HELLENIC REPUBLIC
National and Kapodistrian
University of Athens

Animal models of disease
Introduction to translational medicine

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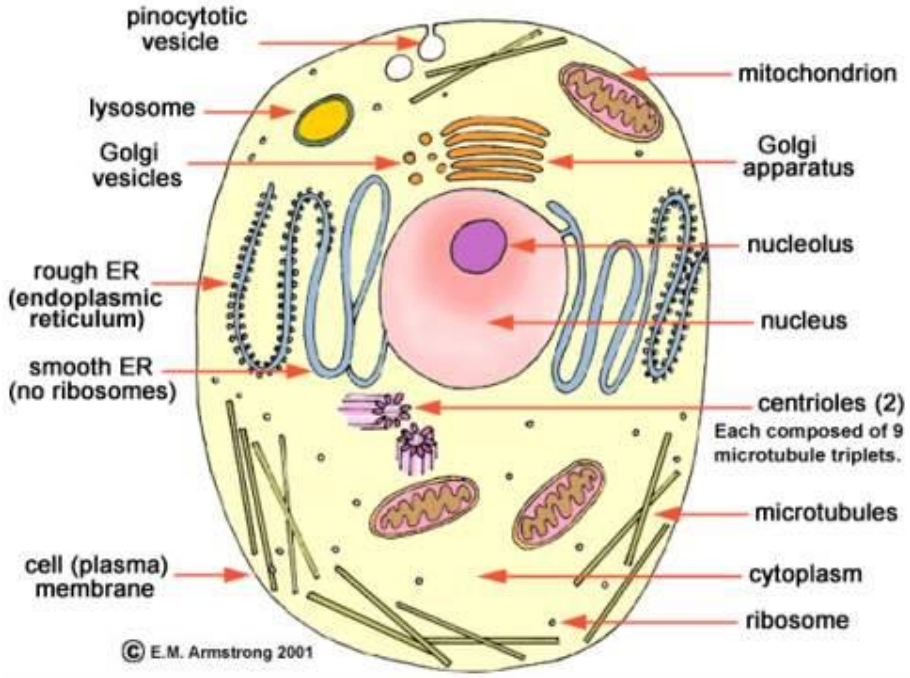
Overview

- Why do we need animal models for research and education?
  - What about alternatives like computers or cell lines?
  - Can results from animal studies really apply to humans?
- What have we learned from animal research?
- Are the animals used in research & education protected?
  - Regulations to protect research animals
- Mouse as an animal model

Why Do We Need To Use Animals for Research & Teaching?

- The functions of cells and organs are basically the same in animals and humans.
- What we learn from animals is useful in human and animal medicine.



Animal cells function in many of the same ways as human cells.

Biologically, humans are in the Animal Kingdom.

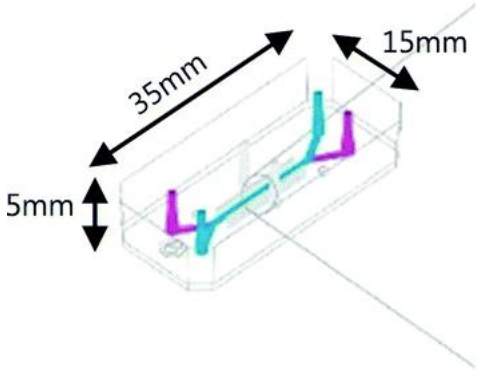
Why do we need animals for research & education?

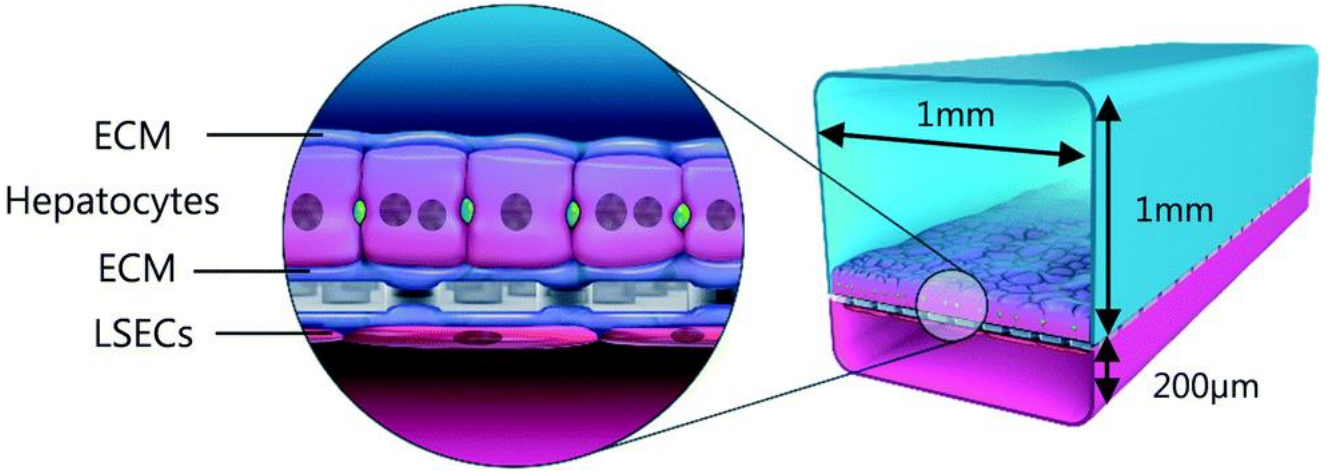
- Animals serve as good models to help us understand how living tissues function and the biology underlying disease.
  - The interaction of cells, tissues and organs within the body is very complex, and can often only be studied in the whole animal.
- Only by understanding how disease or injuries affect living organisms can we develop treatments or cures.
- Animal models are used to:
  - Help researchers understand the fundamental ways in which diseases affect living tissue.
  - Develop and test treatments for illness or injury.
  - Train future scientists and physicians.

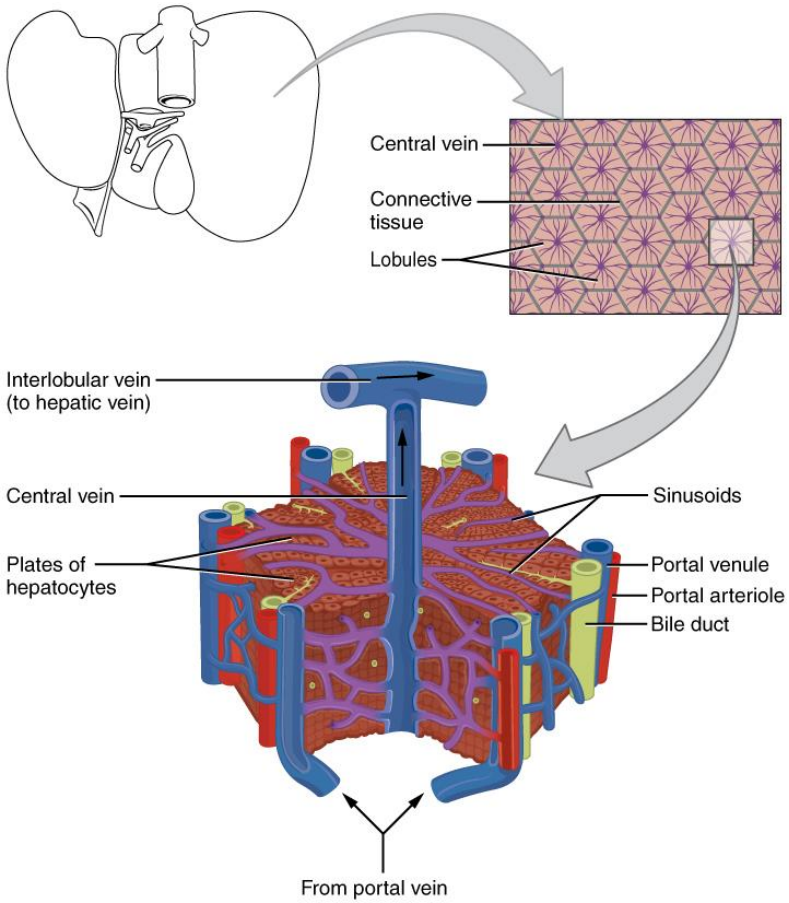
Can’t Computer Models and Cell Cultures Replace Animal Research?

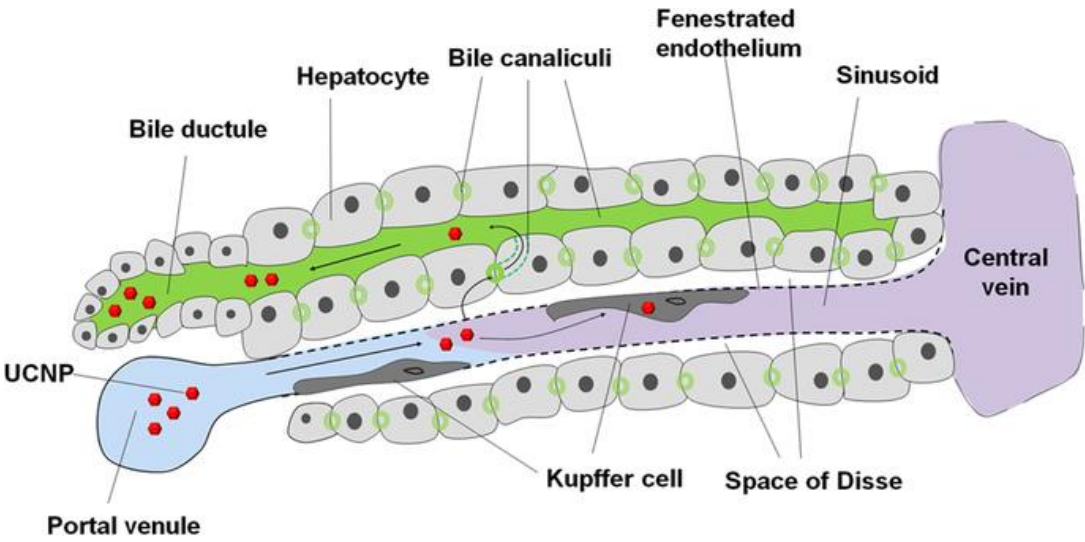
- Computer models and cell cultures are good for screening and are used frequently.
- Such models cannot replicate complicated interactions in the whole system.
- Final testing depends on studies in animals; sometimes it is required by law.
- Animal and non-animal models used in conjunction achieve the best answer.

Liver on a chip

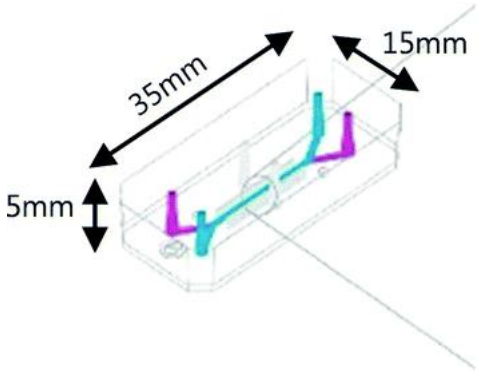


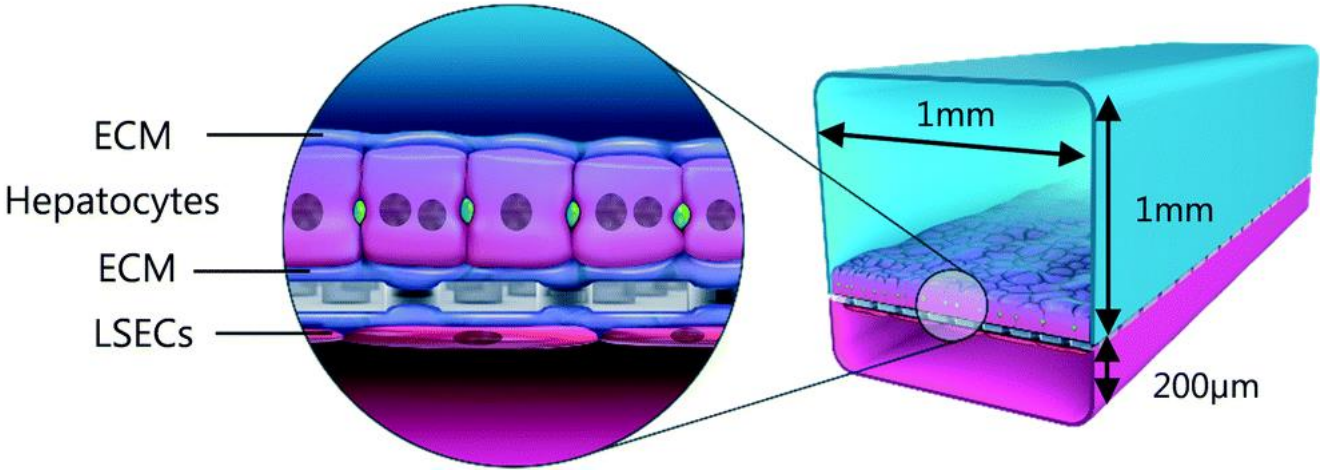






Liver on a chip

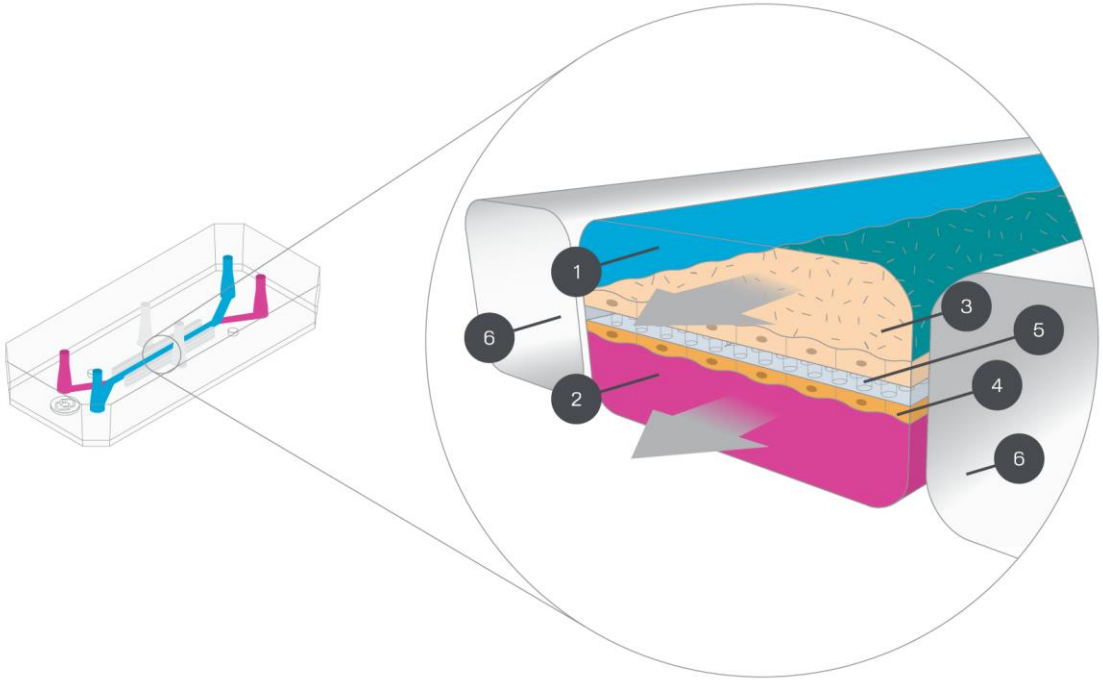




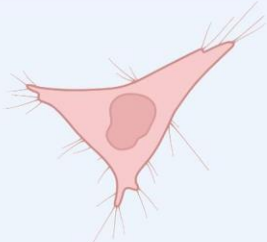
Lung on a chip

LUNG-CHIP

1. Air Channel
2. Vascular Channel
3. Lung Tissue (Epithelial Cells)
4. Capillaries (Endothelial Cells)
5. Membrane
6. Vacuum Channels



Preclinical Models



Cell Lines

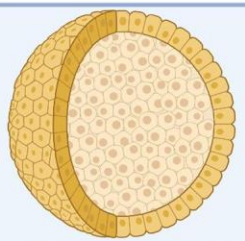
↓↓ *in vivo* recapitulation

↑↑ Scalability potential

Transwell

↓ *in vivo* recapitulation

↑ Scalability potential



Spheroid

↓ *in vivo* recapitulation

↑↑ Scalability potential

in silico

↓ *in vivo* recapitulation

↑↑ Scalability potential

Organoid

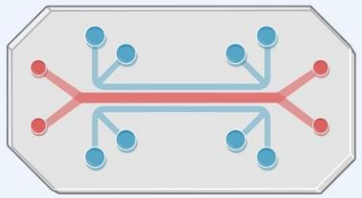
↑ *in vivo* recapitulation

↑↑ Scalability potential

Animal Models

↑↑ *in vivo* recapitulation

↓ Scalability potential



Microfluidic Systems

↑ *in vivo* recapitulation

↓↓ Scalability potential

Tissue Slice

↑↑ *in vivo* recapitulation

↓ Scalability potential

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Can Results from Animal Studies Really Be Applied to Humans?

- There are many similarities between animals and man. Examples include:
  - Immune function in mice
  - Cardiovascular function in dogs

- Animals provide index of safety.
  - Animal studies precede and support human studies.
  - Declaration of Helsinki mandates that medical research on humans must be supported by preceding animal research.

- Nearly all medical advances of the past century started with research in animals.

Limitations of testing in patients

- Ethical concerns with use of poorly tested protocols.
- Practical concerns: organisation, recruitment and cost.
- Requirement ‘to not harm’

What have we learned from animal research?

- Animal research has played a major role in nearly all medical advances for both humans and other animals.
- These include, but are not limited to:
  - Angiograms, X-Rays, CAT scans, blood pressure measurement, cardiac pacemaker, hypertension medications, insulin for diabetes, MRI, numerous vaccines, cardiac catheters, kidney dialysis, cataract surgery, burn treatments, heart valve replacements, artificial hips…. The list goes on and on!!
- So what animals have helped which medical advances?
  - Let's look at some specific examples…

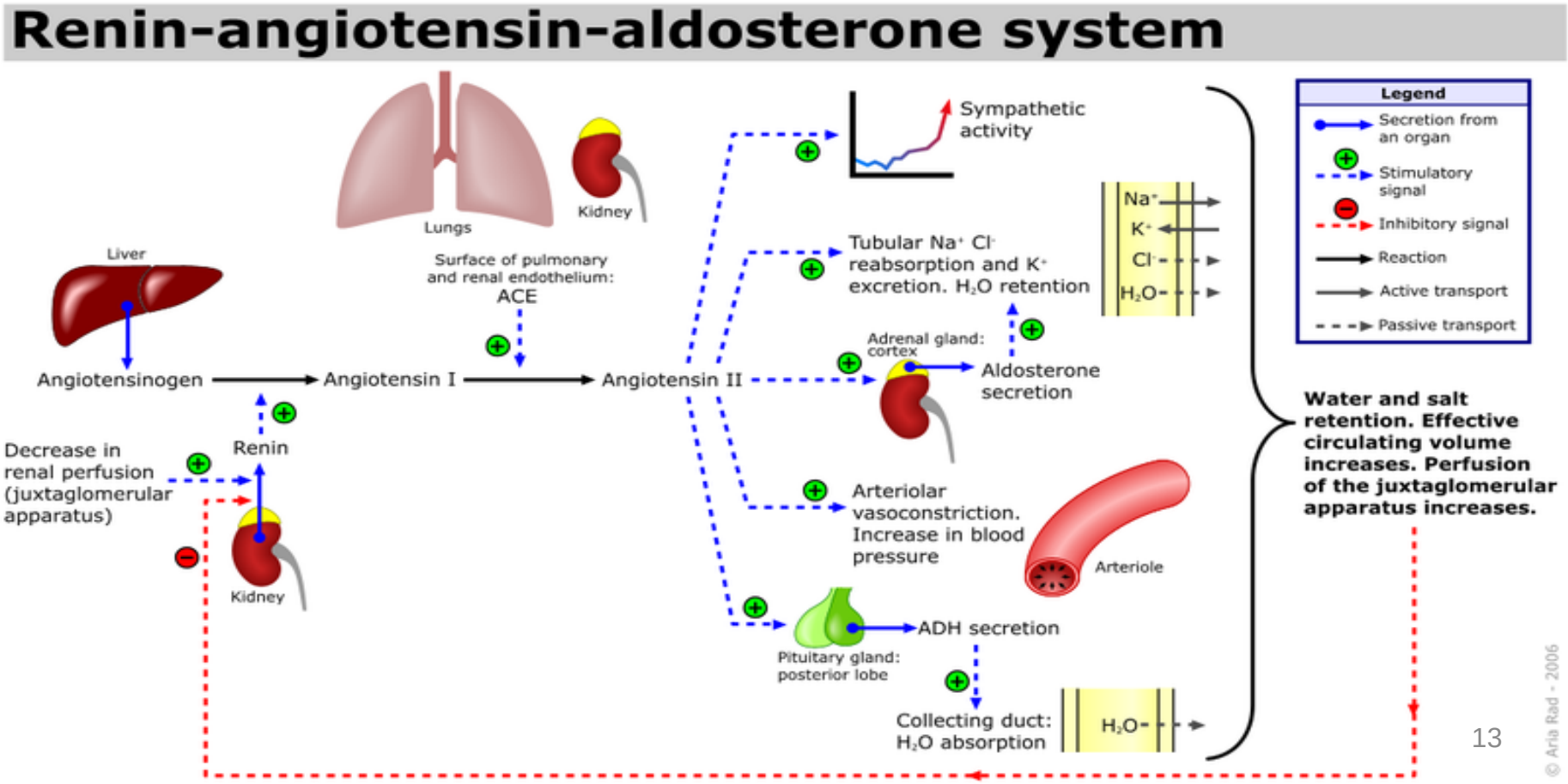
Can Results from Animal Studies Really Be Applied to Humans?

- They CAN and ARE. Virtually all drugs, devices and medical procedures have been developed with some animal research.

This dog, Kodi, underwent hip replacement surgery twice. Hip replacement surgical techniques were tested first on animals and now help both animals and people.

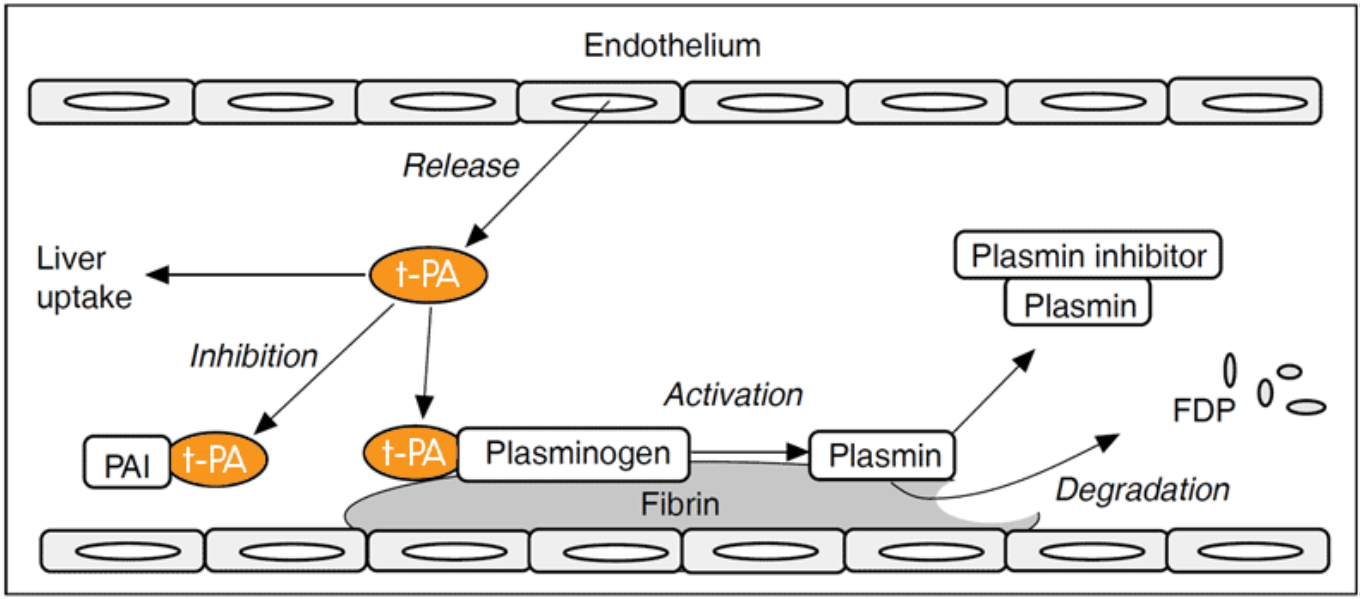
Animal Use in Biomedical Research

High Blood Pressure (HBP): Goldblatt linked HBP to kidneys in rats, cats, and dogs. This research led to treatments for high blood pressure.



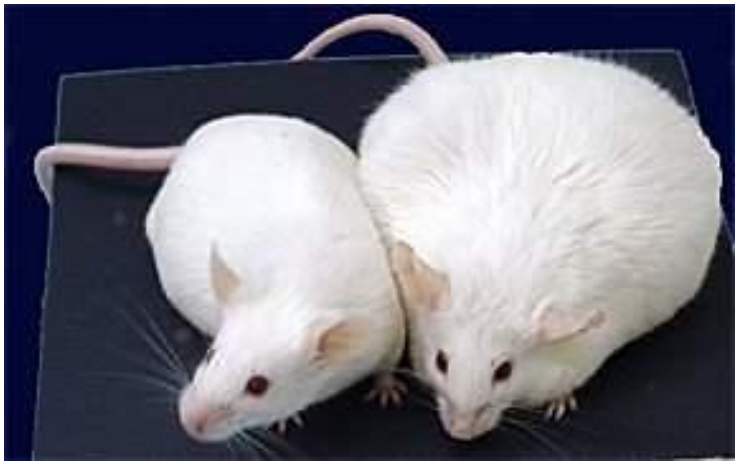
Animal Models for Research & Disease

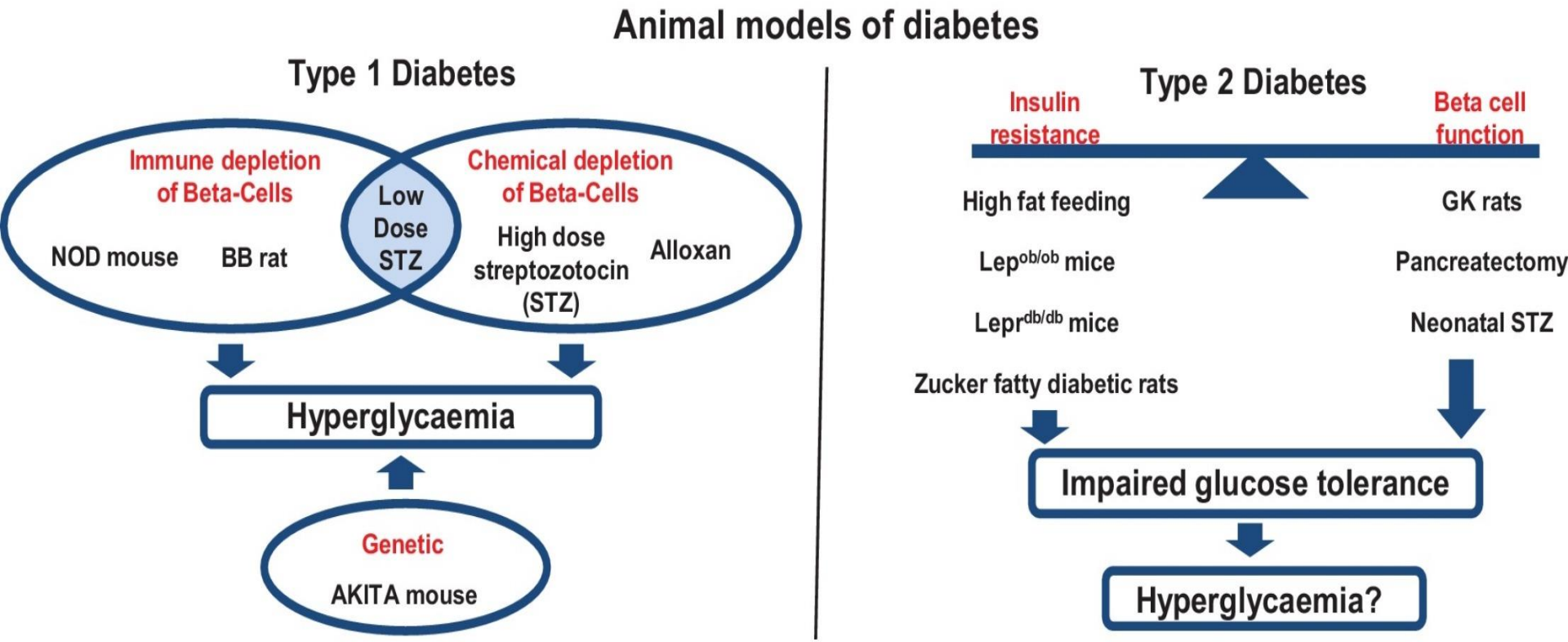
- Stroke
  - Stroke kills over 150,000 people in the U.S. each year and causes major disability that can include paralysis, inability to speak, loss of vision and loss of cognitive function.
  - Tissue plasminogen activator (TPA), a new treatment for acute stroke, and one that can reverse disability due to stroke was first studied in rats with experimental stroke.
  - Other potential treatments, even cures, for stroke are now being studied in experimental animals.



Animal Models for Research & Disease

- Obesity
  - Major risk factor for diabetes mellitus, high blood pressure, heart attack, stroke and certain cancers.
  - Epidemic in the United States: 64% of adults are overweight and 25% are obese.
  - Mouse models and Zucker obese rats shedding new light on causes of overeating, importance of leptin receptors, and ways that obesity leads to disease.

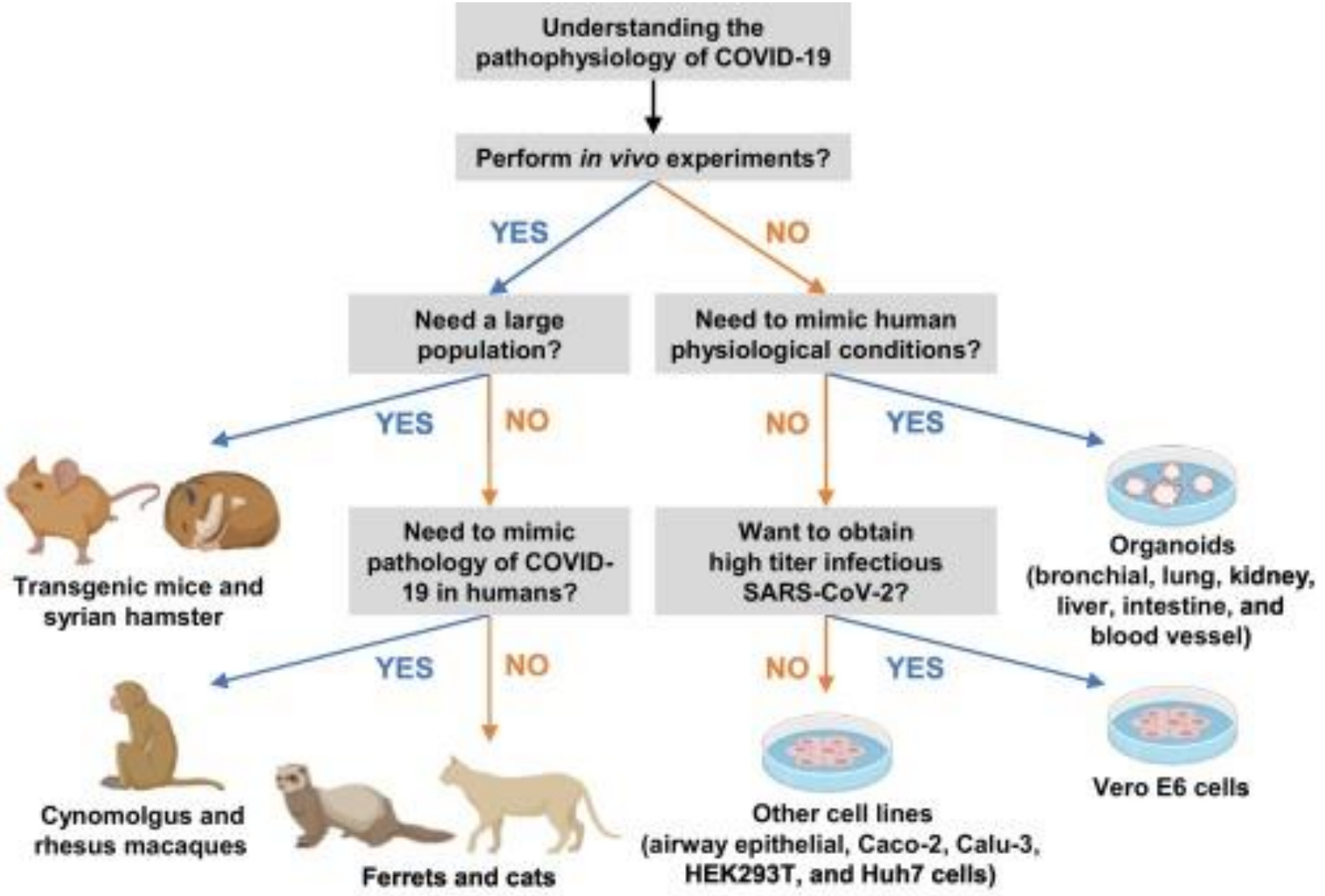




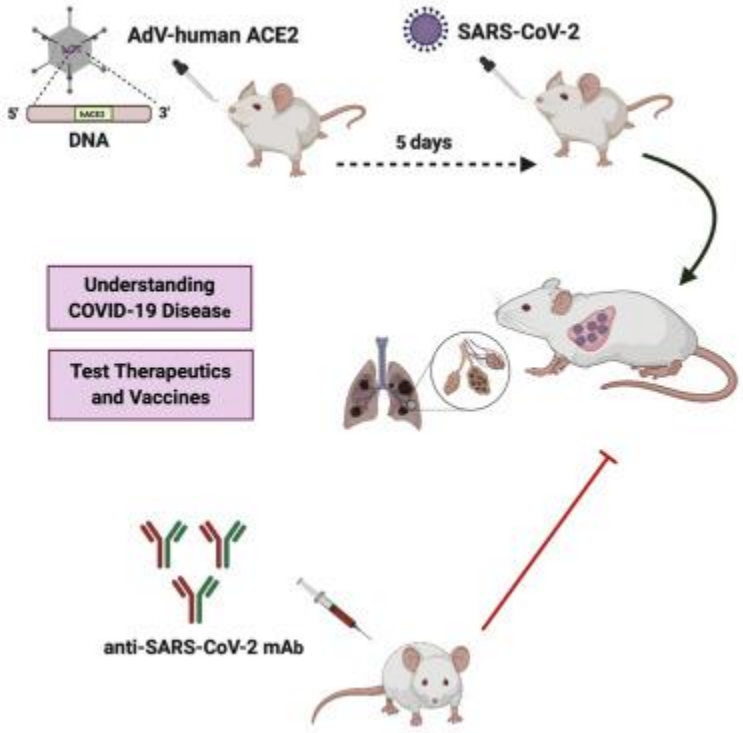
- NOD: polymorphisms in IL-2 gene / mutation in the CTLA-4 gene
- Akita: problem in insulin folding
- BB rat: Gimap5 mutation -> severe T cell lymphopenia -> Treg failure

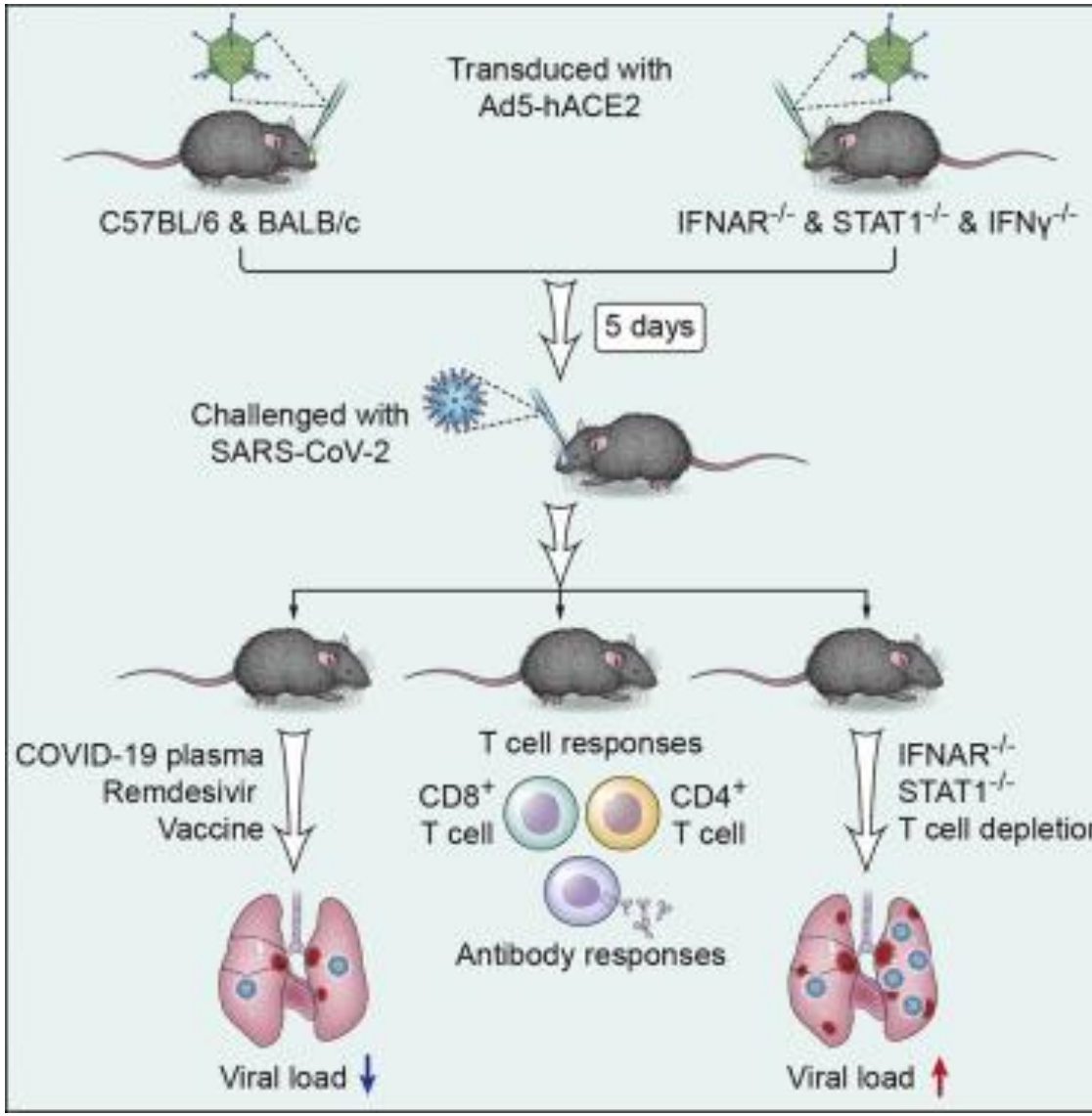
- Zucker: leptin receptor mutation
- GK rats: polygenic diabetes

COVID-19 Research



COVID-19 Research





International estimates of animals used in research

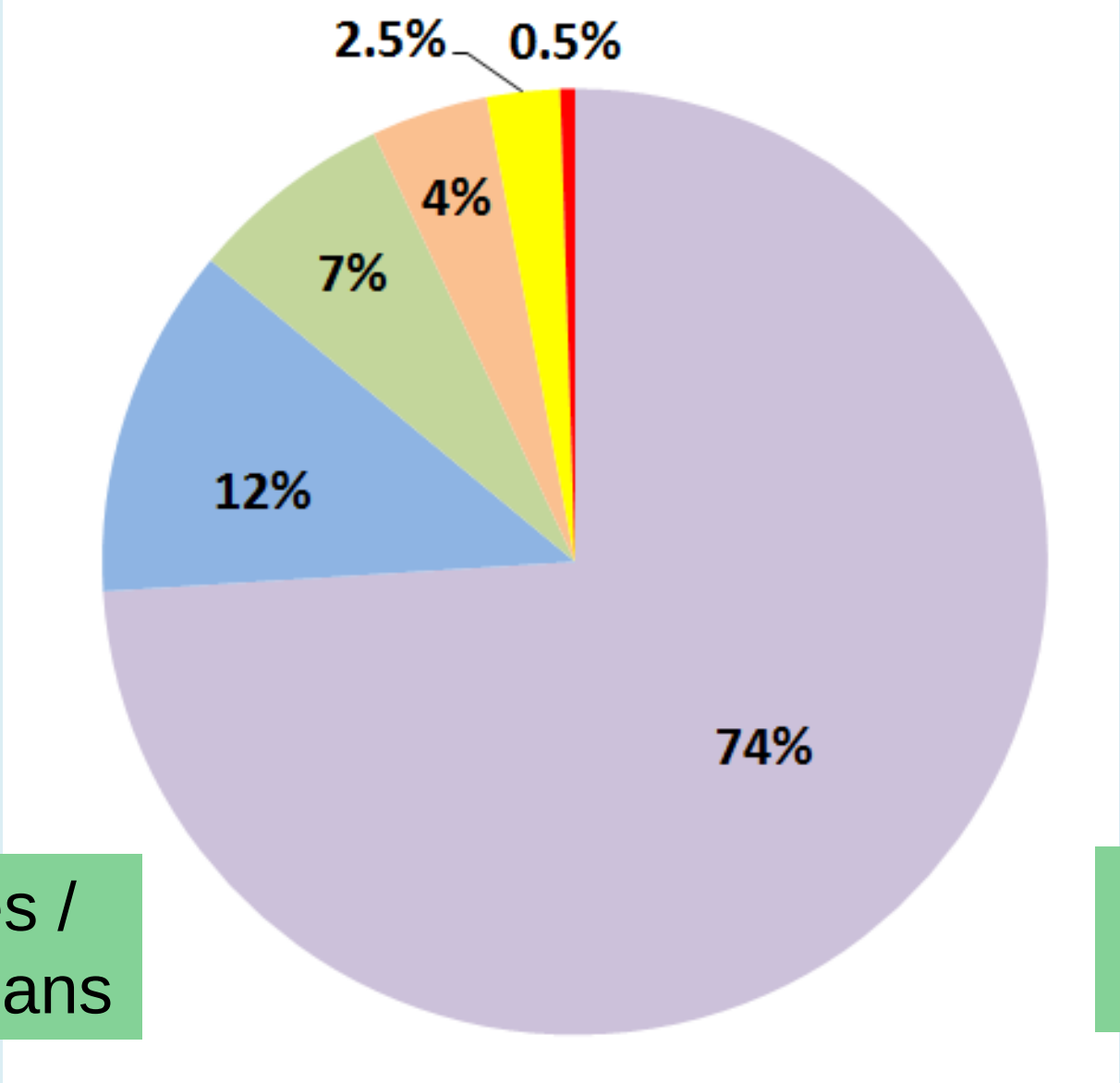
- Many countries do not provide comprehensive statistics.
- The major centres for research:
  - USA (about 15 million procedures)
  - EU (about 11 million procedures)
  - Japan (about 5 million procedures)
  - Canada (2 million), Switzerland (less than 1 million) and Australia (less than 1 million).
- The total worldwide maximum is in the order of 50 million animal procedures per year.

Which Percentage Applies To Which Animals?

Fish

Rats

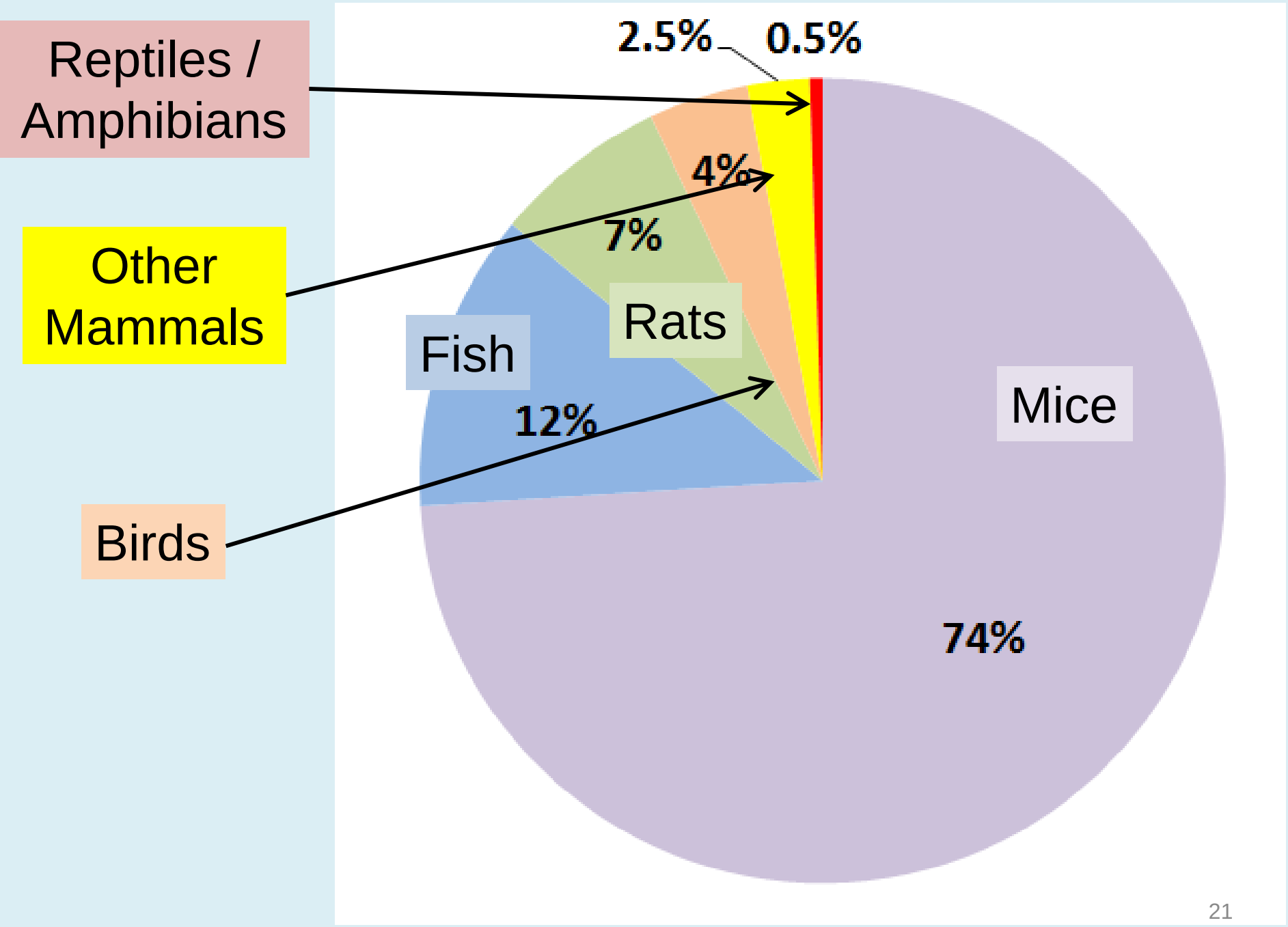
Reptiles / Amphibians



Birds

Mice

Other Mammals

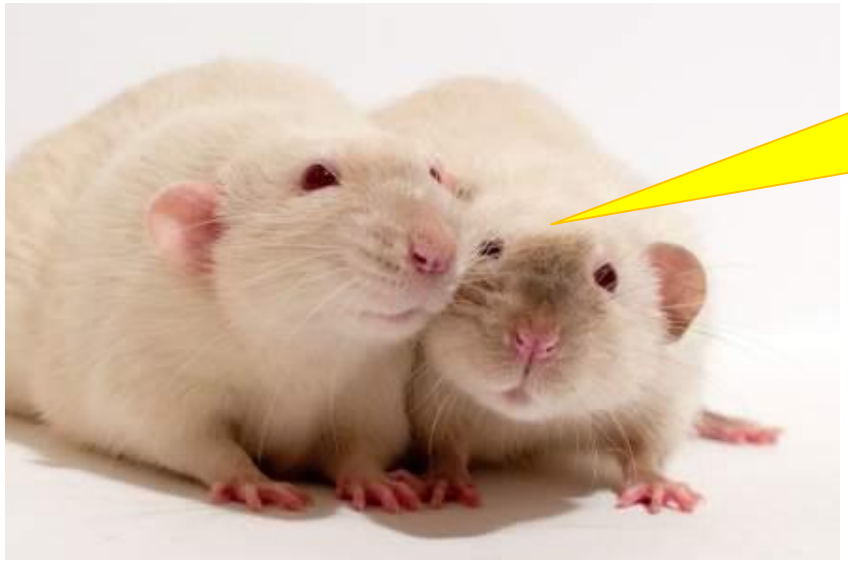

Reptiles / Amphibians
Other Mammals
Birds
2.5%
0.5%
4%
7%
Rats
Fish
12%
Mice
74%

Are the animals used in research & education protected?

- Many federal and local laws ensure animals used in research & education are being treated humanely.
- These include:
  - Animal Welfare Act
  - Public Health Service (NIH)
  - IACUCs (committees that must approve research protocols)
  - AAALAC (agency that accredits animal care facilities)

Animal Welfare Act

- The Animal Welfare Act is a Federal law that Congress passed in 1966 and amended in 1970, 1976, 1985, 1990, and 2002 to protect warm-blooded animals used in research, bred for commercial sale, exhibited to the public, or commercially transported. The law requires standards of animal care to be established and enforced. The Animal Welfare Act applies to dogs, cats, primates, guinea pigs, hamsters, and farm animals.



Animal Welfare Act

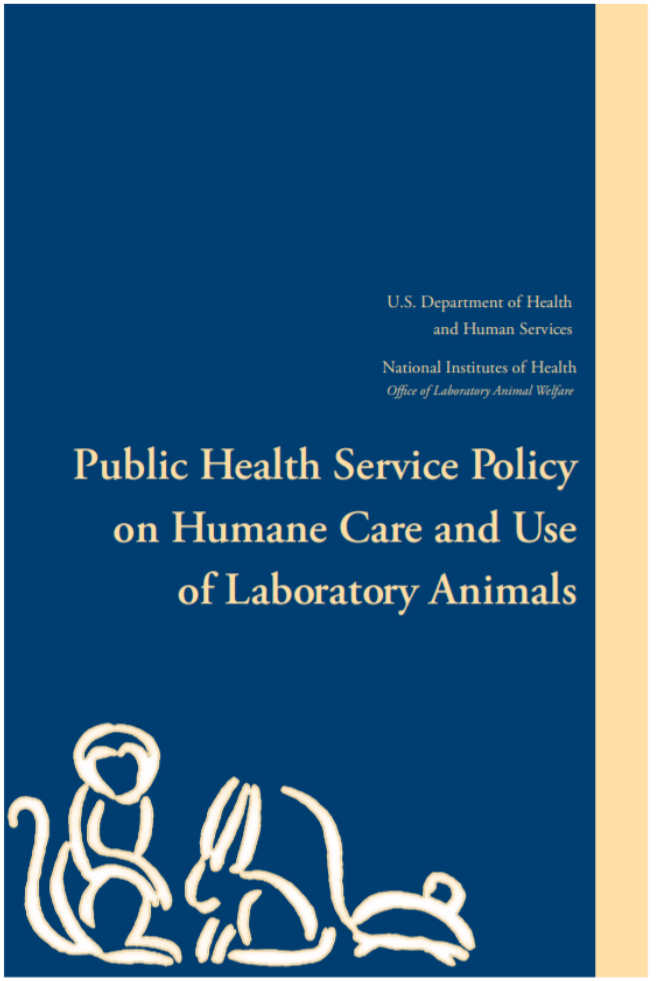
- Includes rules for mandatory surprise inspections of animal research facilities.
- These federal laws & regulations are in place to ensure that all research animals receive:
  - Good veterinary care
  - Appropriate housing
  - Proper Feeding
  - Humane handling
  - Sound sanitation and ventilation

If research animals are not being cared for properly, then the researchers that are responsible are breaking the law!

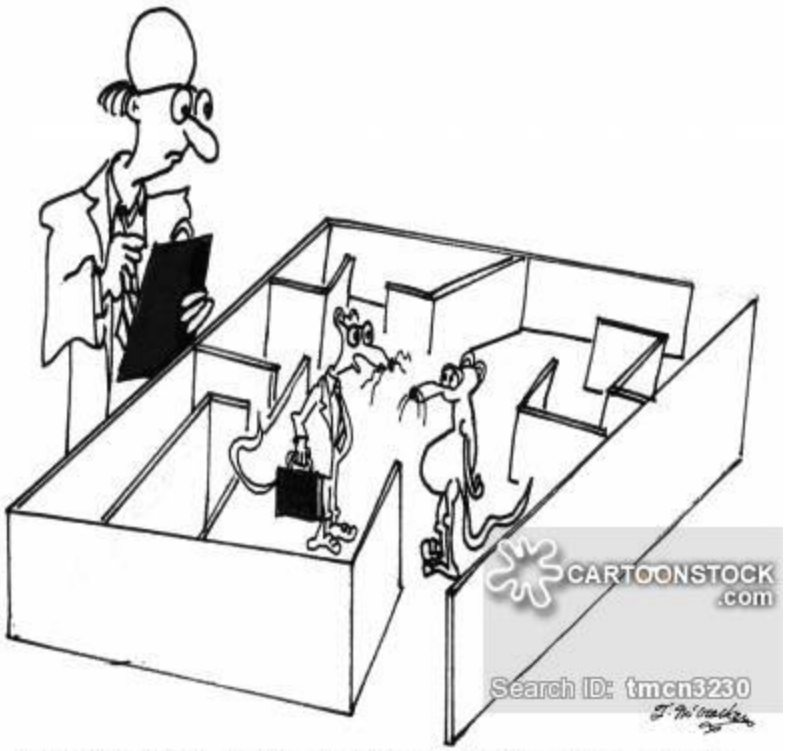
Lab animal care inspection

Public Health Service

- The Public Health Service Policy on Humane Care and Use of Laboratory Animals (updated 2002) requires compliance with federally mandated standards of care in use of laboratory animals for any work funded by the National Institutes of Health.
- Mandated by law, Health Research Extension Act of 1985.
- Administered through the Office of Laboratory Animal Welfare (OLAW) at the U.S. Dept. of Health & Human Services.
- Covers the vast majority of animals used in medical research, including rodents.



Institutional Animal Care & Use Committees (IACUCs)

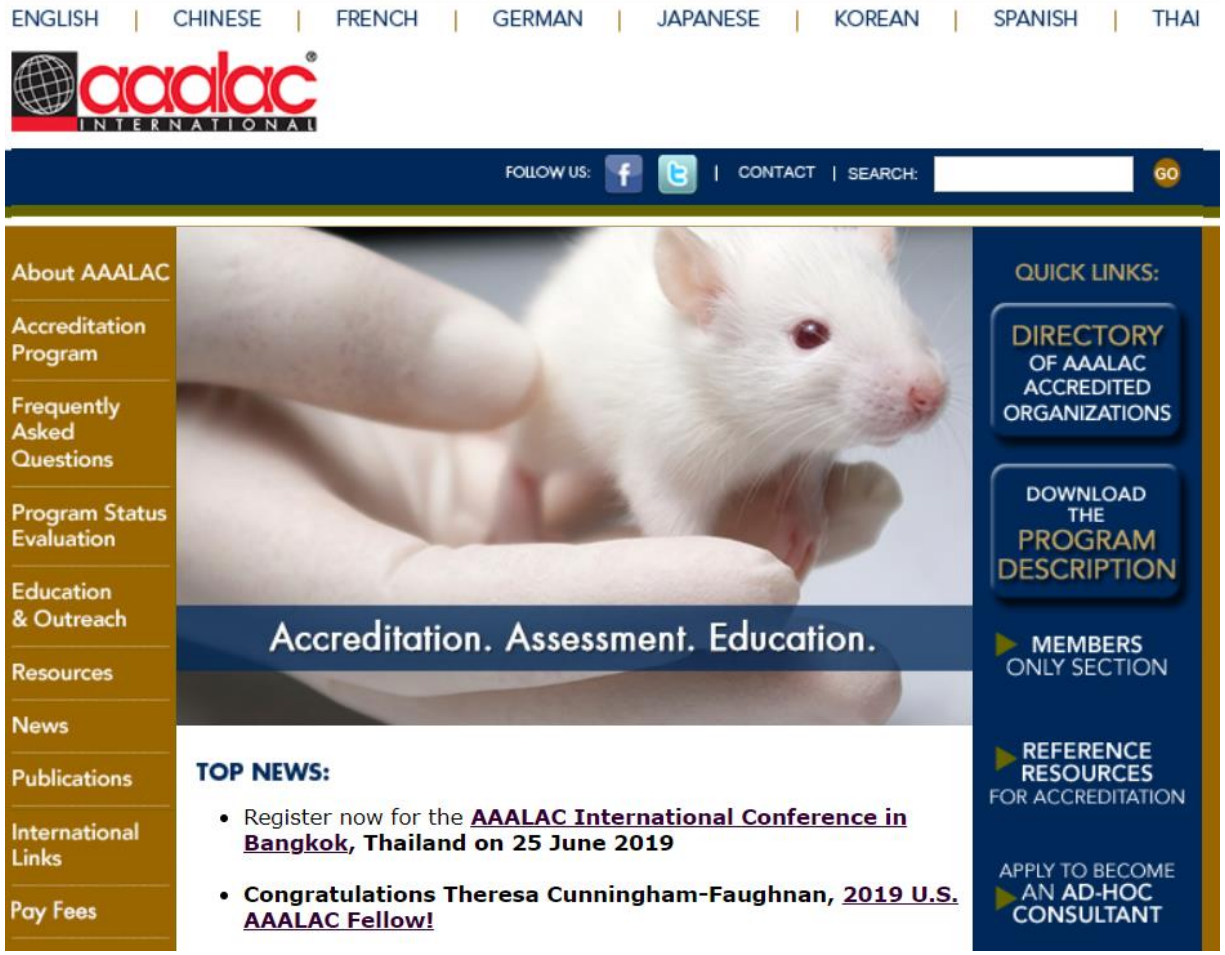


- Required at all research institutions by both AWA and PHS policy.
- Committees consist of veterinarians, scientists, members of the public.
- Without IACUC approval no research using animals may proceed.
- Among IACUC considerations are the measures used to control potential pain and avoid distress as well as the potential value of any scientific outcome from the proposed studies.

Association for the Assessment & Accreditation of Laboratory Animal Care (AAALAC)

- In addition to complying with federal & local laws governing animal care, the majority of research institutions seek voluntary accreditation with AAALAC

- AAALAC accreditation requires that researchers go well above & beyond the minimum standards for lab animal care & welfare mandated by law.



What is AAALAC?

AAALAC International is a private, nonprofit organization that promotes the humane treatment of animals in science through voluntary accreditation and assessment programs.

More than 1,000 companies, universities, hospitals, government agencies and other research institutions in 47 countries have earned AAALAC accreditation, demonstrating their commitment to responsible animal care and use. These institutions *volunteer* to participate in AAALAC's program, in addition to complying with the local, state and federal laws that regulate animal research.

Some of the institutions that have earned AAALAC accreditation include the Sloan-Kettering Cancer Center, St. Jude Children's Research Hospital, The American Red Cross, and the National Institutes of Health.

What about animal research in Europe?

Directive 63/2010/EU

European Commission

ENVIRONMENT

European Commission › Environment › Chemicals › Animals used for scientific purposes

Home | About us | Policies | Funding | Legal compliance | News & outreach

- Animals used for scientific purposes
- Legislation and implementation
- The "Three Rs" and alternative approaches
- Statistics
- Opinions of EU Expert Committees
- Related topics

Animals used for scientific purposes

Introduction

The protection and welfare of animals is an area covered by a wide range of EU legislation. This includes the protection of wildlife, zoo animals, farm animals, animals in transport and animals used for scientific purposes. Animal studies, whether for the development or production of new medicines, for physiological studies, for studying environmental effects or for the testing of chemicals or new food additives, has to be carried out in compliance with EU legislation.

Since 1986, the EU has had in place specific **legislation** covering the use of animals for scientific purposes. On 22 September 2010 the EU adopted **Directive 2010/63/EU** which updates and replaces the 1986 **Directive 86/609/EEC** on the protection of animals used for scientific purposes. The aim of the new Directive is to strengthen legislation, and improve the welfare of those animals still needed to be used, as well as to firmly anchor the principle of the Three Rs, to Replace, Reduce and Refine the use of animals, in EU legislation. Directive 2010/63/EU took full effect on 1 January 2013.

Latest updates

- NEW A new consensus document on the design and functioning of **Animal Welfare Bodies and National Committees has just been published.**
- NEW A new consensus document on the planning and execution of an effective **Inspection and Enforcement**

Federation of European Laboratory Animal Science Associations

History of FELASA

- Federation of European Laboratory Animal Science Associations
- Established in 1978: GV-SOLAS, LASA, Scand-LAS
- Membership is not open to individuals, but to laboratory animal science associations of good standing of nations in Europe
- Since 1991: observer status at CoE/EU levels
- In 2012 composed of 18 constituent associations + 1 affiliate association representing 26 countries + 2 with observer status

Associations represented in FELASA

- AFSTAL - France
- AISAL – Italy
- ARSAL – Romania
- BaltLAS – Latvia, Lithuania
- BCLAS – Belgium
- CLASA – Czech republic
- CroLASA -Croatia
- GV-SOLAS – Germany, Austria
- HLASA – Hungary
- HSBLAS - Greece
- LASA – United Kingdom, Ireland

and others....

Hellenic Society of Biomedical and Laboratory animal science

felasa

Federation of European Laboratory Animal Science Associations

FELASA's mission

- Represent common interests of constituent LAS associations
- Advance and co-ordinate the development of all aspects of LAS and practice in Europe
- Act as a focus for the exchange of information about LAS amongst European states
- Establish and maintain appropriate links with national, international or governmental bodies as well as other organisations concerned with LAS
- Promote the recognition and consultation of FELASA as the specialist European body on LAS and welfare *(observer status rev. ETS123 App A and EU Directive 2010/63)*
- Organise joint scientific meetings of the constituent associations

FELASA Interaction with EU Commission

Expert Working Groups

- Statistical reporting
- Severity classification
- Education & training
- Non-technical project summaries
- Alternatives & the 3Rs

National Contact Point Meetings

- Observer status at regular 6-monthly meetings

FELASA`s role in education & training

FELASA recognises education & training of the LAS community as an important aim of the organisation

To this end it has:

- Developed education & training recommendations for four categories of laboratory animal staff
- Established an accreditation system for teaching programmes for the four categories
- Developed guidelines for continuing education for all persons involved in the care & use of animals

Education & training recommendations – categories of personnel

- **Category A:** persons taking care of animals (caretakers)
- **Category B:** persons carrying out experimental procedures (research technicians)
-
- **Category C:** persons responsible for directing or designing procedures/projects (scientists)
- **Category D:** laboratory animal specialists (specialists)

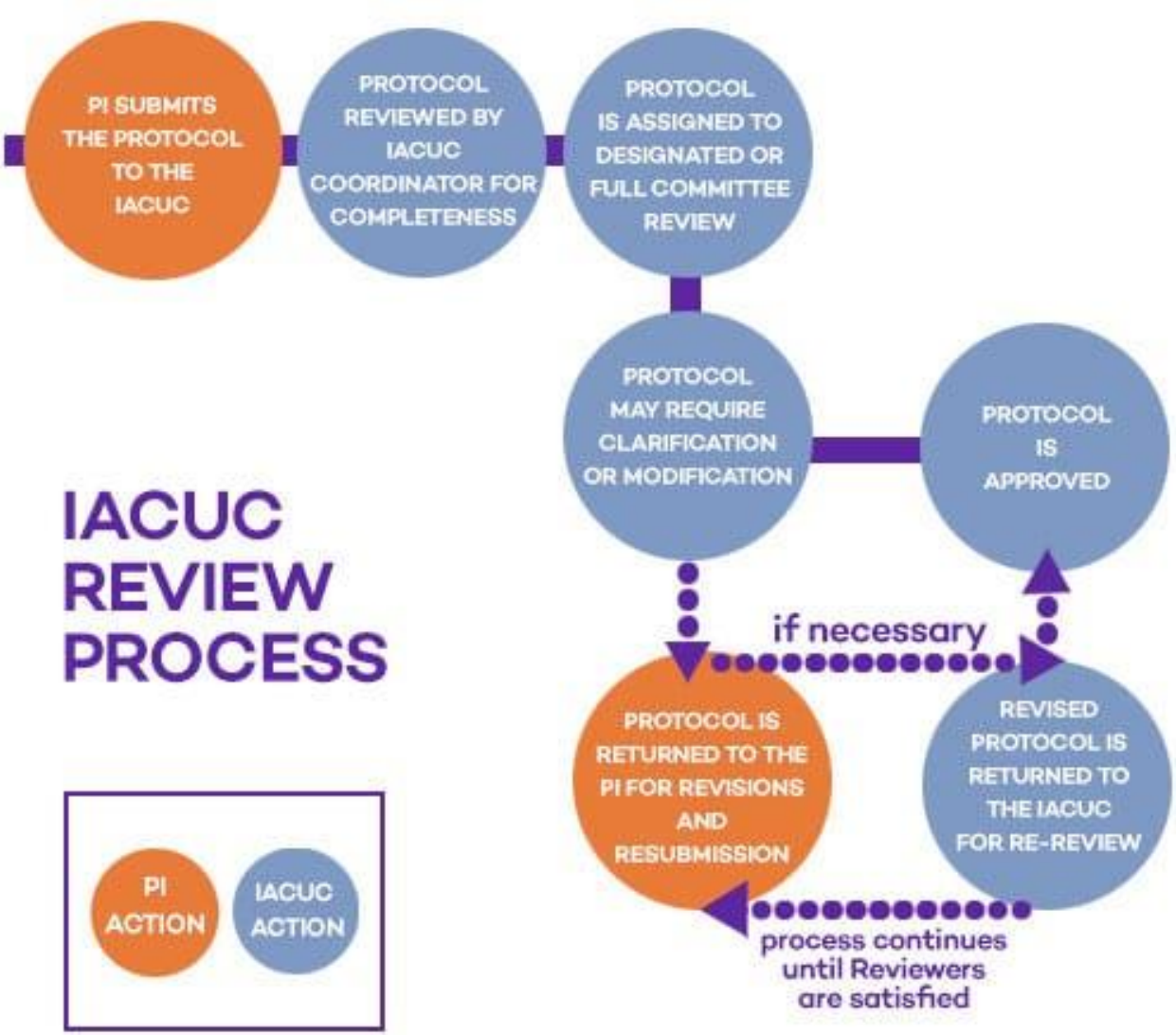
Course Listings

Following EU Directive 2010/63/EU and the working document on the development of a common education and training framework to fulfill the requirements under the Directive (Brussels, 19-20 February 2014), FELASA is accrediting courses using the "Functions' system", which replaces the "Categories' system".

EU Functions:

- EU Function A: carrying out procedures on animals
- EU Function B: designing procedures and projects
- EU Function C: taking care of animals
- EU Function D: killing animals

Animal protocol in practice?



Animal sources?

Other organizations and researchers

Examples of proper animal handling

Training Prior to Work With Animals

- **Training for research staff and animal care staff**
  - Hands on training by PI or designated staff member
  - Documented on-the-job training
  - Certification by AALAS is encouraged

First Impressions

- Clearly represent that animal users are interested in a quality program

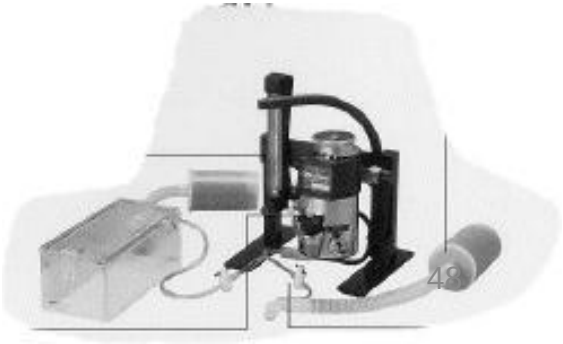
- Keep laboratory neat and organized
  - This says a great deal about the lab
  - Good Animal Care = Good Science

Maintain housekeeping in animal use areas

Storing Equipment & Supplies

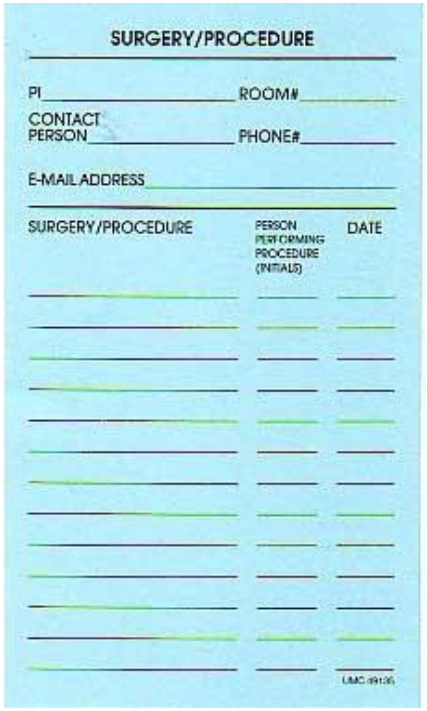
Inhalant Anesthesia

- Use anesthetics only as described in the protocol.
- Gas anesthetic vaporizers must be calibrated every 2 years.
- Gas anesthetic agents must be appropriately scavenged.
- Animals must not come in contact with liquid anesthetic agents such as isoflurane.



Documenting Procedures

Blue Procedure Cards

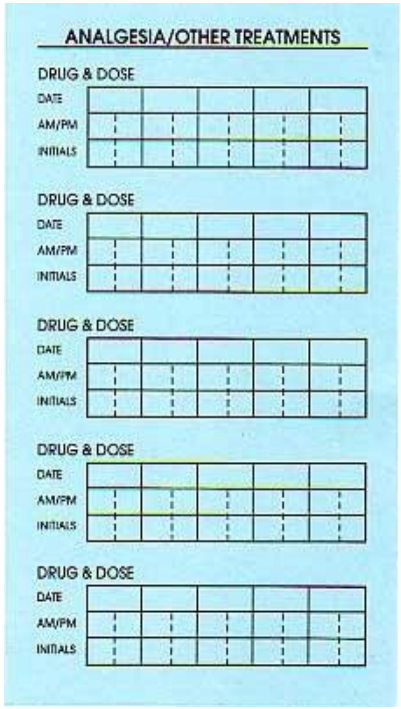

SURGERY/PROCEDURE

PI _____ ROOM# _____

CONTACT PERSON _____ PHONE# _____

E-MAIL ADDRESS _____

SURGERY/PROCEDURE	PERSON PERFORMING PROCEDURE (INITIALS)	DATE


ANALGESIA/OTHER TREATMENTS

DRUG & DOSE

DATE					
AM/PM					
INITIALS					

DRUG & DOSE

DATE					
AM/PM					
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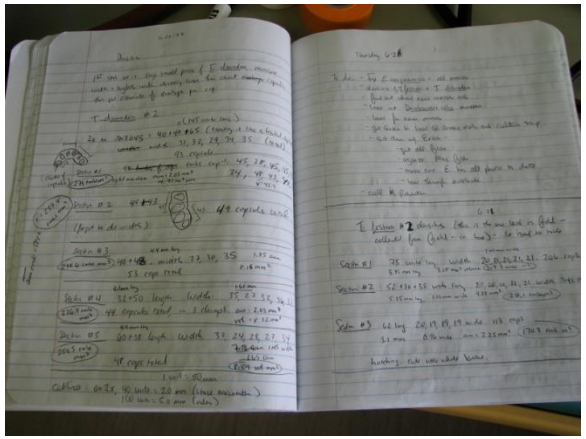
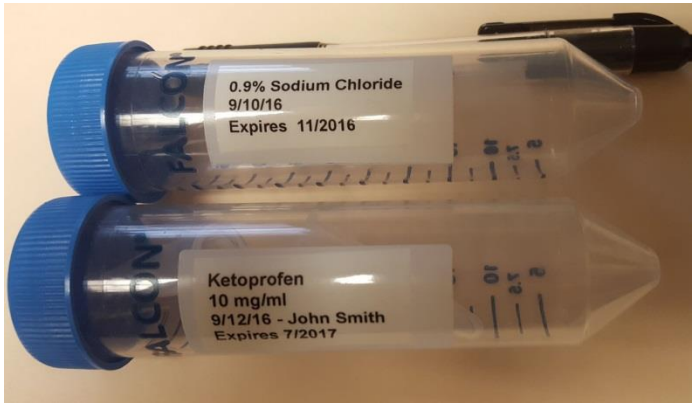
Research Records



Dates, procedures, observations, initials

Write legibly

Labeling Secondary Containers



Animal Transport

- Use only the transport routes approved in the protocol
- Use only approved transport containers
- Refer to animal transport policy

Animal Housing

- Minimum cage space requirements are listed in the Guide, the Animal Welfare Act & Regulations, and summarized in DLAR SOP #112 (*Minimum Space Requirements of Primary Enclosures*) posted on the DLAR website.

- Overcrowded mouse cages primarily result from
  - Overdue weaning (> 28 days)
  - Multiple litters in the same cage

- Managing rodent breeding colony cage density is the PI's responsibility.

Identify Single Housed Animals

Social Housing Key

Protocol Exemption	 blue
Aggressive	 red
Research Attrition	 yellow
Veterinary Exemption*	 pink
Post- Procedure**	 green

*Write date of dispensation expiration

**Write date when animal can return to social housing

Pregnant animals must be labeled with a "pregnant" cage card

- Rationale for single housing must be provided at the cage level.
- Appropriate colored "dot" sticker should be placed on the CAMS card.

Sharps Safety

Sharps injuries are a significant injury and health hazard for researchers and animal care workers

Sharps include:

- Needles, scalpel blades, lancets, razor blades, and broken glass

Sharps Safety

Reducing the hazard from sharps requires the use of:

- Engineering controls
  - Equipment designed to reduce risk
- Work practice controls
  - Handling practices that reduce the risks of exposure

The 3Rs

- Reduce the number of animals used to a minimum
- Refine the way experiments are carried out, to make sure animals suffer as little as possible
- Replace animal experiments with non-animal techniques wherever possible.

Russell and R.L. Burch (1959) The Principles of Humane Experimental Technique.

The Three R's of using animals in research

- The Three R's are principles of good science that scientists must adhere to when conducting animal-based research.

- **Replacement** Using non-animal alternative wherever they exist in order that the only research done using animals is that which can be done no other way.

- Reduction
  - Using as few animals as possible to attain statistically significant results, as well as finding ways to cut down on the number of animals used for any specific piece of research.

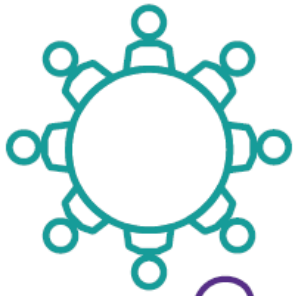
- Refinement
  - Improving animal welfare in laboratories by enhanced lab technician training, better enrichment inside the cages for animals, redesign of an experiment, etc.

THE USE OF ANIMALS IN MEDICAL RESEARCH

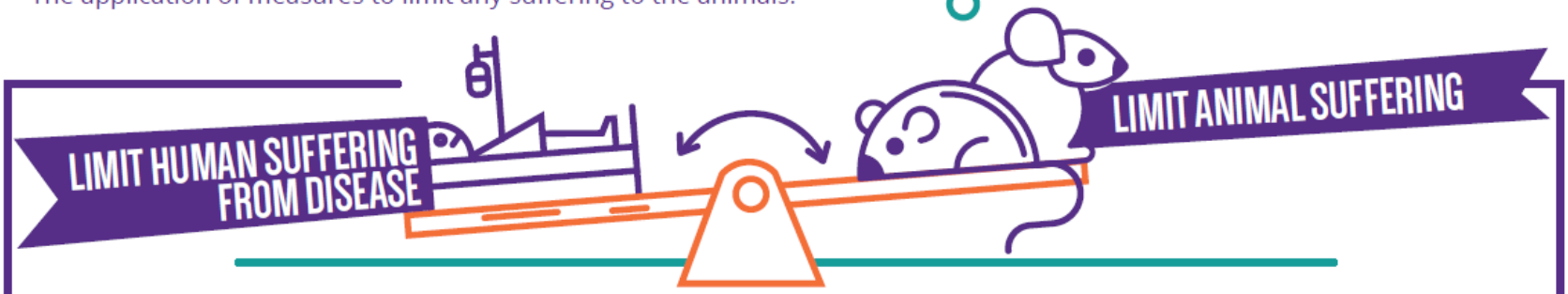
A responsible balance

FACTORS THAT SWING THE BALANCE

- The goal of the research and the prospected human or animal benefit resulting from that research.
- The existence of non-animal alternatives for the research.
- The number of animals used.
- The application of measures to limit any suffering to the animals.



Ethical committees oversee the strict application of the three R's principle and determine which way the balance swings.



REPLACEMENT

If there is a non-animal alternative, it must be used.

REDUCTION

If the experiment can scientifically correct be done with fewer animals, then the number must be reduced.

REFINEMENT

If there are ways to limit any suffering, these measures must be applied.

NO ANIMAL EXPERIMENT WITHOUT APPROVAL FROM AN ETHICAL COMMITTEE