DRAFT OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Test Guideline 451: Carcinogenicity 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 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.

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 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 (6).

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 an OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (6).

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 inhalation (6)(7) and dermal routes (6). 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 carcinogenicity studies covered by this test guideline include:
   - the identification of the carcinogenic properties of a chemical, resulting in an increased incidence of neoplasms compared with concurrent control groups,
   - the identification of target organ(s) of carcinogenicity,
   - 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,
INITIAL CONSIDERATIONS

6. 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 that will assist in the study design includes the identity, chemical structure, and physico-chemical properties of the test article substance; any information on the mode of action; 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 (14)(15)(16)(17).

7. In conducting a carcinogenicity 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 (18), in particular paragraph 62 thereof, should always be followed.

8. 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 (6) as well as two International Life Sciences Institute publications (19)(20). 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.

9. 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 8 and 19-23) 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.

10. Definitions used are given in the Annex.

PRINCIPLE OF THE TEST

11. 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 (paragraphs 3 - 4). 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 also killed and necropsied.
DESCRIPTION OF METHOD

Selection of animal species

12. 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 must 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 (21)(22)(23), 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 an OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (6).

13. 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 OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (6)), 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

14. Animals may be housed individually, or be caged in small groups of the same sex; individual housing should be considered only if scientifically justified (24)(25)(26). 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

15. 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 ± 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

16. 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 recognised 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 an OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (6).

Provision for interim kills and satellite (sentinel) groups

17. 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. 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 (27). Further guidance is provided in an OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (6).

Dose groups and dosage

18. 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. For most chemicals, a limit test is not considered appropriate for an assessment of carcinogenicity.
19. 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 20 below, the highest dose level should be chosen to elicit evidence of toxicity, as evidenced by, for example, depression of body weight gain (approximately 10 per cent).

20. 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.

21. 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 23) 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.

22. 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 (6), but in general the use of factors greater than 10 should be avoided, and must be justified if used.

23. As outlined further in the OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (6), 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.

24. 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.

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

25. 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 (6). 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.

26. 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 possible solution in other vehicles. For vehicles other than water, the toxic characteristics of the vehicle should be known. 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.

27. 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.

28. 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 18 (for mice and hamsters) to 24 months (for rats) (see also paragraph 30). 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.

29. 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

30. The duration of the study will normally be 24 months for rats and 18 months for mice and hamsters, representing the majority of the normal life span of the animals to be used. 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 an OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (6)

- 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

31. All animals should be checked for morbidity or mortality and for specific signs of toxicological relevance (see TG 452, chronic toxicity studies, paragraph 34), usually at the beginning and the end of each working day. Additionally, animals should be checked at least once each weekend day and holiday. Particular attention should be paid to tumour development; the time of onset, location, dimensions, appearance, and progression of each grossly visible or palpable tumour should be recorded.

Body weight, food/water consumption and food efficiency

32. 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, clinical biochemistry and other measurements

33. 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, although this is not obligatory and is 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 an OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (6). If blood samples are taken,
these should be collected at the end of the test period, just prior to or as part of the procedure for killing the animals. They should be taken from a named site, for example by cardiac puncture or retro-orbital sinus, 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 (28).

PATHOLOGY

Gross necropsy

34. All animals in the study except sentinel animals (see paragraph 17) and other satellite animals 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. 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.

35. The following tissues should be preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent histopathological examination (24): 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, 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 (foregut, 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

36. Guidance is available on best practices in the conduct of toxicological pathology studies (29). 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

37. 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.

38. 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 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 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.

39. 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

40. 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:

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;

Clinical findings
  – Include 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, where appropriate.
  – body weights,
  – organ weights,
  – feed consumption (or water consumption)
  – Tumour incidences

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

Conclusions

LITERATURE


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