



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Preclinical Test Methods with Animal Mouse and Rats Biomedical Research: Technical Guideline

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ARTICLE INFO

Keywords:

Methods
Preclinical Testing
Mouse
Rats

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All authors have reviewed and approved the final version of the manuscript.

<https://doi.org/10.32539/bsm.v5i2.232>

ABSTRACT

Trials in pre-clinical trials are very complex multidisciplinary studies. Extrapolating data from animals to humans requires information from many fields of science. From pre-clinical trials, more detailed information and data on efficacy and safety were obtained, especially at doses equivalent to doses in humans, and the presence or absence of cumulative effects and whether these effects could return to normal (reversible) after the administration of the test material was stopped. This test can be used to predict the negative impact on humans if he is exposed to the material for a long time.

1. Introduction

In extrapolating data from animals to humans, many factors must be considered, including the extent or threshold where there is no toxic effect, what is the shape of the dose logarithmic curve and how the manifestation of the toxic effect occurs. The no toxic effect threshold is usually calculated statistically at the 95% confidence level with a 5% probability of error. From this calculation it can be imagined that if a toxic effect occurs only in 0.1% of the tested animals it means that the effect cannot be observed if only 100 animals are used. Likewise, if anomalies can appear spontaneously in control test animals, the anomalies that can arise in animals can be greater and if this data

is directly extrapolated to humans it can be surprising.^{1,2}

The prediction of toxicity in humans based on the toxicity test carried out on tested animals depends on the relationship between the test and humans, the environment and other living things. It is also greatly influenced by heredity, nutrition, general health and the environment.^{3,4}

How to handle and restraint test animals

In conducting research with animals, knowledge and skills are needed about handling test animals so that research can run smoothly according to plan.

Therefore, if you want to do research with test animals, researchers need to pay attention to the things below.⁴

- a. Work calmly, eliminating feelings of fear or tension because if you feel scared or tense we become nervous or hesitant, causing difficulties.
- b. Improper handling of test animals can cause physical injury to researchers, test animals, damage to equipment or cause stress to test animals.
- c. Each step should not be rushed, even if it does not mean it is slow. It is advisable to give any treatment if the test animal has not calmed down.
- d. Test animals should be handled with compassion, gentleness, wholeheartedness and not harshly.

In pre-clinical trials, the most commonly used animals are mice, white or rat, guinea pigs, rabbits, cats and dogs. Pigs and macaques are also sometimes used but not as much as the first mentioned animals. Therefore, the following description will be limited to test animals that are widely used in pre-clinical tests such as mice, white mice, guinea pigs, rabbits, cats and dogs.⁵

a. Mice

1. How to Hold

Generally, mice will try to bite when held. Handling just a few days of softer and gentler mice can be tame. However, we must always be vigilant because mice are still sometimes wild animals. Taking mice from the cage should be done with care because even a little surprise the test animals can be aroused. When taking or entering mice, the cage door should not be fully opened. It must be remembered that mice can jump several meters without having to suffer injury from falling from the jump because of the small body size of mice, the way to hold a mouse is certainly not the same as a mouse. Mice can be raised and kept hanging in the air for a short time by holding the tail at a place between 3-4 cm from the base. This method is only for holding for a short time.⁶ In this way the mice can be placed on the palms of the hands for observation or to move the animal.

2. How to Curb

Mice are test animals that always move when handled or treated, so vigilance is very necessary in handling these test animals.⁷

How to curb and hold a mouse for an action cannot be done by holding the mouse by the tail with the right hand. While still holding the mice, put them in a place with a rough surface, for example, on a cloth or wire mesh at the top of the cage. By slightly tugging at the tail, the mouse will deepen the leg even stronger. In this situation the mice can be grasped by the skin on the neck with the thumb and index finger of the left hand. The little finger can be used to tighten the grip by grabbing and placing the tail of the mouse between the little finger and ring finger of the left hand. This method of holding mice is done when giving the drug with an oral needle or gastric swab, intraperitoneal injection and so on. For intravenous administration or taking blood from the tail vein, the way to hold the mice is with a restraint device.⁸

b. White rat

1. How to hold

White rats or rat, are widely used in pre-clinical trials because in addition to having physiological properties that are closer to humans, they also have a calm nature even though they receive less pleasant treatment.^{9,11}

White rats should not be handled by pulling the tail, as hard tail pulling can cause stress to the animal. Taking rats from their cages and how to pull their tails forcefully can cause injury to test animals. Likewise, if the test animal panics, it can move in circles in the air when our tail is held, and can injure its tail or bite us.¹¹

The white mouse should be held by grasping it by the shoulder area with the thumb on the front and the palm on the back of the rat. Meanwhile, the other four fingers curl on the stomach. The position of the fingers is thus strengthened by placing the thumb on the neck under the chin. If the mouse is big enough, the way to hold it with the right hand needs to be assisted with the left hand to hold the mouse by its pelvis. With this position the rats calmed down.¹⁰

In this tense state, usually the test animal will try to escape. By swinging the animal in the grip back and forth, the test animal calmed down.¹²

The hand strength in holding the test animal must be made so that it is not too strong which could interfere with breathing but also strong enough to overcome the movement of the test animal that wants to escape. Too weak a grip can result in test animals releasing or injuring researchers. This method of handling rats is recommended to take the animal from its cage and put it back in the cage after certain treatment and so on. Never move the white mouse directly from the handle to another person as the test animal could injure us when it got out of the handle.¹⁴

The pregnant mice were also held this way with a loose added grip on the pelvic area. Transfer of the mother who is breastfeeding is done after the mother away from her children. Get rid of the pups first and then try to hold the mother.¹³

2. How to curb

Before taking any action to white rats such as giving the drug by mouth with an oral needle or gastric swab, intraperitoneal injection, giving signs to the back, stomach, ears or other measures, the test animal must be handled in a proper manner.^{15,16}

For the purposes of administering the drug by gastric swab or intraperitoneal injection, the most appropriate way to hold mice is to take advantage of the loose skin on the neck so that it allows us to pinch and hold it with both fingers. The test animal should be held in the left hand so that the right hand can perform the necessary work such as inserting the drug with an oral needle from the syringe into the stomach.^{17,18}

The first time the mouse is taken with the right hand and the head position is held with the thumb on the chin area so that the mouse's head cannot move to bite. Using the looseness of the skin on the nape of the neck, the skin of this area is clamped with the thumb and index finger. The remaining fingers are used to hold the skin on the back. In this position, intraperitoneal injections can be carried out, topical administration of drugs to the eyes and ears, sampling for examination of vaginal swab preparations and so on.¹⁹

For the purpose of administering the drug orally by means of an oral needle or gastric swab, the rat is held by the skin of its nape with the thumb and middle finger of the left hand. The position of the mice was then made to look more upward with the help of pinching the index finger on the back of the scalp in such a way that the head looked up more, ready to be given oral material.^{21,22}

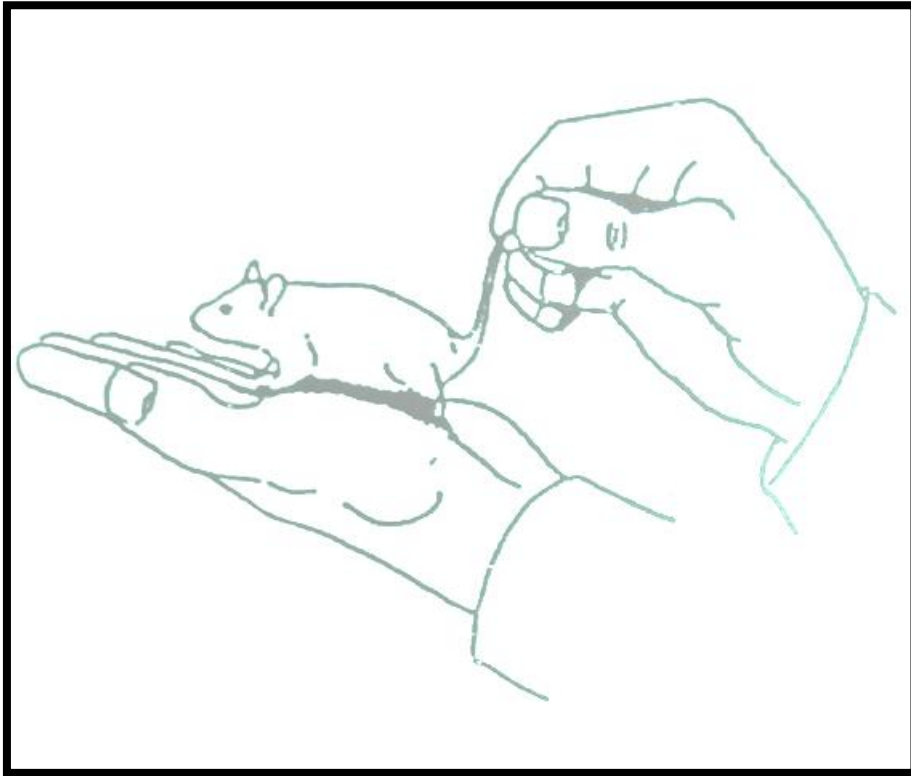


Figure 1. How to hold a mouse.

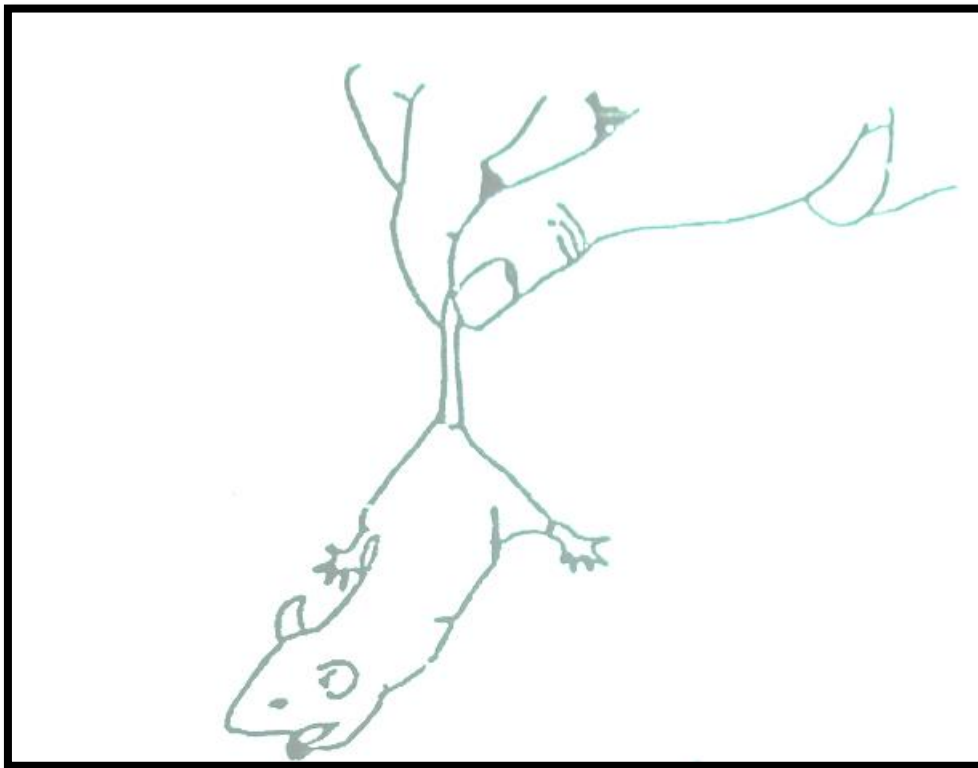


Figure 2. How to hold a mouse in the first step. Hold the tail of the mouse with the thumb and index finger of the right hand.

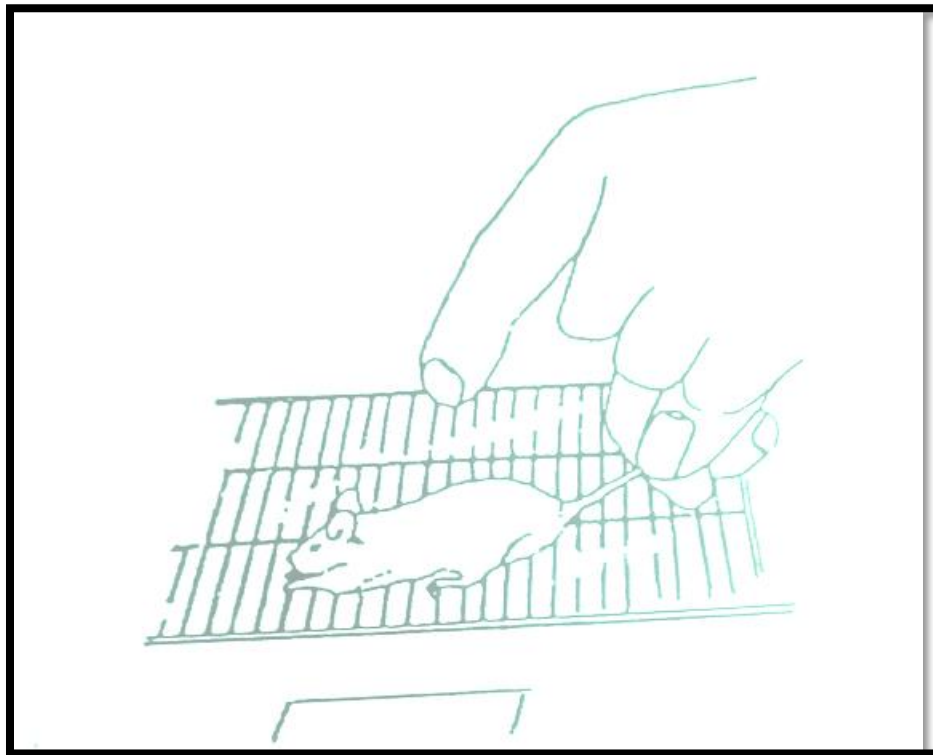


Figure 3 How to hold mice in the second step. While keeping the right hand holding the tail, the mouse is placed on top a rough surface (woven wire) on all four legs.

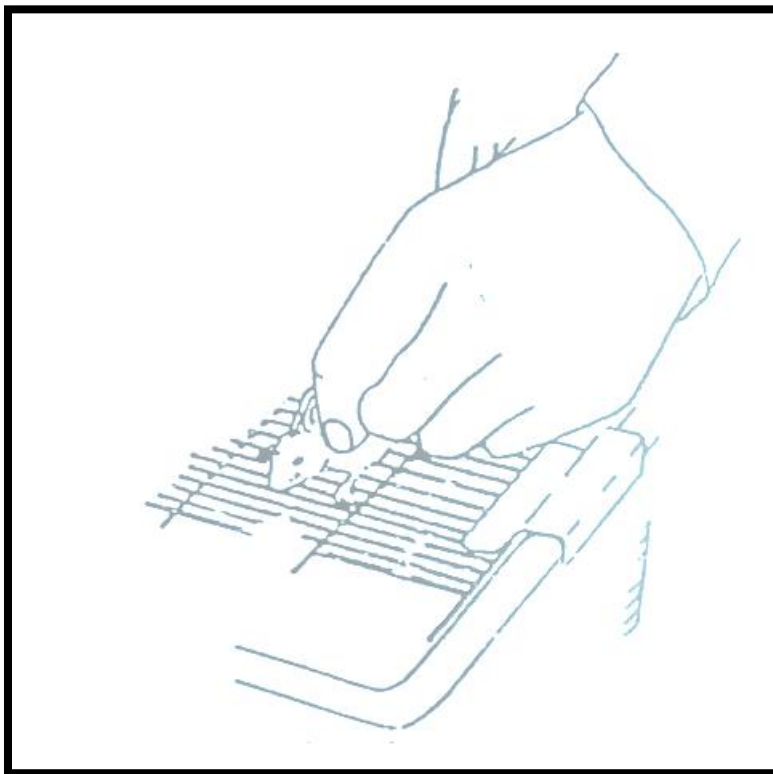


Figure 4 How to hold a mouse in step three. While still holding the tail with your right hand, skin, skin on the tengkung is held with the thumb and index finger of the left hand.



Figure 5 How to hold mice with a tool for drawing blood or giving drugs through the tail.

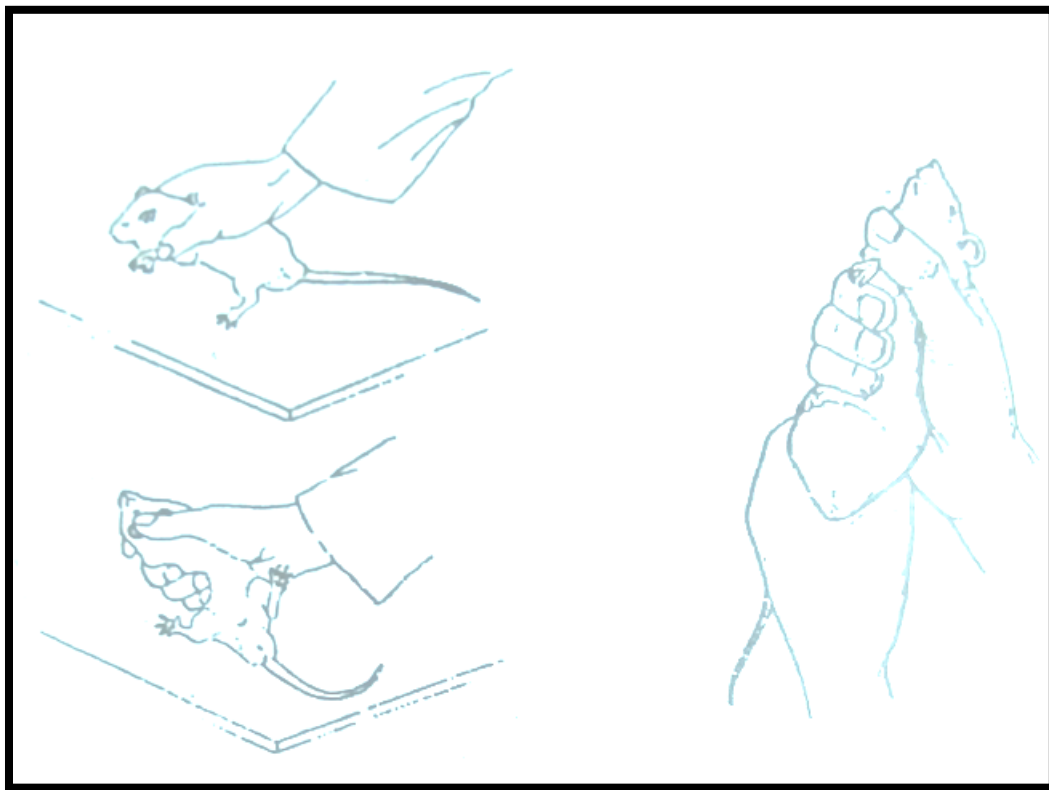


Figure 6. How to hold a white mouse.

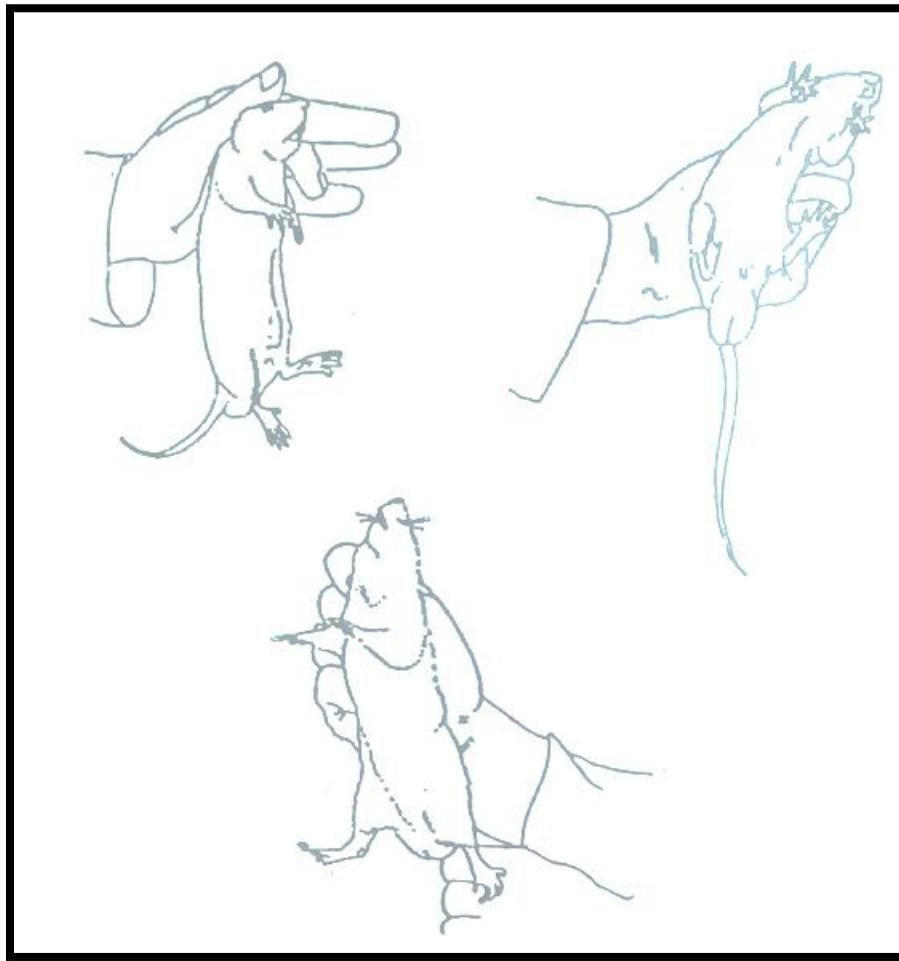


Figure 7. How to hold a white mouse.

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