****

**Welfare assessment and severity classification**

**Welfare assessment and severity classification**

It is almost inevitable that some laboratory animals will experience discomfort due to scientific procedures, but ASPA requires that every effort must be made to identify and minimise the adverse effects caused. Poor welfare and pain can produce a range of undesirable physiological changes, and these changes may affect the experimental results as well as compromising animal welfare. The Animals (Scientific Procedures) Act 1986 requires that anaesthesia or analgesia are used for all procedures unless they would be more traumatic to the animal than the procedure itself, that the most refined research techniques possible are used, that animals in severe pain must be killed in all circumstances, and that the animal’s physiological and behavioural needs are catered for. Failure to provide for these needs can result in an abnormal animal, and abnormal experimental results.

**Why do we need to assess welfare?**

There are many reasons why the degree of suffering that the animals experience is assessed. The reasons for this are:

* We have a moral obligation to animals to minimise harms caused
* It is essential to justify use of animals in biomedical research as part of the harm: benefit balance
* It facilitates decision making about responses to treatment, decisions about euthanasia
* Protocols in project licences are assigned one of four severity categories: non-recovery, mild, moderate and severe. It is important to be able to assess welfare to know if this is exceeded.
* The Animals (Scientific Procedures) Act 1986 requires retrospective assessment of actual severity
* Essential for implementing refinement in an ongoing manner

**What is good welfare?**

Before we can assess welfare, it is important to understand what we are looking for. The aim is to ensure that the welfare of animals is maintained, and that procedures are maximally refined to minimise any pain suffering distress or lasting harm.

The Animal Welfare Act 2006 identifies 5 welfare needs. These are:

* the need for a suitable environment
* the need for a suitable diet
* the need to be able to exhibit normal behaviour patterns
* the need to be housed with, or apart from, other animals
* the need to be protected from pain, suffering, injury and disease.

If these welfare needs are met, it can be assumed that the welfare of the animal is not compromised. Scientific procedures can impact negatively on all these welfare needs. Failure to provide for these needs impacts on both welfare and scientific outputs.

**What are Pain, Suffering, Distress and Lasting Harm?**

Stress can be described as an induced alteration in biological equilibrium caused by internal or external factors, physiological or psychological. Animals are exposed to stressors all the time. Homeostatic mechanisms are then stimulated to counteract the stress. Mostly, these stressors are minor, and responses are within normal physiological limits. The animal uses minimal effort to respond and is unconscious of the stress. Prolonged challenge though will result in overstress, where homeostasis struggles to return the animal completely to normal. This impacts on physiology although the animal may remain unconscious of it. Overstress can be detrimental to biological processes, such as growth, in the longer term. Eventually, prolonged or more significant challenge can lead to distress, where considerable effort is put into the response, of which the animal is aware. This is the point at which the animal experiences **distress**. This is an aversive, negative state in which coping and adaptation processes in response to stressors fail to return an organism to physiological and/or psychological homeostasis. Distress can result in maladaptive behaviours, or an aversive state resulting from maladaptation. Distress occurs if an animal cannot escape from or adapt to the internal or external stressors or conditions that it is experiencing, and this results in negative effects on its well-being.

The point at which stress becomes distress depends on several factors, including the type, duration and intensity of stress, the species of animal, and the capacity of an animal to respond. Animals may deteriorate while appearing otherwise normal- this is common in prey species. At some unknown moment, the animal rapidly deteriorates into a sick or debilitated animal.

**Pain** is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Normal/protective pain is intended to prevent further tissue damage following injury. It results in learned avoidance and modification of behaviour. However, pain quickly becomes non-protective/maladaptive, due to peripheral and central sensitisation, and then pain can persist after the initiating cause has been removed, or the pain experienced is out of proportion to the injury.

**Suffering** can be defined as a negative avoidable state derived from adverse physical, physiological and psychological circumstances in accordance with the cognitive ability of the species and the life experiences of the individual. Suffering may be induced by pain, distress, malaise, boredom, frustration, or even grief.

Laboratory animals may experience both **direct suffering**, due to the effects of the procedures themselves, or **contingent suffering**, due to indirect factors such as single housing, transport etc.

Animals that are experiencing distress or pain will exhibit biological and behavioural responses. For example, there may be:

• Physiological and biochemical changes: heart rate, blood pressure, hormone levels or immunological function.

• Behavioural changes: modification of behaviour to avoid repetition of the painful situation. Behavioural responses may be automatic - reflex responses to protect the animal or a part of it; for example, withdrawal reflex, freeze or flight responses. Alternatively, behavioural changes may be population response, where the animal reacts to convey the experience to others in its group, e.g. by vocalisation or by the release of pheromones. This will influence the behaviour of others in the group. If they are unable to react as their normal behavioural repertoire would demand, for example by escaping to a safer place, this may cause stress to these other animals.

These changes can be monitored, to identify when animals are experiencing adverse effects.

**How do we assess welfare?**

In principle, welfare can be evaluated by looking for responses to pain and distress. However, this is not straightforward, because responses to pain and distress vary between species and among individuals within a species. Additionally, there are no universally agreed criteria for the assessment of welfare. Nonetheless, there are several methods described for welfare assessment in animals. While none are universal, each has its merits.

The first stage in assessing an animal's well-being is to become familiar with what is normal, for the species, the physiological state of the animal, and for the individual. This includes the normal behavioural repertoire, remembering that this may not be the same as the behaviour observed in the laboratory animal house due to the constraints of the local environment. It is important to distinguish between what is ‘normal’, and what is normally seen. Once the observer is familiar with the normal, it is easier to discern when something is abnormal. These abnormalities can then be evaluated and sometimes quantified, to determine the degree of pain suffering or distress being experienced.

**Practical welfare assessment**

When assessing welfare, several factors need to be evaluated, including:

* Individual details, e.g. species, age, and origin. For example: A rabbit is more stoical than a dog and will be better at hiding illness.
* The history of the animal. Have there been any previous problems? The environment in which the animal is kept can influence responses, as can the procedures that have been carried out, and current disease problems. If the animal has recently arrived then it may be stressed from transport and show abnormal responses.
* Current condition of the animal. A clinical examination may be needed. The extent of this will depend on the species. Consider appearance, behaviour, food and water intake, physiological signs (such as heart and respiration rate), biochemical signs or signs of disease.
* Mental status. Take notice if the animal appears dull, depressed, aggressive, or hyperexcitable, especially if such traits are unusual.
* Activity. This may range from total inactivity to maniacal hyperactivity. Notice if there are any changes in gait, posture or facial expression.
* Vocalisation. This depends on the species and there are a wide variety of different noises produced by each species. The sound emitted may be outside the human auditory range and therefore go unnoticed but may be causing distress to others of the same species.
* Response to analgesics. If a dose of an analgesic drug is administered and the animal's condition and demeanour improves, then this may indicate that pain was present.

**Quantitative welfare assessment – distress scoring**

Reliable distress scoring systems can be developed to allow quantitative assessments of the amount of pain or distress an animal is experiencing. Such systems have to be easy to use, consistent, specific and sensitive. Several systems have been described, all typically involve the selection of parameters believed to be indicative of well-being, then assigning a score with the aid of descriptors to indicate whether the animal is normal or abnormal, and evaluating the degree of abnormality.

The use of such systems encourages regular close observation of the animal, leading to improved standards of animal care. If the animal is found to be deteriorating, then steps can be taken to ameliorate this in a timely fashion. It is vital to remember to re-score the animal after giving analgesics or other treatments to ensure that the drugs have had the desired effect and the animal’s condition has improved.

There are limitations with distress scoring systems, due to their subjective nature, and the lack of universally agreed criteria for determining what is, or is not, painful or distressing to an animal. Pain and suffering may be overestimated in some situations and underestimated in others. There can be inter-observer variation, where operators differ in their interpretation of the same signs. Consistency and sensitivity can be improved by having several observers score the animal at each point, or by having all assessments performed by just one or a few, experienced persons. This requires a proactive approach and a great deal of effort to develop consistency. Lack of sensitivity is another problem: ‘general’ score sheets will not be sensitive enough to pick up specific effects, so a tailor-made system specific for each model is needed. Even simple systems can be very time consuming to implement, making them impractical to use on large scale. Use of behavioural indices improves the effectiveness of welfare assessment. Effectiveness also requires detailed species knowledge, including a thorough understanding of the normal/baseline state

**Example 1.** General distress scoresheet. This example was devised by Morton and Griffiths (1985) (Veterinary Record 116, 431-36). The parameters used were:

* Appearance
* Food and water intake
* Clinical signs
* Natural behaviour
* Provoked behaviour

This is a very general method, which may not be sensitive in all situations. It can however form the basis for more sophisticated systems and be developed to fit the model, when specific indicators of distress can be identified. Pilot studies are helpful here, to identify more specific parameters. See appendix 1.

**Example 2.** Evaluation of model specific pain-related behaviours. Rats were scored for specific pain related behaviours after laparotomy (see http://www.digires.co.uk/). It was found that certain behaviours, twitching, back arching and falling over, could be quantified and were correlated with the response to analgesics.

**Example 3.** Facial expression. Several groups have now begun to use facial expression as a tool in assessment of pain (see Langford, D.J., Bailey, A.L., Chanda, M.L., et al. (2010). Coding of facial expressions of pain in the laboratory mouse. Nature Methods, 7:447-449; and Guesgen M.J., Beausoleil N.J., Leach M., Minot E.O., Stewart M., and Stafford K.J (2016). Coding and quantification of a facial expression for pain in lambs. Behavioural Processes, 132: 49-56). Grimace scales have been developed for use in mice, lambs, horses, piglets, rats, and rabbits, and will no doubt follow for other species.

None of these systems are applicable universally, but they provide researchers with a range of tools to aid in assessment of animal welfare.

**End points**

Welfare assessment has to be accompanied by a plan of action, since monitoring without intervention does NOTHING for the animal. Welfare assessment can be used to define predetermined end points, e.g.

* Scientific end point – all required data has been collected.
* Error end point – experiment has gone wrong and data cannot be collected
* Humane end point – animal has reached predetermined limit of acceptable suffering.

Death should be avoided as the endpoint for animal experiments unless scientifically necessary. A defined humane end point may allow for a lower severity category than would be needed if the procedure were allowed to run its full course.

Humane endpoints consist of clear, predictable and irreversible criteria which are used to determine the degree of suffering experienced, and/or are predictive of more severe effects such as advanced pathology or death. When assessment of the animal identifies that the endpoint is reached, action can be taken to minimise pain or distress whilst still meeting experimental objectives, such as administration of analgesics, humane killing or conducting a terminal procedure. Humane endpoints are especially important in studies where potential severe suffering or death may occur.

Criteria which can be used to set a humane endpoint include body temperature, body weight, behavioural changes, pathology observed using imaging technology and blood oxygen saturation.

For examples of humane end points, see https://www.nc3rs.org.uk/humane-endpoints

When designing a humane endpoint, the researcher should first understand the desired scientific outcomes, and identify criteria that allow recognition of when these have been met. Then, the researcher should identify any potential adverse effects, and the times when these may occur. These can then be used to place the potential adverse effects and associated clinical signs in the context of the scientific output and establish criteria for humane endpoints. Pilot studies using small numbers of animals may be helpful to determine the onset and progress of adverse effects and to identify criteria for humane endpoints. Once the end points are set, they should be validated and monitored to ensure they are sufficiently sensitive to minimise suffering without interference with the scientific objectives.

Animals need to be monitored frequently enough to detect signs of distress at an early stage, then ACTION taken to reduce potential suffering. This will reduce the overall severity and lead to refinement. Action taken could include the administration of analgesics, changes to husbandry, euthanasia, or changes to the procedure. There should be appropriate training and assessment of competency for all those engaged in monitoring animals for signs of adverse effects.

**Cumulative severity**

In some cases, it is necessary to make a judgment as to the total lifetime severity an animal may have experienced. This will be needed if an animal is to be re-used, or re-homed. This judgment may need to include consideration of intercurrent problems such as illness, injury, or contingent suffering due to transport, restrictive housing etc, and will usually require an assessment to be made by a veterinary surgeon. Good record keeping is essential to carry out such an assessment.

**Severity categories and retrospective recording of actual severity**

Project licences specify the likely adverse effects to be caused to animals, and the action to be taken if these occur. Each protocol is assigned to one of four **severity categories**, which describes the maximum degree of severity which may be inflicted in pursuit of the scientific goal. The categories are: non-recovery, mild, moderate, or severe. The category reflects the single worst case scenario - the worst degree of pain, suffering, distress or lasting harm expected to be experienced by an individual animal during the course of the procedure. If this severity limit is exceeded, the Home Office must be informed by the PPLh. Animals in severe pain which cannot be alleviated MUST be killed – this is the responsibility of the personal licence holder.

The ASPA 1986 also requires project licence holders to record the **actual severity** caused to animals by the regulated procedures. Non-procedure related harms are not included in this assessment. Procedures are classified as having been either non-recovery, mild, moderate, or severe. In addition, if the regulated procedure is deemed not to have inflicted sufficient harm to have reached the lower threshold for regulation, the actual severity will be recorded as sub-threshold. PILh may be asked to help with classification of procedures. Good, accurate, contemporaneous records are required. The actual severity records are returned to the Home Office by the PPL holder in the annual returns.

**Severity categories**

* **Non-recovery:** Procedures performed entirely under general anaesthesia from which the animal does not recover consciousness.
* **Mild:** Procedures in which the animals experience short-term mild pain, suffering or distress, and procedures with no significant impairment of the animal’s well-being or general condition.
* **Moderate:** Procedures which cause short-term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress. Procedures that cause moderate impairment of the well-being or general condition are also ‘moderate’.
* **Severe:** Procedures which may cause severe pain, suffering or distress, or long-lasting moderate pain, suffering or distress. Procedures causing severe impairment of the well-being or general condition of the animals are also ‘severe’. If death is likely, the prospective severity will be categorised as severe. Animals which die are recorded as having experienced severe actual severity, unless the cause of death was unrelated to the regulated procedures, or an informed decision can be made that the animal did not experience severe suffering prior to death.
* **Sub-threshold**: procedures in which actual severity experienced does not come above the threshold for regulation.

**Assignment criteria**

The severity category is based on all the interventions or manipulations carried out during a defined procedure and reflects the most severe effects likely to be experienced by an individual animal after applying all appropriate refinement techniques.

The factors considered include:

* Procedure related factors
	+ type of manipulation;
	+ nature of pain, suffering, distress or lasting harm caused by the procedure, and its intensity, the duration, frequency and multiplicity of techniques employed;
	+ cumulative suffering within a procedure;
	+ if the animal is to be reused, the actual severity of the previous procedures;
* Husbandry factors
	+ handling;
	+ prevention from expressing natural behaviour including restrictions on the housing, husbandry and care standards.
* Animal related factors
	+ species and genotype;
	+ maturity, age and gender;
	+ training experience of the animal with respect to the procedure;
* Refinements applied
	+ methods used to reduce or eliminate pain, suffering and distress, including refinement of housing, husbandry and care conditions;
	+ frequency of monitoring;
	+ humane end-points;

The actual severity recorded should be the maximum level experienced during the procedure, and may differ significantly from the severity limit allowed by the PPL. In many cases it will be much less: often animals will suffer mild severity on a protocol that has a moderate or severe severity limit.

NOTE: If the suffering is judged to have exceeded the severity limit allowed, the PPL holder must inform he Home Office Inspector of this as soon as possible.

**EXAMPLES**

**Mild:** Administration of anaesthesia except for the sole purpose of killing; subcutaneous injection of substance which causes no adverse effects.

**Moderate:** Surgery under general anaesthesia with appropriate analgesia, associated with some post-surgical pain, or impairment of general condition, e.g. thoracotomy, craniotomy, laparotomy, lymphadenectomy, orthopaedic surgery with effective stabilisation and wound management, organ transplantation with effective management of rejection, surgical implantation of catheters, or biomedical devices (e.g. telemetry transmitters, mini-pumps etc.).

**Severe:** Induction of tumours, or spontaneous tumours, expected to cause progressive lethal disease associated with long-lasting moderate pain, distress or suffering. E.g. tumours causing cachexia, invasive bone tumours, tumours resulting in metastatic spread. Models where mortality is expected.

**More examples can be found in Appendix G of the HO Guidance notes.**

**Summary**

Welfare assessment allows for objective measurements of suffering, and it facilitates evaluation of responses to treatment and decisions about euthanasia. It requires that ‘pain, suffering, distress and lasting harm’ can be recognised. This can be difficult as there are no universally agreed criteria for assessing what is, or is not, painful or distressing to an animal.

To be able to evaluate the welfare of an animal, you must be able to understand the species, know the behaviour of the individual animal, observe it carefully: appearance, behaviour, physiology etc at appropriate intervals, and take ACTION to REFINE your procedures.

**Appendix 1. Example of a general distress scoring sheet**

| **PARAMETER** | **ANIMAL ID:** |  | **DATE/TIME** | **DATE/TIME** |
| --- | --- | --- | --- | --- |
| **APPEARANCE** | Normal | 0 |  |  |
|  | General lack of grooming | 1 |  |  |
|  | Coat staring, ocular and nasal discharges | 2 |  |  |
|  | Piloerection, hunched up | 3 |  |  |
| **FOOD AND**  | Normal | 0 |  |  |
| **WATER**  | Uncertain: body weight --<5% | 1 |  |  |
| **INTAKE** | --intake: body weight –10-15% | 2 |  |  |
|  | No food or water intake | 3 |  |  |
| **CLINICAL**  | Normal T, Cardiac and Resp. rates | 0 |  |  |
| **Signs** | Slight changes | 1 |  |  |
|  | T ± 1oC, C/R rates ↑ 30% | 2 |  |  |
|  | T ± 2oC, C/R rates ↑ 50% or very -- | 3 |  |  |
| **NATURAL** | Normal | 0 |  |  |
| **BEHAVIOUR** | Minor changes | 1 |  |  |
|  | Less mobile and alert, isolated | 2 |  |  |
|  | Vocalisation, self-mutilation, restless/still | 3 |  |  |
| **PROVOKED**  | Normal | 0 |  |  |
| **BEHAVIOUR** | Minor depression or exaggerated response | 1 |  |  |
|  | Moderate change in expected behaviour | 2 |  |  |
|  | Reacts violently, or very weak and precomatose | 3 |  |  |
| **SCORE ADJUSTMENT** | If you have scored a 3 more than once, score an extra point for each 3 | 2-5 |  |  |
|  | **TOTAL** | 0-20 |  |  |

**JUDGEMENT**

0-4 Normal

5-9 Monitor carefully, consider analgesics or other treatments.

10-14 Suffering, provide relief, observe regularly. Seek second opinion from named animal care and welfare officer and/or named veterinary surgeon. Consider termination.

* 1. Severe distress, is this severity limit justified?