# Common Techniques When Using Mice in Cancer Research Part I

## *NCI Mouse 101*

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# Topics Covered : Techniques Part I

- Laboratory Mouse Biology
- Stress, Adaptation, and Distress
- Importance of Animal Facility Practices
- Ordering mice
- Receiving mice from other institutions
- Transportation of mice
- Restraint techniques
- Microisolator technique
- Drug delivery / Oral dosing
- Solvents, injection volumes, needle sizes
- **Injection routes**
- Footpad/hock injections
- Genotyping / tissue collection
- Identification methods
- Nutrition
- Tumor injection/implantation
- Imaging resources
- Irradiation

# Topics Covered (Part II)

- Blood Collection
- Euthanasia techniques
- Tissue collection
- Anesthesia/analgesia
- Surgical Techniques
- Veterinary therapeutics
- Health monitoring
- Emergency contact info
- Summary / Wrap-up

# The Main Take-home Messages

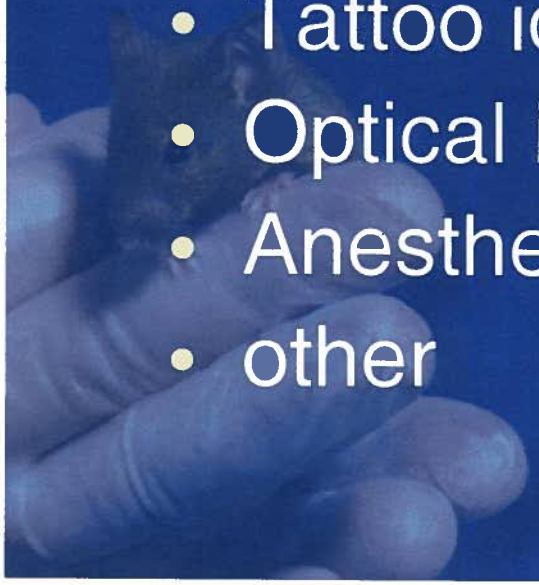
- Get advice from experienced animal handlers about best methods and techniques. Training is available and often *free* .
- Techniques can strongly affect data
- Improper techniques can needlessly harm animals
- Restraint and positioning are critical
- Practice
- Practice
- Practice

# Laboratory Animal Science

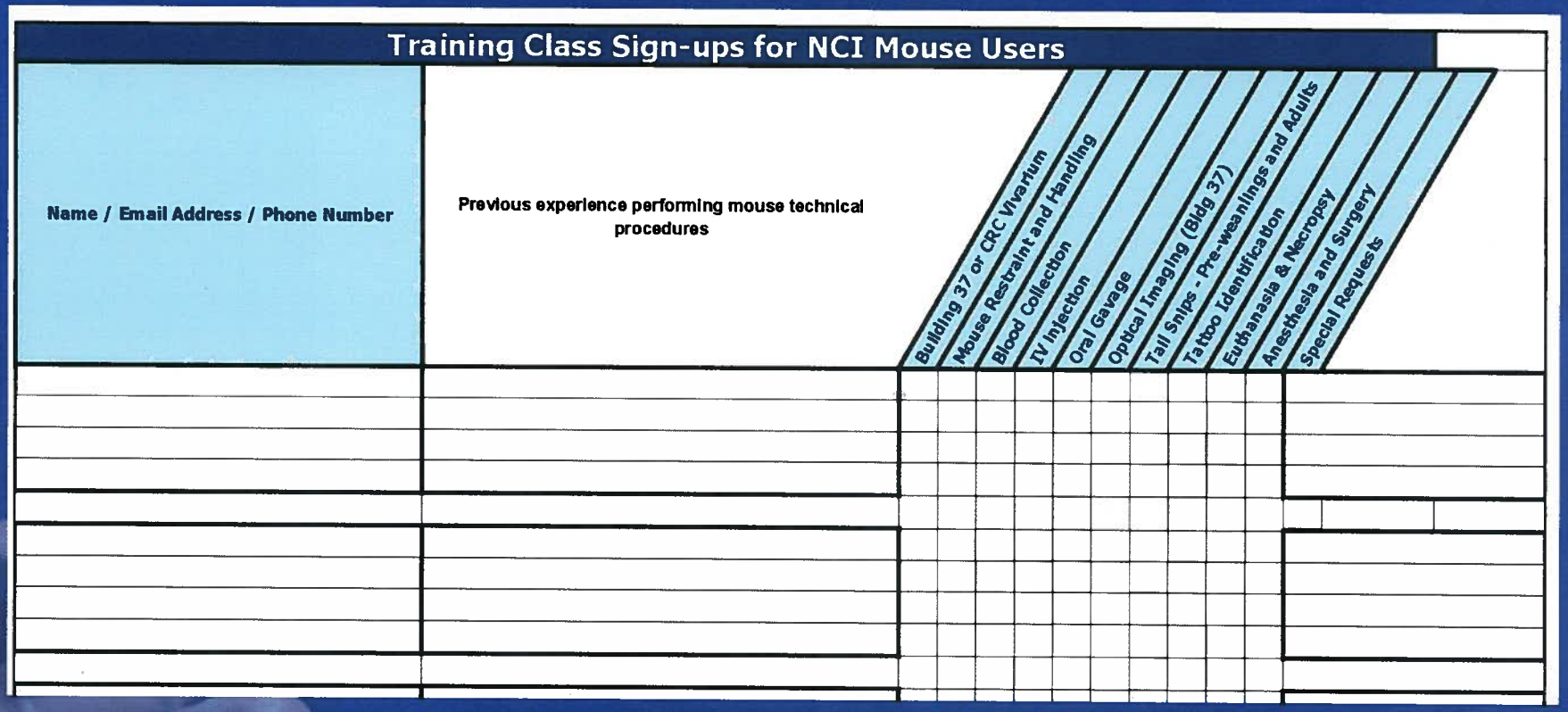
- Technicians, Veterinarians, Biomedical Scientists, Managers, others
- Dedicated Professionals with Specialty training, skills, degrees, certifications
- SAIC-Frederick Laboratory Animal Science Program
- High quality animal research techniques accompany high quality research

# Mouse Technique Wet labs

- Restraint and handling
- Blood collection- mandibular technique
- Injections – IV
- Oral Gavage
- Euthanasia, CO2, CD, and necropsy
- Tattoo identification
- Optical imaging
- Anesthesia and major surgery
- other



# Training Wet Lab Sign-up Form



| Training Class Sign-ups for NCI Mouse Users | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name / Email Address / Phone Number | Previous experience performing mouse technical procedures | Building 37 or CRC Vivarium | Mouse Restraint and Handling | Blood Collection | IV Injection | Oral Gavage | Optical Imaging (Bldg 37) | Tail Snips - Pre-weanlings and Adults | Tattoo Identification | Euthanasia & Necropsy | Anesthesia and Surgery | Special Requests |
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# The House Mouse

- Origin of today's lab mouse
  - Mouse breeders and coat color genetics
  - 100 years of cancer genetics (Tyzzer,1909)
  - Genetically uniform inbreed strains
  - The first NCI grant 1937 : Jackson Lab

"adaptable"

"domestic"

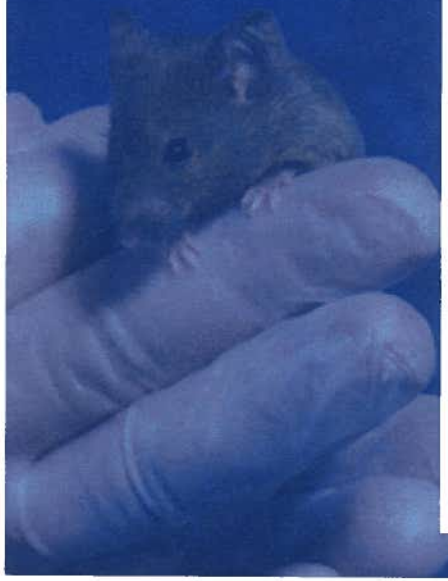
"practical"

"critically important"

# Use of the Mouse in Biomedical Research

- The workhorse species for the NCI
- Small size +/-
- Relative ease of maintenance
- Rapid and high reproductive abilities
- Short life span
- Historical data
- Mouse tumor cell lines
- Immune deficient lines may receive human cell xenografts
- **Genetic manipulation**

# Use of the Mouse in Biomedical Research

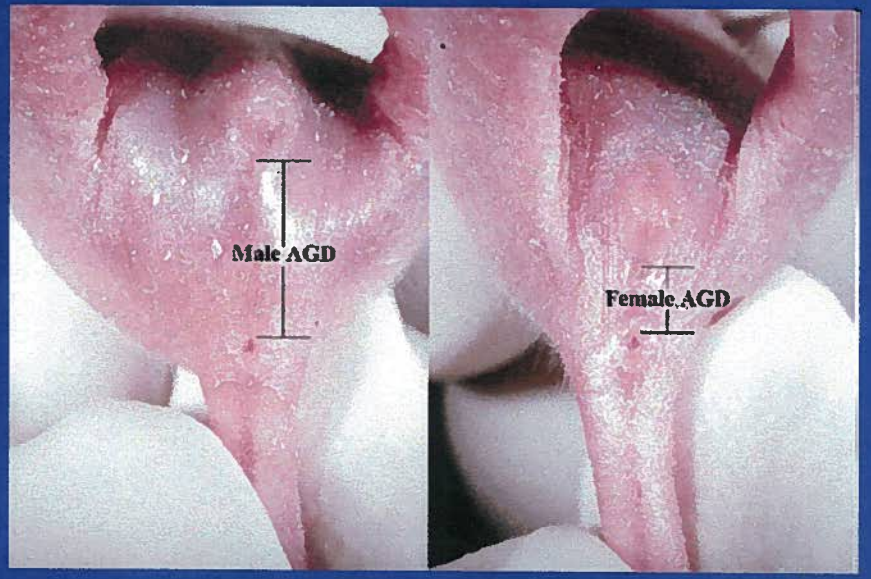


# Mus musculus Physiology

- Adult weight 20-40 g (male) 18-35 g (female )
- Birth Weight 1 g
- Daily water consumption 5-6.7 ml
- Daily food consumption 5 g or 150 g/kg BW
- Body Temp 96.8-99.7 F (35.8- 36.7 C)
- Heart rate 600 bpm (350-750)
- 40 chromosomes
- Blood volume 1.7 ml (30g)
- Sexual maturity 5-8 weeks
- Reproductive pheromones
- Gestation length 19-21 days
- Weaning age :21 days for most
- Nocturnal
- Lifespan 2-3 years (only in the animal facility)

# Mouse Anatomy : Male vs. Female

Anogenital Distance
Masculinization / feminization
Intra-uterine position



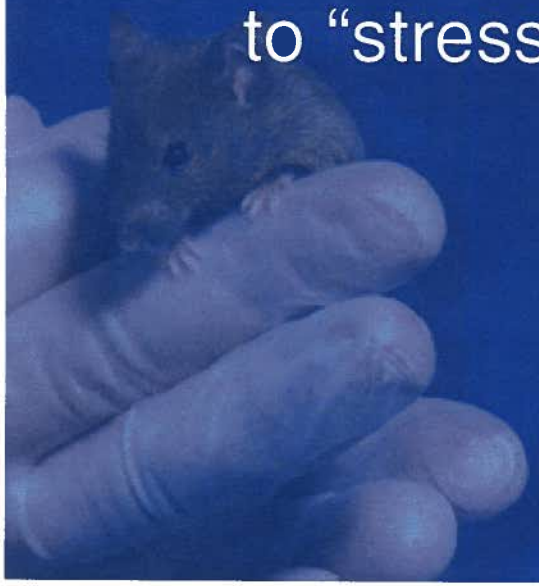
# Test yourself

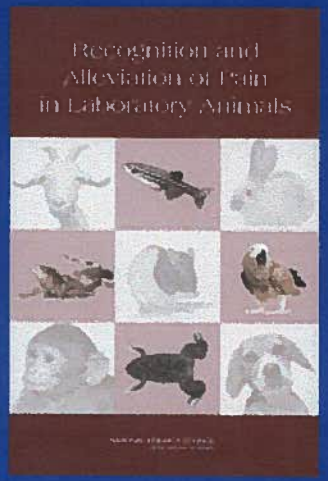
9 days old

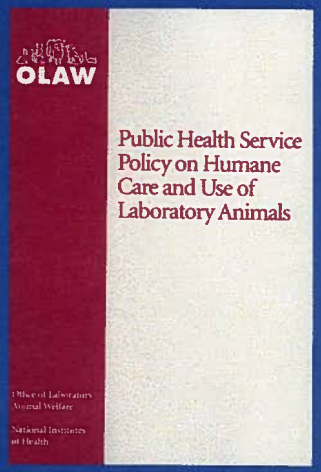
16 days old

# Stress, Pain, and Distress

- Mice are capable of experiencing stress, pain and distress
- Stress can be minor or major, temporary, or prolonged. Stress is a normal part of life. Unrelieved stress or pain can lead to distress.
- Laboratory mice can often adapt or acclimate to "stressors"

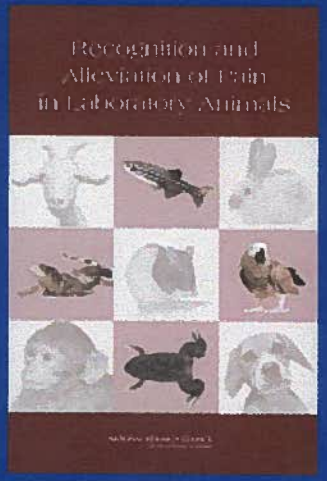


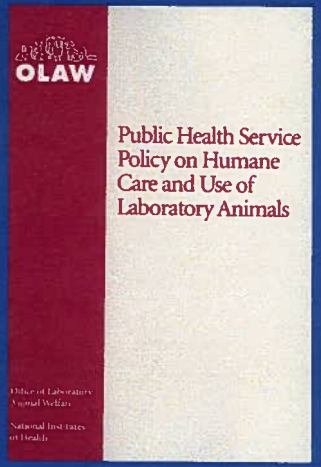


# Stress, Pain, and Distress

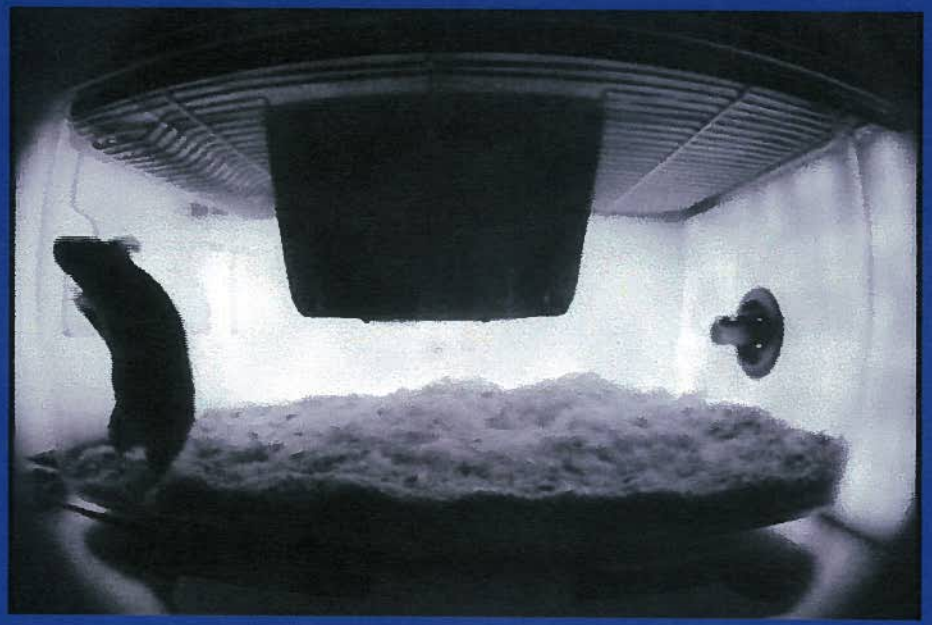
- Proficiency in handling mice and in performing technical procedures will definitely reduce stress and pain and distress
- How does one recognize unrelieved pain or distress in a mouse?



# Answer: Know what is normal for mice

- Nocturnal
- Inactive during day if undisturbed
- Normal behavior and posture



# Which persons are most skilled at recognizing illness in animals

- At ones' home?
- On the farm?
- In the animal facility?

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- On the farm?
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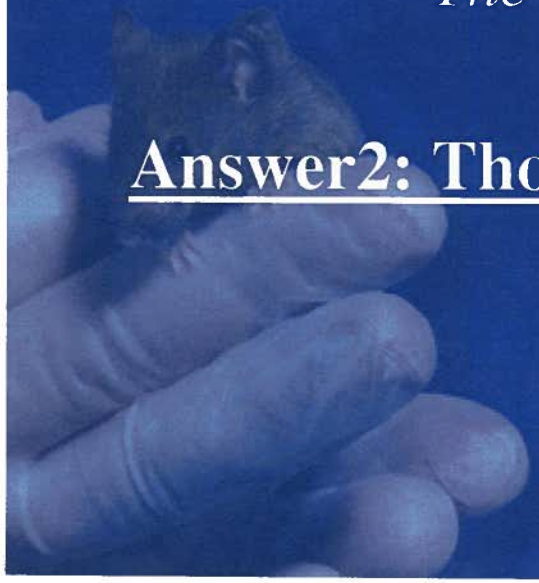
Answer: *The ones that see the animals everyday:*

*The one who takes care of the pets*
*The farmer who knows his/her animals*
*The Animal Care Staff*

# Which persons are most skilled at recognizing illness in animals

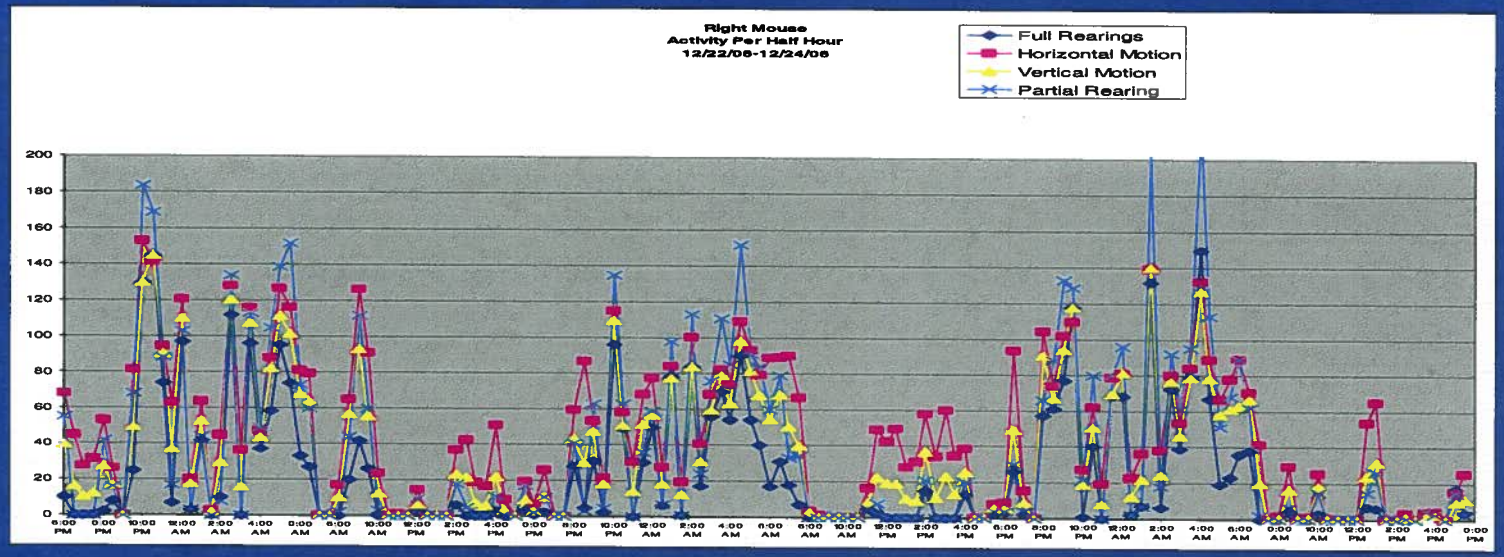
- At ones' home?
- On the farm?
- In the animal facility?

*Answer: The ones that see the animals everyday:*
*The Animal Care Staff*



**Answer2: Those who are actually looking at the animals**

# How to evaluate mice



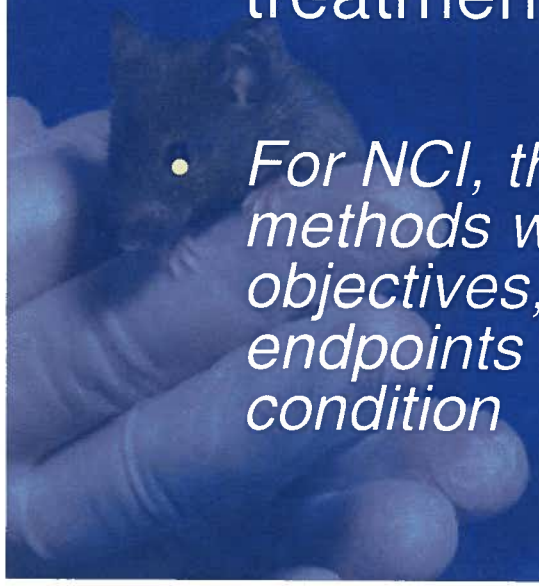

1) Look at the mice *and* the cage contents
2) Move the cage
3) Open the cage
4) Reach in with your hand
5) Compare the mice – responsiveness, velocity, size, etc.
6) Use the wirebar lid
7) Recheck

# Know the signs of Pain and Distress in Mice

- Hunched
- Inactive, because of pain or weakness
- Signs of inability to compensate
  - Dehydration
  - Severe weight loss
  - Hypothermic
  - Progressive worsening disease
- The regulatory rule of thumb:
  - *It it causes severe pain in human beings, it probably causes pain in other species.*

# Animal Pain in Cancer Studies

- Bone metastasis
- Signs of pain
  - Changes in behavior- may be subtle
  - Loss of body weight (not eating)
    - May need to check weights 2x week or more often
  - Generalized humane endpoints
- Pain or distress may be secondary to chemo treatments, surgery, technical problems, etc.



- *For NCI, the expectation is that the most humane methods will be utilized to achieve scientific objectives, and that when possible, experimental endpoints will be selected that precede a moribund condition*

# Mouse Techniques: Use of the Animal Facility

# Access to the Animal Facility (Bethesda)

- Required NIH OACU Training *(online)*
- Name is on approved protocol
- Registered with DOHS as animal user
- Watch the Facility Orientation video
- Access by ID badge and/or Room code
- Tour/orientation by Facility Manager
- *May be accomplished in one week*

# Ordering mice

- Different for NCI facilities and centralized facilities managed by DVR
- All orders placed by facility staff
- Written orders faxed, emailed or delivered to animal facility
- List the strain or stock, sex, number, age, delivery date, facility
- Must include PI, current ASP# and Center #
- Orders from Jax and Taconic must include strain code
- Confirmatory email will be sent to ordering investigator
- Please be sure that name, contact info, and ASP # is accurate.

# Receiving Mice

- Approved sources are the NCI-Frederick Animal Production Area (APA) ,Charles River Labs, Jax, & Taconic
- **Receiving and Quarantine Program at NCI-Frederick** for most other sources
- Quarantine length 3.5-7.5 weeks
- Details and online forms at LASP website
  - http://www.ncifcrf.gov/rtp/lasp/intra/randq/default.asp
- Mice may be moved by LASP staff between compatible facilities
- Health status is first reviewed by Facility Vet
- **Recommended acclimatization: 5-7 days**

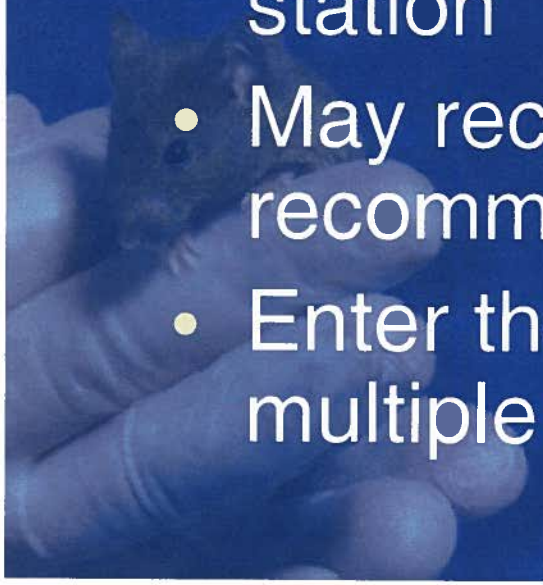
# Using the Animal Facility

- Get to know the staff and room routines
- A shower and change of clothes (and shoes) may be required if you have been in another animal facility that day.
- Lights on - Lights off. 6 AM, 6 PM
- Protective clothing and gloves; Follow instructions for additional practices in Biohazard or Quarantined Rooms.
- Nothing should contact the floor except your feet and the wheels of a cart
- All equipment, supplies should be dedicated to ONE facility and container surfaces should be disinfected during entry

Individually ventilated cages
Automatic watering on most racks and rows
Always Check for proper placement and access to food/$H_2O$

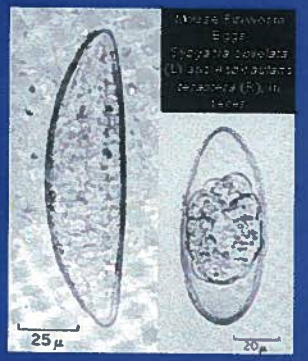
# Special Procedures and Rooms for Immunodeficient Mice

- More susceptible to opportunistic agents that cannot be excluded
- Segregated from other mice
- Sterile cages and autoclaved water
- Always handle in a hood or change station
- May receive prophylactic antibiotics if recommended by facility veterinarian
- Enter these rooms first if working in multiple animal rooms



# Personal Protective Equipment, Rules and Facility Sanitation Routines

- Exclusion of mouse diseases is the goal
- Facility Health Status
  - Movement of rodents, people, and supplies should only be from higher level (clean) to equivalent or lower level health status
  - Does the Facility exclude all known rodent pathogens (including norovirus)?
  - Helicobacter? Parvovirus?
- There are many mouse infectious diseases endemic on the NIH campus, including pinworms, mites, MHV, and others.
- Facility Veterinarian can advise



# Consequences of contamination..

- Alteration of experimental measures, tumor growth rates, immunity, etc.
- Quarantine will limit access to animals
- Movement of animals ceases
- Procedure rooms, MIF, etc. may not be used
- Expensive testing ; expensive extra PPE
- Cull and slowly repopulate with clean stocks
- Reduced ability to share mice with colleagues
- Research progress and productivity may be severely affected

# Personal Protective Equipment:

## Important to protect both mice and the users

- Dander Containment- to minimize allergy development
- Bites – usually very minor, but report…
- Exposure to hazards being used
  - Needlesticks, ocular irritants
  - Human tissues, recombinant viruses
  - Chemical carcinogens – may be excreted into the bedding

# Taking mice to the Laboratory

- Special routes and elevators, esp. in CRC
- Should be rapid and direct
- Provide food, bedding, fresh water source
- Practice containment and minimize stress to animals
- Unless the contrary is approved in the ASP, animals must be euthanized within 12 hours or less

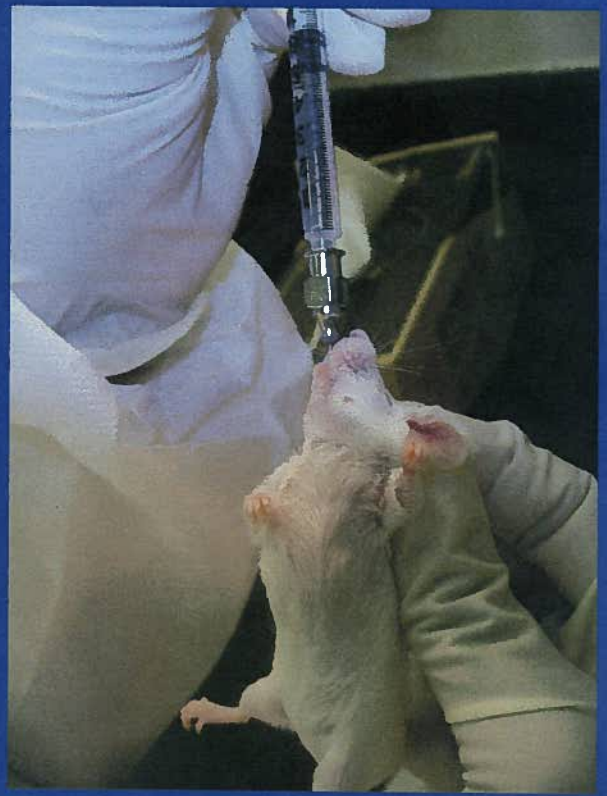
# Restraint of mice

- Domestic mice vs. wild mice
- One hand technique
- Two hand technique
- Firmly in control without causing harm
- Grab near base of tail

*Take advantage of the mouse's normal tendency to pull away from you*

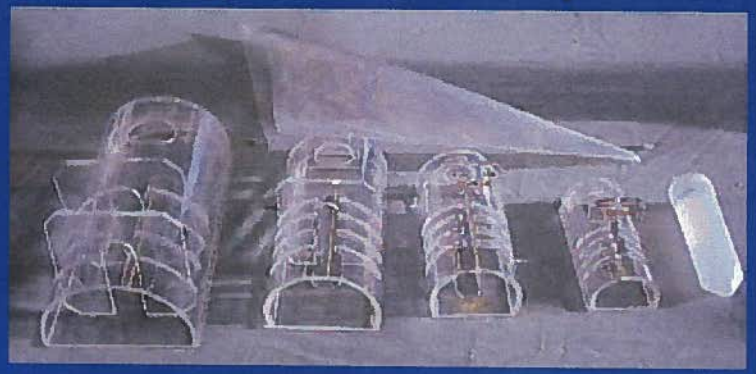
# A properly restrained mouse

- Not trying to escape
- Not likely to get injured



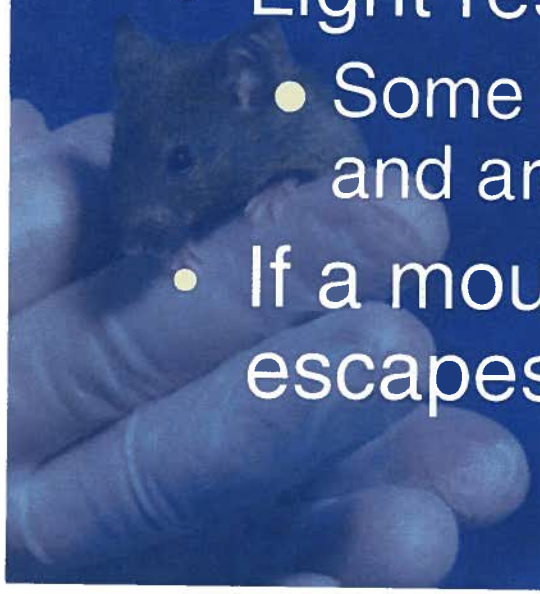
# Mouse restrainers

- Different sizes / adjustable or open/close
- May be commercial or customized, as for local body irradiation
- Brief procedures, involving minimal pain
- Restraint stress : time-dependent
- Adaptation
- Anesthesia would be more appropriate if the procedure is likely to cause pain



# Restraint

- Monitor breathing rate and membrane color (cyanosis)
- Do not attempt injection or gavage if mouse is struggling to escape.
- Do not throw, spin, or threaten mice
- Light restraint is sometimes best
  - Some strains are surprisingly cooperative and are slow in attempt to escape
- If a mouse is dropped on floor or escapes, alert facility supervisor



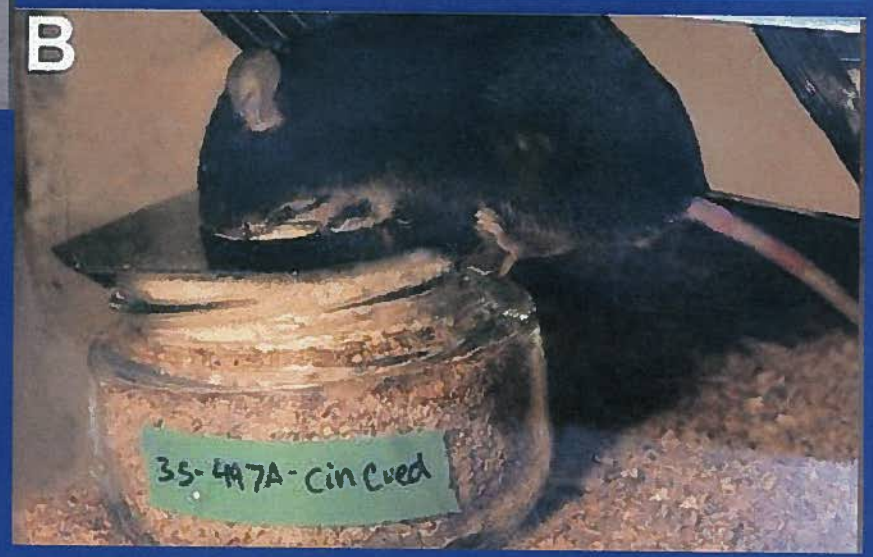
# Microisolator Technique

- Some facilities or rooms require that mouse cages be opened only inside a biosafety cabinet or clean flow work bench.
- All surfaces, including gloves, are disinfected with spray (ex. chlorine dioxide, MB-10) before opening the cage.
- Disinfection of surfaces and materials repeated for each cage
- Handling mice and/or performing technical procedures is a common route of disease transmission

# Drug Delivery – Oral Dosing

- Formulation of custom pellet diets for chronic compound administration
  - Mice eat to meet calorie requirements
  - Consumption depends on activity levels, temperature, etc.
  - Most consumption is at night
  - Powder diets, liquid diets are possible
  - Special diets may be short-dated and expire before one can finish using them
- Addition to drinking water (not accurate, esp if aversive taste; req wide therapeutic range)

# Liquid diets, powder diets




Not commonly used at NCI

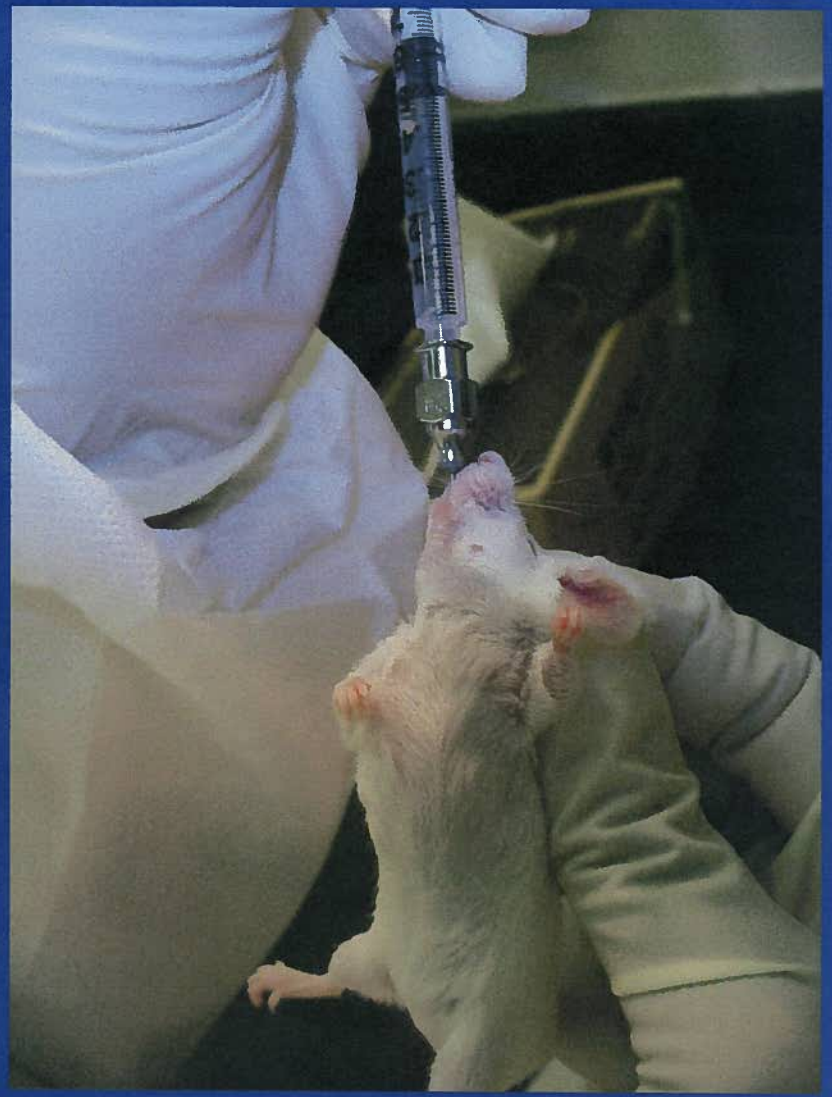
Mice still need to gnaw on something to prevent overgrowth of teeth

# Custom Diet Formulations

- Rodent Feed producers
  - Zeigler, Purina, Harlan, Research Diets
  - Bio-serv
  - Others
- Must be sterile by gamma-irradiation or autoclavable
- Consultation with a diet formulation specialist & facility veterinarian is advisable for new test compounds

# Drug Delivery – Oral Dosing

- Oral gavage
  - Ball-tipped feeding needle
  - Technical expertise required to prevent injury
  - Directly places fixed quantity of compound into stomach
  - Should be very smooth with no resistance



Video – oral gavage

# Volume for Intragastric Delivery

- Safe guideline is 1% body weight (20 g mouse receives 0.2 ml)

**Table 1: Gavage Guidelines in the Adult Mouse**

| Weight (Grams) | Gauge | Length (in) | Ball Diameter (mm) | Shape | Volume (ml) |
|---|---|---|---|---|---|
| up to 14 | 24 | 1 | 1.25 | Straight/Curved | up to 0.14 |
| 15-20 | 22 | 1-1.5 | 1.25 | Curved | up to 0.2 |
| 20-25 | 20 | 1-1.5 | 2.25 | Curved | up to 0.25 |
| 25-30 | 18 | 1.5-2.0 | 2.25 | Curved | up to 0.3 |
| 30-35 | 18 | 2-2.5 | 2.25 | Curved | up to 0.35 |

# Diluents and Vehicles

- Saline or distilled water
  - If ionizable, may require pH adjustment
- Suspension appropriate (oral gavage)
- Soluble in oil ?
- Polyethylene glycol (PEG400)
- DMSO
- Absolute ethanol
- Complex formulations sometimes necessary for iv injections
  - (ex. 10% DMSO, 18% Cremophore RH40, 5% dextrose for small molecules)

# Injection Needle Sizes

- LASP website
- ½ to 1 inch length
- Optimum depends on viscosity and site
- Tail vein 25-30 ga (25)
- SC < 23 ga
- IM < 25 ga (27.5)
- IP 23-27.5 ga
- ID 27.5 - 30 ga
- Risk of aerosolization
  - Lure-lock; eye protection

# Injection routes- IV

- Lateral tail vein
- Vasodilation – warmth, alcohol
  - Heat lamp: Be careful
- Start distal on tail; distal 1/3
  - subsequent injections should be proximal
- 0.1-0.2 ml recommended *
- No resistance should be sensed
- Repeated tail trauma
- Intra-orbital injections are possible
- Arterial injections require surgery

No fooc
prepara

- i.v. injection videos

# Hydrodynamic injections

- 1.0- 3.0 ml injected iv rapidly
- Physiological fluid overload useful for some gene transduction studies
- Requires perfect needle placement
- Tolerated well by some strains, depending on what is injected
- Mice may seem dazed or sleepy for about one hour
- Mice should be observed for signs of acute heart failure, hypothermia

# Subcutaneous injection

- Useful route for some drugs, including analgesics, and parenteral fluids
- Restrain mouse and with thumb and forefinger, make a dorsal tent of skin over scruff, & prep site with 70% ethanol
- Insert needle bevel-up in retracted loose skin over neck and back and aspirate to assure proper placement
- Max volume 2.5 ml
  - Depending on site

# Subcutaneous Fluids

- Very helpful following surgery or whole body irradiation (1.0 cc)
- Should be isotonic NaCL or LRS
- If not prewarmed, may worsen hypothermia

# Intramuscular injection

- Max recommended volume 0.03 ml
- Use the largest muscles of the hind leg
  - (quadriceps is best)
- Avoid sciatic nerve which runs along the femur and back of upper leg
- Not usually done in mice because of small muscle mass, except for orthotopic muscle tumor cell injections
- Absorption rate similar to SC for aqueous drug solutions

# Intraperitoneal injection

- Rapid absorption may closely resemble iv injection kinetics for some compounds
- Use the lower left or right quadrant
- Tilt the head down
- Prep with 70% alcohol
- Insert needle at 30° angle
- Only the tip and bevel need to penetrate abdominal wall
- No more than 2 ml
- Slight negative pressure ensures correct placement

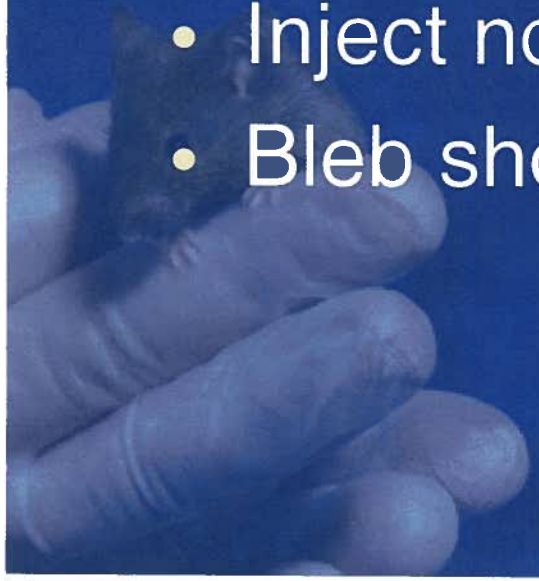
Video ip inj

# Repeat injections with same needle ?

- Passing needle through the skin dulls the tip (esp. tail skin or a rubber stopper)
- Contaminated needle can transmit disease between mice
- No more than a max of 5 injections per needle, depending on needle size
- All those mice should be from same cage

# Intradermal injections

- Mouse should be anesthetized
- Remove hair by shaving or plucking & swab with 70% alcohol
- Insert 30 ga needle into skin, bevel-up, nearly parallel to skin. Do not aspirate.
- Inject no more than 50 μl
- Bleb should be evident



# Footpad injections

- Historically used for inflammation and antibody production studies
- Footpad is extremely sensitive and is weight-bearing structure
- If scientifically justified, a single hind footpad may be used
- Alternative is hock, which drains to same lymph node
  - T. Kamela (2007) J. Immunological Methods http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2464360/
- Discussed in ARAC Guideline on adjuvants

# Hock Injection: A humane refinement for footpad injections

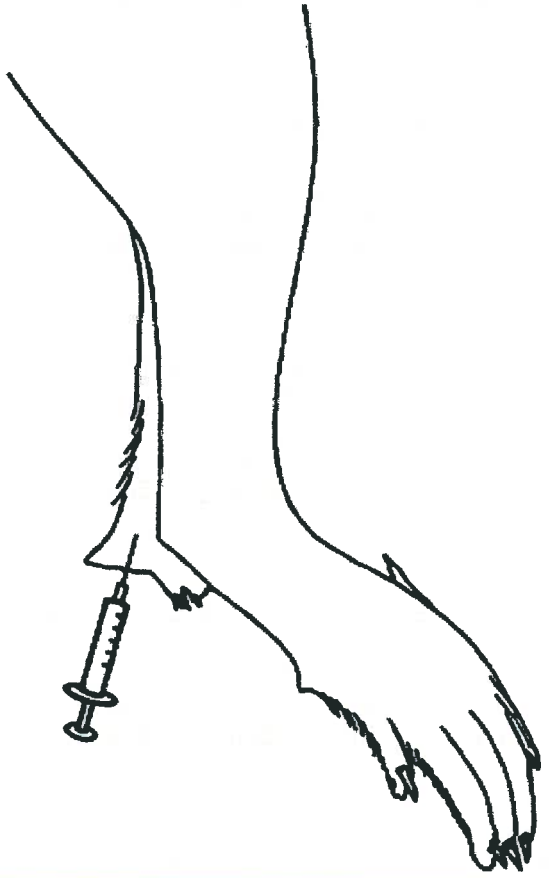
**A** **Immunizations of mice**

**Footpad**

**Hock**

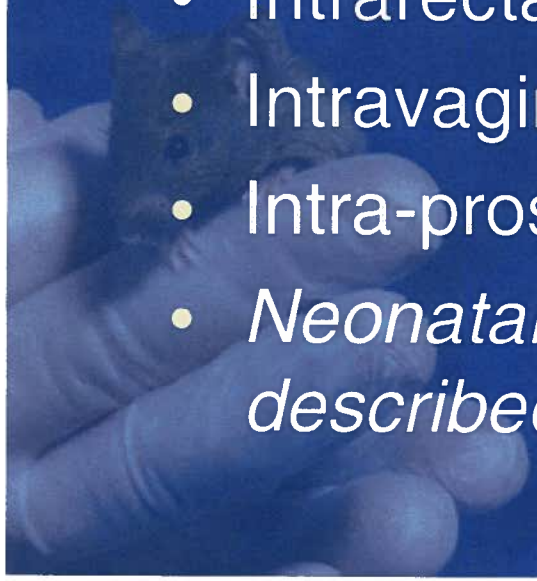
**Withdrawl**

**B** **Hock-immunization schematic**



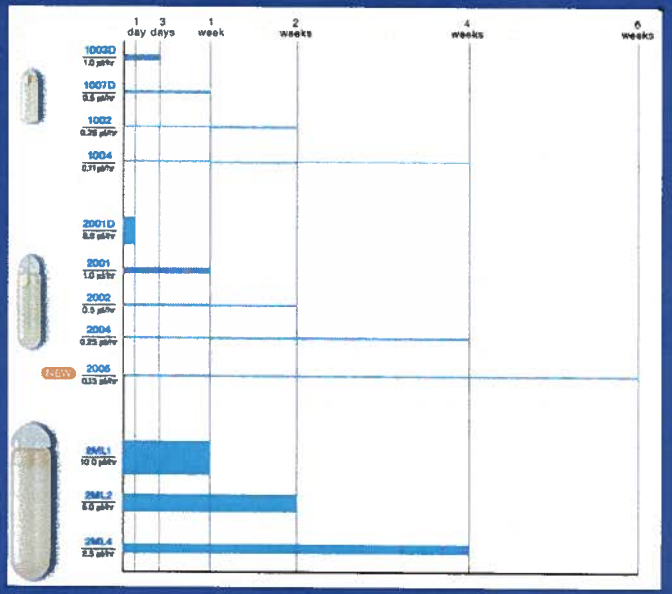
# Other routes

- Intracranial
- Intraosseous
- Intramedullary
- Intranasal
- Intratracheal
- Intrarectal
- Intravaginal
- Intra-prostatic
- *Neonatal procedures have also been described*



# Prolonged parenteral delivery of drugs or test compounds

- Slow-release pellets
  - Innovative Research of America
- Liposome formulations / repeat injections
- Osmotic mini-pumps (surgery required)
  - Interscapular area
  - 7-42 days
  - Can be replaced
  - Size matters



# Topical drug application

- Skin should be shaved but not traumatized (may be done a day before)
- Application with pipette, applicator stick or spatula
- Difficulty is preventing ingestion of compound
- Cover with bandage and consider single housing
- If skin will be irritated or inflamed, consider soft, dust-free bedding

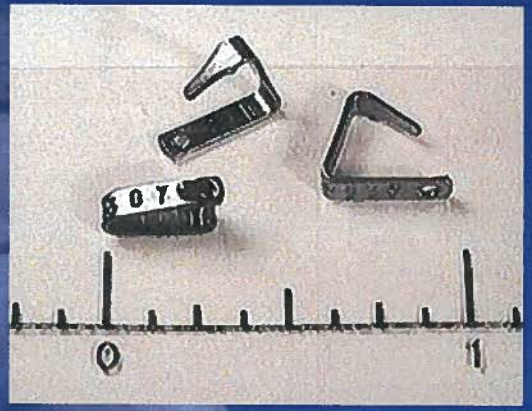
# Tissue collection for genotyping

- NIH ARAC Guidelines
- Not normally considered a surgical procedure
- Ideally tail snip between 10-21 days old
- Analgesia recommended
  - Ethyl chloride spray or ice cold ethanol
- 2-5 mm tail excised
- Disinfect scalpel or scissors
- Older mice: use anesthesia
- Select most practical identification method

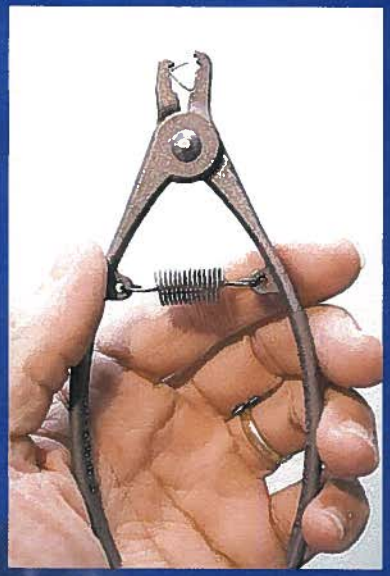
# Identification of mice

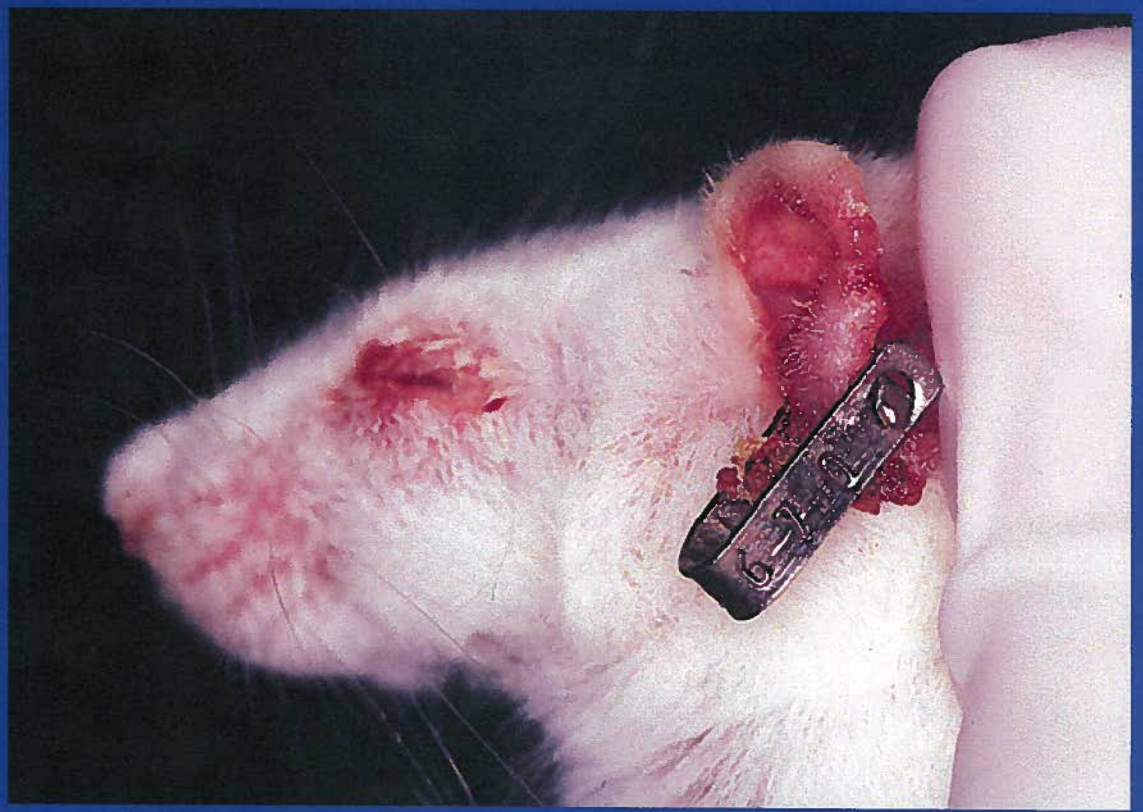
- Critical for genotyping
- 10-15 days of age: ear punch. May shave hair. Tattoo: any age
- Tattoo toes, rather than toe-clip
- Indelible, non-toxic markers (Sharpie) may last 3-4 days; lines or dots on tail
- After 18 days : ear tag, ear punch, microchip transponder or tattoo
- Ear tags should be sanitized and cleaned with alcohol

# Identification of Individual Mice



# Ear tags work well initially, but later…

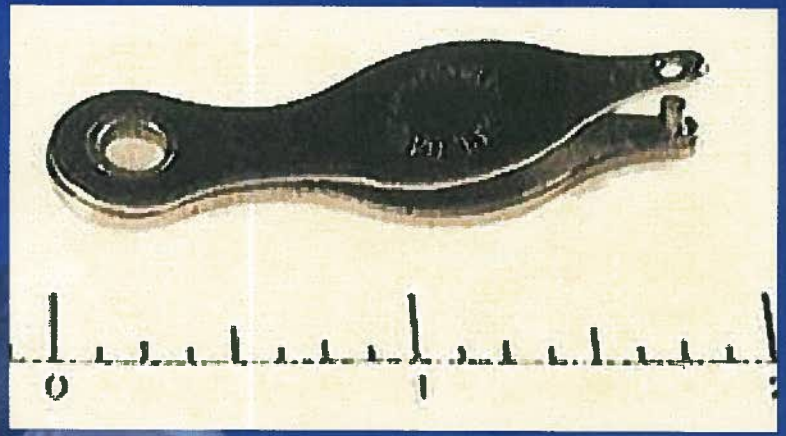


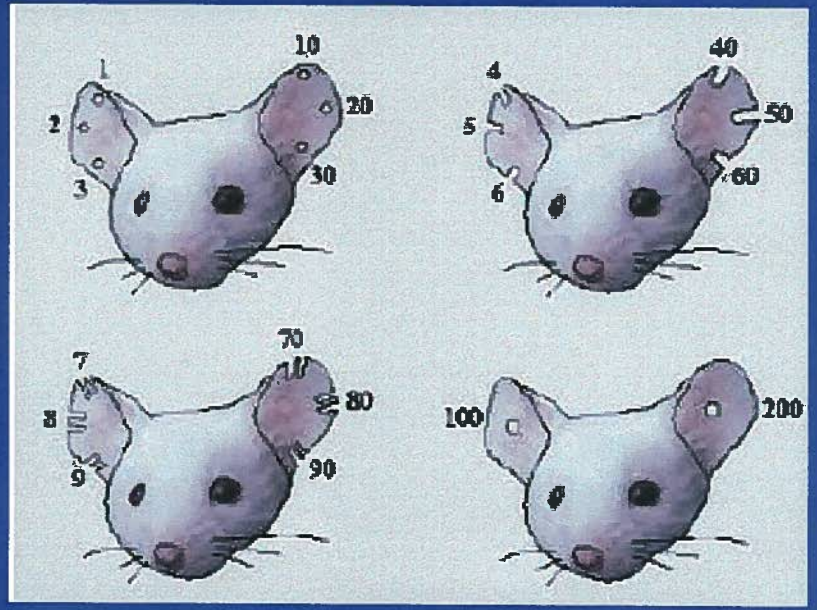


# Identification of Mice

Ear punch or ear notch

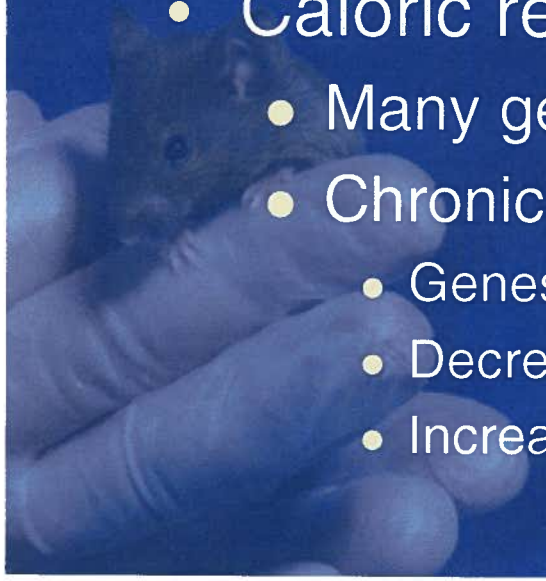
(Record ID numbers on cage card)





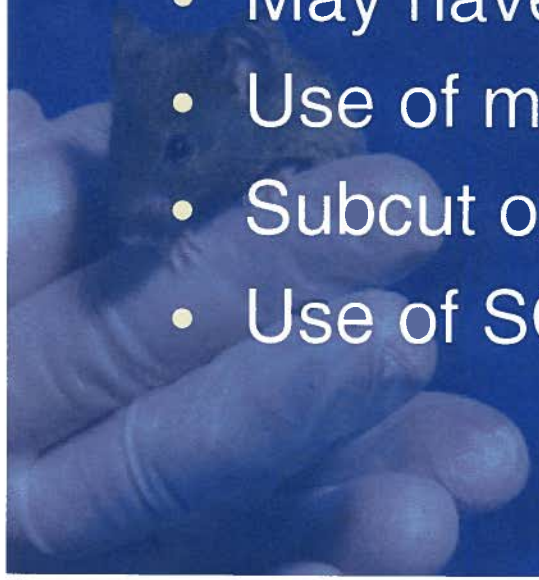
# Nutrition Considerations & Cancer Studies

- NIH-31 rodent diet (autoclaved) *ad lib*
- Fixed ingredient, open formula
- Ingredients can have variable amt's of protein and non-nutrients
  - Nitrosamines, metals, phytoestrogens
- Caloric restriction
  - Many genes affected by an 18 hour fast
  - Chronic caloric restriction
    - Genes for metabolism, mitochondria
    - Decreased inflammation, decreased angiogenesis
    - Increased longevity, Decreased tumor growth



# Tumor Injection/Implantation

- $10^5$ -$10^8$ cells
- Large clumpy cells – low # and higher volume
- Automatic cell counter or hemocytometer
- Orthotopic injections 25 µl
- 50-200 µl for most SC or IV injections
- May have to use 500 µl if cells clump
- Use of matrigel
- Subcut or orthotopic tumor fragments (3 mm)
- Use of SOP for each type of injection



# Intracardiac Injections

- Single cell suspensions
- Typically done percutaneously, based on landmarks
- 100,000 cells into left ventricle
- Nudes much easier than haired mice
- Exact positioning, practice, SOP
- Acute mortality may be seen
- Evaluate for accuracy- imaging
- Ultrasound-guided injections are possible

# Tumor Cell Line Testing Requirements

- PCR-based testing has replaced MAP-testing to a large extent
- Instructions at LASP AHDL web page
- All untested cell lines if they have been passaged through rodents outside of the NCI animal program or exposed to rodent serum or if their history is unknown should be submitted
- Human-derived cells
- Matrigel, serum, or other biologics

# Imaging Resources

- SAIC-Frederick Small Animal Imaging Program
  - http://www.ncifcrf.gov/rtp/lasp/intra/saip/
- MIF and Holding Facility Arrangements
- Optical Imagers in many animal facilities
  - Comparative Molecular Pathology Program
- Specialized Dedicated Resources
- Ultrasound, PET, MRI, CT, Faxitron,…

# Irradiation

- Gamma vs. X-ray irradiator (mice)
- Radiation Safety Training REQUIRED
- Allow mice to acclimate 1 week
- Strain and age effects
- Use of antibiotics not usually necessary if mice receive compatible marrow graft within 24 hours
- Radiation dose and drinking water quality and husbandry may determine need for antibiotics

# Irradiation Dose Effects

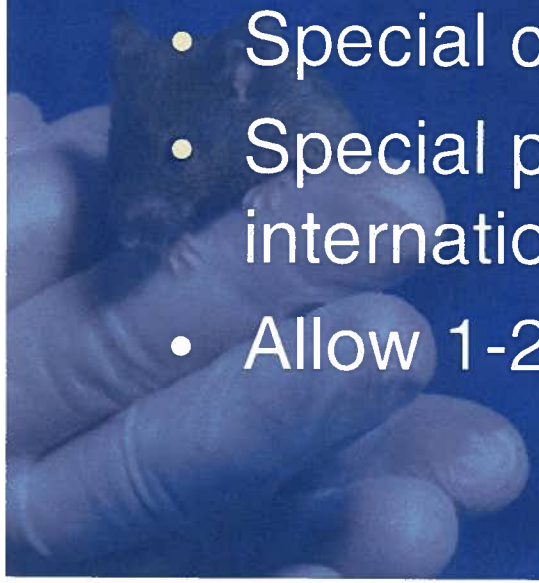
- $LD_{50/30}$ hematopoetic 7.5 gy
- $LD_{50/10}$ gastrointestinal
- Age effects stable by 9 weeks of age
- Dose depends on strain, dose rate/fractionation, health status, etc.
- Balb/c, scid, rag -/- most sensitive
  - Radiation repair defects
- Suppression vs. ablation (9-11 gy)
- Post-irradiation : transgel, gel-meal
  - Will need euth in 2 weeks if not dead
  - If they make it to 3 weeks, they will survive

# Bone Marrow Transplant

- Most important factor is strain compatibility
- Collection of donor bone marrow & processing must maintain sterile conditions for cells
- Cells delivered iv usually or intrafemorally (rarely)
- Use of antibiotics in drinking water – depends on the particular model. In human beings and some mouse models, ciprofloxacin is used.

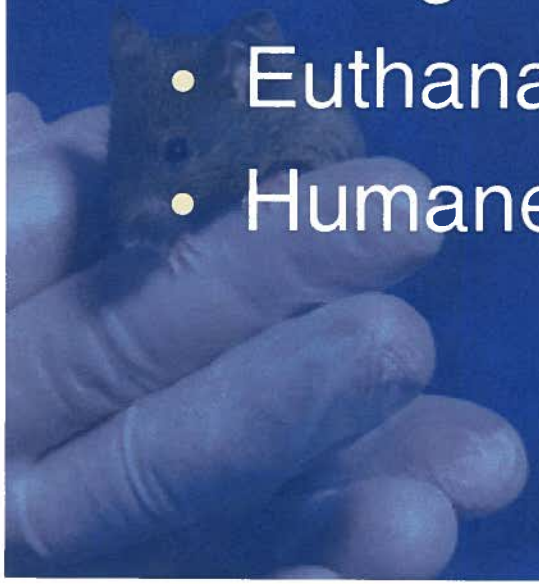
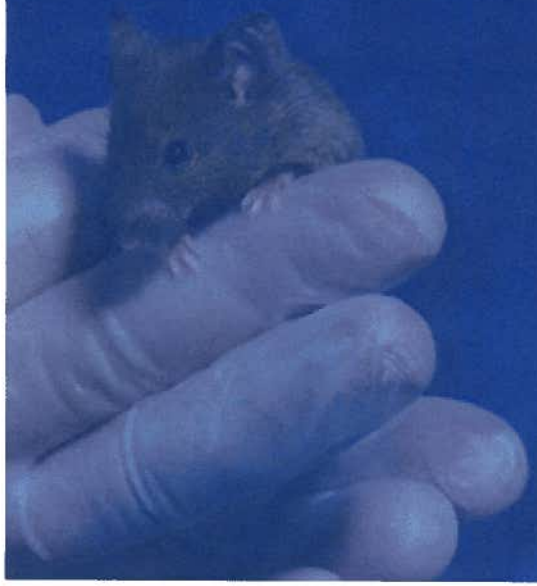
# Exporting Mice

- Transfers within NCI or within NIH
  - Speak with facility veterinarian or transportation coordinator . LASP will relocate the mice.
- Send only healthy young adults
- Requirement for MTA (Material Transfer Agreement)
- Special courier charges
- Special paperwork is required, esp. for international shipments
- Allow 1-2 weeks minimum for processing



# To be continued, in two weeks…

- Blood collection
- Tissue collection / Necropsy
- Anesthesia
- Aseptic Surgery
- Analgesia
- Euthanasia
- Humane endpoints



# Questions?