

Animals Used in Preclinical Trials: A Review of Models, Applications and Welfare Considerations

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Abstract: Before a new drug is tested in people, preclinical studies examine its safety, biological activity and probable efficacy. Animal models are central to this stage because they show how a compound behaves in a whole living system, including absorption, metabolism, toxicity and effect on disease. This review draws on a B. Pharm practice-school project and describes six laboratory animals: mouse, rat, guinea pig, rabbit, hamster and frog. For each, it brings together biological data, housing and handling requirements, experimental uses and practical advantages. Mice and rats serve broad toxicological and pharmacological screening; guinea pigs suit histamine, respiratory and immunological work; rabbits are used for bioassays, pharmacokinetics and ocular and isolated-tissue studies; hamsters are important in infectious-disease and metabolic models; and frogs support isolated-organ and neuromuscular experiments. No model fits every question, and animal results do not guarantee human outcomes. Ethical approval, trained staff, humane endpoints and the 3Rs (Replacement, Reduction, Refinement) should govern every study, and in-vitro, organoid and computational methods should be used wherever they can answer the question.

Keywords: preclinical research; laboratory animals; rodents; toxicology; pharmacokinetics; animal welfare; 3Rs

I. INTRODUCTION

Preclinical trials are the laboratory stage of drug development. They are meant to show whether a candidate is reasonably safe to give to people and whether it has the intended effect. They also help set dosage and formulation, and they produce the data that regulators such as the U.S. Food and Drug Administration expect before human trials, for example in an Investigational New Drug (IND) filing. Finding a safety or efficacy problem at this stage costs far less time and money than finding it in patients.

Preclinical work is usually divided into in-vitro and in-vivo studies. In-vitro ("in glass") studies use isolated cells, tissues or organs in test tubes or dishes. They are cheaper, simpler and tightly controlled, which makes them suitable for early screening and basic mechanistic work. In-vivo studies are done in living organisms. They are more complex, but they capture metabolism, immune response and organ interaction together, so they reveal side effects and interactions that an in-vitro system may miss.

Animals are used in this stage in several ways: to assess safety by watching for toxicity and adverse effects; to test efficacy in animals with a disease resembling the human condition; to study pharmacokinetics (absorption, metabolism, distribution, excretion) and pharmacodynamics (interaction with target molecules and cells); and to explore mechanisms of action at the molecular or cellular level. Ethical guidelines and regulations govern this use, and research into alternatives such as in-vitro tests and computer simulation continues. This paper reviews the animals most often used, what each is good for, and how they should be housed, handled and reported on.

Preclinical results also decide how the later human trials are designed. Clinical trials proceed in phases: the earliest phases look mainly at whether a drug is safe and what side effects it causes, and later phases test whether the new



treatment is better than those already available. The dose range, formulation and safety limits used in those phases come from the preclinical data. The weight of this work explains why the choice of animal species and the quality of the animal study matter so much.

II. LITERATURE SURVEY

Laboratory animals are commonly grouped into rodents (mouse, rat, guinea pig, gerbil, hamster), non-rodents (rabbit, dog, cat, monkey, pig) and miscellaneous species (frog, pigeon, zebrafish, chicken). Standard reference works describe the biology and husbandry of the rodent and rabbit species in detail [1–9,12]. Non-human primates, which are also used in safety testing, need specialised husbandry and welfare provision [10,11].

Three studies cited in the project frame the field. Sreeja discusses how dose fixation in animal studies must allow for species differences, and explains conversion of doses by normalization to body surface area and the calculation of animal equivalent doses [13]. Pierre reviews in-vivo models that mimic pathological and neurological processes in cerebral reperfusion injury, including middle cerebral artery occlusion, embolic stroke, two- and four-vessel occlusion, photochemical stroke and haemorrhage models [14]. Kasture describes preclinical pharmacology and toxicology as an iterative part of drug discovery, in which the pharmacological and drug-like properties of a new chemical entity are optimized and side effects and toxicity are minimized [15]. Together these works show that the choice of model, the dose and the interpretation of results all need care.

III. PROBLEM DEFINITION AND SCOPE OF THE REVIEW

Several laboratory species are available, but their uses overlap only in part. A student or early researcher planning a pharmacological study needs a single concise guide that says which animal suits which type of experiment and what it needs in terms of care. This review addresses that gap.

Aim: to review the animals used in preclinical trials. Objectives: (i) to explain why preclinical studies matter; (ii) to describe the mouse, rat, guinea pig, rabbit, hamster and frog, with their biology and experimental uses; (iii) to summarize their care, handling and ethical requirements; and (iv) to discuss limitations and alternatives. Method: this is a narrative review. The animal-specific data were taken from a B. Pharm practice-school project and checked against standard references where possible. No systematic database search was done and no new experimental data are reported.

IV. RESULTS AND DISCUSSION

4.1 Biology and husbandry of the selected animals

Table 1 sets out basic biological data and husbandry points for the six animals. Mice and rats belong to the family Muridae, the guinea pig to Caviidae, the rabbit to Leporidae, the hamster to Cricetidae and the frog to Ranidae. Size differs widely, from about 25 g for an adult mouse to more than 700 g for a guinea pig, and this affects how much test substance is needed and how animals are housed and restrained.

Table 1. Biological data and husbandry points

Animal	Family	Adult weight	Gestation	Housing and husbandry points from the project
Mouse	Muridae	25–28 g	19–21 days	Room at 20–24 °C, humidity 45–65%, light–dark cycle, low noise; colonies usually kept specific-pathogen-free.
Rat	Muridae	200–500 g	21–24 days	Estrous cycle 4–5 days; laboratory pellet diet; clean cages with bedding and nesting material; good ventilation.
Guinea pig	Caviidae	700–1200 g	Not stated	Humidity about 45%; needs dietary vitamin C and hay or grass; handled with support under the hindquarters.



Rabbit	Leporidae	1000–1200 g*	28–35 days	Humidity 40–50%; hay, grass and leafy greens; lifespan 7–10 years; lift with support under thorax and hindquarters.
Hamster	Cricetidae	85–150 g	15–18 days	Diet at least 16% protein and 5% fat; avoid handling for 12–24 h after arrival; cages with bedding and enrichment.
Frog	Ranidae	Not given	—	Room at 18–25 °C, humidity 70–100%; live insects as food; handled with wet gloves or a damp net.

*Weight as given in the source project; typical adult rabbit weights are higher, so verify against a standard reference before use. Dashes mean no suitable value was reported.

4.2 Experimental uses and advantages

Table 2 lists the uses and advantages described for each species. The uses are not interchangeable. The best model is the one that is scientifically justified for the question and gives the most relevant information at the lowest cost to the animal.

Table 2. Experimental uses and advantages of each animal

Animal	Experimental uses listed in the project	Advantages listed in the project
Mouse	Toxicology; teratogenicity; bioassays; analgesic and anticonvulsant screening; chemotherapeutic screening; CNS drug action; genetics and cancer research.	Small, easy to handle, inexpensive, needs little drug.
Rat	Analgesic and anticonvulsant studies; hormone bioassays (insulin, oxytocin, vasopressin); estrous cycle, mating and lactation; psychopharmacology; muscle grip strength; anti-ulcer and anti-inflammatory screening.	Small; no vomiting centre, so oral dosing is possible; gall bladder absent; small drug quantity.
Guinea pig	Bronchodilator evaluation; anaphylaxis and immunology; digitalis bioassay; local anaesthetics; histamine and antihistamine studies; hearing studies; tuberculosis and ascorbic acid metabolism; isolated ileum, trachea and heart.	Sensitive to histamine and penicillin; susceptible to tuberculosis and anaphylaxis; long-lived.
Rabbit	Pyrogen testing; bioassay of antidiabetic drugs and sex hormones; capillary permeability; glaucoma; antifertility agents; pharmacokinetics; isolated heart, duodenum, ileum and trachea; local anaesthetics, diuretics, miotics and mydriatics.	Lives 7–10 years; liver atropine esterase lets it tolerate large doses of atropine.
Hamster	Cardiovascular and stroke models; immunomodulation; infectious and metabolic disease; anticancer drugs; respiratory disease; hantavirus pulmonary syndrome and leptospirosis; SARS-CoV-2 infection; antiepileptic, anti-ulcer and antiandrogen tests; alcohol research.	Small, easy to maintain and handle; susceptible to infectious disease.
Frog	Isolated heart and rectus abdominis preparations; CNS drugs; neuromuscular junction; Starling's law of the heart and autonomic control; effects of chemicals on organs.	Amphibian that is safe to handle; small.

4.3 Mouse and rat

The mouse is used in toxicology, teratogenicity, bioassay, screening of analgesic, anticonvulsant and chemotherapeutic agents, CNS pharmacology, genetics and cancer research [1–3]. Its small size, low cost and ease of handling are the main practical reasons, and a small amount of test drug is enough. The rat is used for analgesic, anticonvulsant, anti-



ulcer and anti-inflammatory screening, hormone bioassays, and studies of the estrous cycle, mating and lactation [3,4]. Because the rat has no vomiting centre, oral administration is practical. In both species, strain, age, sex, housing and health status can change the result, so controls and study design need to be sound.

4.4 Guinea pig

The guinea pig is especially useful where the study concerns histamine, bronchial or immune responses: bronchodilator and antihistamine evaluation, anaphylaxis, and isolated ileum, trachea and heart preparations [5,6]. It is also used for local anaesthetics, digitalis bioassay and hearing research. It cannot make its own vitamin C, so the diet must supply it, along with hay or grass for normal digestion. Handling should support the hindquarters to avoid back injury.

4.5 Rabbit

Rabbits are used for pyrogen testing, bioassays, pharmacokinetic studies, ocular pharmacology (glaucoma, miotics, mydriatics) and isolated heart, duodenum, ileum and trachea preparations [7–9]. Their hepatic atropine esterase lets them tolerate large doses of atropine. They must be lifted with support under the thorax and hindquarters, because a twisting or jumping rabbit can injure its spine. Where a validated non-animal pyrogen test is accepted, it should be preferred over the rabbit test.

4.6 Hamster

Hamsters are valuable in infection research. The project lists them as a main model for hantavirus pulmonary syndrome and leptospirosis and as a model for SARS-CoV-2 infection, besides stroke, immunomodulation, metabolic disease and anticancer studies [12]. Work with infectious agents calls for biosafety facilities, trained staff and formal oversight. Newly arrived hamsters should be left unhandled for 12 to 24 hours, and their diet should contain adequate protein and fat.

4.7 Frog

The frog remains a classical preparation for isolated heart and rectus abdominis muscle experiments, studies on Starling's law and autonomic control of the heart, and work on the CNS and neuromuscular junction. Frogs need a humid environment, suitable substrate such as moss, soil or water, and wet gloves or a damp net for handling, because dry handling harms the skin.

4.8 Dose selection across species

Because species differ in size and metabolic rate, a dose that is safe in one animal cannot be copied directly to another or to humans. The project's literature stresses that dose fixation must consider pharmacokinetic and pharmacodynamic differences between species. A common approach is to normalize doses to body surface area and calculate an animal equivalent dose, usually by multiplying the animal dose in mg/kg by the ratio of conversion factors for the two species [13]. Such conversion gives a starting point only. Doses are normally refined by pilot work and by the toxicity and pharmacokinetic data from the study itself, and the smaller the animal, the larger the dose per kilogram that it tends to tolerate.

4.9 Diet, handling, housing and monitoring by species

Good animal care is part of good science, because stress, poor diet or hidden illness add variation that can hide or imitate a drug effect. Table 3 summarizes the practical care points that the project gives for each animal. Several themes repeat. Handling should be calm and trained, with proper support for the body. Housing must give space, hygiene and a controlled environment. Health should be watched regularly, with veterinary help available, and reports should be complete.



Table 3. Diet, handling, housing and monitoring

Animal	Diet	Handling and housing	Health monitoring and reporting
Mouse	Diet varies with research aims but must keep the animal healthy and the results valid.	Trained, skilled handling reduces pain and distress; cages well ventilated, softly lit, light-dark cycle, 20–24 °C.	Colonies kept specific-pathogen-free, checked with sentinel animals; report studies fully.
Rat	Laboratory pellets or blocks formulated for rats; occasional fresh fruit and vegetables.	Hold gently but firmly around the shoulders to prevent struggling or biting; clean cages with bedding and nesting material.	Regular checks for illness or distress; isolate and treat sick rats; report care and procedures accurately.
Guinea pig	Hay or grass for digestion, fresh fruit and a vitamin C source.	Support the neck and hindquarters; spacious clean, dry cages that allow natural behaviour.	Regular observation, a veterinary care plan and prompt treatment.
Rabbit	Clean water, good hay and grass, leafy greens and a few pellets.	Lift with support under thorax and hindquarters; appropriately sized enclosure, controlled temperature and lighting.	Regular observation and data collection to detect problems early; ethics approval before work.
Hamster	Fresh vegetables, hay, small amounts of fruit and dry fruit; adequate protein and fat.	Leave alone 12–24 h after arrival; cages with bedding, nesting material and enrichment, cleaned regularly.	Watch for weight loss, abnormal behaviour or visible symptoms; report all outcomes, positive or negative.
Frog	Live moving insects, worms or snails, depending on species.	Wet gloves or damp net; ventilated, temperature-controlled enclosure with suitable substrate.	Record signs of disease, injury or stress; report objectives, methods, results and welfare data.

4.10 Study design, welfare and the 3Rs

Across all six species the project stresses the same points: appropriate cages with room to move and show natural behaviour, a clean dry environment, controlled temperature, humidity and lighting, regular health monitoring with veterinary support, and anaesthesia and analgesia where procedures cause pain. Reporting should be complete and honest, covering animal care and procedures and including negative as well as positive results. A sound study also has a clear hypothesis, controls, predefined outcomes, a justified sample size and, where possible, randomization and blinding.

The 3Rs frame the ethics. Replacement means using cell-based assays, organoids, organ-on-chip systems or computational models when they can answer the question. Reduction means using the fewest animals that give meaningful results. Refinement means changing procedures, housing and handling to lessen pain and distress. Approval must be obtained before work begins. In India, this means the Institutional Animal Ethics Committee (IAEC) and the rules of the CPCSEA, and the application is made on the prescribed protocol form (Form B), which asks for the establishment's registration, the source of animals, the place of study, the type of research and a plan of study. Ethical requirements written for human participants, such as informed consent and anonymity, do not apply to animal experiments.

4.11 Alternatives to animal use

Animal studies are one tool among several, and the choice between methods should be made at the start of a project. Table 4 compares in-vitro and in-vivo approaches. In-vitro assays, organoids and computer simulations can often answer early screening and mechanism questions, so animals are needed only for the questions that require a whole



organism. The project notes that research into such alternatives is continuing and that advances in science and technology are gradually reducing reliance on animals.

Table 4. Comparison of in-vitro and in-vivo studies

Feature	In-vitro studies	In-vivo (animal) studies
Setting	Isolated cells, tissues or organs in test tubes or dishes.	Whole living organism with interacting organs.
Control	High control over single variables.	Lower control; many biological factors interact.
Cost and complexity	Cheaper and simpler.	More costly and complex, and needs ethical approval.
Best suited to	Initial screening and basic mechanistic research.	Safety, efficacy, pharmacokinetics and side effects in a whole system.
Main limitation	Cannot show whole-body metabolism, immune response or organ interaction.	Species differences limit prediction of human outcomes; welfare concerns.

4.12 A practical guide to choosing a model

A model should be chosen from the question, and not from habit or availability. Table 5 links common types of question to the animals described above, using the reasons the project gives. Before settling on a species, the researcher should ask whether a non-animal method is enough, whether published background data exist for the species and strain, how many animals the design truly needs, and whether the unit has the facilities and trained staff to look after that species properly. Where infectious agents are involved, containment and biosafety come first.

Table 5. Matching the research question to the animal

Type of question	Suggested model	Reason given in the project
Broad toxicology, teratogenicity, CNS screening	Mouse	Small, cheap, easy to handle, needs little drug.
Analgesic, anticonvulsant, anti-ulcer, hormone bioassay	Rat	Established screening model; oral dosing practical.
Bronchodilator, histamine, anaphylaxis	Guinea pig	Sensitive to histamine; susceptible to anaphylaxis.
Pyrogen, ocular or pharmacokinetic study	Rabbit	Long-lived; established in ocular and isolated-tissue work.
Infectious disease, stroke, metabolic disease	Hamster	Susceptible to infection; model for several viral and bacterial diseases.
Isolated heart, neuromuscular junction	Frog	Classical isolated preparations; small and safe to handle.

The ethical process adds its own steps. In India, the protocol sent to the Institutional Animal Ethics Committee asks for the title of the project, the principal investigator and co-investigators with their qualifications and experience, the persons authorized to handle animals, the source of funding, the duration of the study, and a plan of study with introduction, methodology and expected outcomes. The application also records the registration of the establishment, the breeder from whom animals will be obtained, and the place where animals are kept and where experiments will be done. Preparing these details early forces a researcher to justify the species and the number of animals before any work begins.

4.13 Limitations

Species differ from humans in physiology, metabolism and disease biology, and a model reproduces only some features of a disease; this limits how far animal results predict human outcomes. Strain, sex, microbiome, housing, handling and dose add further variation. Animal data should therefore be read together with in-vitro, mechanistic, computational and,



where available, human data. The source project is descriptive and contains no experimental results of its own, so this review offers a synthesis and not new findings. Some numerical values in the source (for example the rabbit weight) should be checked against standard references.

V. CONCLUSION

Looking ahead, the main task is balance. Animal models will remain necessary for questions that need a whole living system, but their use should shrink as validated alternatives improve and as study designs become more efficient. Researchers can contribute by justifying every species and sample size, reporting negative results, and sharing methods clearly so that animals are not used to repeat work that has already been done.

Animals continue to support preclinical research on drug safety, pharmacological activity, disease mechanisms and drug disposition. The mouse, rat, guinea pig, rabbit, hamster and frog each suit particular experimental purposes, and their value depends on the fit between model and question and on the quality of the study. Animal results cannot by themselves guarantee safety or benefit in humans. Ethical approval, humane care and the 3Rs should guide all animal work, and continued validation of in-vitro, organoid and computational methods can reduce dependence on animals while keeping drug development reliable.

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