OECD GUIDELINE FOR TESTING OF CHEMICALS

Combined Repeated Dose Toxicity Study with the 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 "Combined Repeat Dose and Reproductive/Developmental Screening Test", that could effectively be utilised in the initial evaluation of existing chemicals (1).

2. This 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). The reproduction/developmental toxicity screening part 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)(4). For the repeated dose toxicity part, it is in concordance with (the current proposal for updating) Guideline 407.

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INITIAL CONSIDERATIONS

3. In the assessment and evaluation of the toxic characteristics of a chemical the determination of oral toxicity using repeated doses may be carried out after the initial information on toxicity has been obtained by acute testing. This study provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time. The method comprises the basic repeated dose toxicity study that may be used for chemicals on which a 90-day study is not warranted (e.g. when the production volume does not exceed certain limits) or as a preliminary study to a long-term study.

4. It further comprises a reproduction/developmental toxicity screening test and, therefore, can also be used to provide initial information on possible effects on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition, either at an early stage of assessing the toxicological properties of chemicals, or on chemicals of concern. This test does not provide complete information on all aspects of reproduction and development. In particular, it offers only limited means of detecting postnatal manifestations of prenatal exposure, or effects that may be induced during postnatal exposure. Due (amongst other reasons) to the selectivity of the end points, and the short duration of the study, this method will not provide evidence for definite claims of no reproduction/developmental 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).

5. The Guideline also places emphasis on neurological effects as a specific endpoint, and the need for careful clinical observations of the animals, so as to obtain as much information as possible, is stressed. The method should identify chemicals with neurotoxic potential, and which may warrant

further in-depth investigation of this aspect. In addition, the method may also give a basic indication of immunological effects.

6. In the absence of data from other systemic toxicity, reproduction/developmental toxicity, neurotoxicity and/or immunotoxicity studies, positive results are useful for initial hazard assessment and contribute to decisions with respect to the necessity and timing of additional testing. The test may be particularly useful as part of the Screening Information Data Set (SIDS) for the assessment of existing chemicals for which little or no toxicological information is available and can serve as an alternative to conducting two separate tests for repeated dose toxicity (Guideline 407) and reproduction/developmental toxicity (Guideline 421), respectively. It can also be used as a dose range finding study for more extensive reproduction/developmental studies, or when otherwise considered relevant.

7. Generally, it is assumed that there are differences in sensitivity between pregnant and non-pregnant animals. Consequently, it may be more complicated to determine dose levels in this combined test that are adequate to evaluate both general systemic toxicity and specific reproduction/developmental toxicity, rather than when the individual tests are conducted separately. Moreover, interpretation of the test results with respect to general systemic toxicity may be more difficult than when conducting a separate repeated-dose study, especially when serum and histopathology parameters are not evaluated at the same time in the study. Because of these technical complexities, considerable experience in toxicity testing is required for the performance of this combined screening test. On the other hand, apart from the smaller number of animals involved, the combined test may offer a better means of discriminating direct effects on reproduction/development from those that are secondary to other (systemic) effects.

8. In this test, the dosing period is longer than in a conventional 28-day repeated dose study. However, it uses fewer animals of each sex per group when compared with the situation where a conventional 28-day repeated dose study is conducted in addition to a Reproduction/Developmental Toxicity Screening Test.

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

10. Definitions used are given in Annex 1.

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PRINCIPLE OF THE TEST

11. 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, 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 particularly 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.

12. 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.

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

14. 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 are necropsied and, at the conclusion of the test, surviving animals are killed and necropsied.

DESCRIPTION OF THE METHOD

Selection of animal species

15. 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. Where the study is conducted as a preliminary study to a long-term or a full-generation study, preferably animals from the same strain and source should be used in both studies.

Housing and feeding conditions

16. The temperature in the experimental animal room should be 22 °C (± 3°). The relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning. 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.

17. 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.

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Preparation of the animals

18. Healthy young adult animals are randomised and assigned to the treatment groups and cages. Cages should be arranged in such a way that possible effects due to cage placements are minimised. 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

19. 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 also be administered via the diet or drinking water.

20. 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 non-aqueous vehicles 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

21. 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. If interim kills are planned, the number should be increased by the number of animals scheduled to be killed before the completion of the study. Consideration should be given to an additional satellite group of five animals per sex in the control and the top dose group for observation of reversibility, persistence or delayed occurrence of systemic toxic effects, for at least 14 days post treatment. Animals of the satellite groups will not be mated and, consequently, are not used for the assessment of reproduction/developmental toxicity.

Dosage

22. Generally, at least three test groups and a control group should be used. If there are no suitable general toxicity data available, a range finding study may be performed to aid the determination of the doses to be used. 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.

23. Dose levels should be selected taking into account any existing toxicity and (toxico-) kinetic data available for the test compound or related materials. It should also be taken into account that there may be differences in sensitivity between pregnant and non-pregnant animals. The highest dose level should be chosen with the aim of inducing toxic effects but not death nor obvious suffering. Thereafter, a descending sequence of dose levels should be selected with a view to demonstrating any dosage related response and no adverse effects at the lowest dose level. Two- to four- fold intervals are frequently optimum 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

24. If an oral study at one dose level of at least 1000 mg/kg body weight/day or, for dietary administration, an equivalent percentage in the diet, or drinking water (based upon body weight determinations), 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 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 exposure.

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Administration of doses

25. 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.

26. 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. Where the combined study is used as a preliminary to a long term or a full reproduction toxicity study, a similar diet should be used in both studies.

Experimental schedule

27. Dosing of both sexes should begin 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. In order to allow for overnight fasting of dams prior to blood collection (if this option is preferred), dams and their offspring need not necessarily be killed on the same day. 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.

28. 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.

29. Animals in a satellite group scheduled for follow-up observations, if included, are not mated. They should be kept at least for a further 14 days after the first scheduled kill of dams, without treatment to detect delayed occurrence, or persistence of, or recovery from toxic effects.

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

Mating procedure

31. 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

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or sperm is found. In case pairing was unsuccessful, re-mating of females with proven males of the same group could be considered.

Observations

32. General clinical observations should be made at least once a day, preferably at the same time(s) each day and considering the peak period of anticipated effects after dosing. The health condition of the animals should be recorded. At least twice daily all animals are observed for morbidity and mortality.

33. Once before the first exposure (to allow for within-subject comparisons), and at least once a week thereafter, detailed clinical observations should be made in all animals. These observations should be made outside the home cage in a standard arena and preferably at the same time, each day. They should be carefully recorded; preferably using scoring systems, explicitly defined by the testing laboratory. Effort should be made to ensure that variations in the test conditions are minimal and that observations are preferably conducted by observers unaware of the treatment. Signs noted should include, but not be limited to, changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g. lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypies (e.g. excessive grooming, repetitive circling), difficult or prolonged parturition or bizarre behaviour (e.g. self-mutilation, walking backwards) should also be recorded.

34. At one time during the study, sensory reactivity to stimuli of different modalities (e.g. auditory, visual and proprioceptive stimuli) (5), (6), (7), assessment of grip strength (8) and motor activity assessment (9) should be conducted in five males and five females, randomly selected from each group. Further details of the procedures that could be followed are given in the respective references. However, alternative procedures than those referenced could also be used. In males, these functional observations should be made towards the end of their dosing period, shortly before scheduled kill but before blood sampling for haematology or clinical chemistry. Females should be in a physiologically similar state during these functional tests and should preferably be tested during lactation, shortly before scheduled kill. In order to avoid hyperthermia of pups, dams should be removed from the pups for not more than 30-40 minutes.

35. Functional observations made once towards the end of the study may be omitted when the study is conducted as a preliminary study to a subsequent subchronic (90-day) or long-term study. In that case, the functional observations should be included in this follow-up study. On the other hand, the availability of data on functional observations from this repeated dose study may enhance the ability to select dose levels for a subsequent subchronic or long-term study.

36. Exceptionally, functional observations may also be omitted for groups that otherwise reveal signs of toxicity to an extent that would significantly interfere with the functional test performance.

37. 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.

38. 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 paragraphs 33 and 34), any abnormal behaviour of the offspring should be recorded.

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Body weight and food/water consumption

39. 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.

40. 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 by that medium.

Haematology

41. Once during the study, the following haematological examinations should be made in five males and five females randomly selected from each group: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leucocyte count, platelet count and a measure of blood clotting time/potential.

42. Blood samples should be taken from a named site. Females should be in a physiologically similar state during sampling. In order to avoid practical difficulties related to the variability in the onset of gestation, blood collection in females may be done at the end of the pre-mating period as an alternative to sampling just prior to, or as part of, the procedure for killing the animals. Blood samples of males should preferably be taken just prior to, or as part of, the procedure for killing the animals. Alternatively, blood collection in males may also be done at the end of the pre-mating period when this time point was preferred for females.

43. Blood samples should be stored under appropriate conditions.

Clinical Biochemistry

44. Clinical biochemistry determinations to investigate major toxic effects in tissues and, specifically, effects on kidney and liver, should be performed on blood samples obtained from the selected five males and five females of each group. Overnight fasting of the animals prior to blood sampling is recommended[1]. Investigations of plasma or serum shall include sodium, potassium, glucose, total cholesterol, urea, creatinine total protein and albumin, at least two enzymes indicative of hepatocellular effects (such as alanine aminotransferase, aspartate aminotransferase and sorbitol dehydrogenase) and bile acids. Measurements of additional enzymes (of hepatic or other origin) may provide useful information under certain circumstances.

45. Optionally, the following urinalysis determinations could be performed in five randomly selected males of each group during the last week of the study using timed urine volume collection; appearance, volume, osmolality or specific gravity, pH, protein, glucose and blood/blood cells.

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(1) For a number of measurements in serum and plasma, most notably for glucose, overnight fasting would be preferable. The major reason for this preference is that the increased variability which would inevitably result from non-fasting, would tend to mask more subtle effects and make interpretation difficult. On the other hand, however, overnight fasting may interfere with the general metabolism of the (pregnant) animals, disturbs lactation and nursing behaviour, and, particularly in feeding studies, may disturb the daily exposure to the test substance. If overnight fasting is adopted, clinical biochemical determinations should be performed after the conduct of functional observations in week 4 of the study.

46. In addition, studies to investigate serum markers of general tissue damage should be considered. Other determinations that should be carried out if the known properties of the test substance may, or are suspected to, affect related metabolic profiles include calcium, phosphate, fasting triglycerides and fasting glucose, specific hormones, methaemoglobin and cholinesterase. These need to be identified on a case-by-case basis.

47. Overall, there is a need for a flexible approach, depending on the observed and/or expected effect with a given compound.

48. If historical baseline data are inadequate, consideration should be given to determination of haematological and clinical biochemistry variables before dosing commences.

Pathology

Gross necropsy

49. All adult animals in the study shall be subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. 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.

50. The testes and epididymides of all adult males should be weighed and the ovaries, testes, epididymides, accessory sex organs, and all organs showing macroscopic lesions of all adult animals, should be preserved.

51. In addition, for five adult males and females, randomly selected from each group, the liver, kidneys, adrenals, thymus, spleen, brain and heart should be trimmed of any adherent tissue, as appropriate and their wet weight taken as soon as possible after dissection to avoid drying. Of the selected males and females, the following tissues should also be preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent histopathological examination: all gross lesions, brain (representative regions including cerebrum, cerebellum and pons), spinal cord, stomach, small and large intestines (including Peyer's patches), liver, kidneys, adrenals, spleen, heart, thymus, thyroid, trachea and lungs (preserved by inflation with fixative and then immersion), uterus, urinary bladder, lymph nodes (preferably one lymph node covering the route of administration and another one distant from the route of administration to cover systemic effects), peripheral nerve (sciatic or tibial) preferably in close proximity to the muscle, and a section of bone marrow (or, alternatively, a fresh mounted marrow aspirate).

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52. 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. The clinical and other findings may suggest the need to examine additional tissues. Also, any organs considered likely to be target organs based on the known properties of the test substance should be preserved.

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

Histopathology

54. Full histopathology should be carried out on the preserved organs and tissues of the selected animals in the control and high dose groups (with special emphasis on stages of spermatogenesis in the male gonads and histopathology of interstitial testicular cell structure). These examinations

should be extended to animals of other dosage groups, if treatment-related changes are observed in the high dose group.

55. All gross lesions shall be examined. To aid in the elucidation of NOAELs, target organs in other dose groups should be examined, particularly in groups claimed to show a NOAEL.

56. When a satellite group is used, histopathology should be performed on tissues and organs identified as showing effects in the treated groups.

DATA AND REPORTING

Data

57. 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, which has proven to be very useful for the evaluation of reproductive/developmental effects, is given in Annex 3.

58. When possible, numerical results should be evaluated by an appropriate and general acceptable statistical method. The statistical methods should be selected during the design of the study. 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. Some of the most widely used methods, especially parametric tests for measures of central tendency, are inappropriate. 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.

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Evaluation of results

59. 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.

60. 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 reproduction effects. The use of historic control data on reproduction/development (e.g. for litter size) where available may also be useful as an aid to the interpretation of the study.

Test report

61. 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 concentration, 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 applicable;
- 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, postnatal growth, etc.;
- nature, severity and duration of clinical observations (whether reversible or not);
- sensory activity, grip strength and motor activity assessments;
- haematological tests with relevant base-line values;
- clinical biochemistry tests with relevant base-line values;
- number of live births and post implantation loss;
- number of 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 histopathological findings;
- absorption data (if available);
- statistical treatment of results, where appropriate.

Discussion of results.

Conclusions.

Interpretation of Results

62. The study will provide evaluations of reproduction/developmental toxicity associated with administration of repeated doses. In particular, since emphasis is placed on both general toxicity and reproduction/developmental toxicity endpoints, the results of the study will allow for the

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This version has been replaced on 28 July 2016 and will be deleted effectively on 23 January 2017.

discrimination between reproduction/developmental effects occurring in the absence of general toxicity and those which are only expressed at levels that are also toxic to parent animals. It could provide an indication of the need to conduct further investigations and could provide guidance in the design of subsequent studies.

LITERATURE

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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) and being related to the gravid state.

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, gm) or 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 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.

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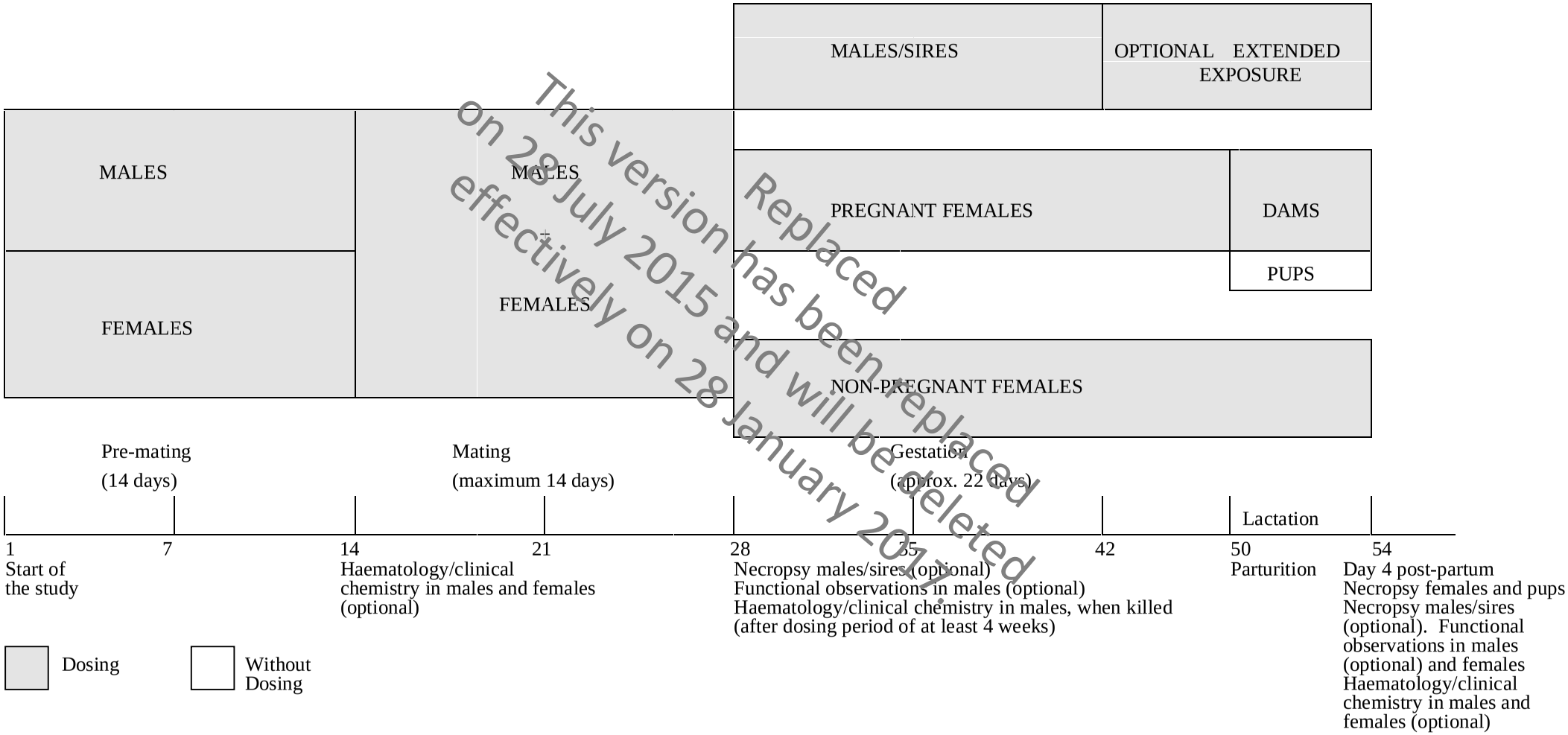
ANNEX 2

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



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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 ³ 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 (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					

Replaced
This version has been replaced on 28 July 2015 and will be deleted effectively on 28 January 2017.

(1) last day of the mating period