
•8. UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia

The original (1988) version of the Guidelines was prepared for the UKCCCR by an *ad hoc* committee comprising:

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Background and scope

While we recognize and encourage the development of alternative research techniques which do not involve animals, we consider that there are many questions in oncology research which can be answered only by the study of tumours growing *in vivo*.

Animals with local or disseminated tumours are likely to experience pain and/or distress, thus justifying special care and attention from both licensees and others involved in their welfare. Associated techniques including surgical preparation, irradiation, and drug administration may increase the severity of an experimental procedure. Recognising this, the United Kingdom Coordinating Committee on Cancer Research (UKCCCR) in 1988 set up an *ad hoc* committee to develop guidelines for research workers using animals in experimental neoplasia. The MCCC (for membership see below*) is charged by the major bodies involved in the funding of cancer research in the UK with the coordination and development of areas in which they have a common interest. The members of the *ad hoc* committee were selected so as to represent a wide range of specialities which make use of animal tumour models in cancer research together with experts in animal husbandry and welfare and an observer from the Home Office Inspectorate. Feedback on the 1988 UMCCR guidelines has indicated that these were well received and have been widely used in the UK, as well as having an influence overseas. It was explicitly stated in the 1988 guidelines that procedures practised upon animals in cancer research, and in particular that humane endpoints used, should be subject to a continuous process of refinement. Indeed the 'Three Rs', that is reduction (in numbers),

* Member Organisations of UKCCCR. Cancer Research campaign, Imperial Cancer Research Fund, Institute of Cancer Research, Leukaemia Research Fund, Ludwig Institute for Cancer Research, Marie Curie Foundation, Medical Research Council, Tenovus Cancer Fund. Observers: Department of Health, Scottish Office Department of Health.

refinement (of methods) and replacement (of animals by other techniques where appropriate) should constantly be borne in mind by all users of experimental animals (Russell and Burch, 1959; Roush, 1966; Balls, 1994; Festing, 1994; Flecknell, 1994). Both science and attitudes to animal work change. Accordingly, it was envisaged in the 1988 guidelines that these would be modified and updated as necessary. The present edition contains a number of changes and will again be modified in the future should this be necessary. To aid this process, feedback on the guidelines is actively encouraged.

As before, particular emphasis within the guidelines is focused on the prediction and recognition of adverse effects and the implementation of humane end points. The majority of work in experimental neoplasia utilises small laboratory animals, particularly rodents. Consequently we have drawn largely on available expertise with these species. However, the general principles are applicable to all species of animal. It should be noted that these guidelines are not intended to apply to the treatment of veterinary patients with spontaneous tumours where different considerations apply. In most instances induced models of neoplastic disease will be less traumatic to the host animal than clinical disease.

The general welfare of laboratory animals and the performance of regulated procedures upon them are both covered in the United Kingdom by the Animals (Scientific Procedures) Act (1986) effective from 1 January 1987. Under this Act all scientific procedures on living vertebrates which may have the effect of causing pain, suffering, distress or lasting harm are controlled by the Home Office and require specific authority through Personal and Project Licences.

Guidance on the operation of the 1986 Act and Codes of Practice for the Housing and Care of Animals have been produced by the Home Office (see Bibliography, page 30). In addition, a number of references which provide useful advice on general animal husbandry and experimental techniques are listed in the Bibliography.

The 1986 Act, together with the Home Office document listed above and the 1987 Royal Society/UFAW Guidelines (see Bibliography), provide a firm basis for experimental practice. We would welcome the publication of further guidelines from expert sources. We envisage that the present guidelines will be of general value to workers carrying out experiments

which involve the growth of tumours in experimental animals, which arise spontaneously (including those in transgenic and gene 'knockout' animals), are produced by transplantation (including routine passage tumours, orthotopic tumours and hybridomas), or are induced by carcinogenic agents. The guidelines may be especially helpful in the completion of Project Licence applications, in particular section 19b (v and vi) which requires that applicants list the **possible adverse effects** and their **likely incidence** as well as the **proposed methods of controlling severity**, eg the use of **analgesia**, regional or local **anaesthesia** and **sedation**, and the implementation of **humane end points**. The guidelines are not mandatory. The term 'should' is used to encourage attainment of desirable standards; the term 'must' is used only where legal obligations apply.

The Recommendations are divided into two parts. The General Recommendations are applicable to all regulated procedures. The Specific Recommendations are more directly targeted to the particular problems of experimental neoplasia. It is important to emphasize that procedural guidelines, especially with respect to implementation of humane end points, must be tailored to the precise nature of each individual experimental neoplasia model. To illustrate this, Appendix 1 gives some examples of criteria for particular tumour systems. More detailed and specific information regarding various procedures is given in Appendices 2-6.

Recommendations

General Recommendations

1. The following recommendations are based on the premise that for each individual study those involved in the procedures will weigh the likely adverse effects on the animals used against the benefits likely to accrue from the work. Cancer is a disease of major unmet medical need and the potential benefits of cancer research are clear. Nevertheless, the feasibility of using alternative methods not involving live animals should always be considered. In vitro cell lines may be appropriate in many instances as illustrated by the decision of the US National Cancer Institute to replace the use of transplantable murine tumours in primary anticancer drug screening with panels of in vitro human tumour cell lines (Boyd, 1986). Further examples are the increasing use of in vitro methods (rather than ascites tumours) for the production of

- monoclonal antibodies, and the development of 'test cascades' for drug discovery in the pharmaceutical industry (see Appendix 4). The use of animals for study of the therapeutic effects of administered substances without prior *in vitro* or *ex vivo* determination of likely biological activity needs specific justification.
2. Where animals must be used, the degree of pain and distress must be minimised by judicious use of anaesthetics and analgesics, the refinement of experimental techniques, and the early implementation of humane end points. Licensees must know the severity limit for each regulated procedure (ie mild, moderate, substantial or unclassified). The severity limit will have been arrived at by agreement between the applicant and the Home Office and takes into consideration details of the procedure itself, the nature and incidence of any likely adverse effects and any practical measures which will be used to minimise severity. Standard conditions controlling the severity of procedures attached to personal and project licences require the Personal Licence holder to notify the Project Licence holder as soon as possible when it appears either that the severity limit of any procedure or the constraints upon the adverse effects described in the protocol sheets have been or are likely to be exceeded. The Project Licence holder must notify the Home Office Inspector of this at the earliest possible opportunity. In addition, there is an inviolable termination condition in all Personal Licences, which requires the Personal Licensee to ensure the immediate humane death of any animal in severe pain or distress which cannot be alleviated.
 3. Where certain procedures cause particular concern, these must be addressed specifically in the Project Licence application. A more detailed justification for the procedure and precise definition of end points will be needed. The Home Office may attach special conditions to such procedures including special reports on the progress of the experiments.
 4. The design of all experiments should meet the highest scientific standards. It is important that pilot experiments should be undertaken on small numbers of animals before new procedures are carried out on a larger scale (see Appendix 2). All available information from other sources should be collected and carefully appraised before the design of (or need for) appropriate pilot experiments is determined. The pilot experiments should identify particular problems, define the time scale of critical events, and help to refine the appropriate end point. The use of new *in vivo* model tumour systems will require a full initial investigation of growth behaviour including patterns of local invasion and/or metastatic spread in a minimal number of animals. In all experiments the numbers of animals used should be restricted to the minimum consistent with the design and purpose of the experiment. Expert statistical advice should be sought, especially by less experienced investigators. In initial drug toxicity studies, 2 mice per group will often be appropriate (Burtles et al, 1995).
 5. All involved staff should be aware of their individual legal and ethical responsibilities and a clear chain of responsibility and consultation should be established. The decision-making process should be designed so that, under all circumstances, appropriate action is taken promptly to deal with any problems which may arise, for example if the clinical condition of a tumour-bearing animal deteriorates unexpectedly or if the individual effects of tumour and therapeutic treatment are difficult to distinguish (see section 3.5). Working protocols, including details of endpoints and signs of adverse effects should be made available to all those concerned with the care or use of tumour-bearing animals.
 6. All involved staff should receive appropriate training and supervision for the required time period such as to be fully competent in the procedures to be used. Systems for documentation of competence in different procedures should be in place under the supervision of the Project Licence holder. Where research workers are using unfamiliar procedures, information and guidance should be obtained from experienced colleagues, as well as from the scientific literature. For particularly skilled procedures, the use of expert outside assistance is recommended.
 7. In the planning of experiments, due attention should be given to whether resources are available such that it may reasonably be expected that an answer to the scientific question will be obtained. Such resources may, for example, include numbers of animals of suitable strain, age and weight, appropriately skilled manpower, and validated analytical methods.

Specific Recommendations

1. Assessment of severity

1.1. Before assessing the severity of any procedure on the wellbeing of an animal, it is essential that the licensees familiarise themselves with the signs of pain, discomfort and distress in the species they are using, by consultation with experienced colleagues, the named Animal Health and Welfare Officer, the named Veterinary Surgeon and by reference to published guides (see Bibliography).

1.2. Particular attention should be paid to those body systems most likely to be affected by the procedure. With solid tumours this will include ulceration, distension of covering tissues and cachexia. In the case of ascitic tumours, abdominal distension, anaemia and cachexia will be important. Lymphatic involvement from lymphoma and neurological disturbance from intracerebral tumours are examples of special complications arising in specific situations.

1.3. Certain deviations from normal well-being may be difficult to observe, for example induction of anaemia or the development of metastases, and special investigations may be required to detect them.

1.4. Appropriate control animals should always be included, so that the individual effects of the tumour and of any treatment can be distinguished.

2. Biology of tumours

2.1. Due consideration should be given to the known biology of the tumour. For spontaneous and transplanted tumours important features will include growth rate, invasion, distension, ulceration, metastases, site, and production of cachectic factors. These features, which define the tumour profile, should be established in pilot experiments. Methods of tumour implantation or induction should be chosen so as to minimise trauma to the host animal.

2.2. In the case of tumours induced by carcinogens, viruses or genetic manipulation, factors such as method of induction may affect the nature and location of resulting tumours. Animals at risk of such tumours should be observed particularly frequently for signs of

possible tumour development or associated disease.

2.3. Contamination of tumour cell lines with viruses and other micro-organisms may compromise experimental results, as well as causing an outbreak of disease among laboratory animals. Screening of cell lines for rodent viruses is strongly recommended. For example, Sendai virus is often used to induce cell fusion in vitro and is pathogenic to mice and rats. A potential hazard exists for research workers from immune-compromised animals receiving human tumour xenografts which may be contaminated with human pathogens including live viruses. In such cases, special facilities should be considered for both tissue preparation and animal containment (eg flexible film isolators).

3. Humane considerations in experimental design

3.1. Considerable care should be given to the judicious choice of end point for tumour growth, bearing in mind the objectives of the experiment and the underlying biology. This should take into account predictable indications of pain, distress or significant deviation from normal behaviour. Unless specified otherwise on the Project Licence, animals should be killed before:

- i. predictable death occurs;
- ii. they get into poor condition;
- iii. the tumour mass becomes overlarge, likely to ulcerate or unacceptably limits normal behaviour.

3.2. In the case of local solid tumours, the required information on response to therapy may be obtained by tumour regrowth delay, clonogenic assay following tumour excision or an appropriate surrogate end point, rather than by tumour weight at a given time. Difficulties may arise with this last method because optimum shrinkage of treated tumours may not be achieved before control tumours become excessively large and/or distressing to the host animal. Where such an assay has to be used, the tumour burden should be regulated as indicated in section 3.6.

3.3. The choice of site for transplantable or carcinogen-induced solid tumours also requires considerable care, and particular attention should be given to avoidance of sites involving the special senses or where the capacity for the tumour to

grow without causing pain or distress is limited. Subcutaneous or intradermal growth on the back or in the flank are considered to cause the least distress, while implantation of tumours in the footpad, tail, brain and eye will require special justification and is strongly discouraged. Distension of musculature is generally painful and this should be considered with intramuscular implants. Extra attention must be paid if multiple sites are used.

- 3.4. The intentional death end point should no longer be used. This applies both to toxicity studies and to therapeutic studies in animals bearing experimental tumours. Animals expected shortly to become moribund should be killed, unless specified otherwise in the Project Licence.
- 3.5. Difficulties may occur where the effects of anticancer agents on tumour growth are being evaluated, and it is essential that the individual toxic effects of the tumour and the treatment are initially determined. The maximum tolerated dose of the therapeutic agent (see Appendix 4b) should not be exceeded. This dose may differ in control and tumour-bearing animals and will require prior investigation.
- 3.6. No precise quantitative guide can be given as to the acceptable upper limit of tumour burden, since the adverse effects on the host will depend on the biology of the tumour, the site and mode of growth, and the nature of associated treatments. However, tumour burden should not usually exceed 5% of the host animal's normal body weight in the case of animals being used for routine tumour passage, or 10% in animals involved in therapeutic experiments. (This latter size, ie 10%, would typically represent a mean subcutaneous flank tumour diameter of 17mm in a 25g mouse or 35mm in a 250g rat). Calibration curves relating tumour weight to measured diameters should be established as part of the initial characterisation of any new tumour system. Consideration should be given to variation in measurement between individual experimenters. Although the sizes given above serve as a maximum guideline, it should be emphasised that problems may arise with much smaller tumour burdens and the clinical condition of the individual animal will always be the over-riding consideration.
- 3.7. In the case of leukaemias, determination of the tumour burden may be difficult. The development and use of appropriate bio-chemical and pathological laboratory methods to determine the onset of leukaemia prior to clinical signs is strongly encouraged.
- 3.8. With all ascitic tumours care should be taken to ensure that the volume of ascitic fluid does not become excessive, causing gross abdominal distension, and that solid deposits and cachexia are not allowed to become clinically significant. Ascitic burden should not usually exceed 10% of normal body weight in mice and rats. In view of the wide availability of *in vitro* methodology, the use of animals for monoclonal antibody production is increasingly difficult to justify. Where authority exists for such use, it should be noted that retired breeders are advantageous, since their abdominal musculature more readily allows larger ascites volumes to be tolerated without discomfort. Ascitic tumours should be drained only after death.
- 3.9. Particular care should be taken with monitoring the development of tumours in transgenic animals. Careful clinical examination should be carried out to allow for the detection of both predicted and unexpected sites of tumour development. This should include measurement of body weight changes, palpation and monitoring for deterioration in clinical condition. Experience suggests that animals should be examined at least twice weekly throughout their life-span.
- 3.10. In tumour therapy experiments with adult rodents, it is recommended that weight loss should not normally exceed 20% of the host body weight at the start of the experiment. For younger animals, failure to maintain the weight gain seen in untreated control animals should be considered as an indication of toxicity.
- 3.11. Care should be taken that general housing conditions are appropriate to the known or anticipated condition of the tumour-bearing animal, for example in terms of appropriate bedding, cage structure and accessibility of food and water.
- 3.12. Humane end points and other procedures should be refined in the light of experience. (Also see section 5.2.)

4. Examination of Animals

- 4.1. The frequency with which animals must be inspected for signs of pain or distress and the extent of each examination will be dictated by:
- i. the known biology of the tumour and/or the effects of the inducing agent;
 - ii. the effect of any associated techniques;
 - iii. the changing clinical status of the animal.
- 4.2. Rapidly growing or invasive tumours will require more frequent attention, and greater care will be required as the tumour burden increases. As a minimum, every tumour-bearing animal should be inspected daily and additional, more detailed, examinations undertaken as appropriate. The frequency of the latter should be increased during critical periods where the potential for animal suffering may be anticipated. The experimental design should ensure that these do not occur when staff are absent. Particular attention should be given to animals in poor health.
- 4.3. Appropriate assessment techniques will include: evaluation of overall clinical condition, including appearance, posture, body temperature, behaviour and physiological responses; assessment of food and water intake; weighing to determine changes in body weight (both positive and negative changes compared to controls can be associated with increasing tumour burden); caliper measurements to determine tumour volume or mass; and inspection and palpation to locate the sites of tumour growth, as well as to assess distension, ulceration and compromised mobility.
- 4.4. Other special examination techniques will be more valuable for specific sites, eg breathing rate for lung deposits, neurological disturbance or irreversible weight loss for brain neoplasms (Redgate et al, 1991) and blood cell counts for leukaemias. Laparotomy or endoscopy may be appropriate in some instances. Estimation of circulating tumour marker substances may also be of value. Consideration should be given to the use of any novel techniques which may be available. Autopsy of animals may expose adverse effects undetected by external examinations.

5. Documentation and publication

- 5.1. It is essential that all animal experiments are carried out and documented in accordance with Home Office regulations and the principles of sharing 'best practice'. Researchers are strongly urged, for each tumour model in use in their laboratory, to document the expected behaviour of the tumour and host animal under various experimental conditions, including therapy. They should also document humane end points to limit severity with regard to acute and delayed toxicity and maximal tumour burden, and indicate any particular problems which may be encountered in the use of each model. Such information should be incorporated into working protocols and widely disseminated for the benefit of others. The appropriate response to problems which have been or may be encountered should be described and the chain of consultation and responsibility clearly defined. Particular care should be taken that all procedures are understood by junior and occasional staff. Consideration should be given to the inclusion of a numerical scoring system to facilitate decision-making, eg when to contact senior staff or to kill an animal. The guidelines for specific tumour models should be readily available to, and agreed between, all research and animal husbandry staff involved with that model. Instructions for the appropriate use of anaesthesia and/or analgesia should be included. Researchers are again urged to share this information with other groups using the same system, for example when providing a tumour cell line to another laboratory.
- 5.2. Researchers are encouraged to publish improvements in humane end points in appropriate journals, so as to ensure wide dissemination of the information.
- 5.3. Encouragement is given to incorporate animal welfare statements into experimental protocols, and in addition to report compliance with these and other appropriate guidelines (including any local ones) when publishing results. Certain journals require or encourage this (eg British Journal of Radiology, British Journal of Cancer, Cancer Chemotherapy and Pharmacology, Cancer Research, and the Journal of the National Cancer Institute) and we would

urge other journals to adopt such a policy.

Summary and concluding remarks

Researchers have a legal and ethical responsibility for the welfare of experimental animals in their care and due consideration should always be given to the 'three R's' (reduction, refinement, replacement). They must decide whether, for each individual experiment, the use of animals is justified to answer a particular question, and, if so, minimise the pain and distress involved. Studies in experimental neoplasia present particular problems. Workers should possess adequate knowledge of the animals and tumour systems to be used. Where unfamiliar procedures are to be employed, information and guidance should be obtained through consultation with experienced colleagues and from the scientific literature. Workers should receive appropriate training and supervision. Pilot experiments should be carried out with small numbers of animals, and numbers should always be restricted to the minimum consistent with the design and purpose of the experiment. Tumour end points should be chosen and refined so as to minimise the adverse effects on the host animal. Death as an end point should no longer be used. The use of animals for monoclonal antibody production is increasingly difficult to justify due to the availability of alternative methods. Ascites tumours should only be drained after the death of the animal. The use of new technologies will present new opportunities and problems which will need to be taken into account. All staff should understand their individual responsibilities, and a clear chain of responsibility and communication should be established so that prompt action can be taken to deal with any problems that arise. Finally, researchers are encouraged to refine end points in experimental neoplasia and to disseminate best practice by publishing such improvements, to incorporate welfare statements in experimental protocols, and to report compliance with appropriate guidelines in publications.

The full references cited in these Guidelines may be obtained from the UKCCCR.

APPENDICES

Appendix 1 - Model Tumour Systems

The following examples of tumour systems are given for illustration.

- a) **A transplantable mouse tumour with a choice of therapeutic endpoints (RIF-1 fibrosarcoma).** This is a transplantable sarcoma of C3H/Km mice which is widely used in radiation and chemotherapy studies (Twentyman et al, 1980). It can be maintained in cell culture and is grown in vivo as a solid tumour by implantation intradermally in the skin of the flank or intramuscularly in the hind leg. The end points used to determine therapeutic effects on the solid tumour are clonogenic survival, regrowth delay and tumour cure. It is common practice to terminate regrowth delay experiments with leg tumours when the maximum limb diameter reaches approximately 15 mm or with subcutaneous tumours in the flank at a mean diameter of 17mm. At this point the tumour mass is about 2.5g or about 10% of the body weight and the host animals are in otherwise normal condition. Growth delay is determined from the time to reach four times the treatment size. Metastases occur late and rarely.
- b) **Rodent tumour metastasis models.** Metastases may be seeded either 'artificially' by intravenous injection of tumour cells, or spontaneously after growth of a solid deposit which can be removed surgically when appropriate. Such models include the B16 and other melanomas and UV-induced fibrosarcomas in mice (Kripke et al, 1978). It may not be necessary to wait until mice develop symptoms of impending morbidity, and the required information may be obtained after humane killing at an earlier stage (see Kripke et al, cited above). Special attention should be directed to detecting signs associated with clinically significant disease in sites particularly susceptible to metastasis, eg dyspnoea due to lung deposits.