

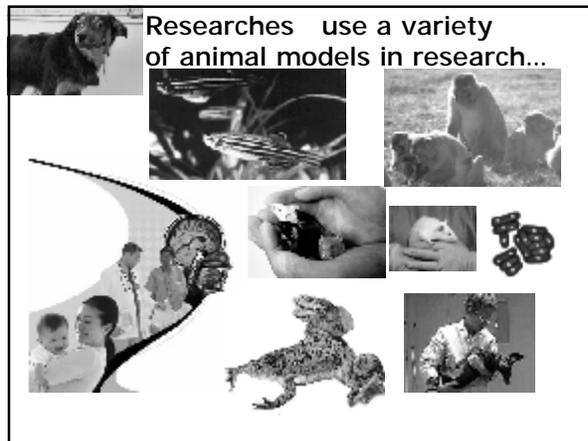
Research on Animals: Methodology, Regulations & Ethics

Khalid Al-Ani, Ph.D.
Department of Clinical Pharmacy
Faculty of pharmacy
Jordan University of Science & Technology

- According to the U.S.D.A, the total number of animals used in 2005 was 1,177,566 .
- 50 to 100 million vertebrate animals used annually worldwide from zebrafish to non-human primates ,

The research is carried out

- inside universities
- medical schools
- pharmaceutical companies,
- farms
- defense-research establishments
- others

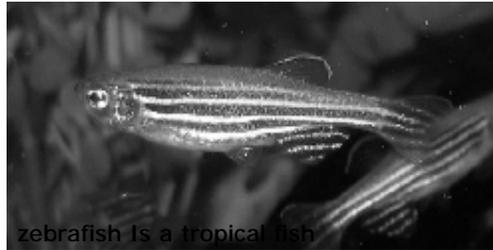


● **Invertebrates**



Drosophila a fruit fly, (and others) are one of the most widely used species for experimentation

● **Fish and amphibians**



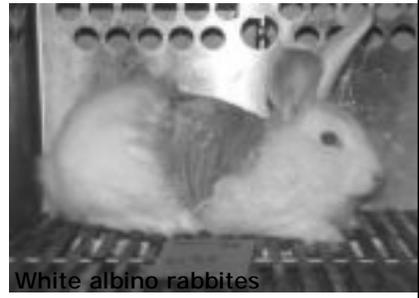
zebrafish is a tropical fish

● **Rodents**

● guinea pigs, hamsters, gerbils, rats and mice



● **Rabbits**



White albino rabbits

Dogs



Beagles are used, largely because they are

- friendly
- gentle, in
 - toxicity tests
 - surgery
 - dental experiments



Non-human primates (NHPs)

- Most of the NHPs used are
 - monkeys,
 - baboons
 - chimpanzees
 - others




Types of experiments

Experiments can be split into three broad, overlapping categories:

- **pure or basic research**,
 - aims to increase knowledge
 - no direct commercial application

Types of experiments-conti

applied research,

to solve specific biological need

- the treatment or cure of disease
- disorder in humans and animals
- develop commercial products, either for medical or non-medical use such as

Types of experiments-conti

toxicology or safety testing,

to measure potential adverse biological reactions to the ingredients.

Drug testing

- *metabolic tests*

- *toxicology tests*

- *efficacy studies*

double-blind controlled trial

dose-response

Cosmetics testing

- Cosmetics testing is particularly controversial
It is banned in the Netherlands, Belgium, and the UK, and in 2002, after 13 years of discussion

- **Products in Europe not tested on animals carry this symbol**



Today's conversation

- The use of animal models is **critical** to biomedical research since scientific progress depends on experiments
 - ┆ animal research facing Social, legal, and ethical context



Science EDITORIAL

Animal Activism: Out of Control

By Richard Stern, Editor in Chief of Science

THE SCIENTIFIC COMMUNITY HAS RESPONDED TO SEVERAL IMPORTANT SCIENCE POLICY issues this year and is getting a little public traction on some, including stem cell research, nuclear and global climate change. We have mostly ignored another, however, and it's a big one. Scientific progress depends on experiment, and in the life sciences that usually entails the use of live animals. But in many countries, animal rights organizations have successfully used extreme tactics to intimidate scientists and their institutions.

Scientists in the United Kingdom have been engaged in this struggle longer than those in the United States, and they appear to have been vigilant enough to secure at least some moderation of the problem. In the United States, however, if you conduct experiments on primate nervous systems, you might have the following experience. Photographs, allegedly of your subjects wearing expressions of extreme pain, are circulated to media outlets. Crowds with banners picket your residence and leaflets declaring that you commit "atrocities" are distributed to your neighbors. Your colleague who works on monkey behavior is the target of a libel suit. This is maliciously placed on a neighbor's porch; the good news is that the fax line is failed, but the Federal Bureau of Investigation (FBI) says the leaflet might well have killed your monkey.



The Washington Post

The Animal Zealotry That Destroyed Our Lab

IOWA CITY, Iowa -USA



- International legal campaigns to give animals rights
- Guardianship and civil court cases



Responsibility of Scientists

- Experimental animals must be housed in facilities designed for animal in acceptable standards
- Using procedures to minimize pain and distress such as
 - use of adequate caging & proper handling by personnel

There is an Ethical issues for using animals in research

1. It is the moral and ethical obligation of each authorized faculty and staff member, and all persons working under their direction, to insure that:
 - a. All animals, regardless of species, are treated humanely

Ethical Expectations for JUST researchers using animals-con

- b. When methods are used that may cause pain, discomfort, or distress, all possible measures should be taken to minimize the pain , including

- the use of anesthetic and analgesic drugs to minimize pain, discomfort or distress.
- Experiments must be of the shortest possible duration for valid results.
- Any animal which exhibits severe pain or distress that cannot be alleviated must be euthanized immediately

Ethical Expectations for JUST researchers using animals-cont

- c. If the induction of hunger or thirst through food and water deprivation is a necessary part of an experiment, it will be conducted in a manner that does not interfere with the normal growth and development or health of the animal.

Ethical Expectations for JUST researchers using animals-cont

- d. Physical restraint will not be used as a substitute for anesthesia.
- e. Methods which cause suffering or distress which cannot be justified by the expected quality of data are not used
- f. The research is designed to utilize the best methods on the smallest number of animals of the appropriate species yielding valid results

● **Why Animal models are critical to the present and future biomedical research?**

Animal models are critical to biomedical research

- Genes
 - Molecular pathways
 - Computer modeling can put the researcher in the picture, but cannot replace an understanding of processes in the whole organism.
- Animal models provide whole-organism knowledge



Animal models are critical to biomedical research

! The use of animal models in basic or clinical research is an appropriate precursor to safe and ethically sound research because

1. It would be unethical to test substances or drugs with potentially adverse side-effects on human beings.
2. Animals are good models because of their similarities to humans.
3. There is no substitute for some clinical trials that require behavioral data such as psychiatric studies e.g., antidepressant or infection of a host with hepatitis, malaria .

4. Some animals (e.g. Drosophila) have shorter life and reproductive spans than humans, meaning that several generations can be studied in a relatively shorter time.

5. Drugs and vaccines produced through animal testing are vital to modern medicine.

6. There have been several examples of substances causing death or injury to human beings because of inadequate animal testing.

- Mice are the most commonly used vertebrate species because of their
- small size
- low cost
- ease of handling, and
- fast reproduction rate.

Mice are widely considered to be the best model of

- inherited human disease

- share 99% of their genes with humans.
- genetically modified mice can be generated



Hairless mice

Handling Techniques

- Rats should be acclimatized to handling (*gentling*) to reduce stress
- Handling is easily accomplished if the operator approaches the animal in a calm and slow approach.
- the animal should be given the opportunity to smell your hand as you approach it.

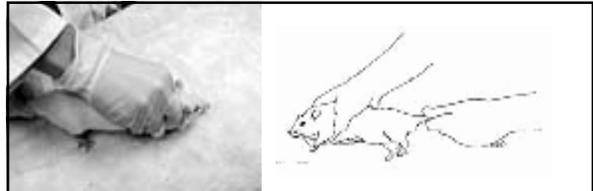
Handling Techniques-conti

- It is recommended that gloves not be used, in that the glove masks the natural human odor of the operator and prevents the animal from becoming familiar with the operator and recognizing him over a period of time when handling may be necessary.

- Rats should be handled at the base of the tail using your fingers



NOTE The rat dislikes being handled by the tail and is much more liable to be aggressive



- Pick up rats by placing the hand firmly over the back and the rib cage and restraining the head with thumb and forefinger immediately behind the mandibles.



- Holding the rat upside down keeps it distracted and reduces the chances of biting.



Husbandry

- Rats should be kept in rooms with the temperature set at about 70F and humidity at 50%.
- Lights should not be too bright since white rats are albinos and too much light damages their retina.
- They are diurnal which means they need about 12 hours of light and 12 hours of darkness each day.

● Husbandry-conti

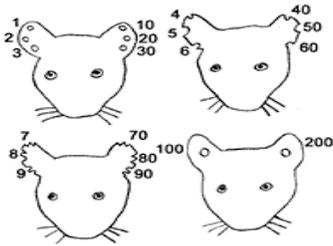
- Bedding can be paper, wood shaving, wood chips or corncob.
(some wood shaving emits hepatotoxic fumes)
- They should have fresh mouse or rat food and water available at all times. Their bedding should be changed 2 or 3 times a week to prevent the build-up of urea

● Identification

- Rats should always be clearly identified on cage cards indicating protocol number, strain (using standard nomenclature), sex, age, supplier, investigator and contact person.

Identification-conti

- Individual rats can be identified using ear punches, ear tags, fur dyes, indelible mark on tail or microchips



Blood sampling

- An adult rat has a circulating blood volume of about 15-35 ml (**5-7% of the body weight**),
- Up to 10% of the circulating blood volume may be taken on a single occasion from a normal healthy animal
- For repeat bleeds at shorter intervals, a maximum of 1% of an animal's circulating blood volume can be removed every 24 hours

Sites of blood collection

Tail

- Restrain the rat in a device for the collection



Sites of blood collection- *Tail*

- warm the tail by exposing it briefly to a heat lamp or placing it in a bowl of **warm water**.



Sites of blood collection- **Tail**

- Blood collected from tail vein or artery by making a cut in terminal ≈5 mm of the tail with a blade

- Use a hematocrit tube or blood-collecting tube to collect blood dripping from the sectioned tail



Sites of blood collection- **Tail**

- Massage the tail by passing the thumb and index finger from the base to the tip of the tail if blood flow is inadequate.
- Or Insert a syringe equipped with a 25 gauge needle at a 45 degree angle toward the vein.

Sites of blood collection- **Tail**

- At the end of the collection apply pressure to the cut end with a gauze bandage and ensure that blood has completely stopped flowing before returning the rat to the cage

● **Retro-orbital sinus**

- The retro orbital sinus is a system of dilated venous channels at the back of the orbit.
- Blood can be collected from this area in anesthetized rats using a micro-hematocrit tube

Pressure down with the thumb and forefinger just behind the eye and pull back on the skin to allow the eyeball to protrude or projected



Position a micro hematocrit tube along the inner corner of the eye (medial canthus) beside the eyeball



Insert the tube gently but firmly through the conjunctiva towards the back of the eye along the orbit



Cardiac puncture

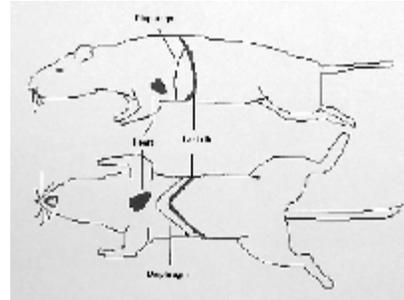
*Up to 10 ml of blood can be obtained from the heart of a **deeply anesthetized** animals*

Cardiac puncture-conti

The needle should be introduced at 10-30 degrees from the horizontal axis of the sternum in order to enter the heart.



- Alternatively approach the heart laterally immediately behind the elbow at the point of maximum heartbeat



- **Blood Collection from the Posterior Vena Cava and others**

Administration of substances

- Materials to be administered to rats can be given orally e.g. in water or feed or
- injected systemically through a variety of routes.

• Administration of substances-conti

- volumes can be injected into rats safely:
- 2-5 ml subcutaneously,
- 0.1-0.2 ml intramuscularly (0.1 ml per site),
- IM injections are usually not recommended in rats because of the small muscle mass)
- 1.5-2.5 ml intravenously,
- 3-5 ml into the stomach
- 3-5 ml intraperitoneally..

Note A fine gauge needle should be used to make injections

Gavage

- ... Oral gavage is performed using a ball ended feeding needle.



- Estimate the distance that the needle needs to be inserted into the rat (usually from the nose to the first rib) and mark it on the needle.

• Gavage=conti

- Introduce the needle in the space between the left incisors and molars, and gently direct it caudally toward the right ramus of the mandible.



• Gavage=conti

- The rat usually swallows as the feeding tube approaches the pharynx, facilitating entry into the esophagus.



- *Gavage=conti*
- *If the animal struggles or appears to be in respiratory difficulty withdraw the tube and begin all over again.*



- ***Intraperitoneal injections IP***
IP are usually made in the lower right quadrant of the abdomen.



- *Intraperitoneal injections IP-conti*
- *After swabbing the lower right quadrant with alcohol,*



- *a fine gauge needle is introduced slowly through the skin, subcutaneous tissue and abdominal wall.*



Picture demonstrating IP injection in a rat

Intraperitoneal injections IP-conti

- *Withdraw the syringe plunger to ensure that you are not in the bladder or intestines.*
- *If nothing is withdrawn inject the material slowly.*
- *If you accidentally enter the bladder or intestines withdraw and discard the needle and syringe*

Intravenous injections

Intravenous injections are usually made into the dorsal tail vein after Warming the tail



Anaesthesia

- **In general inhalant anesthetics are safer than injectable anesthetics.**
- **Halothane and isoflurane are the safest ones to use.**
- **ether is good but its use can be subject to restrictions due to safety concerns.**

Anaesthesia

- **Ketamine and xylazine is a common and relatively common injectable anesthetic combination.**
- **Sodium pentobarbital can be used, but it has a narrow safety margin and is associated with a prolonged recovery period**

Euthanasia الموت الرحيم

Euthanasia in rats is most often performed by

- carbon dioxide asphyxiation or
- overdose of an anesthetic agent.
- Use of cervical dislocation or decapitation in absence of deep anesthesia must be scientifically justified.

Euthanasia الموت الرحيم

- All individuals performing euthanasia must be properly trained.
- Individuals must also ensure that animals are dead before the carcass is disposed.

That's all for this lecture



Questions