TOPIC- REPRODUCTIVE TOXICOLOGY STUDY

CONTENTS

- Introduction
- OECD guideline for testing of chemical on reproductive toxicity
- Male Fertility
- Female Fertility

WHAT IS REPRODUCTIVE TOXICOLOGY

- Reproductive toxicity refers to structural and functional alterations that affect reproductive system in sexually mature males and females.

- Reproductive toxicity includes effects on males fertility and female fertility and Lactation.

OECD GUIDELINES FOR TESTING OF CHEMICALS ON REPRODUCTIVE TOXICOLOGY

- **Principle of the test:**

- The test chemical is administered in graduated doses to several groups of males and females.
- Males should be dosed for a minimum of four weeks and up to and including the day before scheduled kill.

- Pre-mating dosing period in males, fertility may not be a particular sensitive indicator of testicular toxicity.

- Therefore, a detailed histological examination of the testes is essential.
- Histopathology of the male gonads, is considered sufficient to enable detection of the majority of effects on male fertility and spermatogenesis.
- Females should be dosed throughout the study.
- This includes mating, the duration of the pregenancy and including the day before scheduled kill.
- Duration of the study, following acclimatisation and pre-dosing oestrous cycle evaluation, is dependent on the female performance and is approximately 63 day.

DESCRIPTION OF THE METHOD

- Selection of Animal species

- Guideline is designed for use with rat. The rat was the only species used. The test animals should be characterized as to species, strain,sex,weight and age.

Weight variation of the animals used should be minimal and not exceed 20 % of the mean weight of each sex.

Animals from the same strain and source are used in both studies.

HOUSING AND FEEDING

- The temperature in the experimental animal room should be 22 C (+-3). Relative humidity should be at least 30%.
- Lighting should be artificial, the photoperiod being 12 hours light, 12 hours dark
- For feeding, laboratory diets used with an unlimited supply of drinking water.
- No more than five animals should be housed per cage.
- Pregnant females should be caged individually and provided with nesting materials.
- Lactating females will be caged individually with their Offspring.

PREPARATION OF THE ANIMALS

- Healthy young adult animals are randomly assigned to the control and treatment groups.
- Cages should be arranged in such a way that possible effects due to cage placement are minimized.
- The animals are uniquely identified and kept in their cages for at least five days prior to the start of the study to allow for acclimatization to the laboratory conditions
- **Preparation of doses**

- the test chemical is dissolved or suspended in a suitable vehicle.

PROCEDURE

- **Number and sex of animals:**

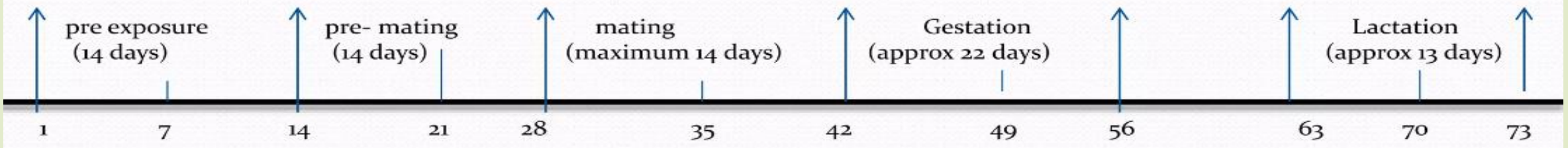
- It is recommended that each group be started with at least 10 males and 12-13 females.

- **Dosage**
- Generally, at least three test groups and a control group should be used.
- If a vehicle is used in administering the test chemical, the control group should receive the vehicle in the highest volume used.
- Dose level should be selected taking into account any existing toxicity and (toxico) kinetic data available.

ADMINISTERATION OF DOSES

- The animals are dosed with the test chemical daily for 7 days a week.
- The volume should not exceed 1 ml/100g body weight, except in the case of aqueous solutions where 2 ml/100g body weight may be used.
- For test chemical administered via the diet or drinking water, it is important to ensure that the quantities of the test chemical involved do not interfere with normal nutrition or water balance.

Experimental schedule



start of study

Necropsy
After dosing
period of
at least 4 weeks

pre exposure
evaluation of
oestrous cycle
Followed by daily
Monitoring of vaginal
Smear from the beginning
Of tratment until
Evidence of mating

EXPERIMENTAL SHEDULE

- Dosing of both sexes should begin at least 2 weeks prior to mating, females have been screened for normal oestrous cycles.
- Dosing is continued in both sexes during the mating period.
- Males should further be dosed after the mating period at least until the minimum total dosing period of 28 days has been completed.

- They are then killed, or, alternatively, are retained and continued to be dosed for the possible conduction of a second mating if considered appropriate

- Daily dosing of the parenteral females should continue throughout pregnancy.

- **MATING PROCEDURE:**
- Normally, 1:1 (one male to one female) mating's should be used in this study
- Each morning the females should be examined for the presence of sperm or a vaginal plug.

IN LIFE OBSERVATIONS

- **Clinical Observation**

- Throughout the test period, general clinical observations should be made at least once a day, and more frequently when signs of toxicity are observed.

- They should be made preferably at the same time(s) each day. All signs of toxicity, including mortality, should be recorded.

- These records should include time of onset, degree and duration of toxicity signs.

BODY WEIGHT AND FOOD/WATER CONSUMPTION

- Males and females should be weighed on the first day of dosing, at least weekly thereafter, and at termination.
- During pregnancy, females should be weighed on days 0,7,14& 20.

- **Oestrous cycles**
- Oestrous cycles should be monitored before treatments starts to select for the study females with regular cycle.
- Vaginal smears should also be monitored daily from the beginning of the treatment period until evidence of mating.
- If there is concern about acute stress effects that could alter oestrous cycles.

OFFSPRING PARAMETERS

- The duration of gestation should be recorded and is calculated from day 0 pregnancy.
- Any abnormal behaviour of the offspring should be recorded.
- **Clinical biochemistry**
- Blood samples from a defined site are taken.
- Plasma samples specifically for hormone determination should be obtained at a comparable time of the day.
- The numerical value obtained when analysing hormone concentration.

PATHOLOGY

- **Gross Necropsy**
- At the time of sacrifice or death during the study, the adult animals should be examined macroscopically for any abnormalities or pathological changes.
- Vaginal smears should be examined in the morning on the day of necropsy to determine the stage of the oestrous cycle and histopathology of ovaries.
- The testes and epididymides of all male adult animals should be weighed.
- **Histopathology**
- Histological examination should be performed on the ovaries, testes and epididymides of the animals of the highest dose group and the control group.

DATA AND REPORTING

- Individual animals data should be provided.
- Additionally, all data should be summarized in tabular form, showing for each test group the number of the animals at the start of the test, the number of animals found dead during the test or killed.
- The time of any death or humane kill, the number of fertile animals, the number of pregnant females.
- The number of animals showing signs of toxicity, a description of the signs of toxicity observed.

EVALUATION OF RESULTS

- The findings of this toxicity study should be evaluated in terms of the observed effects, necropsy and microscopic findings.

- **TEST REPORTS**
- Test chemical:
- Source, limit data for use, if available
- Stability of the test chemical
- Physical appearance, water solubility, and additional relevant physiochemical properties;
- Vehicle

- **Test animals:**
- Species/strain used;
- Number, age and sex of animals;
- Source, housing conditions, diet, etc.;
- Individual weights of animals at the start of the test.

- **Results:**
- Body weight/body weight changes;
- Food consumption, and water consumption if available;
- Toxic response data by sex and dose, including fertility, gestation, and any other signs toxicity;

REPRODUCTIVE TOXICOLOGY STUDIES

- **A) Male fertility**

Method

one rodent species (rat)

3 dose group taken

(each 6 adult males)

Drug treatment by clinical route for 28-72 days

Mixed with female in 1:2 ratio

Female getting pregnant should be examined after 13 days of gestation

All male animals scarified

Weights of testis, epididymis recorded and examined for their histology

Sperms examined for motility and morphology

B) Female Fertility

Drugs administered to both males(28 days) and female (14 days) before mating

Segment I : Fertility and general reproductive Performance study

Segment II : Teratogenicity

Segment III : Perinatal and post-natal study

Perinatal: Fertility and early embryonic development.

Post-natal development (rats) (post natal survival of offspring) growth parameter, vital senses, behavior effect