Severity classification

Severity of animal studies - Prospective estimate, Reporting Retrospective Assessment

Within the European community animal research regulation as the EU directive has defined 3 different severity categories based on the level impact on the animal i.e. mild, moderate and severe [1]. I addition a 4th category "Non recovery" has been defined for procedures which are performed entirely under general anaesthesia from which the animal shall not recover consciousness but will be killed while it is under anaesthesia [1].

There are several good reasons of having a severity classification system. First, to continuously focus on the application of the 3R when planning animal studies. Input from the Animal Welfare Body may be helpful. The severity system improve transparency on actual animals' welfare costs and facilitate communication between those using, caring and monitoring research animals building a common language. All sources of pain, suffering and distress should be identified in the planning and design phase of the study and means to minimise negative effect on the animals (refinement) should be considered. This planning phase should also uncover if there are training needs. There might be a need for specific training of those that will do the daily observation of the animals with focus on what to observe and how to report and respond. Specific assessment protocols or score sheets might be helpful in the monitoring of the animals. As a rule of thumb – the more severe impact on the animals – the more frequent and rigorous monitoring of animals is required. Factors to take into consideration when defining severity level include the procedure, the level of invasiveness, level of restraint, duration of the procedure, whether treatment to reduce pain, suffering or distress can be applied and to what level the animals normal behaviour is affected. This evaluation must also consider the species in questions, how well are they suited to the experimental conditions we provide them including contact with humans. In addition, it must be evaluated if there is a need to repeat procedures during the study and if the animal will be allowed to rest and recover between procedures [1]. All these factors sum up to the cumulative severity for that animal.

Procedures that are classified as severe should be reconsidered for refinement. The severity classification also defines lower and upper limits for what is defined as a procedure, as well as an upper limit for what should never been authorised.

The lover level is defined as pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice [1]. Similarly, an upper limit – that should be prohibited - is defined as procedures that result in severe pain, suffering or distress, which is likely to be long-lasting and that cannot be ameliorated [1].

Examples of each severity category

A complete overview of procedures belonging to the different severity categories has been defined in annex VIII of the EU directive [1]. An expert has provided examples on how to allocate animal procedures to the right category [2]. All example provided are based on the assumption that best practices are strictly adhered to [2].

According to the directive **[1] "Mild severity"** is defined as procedures on animals as a result of which the animals are likely to experience short term mild pain, suffering or distress. Procedures that cause no significant impairment on the wellbeing or general condition of the animals. That also include non-invasive imaging of animals with appropriate sedation or anaesthesia; breeding of genetically altered animals which is expected to result in a phenotype with mild effects and short term (<24 h) restraint in metabolic cages have been defined as mild procedures.

Moderate procedures have been defined as procedures on animals as a result of which the animals are likely to experience short term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress. Examples include surgery that involves penetration of a body cavity, under general anaesthesia and appropriate analgesia, associated with postsurgical the risk of pain, suffering or impairment of general condition. Moderate procedures also include breeding of genetically altered animals which are expected to result in a phenotype with moderate effect. Moderate severity also includes creation of genetically altered animals through surgical procedure and use of metabolic cages involving moderate restriction of movement over a prolonged period (up to 5 days).

Severe procedures on animals as are procedures likely to experience severe pain, suffering or distress, or long-lasting moderate pain, suffering or distress. That includes experiments where death is the end-point, or fatalities are to be expected and severe pathophysiological states are induced. Severe procedures also include use of metabolic cages involving severe restriction of

movement over a prolonged period, inescapable electric shock (e.g. to produce learned helplessness), complete isolation for prolonged periods of social species or immobilization stress to induce gastric ulcers or cardiac failure in rats or forced swim or exercise tests with exhaustion as the end point.

The examples listed here is not complete but can be used as guidance and comparison when studies are planned.

Also, if several mild procedures are performed in the same animal, this might cause the project to be categorized as moderate (a more burdensome category). The same principle applies for animals experience a series of moderate procedures – in such cases the grand score for that project can be severe.

The fourth category, terminal procedures is used for studies where an animal is anesthetized without any prior intervention, the complete study is completed while the animal is in anaesthesia and then euthanizing the animal still under anaesthesia is classified as a separate harm category under European regulations [1]. In this way the animal will not have other experiences of procedures beside the injection of the needle or other method to induce anaesthesia.

Procedures need to be assigned a severity classification prospectively before it is authorised by the authority. The actual severity experienced by each animal during the course of a procedure shall be reported annually after the study and reported in the statistical information made publicly available.

The example sin the directive are rather genera. A guidance document on the severity on fish has been published and include some more examples that are relevant for aquatics [3]. There is also available a publication and severity assessment for genetically altered mice [4].

Reporting actual severity

The actual severity of procedures will be reported by member states in the annual statistical returns. This reflects the highest severity experienced by the animal because of the procedure(s).

The prospective severity described in a project proposal is a best guess based on the guidance in the section above and experience and skills of the research team. [5] This estimate of severity applies for the whole groups of animals in a study.

The actual severity does not always match the prospective severity, as experiments can show to be both less and more severe than anticipated. For example, experimental groups in testing a cure for a disease can advantage from that cure with less severe consequences, while control animals may have no such advantage and the outcome might be more severe for them. In toxicity tests, this may be the opposite outcome.

The actual severity also give direction as on criteria among others on weather an animal can be reused in another procedure as re-use depend on severity of the previous procedure(s). One aim by a common severity classification system is to seek harmonization between European countries. Sufficiently trained and competent staff are an absolute requirement to assess animal welfare during the course of the study and to report actual severity correctly. It needs to be an observational strategy and a common recording system that captures all the necessary data in a consistent format to be able to harmonize the severity category system across the European countries.

Animals may only be re-used provided that the severity of the previous procedure was ‘mild’ or ‘moderate’; that the animal’s general state of health and well-being has been fully restored; that the further procedure is classified as ‘mild’, ‘moderate or ‘non-recovery’ and is in accordance with veterinary advice, taking account the lifetime experience of the animal.

Retrospective severity assessment

The Retrospective severity assessment is useful in a transparent way to identify improvements on the 3Rs as well as evaluating if the model is suitable to achieve expected benefits. The prospective severity classification has impact on obligation for Retrospective Assessment as all projects using non-human primates and projects involving procedures classified as ‘severe’, shall undergo retrospective assessment to clarify whether the objectives of the project were achieved, the harm inflicted on animals, including the numbers and species of animals used, the severity of the procedures any elements that may contribute to the further implementation of the requirement of replacement, reduction and refinement [1]. In addition, the competent authority may decide that also other studies shall undergo retrospective assessment. This can be relevant for new animal models, pilots, newly created genetically

altered animals, new test-substances or classes of compounds or animal use for education and training.

The retrospective assessment provides an opportunity to review if the outcome of the study met the defined objective(s) as well as evaluated if the estimated severity prospectively matches the actual severity. These experiences should be used for consideration of 3R alternatives and best practices for planning similar studies in the future. So, the retrospective assessment is a tool for learning and continuous improvement.

The retrospective assessment should be carried out as soon as the experiment is completed, and both the scientific and animal care and welfare team should be involved and share experiences from the study.

Retrospective assessment of a project gives a further opportunity to review the welfare or harms to the animals, to determine whether the objectives have been met, and to re-consider the appropriateness of the severity classification, prior to any future study. More information on retrospective assessment can be found in the publication on Project Evaluation and Retrospective Assessment from the European Commission [6]

References

1. EuropeanCommission, *Directive 2010/63/EU og the European Parliament anf of the council of 22 September 2010 on the protection of animals used for scientific purposes*, EuropeanCommission, Editor. 2010.
2. EuropeanCommissionExpertWorkingGrouponSeverityAssessment, *Working document on a severity assessment framework*. 2012: http://ec.europa.eu/environment/chemicals/lab_animals/pdf/Endorsed_Severity_Assessment.pdf.
3. Hawkins, P., et al., *Guidance on the severity classification of scientific procedures involving fish: report of a Working Group appointed by the Norwegian Consensus-Platform for the Replacement, Reduction and Refinement of animal experiments (Norecopa).* Laboratory Animals, 2011. **45**(4): p. 219-224.
4. Zintzsch, A., et al., *Guidelines on severity assessment and classification of genetically altered mouse and rat lines.* Lab Anim, 2017. **51**(6): p. 573-582.
5. EuropeanCommissionExpertWorkingGrouponSeverityAssessment, *Expert working group on severity classification of scientific procedures performed on animals*. 2009.
6. EvaluationandRetrospectiveAssessment, E., *Project Evalaution and retrospective assessment - Expert Working group report*. 2013: Brussels.