



**Raj Biotech (India) Pvt. Ltd.**

**Discovery Services  
(Analgesic, Muscle Relaxant  
Efficacy Models)**

# Analgesic Efficacy Models



Raj Biotech conducts pharmacology proof-of-concept studies used in early stage preclinical development to ascertain the efficacy of test compounds.

The various analgesic models of interest for screening test compounds are as follows :

- Hot plate model (Central analgesics)
- Tail Immersion model (Central analgesics)
- Acetic acid writhing model (Peripheral analgesic)
- Formalin model (Chronic pain and central analgesics)

# Analgesic Activity

The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics, whereas peripheral analgesics of the acetylsalicylic acid or phenyl-acetic acid type do not generally affect these responses.

The hot plate test has been used by many investigators and has been found to be suitable for evaluation of centrally but not of peripherally acting analgesics. Mice as well as rats have been used.

**Positive control:** morphine hydrochloride, 30 mg/kg s.c. codeine hydrochloride, 30 mg/kg s.c. pethidine hydrochloride and 400 mg/kg s.c. phenazone



HOT PLATE  
MODEL  
(Rats & Mice)

# Analgesic Activity

## Formalin Model (Rat)

The formalin test in rats has been proposed as a chronic pain model which is sensitive to centrally active analgesic agents. Formalin test may allow a dissociation between inflammatory and non-inflammatory pain, a rough classification of analgesics according to their site and their mechanism of action. Formalin-test is a model of chronic pain whereas most other methods measure only the effect on acute pain.

Male Wistar rats are used where 10% formalin is injected into the dorsal portion of the front paw. The test drug is administered simultaneously either sc. or orally. Each individual rat is placed into a clear plastic cage for observation. Readings are taken at 30 and 60 min and scored according to a pain scale. Pain responses are indicated by elevation or favoring of the paw or excessive licking and biting of the paw. Analgesic response or protection is indicated if both paws are resting on the floor with no obvious favoring of the injected paw.

**Positive controls:** 1.7 mg/kg morphine s.c. & 15 mg/kg s.c. pethidine

# Analgesic Activity

## Tail Immersion Model (Female Wistar Rat)

The model is selective for morphine-like compounds. The procedure is based on the observation that morphine-like drugs are selectively capable of prolonging the reaction time of the typical tail-withdrawal reflex in rats induced by immersing the end of the tail in warm water of 55 °C.

Rats are placed in special restraining cages leaving the tail hanging out freely. The animals are allowed to adapt to the cages for 30 min before testing. The lower 5 cm portion of the tail is marked. This part of the tail is immersed in a cup of freshly filled water of exactly 55 °C. Within a few seconds the rat reacts by withdrawing the tail. The reaction time is recorded in 0.5 s units by a stopwatch. After each determination the tail is carefully dried. The reaction time is determined before and periodically after either oral or subcutaneous administration of the test substance, e.g., after 0.5, 1, 2, 3, 4 and 6 h. The cut off time of the immersion is 15 s. The withdrawal time of untreated animals is between 1 and 5.5 s. A withdrawal time of more than 6 s therefore is regarded as a positive response.

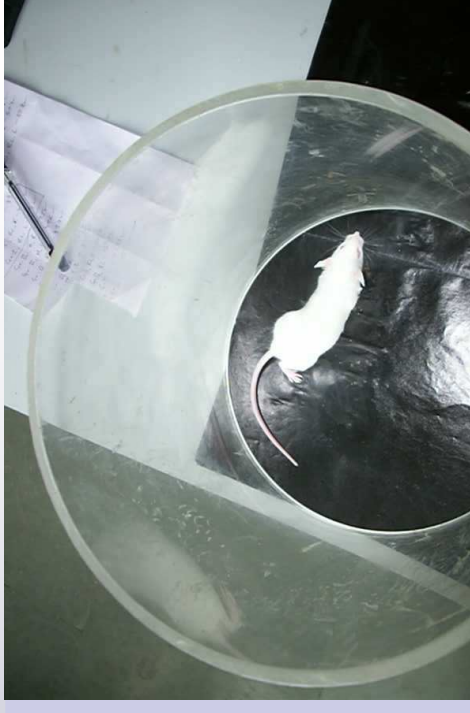
**Positive controls:** Morphine and Methadone



# Analgesic Activity

## Acetic Acid Model (Mouse)

Model is induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behavior which is called writhing.



The test is suitable to detect analgesic activity. Although some psychoactive agents also show activity. An irritating agent such as phenylquinone or acetic acid is injected intraperitoneally to mice and the stretching reaction is evaluated. The reaction is not specific for the irritant

In this test both central and peripheral analgesics are detected. The test, therefore, has been used by many investigators and can be recommended as a simple screening method. However, it has to be mentioned that other drugs such as clonidine and haloperidol also show a pronounced activity in this test. Because of the lack of specificity, caution is required in interpreting the results, until other tests have been performed. Nevertheless, a good relationship exists between the potencies of analgesics in writhing assays and their clinical potencies.

**Positive control:** Indomethacin, 30 mg/kg p.o. Acetylsalicylic acid, 40 mg/kg p.o. Amidopyrine & 80 mg/kg p.o. Phenacetin



# Muscle Relaxant Efficacy

The various muscle relaxant models of interest for screening test compounds are as follows :

- Rota rod Model (Mice)
- Traction Model (Mice)
- Inclined Plane Model (Mice)
- Horizontal Wire Model (Mice)
- Grip Strength (Mice)
- Chimney Test (Mice)

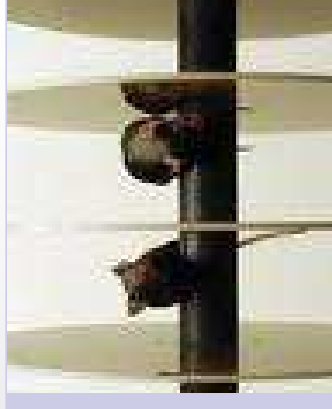
# Muscle Relaxant Activity

## Rota Rod Model (Mice Model)

The test is used to evaluate the activity of drugs interfering with motor coordination mice were placed on a horizontal wooden rod (diameter 3 cm) that was 23 cm above the bench and rotating at a rate of 15 rev/min. After a preliminary run of naive animals, those that did not remain on the rod for 2 consecutive minutes within a period of 5 min were discarded.

Immediately before giving test samples the mice were tested once more and those that did not stay on the rod for 1 min were discarded. The mice were placed on the rotarod at 0, 15, 30, 45, 60 and 75 min after injection. The time taken for each mouse to fall off the rotarod was recorded as the endurance time. If a mouse remained on the rod for more than 10 min, then its endurance time was recorded as 10 min. Total endurance time was calculated during 75 min after test sample administration.

**Positive Controls:** Many central depressive drugs are active in this test. Benzodiazepines, such as diazepam and flurazepam can be used. The activity of neuroleptics, such as chlorpromazine or haloperidol can also be used. This test does not really differentiate between anxiolytics and neuroleptics but can evaluate the muscle relaxant potency in a series of compounds such as the benzodiazepines. Moreover, the test has been used in toxicology for testing neurotoxicity.



# Muscle Relaxant Activity

## Traction Model (Male BALB/c mice)

Forepaws of a mouse were placed on a small twisted wire rigidly supported above a bench top. Normal mice grasped the wire with forepaws and when allowed to hang free, placed at least one hind foot on the wire within 5 seconds. Inability to put up at least one hind foot constituted failure to the traction. The test was conducted in groups of ten previously screened animals. Test compounds are administered 60 and 30 minutes before the test.

Positive Control: Diazepam & Carbenoxolone

# Muscle Relaxant Activity

## Inclined Plane Model (Mice Model)

The method has been proven to be a simple assay for muscle relaxant activity. Although the muscle relaxant tests satisfy the criteria of sensitivity and relative potency compared with clinically effective doses, the effects of anxiolytics are not clearly differentiated from neuroleptics and even from neurotoxic compounds.

The plane consists of two rectangular plywood boards connected at one end by a hinge. One board is the base, the other is the movable inclined plane. Two plywood side panels with degrees marked on their surface are fixed on the base. A rubber mat with ridges 0.2 cm in height is fixed to the inclined plane which is set at 65 degrees.

The test compound or the standard are administered to groups of 10 male mice either i.p. or s.c. or orally. 30, 60 & 90 min thereafter, the mice are placed at the upper part of the inclined plane and are given 30 s to hang on or to fall off. The peak time is determined as the time at which a compound produces the maximum performance deficit.

# Muscle Relaxant Activity

## Horizontal Wire Model (Mice Model)

Mice were lifted by their tails and allowed to grasp a horizontally strung wire (20 cm high, 1 mm diameter, 15 cm long) with their forepaws and then released. The trials were executed two times at 30 min after injection. The number of animals from a total of ten per treatment group that did not grasp the wire with the forepaws or actively grasp the wire with at least one hind paw within 3 s was determined. Methocarbamol and dantrolene sodium salt were used as positive controls/reference compounds.

# Muscle Relaxant Activity

## Grip Strength (Mice Model)

The test is being used to assess muscular strength or neuromuscular function in rodents which can be influenced not only by sedative drugs and muscle relaxant compounds but also by toxic agents.

Male or female mice are tested for their normal reactivity. The animals are exposed to a horizontal thin metallic wire suspended about 30 cm into the air which they immediately grasp with the forepaws. The mouse is released to hang on with its forelimbs. Normal animals are able to catch the threat with the hind limbs and to climb up within 5 s. Only animals who fulfill this criterion are included into the experiment.

After oral or subcutaneous administration the animals are tested every 15 min. Animals which are not able to touch the threat with the hind limbs within 5 s or fall off from the threat are considered to be impaired. The test is continued for 2 h. The animals are observed for their behavior in the cages. Only if their behavior and their motility in the cage seem to be normal the disturbance of the grasping reflex can be considered as caused by central relaxation.

Only simultaneous observation of the animals under normal conditions gives the possibility to distinguish between central relaxation and toxic effects on neuromuscular function



# Muscle Relaxant Activity

## Chimney Test (Mice Model)

This is a simple test for tranquilizing and muscle relaxant activity. The chimney test can be used as an additional test with other tests determining muscle relaxant activity.

Pyrex-glass cylinders 30 cm long are required. Each tube has a mark 20 cm from its base. Initially, the tube is held in a horizontal position. At the end of the tube, near the mark, a male mouse is introduced with the head forward. When the mouse reaches the other end of the tube, toward which it is pushed if necessary with a rod, the tube is moved to a vertical position. Immediately, the mouse tries to climb backwards and performs coordinated movements similar to an alpinist to pass a chimney in the mountains. This gave the name for the test.

The time required by the mouse to climb backwards out at the top of the cylinder is noted and calculated for muscle relaxant activity.





Thank you.

Please visit us at [www.rajbiotech.com](http://www.rajbiotech.com)