to control cracking

Sound / Noise Control

- House larger animals separate/ away from rodents
  - Use full height walls to separate housing groups (walls continue above ceiling to roof)
- Seal all penetrations
- Sound absorbing ceilings in corridors
  - Consider insulated tray ceilings vs. drop panel
- Doors at end of all corridors
- Use non-housing space as buffers
General Recommendations

- Seal junctions and penetrations
  - Caulk around:
    - Ceiling mounted lights and HVAC ducts
    - Conducts into electrical boxes
    - Pipe collars and around pipe penetrations
  - Some penetrations not obvious (e.g., doorframes)
  - Eliminate return grills in doors
- Stainless steel plates on floor to wall junctions with undertam of sealant
- Drop sweeps on doors and gaskets on door / frame strikes
- Use water resistant outlet and switch covers

General Recommendations

- Protect surfaces
  - Corner guards
  - Bull-nose curbing or wall bumper guards
  - Locate wall-mounted fixtures in protected area
- Doors
  - Door strike plates: beware of door bumper guards
    - Double doors - locking
  - Low profile or drop door handles on doors
  - Power doors in high traffic areas
  - Galvanized versus fiberglass doors
  - Viewing windows in doors
  - Door closers important

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Miscellaneous

- Door swings: lose space in arc
  - Doors swing into rooms, not into corridors
- Fire code mandates some door locations
  - Cannot have circuitous exit
- Sinks
  - Decide on how they will be used (e.g., for what purposes)
  - Stainless steel looks good for the first five minutes
  - Ceramic and fiberglass less expensive and replaceable
  - Consider depth if filling cleaning buckets
    - Mop / sink
  - Ease of maintenance

Operating Room / Suite

- Def.: One or more rooms in which the surgical procedure(s) are conducted
- Features:
  - Minimal fixed equipment preferred
  - All surfaces accessible for regular cleaning with aqueous agents and disinfectants
  - Ceiling height should be at least 10 feet (3.0 m)
    - Provide for ceiling-mounted equipment
    - Rodent facilities--8 feet (2.44 m) may be appropriate
  - Location of surgical tables controls this
  - Need to minimize length of connecting cable or lines from service outlet to operating table
  - Ceiling versus wall-mounted service outlets
  - Service outlets located 60 inches (152 cm) off floor by convention

Storage

- Aka. Unassigned space
- Determine what will be stored and in what quantities
- Should be located in reasonable proximity to where the stored materials will be used.
- How will materials be moved in and out of the space?
  - Beware of pallets and forklifts
  - Door protection and swings
  - Provide for orderly storage and easy cleaning of space
    - Cabinets encourage poor sanitation and clutter
    - Open shelving easier to disinfect / clean