OECD GUIDELINE FOR TESTING OF CHEMICALS

Adopted by the Council on 27th July 1995

Reproduction/Developmental Toxicity Screening Test

INTRODUCTION

1. In London, January 1990, an ad hoc Meeting of Experts on Screening Methods for Reproductive Toxicity discussed and agreed on a protocol for a "Preliminary Reproduction Toxicity Screening Test", that could effectively be utilised in the initial evaluation of existing chemicals (1).

2. This screening Test Guideline, which is an updated version of the protocol agreed at the London meeting, is the outcome of a Nominated Experts Meeting on Reproductive Toxicity Screening Methods, held in Tokyo, October 1992 (2). It is based on experience gained in Member countries from using the original method on existing high production volume chemicals and in exploratory tests with positive control chemicals.

3. This Guideline is designed to generate limited information concerning the effects of a test substance on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition. It is not an alternative to, nor does it replace the existing Test Guidelines 414, 415 and 416.

INITIAL CONSIDERATIONS

4. This Screening Test Guideline can be used to provide initial information on possible effects on reproduction and/or development, either at an early stage of assessing the toxicological properties of chemicals, or on chemicals of concern. It can also be used as part of a set of initial screening tests for existing chemicals for which little or no toxicological information is available, as a dose range finding study for more extensive reproduction/developmental studies, or when otherwise considered relevant.

5. This test does not provide complete information on all aspects of reproduction and development. In particular, it offers only limited means of detecting post-natal manifestations of pre-natal exposure, or effects that may be induced during post-natal exposure. Due (amongst other reasons) to the relatively small numbers of animals in the dose groups, the selectivity of the end points, and the short duration of the study, this method will not provide evidence for definite claims of no effects. Although, as a consequence, negative data do not indicate absolute safety with respect to reproduction and development, this information may provide some reassurance if actual exposures were clearly less than the dose related to the No-Observed-Adverse Effect Level (NOAEL). Moreover, in the absence of data from other reproduction/developmental toxicity tests, positive results are useful for initial hazard assessment and contribute to decisions with respect to the necessity and timing of additional testing.

6. This Guideline assumes oral administration of the test substance. Modifications may be required if other routes of exposure are used.

7. Definitions used are given in Annex 1.

PRINCIPLE OF THE TEST

8. The test substance 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 (this includes a minimum of two weeks prior to mating, during the mating period and, approximately, two weeks post-mating). In view of the limited 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. The combination of a pre-mating dosing period of two weeks and subsequent mating/fertility observations with an overall dosing period of at least four weeks, followed by detailed histopathology of the male gonads, is considered sufficient to enable detection of the majority of effects on male fertility and spermatogenesis.

9. Females should be dosed throughout the study. This includes two weeks prior to mating (with the objective of covering at least two complete oestrous cycles), the variable time to conception, the duration of pregnancy and at least four days after delivery, up to and including the day before scheduled kill.

10. Duration of study, following acclimatisation, is dependent on the female performance and is approximately 54 days, [at least 14 days premating, (up to) 14 days mating, 22 days gestation, 4 days lactation].

11. During the period of administration, the animals are observed closely each day for signs of toxicity. Animals which die or are killed during the test period are necropsied and, at the conclusion of the test, surviving animals are killed and necropsied.

DESCRIPTION OF THE METHOD

Selection of animal species

12. This Test Guideline is designed for use with the rat. If other species are used, appropriate modifications will be necessary. Strains with low fecundity or well-known high incidence of developmental defects should not be used. Healthy virgin animals, not subjected to previous experimental procedures, should be used. The test animals should be characterised as to species, strain, sex, weight and/or age. At the commencement of the study the weight variation of animals used should be minimal and not exceed 20% of the mean weight of each sex.

Housing and feeding conditions

13. The temperature in the experimental animal room should be 22 °C (± 3°). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning, the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. The choice of diet may be influenced by the need to ensure a suitable admixture of a test substance when administered by this method.

14. Animals may be housed individually or be caged in small groups of the same sex; for group caging, no more than five animals should be housed per cage. Mating procedures should be carried

out in cages suitable for the purpose. Pregnant females should be caged individually and provided with nesting materials.

Preparation of the animals

15. 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 acclimatisation to the laboratory conditions.

Preparation of doses

16. It is recommended that the test substance be administered orally unless other routes of administration are considered more appropriate. When the oral route is selected, the test compound is usually administered by gavage; however, alternatively, test compounds may be administered via the diet or drinking water.

17. Where necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that, wherever possible, the use of an aqueous solution/suspension be considered first, followed by consideration of a solution/emulsion in oil (e.g. corn oil) and then by possible solution in other vehicles. For vehicles other than water the toxic characteristics of the vehicle must be known. The stability of the test substance in the vehicle should be determined.

PROCEDURE

Number and sex of animals

18. It is recommended that each group be started with at least 10 animals of each sex. Except in the case of marked toxic effects, it is expected that this will provide at least 8 pregnant females per group which normally is the minimum acceptable number of pregnant females per group. The objective is to produce enough pregnancies and offspring to assure a meaningful evaluation of the potential of the substance to affect fertility, pregnancy, maternal and suckling behaviour, and growth and development of the F_1 offspring from conception to day 4 post-partum.

Dosage

19. Generally, at least three test groups and a control group should be used. Dose levels may be based on information from acute toxicity tests or on results from repeated dose studies. Except for treatment with the test substance, animals in the control group should be handled in an identical manner to the test group subjects. If a vehicle is used in administering the test substance, the control group should receive the vehicle in the highest volume used.

20. Dose levels should be selected taking into account any existing toxicity and (toxico-) kinetic data available for the test compound or related materials. The highest dose level should be chosen with the aim of inducing toxic effects but not death or severe suffering. Thereafter, a descending sequence of dose levels should be selected with a view to demonstrating any dosage related response and no-observed-adverse effects (NOAEL) at the lowest dose level. Two to four fold intervals are frequently optimal for setting the descending dose levels and addition of a fourth test group is often preferable to using very large intervals (e.g. more than a factor of 10) between dosages.

Limit test

21. If an oral study at one dose level of at least 1000 mg/kg body weight/day or, for dietary or drinking water administration, an equivalent percentage in the diet, or drinking water using the procedures described for this study, produces no observable toxic effects and if toxicity would not be expected based upon data from structurally related compounds, then a full study using several dose levels may not be considered necessary. The limit test applies except when human exposure indicates the need for a higher oral dose level to be used. For other types of administration, such as inhalation or dermal application, the physical chemical properties of the test substance often may dictate the maximum attainable concentration.

Administration of doses

22. The animals are dosed with the test substance daily for seven days a week. When the test substance is administered by gavage, this should be done in a single dose to the animals using a stomach tube or a suitable intubation cannula. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. The volume should not exceed 1 ml/100 g body weight, except in the case of aqueous solutions where 2 ml/100 g body weight may be used. Except for irritating or corrosive substances which will normally reveal exacerbated effects with higher concentrations, variability in test volume should be minimised by adjusting the concentration to ensure a constant volume at all dose levels.

23. For substances administered via the diet or drinking water, it is important to ensure that the quantities of the test substance involved do not interfere with normal nutrition or water balance. When the test substance is administered in the diet either a constant dietary concentration (ppm) or a constant dose level in terms of the animals' body weight may be used; the alternative used must be specified. For a substance administered by gavage, the dose should be given at similar times each day, and adjusted at least weekly to maintain a constant dose level in terms of animal body weight.

Experimental schedule

24. Dosing of both sexes should begin at least 2 weeks prior to mating, after they have been acclimatised for at least five days. The study should be scheduled in such a way that mating begins soon after the animals have attained full sexual maturity. This may vary slightly for different strains of rats in different laboratories, e.g. Sprague Dawley rats 10 weeks of age, Wistar rats about 12 weeks of age. Dams with offspring should be killed on day 4 post-partum, or shortly thereafter. The day of birth (viz. when parturition is complete) is defined as day 0 post-partum. Females showing no-evidence of copulation are killed 24-26 days after the last day of the mating period. 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.

25. Daily dosing of the parental females should continue throughout pregnancy and at least up to, and including, day 3 post-partum or the day before sacrifice. For studies where the test substance is administered by inhalation or by the dermal route, dosing should be continued at least up to, and including, day 19 of gestation.

26. A diagram of the experimental schedule is given in Annex 2.

Mating procedure

27. Normally, 1:1 (one male to one female) matings should be used in this study. Exceptions can arise in the case of occasional deaths of males. The female should be placed with the same male

until pregnancy occurs or two weeks have elapsed. Each morning the females should be examined for the presence of sperm or a vaginal plug. Day 0 of pregnancy is defined as the day a vaginal plug or sperm is found. In case pairing is unsuccessful, re-mating of females with proven males of the same group could be considered.

Observations

28. 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, considering the peak period of anticipated effects after dosing. Pertinent behavioural changes, signs of difficult or prolonged parturition and all signs of toxicity, including mortality, should be recorded. These records should include time of onset, degree and duration of toxicity signs.

29. The duration of gestation should be recorded and is 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, runts (pups that are significantly smaller than corresponding control pups) and the presence of gross abnormalities.

30. Live pups should be counted and sexed and litters weighed within 24 hours of parturition (day 0 or 1 post-partum) and on day 4 post-partum. In addition to the observations on parent animals (see paragraph 28), any abnormal behaviour of the offspring should be recorded.

Body weight and food/water consumption

31. 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 and 20 and within 24 hours of parturition (day 0 or 1 post-partum) and day 4 post-partum. These observations should be reported individually for each adult animal.

32. During pre-mating, pregnancy and lactation, food consumption should be measured at least weekly. The measurement of food consumption during mating is optional. Water consumption during these periods should also be measured when the test substance is administered via drinking water.

Pathology

Gross necropsy

33. At the time of sacrifice or death during the study, the adult animals should be examined macroscopically for any abnormalities or pathological changes. Special attention should be paid to the organs of the reproductive system. The number of implantation sites should be recorded. The counting of corpora lutea is strongly recommended.

34. The testes and epididymides of all male adult animals should be weighed.

35. Dead pups and pups killed at day 4 post-partum, or shortly thereafter, should, at least, be carefully examined externally for gross abnormalities.

36. The ovaries, testes, epididymides, accessory sex organs and all organs showing macroscopic lesions of all adult animals should be preserved. Formalin fixation is not recommended for routine examination of testes and epididymides. An acceptable method is the use of Bouin’s fixative for these tissues.

Histopathology

37. Detailed histological examination should be performed on the ovaries, testes and epididymides (with special emphasis on stages of spermatogenesis and histopathology of interstitial testicular cell structure) of the animals of the highest dose group and the control group. The other preserved organs may be examined when necessary. Examinations should be extended to the animals of other dosage groups when changes are seen in the highest dose group.

DATA AND REPORTING

Data

38. Individual animal data should be provided. Additionally, all data should be summarised in tabular form, showing for each test group the number of animals at the start of the test, the number of animals found dead during the test or killed for humane reasons, 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, including time of onset, duration, and severity of any toxic effects, the types of histopathological changes, and all relevant litter data. A tabular summary report format that has proven to be very useful for the evaluation of reproductive/developmental effect is given in Annex 3.

39. Due to the limited dimensions of the study, statistical analysis in the form of tests for "significance" are of limited value for many endpoints, especially reproductive endpoints. If statistical analyses are used then the method chosen should be appropriate for the distribution of the variable examined, and be selected prior to the start of the study. Because of the small group size, the use of historic control data (e.g. for litter size), where available, may also be useful as an aid to the interpretation of the study.

Evaluation of results

40. The findings of this 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, incidence and severity of abnormalities, including gross lesions, identified target organs, infertility, clinical abnormalities, affected reproductive and litter performance, body weight changes, effects on mortality and any other toxic effects.

41. Because of the short period of treatment of the male, the histopathology of the testis and epididymus must be considered along with the fertility data, when assessing male reproductive effects.

Test report

42. The test report must include the following information:

Test substance:

- physical nature and, where relevant, physicochemical properties;
- identification data.

Vehicle (if appropriate):

- justification for choice of vehicle if other than water.

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.

Test conditions:

- rationale for dose level selection;
- details of test substance formulation/diet preparation, achieved concentrations, stability and homogeneity of the preparation;
- details of the administration of the test substance;
- conversion from diet/drinking water test substance concentration (ppm) to the actual dose (mg/kg body weight/day), if applicable;
- details of food and water quality.

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 of toxicity;
- gestation length;
- toxic or other effects on reproduction, offspring, post-natal growth, etc.;
- nature, severity and duration of clinical observations (whether reversible or not);
- number of live births and post-implantation loss;
- number pups with grossly visible abnormalities, number of runts;
- time of death during the study or whether animals survived to termination;
- number of implantations, corpora lutea (recommended), litter size and litter weights at the time of recording;
- body weight at sacrifice and organ weight data for the parental animals;
- necropsy findings;
- a detailed description of microscopic findings of the male genital tract and in other tissues, when performed;
- absorption data (if available);
- statistical treatment of results, where appropriate.

Discussion of results.

Conclusions.

Interpretation of results

43. The study will provide evaluations of reproduction/developmental toxicity associated with administration of repeated doses. It could provide an indication of the need to conduct further investigations and provides guidance in the design of subsequent studies.

LITERATURE

(1) OECD, Paris (1990). Room Document No. 1 for the 14th Joint Meeting of the Chemicals Group and Management Committee.

(2) OECD, Paris (1992). Chairman's Report of the ad hoc Expert Meeting on Reproductive Toxicity Screening Methods, Tokyo, 27th-29th October, 1992.

ANNEX 1

DEFINITIONS

Reproduction toxicity represents harmful effects on the progeny and/or an impairment of male and female reproductive functions or capacity.

Maternal toxicity: adverse effects on gravid females, occurring either specifically (direct effect) or aspecifically (indirect effect).

Impairment of fertility represents disorders of male or female reproductive functions or capacity.

Developmental toxicity: the manifestation of reproductive toxicity, representing pre-, peri- post-natal, structural, or functional disorders in the progeny.

Dose is the amount of test substance administered. Dose is expressed as weight (g, mg) as weight of test substance per unit weight of test animal (e.g. mg/kg), or as constant dietary concentration (ppm).

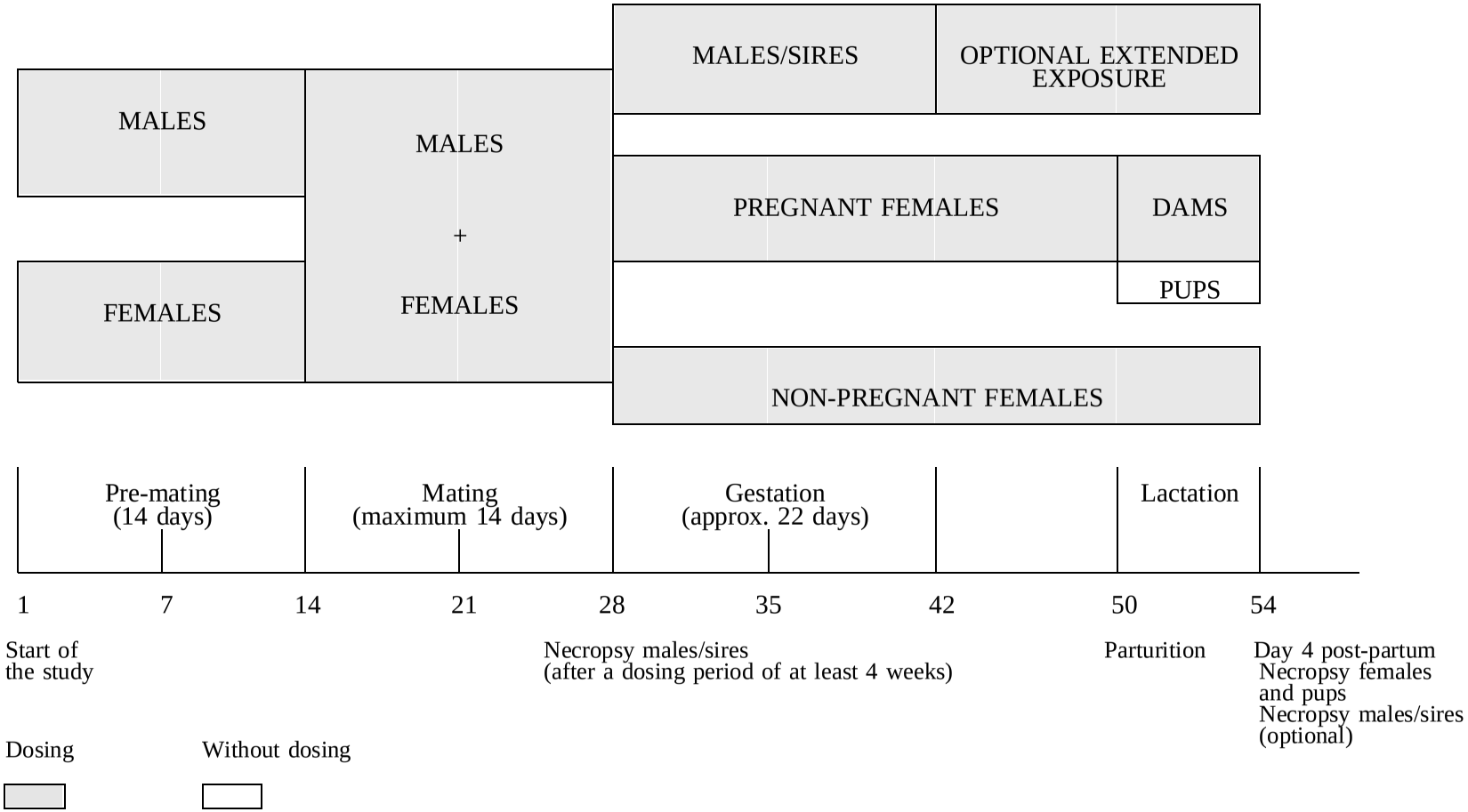
Dosage is a general term comprising of dose, its frequency and the duration of dosing.

Evident toxicity is a general term describing clear signs of toxicity following administration of test substance. These should be sufficient for hazard assessment and should be such that an increase in the dose administered can be expected to result in the development of severe toxic signs and probable mortality.

NOAEL is the abbreviation for no-observed-adverse effect level and is the highest dose level where no adverse treatment-related findings are observed.

ANNEX 2

DIAGRAM OF THE EXPERIMENTAL SCHEDULE INDICATING THE MAXIMUM STUDY DURATION, BASED ON A FULL 14-DAY MATING PERIOD



ANNEX 3

TABULAR SUMMARY REPORT OF EFFECTS ON REPRODUCTION/DEVELOPMENT

OBSERVATIONS	VALUES				
Dosage (units).....	**0 (control)**	**. . .**	**. . .**	**. . .**	**. . .**
Pairs started (N)					
Females showing evidence of copulation (N)					
Females achieving pregnancy (N)					
Conceiving days 1 - 5 (N)					
Conceiving days 6 - . . .[(1)] (N)					
Pregnancy ≤ 21 days (N)					
Pregnancy = 22 days (N)					
Pregnancy ≥ 23 days (N)					
Dams with live young born (N)					
Dams with live young at day 4 pp (N)					
Corpora lutea/dam (mean)					
Implants/dam (mean)					
Live pups/dam at birth (mean)					
Live pups/dam at day 4 (mean)					
Sex ratio (m/f) at birth (mean)					
Sex ratio (m/f) at day 4 (mean)					
Litter weight at birth (mean)					
Litter weight at day 4 (mean)					
Pup weight at birth (mean)					
Pup weight at day 4 (mean)					
ABNORMAL PUPS					
Dams with 0					
Dams with 1					
Dams with ≥ 2					
LOSS OF OFFSPRING					
Pre-implantation (corpora lutea minus implantations)					
Females with 0					
Females with 1					
Females with 2					
Females with ≥ 3					
Pre-natal/post-implantations (implantations minus live births)					
Females with 0					
Females with 1					
Females with 2					
Females with ≥ 3					
Post-natal (live births minus alive at post-natal day 4)					
Females with 0					
Females with 1					
Females with 2					
Females with ≥ 3					

(1) last day of the mating period