

# FACTORS AFFECTING RESEARCH RESULTS



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What is the single largest factor affecting research outcomes?

- Researcher bias:
  - How clean/pure should the glassware or reagents be?
  - What is (or is not) important to the research outcomes?
- 1<sup>st</sup> question researchers should ask:
  - What am I doing that may impact the laboratory also sharing this holding room, procedure room, or equipment?



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## FACTORS AFFECTING RESEARCH

**INTRINSIIC:** Inherent to the animal

Genetics, age, sex, health, nutritional status, immune status, circadian rhythms, endocrine factors

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## INTRINSIC FACTORS AFFECTING RESEARCH Genetics

- OUTBRED ANIMALS: Problems occur when:
  - Colony decreases in size insufficient to maintain heterogeneity
  - Management techniques do not ensure genetic variability
- INBRED ANIMALS: Problems occur when there are:
  - Incomplete inbreedings
  - Mismatings
  - Inadvertent outcrossings with other strains
  - Spontaneous mutations
  - Chromosomal aberrations
  - Residual heterozygosity

**TAKE HOME MESSAGE: GENETIC INTEGRITY IS NOT GUARANTEED BY ITS NOMENCLATURE!**

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## INTRINSIC FACTORS AFFECTING RESEARCH Genetics

- Spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) rats:
  - Genetically disparate, while from the same parental Wistar stock.
  - WKY, used as controls, were discovered to share only approximately 50% of their DNA fingerprint bands in common with SHR.
- Heterogeneity between Dahl salt-sensitive rats (SS/Jr) and Dahl salt-resistant rats (SR/Jr) is small (80% common DNA) .
- Variable metabolism due to qualitative and quantitative enzyme differences between species and strains:
  - Cytochrome P450 concentration and competing isoenzymes
  - Defective/absent enzymes or presence of unique enzymes

**TAKE HOME MESSAGE: SELECT THE STRAIN CAREFULLY!**

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## INTRINSIC FACTORS AFFECTING RESEARCH Age

- Factor in rodent carcinogenicity studies.
- Neonates have immature body systems compared to adults.
- Older animals may develop deficiencies as previously normally functioning systems fail.
- On occasion the response may be strain related!

**TAKE HOME MESSAGE: MATCH THE AGE TO THE NEED!**

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### INTRINSIC FACTORS AFFECTING RESEARCH

#### Gender

- Marked differences in pharmacologic and toxicologic responses to xenobiotics has been demonstrated between male and female rats.
- Sex-related differences in DMBA-induced mammary tumors:
  - Wistar-Furth rats: 100% in females; 19% in males.
  - Copenhagen rats: No difference between males and females

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### INTRINSIC FACTORS AFFECTING RESEARCH

#### Immune Status

- Immunologic dysfunction, including hypersensitivity and allergy, autoimmunity and immunodeficiency may influence experimental outcome.
- Agents that alter immune function:
  - Age
  - Nutritional status
  - Chemicals
  - Drugs
  - Food additives
  - Metals
  - Microbes



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### INTRINSIC FACTORS AFFECTING RESEARCH

#### Immune Status

- Resistance to infectious agents may be dependent on the type of immune response that the host generates:
  - CBA/J mice are highly resistant to *L. major* as they develop a cell-mediated response, vs. the humoral response of the non-resistant BALB/c
  - 100% incidence of leukemia in response to murine leukemia virus by BALB.K mice (humoral) vs. resistance by B10.BR mice (cellular)

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## INTRINSIC FACTORS AFFECTING RESEARCH

### Immune Status

- Nutritional status is dependent on:
  - Type of feed provided
  - Method and amount of feeding
  - Appetite
  - Age
- Various dietary conditions such as mineral, vitamin and protein deficiencies, lipid composition, and the composition of the diet alter the biotransformation of xenobiotics.



TAKE HOME MESSAGE: KNOW WHAT YOU ARE FEEDING!

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## INTRINSIC FACTORS AFFECTING RESEARCH

### Circadian Rhythms

- Many behavioral, biochemical and physiologic parameters (daily, rhythmic, minima and maxima) occur at specific times:
  - Blood counts and coagulation times
  - [CD-3 molecules] on T cell surface
  - T cell response to mitogens
  - Plasma steroid
  - Body temperature
  - Sensitivity to audiogenic seizure induction
  - Drug metabolism and toxicity
  - Susceptibility to neoplasia
  - DNA, protein synthesis

TAKE HOME MESSAGE: BE CONSISTENT WITH DATA COLLECTION!

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## INTRINSIC FACTORS AFFECTING RESEARCH

### Endocrine Factors

- Sex hormones are important determinants of cytochrome P<sub>450</sub> enzyme activity.
- Testosterone administered to female rats increases their ability to biotransform xenobiotics
- Castrating male rats decreases the ability to biotransform xenobiotics.

TAKE HOME MESSAGE: SEX IS IMPORTANT! CHOOSE WISELY.

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**INTRINSIC FACTORS AFFECTING RESEARCH**  
**Endocrine Factors**

- Gonadectomy, hypophysectomy and adrenalectomy will alter hormonal levels
- Neonatal gonadectomy of CE and DBA strains of mice leads to high incidence of estrogen-secreting adrenal tumors
- Postulated that hypersecretion of trophic pituitary hormones in gonadectomized ferrets may result in stimulation of the adrenal gland, producing adrenal gland tumors

TAKE HOME MESSAGE: SEX IS IMPORTANT! CHOOSE WISELY.

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**INTRINSIC FACTORS AFFECTING RESEARCH**  
**Endocrine Factors**

- Prolactin-secreting pituitary adenomas may develop in nulliparous rabbits because of exposure of pituitary acidophils to high concentrations of plasma estrogens secreted by ovarian follicles.
- Prolactin-secreting tumors also develop in rats following prolonged administration of natural or synthetic estrogens.

TAKE HOME MESSAGE: SEX IS IMPORTANT! CHOOSE WISELY.

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**FACTORS AFFECTING RESEARCH**

EXTRINSIC: External to the animal

Physical factors, chemical factors,  
microbial agents, stressors

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## EXTRINSIC FACTORS AFFECTING RESEARCH

- PHYSICAL
- CHEMICAL
  - Air
  - Water
  - Diet
  - Drugs
- MICROBIAL AGENTS
- STRESSORS



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## EXTRINSIC FACTORS AFFECTING RESEARCH Physical Factors

- ENVIRONMENTAL
  - Macro vs. Micro Environment
  - Cage Design
  - Caging Accessories
  - Temperature
  - Humidity
  - Ventilation
  - Illumination
  - Noise



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## EXTRINSIC FACTORS AFFECTING RESEARCH Physical Factors

- MICROENVIRONMENT
  - The physical environment immediately surrounding the animal.
  - The primary enclosure
- MACROENVIRONMENT
  - The physical environment of the room, cubical, etc.
  - The secondary enclosure



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### EXTRINSIC FACTORS AFFECTING RESEARCH Cage Design



- Amount of air, light and sound the animal receives.
- Amount of heat, humidity and gaseous waste dissipated into the macroenvironment.

TAKE HOME MESSAGE: RECORD CAGE DESIGN AS A VARIABLE!

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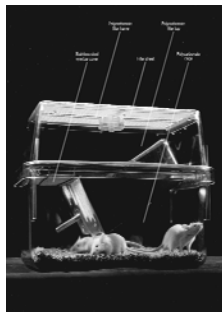
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### EXTRINSIC FACTORS AFFECTING RESEARCH Cage Design



- Solid bottom caging for rodents
- Behavioral characteristics
- Recovery cages
- Mouse urologic syndrome
- Randomization of cage location

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### EXTRINSIC FACTORS AFFECTING RESEARCH Cage Design - Rack placement

- Cage placement on the rack may be important!
- Rack vendors work hard to make all slots equal, but ...
- Rack location may impact study outcomes.



TAKE HOME MESSAGE: ROTATE CAGE PLACEMENT ON RACKS!

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**EXTRINSIC FACTORS AFFECTING RESEARCH  
Cage Design – a few facts ...**

- 68 F (20 C) air moving at 60 liner feet per minute has a cooling effect of 45 F (7 C).
- ACH has a wash-out effect upon pheromones
- Study: Effect of IVC air flow on rats:
  - 60 ACH did not impair the reproductive performance
  - Higher air flow increased the survivability of litters and ...
    - those litters were more uniform in size and viability over the control in an open topped cage.
- Study: Comparing mice housing in static and IVC
  - IVCs had lower relative humidity, lower NH<sub>3</sub> levels, lower CO<sub>2</sub> levels, higher body weight gain, and higher water consumption .

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**EXTRINSIC FACTORS AFFECTING RESEARCH  
Cage Design – a few more facts ...**

- Study: Measuring air quality in IVC versus static:
  - IVCs: Significantly lower ammonia, relative humidity, and carbon dioxide values.
- Study: Measuring noise:
  - The overall mean MICROENVIRONMENT decibel (dB) levels, as determined from a range of octaves, ventilated systems were associated with significantly higher mean noise levels, compared with room background levels.
  - There was no significant difference in noise detected among three vendor systems.

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**EXTRINSIC FACTORS AFFECTING RESEARCH  
Cage Design – a few more facts ...**

- Study: Measuring MICRO and MACRO noise:
  - Significant differences between macro- and microenvironmental dB levels.
  - In addition, microenvironmental noise levels were significantly higher than room background levels in the majority of octaves tested for each caging system.
- Study: Effect of Failed Air Supply to IVCs (rats):
  - Greater than 30% mortality of rat pups of weaning age
  - Oxygen concentrations in the air in individual cages were rapidly depleted to less than 10% in 1 h.
  - Animals died within 60 min of loss of power to IVC.

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### EXTRINSIC FACTORS AFFECTING RESEARCH Cage Design - a few facts ...

- 68 F (20 C) air moving at 60 liner feet per minute has a cooling effect of 45 F (7 C).
- ACH has a wash-out effect upon pheromones



TAKE HOME MESSAGE: IVCs ARE GREAT, BUT CAN HURT OUTCOMES!

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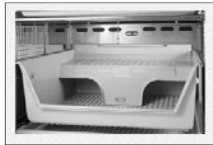
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### EXTRINSIC FACTORS AFFECTING RESEARCH Cage Design

- ACCEPTABLE PRIMARY ENCLOSURES
  - Normal physiologic and behavioral needs
  - Conspecific social interaction
  - Development of hierarchies within or between enclosures
  - Clean and dry
  - Adequate ventilation
  - Access to food and water
  - Secure environment
  - Free of sharp edges
  - Observe with minimal disturbance



TAKE HOME MESSAGE: BE CONCERNED WITH MICROENVIRONEMNT!

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### EXTRINSIC FACTORS AFFECTING RESEARCH Cage Bedding



- TYPES
  - Softwood
  - Hardwood
  - Corn cob
  - Paper products



- IDEAL
  - Dustfree
  - Nonpalatable
  - Absorbent
  - Free of contaminants
- Dependent upon species and experimental design

TAKE HOME MESSAGE: BEDDING IS A CONTROLABLE FACTOR!

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### EXTRINSIC FACTORS AFFECTING RESEARCH Cage Bedding

- Autoclaving reduces fungal contamination risk
- Size and manipulability of bedding determine preference
- Bedding choices may influence the immune system
- Bedding choice influences ammonia level in the cage
  - (MOST) Aspen shavings > pine shavings > reclaimed wood pulp bedding > virgin pulp loose > bedding hardwood chip bedding > recycled paper bedding > virgin cellulose > pelleted paper bedding > corn cob bedding (LEAST)

**TAKE HOME MESSAGE: BEDDING IS A CONTROLABLE FACTOR!**

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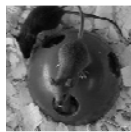
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### EXTRINSIC FACTORS AFFECTING RESEARCH Cage Accessories

- MATERIALS
  - Nonreactive
  - Uningestible
  - Smooth and impervious
  - Durable
  - Corrosion resistant
  - Sturdy - withstand rough handling
  - Wood may be used
  - Galvanized metal and rubber stoppers



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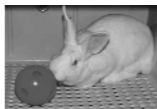
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### EXTRINSIC FACTORS AFFECTING RESEARCH Enrichment



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### EXTRINSIC FACTORS AFFECTING RESEARCH Watering

- Too little water is not good, and ...
- **Too** much water is not good
- Water supply **MUST** be checked daily
- Are there advantages of auto water? Maybe
- Strain differences may affect wet bedding
- Cage design may affect wet bedding
- Enrichment devices may affect wet bedding



TAKE HOME MESSAGE: WATER MUST BE WATCHED CLOSELY!

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### EXTRINSIC FACTORS AFFECTING RESEARCH Feeders

- Type depends upon species, diet used and primary enclosure
- Animals must have easy access to food
- Minimize contamination with feces and urine
- Group housing considerations
  - Multiple feeding and watering points
- Diet Optimization



TAKE HOME MESSAGE: YOUR RESEARCH IS WHAT THEY EAT!

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### EXTRINSIC FACTORS AFFECTING RESEARCH Temperature and Humidity



- Depend upon husbandry practices and housing design
- Macro and microenvironment may differ due to:
  - Housing material, construction, and use
  - Forced ventilation (or not)
  - Filter tops
  - Number of animals per cage
  - Activity level of the animals
  - Cage location within the rack or room (if static)
  - Frequency of bedding changes
  - Bedding type

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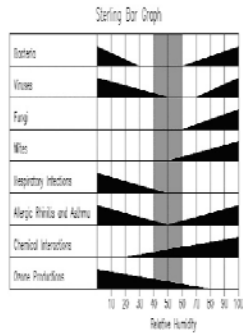
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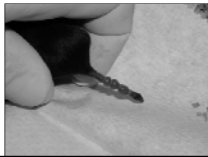
## EXTRINSIC FACTORS AFFECTING RESEARCH Temperature and Humidity

- Airborne particles, humidity and respiratory disease:
  - As RH increases, adhesiveness of particles increases leading to larger particle size
  - As RH decreases, electrostatic forces increase



## EXTRINSIC FACTORS AFFECTING RESEARCH Temperature and Humidity

- Lactating rats exposed to 95°F (35°C) for six hours daily produced less milk than rats housed at 72°F (22°C)
- Reproduction in rats decreases markedly at 90°F (32°C)
- High mortality and retarded testicular development in rats occurred after accidental exposure to high temps for prolonged periods
- Mice housed at 95°F (36°C) at 22% RH were more susceptible to influenza virus than mice housed at 97°F (35.6°C) and 90% RH
- Ringtail in rats, South African hamsters and mice is associated with the inability to control heat loss in environments of 40% RH or lower and results annular constrictions of the tail.



## EXTRINSIC FACTORS AFFECTING RESEARCH Temperature and Humidity

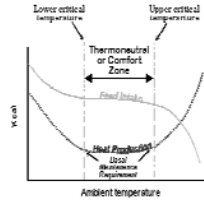
- Guide recommends RH between 30 and 70%
- Variations in RH are better tolerated at lower temps due to heat loss mechanisms of most animals
- Little evidence for strict RH control



TAKE HOME MESSAGE: WORK FOR STABLE 30%-70% HUMIDITY!

## EXTRINSIC FACTORS AFFECTING RESEARCH Thermal Neutral Zone

- Species differences
- No energy expended to either cool or warm itself
- Does not necessarily equate to comfort
- Measurements do not take into account differences in activity or metabolism
- Exposure of unadapted animals to temperature  $>85^{\circ}\text{F}$  or  $<40^{\circ}\text{F}$  without access to shelter may produce clinical effects which could be life threatening. (Guide)




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## EXTRINSIC FACTORS AFFECTING RESEARCH Thermal Neutral Zone - Adaptive Mech.

- Behavioral: If temp change is short / low magnitude
  - Huddling
  - Curling up
  - Nest building
  - Increased activity
- Physiological: Altered metab. rate, food/water consump., growth rate
  - Hibernation or estivation
  - Nonshivering thermogenesis
  - Peripheral vascular changes
  - Piloerection
- Morphological: Profound temperature changes for at least 14 - 21 days
  - Fat stores
  - Hair coat thickens
  - Heat radiating structures




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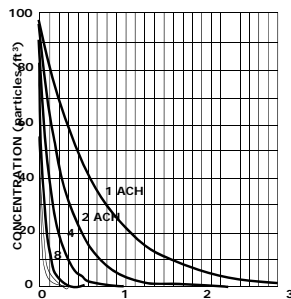
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## EXTRINSIC FACTORS AFFECTING RESEARCH Ventilation

- Purposes
  - Supply adequate oxygen
  - Remove thermal loads
  - Dilute gaseous and particulate contaminants
  - Adjust moisture content
  - Create pressure differentials
- Guideline of 10-15 fresh air changes/hour
  - refers to macroenvironment
  - doesn't take into account heat load




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### EXTRINSIC FACTORS AFFECTING RESEARCH Ventilation

- Adequate room ventilation does not ensure adequate ventilation of microenvironment!
  - Twelve air changes/hour (12 ach) equals:
    - Empty cage: Turnover rate is once every 4.5 minutes
    - Flexible film isolator cage: Turnover rate is every 6 minutes
    - Cage with Filter Top: Turnover rate is every 38 minutes

TAKE HOME MESSAGE: ROOM VENTILATION MAY NOT BE THE ANSWER!

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### EXTRINSIC FACTORS AFFECTING RESEARCH Ventilation

- Excessive odor first indication to humans
- Concentrations capable of causing damage to animals are much lower (10 PPM) than human threshold level for ammonia (25 PPM)
- Best expressed in terms of volumetric air exchange rate/occupant versus room air changes per hour



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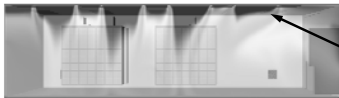
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### EXTRINSIC FACTORS AFFECTING RESEARCH Ventilation

- Heat load dependent on:
  - Species size
  - Number of animals
  - Type of bedding used
  - Frequency of changing bedding
  - Room dimensions
  - Efficiency of air distribution from secondary to primary enclosure

HIGH SUPPLY & LOW EXHAUST



Note influence of bio-safety cabinet and transfer station

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## EXTRINSIC FACTORS AFFECTING RESEARCH Ventilation

- Filtered one-pass air is preferred, but ...
- Filtered Recycled air: HEPA and charcoal if from animal facility
- Filtered recycled air : from non-animal use areas



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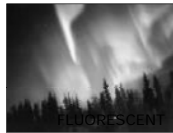
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## EXTRINSIC FACTORS AFFECTING RESEARCH Illumination



- Definitions:
  - Crepuscular: Active at dusk / dawn
  - Nocturnal: active at night
- Light intensity = illumination
  - Foot candles (fc) = lumens/ft<sup>2</sup>
  - Lux = lumens/m<sup>2</sup>
- Quality = color
  - Wavelength
  - Angstrom (A)
- Photoperiod or duration of light (L:D)
  - Hours of light (L) versus hours of dark (D) in a 24 hour day
  - Rodents in research: 12/12
  - Breeding rodents: 12/12 or 14/10



TAKE HOME MESSAGE: DARK CYCLE INTERRUPTUONS MOST DISRUPTIVE!

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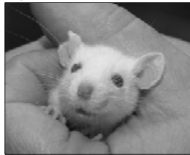
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## EXTRINSIC FACTORS AFFECTING RESEARCH Illumination



- Albino rat is more susceptible to phototoxic retinopathy than other species
- Intensity and photoperiodicity can affect physiology, morphology, behavior
  - Hepatic enzyme activity
  - Metabolism of compounds
  - Rats given a fixed dose of hexobarbital:
    - Light cycle: longer sleep
    - Dark cycle: less sleep
- Lab housing MAY cause deviation form seasonal norms



TAKE HOME MESSAGE: LIGHT LEVELS & SEASONS MAY AFFECT RESULTS!

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## EXTRINSIC FACTORS AFFECTING RESEARCH Illumination



- Guide: light at cage level = 130 and 325 Lux
- Light levels of 325 Lux (30fc) about 1.0 m (3.3 ft) above the floor appear to be sufficient for animal care and do not cause clinical phototoxic retinopathy in albino rats
- Animal facilities should be windowless
- Cage position on rack is important
- Light intensity decreases with square of the distance from the light source
- Light at the top of a rack may be 80 times more intense than at the bottom of the rack

TAKE HOME MESSAGE: LOCATION, LOCATION, LOCATION!

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## EXTRINSIC FACTORS AFFECTING RESEARCH Illumination



- Circadian rhythms are important
- Biorhythms are modulated by light
- Things YOU can do:
  - Rotate cage position
  - Provide hiding places
  - Choose colored manipulanda (yellow or red = dark)
  - Assure automatic light timers with variable intensity controls ARE WORKING!!!



TAKE HOME MESSAGE: DURATION AND INTENSITY MATTER!

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## EXTRINSIC FACTORS AFFECTING RESEARCH Illumination



- Biorhythms types
  - Circadian: ~ 24 hours
  - Ultradian: < 24 hours
  - Infradian: > 24 hours
  - Circaseptadian: weekly



TAKE HOME MESSAGE: RESULTS MAY BE EFFECTED BY B-RHYTHM?

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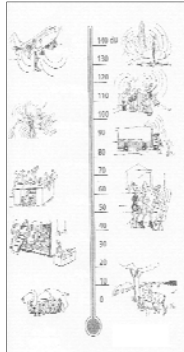
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### EXTRINSIC FACTORS AFFECTING RESEARCH Noise

- Measurements
  - Frequency = hertz (Hz)
  - Pressure = decibels (dB)
- Sounds > 20 kHz = ultrasound
- Most animals hear higher frequency sounds as compared to man
  - Rats - optimal hearing = 40kHz
  - Mice – optimal hearing = 15-20 kHz




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### EXTRINSIC FACTORS AFFECTING RESEARCH Noise

- Noise levels in animal facilities should not exceed 85dB (human guidelines)
- No guidelines on frequency limits
- Auditory effects of noise at >85 dB
  - Destruction of sensory hairs and supporting cells
  - Mechanical damage in rats at 160 dB
  - Pain in rats at 140 dB
  - Inner ear damage in rats after prolonged exposure to 100 dB




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### EXTRINSIC FACTORS AFFECTING RESEARCH Noise

- Eosinopenia
- Increased serum cholesterol, adrenal weights, adrenal ascorbic acid in rats exposed to 83 dB and intermittent sound of 114 dB
- Audiogenic stress due to pulsed noise exceeding 83 dB may cause reduced fertility in rodents
- Audiogenic seizures in gerbils, DBA/2, C57, AKR, BALB/c, and CBA.




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### EXTRINSIC FACTORS AFFECTING RESEARCH Noise

- Radios, alarms, timers
- Guide - these devices should not be used unless part of an approved protocol or enrichment
- UFAW Handbook states use of artificial background noise may be useful in masking sudden unexpected noise
- Masking noise = one which is intense enough to render inaudible or unintelligible another sound which is simultaneously present - potential danger = adverse effect on animals

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### EXTRINSIC FACTORS AFFECTING RESEARCH Noise

- Separate human and animal areas
- Separate noisy species from quieter species
- Loud, unexpected and unfamiliar sounds are probably more disruptive and harmful than constant sound



TAKE HOME MESSAGE: SOUNDS MAY CONFUSE OUTCOMES!

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### EXTRINSIC FACTORS AFFECTING RESEARCH Transportation

- Acclimation period after shipment
- Length of time required depends upon:
  - Type and duration of transport
  - Species
  - Intended use
- Adolescent rats recovery period = 1 - 5 days
- Decrease stress by:
  - Vendor supplying 1 - 2 weeks of food
  - Use of similar cage and watering system

TAKE HOME MESSAGE: ACCLIMATE PRIOR TO USE!

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### EXTRINSIC FACTORS AFFECTING RESEARCH Overcrowding & Isolation

- Effects behavior, reproduction, immune system, metabolism
- Cage size requirements depend upon:
  - Species
  - Size
  - Number of animals/cage
  - Breeding status



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### EXTRINSIC FACTORS AFFECTING RESEARCH Overcrowding & Isolation

- Aggressive behavior can be strain specific
- Group housed mice show marked adrenal response that is directly proportional to the animal density
  - When housed in pairs, the subordinate has the higher adrenal weight and plasma cortisone level



**TAKE HOME MESSAGE: MAINTAIN ACCEPTABLE POPULATION DENSITY!**

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### EXTRINSIC FACTORS AFFECTING RESEARCH Social Ranking

- Consider species
- Social animals may fight if placed in new groups or new member placed in established group
- Breeding activity:
  - Can be significantly modified by group housing
  - Female mice
    - Group housed = anestrus
    - Male introduced = synchronized estrus



**TAKE HOME MESSAGE: SOCIAL RANKING IS IMPORTANT!**

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### EXTRINSIC FACTORS AFFECTING RESEARCH Handling

- Regular handling may reduce stress
- Correct handling/restraint methods important
- Gentling studies in rats and rabbits
- Caretaking staff changes



TAKE HOME MESSAGE: HANDLE GENTLY AND SLOWLY!

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### EXTRINSIC FACTORS AFFECTING RESEARCH Chemicals

- Sources
  - Air, feed, water, bedding, caging materials
  - Inherently toxic or metabolites are toxic
- Portals of entry
  - Skin, GI tract, respiratory tract
- May affect hepatic microsomal enzymes
  - Biotransformation of drugs and chemicals
  - Regulation of oxygen radical removal



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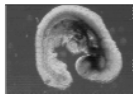
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### EXTRINSIC FACTORS AFFECTING RESEARCH Chemicals

- Serve as local irritants
- Produce generalized disease
- Alter immune functions
- Allergens
- Mutagens
- Teratogens



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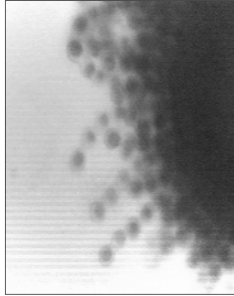
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## EXTRINSIC FACTORS AFFECTING RESEARCH Chemicals

- Examples
  - Nitrates in drinking water - carcinogenic nitrosamines
  - Aflatoxins in feed - carcinogenic
  - Aromatic hydrocarbons - hepatic microsomal enzymes
  - Insecticides - lymphopenia
  - Heavy metals - alter immune response



TAKE HOME MESSAGE: KNOW WHAT IS BEING USED IN YOUR AREA!

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## EXTRINSIC FACTORS AFFECTING RESEARCH Air Quality

- Air quality of macroenvironment affects the air quality of microenvironment
  - Ozone - depresses hepatic microsomal enzymes
- Microenvironmental contaminants
  - Ammonia - urease positive bacteria
  - Carbon dioxide




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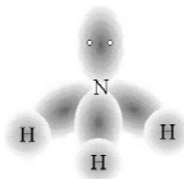
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## EXTRINSIC FACTORS AFFECTING RESEARCH Air Quality

- Ammonia
  - Acceptable level < 25 PPM
  - M. pulmonis + ammonia enhances:
    - lesion severity
    - bacterial growth
    - bacterial adherence



TAKE HOME MESSAGE: KNOW IF THERE HAVE BEEN FAILURES!

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## EXTRINSIC FACTORS AFFECTING RESEARCH Drinking Water

- Chemical impurities
  - Suspended solids, organic and inorganic solutes
- Trihalomethanes present in most chlorinated water
  - Potential carcinogens
- High levels of chlorine may affect immune system
- Nitrates - common contaminants
- Guide - periodic monitoring
  - pH, hardness, microbial or chemical



TAKE HOME MESSAGE: KNOW THE QUALITY BEING PROVIDED!

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## EXTRINSIC FACTORS AFFECTING RESEARCH Diets

- Variations in quantity or quality of essential vitamins or minerals may:
  - Alter drug metabolizing systems
  - Affect membrane integrity
  - Predispose to effects of carcinogens
- Raw materials may contain:
  - Nitrates and amines - form nitrosamines *in vivo*
  - Fish meal high in nitrosamines

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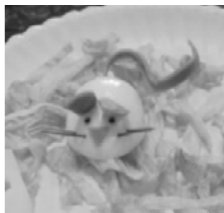
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## EXTRINSIC FACTORS AFFECTING RESEARCH Diets

- Feed contaminants
  - Chlorinated hydrocarbons, organophosphates, PCBs, heavy metals, aflatoxins, estrogenic compounds
- Guide:
  - Date of manufacture
  - Feed analysis for critical nutrients
  - Minimize abrupt diet changes
  - Record date of autoclaving
  - Use Purified and chemically defined diets
  - Vitamin C in select species



TAKE HOME MESSAGE: KNOW WHAT IS BEING FED!

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### EXTRINSIC FACTORS AFFECTING RESEARCH Diseases – What’s Common?

- ♦ MHV – 2%
- ♦ Parvoviruses
  - Mouse – 2%
  - Rat – 4%
- ♦ Rotavirus – 0.7%
- ♦ Norovirus - 30%
- ♦ RRV – 7%
- ♦ Theilovirus
  - Mouse – 0.3%
  - Rat – 1.4%
- ♦ Helicobacter spp. – 15%
- ♦ P. pneumotropica
  - Mouse – 15%
  - Rat – 5%
- ♦ C. bovis – 3%
- ♦ Pneumocystis carinii – 2%
- ♦ Pinworms –
  - Mouse – 0.3%
  - Rat – 1.3%
- ♦ Mites – 0.1%

TAKE HOME MESSAGE: IS THERE A SAFE DISEASE?

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### EXTRINSIC FACTORS AFFECTING RESEARCH Diseases – Mouse Hepatitis Virus (MHV)

- ✓ Degree of mortality can be strain related:
  - BALB/c mice more susceptible than C57BL/6 mice
  - IFN- $\gamma$  KO BALB/c mice had higher mortality than IFN- $\gamma$  KO C57BL/6 mice (polytropic)
- ✓ May contaminate in-vitro hepatocyte cultures
- ✓ Viral clearance:
  - Varies with strain of mouse and virus
    - 2-3 weeks in C57BL/6J (enterotropic)
    - 4 weeks in BALB/cJ (enterotropic)
    - 8 weeks in Hsd:ICR (polytropic)
  - Not cleared in many GM lines!

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### EXTRINSIC FACTORS AFFECTING RESEARCH Diseases – Mouse Hepatitis Virus (MHV)

- ✓ Prolonged immunologic effects:
  - MHV depletes NK cells through apoptosis and syncytia formation
  - T-cells, B-cells modulation
  - Infects monocytes, macrophages, bone marrow dendritic cells
  - Delayed allogeneic graft rejection
- ✓ MHV may confuse infection studies!
  - Co-infections on IFN- $\gamma$  deficient (KO) mice on C3H background developed a wasting syndrome with granulomatous peritonitis
  - Co-infection with H. hepaticus and enterotropic MHV-G reduced mortality and lesion incidence and severity relative to MHV alone during first week, BUT had more severe hepatitis and meningitis at 28d!

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### EXTRINSIC FACTORS AFFECTING RESEARCH Diseases – Parvovirus (PV)

- ✓ Several variants (MPV-1, -2, -3, -4, -?)
  - ✓ Cause persistent infection, even with seroconversion in some cases
  - ✓ No anatomic lesions, even in scid mice
  - ✓ C57BL/6 require 10-100x infective dose; DBA/2 only slightly better
  - ✓ MPV-1a (cell culture adapted) modulates immune response
  - ✓ Suppression of T cell response in vitro
  - ✓ CD8+ T lymphocyte clones lose function and viability
  - ✓ Cytokine- and antigen-induced T cell proliferation in vitro suppressed after exposure to MPV-1a
  - ✓ Potentiates allograft rejection
  - ✓ Induces isograft rejection

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### EXTRINSIC FACTORS AFFECTING RESEARCH Diseases – Parvovirus in Rats

- ✓ RV - Rat Virus (previously KRV, Kilham Rat Virus)
  - ✓ Natural infections usually asymptomatic, but persistent
  - ✓ Infects rapidly growing cells: vascular endothelium, lymphoreticular and hematopoietic tissues, developing cerebellum and liver
  - ✓ Rare epizootic disease in fetal/neonatal rats: Cerebellar hypoplasia, anemia, thrombocytopenia
  - ✓ Very rare disease in older rats: Hemorrhagic disease

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### EXTRINSIC FACTORS AFFECTING RESEARCH Diseases – Norovirus (MNV)

- Impact upon research:
- ✓ Immunocompetent mice – No clinical signs, but ...
    - Minimal change in inflammatory cells in lamina propria of small intestine at 24 hours post infection
    - Increased nuclear staining in red pulp (spleen); no change cell nos.
    - Viral nucleic acid found in small intestine, spleen, mesenteric lymph nodes, liver
    - Anecdotal reports of decreased "stool contents" at 3 days
  - ✓ Immunosuppressed mice:
    - Lethal infection in STAT1 -/- (with or without RAG2 and PKR), and IFN  $\alpha\beta$  -/-
    - Hepatitis, interstitial pneumonia
    - Encephalitis only with intracerebral inoculation
    - Virus present in dendritic cells

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### EXTRINSIC FACTORS AFFECTING RESEARCH Diseases – *Helicobacter*

- ✓ Chronic hepatitis (necrosis, hepatocytomegaly, biliary proliferation, nonsuppurative inflammation) in A/J, C3H/HeN, and some other immunocompetent strains
- ✓ Hepatitis may be particularly necrotizing in immunodeficient strains
- ✓ Hepatocellular carcinomas in A/J, C3H/HeN, SCID mice
- ✓ Colon carcinoma in SMAD-3 deficient mice
- ✓ Increased incidence of mammary carcinoma in RAG2-/- Apc(min/+) mice (secondary to inflammation)
- ✓ C57 resistant to disease, but can carry high level of colonization
- ✓ Proliferative typhlocolitis in athymic mice and rats
- ✓ Rectal prolapse
- ✓ Mild chronic hepatitis in immunocompetent mice (low incidence)

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### EXTRINSIC FACTORS AFFECTING RESEARCH Diseases – *Pasteurella pneumotropica*

- ✓ Lesions in immunocompetent rodents:
  - Mastitis
  - Conjunctivitis / Dacryoadenitis
  - Metritis
- ✓ Lesions in Immunodeficient rodents:
  - Necrotizing, suppurative dacryoadenitis
  - Suppurative dermatitis (abscesses)
  - Metritis

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### EXTRINSIC FACTORS AFFECTING RESEARCH Diseases – Oxyurid nematodes (Pinworms)

- ✓ Induced Th2 response
- ✓ ↑ IgG, interleukins
- ✓ ↑ Ab to Sheep RBC (used in bench top tests)
- ✓ ↑ allergic response to ovalbumin challenge
- ✓ Rectal prolapse – did not exclude *Citrobacter* or *Helicobacter*
- ✓ Slow growth
- ✓ Altered electrolyte transport
- ✓ Depressed exploratory behavior



Tape test  
*Syphacia* sp.



Fecal Exam  
*Aspicularis tetrapectera*

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