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EU GUIDANCE ON THE IMPLEMENTATION OF DIRECTIVE 2010/63/EU ON PROTECTION OF ANIMALS USED FOR SCIENTIFIC PURPOSES

SEVERITY ASSESSMENT – A CONTINUOUS PROCESS

LEGAL REQUIREMENTS – SEVERITY ASSESSMENT

Directive 2010/63/EU on the protection of animals used for scientific purposes requires that a prospective assessment is made on the severity of each procedure in a Project (Article 15) and that a severity classification is assigned, which may be either "*non-recovery*", "*mild*", "*moderate*" or "*severe*". Annex VIII provides guidance on the factors to be taken into account in the consideration of prospective severity and provides some examples in each severity category.

Article 54 on reporting requires that for statistical information, the actual severity of the pain, suffering, distress or lasting harm experienced by the animal must be reported (in contrast to the prospective assessment, or prediction, of severity made at the time of the project evaluation). In addition, the actual severity of any previous procedures will be a key consideration in determining whether or not an animal can be reused in further procedures (Article 16).

BENEFITS OF A CONTINUOUS SEVERITY ASSESSMENT PROCESS

Main benefits of prospective assessment, monitoring, assessing and recording actual severity include:

- Opportunities in particular to implement Refinement and reduce suffering, although prospective discussions will generally also provide an opportunity to consider whether or not animal use is necessary (Replacement) and the study design is appropriate to minimise animal use (Reduction);
- Improved animal welfare, e.g. if suffering is recognised and alleviated sooner;
- Improved transparency, as statistics should better reflect the actual welfare costs to animals;
- Improved communication between those responsible for using, caring for and monitoring animals;
- Input to retrospective project assessment when this is carried out (Article 39);
- Improved scientific data quality due to better welfare;
- Increased knowledge about assessing severity and clinical signs, which will promote greater consistency in assessments - provided that approaches and results are disseminated, e.g. via journals;
- Input into training courses for researchers, animal technologists and laboratory animal veterinarians;
- Evidence-based information that can be used in prospective harm-benefit assessments for similar, future projects.

SEVERITY ASSESSMENT – A CONTINUOUS PROCESS

PROJECT PLANNING

- DEVELOP PROJECT, SPECIES AND STRAIN SPECIFIC SEVERITY ASSESSMENT
- DECIDE ON MONITORING TOOLS, FREQUENCY, TYPE OF SCORING
- AGREE ON ACTIONS WHEN SIGNS OF PAIN, DISTRESS OR SUFFERING OBSERVED
- ENSURE PERSONNEL WITH ALL NECESSARY SKILLS ARE INCLUDED IN THE PROCESS

DURING THE PROJECT

- CONSISTENCY IN OBSERVATIONS / TRAINED STAFF
- EFFECTIVE DAY-TO-DAY MONITORING
- GOOD COMMUNICATION AMONG ALL INVOLVED
- ONGOING REVIEW OF ASSESSMENT PROTOCOL AS NECESSARY

AFTER THE PROJECT ANALYSIS AND FEEDBACK

- ASSESSMENT AND SCORING OF ACTUAL SEVERITY
- ▸ STATISTICAL INFORMATION
- ▸ RETROSPECTIVE PROJECT ASSESSMENT
- ▸ FEEDBACK FOR FUTURE STUDIES
- ▸ REFLECT ON FURTHER OPPORTUNITIES TO IMPLEMENT THREE Rs
- ▸ INPUT TO THEMATIC REVIEWS

Legend: red boxes: practical elements Blue boxes: benefits / outcomes

Example(s) of project/procedure specific severity assessment including the day-to-day assessment sheets, scoring tools, choices of monitoring methods and final assessment and other full document available at: http://ec.europa.eu/environment/chemicals/lab_animals/interpretation_en.htm .

EFFECTIVE SEVERITY ASSESSMENT REQUIRES

- A 'team' approach of people with different expertise, experience and priorities, e.g. researchers, animal technologists and care staff, the veterinarian;
- Appropriate continuing education and training of all personnel involved;
- Day-to-day severity assessment systems that are appropriately tailored to the species, strain and project, including informed and structured observations of animals at appropriate intervals (e.g. frequency increased during and after procedures);
- Well-informed, effective protocols for assessing behaviour and clinical signs;
- Analysis of the observations to make an informed judgement on the nature and level of suffering;
- Awareness of the severity of each procedure and what action to take if this is reached or exceeded;
- A consistent approach to overall judgements on actual suffering (mild, moderate, severe) for statistical reporting;
- Reflection upon how effectively the Three Rs were implemented and whether improvements could be made for future studies.

A number of illustrative examples of severity assessment have been prepared to assist in the understanding of the different elements within the process. (http://www.acceptance.ec.europa.eu/environment/chemicals/lab_animals/pdf/examples.pdf)

One example is given on the right:

EXAMPLE: EFFICACY OF NOVEL PHARMACEUTICAL AGENTS ON TUMOUR GROWTH - MULTI-STEP PROCEDURE

The study is intended to assess the efficacy of novel agents at reducing or arresting growth of tumour cells. The tumour needs to be well established before treatment can begin (usually 0.5 cm) – due to the duration of the study some tumours may develop up to a maximum of 1.2 cm in diameter, usually in the vehicle control group. Cytotoxic drugs are likely to cause some adverse welfare effects.

30 male BALB/C nude mice will be injected with slowly growing tumour cells. Animal welfare will be assessed daily and animals will be weighed once a week for 3 weeks. Tumour growth will be measured on day 7, 14 and 20 and animals then be randomized and treatment started in the form of twice-daily intra-peritoneal injections for 7 days.

INITIAL PROSPECTIVE ASSESSMENT AND CONSIDERATION OF SPECIFIC REFINEMENTS AND HUMANE END-POINTS

What does this study involve doing to the animals?	What will the animals experience? How much suffering might it cause? What might make it worse?	How will suffering be reduced to a minimum?	
	Adverse effects	Methodology and interventions	End-Points
Maintenance of immune-compromised mice	Animals are susceptible to infection	Housed in IVCs and husbandry practices tailored to minimise risk of contamination Animals group housed and environmental enrichment provided to reduce stress Husbandry and care will be reviewed if any signs of distress, aggression or abnormal behaviours observed	Any animal showing signs of inter-current disease will be killed
Sub-cutaneous injection of tumour cells	Transient discomfort following injection	Injection performed once only Appropriate volume will be injected (maximum of 0.2ml) Animals will be closely monitored during immediate post injection period	Animals will be humanely killed if more than mild distress or discomfort, without rapid recovery, observed following injection (very rare)
Growth of tumour	May cause discomfort or affect normal behaviour or locomotion Tumour used may become infected or ulcerate (but should not metastasise)	Daily observation of animals, regular monitoring of general health and tumour growth Monitoring scheme will include careful observation of posture, gait and tumour size and condition Pharmaceutical interventions will begin when tumour reaches 0.5 cm in diameter (measured by callipers)	Animal will be killed if tumour ulcerates, or interferes with normal behaviour, posture or locomotion, or exceeds 1.2cm in diameter (Workman et al. 2010)
Intraperitoneal injection of novel pharmaceutical agent	Transient discomfort following injection Cytotoxic drugs may cause diarrhoea, weight loss, anorexia or lethargy	Animals will be closely monitored during immediate post injection period Maximum volume of 10ml/kg daily for 7 days Minimum dose levels will be used (determined following dose ranging studies) Clinical scoring system will be used to assess welfare	Animals will be killed if weight loss exceeds 20% of initial body weight Animals not eating or having diarrhoea for more than 48 hours will be killed An upper limit for a clinical score will be set as a humane endpoint

ANALYSIS

As a consequence of the tumour size, the increased potential for ulceration, the frequency of injections and the adverse effects of the drugs given, a **prospective severity classification of MODERATE** is appropriate in this case.

CLINICAL OBSERVATIONS

An example of an observation sheet and a sample score sheet are included below.

EXAMPLE OF A SCORE SHEET

Animal no.				
Date	1-june	2-june	3-june	4-june
Appearance				
Body weight				
Coat condition				
Body function				
Dyspnoea and/or tachypnoea				
Food intake				
Environment				
Loose stools or diarrhoea				
Blood in diarrhoea				
Behaviours				
Handling				
Aggression				
Abnormal gait				
Abnormal posture				
Reluctance to move				
Procedure-specific indicators				
Tumour size				
Ulceration of tumour				
Tumour impeding movement				
Total score				
Any other observations				

EXAMPLES OF CLINICAL SCORES

Appearance	Score
Bodyweight	
5-10% weight loss	1
11-15 % weight loss	2
16-20% weight loss	3
20% + weight loss	HEP
Coat Condition	
Coat slightly unkempt	1
Slight piloerection	2
Marked piloerection	3
Body Function	
Tachypnoea (fast breathing)	1
Dyspnoea (difficulty breathing)	3
Environment	
Loose stools or diarrhoea	1
Blood in diarrhoea	HEP
Behaviour	
Tense and nervous on handling	1
Markedly distressed on handling, e.g. shaking, vocalizing, aggressive	3
Locomotion	
Slightly abnormal gait/posture	1
Markedly abnormal gait/posture	2
Significant mobility problems / reluctance to move	3
Immobility >24h	HEP
Procedure Specific Indicators	
Tumour size >1.2cm	HEP
Tumour ulceration	HEP
Tumour impeding movement	HEP

RESULTS

Of the 30 male BALB/C mice, 25 were used for efficacy evaluation; 10 animals received drug B at dose H, 10 drug B at dose X and 5 drug C at dose Y.

ASSESSMENT OF ACTUAL SEVERITY - EXAMPLES

- 10 animals receiving drug B at dose H had tumours that remained relatively small, with no significant BW loss and no clinical signs – **MILD**
- 7 animals receiving drug B at dose X had a decrease in tumour size, a BW loss of 15% and presence of loose stools, but were kept until the end of the experiment – **MODERATE**
- 5 Animals receiving drug C at dose Y had a continued increase in tumour size, body weight increased, no clinical signs apart from tumour growth. These animals were euthanized when the tumour size exceeded 1.2 cm - **MODERATE**
- 3 animals receiving drug B at dose X had a decrease in tumour size, a BW loss of 15%, presence of loose stools, anorexia and were very lethargic; these were humanely killed on day 25 – **SEVERE**

Source: http://ec.europa.eu/animals-in-science

European Commission