

# DRAFT OECD GUIDELINE FOR THE TESTING OF CHEMICALS

## Test Guideline 452: Chronic Toxicity Studies

### INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress, changing assessment practices and animal welfare considerations. The original Guideline 452 was adopted in 1981. Development of a revised TG 452 was considered necessary in order to reflect recent developments in the field of animal welfare and regulatory requirements (1)(2)(3)(4). The updating of TG 452 has been carried out in parallel with revisions of the Test Guidelines 451, Carcinogenicity Studies and 453, Combined Chronic Toxicity/Carcinogenicity studies, with the objective of obtaining additional information from the animals used in the study and providing further detail on dose selection.
2. The majority of chronic toxicity studies are carried out in rodent species, and this Test Guideline is intended therefore to apply primarily to studies carried out in these species. Should such studies be required in non-rodent species, the principles and procedures outlined may also be applied, with appropriate modifications, as outlined in an OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (5).
3. The three main routes of administration used in chronic toxicity studies are oral, dermal and inhalation. The choice of the route of administration depends on the physical and chemical characteristics of the test substance and the predominant route of exposure of humans. Additional information on choice of route of exposure is provided in an OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (5).
4. This Guideline focuses on exposure via the oral route, the route most commonly used in chronic toxicity studies. While long-term chronic toxicity studies involving exposure via the dermal or inhalation routes may also be necessary for human health risk assessment and/or may be required under certain regulatory regimes, both routes of exposure involve considerable technical complexity. Such studies will need to be designed on a case-by-case basis, although the Guideline outlined here for the assessment and evaluation of chronic toxicity by oral administration could form the basis of a protocol for inhalation and/or dermal studies, with respect to recommendations for treatment periods, clinical and pathology parameters, etc. OECD Guidance is available on the administration of test substances by the inhalation (5)(6) and dermal routes (5). The updated Guidelines TG 412, Subacute inhalation toxicity: 28 day study (8) and TG 413, Subchronic Inhalation Toxicity: 90-Day Study (9), together with the associated OECD Guidance Document on acute inhalation toxicity testing (7), should be specifically consulted in the design of longer term studies involving exposure via the inhalation route.
5. The objectives of chronic toxicity studies covered by this test guideline include:
  - the identification of the hazardous properties of a chemical,
  - the identification of target organs,
  - characterisation of the dose:response relationship,
  - identification of a no-observed-adverse-effect level (NOAEL) or point of departure for establishment of a Benchmark Dose (BMD),
  - the prediction of chronic toxicity effects at human exposure levels,
  - provision of data to test hypotheses regarding mode of action (5).

## INITIAL CONSIDERATIONS

6. In the assessment and evaluation of the toxicological characteristics of a chemical, all available information on the test substance should be considered by the testing laboratory prior to conducting the study, in order to focus the design of the study to more efficiently test for chronic toxicity potential and to minimize animal usage. Information that will assist in the study design includes the identity, chemical structure, and physico-chemical properties of the test substance; any information on the mode of action; results of any *in vitro* or *in vivo* toxicity tests; anticipated use(s) and potential for human exposure; available (Q)SAR data and toxicological data on structurally-related substances; available toxicokinetic data (single dose and also repeat dose kinetics where available) and data derived from other repeated exposure studies. The determination of chronic toxicity may be carried out after initial information on toxicity has been obtained from repeated dose 28-day and/or 90-day toxicity tests. A phased testing approach to chronic toxicity testing should be considered as part of the overall assessment of the potential adverse health effects of a particular chemical (9)(10)(11)(12).
7. The chronic toxicity study provides information on the possible health hazards likely to arise from repeated exposure over a considerable part of the entire lifespan (in rodents). The study will provide information on the toxic effects of the substance, indicate target organs and the possibility of accumulation. It can also provide an estimate of the no-observed-adverse effect level which can be used for establishing safety criteria for human exposure. The need for careful clinical observations of the animals, so as to obtain as much information as possible, is also stressed.
8. In conducting a chronic toxicity study, the guiding principles and considerations outlined in the OECD Guidance Document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation (13), in particular paragraph 62 thereof, should always be followed.
9. Detailed guidance on and discussion of the principles of dose selection for chronic toxicity and carcinogenicity studies can be found in an OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (5) as well as two International Life Sciences Institute publications (14)(15). The core dose selection strategy is dependent on the primary objective or objectives of the study (paragraph 5). In selecting appropriate dose levels, a balance has to be achieved between hazard screening on the one hand and characterisation of low-dose responses and their relevance on the other. This is particularly relevant in the situation where a combined chronic toxicity and carcinogenicity study (TG 453) is to be carried out (paragraph 10).
10. Consideration should be given to carrying out a combined chronic toxicity and carcinogenicity study (TG 453), rather than separate execution of a chronic toxicity study (TG 452) and carcinogenicity study (TG 451). Careful consideration should however be given to the principles of dose selection (paragraphs 9 and 20-24) when undertaking a combined chronic toxicity and carcinogenicity study (TG 453), and it is also recognised that separate studies may be required under certain regulatory frameworks.
11. Definitions used are given in the Annex.

## PRINCIPLE OF THE TEST

12. The test substance is administered daily in graduated doses to several groups of experimental animals for a period of 12 months, although longer or shorter durations may also be chosen (see paragraph

30). This duration is chosen to be sufficiently long to allow any effects of cumulative toxicity to become manifest, without the confounding effects of geriatric changes. Deviations from the exposure duration of 12 months must be justified, particularly in the case of shorter durations. The test substance is normally administered by the oral route although testing by the inhalation or dermal route may also be appropriate (paragraphs 3-4). The study design may also include one or more interim kills, e.g. at 3 and 6 months, and additional groups of animals may be included to accommodate this (see paragraph 18). During the period of administration the animals are observed closely 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 also killed and necropsied.

## **DESCRIPTION OF THE METHOD**

### **Selection of animal species**

13. This Guideline primarily covers assessment and evaluation of chronic toxicity in rodents (see paragraph 2) although it is recognised that similar studies in non-rodents may be required under certain regulatory regimes. The choice of species must be justified. The design and conduct of chronic toxicity studies in other non-rodent species, when required, should be based on the principles outlined in this Guideline together with those in OECD TG 409, Repeated Dose 90-day Oral Toxicity Study in Non-Rodents. Additional information on choice of species and strain is provided in an OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (5).
14. In this Guideline, the preferred rodent species is the rat, although other rodent species, e.g., the mouse, may be used. Rats and mice have been preferred experimental models because of their relatively short life span, their widespread use in pharmacological and toxicological studies, their susceptibility to tumour induction, and the availability of sufficiently characterised strains. As a consequence of these characteristics, a large amount of information is available on their physiology and pathology. Young healthy adult animals of commonly used laboratory strains should be employed. The chronic toxicity study should be carried out in animals from the same strain and source as those used in preliminary toxicity study(ies) of shorter duration. The females should be nulliparous and non-pregnant.

### **Housing and feeding conditions**

15. Animals may be housed individually, or be caged in small groups of the same sex; individual housing should be considered only if scientifically justified (16)(17)(18). Cages should be arranged in such a way that possible effects due to cage placement are minimised. The temperature in the experimental animal room should be 22°C ( $\pm$  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 diet should meet all the nutritional requirements of the species tested and the content of dietary contaminants including but not limited to pesticide residues, persistent organic pollutants, phytoestrogens, heavy metals and mycotoxins, that might influence the outcome of the test, should be as low as possible. Analytical information on the nutrient and dietary contaminant levels should be generated periodically, at least at the beginning of the study and when there is a change in the batch used, and should be included in the final report. Analytical information on the drinking water used in the study should similarly be provided. The choice of diet may be influenced by the need to ensure a suitable admixture of a test substance and to meet the nutritional requirements of the animals when the test substance is administered by the dietary route.

## **Preparation of animals**

16. Healthy animals, which have been acclimated to laboratory conditions for at least 7 days and have not been subjected to previous experimental procedures, should be used. In the case of rodents, dosing of the animals should begin as soon as possible after weaning and acclimatisation and preferably before the animals are 8 weeks old. The test animals should be characterised as to species, strain, source, sex, weight and age. At the commencement of the study, the weight variation of animals used should be minimal and not exceed  $\pm 20\%$  of the mean weight of all the animals within the study, separately for each sex. Animals should be randomly assigned to the control and treatment groups. After randomisation, there should be no significant differences in mean body weights between groups within each sex. If there are statistically significant differences, then the randomisation step should be repeated, if possible. Each animal should be assigned a unique identification number, and permanently marked with this number by tattooing, microchip implant, or other suitable method.

## **PROCEDURE**

### **Number and sex of animals**

17. Both sexes should be used. A sufficient number of animals should be used so that at the end of the study enough animals in every group are available for thorough biological and statistical evaluation. Normally, at least 20 animals per sex per group should be used at each dose level. In studies involving mice, additional animals may be needed in each dose group to conduct all required haematological determinations. Depending on the aim of the study, it may be possible to increase the statistical power of the key estimates by allocating animals unequally to the different dose levels.

### **Provision for interim kills, satellite groups and sentinel animals**

18. The study may make provision for interim kills, e.g. at 6 months, to provide information on progression of toxicological changes and mechanistic information, if scientifically justified. Satellite groups may also be included to monitor the reversibility of any toxicological changes induced by the chemical under investigation; these will normally be restricted to the highest dose level of the study plus control. An additional group of sentinel animals (typically 5 animals per sex) may also be included for monitoring of disease status, if necessary, during the study (19). If interim kills or inclusion of satellite or sentinel groups are planned, the number of animals included in the study design should be increased by the number of animals scheduled to be killed before the completion of the study. These animals should normally undergo the same observations, including body weight, food/water consumption, haematological and clinical biochemistry measurements and pathological investigations as the animals in the chronic toxicity phase of the main study, although provision may also be made (in the interim kill groups) for measurements to be restricted to specific, key measures such as neurotoxicity or immunotoxicity.

### **Dose groups and dosage**

19. At least three dose levels and a concurrent control should be used, except where a limit test is conducted (see paragraph 26). Dose levels will generally be based on the results of shorter-term repeated dose or range finding studies and should take into account any existing toxicological and toxicokinetic data available for the test substance or related materials.
20. Unless limited by the physical-chemical nature or biological effects of the test substance, the highest dose level should be chosen to identify the principal target organs and toxic effects while avoiding

suffering, severe toxicity, morbidity, or death. While taking into account the factors outlined in paragraph 21 below, the highest dose level should be chosen to elicit evidence of toxicity, as evidenced by, for example, alterations in serum enzyme levels or depression of body weight gain (approximately 10 per cent).

21. However, dependent on the objectives of the study (see paragraph 5), a top dose lower than the dose providing evidence of toxicity may be chosen, e.g. if a dose elicits an adverse effect of concern that nonetheless has little impact on lifespan or body weight. The top dose should not exceed 1000 mg/kg body weight/day (limit dose, see paragraph 26).
22. Dose level spacing should be designed to demonstrate a dose:response and to establish a no-observed-adverse-effect level (NOAEL) or other intended outcome of the study, e.g. a BMD (see paragraph 24) at the lowest dose level. Factors that should be considered in the placement of lower doses include the expected slope of the dose–response curve, the doses at which important changes may occur in metabolism or mode of toxic action, where a threshold is expected, or where a point of departure for low-dose extrapolation is expected.
23. The dose level spacing selected will depend on the characteristics of the test substance, and cannot be prescribed in this Guideline, but 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. Further guidance on dose selection and dose level spacing is provided in an OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (5), but in general the use of factors greater than 10 should be avoided, and must be justified if used.
24. As outlined further in the OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (5), points to be considered in dose selection include:
  - known or suspected nonlinearities or inflection points in the dose–response;
  - pharmacokinetics, and dose ranges where metabolic induction, saturation, or nonlinearity between external and internal doses does or does not occur;
  - precursor lesions, markers of effect, or indicators of the operation of key underlying biological processes;
  - key (or suspected) aspects of mode of action, such as doses at which cytotoxicity begins to arise, hormone levels are perturbed, homeostatic mechanisms are overwhelmed, etc.;
  - regions of the dose–response curve where particularly robust estimation is required, e.g., in the range of the anticipated BMD or a suspected threshold;
  - consideration of anticipated human exposure levels.
25. The control group shall be an untreated group or a vehicle-control group if a vehicle is used in 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 among the dose groups. If a test substance is administered in the diet, and causes reduced dietary intake (of the order of 20% or more) due to the palatability of the diet, an additional pair-fed control group may be useful to allow for this.

26. If it can be anticipated, based on information from preliminary studies, that a test at one dose level, equivalent to at least 1000 mg/kg body weight/day, using the procedures described for this study, is unlikely to produce adverse effects and if toxicity would not be expected based upon data from structurally related substances, then a full study using three dose levels may not be considered necessary. The limit of 1000 mg/kg body weight/day applies except when human exposure indicates the need for a higher dose level to be used.

#### **Preparation of doses and administration of test substance**

27. The test substance is normally administered orally, by gavage or via the diet or drinking water. Additional information on routes and methods of administration is provided in an OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (5). The route and method of administration is dependent on the purpose of the study, the physical/chemical properties of the test substance, its bioavailability and the predominant route and method of exposure of humans. A rationale should be provided for the chosen route and method of administration. In the interests of animal welfare, oral gavage should normally be selected only for those agents for which this route and method of administration reasonably represent potential human exposure (e.g., pharmaceuticals). For dietary or environmental chemicals including pesticides, administration should be via the diet or drinking water.
28. Where necessary, the test substance is dissolved or suspended in a suitable vehicle. 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. 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 should be known. The stability of the test substance under the conditions of administration (e.g., diet) should be determined. The homogeneity of dosing solutions or diets containing the test article (as appropriate) should be confirmed analytically before the start of the study, and periodically (as appropriate) throughout the study, if the dose preparation procedure remains unchanged and if necessary, based on characteristics of the test article and the dosing vehicle.
29. 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. In long-term toxicity studies using dietary administration, the concentration of the chemical in the feed should not normally exceed an upper limit of 5% of the total diet, in order to avoid nutritional imbalances. When the test substance is administered in the diet, either a constant dietary concentration (mg/kg or ppm) or a constant dose level in terms of the animal's body weight, calculated on a weekly basis, may be used; the alternative used should be specified.
30. In the case of oral or dermal administration, the animals are dosed with the test substance daily (seven days each week), normally for a period of 1 year (see also paragraph 31). Any other dosing regime, e.g., five days per week, needs to be justified. Dosing by the inhalation route is carried out for 6 hours per day, 5 days per week.
31. When the test substance is administered by gavage to the animals this should be done using a stomach tube or a suitable intubation cannula, at similar times each day. Normally a single dose will be administered once daily, where for example a compound is a local irritant, it may be possible to

maintain the daily dose-rate by administering it as a split dose (b.i.d). The maximum volume of liquid that can be administered at one time depends on the size of the test animal. Normally the volume should be kept as low as practical, and should not exceed 1 ml/100g body weight, except in the case of aqueous solutions where 2 ml/100g body weight may be used. Variability in test volume should be minimised by adjusting the concentration to ensure a constant volume at all dose levels. Potentially corrosive or irritant substances are the exception, and need to be diluted to avoid severe local effects. The pH of dosing solutions should normally lie in the range of 4 to 9.

### **Duration of study**

32. While this Test Guideline primarily is designed as a 12 month chronic toxicity study, the study design also allows for and can be applied to either shorter (e.g. 6 or 9 months) or longer (e.g., 18 or 24 months) duration studies, depending on the requirements of particular regulatory regimes or for specific mechanistic purposes. Deviations from the exposure duration of 12 months must be justified, particularly in the case of shorter durations. Satellite groups included to monitor the reversibility of any toxicological changes induced by the chemical under investigation should be maintained without dosing for a period not less than 4 weeks and not more than one third of the total study duration after cessation of exposure. Further guidance, including consideration of survival in the study, is provided in an OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (5).

### **OBSERVATIONS**

33. All animals should be checked for morbidity or mortality and for specific signs of toxicological relevance, in particular for neurofunctional and neurobehavioural signs (20), usually at the beginning and the end of each day. Additionally, animals should be checked at least once each weekend day and holiday. 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.
34. Detailed clinical observations should be made on all animals at least once prior to the first exposure (to allow for within-subject comparisons), at the end of the first week of the study and monthly thereafter. The protocol for observations should be arranged such that variations between individual observers are minimised and independent of test group. 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. Efforts 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, 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) or bizarre behaviour (e.g., self-mutilation, walking backwards) should also be recorded (20).
35. Ophthalmological examination, using an ophthalmoscope or other suitable equipment, should be carried out on all animals prior to the first administration of the test substance. At the termination of the study, this examination should be preferably conducted in all animals but at least in the high dose and control groups. If treatment-related changes in the eyes are detected, all animals should be examined. If structural analysis or other information suggests ocular toxicity, then the frequency of ocular examination should be increased.

36. For chemicals where previous repeated dose 28-day and/or 90-day toxicity tests indicated the potential to cause neurotoxic effects, sensory reactivity to stimuli of different types (20) (e.g., auditory, visual and proprioceptive stimuli) (21), (22), (23), assessment of grip strength (24) and motor activity assessment (25) may optionally be conducted before commencement of the study and then at 3, 6, 9 and 12 months. 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.
37. For chemicals where previous repeated dose 28-day and/or 90-day toxicity tests indicated the potential to cause immunotoxic effects, further investigations of this endpoint may optionally be conducted at 12 months.

### **Body weight, food/water consumption and food efficiency**

38. All animals should be weighed at the start of treatment, at least once a week for the first 13 weeks, and at least monthly thereafter. Measurements of food consumption and food efficiency should be made at least weekly for the first 13 weeks and at least monthly thereafter. Water consumption should also be considered for studies in which drinking activity is altered, and should be measured at least weekly for the first 13 weeks and at least monthly thereafter, when the substance is administered in drinking water.

### **Haematology and clinical biochemistry**

39. In studies involving rodents, haematological examinations should be carried out in at least 10 male and 10 female animals per group, at 3, 6, and 12 months, using the same animals throughout. In mice, satellite animals may be required in order to conduct all required haematological determinations (see paragraph 17). In non-rodent studies, samples will be taken from smaller numbers of animals (e.g. 4 animals per sex and per group in dog studies). Measurements at 3 months need not be conducted if no effect was seen on haematological parameters in a previous 90 day study carried out at comparable dose levels. Samples should be collected at the end of the designated test period, namely from animals on study at 3 and 6 months, and at 12 months just prior to or as part of the procedure for killing the animals. Blood samples should be taken from a named site, for example by cardiac puncture or retro-orbital sinus, and stored, if applicable, under appropriate conditions. The following list of parameters should be investigated (26): Total and differential leukocyte count, erythrocyte count, platelet count, haemoglobin concentration, haematocrit (packed cell volume), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), prothrombin time, and activated partial thromboplastin time. In addition Heinz bodies or other atypical erythrocyte morphology and methaemoglobin should be investigated if there is any indication of anaemia or other haematopoietic disorders. If the chemical has an effect on the haematopoietic system, reticulocyte counts and bone marrow cytology may also be indicated, although these need not be routinely conducted.
40. 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 at least 10 male and 10 female animals per group at the same time intervals as specified for the haematological investigations, using the same animals throughout. In mice, satellite animals may be required in order to conduct all required clinical biochemistry determinations. Measurements at 3 months need not be conducted if no effect was seen on clinical biochemistry parameters in a previous 90 day study carried out at comparable dose levels. Overnight fasting of the animals (with the exception of mice) prior to blood sampling is recommended. The following list of parameters should be investigated (26): glucose, urea (urea nitrogen), creatinine, total protein, albumin, calcium, sodium, potassium, total

cholesterol, at least two appropriate tests for hepatocellular evaluation (alanine aminotransferase, aspartate aminotransferase, glutamate dehydrogenase, total bile acids)(27), and at least two appropriate tests for hepatobiliary evaluation (alkaline phosphatase, gamma glutamyl transferase, 5'-nucleotidase, total bilirubin, total bile acids)(27). Other clinical chemistry parameters such as fasting triglycerides, specific hormones and cholinesterase may be measured as appropriate, depending on the toxicity of the substance. Overall, there is a need for a flexible approach, depending on the observed and/or expected effect from a given substance.

41. Urinalysis determinations should be performed on at least 10 male and 10 female animals per group on samples collected at the same intervals as for haematology and clinical chemistry. Measurements at 3 months need not be conducted if no effect was seen on urinalysis in a previous 90 day study carried out at comparable dose levels. The following list of parameters was included in a recent expert recommendation on clinical pathology studies (26): appearance, volume, osmolality or specific gravity, pH, total protein, and glucose. Other determinations include ketone, urobilinogen, bilirubin, and occult blood. Further parameters may be employed where necessary to extend the investigation of observed effect(s).
42. It is generally considered that baseline haematological and clinical biochemistry variables need not be determined before treatment (26). However, if historical baseline data (see paragraph 47) are inadequate, consideration should be given to generating such data.

## **Pathology**

### **Gross necropsy**

43. All animals in the study shall normally 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. However provision may also be made (in the interim kill or satellite groups) for measurements to be restricted to specific, key measures such as neurotoxicity or immunotoxicity (see paragraph 18). These animals need not be subjected to necropsy and the subsequent procedures described in the following paragraphs. Sentinel animals may require necropsy on a case-by-case basis, at the discretion of the study director.
44. Organ weights should be collected from all animals, other than those excluded by the latter part of paragraph 42. The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thyroid (weighed post-fixation, with parathyroids), and uterus of all animals (apart from those found moribund and/or intercurrently killed) should be trimmed of any adherent tissue, as appropriate, and their wet weight taken as soon as possible after dissection to prevent drying. In the case of paired organs, e.g., kidney, adrenal, both organs should be weighed separately. In a study using mice, weighing of the adrenal glands is optional.
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 (27): all gross lesions, adrenal gland, aorta, brain (including sections of cerebrum, cerebellum, and medulla/pons), caecum, cervix, coagulating gland, colon, duodenum, epididymis, eye (including retina), [femur with joint] gall bladder (for species other than rat), Harderian gland, heart, ileum, jejunum, kidney, lacrimal gland (exorbital), liver, lung, lymph nodes (both superficial and deep), female mammary gland, [nasal tissue], oesophagus, [olfactory bulb], ovary, pancreas, parathyroid gland, peripheral nerve, pituitary, prostate, [rectum], salivary gland, seminal vesicle, skeletal muscle, skin, spinal cord (at three levels: cervical, mid-thoracic, and lumbar), spleen, [sternum], stomach (forestomach, glandular stomach), [teeth], testis, thymus, thyroid gland, [tongue], trachea, urinary bladder, uterus (including cervix),

[ureter], [urethra], vagina, and a section of bone marrow and/or a fresh bone marrow aspirate). Tissues in square brackets are optional. In the case of paired organs, e.g., kidney, adrenal, both organs should be preserved. 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. In studies involving the dermal route of administration, the list of organs as set out for the oral route should be preserved, and specific sampling and preservation of the skin from the site of application is essential. In inhalation studies, the list of preserved and examined tissues from the respiratory tract should follow the recommendations of Test Guideline 412. For other organs/tissues (and in addition to the specifically preserved tissues from the respiratory tract) the list of organs as set out for the oral route has to be examined.

### **Histopathology**

46. Guidance is available on best practices in the conduct of toxicological pathology studies (27). The minimum histopathological examinations should be:
- all tissues from the high dose and control groups;
  - all tissues from animals dying or killed during the study;
  - all tissues showing macroscopic abnormalities;
  - target tissues, or tissues which showed treatment-related changes in the high dose group, from all animals in all other dose groups;
  - in the case of paired organs, e.g., kidney, adrenal, both organs should be examined.
47. Individual animal data should be provided for all parameters evaluated. 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 and the time of any death or humane kill, the number showing signs of toxicity, a description of the signs of toxicity observed, including time of onset, duration, and severity of any toxic effects, the number of animals showing lesions, the type of lesions and the percentage of animals displaying each type of lesion.
48. In addition to data obtained from the concurrent controls used in the study, the use of historical control data may be valuable in the interpretation of the results of the study. This is particularly the case where there are indications that the data provided by the concurrent controls are substantially out of line compared to recent data from control animals from the same test facility colony. Historical control data should be used only if concurrent controls appear to be significantly different; the priority should be placed on use of concurrent control over historical control data. Historical control data, if evaluated, should be submitted from the same laboratory, strain, species and specific ranges should be provided. The historical control data should be separated by sex and malignant and benign lesions should be presented separate and combined, where appropriate, and preferably by individual study. The use of historical data should be restricted to data generated during the five years preceding the study in question.
49. 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. Selection should make provision for survival adjustments, if needed.

### **TEST REPORT**

49. The test report should include the following information:
- Test substance:

- physical nature, purity, and physicochemical properties;
  - identification data;
  - source of substance;
  - batch number
- Vehicle (if appropriate):
  - justification for choice of vehicle (if other than water).
- Test animals:
  - species/strain used and justification for choice made;
  - number, age, and sex of animals at start of test;
  - source, housing conditions, diet, etc.;
  - individual weights of animals at the start of the test.
- Test conditions:
  - rationale for route of administration and dose selection;
  - when applicable, the statistical methods used to analyse the data;
  - details of test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation;
  - route of administration and details of the administration of the test substance;
  - for inhalation studies, whether nose only or whole body;
  - actual doses (mg/kg body weight/day), and conversion factor from diet/drinking water test substance concentration (mg/kg or ppm) to the actual dose, if applicable;
  - details of food and water quality.
- Results:
  - survival data;
  - body weight/body weight changes;
  - food consumption, calculations of food efficiency, if made, and water consumption if applicable;
  - toxic response data by sex and dose level, including signs of toxicity;
  - nature, incidence (and, if scored, severity), and duration of clinical observations ((whether transitory or permanent);
  - ophthalmological examination;
  - haematological tests;
  - clinical biochemistry tests;
  - urinalysis tests;
  - outcome of any investigations of neurotoxicity or immunotoxicity
  - terminal body weight;
  - organ weights (and their ratios, if applicable);
  - necropsy findings;
  - a detailed description of all treatment-related histopathological findings;
  - absorption data if available;
- Statistical treatment of results, where appropriate.
  - body weights,
  - organ weights,
  - feed consumption (or water consumption) and food efficiency.

- Discussion of results including:
  - Dose:response relationships
  - Consideration of any mode of action information
  - Discussion of any modelling approaches
  - Historical control data
  - Relevance for humans
  
- Conclusions

## LITERATURE

1. OECD (Rome, 1995). Report of the Consultation Meeting on Sub-chronic and Chronic Toxicity/Carcinogenicity Testing.
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## ANNEX

### DEFINITIONS

**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).

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

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

*To be expanded as appropriate*

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