Protocol for bio-rodenticide lab experiments

This protocol describes all procedures to conduct experiments with caged rats for the purpose of testing new biological rodenticides. The purpose is to firstly determine its effectiveness. More specific testing can be done to determine palatability and lethal dose, this requires a different experimental set-up.

In this protocol the purpose of the experiments is to analyse its effectiveness. This means that through trials it is determined if a botanical treatment sickens or kills animals, and if so, how many. With this data the sickness and mortality rates can be determined.

This protocol is divided in four parts.

1. Key words and coding
2. Before the start of the experiment
3. During the experiment
4. After the experiment

2 annexes are part of this protocol:

- Annex 1: data recording template in excel
- Annex 2: production protocol for making a powder/paste out of a plant part

1. Key words and coding

Body Weight (g):
I.Wt. = Initial (market) weight of the animal
T.Wt. = Weight of the animal when treatment started (end of acclimatization)
96hrs .Wt. = Weight of the animal at the end (final) of the treatment
T-96hr = weight difference between start and end of the experiment
96hr-F wt. =weight difference between end of the experiment and final weight (post experiment)

Response of animals to treatment:
A = Animal in 'Active' state
S = Animal in 'Sick' state
M = Animal 'Mortality'

Diet/treatment in take (g):
4 = all treatment/feed consumed by the animal
3 = ~75% of the treatment/feed consumed by the animal
2 = ~50% of the treatment/feed consumed by the animal
1= ~25% of the treatment/feed consumed by the animal
0 = no treatment/feed consumed by the animal

Post-mortem observation

- ***Coding for Liver and Heart affected:*** **1** = Affected by the treatment; **0** = Unaffected by the treatment.

- **Coding for Stomach and Intestine:** **0** = Empty; **1** = Partially with food (less than the control gut); **2** = Full food (like the control gut).
- Do **RBC Count** for controls as well and compare with treated animals.

Indicators of affected animals:
Normal = Similar to the control (not affected by the treatment)
Little Black colour = Partially **Black colour** (discoloured) organ compared to the colour of the control organ
Black colour = **Fully Black** coloured (discoloured) organ compared to the colour of the control organ
Full of clotted blood = Heart with **Black colour** clotted blood (semisolid blood) compared the control heart
Full of food = Stomach and Intestine with full food like the control rats
Few food = Stomach and Intestine with small food, less than the amount of food in the gut of the control rats
Empty = Stomach and Intestine with no food

2. Before the start of the experiment

Preparation of the lab and cages:

- Make sure you have standard rat cages enough at least to complete one experiment. The number of cages is based on the number of individual rats that will be treated.
- Obtain feeding pots (it is advised to have a pot to limit spill-over)

Figure 1 experiment set-up for rodent treatment trials in MU lab

Capturing and caging rats:

- Field capture test animals. This can be done with Sherman traps for instance. The species of rats needed determine where and when to place traps. E.g. day or night, crop fields or urban areas.
- Consider 10 test animals per one treatment and the same number of animals for control.
- Make sure you have the data entry table ready (both on paper and on excel file). Find this in annex 1.
- Note the information of the different animals, capture the following information:
  - ✪ Sex of the animal
  - ✪ Initial weight of the animals when they are put in a cage
  - ✪ Date of capture
  - ✪ Use the data table to record the information (it is advised to note down this brief data on the cage of each animal)

Acclimatization management:

- Acclimatize the caged animals for 4-5 days (or for few more days if the animals still display anxiety and very low food/water intake) before the start of the test.
  - ✪ Note that, in acclimatization the assumption is that the animals will display anxiety and low food/water intake for the first few days of caging until they get used to the caging conditions. They are considered as 'comfortable with the caging conditions and ready for the test' when they stop displaying signs of anxiety and demonstrate constant food/water intake for at least 2 consecutive days.
  - ✪ Others determine the end of acclimatization period based on changes in the animals' body weight. In this case you need to record the weight of the animals every 24 hrs for 2-4 consecutive days and map the body weight measurements of each animal against the days (i.e., weight (y-axis) vs days (x-axis). Then, animals exhibiting constant body weight for 2-4 consecutive days are considered as 'comfortable with the caging conditions and ready for the test', signalling the end of the acclimatization period. Since we have observed considerable amount of individuals of different species in acclimatization, and because weighing the animals adds to their anxiety; Mekelle University does not use this in the lab. This is up to each lab to decide.
- Feed the animals with standard rat pellet feed (or similar diet) *ad libitum.*
- Provide water also *ad libitum.*
- Clean the cages, the feeding plates (pots) and the lab every morning (at least once per day)
- The control animals will also be treated in the same manner
- IMPORTANT: food and water will be withheld overnight (~12 hrs) before the start of the treatment.
- Ensure a mixed number of males and females in every experiment. Also try to have a similar weight-range of the animals over different experiments. In order not to have only lightweight animals in one experiment, and heavyweight animals in the other. This grouping of animals into different experiment groups and control groups can already be done in the acclimatization period.

3. During the experiment (runs for 4 days)

The table below gives an example of an experiment, considering different treatments and concentrations, various rat species and the number of cages required. This serves as an example and will change depending on the experiment specifics.

Table 1 example schedule for an experiment

Rodent species	**July**	**August**	**September**	**October**
	Treatment A (100% concentration, without linseed)	Treatment A (75% concentration, with 25% linseed)	Treatment B (100% concentration, without linseed)	Treatment B (75% concentration, with 25% linseed)
Arvicanthis* + *Rattus	A total of 10 animals (1 indv/cage)	A total of 10 animals (1 indv/cage)	A total of 10 animals (1 indv/cage)	A total of 10 animals (1 indv/cage)
Steno* + *Masto	A total of 10 animals (1 indv/cage)	A total of 10 animals (1 indv/cage)	A total of 10 animals (1 indv/cage)	A total of 10 animals (1 indv/cage)
Control	A total of 10 animals (1 indv/cage)	A total of 10 animals (1 indv/cage)	A total of 10 animals (1 indv/cage)	A total of 10 animals (1 indv/cage)
# Cages required	**30**	**30**	**30**	**30**

Treatment preparation

- Prepare the treatments (in this case the botanical mix) – advisable to do this the day before the first treatment day to quick start the treatment the next day.
    - The method for processing a botanical into a powder/paste is given in a separate document, see annex 2. The process depends largely on the type of plant part, e.g. bulb, leaf, stem, seed.
    - Different experiments have different concentrations of treatment, the botanical that is used as poison is complemented with a bait. As bait linseed-powder is best to be used.
    - For one experiment, multiple concentrations of treatment should be tested to determine the optimal concentration. One could start with a pure treatment of 100% botanical, then 75% botanical, and 50% botanical.
    - It is advisable to prepare enough treatment to run the entire experiment for x number of animals for x amount of days. This means to prepare a 100%, 75%, or 50% treatment in this case, a mix of botanical powder with linseed powder. The prepared treatments should be kept in an air-tight jar or box and kept in a fridge.
    - For each animal the total amount of daily treatment is based on body weight. Daily food supply should be 10% of body weight. In order to ensure the animals have enough treatment/food available, this is set at 12 grams per 24h period. This is provide in two times 6 gram at every 12hr interval.

IMPORTANT: Weighing moments

- There are 4 moments of weighing each individual animal (in grams) these are:
    - I. Wt: Initial weight when animals are transferred to a cage
    - T. Wt: Weight at the start of the treatment (end of acclimatization)

- 96hrs Wt: weight at the moment when 96hrs (4 days) treatment is completed
- F. Wt: post-mortem weight, taken as soon as possible upon death

Running the experiment:

- Clean the cages prior to the start of the experiment, the feeding pots and the lab to avoid (direct/cross) contamination.
- Ensure equivalent data recording for treatment and control animals for the entire duration of the experiment.
- Make sure you have the data entry table ready (both on paper in the lab and as an excel file)
- Data recording:
    - Record body weight at the start of the experiment of each animal as 'treatment weight'
- IMPORTANT: Data recording
    - Ensure same recording for treatment and control animals at all stages of the experiment
    - Record data every 12 hrs
    - Data recording should be done consistently, at the same time every day using the same parameters
    - Be keen to observe the rats and indicate possible behavioural changes
- Provision of treatment/feed: we suggest dividing the daily treatment mix amount for an animal into two and providing it twice a day (e.g. morning and evening). This will help reduce the quantity of spill-over at a time – the more treatment mix provided to an animal at once, the higher the quantity of spill over.
    - Provide new treatment/feed every 12 hours
    - Provide 10 gram every 12 hours
    - Record treatment/feed intake and spill-over
    - Empty and clean the pot and cage, refill with fresh treatment/feed in exact same amount of 6 gram.
    - Repeat this process every 12 hours
    - Record
- Data recording: see annex 1
    - Treatment/feed intake and spill-over. The amount of daily spill-over and left-over treatment/feed is weighed/estimated every 12 hours. So at every occasion of providing new treatment/feed. In this way the intake of treatment/feed is determined.
    - We have chosen to estimate the treatment/feed intake indicating this amount with numbers 0-4. 4 indicating all treatment/feed is consumed by the animal, 0 indicating no treatment/feed is consumed by the animal. See chapter 1 for detailed description.
- Data recoding during experiment: see annex 1
    - Record behaviour of the rats in the designated column, this can be done in narrative, using key words. Note the date.
    - Record state of the animal, active, sick or dead (mortality).
- Data recording at the end of the experiment: see annex 1
    - Take blood sample from the end of the tail (tail tip) for RBC count when an animal dies. Otherwise this will be done immediately post-mortem.

- IMPORTANT: If you encounter a dead animal during the treatment period, quickly wrap and label it in an individual zip-lock bag and refrigerate it for later necropsy. Remember to

record all your observations about the animal along with weight, sex and treatment type using the data table. Quickly take blood sample from the tail tip for RBC count before doing the necropsy procedure. See below (chapter 4) for more on necropsy (post-mortem) analysis.

- Keep the control animals on a clean rat feed diet and water *ad libitum* for 4 days and record the same data as the treatment animals, including RBC count.
- Also record exactly the same data on treatment/feed spill-over, left-over and resulting intake.

4. Post-experiment (runs for 4 days)

- Surviving rodents at the end of the treatment period will be maintained **on a clean rat feed diet and water** *ad libitum* for 4 days additionally. This means after the experiment period of 4 days is finished, the rats that are alive are kept in their cages receiving common rat feed, so no poisonous treatment!
    - Do this for 4 days directly following the experiment period.
    - The feeding process will be exactly similar, so new feed every 12 hours, cleaning of pots/cages.
    - Recording for the post-experiment period is less compared to during the experiment. It is important to keep recording behaviour of the animals and to note the activity (active, sick or mortality). Food intake will not be measured. The other recording will be done post-mortem.
- Then, sacrificed and necropsied for examination of signs of treatment toxicity. Any mortality/sickness that occurred during the post-treatment observation period will be recorded and recognized as part of the test procedure.
- During necropsy, animals will be weighed, sexed, and examined for signs of toxicity. Record all your observations about the animals, including recoveries from the treatment effect; quickly take blood samples for RBC count, and do the necropsy for post-mortem analysis focusing on liver and heart colourations (compared with the liver and heart colourations of the control animals which are sacrificed for this purposes). Please also observe the stomach and intestine for the amount of food content and signs of diarrhoea, compared with those of the control animals.
- See the data table for more on post-mortem data recording.