What Graduate Students Need to Know About the Use of Animal and Human Subjects or Infectious Agents

Slides are courtesy of Kathy Partin, GRAD544 and used with permission
They have been modified for ATS 786
Issues for CSU Students

- Do you know what kind of animal research happens on this campus? Should you?

- What has to happen before an animal is used in an experiment, for a 4H display, or at a football game?

- The CSU IACUC is not some compliance-driven body of deadwood faculty; it is a group passionately involved with a number of processes designed to assure that animals are used ethically at CSU. It is a bioethical committee.

- You can become engaged in the processes in place to make CSU the kind of place you would be proud to be an alumnus of by working with the IACUC.
When do you need to engage the IACUC about your activities?

- Before you do research or testing with animals
- Before you use animals to teach or demonstrate anything
- Before you conduct exhibits with animals
- Before using animals in sports or competition
- When you have concerns about the welfare of animals used in any of the above
First things first

- Get help before you get started with your research
- You will need IACUC approval of a “protocol”
  - Graduate students may not be the PI on a protocol
  - All personnel on the protocol must demonstrate their training qualifications
  - Ask your advisor for help or ask a colleague for help
  - Call RICRO and speak with Molly or Bill
Animal Protocols

- Submitted online at https://csu.keyusa.net/
- Reviewed once a month by the IACUC (Institutional Animal Use & Care Committee)
- Peer reviewed to reduce, refine, and replace animal use
- Address issues that, when they go wrong, could be viewed by others as having contributed to “animal abuse”
What is an “Animal”?  

- **PHS**—”any LIVE, VERTEBRATE animal used or intended for use in research, research training, experimentation, or biological testing or for related purposes” 

- **USDA**—“any LIVE or DEAD dog, cat, nonhuman primate, guinea pig, hamster, rabbit, or any other warm-blooded animal which is being used or intended for use for research, teaching, experimentation, or exhibition purposes or as a pet. This term **EXCLUDES** birds, rats of the genus *Rattus* and mice of the genus *Mus* BRED for use in research and horses not used for research purposes and other farm animals…used for food or fiber… or for improvement of animal nutrition, breeding, management, or production efficiency… 

  Dogs = all dogs including those used for hunting, security, or breeding…  

- **AAALAC**—includes care of INVERTEBRATES
You Will Need to Understand “Pain Categories”

<table>
<thead>
<tr>
<th>USDA Category B</th>
<th>USDA Category C</th>
<th>USDA Category D</th>
<th>USDA Category E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals being bred, acclimatized, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes. Non-invasive observation only of animals in the wild.</td>
<td>Animals that are subject to procedures that cause no pain or distress, or only momentary or slight pain or distress and do not require the use of pain-relieving drugs.</td>
<td>Animals subjected to potentially painful or stressful procedures for which they receive appropriate anesthetics, analgesics and/or tranquilizer drugs.</td>
<td>Animals subjected to potentially painful or stressful procedures that are not relieved with anesthetics, analgesics and/or tranquilizer drugs. Withholding anesthesia/analgesia must be scientifically justified in writing and approved by the IACUC.</td>
</tr>
</tbody>
</table>

**Example**

1. Animals being bred or housed, without any research manipulation, prior to euthanasia or transfer to another protocol
2. Observation of animal behavior in the wild without manipulating the animal or its environment

**Example**

1. Holding or weighing animals in teaching, outreach or research activities
2. Observation of animal behavior in the lab
3. Ear punching of rodents
4. Tail snips in mice ≤ 21 days old
5. Peripheral Injections, blood collection or catheter implantation
6. Feed studies, which do not result in clinical health problems
7. Routine agricultural husbandry procedures approved by the IACUC in a protocol or SOP
8. Live trapping
9. Positive reward training or

**Example**

1. Survival surgery
2. Non-survival surgical procedures
3. Laparoscopy or needle biopsies
4. Retro-orbital blood collection
5. Exposure of blood vessels for catheter implantation
6. Induced infections or antibody production
7. Tattooing
8. Exposure of skin to UV light to induce sunburn
9. Tail snips in mice > 21 days old

**Example**

1. Toxicological or microbiological testing, cancer research or infectious disease research that requires continuation after clinical symptoms are evident without medical relief or require death as an endpoint
2. Ocular or skin irritancy testing
3. Food or water deprivation beyond that necessary for ordinary pre-surgical preparation
4. Application of noxious stimuli such as electrical shock that the animal cannot avoid/escape
Spill Your Guts to the Committee!

- Too much information is better than too little
- If you have a dilemma, explain it to them
- If you need technical advice, ask for it
- Don’t fudge, fabricate, prevaricate, obfuscate, eliminate, neglect or pretend
- Establish a collegial, respectful relationship; it will pay off in spades
What is the Science?

- The IACUC must make a determination that the proposed use of animals is scientifically justified. That is, that the research question is novel enough, important enough, and tractable enough to warrant the use of animals.
- You may need to convince them using scientific citations.
- They will likely not approve research that is “duplicative.”
- In addition, you must write a “lay summary” that explains all of this in terms that could be directly printed in the Coloradoan.
- This information may become public.
Are there any valid alternatives to your approach?

- Why are living animals needed?
- Are there *in vitro* or *in silico* experiments that could be done first?
- Is there emerging or new technology, or existing reagents, that could be used instead of animals?
- They are going to be looking for documentation of this in the Literature Search sections.
What species was chosen?

- You must justify the use of the species, and the closer the species is to human, the more you have to justify.
- The use of animals which are thought of as “companion animals” is also highly scrutinized.
- You must be able to demonstrate that the animal you are proposing to use is a valid animal model for the physiology and/or disease processes you are studying.
How many animals do you need to use?

- Proposing too few is just as bad as proposing too many. Why should any animal be sacrificed if you cannot use the data because the power is too low?
- “Dreaded statistics” feels like compliance-for-compliance-sake, but really, this is the most rigorous tool for animal numbers justification.
- We have a great statistician who can help you! (Jens Eickhoff, Dept. of Statistics).
- There are ways to describe animal use numbers that do not require a power analysis (for example, when you are just going to get a qualitative answer or doing a pilot study). Get some help to apply these ways.
How are you minimizing the animals’ pain & distress?

° Consult with a veterinarian (The University Veterinarian is Dr. James Owiny; the Director of Laboratory Animal Resources is Dr. Sue VandeWoude; also can ask Drs. Kendall or Kesel).
° Consider explicitly the shortcomings of alternative experimental methods.
° Know the difference between anesthesia and analgesia.
° Be prepared to provide pre-emptive anesthesia if you are performing a painful procedure.
° Be prepared to develop an objective scoring system for pain, distress and lameness.
° Be prepared to frequently observe animals during the window of time that they might experience pain.
You Have Been Indoctrinated into “The 3Rs”!

REDUCTION, REFINEMENT, REPLACEMENT
Truth in Advertising

• The problem is, your only avenue to providing this information is a “form.”
• It is not fun to fill out the form.
• Even after you fill out the form, more unfun ensues (“Your protocol requires modification…”).
• Still, doing a good job of filling out the form is **THE FIRST THING** you, as a graduate student, can do to protect animals from abuse while you are at CSU.
After the Protocol is Submitted

- Monthly review
  - The default is Full Committee Review
  - For Pain C, you can request Designated Committee Review (faster)
- Likely to “Require Modifications,” which are reviewed by the IACUC
- Once approved, you will get an approval form and protocol #, good for 3 years.
- That protocol will be renewed annually, or may be amended or closed.
When Do I Need an Amendment?

- Any time you change approved animal manipulations
- Increased pain, distress or mortality
- Change in specific aims
- Adding surgery
- Changing from “terminal” to “survival surgery”
- Changing species
- Using more animals
- Performing multiple procedures on the same animal
- Adding or deleting personnel
- Changing the location of procedures
Let’s Try Some Exercises
Scenario - Wildlife

- You are going to watch wildlife at RMNP – need a protocol?
- You are going to observe animals at RMNP and publish your findings.
- You are going to observe animals at RMNP when you introduce a predator.
- You are going to inject a sterilizing agent at RMNP to control herd size.
Human Subjects
Jessie Gelsinger

- Had a liver illness (ornithine transcarbamylase deficiency) controlled by diet and drugs
- Volunteered to be a human subject for a gene therapy trial, to test the safety of the adenoviral vector for infants
- Died within 4 days of delivery
- 18 years old
Subsequent review found that...

- PI Wilson, U. Penn: “The technologies we had at the time were inadequate”
- Over-worked researchers
- Lack of research funding
- Failure of “informed consent” disclosure to volunteers about adeno toxic effects, deaths of monkeys in preclinical trials, PI’s financial ties
- Lab workers failed to follow their IRB protocol, which called for elimination from the study of volunteers with high ammonia levels
- Gelsinger’s death “derailed” the gene therapy field
Ellen Roche

- Healthy 24 year old volunteer
- Lab worker at JHU asthma clinic
- Participated in a study at JHU (PI, Togias) on inhaled hexamethonium
- Compound restricts airways; induces asthma, allows doctors to look at asthma treatments
- Compound had previously been delivered by inhalation on only 20 subjects
- Roche developed acute lung disease and died 30 days after inhaling the compound
Subsequently found …

- The IRB should have asked for additional toxicity data prior to approval
- The IRB should have gotten an opinion from the FDA about whether there was a need for an IND*
- The consent form did not disclose known health issues with the compound, but should have
- Negative effects (to the lung) on the first subject were not reported to the IRB
- The investigator did not follow his own protocol on minor issues that would certainly have been approved

*Investigational New Drug
If you look at the many cases of serious adverse events in human subjects research... 

- … a common thread is poor communication between the investigator, his/her team, and the IRB
- **You** can play an important role in strengthening the communication between the researchers and the IRB
IRB: What is research?

A *systematic investigation*, including research development, testing and evaluation, designed to develop or contribute to *generalizable knowledge*.

[might be considered research if data are collected for demonstration and service, even in the absence of a “research program”]
IRB: What is a human subject?

(1) living individual (2) about whom an investigator… conducting research obtains

(3) data through intervention or interaction with the individual,

or obtains

(4) identifiable private information.

The first two criteria and at least one of the bottom two criteria are needed to be determined a human subject.
IRB Protocols

• If your project is “research” and research with a “human subject”, you will need to submit a protocol
• Prior to submitting a protocol, you will need formal IRB training
• Like the IACUC, IRB protocols are submitted and reviewed online, using eProtocol
• If you are asking a person about themselves, and you think you are going to want to use the information in a poster, talk or publication, call us!

• If you are asking a person about something other than themselves (not asking their opinion), you probably do not need approval (but call us anyway).
Responsibility to Participants

Protection
Principles of the “Belmont Report”:
  Justice, beneficence, respect for person
Protection includes everything from recruitment to safety measures to transparent communication.

Confidentiality/Anonymity
But, again, the initial way the IRB assesses this information is through a form
Before you get started with your IRB Protocol

- Get Help!!!
  - Review an accepted protocol in your field
  - Ask a colleague to go over your draft
  - Ask RICRO (Janell or Evelyn) to administratively pre-review your protocol
  - Get IRB training
Types of Reviews

- **Exempt**
  - Can be submitted any time
  - Exempt determination made by IRB
  - 7-10 day turn around

- **Expedite**
  - Can be submitted any time
  - Expedite determination made by IRB
  - 7-10 day turn around

- **Full**
  - Due @ Noon on the 2nd Thursday of each month.
  - Response within 7-10 days after full committee meeting
<table>
<thead>
<tr>
<th>Name</th>
<th>Title/Role</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Matthew Hickey</td>
<td>Professor</td>
<td>Health and Exercise Science Department</td>
</tr>
<tr>
<td>Dr. Timothy Davies</td>
<td>Professor</td>
<td>School of Education</td>
</tr>
<tr>
<td>Dr. Roe Bubar</td>
<td>Associate Professor (community member)</td>
<td>School of Social Work</td>
</tr>
<tr>
<td>Dr. Bruce Cooper</td>
<td>Physician (community member)</td>
<td>Medical Director, Health District of Northern</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Larimer County</td>
</tr>
<tr>
<td>Mr. Ken Gorman</td>
<td>Interim Pastor (community member)</td>
<td>Timnath Presbyterian Church</td>
</tr>
<tr>
<td>Dr. John Littrell</td>
<td>Professor</td>
<td>School of Education</td>
</tr>
<tr>
<td>Dr. Stephen Matthews</td>
<td>Physician</td>
<td>Hartshorn Health Center</td>
</tr>
<tr>
<td>Mr. Joel Painter</td>
<td>Adjudicated youth advocate, prisoner</td>
<td>Director, Jacob Center</td>
</tr>
<tr>
<td></td>
<td>representative (community member)</td>
<td></td>
</tr>
<tr>
<td>Dr. Ketul Popat</td>
<td>Assistant Professor</td>
<td>Mechanical Engineering</td>
</tr>
<tr>
<td>Dr. Bernard Rollin</td>
<td>Professor</td>
<td>Philosophy Department, University Bioethicist</td>
</tr>
<tr>
<td></td>
<td></td>
<td>University Distinguished Professor</td>
</tr>
<tr>
<td>Dr. Nancy Rowe</td>
<td>(community member)</td>
<td>Retired teacher</td>
</tr>
</tbody>
</table>
Approval Process

- Review committee will send their comments back to you on eProtocol; please respond to these comments in a timely manner.
- If approved, they will issue a protocol number.
- The protocol may be amended, closed or renewed (yearly renewals!) online.
- Serious adverse events must be reported.
- Extenuating circumstances- explain to a coordinator.
Let’s Try Some Exercises
Scenario – Course Evaluations

- You survey the students in your class to see how you are doing as a GTA – need IRB approval?
- Are the students human subjects? Are you doing research?
- Does it matter if you collect identifiable information about the students within the survey?
- You have done this now for several years, and see an interesting trend. You want to write up the anonymous survey results. Can you? Need IRB approval?
Scenario - RMNP

- You are planning on interviewing hikers in Rocky Mountain National Park asking their opinion on noise pollution in the park for a Plan A thesis.
- Are the hikers human subjects?
- Are you performing research?
  Do you need IRB approval?
Closing Thoughts

- Abuse of human and animal subjects was a problem in the past, leading to public outcry and external regulation.
- There is external scrutiny of academic institutions who receive federal grant money.
- If we, as a community, fail to meet their standards, they may shut down research activity. These are compliance issues.
- None of this matters more than our ethical obligation to protect the vulnerable.
Lab Safety

- Environmental Health & Safety
  - Occupational Health and Safety Program
  - Hazardous Waste
  - Radiation Control Office
  - Ergonomics
About Using Infectious Agents or rDNA

- Smith discusses controversy when first rDNA studies were undertaken
- Presently, guidelines exist (rather than the regulations governing use of human and animal subjects)
- Institutional Biosafety Committee
  http://web.research.colostate.edu/ricro/ibc/about.aspx

  “Reviews are conducted in accordance with the [NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)](http://www.neij.gov) and the [CDC/OHS Biosafety in Microbiological and Biomedical Laboratories (BMBL)](http://www.cdc.gov). This review process ensures that all activities involving these materials and the facilities used to conduct such work are in compliance with current federal regulations and applicable CSU policies.”
Research with Chemical Hazards

Hazardous Chemicals Slides were modified from:
http://www.lni.wa.gov/Safety/TrainTools/Trainer/Kits/hazcom/#SlideShowFiles

And

Laboratory Safety
What is a “hazardous chemical”? 

A hazardous chemical is any chemical that can do harm to your body.

Most industrial chemicals can harm you at some level.

It depends how much gets into your body.
How Chemicals Enter the Body

There Are Three Routes of Entry:

Ingestion – swallowing the chemical

Inhalation – breathing in the chemical

Absorption – the chemical soaks through the skin
The Three Forms of Chemicals

All chemicals exist in one of three forms:

Solid

Liquid

Gas
Some Categories of Hazardous Chemicals

- Carcinogens
- Teratogens
- Mutagens
- Sensitizers
- Corrosives
- Flammables
- Explosives
General Laboratory Safety Rules

• No food or drink or related utensils in the lab
• Do not insert or remove contact lenses in the lab
• Do not apply makeup or lip balm in the lab
• Do not wear open toed shoes or shorts
• Use personal protective equipment (PPE) as needed
• Wash hands before leaving the laboratory
• Familiarize yourselves with the location of the shower, eyewash, and first aid kit
General Laboratory Safety Rules

- Keep fume hoods free of clutter
- Be aware of your surroundings
- A good resource for general rules:
  - http://www.chem.duke.edu/safety/
- CSU’s Environmental Health Services also has extensive resources for Lab and general safety.
  - http://www.ehs.colostate.edu/
Chemicals

• Be familiar with the properties of chemicals you are using (Read the Material Safety Data Sheet (MSDS)).
• Chemicals should be stored in their proper locations when not in use.
• Always properly labeled!
• Proper procedures should be followed when discarding chemicals. Training by EHS is required:

Source: http://www.ehs.colostate.edu/WHazWaste/Home.aspx
# Sample MSDS

**Google “MSDS Benzene”**

**HOVENSA**

**MATERIAL SAFETY DATA SHEET**

**Benzene**

**MSDS No. 1785**

## EMERGENCY OVERVIEW

**DANGER!**

- FLAMMABLE - BLOOD TOXIN AND CARCINOGEN - ABSORBED THROUGH THE SKIN - CENTRAL NERVOUS SYSTEM - HARMFUL OR FATAL IF SWALLOWED - ASPIRATION HAZARD

High fire hazard. Keep away from heat, spark, open flame, and other ignition sources.

If ingested, do NOT induce vomiting, as this may cause chemical pneumonia (fluid in the lungs). Contact may cause eye, skin and mucous membrane irritation. If absorbed through the skin. Avoid prolonged breathing of vapors or mists. Inhalation may cause irritation, anesthetic effects (dizziness, nausea, headache, intoxication), and respiratory system effects.

Long-term exposure may cause blood disease, including anemia and leukemia.

## 1. CHEMICAL PRODUCT AND COMPANY INFORMATION

**HOVENSA L.L.C.**

1 Estate Hope
Christiansted, VI 00820-5652

**EMERGENCY TELEPHONE NUMBER (24 hrs):** CHEMTREC (800) 424-9300

**COMPANY CONTACT (business hours):** Safety Department (340) 692-3000

**SYNONYMS:** Benzol; Coal Naphtha; coal tar naphtha; Cyclohexatriene; Phenyl hydride

See Section 16 for abbreviations and acronyms.

## 2. COMPOSITION and CHEMICAL INFORMATION ON INGREDIENTS

<table>
<thead>
<tr>
<th>INGREDIENT NAME (CAS No.)</th>
<th>CONCENTRATION PERCENT BY WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene (71-43-2)</td>
<td>100</td>
</tr>
</tbody>
</table>

## 3. HAZARDS IDENTIFICATION

[Blank]
 Radiation Control Office

- [http://www.ehs.colostate.edu/WRad/Home.aspx](http://www.ehs.colostate.edu/WRad/Home.aspx)
- We use Kr-85 and Po-210 in our aerosol laboratories
  - Labs posted with signs
  - Need Module 0 (online) training to be in the lab, e.g. for ATS 631
  - Need additional training to work with instruments that contain the sources
How to Report an Emergency?

What Constitutes an Emergency?
An emergency is defined as a fire, explosion or release of hazardous material that could threaten human health or the environment.

Call the CSU Police Department (911) and be sure to give the operator the following information:

• Your name and phone number
• The location (building and room number)
• A description of the incident

Reporting of Non-Emergency Situations
Call 491-6745

Ask for one of the listed reporting individuals below or describe the incident to the operator to get transferred.

<table>
<thead>
<tr>
<th>Type of Complaint</th>
<th>Who to Call</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indoor Air/Animal/Water Quality (Pools)</td>
<td>Jeannine Riess</td>
</tr>
<tr>
<td>Asbestos</td>
<td>Eric March</td>
</tr>
<tr>
<td>Biohazardous Materials</td>
<td>Robert Ellis</td>
</tr>
<tr>
<td>Hazardous Materials</td>
<td>Chris Giglio</td>
</tr>
<tr>
<td>Confined Space</td>
<td>Eric March</td>
</tr>
<tr>
<td>Fire</td>
<td>Ken Quintana</td>
</tr>
<tr>
<td>Injuries/Accidents</td>
<td>Alexander, Sally L</td>
</tr>
<tr>
<td>Radiological Hazards</td>
<td>Jim Abraham, Joe Tessari</td>
</tr>
<tr>
<td>Mixed Waste</td>
<td>Jim Abraham, Joe Tessari</td>
</tr>
<tr>
<td>Petroleum Fuel or Oils (SPCC)</td>
<td>James Graham</td>
</tr>
<tr>
<td>Mold/Water Damage</td>
<td>Doug Rice</td>
</tr>
</tbody>
</table>
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  - Robert.Ellis@ColoState.edu

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  - Dr. Matthew Hickey, Health & Exercise Sciences (IRB)
    - Matthew.Hickey@ColoState.EDU
  - Dr. Rick Allen, VTH (DRC)
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