B. 34. ONE-GENERATION REPRODUCTION TOXICITY TEST

1. METHOD

1.1. Introduction

See General Introduction Part B.

1.2. Definitions

See General Introduction Part B.

1.3. Reference substances

None.

1.4. Principle of the test method

The test substance is administered in graduated doses to several groups of males and females. Males should be dosed during growth and for at least one complete spermatogenic cycle (approximately 56 days in the mouse and 70 days in the rat) in order to elicit any adverse effects on spermatogenesis by the test substance.

Females of the parental (P) generation should be dosed for at least two complete oestrous cycles in order to e adverse effects on oestrus by the test substance. The animals are then mated. The test substance is administered to both sexes during the mating period and thereafter only to females during pregnancy and for the duration of the nursing period.

For administration by inhalation the method will require modification.

1.5. Quality criteria

None.

1.6. Description of the test method

Preparations

Before the test, healthy young adult animals are randomized and assigned to the treated and control groups. The animals are kept under the experimental housing and feeding conditions for at least five days prior to the test.

It is recommended that the test substance be administered in the diet or drinking water. Other routes of administration are also acceptable. All animals should be dosed by the same method during the appropriate experimental period. If a vehicle or other additives are used to facilitate dosing, they should be known not to produce toxic effects.

Dosing should be on a seven-day per week basis.

Experimental animals

Selection of species

The rat or mouse are the preferred species. Healthy animals, not subjected to previous experimental procedures, should be used. Strains with low fecundity should not be used. The test animals should be characterized as to species, strain, sex, weight and/or age.

For an adequate assessment of fertility, both males and females should be studied. All test and control animals should be weaned before dosing begins.

Number and sex

Each treated and control group should contain a sufficient number of animals to yield about 20 pregnant females at or near term.

The objective is to produce enough pregnancies and offspring to assure a meaningful evaluation of the potential of the substance to affect fertility, pregnancy and maternal behaviour in P generation animals and suckling, growth and development of the F_1 offspring from conception to weaning.

Test conditions

Food and water should be provided ad libitum. Near parturition, pregnant females should be caged separately in delivery or maternity cages and may be provided with nesting materials.

Dose levels

At least three treated groups and a control group should be used. If a vehicle is used in administering the test substance, the control group should receive the vehicle in the highest volume used. If a test substance causes reduced dietary intake or utilization, then the use of a paired fed control group may be considered necessary. Ideally, unless limited by the physical/chemical nature or biological effects of the test substance, the highest dose level should induce toxicity but not mortality in the parental (P) animals. The intermediate dose(s) should induce minimal toxic effects attributable to the test substance, and the low dose should not induce any observable adverse effects on the parents or offspring. When administered by gavage or capsule the dosage given to each animal should be based on the individual animal's body weight and adjusted weekly for changes in body weight. For females during pregnancy, dosages may be based on the body weight at day 0 or 6 of the pregnancy, if desired.

Limit test

In the case of substances of low toxicity, if a dose level of at least 1000 mg/kilogram produces no evidence of interference with reproductive performance, studies at other dose levels may not be considered necessary. If a preliminary study at the high-dose level, with definite evidence of maternal toxicity, shows no adverse effects on fertility, studies at other dose levels may not be considered necessary.

Performance of the test

Experimental schedules

Daily dosing of the parental (P) males should begin when they are about five to nine weeks of age, after they have been weaned and acclimatized for at least five days. In rats, dosing is continued for 10 weeks prior to the mating period (for mice, eight weeks). Males should be killed and examined either at the end of the mating period or, alternatively, males may be retained on the test diet for the possible production of a second litter and should be killed and examined at some time before the end of the study. For parental (P) females dosing should begin after at least five days of acclimatization and continue for at least two weeks prior to mating. Daily dosing of the p females should continue throughout the three-week mating period, pregnancy and up to the weaning of the F_1 offspring. Consideration should be given to modification of the dosing schedule based on other available information on the test substance, such as induction of metabolism or bioaccumulation.

Mating procedure

Either 1:1 (one male to one female) or 1:2 (one male to two females) mating may be used in reproduction toxicity studies.

Based on 1:1 mating, one female should be placed with the same male until pregnancy occurs or three weeks have elapsed. Each morning the females should be examined for presence of sperm or vaginal plugs. Day 0 of pregnancy is defined as the day a vaginal plug or sperm is found.

Those pairs that fail to mate should be evaluated to determine the cause of the apparent infertility. This may involve such procedures as providing additional opportunities to mate with other proven sires or dams, microscopic examination of the reproductive organs, and examination of the oestrous cycle or spermatogenesis.

Litter sizes

Animals dosed during the fertility study are allowed to litter normally and rear their progeny to the stage of weaning without standardization of litters.

Where standardization is done, the following procedure is suggested. Between day 1 and day 4 after birth, the size of each litter may be adjusted by eliminating extra pups by selection to yield, as nearly as possible, four males and four females per litter. Whenever the number of male or female pups prevents having four of each sex per litter, partial adjustment (for example, five males and three females) is acceptable. Adjustments are not applicable for litters of less than eight pups.

Observations

Throughout the test period, each animal should be observed at least once daily. Pertinent behavioural changes, signs of difficult or prolonged parturition, and all signs of toxicity, including mortality, should be recorded. During pre-mating and mating periods, food consumption may be measured daily. After parturition and during lactation, food consumption measurements (and water consumption measurements when the test substance is administered in the drinking water) should be made on the same day as the weighing of the litter. P males and females should be weighed on the first day of dosing and weekly thereafter. These observations should be reported individually for each adult animal.

The duration of gestation should be calculated from day 0 of pregnancy. Each litter should be examined as soon as possible after delivery to establish the number and sex of pups, still births, live births and the presence of gross anomalies.

Dead pups and pups sacrificed at day 4 should be preserved and studied for possible defects. Live pups should be counted and litters weighed on the morning after birth and on days 4 and 7 and weekly thereafter until the termination of the study, when animals should be weighed individually. Physical or behavioural abnormalities observed in the dams or offspring should be recorded.

Pathology

Necropsy

At the time of sacrifice or death during the study the animals of the P generation should be examined macroscopically for any structural abnormalities or pathological changes, with special attention being paid to the organs of the reproductive system. Dead or moribund pups should be examined for defects.

Histopathology

The ovaries, uterus, cervix, vagina, testes, epididymes, seminal vesicles, prostate, coagulating gland, pituitary gland and target organ(s) of all P animals should be preserved for microscopic examination. In the event that these organs have not been examined in other multiple-dose studies, they should be microscopically examined in all high-dose and control animals and animals which die during the study where practicable.

Organs showing abnormalities in these animals should then be examined in all other P animals. In these instances, microscopic examination should be made of all tissues showing gross pathological changes. As suggested under mating procedures, reproductive organs of animals suspected of infertility may be subjected to microscopic examination.

2. DATA

Data may be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of fertile males, the number of pregnant females, the types of changes and the percentage of animals displaying each type of change.

When possible, numerical results should be evaluated by an appropriate statistical method. Any generally accepted statistical method may be used.

3. REPORTING

3.1. Test report

The test report shall, if possible, contain the following information:

-species/strain used;

-toxic response data by sex and dose, including fertility, gestation and viability,

-time of death during the study or whether animals survived to time of scheduled sacrifice or to termination of the study,

-table presenting the weights of each litter, the mean: pup weights and the individual weights of the pups at termination,

-toxic or other effects on reproduction, offspring and postnatal growth,

-the day of observation of each abnormal sign and its subsequent course,

-bodyweight data for P animals,

-necropsy findings,

-a detailed description of all microscopic findings,

-statistical treatment of results, where appropriate,

-discussion of the results,

-interpretation of the results.

3.2. Evaluation and interpretation

See General Introduction Part B.

4. REFERENCES

See General Introduction Part B.