the potential of the substance to affect fertility, pregnancy and maternal behaviour and suckling, growth and development of the F_1 offspring from conception to maturity, and the development of their offspring (F_2) to weaning.

Housing and feeding conditions

The temperature in the experimental animal room should be 22°C (± 3°) and the relative humidity 30 to 70 per cent. When the lighting is artificial the sequence should be 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Pregnant females should be caged individually and may be provided with nesting materials.

- Test conditions

Dose levels

At least three treatment 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 utilisation, 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. Ideally, the intermediate dose(s) should induce minimal 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 dose given to each animal should be based on the individual animal's body weight and adjusted weekly. For females during pregnancy the dosage may be based on the daily body weight or the body weight at day 0 or 6 of pregnancy, if desired.

- Performance of the test

Experimental schedules

Daily dosing of the parental (P) males should begin when they are about five to nine weeks old, after they have been weaned and acclimatised for at least five days. In rats, dosing

is continued for ten 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 test diet for the possible production of a second litter and should be killed and examined at some time before the end of the experiment. For parental (P) females, dosing should begin after at least five days of acclimatisation and continue for at least two weeks prior to mating. Daily dosing of the P females should continue throughout the 3-week mating period, pregnancy and up to the weaning of the F_1 offspring. Consideration should be given to modifications in the dosing schedule based on available information on the test substance, such as induction of its metabolism or bioaccumulation.

Dosing of the F_1 animals begins at weaning and ends when they are killed.

Mating procedure

Either 1:1 (one male to one female) or 1:2 (one male to two females) matings may be used in this study.

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 are found.

Taking into account spermeogenesis, the F_1 offspring should not be mated until they are at least 11 weeks of age for mice or 13 weeks of age for rats. For mating the F_1 offspring, one male and one female are randomly selected from each litter for cross-mating with a pup of another litter of the same dose group to produce the F_2 generation. F_1 males and females not selected for mating are killed upon weaning.

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

In certain instances, such as poor reproductive performance in the controls, consideration should be given to the production of two litters per generation.

Litter size

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

If standardisation is done, the following procedure is suggested. On 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. Elimination of runts only is not appropriate. 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. Adjustment of the F_2 litters is conducted in the same manner.

- 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 should be measured weekly. Optionally, during pregnancy, food consumption may be measured daily. After parturition, and during lactation, food consumption measurements should be made on the same day as the weighing of litters. Parental animals (P and F_1) 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, stillbirths, live births and the presence of gross anomalies. Dead pups and pups killed 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 termination of the study, when animals should be weighed individually. Physical or behavioural abnormalities observed in the dams or offspring should be recorded.

- Pathology

All P and F_1 adult animals should be killed when they are no longer necessary for assessment of reproductive effects. F_1 offspring not selected for mating and all F_2 offspring should be killed when weaned.

Gross necropsy

At the time of sacrifice or death during the study all parental animals (P and F_1) 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, epididymides, seminal vesicles, prostate, coagulating gland, pituitary gland and target organ(s) of all P and F_1 animals selected for mating should be preserved for microscopic examination, if necessary. In the event that these organs have not been examined in other mutliple-dose studies, they should be examined microscopically in all high-dose and control P and F_1 animals selected for mating and animals which die during the study, where practicable. Organs showing abnormalities in these animals should be examined in animals from the other dose groups. 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.

3. DATA AND REPORTING

- Treatment of results

Data may be summarised in tabular form, showing for each test group the number of animals at the start of the test, the number of fertile males and 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. A generally accepted statistical method should be used; the statistical methods should be selected as part of the design of the study.

• Evaluation of results

The findings or a reproduction toxicity study should be evaluated in terms of the observed effects, necropsy and microscopic findings. The evaluation will include the relationship between the dose of the test substance and the presence or absence, the incidence and severity, of abnormalities, including fertility, clinical abnormalities, body weight changes, effects on mortality and any other toxic effects. A properly conducted reproduction test should provide a satisfactory estimation of a no-effect level and an understanding of adverse effects on reproduction, parturition, lactation and postnatal growth.

• Test report

The test report should also include the following information:

- species/strain used;
- toxic response data by sex and dose, including fertility, gestation and viability indices;
- time of death during the study or whether animals survived to termination;
- 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, postnatal growth, etc.;
- the day of observation of each abnormal sign and its subsequent course;
- body weight data for P and F_1 animals selected for mating;
- necropsy findings;
- a detailed description of microscopic findings, when performed; and
- statistical treatment of results, where appropriate.

• Interpretation of results

A two-generation reproduction study will provide information on the effects of repeated oral exposure to a substance. The results of the study should be interpreted in conjunction with

the findings of subchronic, teratogenic and other studies. Extrapolation of the results of the study to man is valid to a limited degree, although it can provide useful information on no-effect levels and permissible human exposure.

4. LITERATURE

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