OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Repeated Dose 90-day Oral Toxicity Study in Rodents

INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress, changing regulatory needs, and animal welfare considerations. The original guideline 408 was adopted in 1981. In 1998 a revised version was adopted, to obtain additional information from the animals used in the study, based on the outcome of an OECD Consultation Meeting of Experts on Sub-chronic and Chronic Toxicity Testing held in Rome in 1995 (1).

2. This Test Guideline (TG) was updated in 2018 to add endocrine-sensitive endpoints intended to improve detection of potential endocrine activity of test chemicals and mirrors updates to TG 407 (Repeated Dose 28-Day Oral Toxicity Study in Rodents).

INITIAL CONSIDERATIONS

3. In the assessment and evaluation of the toxic characteristics of a chemical, the determination of sub-chronic oral toxicity using repeated doses may be carried out after initial information on toxicity has been obtained from acute or repeated dose 28-day toxicity tests. The 90- day study provides information on the possible health hazards likely to arise from repeated exposure over a prolonged period of time covering post-weaning maturation and growth into adulthood of the test animals. The study will provide information on the major toxic effects, indicate target organs and the possibility of accumulation of test chemical, and can provide an estimate of a no-observed-adverse-effect level (NOAEL) of exposure which can be used in selecting dose levels for chronic studies and for establishing safety criteria for human exposure. Alternatively, this study yields dose related response data that may be used to estimate point of departure for hazard assessment using appropriate modelling methods (e.g. benchmark dose analysis).

4. The revised Guideline places additional emphasis on endocrine endpoints to combine with the existing sensitivity to neurological and immunological and reproductive effects. The need for careful clinical observations of the animals, so as to obtain as much information as possible, is also stressed. Required endpoints include the measurement of thyroxine (T4), triiodothyronine (T3) thyroid stimulating hormone (TSH) and thyroid gland weight, which are responsive to thyroid pathway perturbation (2). In addition, serum total cholesterol, low-density lipoproteins (LDL) and high-density lipoproteins (HDL) should also be determined as these levels are directly controlled by thyroid hormone action and contribute to evidence of thyroid effects. (3). Optional endpoints include thyroid gland weight and other hormone measurements, as well as assessments of spermatogenesis and oestrus cycle. Required and optional measures that may be altered by endocrine effects are listed in Annex 2. Assessment of the optional measures may be considered if existing information for the test chemical or similar chemicals suggests potential to influence these or can be triggered

by observations from required measures collected as part of this guideline. This study should allow for the identification of chemicals with the potential to cause neurotoxic, endocrine, immunological or reproductive organ effects, which may warrant further in-depth investigation.

5. The results obtained for the endocrine related parameters should be evaluated in the context of the "OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals" (4). In this Conceptual Framework, TG 408 is included in level 4 as an *in vivo* assay providing data on adverse effects on endocrine relevant endpoints. A significant effect on an endocrine endpoint might not, however, be considered sufficient evidence on its own to conclude that the test chemical is an endocrine disruptor (e.g. see par 17).

6. It is acknowledged that all animal-based procedures will conform to local standards of animal care; the descriptions of care and treatment set forth below are minimal performance standards, and will be superseded by local regulations where more stringent. Further guidance of the humane treatment of animals is given by the OECD (19).

7. Definitions used in this test guideline are provided in the Annex.

PRINCIPLE OF THE TEST

8. The test substance is orally administered daily in graduated doses to several groups of experimental animals, one dose level per group for a period of 90 days. During the period of administration, the animals are observed closely for signs of toxicity as recommended by the OECD (19). Animals which die or are humanely killed during the test are necropsied and at the conclusion of the test, remaining animals are also humanely killed and necropsied after the full dosing period.

DESCRIPTION OF THE METHOD

Selection of animal species

9. The preferred species is the rat, although other rodent species (e.g., the mouse) may be used. If the parameters specified within this TG 408 are investigated in another rodent species, a detailed justification should be given. Although it is biologically plausible that other species should respond to toxicants in a similar manner to the rat, the use of smaller species may result in increased variability in endpoint measurements due to technical challenges of dissecting smaller organs. Commonly used laboratory strains of young healthy adult animals should be employed. The females should be nulliparous and non-pregnant. Dosing should begin as soon as possible after weaning and, in any case, before the animals are nine weeks old. 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 preliminary to a long term chronic toxicity study, animals from the same strain and source should be used in both studies.

Housing and feeding conditions

10. All procedures should conform to local standards of laboratory animal care. The temperature in the experimental animal room should be 22°C (± 3°C). 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. Care should be taken to avoid diets or animal bedding that may contain hormonally active substances (e.g. phytoestrogens). Animals should be housed in small groups of the same sex. Animals may be housed individually if scientifically justified and the duration of single housing shall be limited to the minimum period necessary. (5), (6), (7).

Preparation of animals

11. Healthy animals, which have been acclimated to laboratory conditions for at least 5 days and have not been subjected to previous experimental procedures, should be used. The test animals should be characterised as to species, strain, source, sex, weight and/or age. Animals should be randomly assigned to the control and treatment groups. Cages should be arranged in such a way that possible effects due to cage placement are minimised. Each animal should be assigned a unique identification number. The least invasive method of uniquely identifying animals must be used. Appropriate methods include ringing, tagging, micro-chipping and biometric identification.

Preparation of doses

12. The test compound is administered by gavage, incorporated in the diet or dissolved in drinking water. The method of oral administration is dependent on the purpose of the study and the physical/chemical properties of the test material.

13. 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 homogeneity and stability of the test substance under the conditions of administration should be determined.

PROCEDURE

Number and sex of animals

14. At least 20 animals (ten female and ten male) should be used at each dose level. 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. Based on previous knowledge of the chemical or a close analogue, consideration should be given to including an additional satellite group of ten animals (five per sex) in the control and in the top dose group for observation after the treatment period, for the potential reversibility or persistence of any toxic effects. The duration of this post-treatment period should be fixed appropriately with regard to the effects observed.

Dosage

15. At least three dose levels and a concurrent control shall be used, except where a limit test is conducted (see paragraph 19). Dose levels may be based on the results of repeated dose or range finding studies and should take into account any existing toxicological and toxicokinetic data available for the test compound or related materials. Unless limited by the physical-chemical nature or biological effects of the test substance, the highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering (see GD 19). A descending sequence of dose levels should be selected with a view to demonstrating any dosage related response and a 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 about 6-10) between dosages.

16. The control group shall be an untreated group or a vehicle-control group if a vehicle is used for administering the test substance. Except for treatment with the test substance, animals in the control group should be handled in an identical manner to those in the test groups. If a vehicle is used, the control group shall receive the vehicle in the highest volume used. If a test substance is administered in the diet, and causes reduced dietary intake, then a pair-fed control group may be useful in distinguishing between reductions due to palatability or toxicological alterations in the test model.

17. Consideration should be given to the following characteristics of the vehicle and other additives, as appropriate: effects on the absorption, distribution, metabolism, or retention of the test substance; effects on the chemical properties of the test substance which may alter its toxic characteristics; and effects on the food or water consumption or the nutritional status of the animals.

Limit Test

18. Using the methods described for this study, if a test at one dose level equivalent to at least 1000 mg/kg body weight/day produces no observed adverse effects and if toxicity would not be expected based upon data from structurally-related compounds, then a full study using three 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.

Administration of doses

19. The animals are dosed with the test substance daily seven days each week for a period of 90 days. Any other dosing regime (e.g., five days per week) needs to be justified. 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/100g body weight, except in the case of aqueous solutions where 2 ml/100g 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.

20. 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 animal's body weight (e.g. mg/kg body weight/day) 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 as necessary to maintain a constant dose level in terms of animal body weight. Where a 90-day study is used as a preliminary test to a long term chronic toxicity study, a similar diet should be used in both studies.

Observations

21. The observation period should be at least 90 days. Animals in a satellite group scheduled for follow-up observations should be kept for an appropriate period without treatment to detect persistence of, or recovery from toxic effects.

22. General clinical observations should be made at least once a day, preferably at the same time(s) each day, taking into consideration the peak period of anticipated effects after dosing. The clinical condition of the animals should be recorded. At least twice daily, usually at the beginning and end of each day, all animals are inspected for signs of morbidity and mortality (19).

23. At least once prior to the first exposure (to allow for within-subject comparisons), and once a week thereafter, detailed clinical observations should be made in all animals. These observations should be made outside the home cage, preferably in a standard arena and at similar times on each occasion. 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 observation conditions are minimal. 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, pilo-erection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypes (e.g., excessive grooming, repetitive circling) or bizarre behaviour (e.g., self-mutilation, walking backwards) should also be recorded (8, 19).

24. Ophthalmological examination, using an ophthalmoscope or equivalent suitable equipment, should be made prior to the administration of the test substance and at the termination of the study, preferably in all animals but at least in the high dose and control groups. If changes in the eyes are detected all animals should be examined.

25. Towards the end of the exposure period and not earlier than in week 11, sensory reactivity to stimuli of different types (5) (e.g., auditory, visual and proprioceptive stimuli) (9), (10), (11), assessment of grip strength (12) and motor activity assessment (13) should be conducted. 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.

26. Functional observations conducted towards the end of the study may be omitted when data on functional observations are available from other studies or when daily clinical observations did not reveal any functional deficits.

27. 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.

28. At necropsy, the oestrus cycle of all females could be determined (optional) by taking vaginal smears. These observations will provide information regarding the stage of oestrus cycle at the time of sacrifice and assist in histological evaluation of estrogen sensitive tissues (see guidance on histopathology (17)).

Body weight and food/water consumption

29. All animals should be weighed at least once a week. Measurements of food consumption should be made at least weekly. If the test substance is administered via the drinking water, water consumption should also be measured at least weekly. Water consumption may also be considered for dietary or gavage studies during which drinking activity may be altered.

Haematology and Clinical Biochemistry

30. Blood samples should be taken from a named site and stored, if applicable, under appropriate conditions. At the end of the test period, samples are collected just prior to or as part of the procedure for killing the animals.

31. The following haematological examinations should be made at the end of the test period and when any interim blood samples may have been collected: haematocrit, haemoglobin concentration, erythrocyte count, reticulocyte count, total and differential leukocyte count, platelet count and a measure of blood clotting time/potential.

32. 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 each animal just prior to or as part of the procedure for killing the animals (apart from those found moribund and/or intercurrently killed). In a similar manner to haematological investigations, interim sampling for clinical biochemical tests may be performed. Overnight fasting of the animals prior to blood sampling is recommended[1]. Determinations in plasma or serum should include sodium, potassium, glucose, total cholesterol, HDL, LDL, urea, blood urea nitrogen, creatinine, total protein and albumin, and more than two enzymes indicative of hepatocellular effects (e.g., alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, and sorbitol dehydrogenase). Measurements of additional enzymes (of hepatic or other origin) and bile acids, which may provide useful information under certain circumstances, and bilirubin may also be included.

[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 animals 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 of the study.

33. Clinical biochemistries should be evaluated as potential markers of general tissue damage. 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, phosphorus, fasting triglycerides, specific hormones, methaemoglobin and cholinesterase. These need to be identified for chemicals in certain classes or on a case-by-case basis.

34. Serum total T4, T3 and TSH should be measured at study termination. Other hormones, e.g. testosterone, oestradiol, follicle stimulating hormone (FSH), luteinizing hormone (LH) should be considered as on a case by case basis). Serum may be stored frozen to determine the most informative hormone analyses based on results observed for other endpoints (e.g. organ weight and histology).

35. The following factors might influence the variability and absolute concentration of the hormone determinations:

- time of sacrifice because of diurnal variation of hormone concentration
- method of sacrifice to avoid undue stress to the animals that may affect hormone concentrations
- test kits for hormone determinations that may differ by their standard curves

36. Blood samples specifically intended for hormone determination should be obtained at a comparable time of the day. The numerical values obtained when analysing hormone concentrations differ with various commercial assay kits. Consequently, it may not be possible to provide performance criteria based upon uniform historical data. Alternatively, laboratories should strive to keep control coefficients of variation below 25 for T_3 and T_4 and below 35 for TSH. All concentrations are to be recorded in ng/ml.

37. Optionally, the following urinalysis determinations could be performed 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.

38. If historical baseline data are inadequate, consideration should be given as to whether haematological and clinical biochemistry variables need to be determined before dosing commences; however, it is generally not recommended that these data be generated before treatment (14).

Pathology

39. At termination, testis and epididymis weights are recorded for all males. At least one epididymis from each male should be reserved for histopathological examination. The remaining epididymis may be used for enumeration of cauda epididymis sperm reserves sperm morphology (optional) or motility (optional) (15).

40. For the optional evaluation of sperm morphology, an epididymal (or vas deferens) sperm sample should be examined as fixed or wet preparations and at least 200 spermatozoa per sample classified as either normal (both head and midpiece/tail appear normal) or abnormal. Examples of morphologic sperm abnormalities would include fusion, isolated heads, and misshapen heads and/or tails. Misshapen or large sperm heads may indicate defects in spermiation. Sperm motility is an optional endpoint. The percentage of progressively motile sperm could be determined either visually or by computer-assisted motion analysis

41. If sperm samples are frozen and/or smears fixed (or optional sperm motility is recorded), subsequent analysis may be restricted to control and high-dose males. However, if treatment-related effects are observed, the lower dose groups should also be evaluated.

42. At necropsy, the oestrus cycle of all females could be determined (optional) by taking vaginal smears. These observations will provide information regarding the stage of oestrus cycle at the time of sacrifice and assist in histological evaluation of estrogen sensitive tissues (see guidance on histopathology (17 part 3)).

Gross necropsy

43. All 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. The liver, kidneys, adrenals, testes, epididymides, prostate + seminal vesicles with coagulating glands as a whole (alternatively, first weight the entire prostate with seminal vesicles/coagulation glands together, then dissect and weigh the prostate gland separately), uterus, ovaries, thymus, spleen, brain, pituitary gland, and heart of all animals (apart from those found moribund and/or killed before study completion) should be trimmed of any adherent tissue, as appropriate, and their wet weight taken as soon as possible after dissection to avoid drying. Care must be exercised when trimming the prostate complex to avoid puncture of the fluid filled seminal vesicles. Alternatively, seminal vesicles and prostate may be trimmed and weighed after fixation.

44. Weighing of the thyroid gland must be performed with extreme care as this tissue is easily damaged (for guidance, see 20). Tissue damage could compromise histopathology analysis Therefore, the thyroid trimming and weighing should be done very carefully and should preferably be determined after fixation in order to avoid tissue damage.

45. The following tissues should be preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent histopathological examination (16, 17): all gross lesions, brain (representative regions including cerebrum, cerebellum and medulla/pons), spinal cord (at three levels: cervical, mid-thoracic and lumbar), pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines (including Peyer's patches), liver, pancreas, kidneys, adrenals, spleen, heart, trachea and lungs (preserved by inflation with fixative and then immersion), aorta, ovaries, uterus, cervix, vagina, testes, epididymides, prostate, seminal vesicles, coagulation glands, mammary gland (male and female), urinary bladder, gall bladder (mouse), 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), skeletal muscle, and bone, with bone marrow (section or alternatively, a fresh bone marrow aspirate), skin and eyes (if

changes were observed during ophthalmological examinations). It is recommended to preserve testes by immersion in Bouin's or modified Davidson's fixative and histopathological assessment should consider staging of seminiferous tubule cross sections as described (16). See also OECD GD 106 (17) for fixation and histological evaluation of endocrine organs. 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 evaluated.

46. The following tissues may give valuable indication for endocrine-related effects: gonads (ovaries and testes), accessory sex organs (uterus, cervix, vagina, epididymides, seminal vesicles with coagulation glands, dorsolateral and ventral prostate), pituitary, mammary gland (in both sexes), thyroid gland and adrenal gland. In the validation of the repeated dose 28 day toxicity test guideline (TG 407), histological changes were observed in mammary glands of male animals exposed to chemicals with a low potency for affecting estrogen homeostasis suggesting high sensitivity to endocrine changes (18). Although, this assessment was only evaluated by a single laboratory as part of this large exercise, the histopathological examination of male mammary glands is encouraged (see (17) for guidance on histopathology).

47. In the international validation for TG 407, some evidence was obtained that subtle endocrine effects by chemicals with a low potency for affecting sex hormone homeostasis may be identified by disturbance of the synchronisation of the oestrus cycle in different tissues, rather than frank histopathological alterations in female sex organs (17 part 3, 18). Although no definitive proof was obtained for such effects, it is recommended that evidence of possible asynchrony of the oestrus cycle should be taken into account in interpretation of the histopathology of the ovaries (follicular, thecal, and granulosa cells), uterus, cervix and vagina. If assessed, the stage of cycle as determined by vaginal smears could be included in this comparison, as well.

48. Histopathological assessment of the testes should be performed with attention to stage specific changes as described (14b and 14c). Detailed histopathological examination should be conducted in order to identify treatment related effects such as retained spermatids, missing germ cell layers or types, multinucleate giant cells or sloughing of spermatogenic cells into the lumen (21). See also OECD GD 106 for fixation and histological evaluation of endocrine organs.

Histopathology

49. Full histopathology should be carried out on the preserved organs and tissues of all animals in the control and high dose groups. These examinations should be extended to animals of all other dosage groups, if treatment-related changes are observed in the high dose group.

50. All gross lesions should be examined.

51. 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

52. Individual data should be provided. Additionally, all data should be summarized in tabular form showing for each test group the number of animals at the start of the test; number of animals found dead during the test or killed for humane reasons and the time of any death or humane kill; 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; and the number of animals showing lesions, the type of lesions and the percentage of animals displaying each type of lesion.

53. When applicable, numerical results should be evaluated by an appropriate and generally acceptable statistical method. The statistical methods and the data to be analysed should be selected during the design of the study.

54. For quality control it is proposed that control data are compared to historical control values and coefficients of variation are calculated for the parameters for endocrine activity. These data can be used for comparison among studies. Differences between rat strains should be taken into account when evaluating historical control data.

Test report

1. The test report must include the following information:

Test substance:

- physical nature, purity and physico-chemical properties;
- identification data.

Vehicle if appropriate

- justification for choice of vehicle, if other than water.

Test animals:

- species and strain used;
- number, age and sex of animals;
- source, housing conditions, diet, etc.;
- individual weights of animals at the start of the test.
- Justification for species if not rat.

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;
- actual doses (mg/kg body weight/day), and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable;
- details of food and water quality

Results:

- body weight and body weight changes;
- food consumption, and water consumption, if applicable;
- toxic response data by sex and dose level, including signs of toxicity;
- nature, severity and duration of clinical observations (whether reversible or not);
- results of ophthalmological examination;
- sensory activity, grip strength and motor activity assessments (when available);
- haematological tests with relevant base-line values;
- clinical biochemistry tests with relevant base-line values;
- circulating thyroxine (required) and other hormone measures (optional)terminal body weight, organ weights and organ/body weight ratios;
- necropsy findings;
- terminal vaginal cytology (optional)
- a detailed description of all histopathological findings;
- Sperm number, morphology results (optional)
- Sperm motility (optional)
- absorption data if available;
- statistical treatment of results, where appropriate.

Discussion of results.

Conclusions.

LITERATURE

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ANNEX 1

DEFINITIONS

Androgenicity is the capability of a chemical to act like a natural androgenic hormone (e.g. testosterone) in a mammalian organism.

Antiandrogenicity is the capability of a chemical to suppress the action of a natural androgenic hormone (e.g. testosterone) in a mammalian organism.

Antioestrogenicity is the capability of a chemical to suppress the action of a natural oestrogenic hormone (e.g. oestradiol 17ß) in a mammalian organism.

Antithyroid activity is the capability of a chemical to suppress the action of a natural thyroid hormone (e.g. T_3) in a mammalian organism.

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

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

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.

HDL High density lipoprotein

LDL Low Density lipoprotein

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

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

Oestrogenicity is the capability of a chemical to act like a natural oestrogenic hormone (e.g. oestradiol 17ß) in a mammalian organism.

T3 Tri-iodothyronine – the active form of thyroid hormone

T4 Thyroxine – The main circulating thyroid gland product is converted to T3

Thyroid activity is the capability of a chemical to act like a natural thyroid hormone (e.g. T_3) in a mammalian organism.

TSH Thyroid Stimulating Hormone – pituitary hormone that thyroid hormone production and release from the thyroid gland

Validation is a scientific process designed to characterise the operational requirements and limitations of a test method and to demonstrate its reliability and relevance for a particular purpose.

ANNEX 2

Endpoints recommended for detection of endocrine activity

Required Measures	**Optional Measures**
Organ Weights	
-Testes - Epididymides - Adrenal glands - Prostate + seminal vesicles with coagulating glands as a whole complex Uterus Ovaries Pituitary gland Thyroid gland	
Histopathology	
Thyroid and Parathyroid glands Adrenal Glands Pituitary gland[2] Testis Epididymides Ventral and dorsolateral prostate Seminal vesicles and coagulating glands Ovaries[2] Cervix[2] Vagina[2] Uterus[2] Mammary glands (female and male)[2]	Vaginal Smear (Collected at necropsy) to determine stage of oestrus cycle[2] Pancreatic islets
Serum/Plasma Biochemistry	
Total Cholesterol HDL LDL	
Serum/Plasma Hormone Analyses	
Thyroxine (T4) TSH T3	FSH LH Oestradiol Testosterone
Sperm Measures	
Cauda epididymis sperm reserves	Sperm motility Sperm morphology

[2] The condition of the estrogen-sensitive organs in the female should be assessed with reference to the stage of oestrus cycle at termination as endocrine active test agents may cause histological changes that, while not overtly pathological, may differ from the condition anticipated based on the stage of ovarian cycle (17 parts 3, 4).