Template for the **3Rs AGENT** - **3Rs** **A**ssessment of **GEN**etically altered animals-**T**ool

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Component** | **Describe harm causing procedure or factor of harm**  | **Uncertainty factor regarding harm[[1]](#footnote-1)**  | **Modulating factors of harm that influence severity[[2]](#footnote-2)** | **Severity classification[[3]](#footnote-3)** |
| **Genetic engineering***Which technique of genetic engineering is used and are off-target effects of the technique expected?* |  | [ ]  low[ ]  medium[ ]  high |  |  |
| **Sterile males***Which method is used to produce sterile males?* |  | [ ]  low[ ]  medium[ ]  high |  | [ ]  non-harmful[ ]  mild[ ]  moderate[ ]  severe |
| **Production of blastocystes***Which method is used to obtain blastocystes?* |  | [ ]  low[ ]  medium[ ]  high |  | [ ]  non-harmful[ ]  mild[ ]  moderate[ ]  severe |
| **Embryo transfer***Which method is planned for embryo transfer?* |  | [ ]  low[ ]  medium[ ]  high |  | [ ]  non-harmful[ ]  mild[ ]  moderate[ ]  severe |
| **Phenotypic characteristics***Which phenotype is expected?* |  | [ ]  low[ ]  medium[ ]  high |  | [ ]  non-harmful[ ]  mild[ ]  moderate[ ]  severe |
| **Hygienic and husbandry conditions** *How do local hygienic and husbandry conditions influence phenotypic characteristics?* |  | [ ]  low[ ]  medium[ ]  high |  | [ ]  non-harmful[ ]  mild[ ]  moderate[ ]  severe |
| **Breeding scheme and surplus animals***Which breeding scheme is planned to use? How will surplus animals be handled?* |  | [ ]  low[ ]  medium[ ]  high |  |  |
| **Genotyping and tissue sampling** *Which method for tissue sampling will be used?* |  | [ ]  low[ ]  medium[ ]  high |  | [ ]  non-harmful[ ]  mild[ ]  moderate[ ]  severe |

Note: fields highlighted in grey colour are not applicable.

**Prospective severity classification for the project (overall severity[[4]](#footnote-4)) With overall uncertainty of**

[ ]  mild [ ]  moderate [ ]  severe [ ]  low [ ]  medium [ ]  high

|  |  |
| --- | --- |
| **Plan for welfare assessment (see Table 2)** | Structured welfare assessment planned[ ]  yes [ ]  noIf yes, describe your plan |
| **Retrospective evaluation is planned** | [ ]  yes [ ]  no |

**Table 1.** Assigning an uncertainty factor

|  |  |  |  |
| --- | --- | --- | --- |
| **Uncertainty factor** | **Definition** | **Prospective severity classification** | **Retrospective assessment** |
| **Low** | Extensive literature (e.g., scientific articles or databases of genetically altered (GA) animals) or experience with the method or phenotype is available that provides a clear picture of what is expected | Possible  | Recommended but not required |
| **Medium** | Some literature or experience with the method or phenotype is available that provide indicators for severity assessment, but is still vague | Possible, but vague | Strongly recommended to confirm or revise the prospective severity assessment |
| **High** | No literature or experience with the method or phenotype is available | Not possible | Retrospective assessment mandatory |

**Table 2.** Consequences of uncertainty: How to plan a structured welfare assessment and appropriate monitoring**.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Uncertainty factor** | **Welfare assessment factors****1) Time points for assessment** | **2) Assessment/examination parameters[[5]](#footnote-5)** | **3) Assessors** |
| **Low** | Animals are assessed at defined time points, from onset of disease until the end of lifespan; frequency according to expected phenotype, e.g., progression of disease. | Adapt the general welfare assessment scheme according to the clinical signs expected. | Scientist and/or animal caretakers; involve veterinarian or AWO if needed. |
| **Medium** | Animals are assessed at time points according to expected onset of disease until the end of lifespan; additional time points before and between expected time points should be defined to recognize unexpected phenotypes (all lifestages should be covered). | Adapt the general welfare assessment scheme according to the clinical signs expected. Include general welfare criteria, e.g. measurement of body weight to recognize unexpected events as soon as possible, and postmortem examination. | Scientist and/or animal caretakers; involve veterinarian or AWO if unexpected phenotypes occur and for the final assessment of the line. Discuss refinement and degree of severity with veterinarian or AWO. |
| **High** | All livestages should be covered. | Use the general welfare assessment scheme. Include postmortem examination. | Assessment should be carried out by two experienced persons; involve veterinarian or AWO for ongoing monitoring and for the final assessment of the line. Discuss refinement and degree of severity with veterinarian or AWO. |

1

1. See Table 1. for the assignment of an uncertainty factor [↑](#footnote-ref-1)
2. E.g. Refinement, for more details see *3RsAGENT: Supplementary information and practical guidance* [↑](#footnote-ref-2)
3. Consider modulating factors of harm. The classification “non-harmful” may apply only for single procedures but is not applicable to the legal requirement of assigning an overall severity for the project. [↑](#footnote-ref-3)
4. The recommended prospective severity classification assigned to procedures should be based on the highest severity anticipated for any animal on the study (see European Commission Expert Working Group. Working Document on Genetically Altered Animals-Corrigendum of 24 January 2013.2013. Available online: http://ec.europa.eu/environment/chemicals/lab\_animals/pdf/corrigendum.pdf). [↑](#footnote-ref-4)
5. Assessment should be based on observational parameters and should not involve interventions that may cause additional pain, suffering, or distress. If the characterization of severity of a phenotype requires invasive methods, that should be covered under project authorization. [↑](#footnote-ref-5)