A guide to defining and implementing protocols for the welfare assessment of laboratory animals:

Eleventh report of the
BVAAWF/FRAME/RSPCA/UFAW
Joint Working Group on Refinement

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The UK Joint Working Group on Refinement (JWGR) was established in 1989 by the British Veterinary Association Animal Welfare Foundation (BVAAWF), the Fund for the Replacement of Animals in Medical Experiments (FRAME), the Royal Society for the Prevention of Cruelty to Animals (RSPCA) and the Universities Federation for Animal Welfare (UFAW).

The aim is to provide up to date practical information on refinement. The JWGR prepares reports on specific topics, drawing together experts in each field to define contemporary best practice and ideals to aspire to. Professor David Morton chairs the Group and the secretariat is provided by the RSPCA. This report on defining and implementing protocols for the welfare assessment of laboratory animals is the eleventh in the series. (http://www.rspca.org.uk/sciencegroup/researchanimals/implementing3rs/refinement)

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Contents

Abstract

The refinement of husbandry and procedures to reduce animal suffering and improve welfare is an essential component of humane science. Successful refinement depends upon the ability to assess animal welfare effectively, and detect any signs of pain or distress as rapidly as possible, so that any suffering can be alleviated.

This document provides practical guidance on setting up and operating effective protocols for the welfare assessment of animals used in research and testing. It sets out general principles for more objective observation of animals, recognising and assessing indicators of pain or distress, and tailoring these to individual projects. Systems for recording indicators, including score sheets, are reviewed and guidance is set out on determining practical monitoring regimes that will be more effective in detecting any signs of suffering. Disseminating information about welfare assessment and aspects of training those responsible for monitoring and assessing animals are also addressed. This guidance is intended for all staff required to assess or monitor animal welfare, including animal technologists and care staff, veterinarians and scientists. It will also be of use to members of ethics or animal care and use committees. An abridged version of this document is published in *Laboratory Animals* 2011;45:1-13.

1 Introduction and aims

Reducing animal suffering through refinement of husbandry and procedures is an important component of good science[1-6]. It is also essential for humane reasons and is a specific requirement of legislation in some countries. If reducing animal suffering is to be effectively achieved, suffering must be detected as rapidly as possible so that appropriate action may be taken (such as providing analgesia, applying a humane endpoint, reviewing husbandry and enrichment, or euthanasing the animal[7]).

Some signs of animal suffering are relatively easy to identify and assess and many papers have been published on the objective assessment of welfare, both in general and following specific procedures[8-12]. Despite this, there is still much reliance on subjective assessments and individual opinion[13].

Discussion of these issues with veterinarians, animal care staff and scientists from a number of facilities in the UK established that it would be helpful to have further advice on objective methods for predicting and assessing welfare and animal suffering[13]. The aim of this document, therefore, is to provide practical guidance on setting up and operating effective protocols for the welfare assessment of animals within individual projects. It can also be used to assist in project design, as a discussion document for ethics or animal care and use committees, and to help funding bodies and regulators wishing to ensure that welfare will be properly assessed and suffering minimised in projects that they support or license.

The guidance should prove useful for all staff required to assess or monitor animal welfare, including animal technologists and care staff, veterinarians, scientists, and members of ethics or animal care and use committees. Although it was produced in the UK, the issues and guidance apply worldwide.

2 General principles for an effective welfare assessment scheme

The best approach to welfare assessment for each project depends on the type of establishment and its particular working practices, the nature of the research or testing, and the species and numbers of animals involved. However, there are some fundamental principles which should underpin all welfare assessment schemes. These are set out in Table 1 and explained more fully in the rest of the document.

Table 1: General requirements for effective welfare assessment

A team approach	A team approach is the most effective way to ensure consistency and effectiveness[13]. The team should include people with complementary roles and expertise who are prepared to work constructively together[4].
Appropriate welfare indicators	An animal's welfare state cannot be directly measured, but it can be inferred by monitoring appropriate behavioural and physiological parameters that can be used as welfare indicators. It is critical to define and monitor the right types and number of indicators – too many and the system will take too long to implement, too few and it may be inaccurate and misleading.
A sound understanding of good welfare and the "normal" animal	Effective welfare assessors must be able to recognise a "normal" animal, with good welfare, in order to detect early signs of adverse effects. The definitions of both "good welfare" and "normal" need to be carefully considered. This is explained in section 2.2.
Recognition of all potential adverse effects from all sources	There are many potential causes of adverse effects during the lifetime experience of each animal, i.e. not just the scientific procedures but other factors such as husbandry, handling and transport. An effective welfare assessment scheme will consider all sources of potential harms and all of the adverse effects associated with them.
Consistency for all species	Ideally, welfare assessment protocols should pay the same level of attention to all species, regardless of the numbers of animals used or perceptions about their cognitive capacity or ability to suffer.
Consistency between observers	Minimising variation between assessors' observations is essential. Differences in observational skills and subjective interpretations can be reduced by effective training and teamwork, and also by ensuring that observations are adequately described and recorded in a useful and accessible way.
Appropriate recording systems	Data should be captured using a consistent language and format, with the most appropriate recording system for each establishment, species, project and group of personnel.

2.1 A team approach

A team approach to welfare assessment is highly effective because it allows input from people with different expertise, priorities and responsibilities. This should enable animal welfare to be given due priority, while also taking into account scientific requirements and the resources available for animal monitoring. Who is involved in welfare assessment, and how the process operates for each project, will depend on the nature of the individual project and establishment and the experience, expertise and resources available in-house.

The end result could be a fixed group or a more fluid association of people, or the welfare assessment protocol may form part of the remit of an existing committee. It is most effective to take a flexible, tailored approach and consider the skills, knowledge, experience, motivation and authority that are required before identifying the team members who can bring them.

Whatever the structure of the team, the competencies that are invaluable in establishing it are listed in Table 2 below. There are ten competencies, but this does not mean that ten people are required, as there may be multiple people who could fulfil each role and members with more than one area of expertise.

Table 2: Competencies for a successful welfare assessment team

The team should include members who:
✓ can recognise a "normal" animal and understand "normal" behaviour for the species and strain, in the laboratory environment
✓ are familiar with the science relating to the project
✓ can recognise all the potential welfare issues, throughout the animals' lives
✓ can recognise and identify animals showing adverse effects as a result of scientific procedures
✓ can identify an animal who is not "normal" but where this deviation from normality is not an adverse effect resulting directly from scientific procedures*
✓ can assess and interpret welfare indicators
✓ are able to give advice on ameliorating (or avoiding) adverse effects
✓ bring in comparative knowledge across different species and institutes
✓ are able to address management and resource issues, such as staffing levels, either directly or by communicating with management
✓ will take ultimate responsibility for acquiring up to date information on welfare assessment
Advice may sometimes be necessary from people with expertise in:
✓ statistics – in relation to ensuring consistency between observers, comparing predicted with observed severity etc.
✓ ethology – for a deeper understanding of animal behaviour
✓ animal welfare science – for interpretation of how behaviour, physiology, psychology, immunology, neuroendocrinology etc. reflect welfare and how to assess these aspects
✓ setting out strategies for implementing and evaluating refinement

Good communication and constructive working relationships should be promoted, both within the team and with other relevant groups or committees at the establishment (such as ethics or animal care and use committees or Three Rs groups).

A welfare assessment protocol should be developed for each specific project. This should be initiated early in the project planning stage, before the project has been before regulators or review committees, so that welfare assessment is taken into account within the experimental design.

2.2   Definition of good welfare

A baseline standard of good welfare should first be defined, to act as the point of reference for the species (and strain, where applicable) to be used in the study. This standard may apply establishment-wide for particular species or strains. A sound understanding of animal behaviour will help to ensure that the baseline is appropriately defined. For example, genetically altered (GA) mice with vestibular abnormalities spend much time circling in their cage. This is normal behaviour for these animals, but is not necessarily desirable from a welfare point of view. Similarly, understimulating housing can cause stereotypic behaviour[14], which is normal in such environments, but can indicate a serious welfare problem. The term 'natural' is sometimes used instead of normal, but this is no more descriptive – what is normal behaviour for a laboratory animal is not necessarily natural[5].

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* Two examples of adverse effects that are not directly caused by procedures are animals on a long term study becoming "institutionalised" and spontaneous occurrences of hydrocephalus in C57BL/6 mice.

A more useful reference point for the welfare assessment protocol is to define a hypothetical 'ideal' level of welfare. This can be defined as: *the state of being in animals when their nutritional, environmental, health, behavioural and mental needs are met*[15]. There are three key components to this ideal, set out in Table 3.

Table 3: Components of an 'ideal' welfare state and examples of indicators associated with them

Component	Characteristics	Examples of indicators
Physical state	Good level of physiological fitness, with no physical disabilities that either cause discomfort or pain, or that have an impact on physical function that could cause suffering or distress	Indicators relating to the observable physical condition of the animal e.g. body weight, state of the coat, posture, lameness, excessive attention to surgical sites
Physiological/ biochemical state	Levels of stress and distress do not exceed those that would occur during the course of normal social interactions, for example. If parameters such as heart rate or blood pressure were to be measured, they would not be expected to indicate significant stress	Physiological parameters such as heart rate, respiratory rate, levels of stress hormones such as corticosteroids
Psychological state	The animal displays an "appropriate" range of behaviours, according to what is known about the species and strain	Changes in behaviour such as increased aggression to cage mates, withdrawal, apathy, stereotypies, changes in use of enrichment or other changes to behavioural time budget

There is not usually any need to measure all of these parameters. The ideal state can be assumed to exist if the team is confident that animal housing and care is consistent with good practice, that the animals are healthy, behaving according to an appropriate time budget and that they are fully habituated to their accommodation and to husbandry routines.

Taking all of this into account and assuming that welfare is good, any deviation from the 'ideal' level of welfare described in Table 3 could indicate a welfare problem and should be investigated as such.

There are many potential causes of suffering during the animals' lives that may impact on current or future welfare assessments. Causes of deviation from the ideal welfare state may include early separation from the mother, transport, trapping, inappropriate housing, inadequate health care, scientific procedures and their after effects (expected and unexpected), husbandry procedures (such as cleaning out and identification) and euthanasia or release[2,14-17]. These events can interact with one another. For example, stress due to early separation from the dam can influence nociception in rats[18].

Taking all of this into account and assuming that the baseline standard is good, any deviation from this ideal state could indicate a welfare problem and should be investigated as such. Note, however, that some physiological parameters can alter in association with positive excitement, such as play, as well as with negative stimuli. Furthermore, many commonly used species, particularly rodents, do not always display behavioural signs of suffering that can easily be detected by human observers. These issues can be overcome by thoughtful selection and interpretation of welfare indicators.

2.3 Selection of appropriate welfare indicators

Key to the success of the welfare assessment scheme is the selection of welfare indicators that:
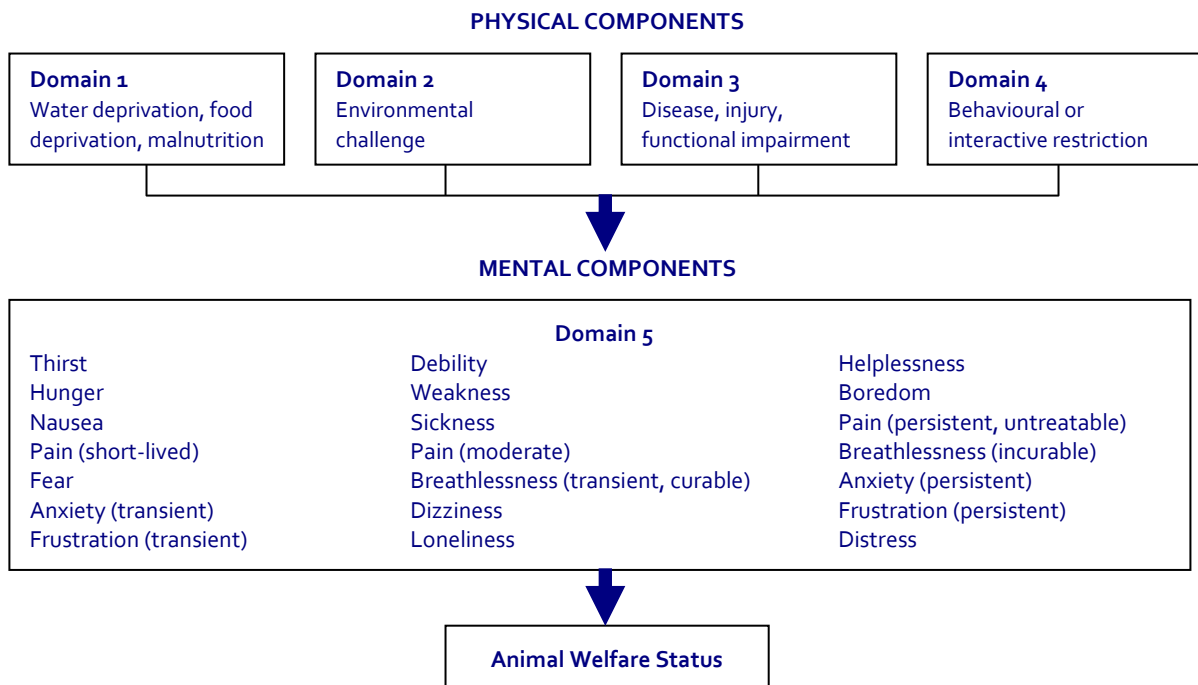
- are readily and reliably recognisable;

- are effective at providing good measures of welfare;

- are relevant to the project and species;

- are practical to carry out and do not overly disturb the animal;

- take the experimental design into account; and

- lend themselves to consistent measurement, interpretation and analysis[4,19].

It is preferable to use a combination of behavioural and physiological indicators, drawing on each of the categories listed in Table 3, to overcome difficulties with interpretation and to provide a more detailed and complete picture of an animal's welfare[4-8,13,20].

The concept of the five domains of potential welfare compromise is also helpful when predicting the potential impact of the planned procedures[15,21,22] (summarised in Figure 2). Under this scheme, compromise in the four physical domains is usually registered in welfare terms in the fifth domain, which represents the components of suffering. The fifth domain can also be the primary focus of welfare compromise as well as a product of the physical components. This concept can provide a useful tool for considering the potential adverse effects more broadly, to include each animal's entire lifetime experience (see box on defining adverse effects).

Figure 2: The five domains of potential welfare compromise (reproduced from *The Sciences of Animal Welfare* by DJ Mellor et al. (2009)[22] with permission from Wiley-Blackwell)

PHYSICAL COMPONENTS

Domain 1 Water deprivation, food deprivation, malnutrition	**Domain 2** Environmental challenge	**Domain 3** Disease, injury, functional impairment	**Domain 4** Behavioural or interactive restriction

MENTAL COMPONENTS

Domain 5

Thirst	Debility	Helplessness
Hunger	Weakness	Boredom
Nausea	Sickness	Pain (persistent, untreatable)
Pain (short-lived)	Pain (moderate)	Breathlessness (incurable)
Fear	Breathlessness (transient, curable)	Anxiety (persistent)
Anxiety (transient)	Dizziness	Frustration (persistent)
Frustration (transient)	Loneliness	Distress

Animal Welfare Status

2.3.1 General indicators

A list of simple, objective welfare indicators, such as body weight and condition (physical state in Table 3), measured body temperature (physiological state) and food and water consumption (this may fall into any of the three categories describing the ideal welfare state), can be drawn up for use in most projects. These indicators can be directly and objectively measured, providing clear indicators that an animal's welfare may be compromised.

The interpretation of these indicators can depend on the husbandry and experimental protocol. For example, tumour mass can increase body weight, even though the animal's body condition may be poor. Group housing can confound food and water uptake data, so that only an average value can be obtained[*]. The approach of determining the general list, while understanding and accounting for its limitations, is fundamental to an effective welfare assessment system.

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[*] Many people would take the view that this is outweighed by the benefits of group housing social animals.

2.3.2 Indicators specific to the project

The next stage is to consider what will be done to the animals and predict the likely adverse effects, so that a list of welfare indicators can be produced that is tailored to the study.

For example, following vasectomy surgery mice would be expected to experience a degree of pain associated with the wound site as an adverse effect. Indicators of this adverse effect would include body weight loss (a general indicator) and behavioural indicators specific to the project such as lifting a hind leg or pressing the abdomen to the cage floor[23]. These behaviours can be used as indicators for welfare assessment post-vasectomy.

> **Defining adverse effects**
> The term *adverse effect* can mean different things to different people. For example, to some it can mean unexpected adverse effects only. To others, it means acute discomfort or pain caused directly by procedures, not taking into account any after-effects of procedures or psychological harms. From an animal's point of view, however, adverse effects include all potential causes of suffering during their lifetime experience from sourcing through to euthanasia, rehoming or release. This may include early separation from the mother, transport, trapping, inappropriate housing, inadequate healthcare, scientific procedures and their after effects (expected and unexpected), and husbandry procedures (such as cleaning out and identification)[2,17,24-25].

The list of potential adverse effects could include items such as discomfort or pain in specific areas of the body, nausea or other toxicological effects, anxiety or reduced physical ability. Table 4 lists potential sources of information and guidance that can be used to predict adverse effects.

Table 4: Sources of information and guidance on predicting potential adverse effects

- Information from care staff (and breeders) on sourcing, transport, identification methods, husbandry regimes etc. and their welfare implications

- Harm-benefit analyses that may have already been carried out

- Past experience, if other, similar studies have been carried out using the same species or strain

- Results of *in vitro* and *in silico* studies, such as Quantitative Structure-Activity Relationship (QSAR) where chemical structure is correlated with biological activity

- The literature on similar studies

- In a contract research setting, information obtained during the testing of other compounds (in the case of agrochemicals for example, signs may be comparable between similar classes of compounds), chemical structure or information supplied with test compounds

- Information from pilot studies, for example data on absorption (Section 2.3.4)

- In pharmaceutical research and development and safety assessment, information on the target, or predicted pharmacological action, not only of the parent compound but also of any metabolites that may be formed*

- For some models of animal diseases, for example where using the same species as the target species, clinical signs of the disease in clinical cases

- For models of human disease, clinical signs and symptoms of the disease in humans can provide pointers, but bear in mind that clinical signs may differ in animals; research projects are usually only modelling certain aspects of a condition[26]

- Contact with other researchers using the same or similar models, possibly using online discussion groups such as Compmed (http://www.aalas.org/online_resources/listserves.aspx)

- Published resources on predicting adverse effects, including those listed in the Appendix

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* Commercial computer databases (e.g. DEREK®) are excellent resources for accessing known information on chemical structure and activity of parent compounds and their metabolites. They can also provide very good predictive activities for naïve compounds, although these predictive systems are not perfect.

Behavioural indicators

Behaviour is a particularly valuable indicator of affective (emotional) state, which is a component of welfare. Assessing behaviour is non-invasive, can be non-disruptive (for example cage-side observation) and can provide a more accurate interpretation of animal affective state than some physiological measures. Commonly used behavioural indicators of affect include vocalisations, general activity (such as sleep duration), maintenance behaviour (such as grooming), social behaviour and abnormal behaviour including stereotypies. Interactions with the environment may also be used, for example nest building behaviour20. Some behaviours are considered 'key' indicators, whose presence almost always indicates either good welfare – such as play – or bad welfare, like self-mutilation. Other behaviours may be extremely subtle or much harder to interpret. Advice from ethologists and adequate training in interpreting animal behaviour is essential.

Physiological indicators

Physiological indicators can provide an extremely useful back-up to behavioural observations. Commonly used physiological parameters include heart rate, blood pressure or biochemical levels of relevant substances such as blood glucose or circulating levels of stress hormones (such as corticosteroids). However, obtaining the necessary samples or data can cause stress and discomfort. This should be considered on a case-by-case basis against the improved ability to monitor animals and to implement humane endpoints in protocols where there is a high risk of suffering[28]. Signs such as heart rate or body temperature are usually monitored using telemetry and transponders, thus reducing invasiveness and causing less overall distress to the animal once devices have been fitted. Alternatively, it may be possible to obtain telemetered data at no additional cost to the animal if it is being collected as part of the scientific project[29].

The next step in the process is to consider what the behavioural or physiological indicators of these adverse effects might be. Examples of indicators associated with adverse effects are arching the back, twitching and writhing and/or drawing in of the flank in rodents, slack muscle tone around the eyes in birds, skin colour changes in fish or reluctance to move in many species. The list of indicators for all species is continually expanding; a recent addition is the use of facial expressions in the mouse[27]. Note that interactions with the environment, such as changes in gnawing on chew blocks or nest building, can provide extremely useful indicators, which is another reason for providing a structured environment in addition to the animal welfare benefits[20].

Table 5 sets out an approach for identifying indicators and considering how each indicator might best be monitored and assessed.

Table 5: Identifying indicators for each adverse effect – questions to consider

- What might the observable or measurable indicators be in an animal experiencing this effect? How should they be described?

- How frequently should animals be monitored, and at what times, to ensure that the indicators will be picked up?

- How could the indicators be assessed and which method is preferable and most feasible?

  ➜ measured objectively?

  ➜ observed and marked as present or absent?

  ➜ assigned a numerical score?

- Will the benefits of monitoring outweigh any disturbance that may be caused? Or, could disturbance be minimised by including welfare assessment when the animals will be disturbed anyway, for example at a project driven bodyweight check?

- Will invasive techniques be involved, such as blood sampling or implanting telemetry devices solely for monitoring purposes[*]?

- Will measuring the indicators adversely affect the scientific outcome? Or, conversely, could data gathered for scientific purposes also be used to assess welfare?

- Can any environmental indicators be used, such as interaction with enrichment items such as climbing resources or nesting material?

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[*] The use of invasive procedures for welfare monitoring or implementing humane endpoints requires a carefully considered harm-benefit assessment and consultation with veterinarians and regulators (the latter with respect to any legal implications). It may also affect the harm-benefit assessment of the project as a whole[29]

The Appendix lists sources of further examples of welfare indicators. For a more in depth approach to estimating the probability of pain and distress, its consequences and associated risk levels, see National Health and Medical Research Council (2008)[4] and for training in all of the above see Assessing the Health and Welfare of Laboratory Animals (http://ahwla.org.uk/).

It may be possible to reduce the number of indicators by using so-called 'iceberg indicators' that summarise other areas of welfare and are easy to understand[30]. One such indicator is lack of grooming after surgery, which could indicate an inability to coordinate grooming movements, postoperative pain or, if shortly after surgery, the side effects of anaesthetic or analgesic agents.

In projects that use new species, it might be necessary to obtain baseline data from animals before experiments, or to use physiological or behavioural data from wild-type animals. There may be information in the literature on the basic biology of the species or a closely related species, preferably of the same genus, that may be applicable. More closely related species should share more behavioural and physiological adaptations and will provide more predictable references for one another. Comparative behavioural or physiological studies exist for many vertebrate taxa that may also prove useful. It may also be that little is known of the natural behaviour of the species, or similar species, or what suitable criteria for welfare assessment will be. Under such circumstances it is necessary to consult more widely with outside experts and possibly to conduct a pilot study (see 2.3.4 below).

Adverse effects and their behavioural indicators can sometimes be completely unpredictable, especially when testing novel compounds or in mutagenesis projects. In such cases, there will be some knowledge gaps and welfare assessment is approached blind, so there is a strong case for including as many potential indicators as is workable in the initial assessment.

The list of indicators can be reviewed and updated as the work progresses, according to the validity of each indicator and the information it can provide. A useful approach is the SPIDER model; Setting Goals, Planning, Implementing, Documenting, Evaluating and Readjusting[31]. The ultimate aim is to include the minimum number of parameters necessary to detect adverse effects rapidly and effectively, yet not waste time gathering data that have no added value.

2.3.3 Intervention points

It is essential for animal welfare, ethical and often legal reasons that clearly defined intervention points are set for each project. Suitable interventions when key signs appear, or reach a threshold level, should be defined at the time when indicators are discussed[7]. For example, fluid therapy could be initiated as soon as signs of dehydration appear, or an intervention for weight loss of ten per cent in rats with a degenerative condition could be providing wet mash at floor level. Humane endpoints, in which the animal is temporarily or permanently removed from the study, should also be defined at this stage.

> **Extrapolating from the human experience**
>
> Where animals are being used to model human diseases, it can be useful to consider the symptoms in humans to define welfare assessment criteria[32]. The approach begins with the basic question "Would a human being with the same disease or pathology suffer, and in what way?". This is then qualified by considering how the biology of the species (and strain) might affect whether and how the animals could suffer. It is essential to strike an informed balance between assuming that what causes suffering in humans will also cause suffering in animals – which can be the case – and taking the biology of the animal into account[33]. Failing to acknowledge species differences can actually be detrimental to animal welfare.
>
> For example, humans with Williams syndrome may have hypercalcaemia, leading to constipation. Genetically altered mouse models of Williams syndrome may have hypercalcaemia, in which case they are treated for this in the same way as humans by giving low calcium milk and laxatives if they are constipated. However, some human patients have defective vision, which is less likely to present problems for mice (M Maconochie, pers. comm.).

Thresholds should be established that allow minimal animal suffering whilst still attaining the objective of the study, so any intervention and its timing will need to be agreed with the researcher and regulator. Some clinical signs may be expected as part of the model. One such case is pale extremities in animals used to study cardiovascular disease. The welfare assessment system should include clear guidance on those effects that are to be expected as part of the model, and what the endpoint is for each one.

2.3.4 Pilot studies

Pilot studies using a small number of animals can provide useful guidance on welfare indicators where these are difficult to predict, for example for the testing of novel compounds or for newly developed experimental designs. Results of pilot studies can not only provide the indicators for the final project, but also help to guide refinements, including intervention points and humane endpoints. Note that there are legal requirements for authorisation of pilot studies, just as with full projects, in many countries including the UK.

The first animals in a pilot study should be monitored especially carefully, using frequent sampling and a broad range of indicators, so as to gain as much information as possible about potential adverse effects and their progression. In the case of pilot studies where effects are highly unpredictable or potentially severe, it is advisable for an experienced animal technologist and/or the attending veterinarian to be present to assist with monitoring.

A successful pilot study will enable a list of welfare indicators to be drawn up for use in the full project. It may also be appropriate for the ethics or animal care and use committee to reconsider the benefits, including scientific justification, against the newly defined harms of the experiment.

2.3.5 Indicators of positive welfare

Welfare assessment generally focuses on negative rather than positive welfare. It is also desirable to improve the animals' quality of life as well as to minimise suffering, so the potential to define signs of positive welfare and the addition of these to the welfare assessment system should be considered. It is unrealistic to expect that any animal, in any context, will be in a wholly positive state all of the time. The aim should be to maximise the presence of indicators of positive welfare and minimise negative welfare. Some general examples of behaviours to consider are listed in Table 6.

Table 6: Examples of behaviours that can indicate positive welfare states

- ✓ Good self-care, including grooming and comfort behaviours
- ✓ Normal activity levels and time budget, including sleep patterns[34]
- ✓ Seeking interactions with humans
- ✓ Curiosity and interest in exploring
- ✓ Appropriate social interactions with conspecifics, including allogrooming
- ✓ Mating
- ✓ 'Anticipatory' behaviour[35]
- ✓ Using enrichment items, especially for "luxury" behaviours
- ✓ Interest in food treats
- ✓ Play[36,37]
- ✓ Vocalisations associated with positive welfare
- ✓ Normal learning and cognitive functions

Positive welfare signs will vary with species, strain, life experience and individual temperament of animals and need careful interpretation, using the animal behaviour literature and advice from ethologists. For example, some strains of rodent are passive or have low activity levels, but this does not necessarily relate to individuals' wellbeing. Some behaviours, such as tail chasing in rats, can mistakenly be believed to be play when the behaviour is in fact a "self-directed activity" in response to social isolation[38].

There are other issues that require consideration; for example, play fighting is regarded as beneficial in dogs but there can be a continuum from play to harmful aggression. Decisions must be made on intervention strategies, such that play is not stopped too early but humans do not become habituated to harmful levels of aggression. There can also be a fine line between positive excitement or anticipation and frustration in many species.

Knowledge about reliable signs of positive welfare is limited for many species at the time of writing and should be kept under review. For further information, see Boissy et al. (2007)[36], Kirkwood et al. (2007)[39], Wemelsfelder (2007)[40] and Yeates & Main (2008)[41]. Given the current state of knowledge about these behaviours and their significance, the focus should be on trying to ensure the ideal level of welfare described in section 2.2 and monitoring for the presence of the kinds of behaviours outlined in Table 6.

2.4   Animal welfare indicator record systems

There are different systems in common use for recording welfare indicators. We have broadly categorised them as:

- relatively unstructured records, with a small number of objective signs and a reliance on written descriptions of adverse effects (free text), or

- more organised animal welfare assessment sheets with predetermined, but flexible, lists of indicators and minimal free text.

The latter may be either numerical or binary score sheets. Numerical sheets aim to quantify the severity of adverse effects; binary systems simply note whether or not the adverse effects are present.

Relatively simple records with free text may be most appropriate where there is a requirement for a flexible and exploratory approach, for example during pilot studies. They are also used where adverse effects are highly unpredictable and animals are monitored very closely. The language used to describe the appearance and behaviour of animals should be consistent, both within the welfare assessment team and between different establishments.

Consistency and objectivity are best achieved by using organised sheets[6] and keeping free text to a minimum, although all

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Consistency in observations and language

Achieving consistency in the language used to describe the appearance and behaviour of animals is critically important. For example, GA mouse lines may be used by multiple groups, in which case facilities will wish to pass on knowledge that could help to improve welfare, minimise suffering or successfully rear and maintain stock. This information should be expressed in a standard, descriptive language and stored in an accessible, searchable format for future referencing and analysis.

The Medical Research Council (MRC) Harwell and the Wellcome Trust Sanger Institute have been compiling mouse welfare terms that provide controlled language, in the form of a list of terms, for assessors to describe what they see. The terms form a hierarchy, with a glossary for (i) the health concern, (ii) the body system and (iii) components of the body system. The system is still evolving and the current terms can be viewed on an interactive website with the facility for suggestion, corrections and developments (www.mousewelfareterms.org). The project was initiated with respect to GA mice, but the mouse welfare terms will also apply to all strains and the approach can be used for other species.

Consistency can also be achieved by sound teamwork and careful communication. Studies have found that subjective judgements about animals' wellbeing can be consistent, provided that the observers possess appropriate experience, empathy and knowledge about species-specific behaviour[40,42,43]. In the case of farm animals, qualitative observations have been found to correlate well between observers and with physiological data such as heart rate[40]. The balance between using a standardised lexicon, as in the MRC/Wellcome project, or a carefully evaluated qualitative approach depends on a number of factors including animal numbers and species.

systems should include the facility to record unforeseen signs as free text (Table 7). It is also useful to record disturbances such as visits by unfamiliar people, lighting system failures, or building noise in the free text box as these can affect both welfare and experimental results[24].

Table 7: Advantages of structured animal welfare assessment sheets

- Signs are recorded consistently using agreed, defined terms, so assessments of welfare should be more objective

- The system is flexible and can be made species-, strain- and model-specific

- Experienced persons can use the outcomes to illustrate to less experienced persons the reasons why an animal is "not right"

- The system can be set up so that single signs, or a combination of signs, can be used to indicate the overall severity of a procedure

- The effectiveness of any therapy intended to relieve adverse effects can be determined

- Procedures that are likely to affect welfare can be indicated, so that interventions in response to predictable adverse effects or welfare issues can be agreed in advance, and action taken without delay

- The impact of scientific procedures on animals can be measured more meaningfully and the effectiveness of refinement strategies can be compared

- Free text boxes are still included so that unforeseen signs and disturbances can be noted

Numerical score sheets

In the case of numerical score sheets, a number of clinical signs, physical indicators and behavioural parameters are assessed and given a score according to their apparent severity. For example, unaffected would score zero, mild deviation from normal might score 1, moderate deviation from normal 2, and substantial deviation 3. Scores are often added up and the result used to determine whether action is needed (such as analgesia) according to a predetermined key attached to the sheet (Figure 3). Numerical scoring can also form the foundation of more complex assessments of welfare, for example in assessing cumulative suffering[44].

Figure 3: Example taken from a numerical score sheet for rats used in inflammatory bowel disease studies (NB this is not a complete sheet.)

Component (see Table 3)	Animal ID	Score	Date/time	Date/time
Body weight	Normal or up to 5% loss	0		
	5 to 10% loss	1		
	Over 10% loss	2		
Physical state	Normal	0		
	General lack of grooming	1		
	Staring coat and/or ocular or nasal discharge	2		
	'Pinched' features, ridge lines in skin	4		
	Faeces normal to slightly soft	0		
	Diarrhoea	1		
	Soft, distended gut, no faeces	2		
	Hard, hot, distended gut	4		
Physiological state	Normal breathing	0		
	Slightly laboured breathing	1		
	Notably laboured breathing	3		
Psychological state	Normal provoked behaviour	0		
	Slightly subdued	1		
	Moderate change in behaviour and/or apart from cage mates	2		
	Reacts violently/vocalisation	6		
Other observations				
TOTAL				

Interventions:

0	Normal
1 or 2	Monitor more closely
3 to 5	Monitor closely, consider analgesia, notify researcher
6 to 10	Significant suffering likely, provide appropriate relief, observe frequently, consider euthanasia
Over 10	Severe suffering, euthanase

However, numerical scores need careful interpretation. Simple addition may be justified in some cases but not others, and some parameters may need to be weighted. In the example shown in Figure 3, a score of 2 for staring coat and 2 for isolation from cage mates does not "equal" a 4 for a hot, distended gut; an animal with a score of 4 is not suffering twice as much as an animal with a score of 2. Intensity can also be subjective and observer-dependent. It is conceivable that an animal may be experiencing suffering yet have a score that does not require action, although empathy and common sense on the part of the assessor should protect the animal from avoidable suffering in such cases.

Binary sheets

In contrast, the binary system records either a "yes" (present) or "no" (absent) depending on whether the behaviour or effect is seen or not, often with no description of its intensity. Boxes are ticked when indicators are observed and appropriate action taken when ticks begin to appear. Core parameters such as body weight are also usually measured and recorded. The binary system is generally regarded as being more objective than the numerical system, as value judgements on severity are not required. Objectivity should not be taken for granted; checks should still be made that people are using the system consistently.

The numerical and binary systems each have their own strengths and weaknesses and will be appropriate in different contexts (Table 8).

Table 8: **Comparison of numerical and binary observation systems**

Numerical system	Binary system
Advantages	**Advantages**
Consistent, provided that guidance is clear and scoring options limited	Potentially more objective assessments – simply need to agree when indicators are present or absent
Considerable amount of data can be collected; data can be statistically analysed	Less time-consuming as judgement on numerical scores not required
Disadvantages	**Disadvantages**
Element of subjectivity in assigning scores	Less data collected
Can be time-consuming	Need to consider threshold for marking as present, which still takes some time and can be subjective
Potentially not sensitive enough to detect any subtle changes	Cannot assign clear intervention points on the basis of a numerical score, although presence of some clinical signs can denote endpoints
Accumulated scores can over or under estimate severity. Some changes may need to be weighted as more indicative than others, and the significance of certain criteria coinciding should be considered	No description of the intensity of each effect, unless this is built in (for example 'pronounced piloerection')

Whichever recording system is chosen, it should be adapted for specific studies and then regularly revised and modified if necessary[31]. Indicators that occur infrequently may be deleted, or if they are vital to the assessment, sampling frequency should be altered to ensure that they are picked up. Conversely, unexpected adverse effects may occur (as noted in the free text boxes) and indicators for these should be added.

As a final note of caution, is should not be assumed that any welfare assessment system is infallible. There is always the potential for unpredicted adverse effects to occur, or for a particularly empathetic assessor to detect very subtle and previously unrecognized clinical signs. Balance is essential between striving for objectivity in welfare assessment on the one hand, and trusting the judgement of empathetic individuals on the other.

2.4.1 Recording media

Data should be recorded accurately, so that welfare assessment teams can search it when developing welfare assessment protocols. The use of databases, either electronic or on paper, to record welfare information allows effective retrospective assessment of suffering.

At the time of writing, most welfare assessments are recorded using paper sheets and/or modified software packages. Historically, paper records have been completed at the cage side, so recorded welfare assessments have to be transferred onto computer spreadsheets if electronic storage or analysis is required. It is becoming increasingly possible for welfare assessment data and observations to be recorded using electronic systems and the development of wireless, handheld systems will inevitably lead to electronic recordings at the time of observation.

All recording systems, whether they are paper records or laboratory information management systems (LIMs) with bespoke designed welfare assessment modules, should be:

- Stable (e.g. databases and spreadsheets are regularly backed up)

- Accessible for all relevant personnel

- Transferable between laboratories in order to disseminate welfare information

- Recorded in a consistent way using the same language for the same welfare concern

- Complete and comprehensive

New applications are continually being developed, such as the primate-specific welfare assessment module within the Primate Systems Management package produced by Brain & Software International (http://www.bsi-usa.com/), which should soon be available for use on a hand-held device. It is good practice for the welfare assessment team to keep up with developments such as these.

2.5 Assessment timing, duration and frequency

Once the recording system has been designed, a monitoring protocol can be set out that includes how often to assess animals, at what point in their activity phase, and how long to spend on the assessment. The details of implementation will depend on the factors set out below[6]. Clearly, the more animals that need to be assessed, the more time is needed and it is essential that adequate resources are in place to allow effective monitoring.

2.5.1 Species and strain

Ideally, all species should be regarded as capable of suffering and should be given equal consideration. There are some practical issues that influence the level of complexity of the assessment and the time that it takes to conduct (and analyse) it. There are more measures of welfare available for some species than others, which can impact on the time required to carry out welfare assessment. Some species display behaviours that are comparatively easy for humans to detect and interpret. A depressed primate displays a characteristic hunched posture with which it is easy for most observers to recognise and empathise[45]. However, the signs of poor welfare may be more difficult and time consuming to recognise in other species such as small rodents and non-mammals; there are also likely to be differences between strains in both normal behaviour and responses to pain, suffering or distress.

Consistent consideration for all animals is more likely if there is a good level of awareness of all of the above issues relating to actual and perceived difficulties in assessing different species and strains. Consultation with ethologists and other relevant experts is especially useful in improving consistency[46].

With respect to GA animals, many gene manipulations have both predictable and unpredictable characteristics that affect welfare, but which may not always be detected using standard

phenotyping protocols[47]. The welfare of founder animals should be carefully assessed over an extended period, beginning at neonatal stages, so that any adverse effects can be identified and appropriate indicators used in the routine welfare assessment of the line. It is also good practice to extend this comprehensive inspection to genetic alterations being bred onto different background strains for the first time or being bred to homozygosity (this is especially relevant in mice).

2.5.2 The experimental design

The nature of the procedures will influence the timing, duration and frequency of observations. Those at higher levels of severity require animals to be monitored more frequently, as will protocols where a rapid onset of adverse effects is expected. The time scale of adverse effects can be more predictable if they have been scientifically evaluated (for example pertussis vaccine potency testing[48]) or there is a body of experience relating to a particular technique.

The timing of procedures should also be taken into account. There will obviously be a risk of discomfort or pain in the immediate post-operative period. For example, rats immediately following various types of abdominal surgery such as laparotomy, adrenalectomy and bladder manipulations have been found to display specific behaviours including twitching (usually observed as rapid fur movements on the back), back arching, belly pressing and writhing[11]. Such behaviours have been found to occur at varying frequencies depending on the level of discomfort and it is necessary to observe each rat for at least five minutes to ensure that they are detected. Left untreated, these behaviours can be present for up to 24 hours following the procedure, which is a welfare issue as pain relief is required.

Animals would need to be assessed more frequently post-surgery to assess whether analgesics were effective. Observations could then be made less frequently provided that there were no complications.

2.5.3 Housing environment

The time allowed for welfare assessment should take account of environmental stimulation such as nesting material, refuges and other structures provided. It may be necessary to move enrichment items or open nests to observe animals properly. Note that changes in interactions with the environment, such as gnawing on chew blocks, foraging or nest building, provide extremely useful information about the animals' welfare state. This is another reason for providing a structured environment, as an indirect welfare indicator in addition to the benefits for animal wellbeing[20].

2.5.4 Husbandry practices

Disturbance caused by husbandry procedures such as cage cleaning can have a significant effect on animal behaviour and physiology[17]. Following cage cleaning in the rat, exploratory behaviours, shelter use, heart rate and blood pressure all increase significantly[17,49-51]. Behavioural and physiological parameters can take up to two hours to return to pre-cage change levels in rodents[50], during which time these responses may mask critical indicators used in the welfare assessment. It may be advisable to conduct welfare assessment an hour or two after husbandry procedures such as cage change, unless the potential severity of the procedure requires more frequent monitoring.

2.5.5 The animals' normal circadian rhythm

It is preferable to observe animals during the time when they would usually be most active, since assessing awake, active animals will reduce the likelihood of missing essential signs. In the case of most rodents this means conducting welfare assessment during the dark period, when they are predominantly active[11,13]. An exception is where there is a potential for sleep disturbance as an adverse effect, in which case nocturnal animals would be observed during the light phase. There are obvious human resource issues associated with observing animals at night, but animals can be housed on an altered or reversed light regime.

Disruptions to the circadian cycle can also be used as a welfare indicator. For example, sleep disruption, reduced activity or responsiveness during periods of normally high activity, or increased activity when animals should be inactive, may indicate adverse effects[34,51]. Care staff may notice changes in circadian behaviour patterns, or it may be appropriate to use sophisticated monitoring techniques such as video monitoring (which can be sped up or sampled for analysis), digital imaging or automated behaviour recognition systems (for example Observer®, http://www.noldus.com/, Trafficage®, http://www.newbehavior.com, or HomeCageScan®, Clever Systems Inc., http://www.cleversysinc.com[52].

3 Practical welfare assessment

This section provides generic guidance on welfare assessment in practice, which can be applied not only to domesticated mammals, but also to wild animals, fish, reptiles, birds and amphibia. Before implementing the welfare assessment protocol, it may be useful to go through the checklist in Table 9.

Table 9: Points to check before using the welfare assessment protocol

- The welfare assessment system is appropriate and tailored to the species, strain and experimental protocol

- Everyone is clear about the purpose of the experiment and the scientific objectives

- Everyone knows what will actually be done that day to the animal(s), with respect to the scientific procedures that will be carried out (including timing and numbers of animals), and are familiar with the signs that they may expect to see and what the endpoints are

- All relevant personnel (such as animal technologists and care staff, scientists) know how to use the system and can recognise the signs and interpret them clearly into intervention points

- All other relevant staff, who are not part of the welfare assessment team but who are directly or indirectly involved in animal care, are informed about the project and assessment protocol to an appropriate level

- If appropriate, the assessment sheets have been updated on the basis of new signs or combinations of signs observed – they are living documents

- Lines of communication are clear to report any concerns about animals or personnel to responsible persons (for example the scientist, veterinarian, senior animal technologist or carer)

3.1 Making observations

Having the same person, or a very small number of people, observing the animals wherever possible can facilitate consistency and enable assessors to follow the progression of an animal's condition more accurately[53]. Animals may also be able to tell the difference between different people (for example on the basis of individual odours), so they may benefit from contact with familiar staff as opposed to strangers[54]. Consistency of staff also enhances job satisfaction for animal technologists and carers, many of whom prefer to be responsible for the same animals throughout a study.

3.1.1 Observation from outside the enclosure

Assessing general appearance, posture and behaviour without provoking any responses provides useful information[6], so animals should first be observed without moving, approaching or entering the enclosure or opening the cage. This enables the observer to see whether there are unprovoked behaviours that could indicate welfare problems, such as social animals isolated from conspecifics, or nocturnal animals immobile but out of the nest. Alternatively, individuals may be playing, foraging or allogrooming, indicating that welfare is probably good. Animals should be observed for the predetermined time period to ensure that relevant indicators are more likely to be detected.

The enclosure should also be observed to see whether activities such as nest building, foraging or gnawing are reduced, or if there is evidence of health problems including bleeding, vomiting or abnormal faeces (see Figure 4). Clinical or behavioural indicators that can be observed without touching the animals or influencing their behaviour should be noted onto the assessment sheet

at this stage. For example these may include piloerection, postural changes, altered opercular beat frequency in fish[55], erratic movements and freezing in zebra fish[56], escape behaviour in *Xenopus*[57], ear posture changes in sheep[58], reduced mobility or favouring a surgical site in any species. These signs may be either specific to the project or unexpected, in which case they should be entered into the free text box if a structured sheet is being used.

Figure 4: Cage appearance in male HsdHan:NMRI mice with and without post-laparotomy pain
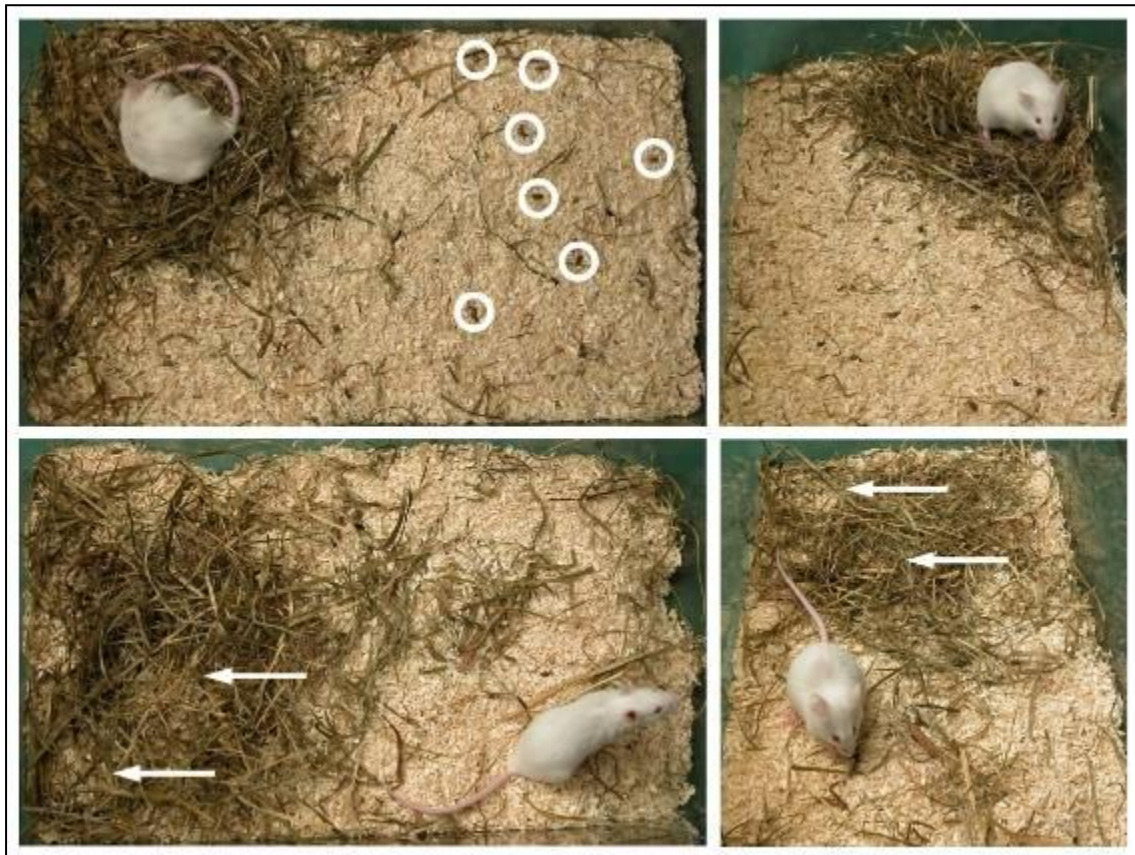
Mice in the upper two pictures have built well-structured nests and are defaecating in a separate area (circles), as expected for the strain. Mice in the lower two pictures are experiencing mild to moderate post-laparotomy pain; the cage area is unstructured without a separate area for defaecation and there are two nest-like resting places (arrows). Reproduced with permission from Arras et al. (2007)[20].

3.1.2 Opening the cage or entering the enclosure

The next stage is to examine the enclosure in more detail by removing the cage lid or entering the enclosure (or closely approaching it as appropriate). Enrichment items or nesting material should be removed or moved aside if necessary, and the animals' reactions to this – and the observer – should be watched for a suitable period of time, as previously determined. Most species would normally respond with increased activity followed by a settling down period. Any specific or unexpected signs should be recorded, as above.

3.1.3 Handling the animals

After completing the above initial checks, terrestrial animals should be individually caught and handled to measure and score relevant core criteria such as body weight, body condition and temperature. This is also the time to assess those specific criteria that require handling, for example, skin tenting, sensitive areas, tumour measurement or parameters that require blood sampling.

It is not always appropriate to handle animals. Handling may cause discomfort or pain following certain procedures, or removing animals from their housing would cause excessive distress under some circumstances, as in aquatic animals or some breeding females. In such cases, extra time should be allowed for careful visual observations.

3.2 Highlighting potential welfare issues

Carrying out observations and noting them onto the assessment sheets, as set out above, may signify that there is a welfare problem. This may be due to the presence of a key indicator, because the animal's score has reached a threshold level, or simply because someone feels that something may be wrong. Actions to take if animals are (or may be) suffering should have already been agreed and understood by all. There should be a clear line of reporting and everyone should know his or her responsibilities within it.

There should also be a failsafe system for flagging up enclosures containing animals that give cause for concern for any reason. All staff should know that animals housed in enclosures highlighted in this way require special attention and additional monitoring[13]. Examples of methods used to draw attention to animals with welfare problems include message boards outside the room and coloured pegs on enclosures.

3.3 Resources for effective welfare assessment

Introducing an effective welfare assessment system will require resources, both in developing the system and in the time taken to observe the animals. However, as good animal welfare is an intrinsic part of successful science[2,5,59-61], the benefits of setting up and implementing proper welfare assessment outweigh the cost in terms of economics and human resources. In the longer term, accurate, objective welfare assessment also enables animal research facilities to show how effectively their refinement initiatives are working. This not only demonstrates compliance with the letter and spirit of animal use regulations but also facilitates a more accurate, ongoing harm-benefit assessment of the research programme.

The financial cost for effective welfare assessment and health checking animals should be factored into the research budget as appropriate, for example by adding it in to grant applications or the cost to the client as applicable.

The Working Group believes that any economic cost will be repaid in improved welfare, reduced suffering and more robust data, that is, better quality science. Scientific results are less likely to be affected if adverse effects are detected earlier, or it will be easier to ameliorate adverse effects without affecting the science.

4   Reviewing welfare records

Timely reviews of welfare assessment records, during and after projects, are essential to ensure that welfare assessment systems are operating effectively; take account of any changes in the adverse effects noted; and ensure that any changes in the nature of the project, knowledge about animal behaviour or new assessment techniques are taken into account[4].

4.1 Reviewing adverse effects during projects

The key aim of welfare assessment review is to examine how well any adverse effects are being predicted, recognised and alleviated (Table 10). This could be done at one or more set points during a project, and/or in response to specific concerns about animal welfare or the effectiveness of the welfare assessment system that may arise. An advantage of setting appropriate interim review points during the life of a project is that initial, far-reaching systems of observations (for example in the case of a pilot study or a founder GA animal) can be refined to include only the most relevant parameters or time points. This makes for more efficient use of resources as well as more effective welfare assessment.

Table 10: Points to include in interim reviews

- ✓ Assessment of the welfare indicators; that is, the frequency with which they have been observed, with the aim of removing any that are redundant or adjusting the observation protocol

- ✓ Review of observations recorded in free text boxes, to see whether any new indicators should be added

- ✓ Review of interventions, including humane endpoints, and whether any animals were found dead; correlation of these with data from the sheets

- ✓ Review of the timing of observations and interventions, to ensure that the frequency of assessment is appropriate throughout the project

- ✓ Checks on consistency between observers, using external expertise where necessary. Statistical analysis can be used to confirm consistency in some circumstances[53,62].

- ✓ Comparison between the predictions of severity made at the project planning stage and the level of severity observed in practice.

Interim review should also help to detect any drift in the welfare consequences and the related clinical or behavioural indicators seen over time. This may be due to factors such as changes in the genotype or strain of animal used, changes in personnel performing procedures, or in the duration of the study, including the aging of the animals in long term studies. It may be appropriate to report the outcome of interim reviews to the ethics or animal care and use committee.

4.2 Retrospective review

There should always be a retrospective review of welfare assessment records once a project has been completed, which should contribute to ethical and scientific reviews of the completed project[63]. This should include the elements of interim review listed in Table 10.

Retrospective evaluation of welfare assessment records may be performed to inform ethical or animal care and use committees, and regulators where required, as to the actual adverse effects and severity of the animal model, including the implementation of humane end-points. This is essential for retrospective assessment of refinement, harm-benefit and the justification for projects (see Jennings & Howard, 2004[63]). Feedback to committees and regulators on these issues is good practice after projects and may also be provided at an appropriate, early, point in model development, such as after an initial pilot study.

A number of other bodies can benefit from the results of welfare assessment reviews. For example, it may well be possible and beneficial to exchange information with other research teams working on similar models. Welfare assessment protocols and data or results should be included if animals are passed to other institutes, in a similar manner to GA animal passports[64].

4.3 Reviewing the approach to welfare assessment

It is good practice regularly to review the effectiveness of welfare assessment at a species/strain level and an establishment level to evaluate how well the system works within the facility as a whole. The welfare assessment team may carry out this review, in conjunction with ethical or animal care and use committees, regulators and other relevant staff at the establishment as appropriate.

The review can take a number of approaches, such as analysing welfare assessment records of individual projects; discussion within the welfare assessment team, focusing on those who have been implementing welfare assessment; reviewing the membership of the team; reviewing the contribution of pilot studies and so on. Suitable topics for review are set out in Table 11.

Table 11: Questions to address when reviewing welfare assessment systems

- Is the system as a whole detecting welfare problems with acceptable efficiency over a range of projects? For example, do observations support subsequent pathological data; are mild effects being detected and leading to interventions that prevent further suffering; are animals being found dead and if so why?

- If the system is not working efficiently, can the reasons for any problems be identified and the problems rectified? At which stages are difficulties experienced?

- Are there issues with particular projects or species? Might external consultation or liaison be necessary?

- How have people adapted to using the system and how do they feel about it? Is everyone fulfilling his or her role?

- How is the welfare assessment system perceived by those not on the team, such as academic staff? Is there a need to better inform others about the team and its function?

- Is the welfare assessment system affecting animals, for example are they being excessively disturbed? Are there any husbandry issues that are making assessment difficult? Does there need to be a compromise between environmental enrichment and welfare assessment?

- Are there any new assessment techniques or knowledge that can be brought in?

- Is communication and liaison between the welfare assessment team and the various committees and regulators running smoothly, or are there any difficulties in communication? Is there any need for help with conflict resolution?

As with project reviews, the frequency of these more fundamental reviews will depend on the size and type of the establishment and the nature of the projects conducted within it. Reviews can be carried out at an appropriate pre-determined frequency and also in response to concerns about welfare assessment relating to any of the points above.

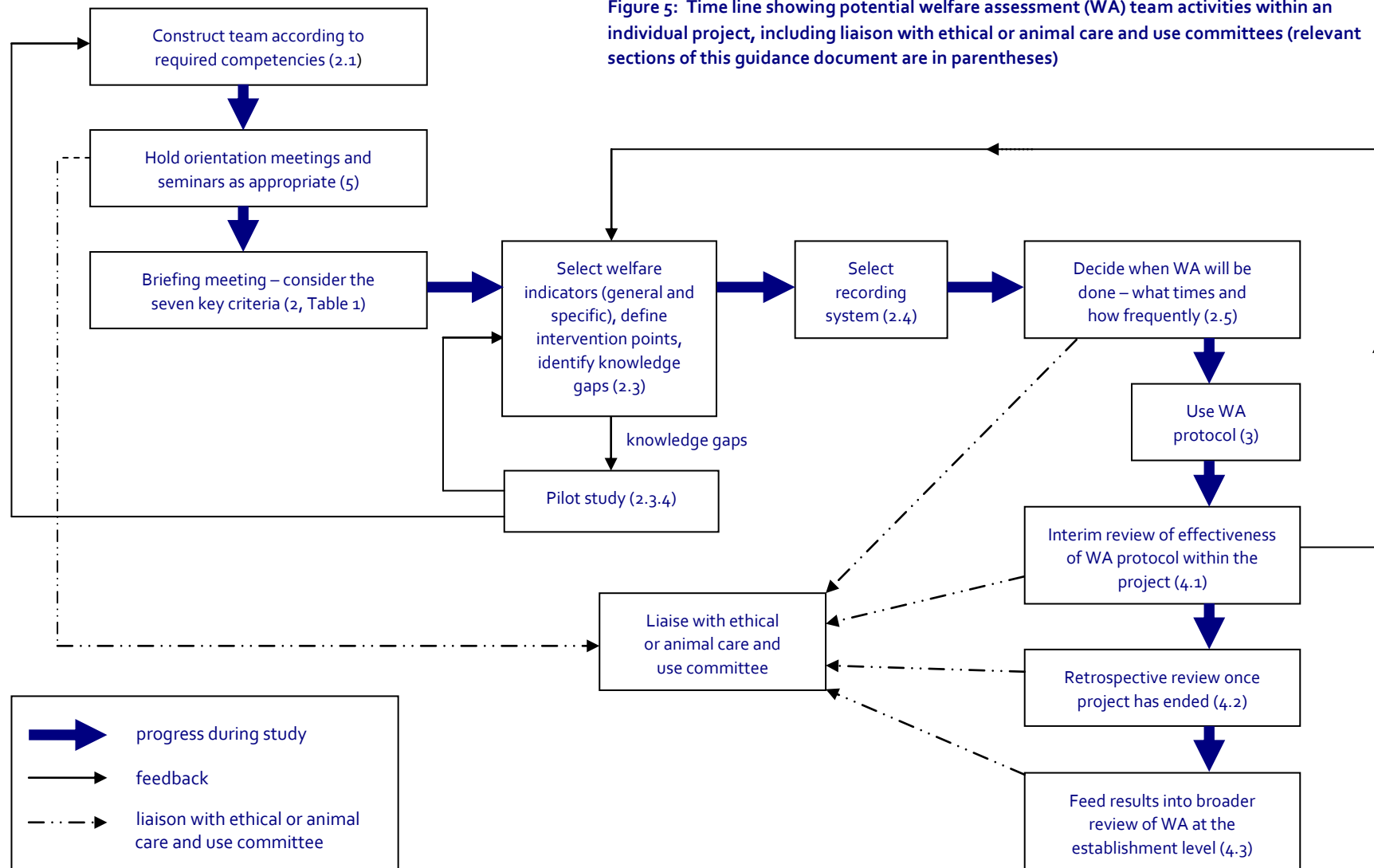# 5 Liaison with ethics or animal care and use committees

There are several points within the life of a project where the welfare assessment team may liaise with the ethics and/or animal care and use committees throughout the project, including interim reviews as appropriate, retrospective reviews upon completion and establishment level reviews of the approach to welfare assessment (Figure 5).

It is especially useful for the welfare assessment team to liaise with relevant ethics or animal care and use committees at the planning stage, to keep the committees informed about the welfare assessment protocol and to seek additional guidance on its effectiveness and acceptability with respect to the local culture of care at the establishment. This will facilitate discussion of the welfare assessment protocol and may enable it to be further refined before it is implemented for the first time. Ensuring that animal welfare and any pain, suffering or distress are adequately recognised and assessed is relevant to a number of the seven core functions of the UK Ethical Review Process (ERP)[65,66].

Depending on the nature and role of the committee, topics for discussion may include:

- What will happen to each of the animals throughout the project, from sourcing to euthanasia, reuse, release or rehoming

- What each animal will experience and where adverse effects on welfare are possible – including, but not only as a result of, experimental procedures

- Which parameters will be monitored during the welfare assessment and how they were decided

- How frequently animals will be assessed, when and why

- How observations will be recorded and analysed

- Explanation of the humane endpoints, how these were set and what will happen if they are exceeded

Figure 5: Time line showing potential welfare assessment (WA) team activities within an individual project, including liaison with ethical or animal care and use committees (relevant sections of this guidance document are in parentheses)

Construct team according to required competencies (2.1)

Hold orientation meetings and seminars as appropriate (5)

Briefing meeting – consider the seven key criteria (2, Table 1)

Select welfare indicators (general and specific), define intervention points, identify knowledge gaps (2.3)

knowledge gaps

Pilot study (2.3.4)

Select recording system (2.4)

Decide when WA will be done – what times and how frequently (2.5)

Use WA protocol (3)

Interim review of effectiveness of WA protocol within the project (4.1)

Retrospective review once project has ended (4.2)

Feed results into broader review of WA at the establishment level (4.3)

Liaise with ethical or animal care and use committee

progress during study

feedback

liaison with ethical or animal care and use committee

This time line depicts the most diverse scenario, where a WA team is initially set up, for a new project where a pilot study is necessary. It also encompasses maximum liaison with the ethical or animal care and use committee and full review of the effectiveness of the protocol, including feeding into review at the establishment level (section 4.3). How much of this is necessary for every project will depend upon factors such as the size of the facility, the nature of the research and testing and whether or not the experimental protocols are well established.

6   Information sharing

Welfare assessment is a rapidly developing field and the welfare assessment team needs to be aware of information on new scientific, technical and practical developments. Communicating with other establishments and research teams about experiences with different systems can help to disseminate good practice and facilitate the exchange of ideas.

6.1   Gathering information

It is good practice to keep up with the literature on welfare assessment and to look elsewhere, as useful information can be gained from other fields. The Appendix lists journals that often publish relevant papers and examples of keywords relating to welfare assessment.

Keeping fully up to date with the most current developments in welfare assessment requires a person with the time to do so, with appropriate skills in information retrieval and access to the sources of information. Ideally, each team would include such a person and they would hold direct responsibility for monitoring the literature. They may also be able to contribute to critically assessing the validity of new approaches and their suitability within the establishment.

In universities and large-scale research establishments, library and information staff can provide training and guidance on database searching, and in many cases can help to develop effective search strategies which can be saved and re-run at regular intervals. For example, these could be project-specific search strategies, using search terms related to the particular technique, species, and research objective, with terms related to welfare aspects such as housing, enrichment and analgesia. Library and information staff can also advise on the range of databases available and their coverage, and on how to formulate searches including the use of Boolean search terms (AND, OR and NOT) and the use of thesauri such as Mesh Terms in Medline (http://www.nlm.nih.gov/databases/databases_medline.html), or the CAB thesaurus (http://www.cabi.org/cabthesaurus/), to identify preferred terms (or keywords) to refine results. They may be able to provide training on the use of reference management software that can be used to build up an in-house reference library of materials.

Other members of the welfare assessment team or committees may keep up with the literature out of interest or in response to particular project proposals. For example, veterinarians, welfare specialists, ethologists, animal technologists and carers can be valuable sources of advice. Good communication with regulators such as the UK Home Office is also of great value, as Inspectors can bring in expertise that they have gathered across a range of establishments, species and projects.

6.2   Communicating about welfare assessment

Disseminating information on welfare assessment can help to progress science and raise the profile of animal welfare, by allowing people to compare new welfare assessment techniques, update methods and generally make welfare assessment more effective. Information on welfare assessment can be shared in a number of ways including papers, posters, presentations and websites.

Publishing information on welfare assessment
It should be possible to include brief details of the welfare assessment protocol within materials and methods sections of scientific papers. As an example:

"Animals were monitored continuously for the first hour following surgery and then hourly for the following six hours, using a binary welfare assessment sheet tailored to the project. The most significant indicators of adverse effects were reduced body mass, heart rate variability, piloerection, flank twitch and reduced rearing behaviour. Animals were treated with analgesics until no further signs of adverse effects were observed."

This uses fewer than 70 words to provide information that would help others to carry out effective welfare assessment of animals undergoing similar procedures (animal welfare benefit), and illustrate that animals had fully recovered before procedures (scientific benefit). The example above is fairly minimal and more detail would be useful if possible. It is also helpful to include the assessment sheet as a figure or appendix within the published paper or as online supplementary material.

A description of the welfare assessment protocol should be included in the methods section of peer-reviewed journal papers as part of the scientific method (see box). This provides information that will allow a better assessment of the scientific validity of the data, because welfare status reflects the psychological, physiological and neurological state of the animal. In addition, more published data on the animals (including species, strain, age, sex, reproductive and dominance status or temperament) and different husbandry systems or experimental procedures can help readers to improve not only their understanding and assessment of the study but also their own welfare assessment and progress with refinement. Interest in ethical publication policies is increasing and some journals have set their own policies and guidelines in this respect[64,67,68].

Posters also present opportunities to provide information on welfare assessment techniques in methods sections. Although the amount of text on a poster is limited, it is possible to include more information on a flyer to accompany the poster. Presentations at scientific or animal welfare meetings can also be used to inform other delegates about welfare assessment techniques; just one slide can make a difference and stimulate discussion.

Other ways of disseminating information include contributing to discussions at workshops and meetings such as industry discussion groups, visiting other establishments, including within employee exchange programmes, secure email forums and meetings for users of particular species or techniques.

6.3 Requirements for external reporting

Welfare records can be used to fulfil external reporting requirements, such as reviews for research funders, which may require evidence that any conditions they have set relating to the Three Rs or severity have been met. Regulators, funding bodies or committees may also require detailed information on severity, for example when considering an application for a new programme of work following on from a previous project or research grant.

At the time of writing, the recently finalised Directive regulating animal research and testing in the EU includes a requirement for the retrospective reporting of the actual severity experienced by each animal. In the UK, a working group set up by the Laboratory Animal Science Association (LASA) and the Animal Procedures Committee (which advises on the implementation of the law regulating animal use in the UK) has also recommended retrospective reporting[69]. It is therefore good practice to make sure that welfare assessment records are collected and stored so as to facilitate effective reporting when required.

The public is also an audience for external reporting, whether this is through the medium of the regulator (through the UK Home Office annual statistics and abstract database) or directly, via a company or other organisation's website. The public is concerned about the potential for suffering in animal research and testing, and directly or indirectly funds much of it. Transparency and accurate reporting are therefore important from a public perspective[2,69].

7 Training

The development and implementation of welfare assessment protocols requires a team of staff who between them have all the relevant knowledge and practical skills required. Some knowledge and skills will be acquired during professional laboratory animal science training (including that for veterinarians and animal technologists) or in mandatory training courses such as UK modular training[70] or the Federation of European Laboratory Animal Science Associations (FELASA) training scheme[71]. However, it is likely that additional, more in-depth training specifically tailored to the species, projects and welfare assessment processes of an individual establishment will also be necessary.

It is difficult to specify the training requirements for individual members of the welfare assessment team since their roles and background experience will differ. However, there are some key competencies that apply to all team members, which training (either locally or as part of the professional or mandatory courses mentioned above) should aim to develop. These are summarised in Table 12.

Table 12: Key welfare assessment competencies

The ability to understand and recognise:
• good welfare for the species (strain and individual, if appropriate) used in a study
• deviations from good welfare and how these present themselves, that is, the general signs of poor health or poor welfare
• specific signs of adverse effects (including early clinical signs and subtle behavioural changes) relating to each experimental protocol with which the trainee/team will be involved
The ability to understand:
• welfare assessment schemes in general (including different recording systems)
• the specific welfare assessment systems used at the establishment and how to implement these

The topics that training should cover to develop the competencies above are shown in Table 13, together with supporting comment about why each is important. Welfare assessment teams will benefit from having someone who understands the likely training needs of individual team members and can ensure that these needs are met.

Table 13: Training topics

Topic	Comments
Biology and behaviour of the species (and strain)	• Helps staff to recognise animals with good welfare and quality of life • Generates more interest in the animal and a greater ability to interpret behaviour, including recognition of deviations from normal • Gives an understanding of what the species requires and why, which helps to provide good housing and care and ameliorate welfare problems • Can encourage greater empathy for animals as individuals
Species and strain differences	• Discourages trainees from generalising between species and strains, which can lead to assumptions about behaviour and to adverse effects being overlooked
Potential causes of pain, suffering, stress and distress	• Facilitates more effective prediction of adverse effects and associated indicators • Improves the ability to devise effective welfare assessment protocols, ensuring that animals are observed when suffering is most likely to occur
Practical exposure to "welfare neutral" animals, those with poor health/welfare and those with adverse effects specific to the model	• Trainees need to see and handle (where appropriate) these animals, to gain competence and fully understand what they are looking for • This may need to be revisited at intervals to ensure that people do not become habituated to what they see
The science behind, and justification for, the animal procedures, at an appropriate depth	• Boosts the status of animal technologists and carers and promotes equal status of team members • Helps to make clear how end-points were decided and may enable staff to suggest earlier end-points
The law (and guidance) relating to severity limits and humane end-points and how it applies to the study	• Ensures that everyone can interpret and apply end-points and helps to prevent avoidable suffering and infringements
Perspectives on suffering in the context of the projects carried out at the establishment	• Acknowledging that the suffering may occur can make people more open to recognising it • Will help to identify those who are not comfortable with what they are being asked to do
Practical use of the welfare assessment systems employed at the establishment	• A sound understanding of the systems the trainee will go on to use is essential

As with other training there are a number of ways of tackling the topics in the table, but many of the issues benefit from a practical, hands-on approach. For example, it is much more meaningful for trainees to actually see animals with good and poor health and welfare and those with adverse effects specific to the model and/or

procedure, rather than just hearing about these in a seminar or lecture[*]. Video clips are a useful training aid in this respect, but visits to their own and other animal houses are essential[72,73].

People naturally feel uncomfortable about causing animal suffering and this can affect their ability to acknowledge and therefore recognise suffering, particularly when the effects are subtle or the animal attempts to hide it. Acknowledging that suffering can occur may make people more open, and able, to recognise it so discussion of these issues (preferably in small groups) is an integral part of the training for welfare assessment. This should also help identify those who are not comfortable with what they are being asked to do.

7.1 Developing competence

Ideally, trainees need to see and handle (where appropriate) animals, to gain competence and fully understand what they are looking for when assessing and recording welfare. They should also have the opportunity to gain competence in using the welfare assessment system and reviewing and acting upon the results of assessments. For staff new to welfare assessment (such as animal care staff starting work in the animal unit or new researchers) it can be helpful to assign an appropriately qualified mentor for the training and initial working period. This provides essential on-the-job training and a friendly face to ask any questions or advice from in a critical learning period.

It is important to recognise that a trainee does not have to be competent in performing a particular technique in order to assess the welfare of the animals involved. For example, it is possible to be competent in assessing the welfare of animals following microsurgery without being able to perform the operation. In fact, this can be an advantage, because post-operative welfare is often used as a benchmark for surgical competence. If the welfare assessor has not performed the surgery, they will not feel that they are calling their own ability into question when observing the animal.

7.2 Assessment of competence

Training (either formal course work or on-the-job) should aim to produce competent, confident welfare assessors who possess the necessary knowledge, experience and empathy to be able to recognise and deal with adverse effects and maximise good welfare for the animals on the project. Not everyone will be able to achieve this, no matter how good their training is, and in such cases, the trainee cannot be certified as competent and should not be entrusted with assessing welfare[*].

Some of the elements in Table 13 can be taught and assessed in the same way as other kinds of factual knowledge. However, it is more difficult to set and assess a standard of empathy with animals. People who cannot empathise with animals clearly should not be entrusted with assessing their welfare. Conversely, those who are highly sensitive to animal suffering may prefer not to be responsible for welfare assessment. These issues should generally become apparent during the discussions recommended on page 21.

Evaluating the ability to conduct welfare assessments is especially complex because the trainee has to demonstrate that they can assess consistently in comparison with other people. For example, it may be that someone is capable of recognising signs of adverse effects but is not good at quantifying the level of suffering, which is a disadvantage with some welfare assessment systems. Such a person may not be deemed sufficiently competent and suitable for the welfare assessment team. It may be helpful to ask each trainee to give an opinion on their own competence and comfort with what they are being trained to do.

Under any training and evaluation system, levels of competence can be thought of as "in training", "trained" or "trained to train". Only those who are "trained to train" can gauge the competence of others.

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[*] Availability of material depicting animal suffering is scarce, because of the legal requirements to minimise suffering and because people do not want to prolong animal suffering in order to record it (and/or may be concerned about traceability). There are also obvious legal, ethical and welfare issues associated with keeping suffering animals alive for trainees to view them, which the Working Group assumes will be taken into account when using this table.

[*] This is not unique to welfare assessment, for example some people are unable to acquire the necessary skills for procedures such as surgery or blood sampling.

7.3 Continuing Professional Development (CPD) and maintaining standards

It is good practice for each member of staff to have a personal training record in which their training, supervision needs and competence is recorded, with recommendations for Continuing Professional Development (CPD). The nature of CPD required will depend upon the individual's role within the welfare assessment team and on factors such as changes in the nature and severity of the technique(s), the species and strains involved and developments within science, assessment technology and the establishment itself. Whatever form of CPD or additional education and training is in place, an essential objective is to check that people are not becoming habituated to the adverse effects that they are observing (as in "normal is what you normally see").

Because welfare assessment is a rapidly developing field, the in-house training provided should itself be regularly reviewed and updated to take account of new information in areas such as animal behaviour, biology and welfare assessment, changes in animal models and new techniques for recognising and recording signs of positive and negative welfare. This also applies to national standards and the Working Group recommends that welfare assessment be addressed in more detail in, for example, the modular training given to meet the requirements within the ASPA and by FELASA courses[70,71].

8 Summary recommendations

Setting up the welfare assessment system

- Recognise that good practice in welfare assessment is necessary for good science as well as animal welfare.

- Take a team approach to welfare assessment, considering the team membership in terms of fulfilling appropriate competencies.

- Promote good team cohesion and constructive working relationships, both internally and between the welfare assessment team and other ethics and animal care and use committees.

- Provide education and training for team members as necessary, and ensure their competence in welfare recognition and assessment.

- Carefully consider how to define the baseline standard of good welfare for each project.

- Use a general list of simple, objective indicators as the basis of the assessment system.

- Set out a list of potential specific adverse effects tailored to each project, using a wide range of information sources.

- Predict the clinical or behavioural signs that may be associated with each adverse effect, taking into account how easy it will be to recognise and assess each one.

- Define intervention points and humane endpoints as part of the process of setting up the assessment system, at the project planning stage.

- Conduct pilot studies to define welfare indicators if necessary.

- Research the current understanding of indicators of positive welfare and consider adding these to the protocol if appropriate.

- Take a flexible approach to the choice of recording system.

- Consider using organised Animal Welfare Assessment Sheets to reduce subjectivity.

- Take care when interpreting numerical score sheets, as weighting and addition are not always straightforward.

- Strike a balance between relying on objective assessment schemes and experienced human judgement – both are necessary and complementary.

- Minimise the risk of missing essential signs by considering very carefully when animals will be assessed, how long for, and how often.

- Discuss the proposed welfare assessment protocol with all relevant committees; to explain how welfare will be assessed and suffering reduced, and to obtain their input and advice if this is part of their remit.

Using the welfare assessment system

- Make sure that the welfare assessment team has been properly briefed and trained, and everyone knows what they are supposed to be doing.

- Ensure that the recording systems, such as assessment sheets, have been updated if necessary.

- Observe the animals and their enclosure from a distance before opening the cage or entering the enclosure and handling them.

- Have clear systems in place for highlighting welfare concerns if suffering is believed to be significant or reaches pre-defined limits.

- Make sure that everyone is aware of requirements for interventions and humane endpoints and knows how to act on them.

- Maintain excellent communication and teamwork between all persons involved with the study and the welfare assessment team. This will ensure that the entire team works smoothly together to resolve issues rapidly if interventions are required.

- Accept that effective welfare assessment will require resources in terms of financial costs and staff time.

- Ensure that there are sufficient staff to monitor animals properly, however long this may take.

- Always give animals the benefit of the doubt with respect to whether or not they are experiencing suffering.

Reviewing the welfare assessment system

- Conduct both planned and ad hoc interim reviews as appropriate.

- Relate observations made in practice to the predictions of the level and nature of adverse effects made during project planning.

- Always perform a retrospective review of welfare assessment records when projects are completed, in conjunction with ethical and scientific reviews and reporting requirements as appropriate. Communicate the results to all those who would benefit.

- Regularly review welfare assessment within the facility as a whole, to check that it is working effectively and that people are comfortable with the system.

Information sharing

- Review and incorporate new knowledge about behaviour and welfare assessment into welfare assessment protocols and encourage others to do so.

- Appoint someone with the time and necessary skills in information retrieval to keep up to date with developments in welfare assessment and other relevant areas.

- Share details of the welfare assessment method used in projects whenever possible, in papers, posters, presentations, at meetings and so on.

- Consider how welfare assessment schemes could relate to severity reporting requirements.

Training

- Provide in-house training in welfare assessment, tailored to the trainees' future areas of work as far as possible.

- Take every step possible to ensure that only those with the appropriate level of empathy with animals are allocated to welfare assessment.

- Provide a syllabus that includes all elements that are important for effective welfare assessment.

- Do not entrust anyone with assessing animal welfare unless they have attended and passed a training course and are fully competent.

- Ensure that training courses are regularly updated to take account of new knowledge and techniques.

- Include provision for CPD and regular refresher courses within the training scheme.

Acknowledgements

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10   Appendix: Resources

This Appendix sets out useful journals, web-based resources and discussion forums relating to welfare assessment at the time of writing.

Journals and keywords
These journals frequently publish papers relevant to welfare assessment:

Alternatives to Laboratory Animals (*ATLA*)	http://www.frame.org.uk/index.php
Applied Animal Behaviour Science	http://www.applied-ethology.org/thejournalaabs.htm
Animal Technology and Welfare	http://www.iat.org.uk/publications/atw.htm
Animal Welfare	http://www.ufaw.org.uk/animal.php
Contemporary Topics in Laboratory Animal Science	http://www.aalas.org/index.aspx
Journal of Applied Animal Welfare Science	http://www.psyeta.org/jaaws/
Lab Animal and *Lab Animal Europe*	http://www.labanimal.com/laban/index.html http://www.labanimaleurope.eu/
Laboratory Animals	http://la.rsmjournals.com/
Physiology and Behavior	http://www.elsevier.com/

The following keywords are helpful when searching for information on welfare assessment:			
affect	harm benefit assessment	positive indicators	severity scale
animal welfare	humane endpoints	positive welfare	sickness behavio(u)r
animal suffering	needs	qualitative behavio(u)r assessment	stress
assessment	objective assessment	quality of life	suffering
discomfort	pain	refinement	welfare assessment
distress	pain assessment	score sheets	welfare indicator
harm assessment	pain measurement	scoring system	welfare outcomes

Recommended reading and web-based resources

Background reading

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Discussion forums

American Association for Laboratory Animal Science (AALAS) discussion groups: CompMed™ for veterinarians, animal technologists and care staff, animal facility managers, researchers, and graduate/veterinary students; TechLink for animal technologists and care staff who are AALAS members; and IACUC-Forum for AALAS members and their IACUC members and staff. See http://www.aalas.org/online_resources/listserves.aspx

Laboratory Animal Refinement and Enrichment Forum (LAREF) is open to animal technologists and care staff, students, attending veterinarians and researchers who have or had first-hand experience in the care of animals kept in laboratories. See the Animal Welfare Institute website; http://www.awionline.org/ and search for "LAREF".

Guidelines

American College of Laboratory Animal Medicine (ACLAM). *Guidelines for the Assessment and Management of Pain in Rodents and Rabbits*, 2006*.* See http://tinyurl.com/65ez5vh

Companion Animal Welfare Council (CAWC). *Companion Animal Welfare Assessment.* Sidmouth, Devon, CAWC, 2009. See http://www.cawc.org.uk/reports

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Examples of welfare indicators

ACLAM (American College of Laboratory Animal Medicine). *Guidelines for the Assessment and Management of Pain in Rodents and Rabbits.* ACLAM, 2006. See http://tinyurl.com/65ez5vh

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Institute for Laboratory Animal Research (ILAR). *Recognition and Alleviation of Pain in Laboratory Animals.* Washington, DC, National Academies Press, 2009. See http://dels.nas.edu/animal_pain/

Links to other resources

Altweb section on refinement and welfare assessment http://altweb.jhsph.edu/

Canadian Council on Animal Care (CCAC) Welfare assessment. See http://www.ccac.ca/en/alternatives/ and click on "Refinement alternatives" and "Welfare assessment".

National Centre for the Three Rs (NC3Rs) Welfare assessment. See http://www.nc3rs.org.uk/welfareassessment

Training

Assessing the Health and Welfare of Laboratory Animals (AHWLA) http://www.ahwla.org.uk/index.html

Retrospective review

Jennings M, Howard B. *Guidance Notes on Retrospective Review: A Discussion Document Prepared by the LASA Ethics and Training Group.* Tamworth, LASA, 2004. See http://www.lasa.co.uk/publications.html

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Karas AZ. Barriers to assessment and treatment of pain in laboratory animals. *Lab Animal* 2006;**35:**38-45

Mayer J. Use of behaviour analysis to recognize pain in small mammals. *Lab Animal* 2007;**7:**16-26

Resources for ethical and animal care and use committees

The RSPCA Research Animals Department website includes resources for members of local Ethical Review Processes (ERPs) in the UK that are also useful for other forms of ethical or animal care and use committee wolrdwide. It addresses a number of relevant topics including *Severity of suffering*. http://www.rspca.org.uk/ethicalreview