Adopted:
7 September 2009

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Carcinogenicity Studies

INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals (TGs) are periodically reviewed in the light of scientific progress, changing assessment practices and animal welfare considerations. The original Test Guideline 451 on Carcinogenicity Studies was adopted in 1981. Development of a revised TG 451 was considered necessary, in order to reflect recent developments in the field of animal welfare and regulatory requirements (1) (2) (3) (4) (5). The updating of TG 451 has been carried out in parallel with revisions of the Test Guidelines 452, Chronic Toxicity Studies, and 453, Combined Chronic Toxicity\Carcinogenicity Studies**,** and with the objective of obtaining additional information from the animals used in the study and providing further detail on dose selection. This Test Guideline is designed to be used in the testing of a broad range of chemicals, including pesticides and industrial chemicals. It should be noted however that some details and requirements may differ for pharmaceuticals (see International Conference on Harmonisation (ICH) Guidance S1B on Testing for Carcinogenicity of Pharmaceuticals).

2. The majority of carcinogenicity 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 in this Guideline together with those outlined in OECD TG 409, Repeated Dose 90-day Oral Toxicity Study in Non-Rodents (6) should be applied, with appropriate modifications. Further guidance is available in the OECD Guidance Document No. 116 on the Design and Conduct of Chronic Toxicity and Carcinogenicity Studies (7).

3. The three main routes of administration used in carcinogenicity 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 Guidance Document No. 116 (7).

4. This Guideline focuses on exposure via the oral route, the route most commonly used in carcinogenicity studies. While carcinogenicity 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 carcinogenicity 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 dermal (7), and inhalation routes (7) (8). TG 412 (9) and TG 413 (10), together with the associated OECD Guidance Document on acute inhalation testing (8), should be specifically consulted

in the design of longer term studies involving exposure via the inhalation route. TG 410 (11) should be consulted in the case of testing carried out by the dermal route.

5. The carcinogenicity study provides information on the possible health hazards likely to arise from repeated exposure for a period lasting up to the entire lifespan of the species used. The study will provide information on the toxic effects of the substance including potential carcinogenicity, and may indicate target organs and the possibility of accumulation. It can provide an estimate of the no-observed-adverse effect level for toxic effects and, in the case of non-genotoxic carcinogens, for tumour responses, 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.

6. The objectives of carcinogenicity studies covered by this test guideline include:

- The identification of the carcinogenic properties of a chemical, resulting in an increased incidence of neoplasms, increased proportion of malignant neoplasms or a reduction in the time to appearance of neoplasms, compared with concurrent control groups,
- The identification of target organ(s) of carcinogenicity;
- The identification of the time to appearance of neoplasms;
- Characterisation of the tumour dose-response relationship;
- Identification of a no-observed-adverse-effect level (NOAEL) or point of departure for establishment of a Benchmark Dose (BMD);
- Extrapolation of carcinogenic effects to low dose human exposure levels;
- Provision of data to test hypotheses regarding mode of action (2) (7) (12) (13) (14) (15).

INITIAL CONSIDERATIONS

7. In the assessment and evaluation of the potential carcinogenicity 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 carcinogenic potential and to minimize animal usage. Information on, and consideration of, the mode of action of a suspected carcinogen (2) (7) (12) (13) (14) (15) is particularly important, since the optimal design may differ depending on whether the substance is a known or suspected genotoxic carcinogen. Further guidance on mode of action considerations can be found in Guidance Document No.116 (7).

8. Information that will assist in the study design includes the identity, chemical structure, and physico-chemical properties of the test substance; results of any *in vitro* or *in vivo* toxicity tests including genotoxicity tests; anticipated use(s) and potential for human exposure; available (Q)SAR data, mutagenicity/genotoxicity, carcinogenicity and other 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. Assessment of carcinogenicity should be carried out after initial information on toxicity has been obtained from repeated dose 28-day and/or 90-day toxicity tests. Short-term cancer initiation-promotion tests could also provide useful information. A phased testing approach to carcinogenicity testing should be considered as part of the overall assessment of the potential adverse health effects of a particular chemical (16) (17) (18) (19).

9. The statistical methods most appropriate for the analysis of results, given the experimental design and objectives, should be established before commencing the study. Issues to consider include

whether the statistics should include adjustment for survival, analysis of cumulative tumour risks relative to survival duration, analysis of the time to tumour and analysis in the event of premature termination of one or more groups. Guidance on the appropriate statistical analyses and key references to internationally accepted statistical methods are given in Guidance Document No.116 (7), and also in Guidance Document No. 35 on the analysis and evaluation of chronic toxicity and carcinogenicity studies (20).

10. In conducting a carcinogenicity study, the guiding principles and considerations outlined in the OECD Guidance Document No. 19 on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation (21), in particular paragraph 62 thereof, should always be followed. This paragraph states that "*In studies involving repeated dosing, when an animal shows clinical signs that are progressive, leading to further deterioration in condition, an informed decision as to whether or not to humanely kill the animal should be made. The decision should include consideration as to the value of the information to be gained from the continued maintenance of that animal on study relative to its overall condition. If a decision is made to leave the animal on test, the frequency of observations should be increased, as needed. It may also be possible, without adversely affecting the purpose of the test, to temporarily stop dosing if it will relieve the pain or distress, or reduce the test dose.*"

11. Detailed guidance on and discussion of the principles of dose selection for chronic toxicity and carcinogenicity studies can be found in Guidance Document No.116 (7) as well as two International Life Sciences Institute publications (22) (23). The core dose selection strategy is dependent on the primary objective or objectives of the study (paragraph 6). In selecting appropriate dose levels, a balance should be achieved between hazard screening on the one hand and characterization 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 12).

12. 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). The combined test provides greater efficiency in terms of time and cost compared to conducting two separate studies, without compromising the quality of the data in either the chronic phase or the carcinogenicity phase. Careful consideration should however be given to the principles of dose selection (paragraphs 11 and 22-25) 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.

13. Definitions used in the context of this Test Guideline can be found in Guidance Document No. 116 (7).

PRINCIPLE OF THE TEST

14. The test substance is administered daily in graduated doses to several groups of test animals for the majority of their life span, normally by the oral route. Testing by the inhalation or dermal route may also be appropriate. The animals are observed closely for signs of toxicity and for the development of neoplastic lesions. 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 METHOD

Selection of animal species

15. This Guideline primarily covers assessment and evaluation of carcinogenicity in rodents (paragraph 2). The use of non-rodent species may be considered when available data suggest that they are more relevant for the prediction of health effects in humans. The choice of species should be justified. The preferred rodent species is the rat, although other rodent species, e.g., the mouse, may be used. Although the use of the mouse in carcinogenicity testing may have limited utility (24) (25) (26), under some current regulatory programmes carcinogenicity testing in the mouse is still required. 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. Additional information on choice of species and strain is provided in Guidance Document No.116 (7).

16. Young healthy adult animals of commonly used laboratory strains should be employed. The carcinogenicity study should preferably be carried out in animals from the same strain and source as those used in preliminary toxicity study(ies) of shorter duration although, if animals from this strain and source are known to present problems in achieving the normally accepted criteria of survival for long-term studies (see Guidance Document No. 116 (7)), consideration should be given to using a strain of animal that has an acceptable survival rate for the long-term study. The females should be nulliparous and non-pregnant.

Housing and feeding

17. Animals may be housed individually, or be caged in small groups of the same sex; individual housing should be considered only if scientifically justified (27) (28) (29). 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 (± 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

18. 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 for each sex of animal used should be minimal and not exceed ± 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

19. Both sexes should be used. A sufficient number of animals should be used so that a thorough biological and statistical evaluation is possible. Each dose group and concurrent control group should therefore contain at least 50 animals of each sex. Depending on the aim of the study, it may be possible to increase the statistical power of the key estimates by differentially allocating animals unequally to the various dose groups, with more than 50 animals in the low dose groups; e.g., to estimate the carcinogenic potential at low doses. However it should be recognized that a moderate increase in group size will provide relatively little increase in statistical power of the study. Further information on statistical design of the study and choice of dose levels to maximise statistical power is provided in Guidance Document No. 116 (7).

Provision for interim kills and satellite (sentinel) groups

20. The study may make provision for interim kills, e.g., at 12 months, to provide information on progression of neoplastic changes and mechanistic information, if scientifically justified. Where such information is already available from previous repeat dose toxicity studies on the substance, interim kills may not be scientifically justified. If interim kills are included in the study design, the number of animals in each dose group scheduled for an interim kill will normally be 10 animals per sex, and the total 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. An additional group of sentinel animals (typically 5 animals per sex) may be included for monitoring of disease status, if necessary, during the study (30). Further guidance is provided in Guidance Document No. 116 (7).

Dose groups and dosage

21. Guidance on all aspects of dose selection and dose level spacing is provided in Guidance Document No. 116 (7). At least three dose levels and a concurrent control should be used. 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.

22. 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 23 below, the highest dose level should normally be chosen to elicit evidence of toxicity, as evidenced by, for example, depression of body weight gain (approximately 10%). However, dependent on the objectives of the study (see paragraph 6), 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.

23. Dose levels and dose level spacing may be selected to establish a dose-response and, depending on the mode of action of the test substance, a NOAEL or other intended outcome of the study, e.g. a BMD (see paragraph 25) 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.

24. 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 frequently provide good test performance 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. In general, the use of factors greater than 10 should be avoided, and should be justified if used.

25. As discussed further in Guidance Document No. 116 (7), points to be considered in dose selection include:

- Known or suspected nonlinearities or inflection points in the dose–response;
- Toxicokinetics, 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 needed, e.g., in the range of the anticipated BMD or a suspected threshold;
- Consideration of anticipated human exposure levels.

26. 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 significantly reduced dietary intake due to the reduced palatability of the diet, an additional pair-fed control group may be useful, to serve as a more suitable control.

Preparation of doses and administration of test substance

27. The test substance is normally administered orally, via the diet or drinking water, or by gavage. Additional information on routes and methods of administration is provided in Guidance Document No. 116 (7). 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 interest 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 is typically via the diet or drinking water. However, for some scenarios, e.g., occupational exposure, administration via other routes may be more appropriate.

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. Information should be available on the stability of the test substance and the homogeneity of dosing solutions or diets (as appropriate) under the conditions of administration (e.g., diet).

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 diet or ppm) or a constant dose level in terms of the animal's body weight (mg/kg body weight), calculated on a weekly basis, may be used. The alternative used should be specified.

30. In the case of oral administration, the animals are dosed with the test substance daily (seven days per week), normally for a period of 24 months for rodents (see also paragraph 32). Any other dosing regime, e.g., five days per week, needs to be justified. In the case of dermal administration, animals are normally treated with the test substance for at least 6 hours per day, 7 days per week, as specified in TG 410 (11), for a period of 24 months. Exposure by the inhalation route is carried out for 6 hours per day, 7 days per week, but exposure for 5 days per week may also be used, if justified. The period of exposure will normally be for a period of 24 months. If rodent species other than rats are exposed nose-only, maximum exposure durations may be adjusted to minimise species-specific distress. A rationale should be provided when using an exposure duration less than 6 hours per day. See also TG 412 (9).

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 (twice a day). The maximum volume of liquid that can be administered at one time depends on the size of the test animal. The volume should be kept as low as practical, and should not normally exceed 1 ml/100g body weight for rodents (31). 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. Testing at concentrations that are likely to be corrosive or irritant to the gastrointestinal tract should be avoided.

Duration of study

32. The duration of the study will normally be 24 months for rodents, representing the majority of the normal life span of the animals to be used. Shorter or longer study durations may be used, dependent on the lifespan of the strain of the animal species in the study, but should be justified. For specific strains of mice, e.g., AKR/J, C3H/J or C57BL/6J strains a duration of 18 months may be more appropriate. The following provides some guidance on duration, termination of the study and survival;

further guidance, including consideration of the acceptability of a negative carcinogenicity relative to survival in the study, is provided in the OECD Guidance Document No. 116 on the Design and Conduct of Chronic Toxicity and Carcinogenicity Studies(7).

- Termination of the study should be considered when the number of survivors in the lower dose groups or the control group falls below 25 per cent.
- In the case where only the high dose group dies prematurely due to toxicity, this should not trigger termination of the study.
- Survival of each sex should be considered separately.
- The study should not be extended beyond the point when the data available from the study are no longer sufficient to enable a statistically valid evaluation to be made.

OBSERVATIONS

33. All animals should be checked for morbidity or mortality, usually at the beginning and the end of each day, including at weekends and holidays. Animals should additionally be checked once a day for specific signs of toxicological relevance, taking into consideration the peak period of anticipated effects after dosing in the case of gavage administration. Particular attention should be paid to tumour development; and the time of tumour onset, location, dimensions, appearance, and progression of each grossly visible or palpable tumour should be recorded.

Body weight, food/water consumption and food efficiency

34. 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 be measured at least weekly for the first 13 weeks and at least monthly thereafter when the substance is administered in drinking water. Water consumption measurements should also be considered for studies in which drinking activity is altered.

Haematology, clinical biochemistry and other measurements

35. In order to maximise the information obtained from the study, especially for mode of action considerations, blood samples may be taken for haematology and clinical biochemistry, and this at the discretion of the study director. Urinalysis may also be appropriate. Further guidance on the value of taking such samples as part of a carcinogenicity study is provided in Guidance Document No. 116 (7). If considered appropriate, blood sampling for haematological and clinical chemistry determinations and urinalysis may be conducted as part of an interim kill (paragraph 20) and at study termination on a minimum of 10 animals per sex per group. Blood samples should be taken from a named site, for example by cardiac puncture or from the retro-orbital sinus under anaesthesia, and stored, if applicable, under appropriate conditions. Blood smears may also be prepared for examination, particularly if bone marrow appears to be the target organ, although the value of such examination for the assessment of carcinogenic/oncogenic potential has been questioned (32).

PATHOLOGY

Gross necropsy

36. All animals in the study except sentinel animals (see paragraph 20) and other satellite animals should 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. Sentinel animals and other satellite animals may require necropsy on a case-by-case basis, at the discretion of the study director. Organ weights are not normally part of a carcinogenesis study, since geriatric changes and, at later stages, the development of tumours confounds the usefulness of organ weight data. They may, however, be critical to performing a weight of evidence evaluation and especially for mode of action considerations. If they are part of a satellite study, they should be collected at no later than one year after initiation of the study.

37. The following tissues should be preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent histopathological examination (33) (tissues in square brackets are optional):

all gross lesions	heart	pancreas	stomach (forestomach, glandular stomach)
adrenal gland	ileum	parathyroid gland	[teeth]
aorta	jejunum	peripheral nerve	testis
brain (including sections of cerebrum, cerebellum, and medulla/pons)	kidney	pituitary	thymus
caecum	lacrimal gland (exorbital)	prostate	thyroid
cervix	liver	rectum	[tongue]
coagulating gland	lung	salivary gland	trachea
colon	lymph nodes (both superficial and deep)	seminal vesicle	urinary bladder
duodenum	mammary gland (obligatory for females and, if visibly dissectable, from males)	skeletal muscle	uterus (including cervix)
epididymis	[upper respiratory tract, including nose, turbinates, and paranasal sinuses]	skin	[ureter]
eye (including retina)	oesophagus	spinal cord (at three levels: cervical, mid-thoracic, and lumbar)	[urethra]
[femur with joint]	[olfactory bulb]	spleen	vagina
gall bladder (for species other than rat)	ovary	[sternum],	section of bone marrow and/or a fresh bone marrow aspirate
Harderian gland			

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 TG 412 (9) and TG 413 (10). 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 should be examined.

Histopathology

38. Guidance is available on best practices in the conduct of toxicological pathology studies (33). The minimum tissues examined should be:

- All tissues from the high dose and control groups;
- All tissues of animals dying or killed during the study;
- All tissues showing macroscopic abnormalities including tumours;
- When treatment-related histopathological changes are observed in the high dose group, those same tissues are to be examined from all animals in all other dose groups;
- In the case of paired organs, e.g., kidney, adrenal, both organs should be examined.

DATA AND REPORTING

Data

39. 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. Summary data tables should provide the means and standard deviations (for continuous test data) of animals showing toxic effects or lesions, in addition to the grading of lesions.

40. Historical control data may be valuable in the interpretation of the results of the study, e.g. in the case when there are indications that the data provided by the concurrent controls are substantially out of line when compared to recent data from control animals from the same test facility/colony. Historical control data, if evaluated, should be submitted from the same laboratory and relate to animals of the same age and strain generated during the five years preceding the study in question.

41. 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 (paragraph 9). Selection should make provision for survival adjustments, if needed.

Test report

42. The test report should include the following information:

Test substance:

- physical nature, purity, and physicochemical properties;
- identification data;

- source of substance;
- batch number;
- certificate of chemical analysis;

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.
- analytical data on 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 (summary tabulated data and individual animal data should be presented)

General

- survival data;
- body weight/body weight changes;
- food consumption, calculations of food efficiency, if made, and water consumption, if applicable;
- toxicokinetic data if available;
- opthalmoscopy (if available);
- haematology (if available);
- clinical chemistry (if available);

Clinical findings

- Signs of toxicity;
- Incidence (and, if scored, severity) of any abnormality;
- Nature, severity, and duration of clinical observations (whether transitory or permanent);

Necropsy data

- Terminal body weight;
- Organ weights and their ratios, if applicable;
- Necropsy findings; Incidence and severity of abnormalities;

Histopathology

- Non neoplastic histopathological findings,;
- Neoplastic histopathological findings;
- Correlation between gross and microscopic findings;
- Detailed description of all treatment-related histopathological findings including severity gradings;
- Report of any peer review of slides;

Statistical treatment of results, as appropriate

Discussion of results including

- Discussion of any modelling approaches;
- Dose-response relationships;
- Historical control data;
- Consideration of any mode of action information;
- BMD, NOAEL or LOAEL determination;
- Relevance for humans;

Conclusions

LITERATURE

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