Discussion paper
for the purposes of promoting consistent reporting of statistical data (actual severity and animal numbers) under Article 54(2) of Directive 2010/63/EU and Commission Implementing Decision 2012/707/EU
- January 2016 -

This discussion paper has been developed in response to difficulties reported in three areas with the assessment of actual severity, and which are likely to result in non-uniform reporting of statistical data. It is intended to further promote a common understanding of the issues and consistent statistical reporting.

It includes the relevant legal background and extracts from previously endorsed guidance together with some further explanation and practical examples developed from material provided by some individual MSs. The document provides a reflection as to how these questions could be approached. In addition, it includes a section on expandable miscellaneous points requiring further clarification.

In some cases, this document makes reference to project authorisation. This is relevant as *only* animals used under *authorised work* need to be reported. However, *not all* animal use under project authorisation is reported. For example, in the maintenance of colonies of harmful phenotype GA lines, animals are not reported if they have *not* exhibited adverse effects or been subject to another procedure (e.g. invasive genotyping).

1. Reporting of Genetically Altered (GA) animals

Commission Implementing Decision 2012/707/EU states the following in Annex II, Part B, point A:

> *"1. For the purposes of statistical reporting, "genetically altered animals" include genetically modified (transgenic, knock- out and other forms of genetic alteration) and naturally occurring or induced mutant animals.*
>
> *2. Genetically altered animals are reported either*
>
> *a) when used for the creation of a new line;*
>
> *b) when used for the maintenance of an established line with an intended and exhibited harmful phenotype; or*
>
> *c) when used in other (scientific) procedures (i.e. not for creation or for the maintenance of a line)."*

Reporting animals during creation of new line

"3. All animals carrying the genetic alteration should be reported during the creation of a new line. In addition, those used for superovulation, vasectomy, embryo implantation should equally be reported (these may or may not be genetically altered themselves). Genetically normal animals (wild type offspring) produced as a result of creation of a new genetically altered line should not be reported.

4. In the category 'Purposes', the animals used for the ***creation*** *of a new genetically altered line should be reported under 'basic research' or 'translational and applied research' in the* ***respective category the line is being created for****."*

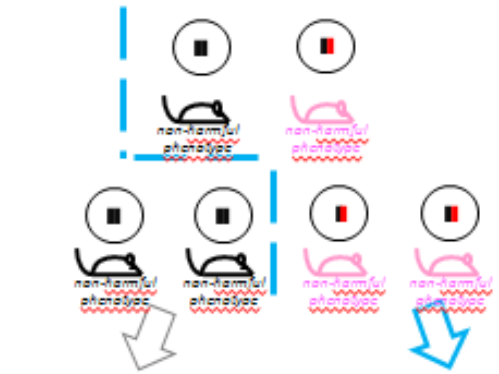
Creation of new GA line

Project approval required (severity unknown, to be established within this project)

Example 1

non-GA parent +
GA parent

non-GA offspring +
GA offspring



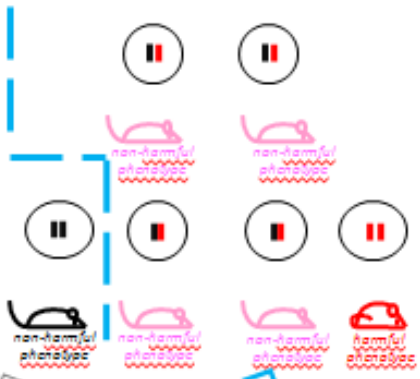
5-year report:*
wt offspring

annual statistics:
1 parent non-GA, non-harmful phenotype, severity „mild up to and including"
3 animals GA, non-harmful phenotype, severity = „mild up to and including"

Example 2

GA parents
(*recessive harmful phenotype*)

GA offspring (*recessive harmful phenotype*) +
wt offspring



5-year report:*
wt offspring

annual statistics :
4 GA animals, non-harmful phenotype, severity = „mild up to and including";
1 GA animal, harmful phenotype, severity as observed

**for the first time in 2017*

Reporting animals from an established line

> *"5. **A new strain or line of genetically altered animals is considered to be 'established'** when transmission of the genetic alteration is stable, which will be a minimum of two generations, and a welfare assessment has been completed.*
>
> *6. The welfare assessment will determine if the newly created line is expected to have an intended harmful phenotype and, if this is the case, the animals from this point onwards shall be reported under category 'Maintenance of colonies of established genetically altered animals, not used in other procedures' — or, if appropriate, in the other procedures they are being used for. If the welfare assessment concludes that the line is not expected to have a harmful phenotype, its breeding falls outside the scope of a procedure and no longer needs to be reported.*
>
> *7. '**Maintenance of colonies of established genetically altered animals, not used in other procedures**' contains the animals required for the maintenance of colonies of genetically altered animals of established lines with an intended harmful phenotype and which have exhibited pain, suffering, distress or lasting harm as a consequence of the harmful genotype. The intended purpose for which the line is being maintained for is not recorded."*

A practical problem has been found that on the basis of the above and in the absence of an animal welfare assessment on some occasions animals are reported under "creation", even if in practice they are actually being used for "maintenance" or in other procedures.

In line with Article 17 of the Directive regarding the creation of a new genetically modified animal line, **the procedure ends when the progeny is no longer observed or expected to experience adverse effects**. Only the breeding of a harmful phenotype line shall require project authorisation. Consequently, only the animals from such lines exhibiting adverse effects should be included in the annual statistical reporting under "maintenance", in line with the Commission Implementing Decision. Should animals remain indefinitely under a project authorisation for a **creation** of a new genetically altered line, there would be significant impacts on the accuracy of statistical reporting:

- Over-reporting the numbers of animals under creation in the different sub-categories of 'basic research' and 'translational and applied research' and
- Under-reporting of animals required for the maintenance of colonies of established genetically altered animals, not used in other procedures.

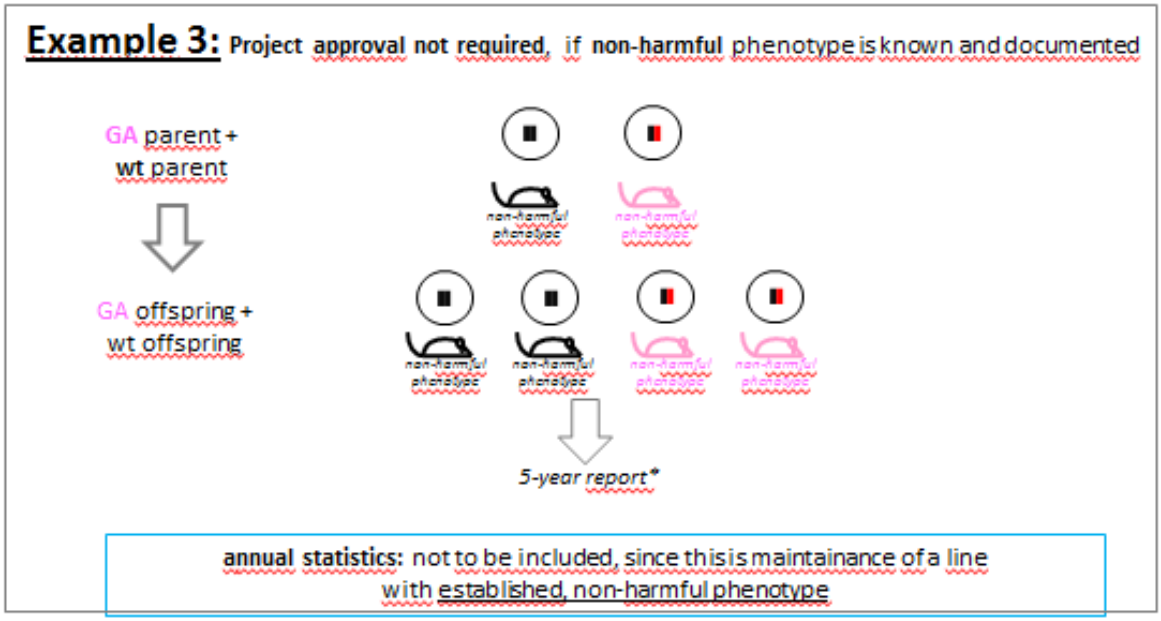
Equally, breeding of animals from a line with ***non***-harmful phenotype does not require project authorisation, and subsequently no reporting under annual statistics.

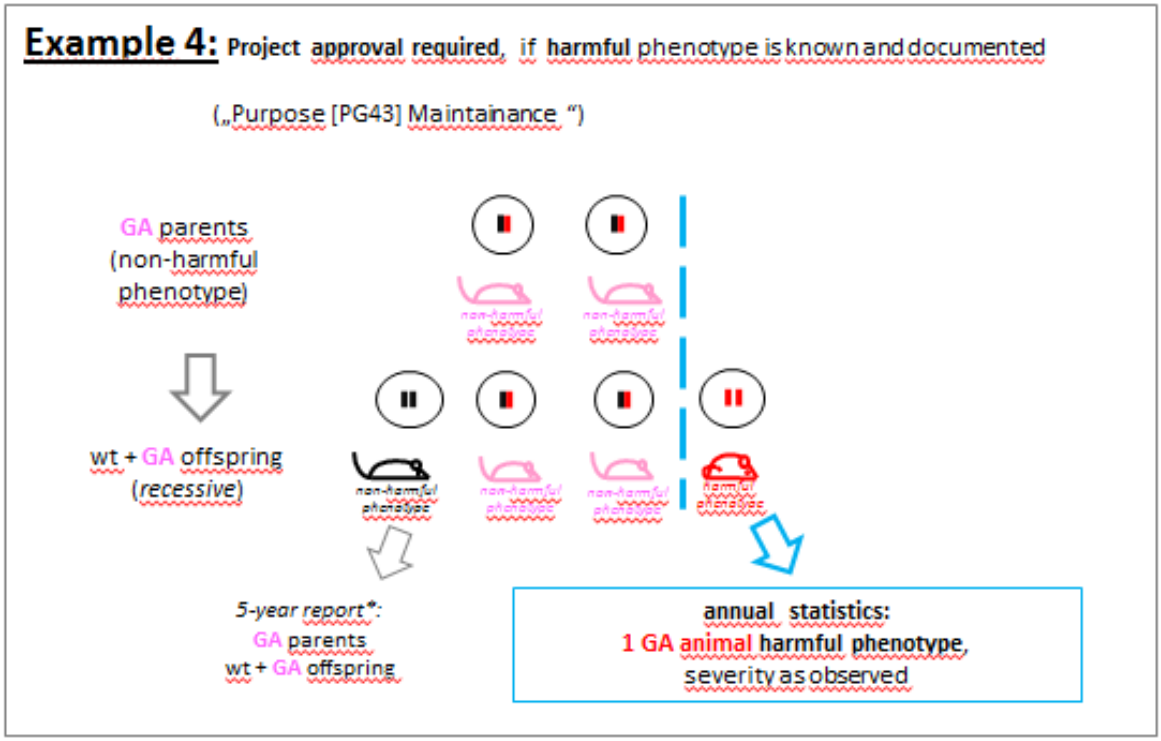
The wording of point 6 above reflects that animals are used in procedures or for maintenance once the line is established ("… ***from this point onwards*** *shall be reported under category "Maintenance …* ***or*** *if appropriate,* ***in the other procedures they are being used for*** *…").*

Reporting should reflect the reality of what the animals are used for. If **animals from the same litter are being *'used'* (**by the same or a different user) for the purposes of a specific procedure (not creation) it follows that these animals (and their siblings) have reached that 'point in time' when the line should have been considered "established".

Maintainance of an established GA line

Maintainance breeding: severity known and documented





Reporting GA animals used in other procedure

*"8. **All genetically altered animals which are used in other procedures** (not for the creation or maintenance of a genetically altered line) should be reported under their respective purposes (the same way as any non-genetically altered animal). These animals may or may not exhibit a harmful phenotype. "*

Reporting GA animals killed for their organs or tissue

"9. Genetically altered animals, expressing a harmful phenotype, and killed for their organs and tissue, should be reported under the respective primary purposes for which the organs/tissue were used."

2. Assigning actual severity if animals are found dead

- Article 54(2) requires reporting of the actual **severity of the procedures** where **animals are used.**
- The endorsed consensus document on Severity Assessment Framework (http://ec.europa.eu/environment/chemicals/lab_animals/pdf/guidance/severity/en.pdf) further states that

  *"For the purposes of statistical reporting, **actual severity should primarily relate to the severity of the experimental procedures and not unrelated incidents** such as disease outbreaks or cage flooding. These types of incident relate to health problems or to husbandry and care practices, not harms due to procedures, however, they should be recorded, investigated further and followed up to prevent recurrence.",*

- In this context it is also important to note the guidance on assessment of actual severity:

  *"The actual severity to be reported for the individual animal should be **the highest level experienced during the course of the procedur**e and not based on the severity at the end of the procedure. Nor should the evaluation be considered a simple additive process e.g. a number of mild procedures = moderate severity. It should be based on an overall assessment of the animal's experience from the start of the procedure to the end."*

On these basis, a following decision tree could be proposed:

1. Is the death <u>unrelated</u> or <u>related</u> to <u>the procedure</u> the animal was under-going?

1.1 **<u>Unrelated</u>**

Examples of unrelated deaths:

- Deficiencies in equipment or environmental controls such as cage flooding, heating/ventilation malfunction;
- Inappropriate husbandry or care practices such as failure to provide adequate diet (e.g. inappropriately balanced) or diet contaminated (e.g. poor storage);
- Aggression between animals in a group housing;
- Unrelated disease and infections;
- <u>Ageing animals</u>: deaths in animals on long-term studies should be evaluated to **clearly differentiate** deaths as a **result of the procedure** from those as a **consequence of the natural ageing process**. Deaths in such studies should not be automatically classed as severe, and the clinical history and condition of the animal at the time of the last observation should be given due consideration;
- In the case of <u>GA breeding of an established line</u>, when the **genetic alteration** is ***not* considered to cause any mortalities** on the basis of the welfare assessment performed on the established line, therefore, it is unlikely that deaths during the breeding programme are due to the genetic alteration**.**

> ➢ The actual severity for the animal should reflect the highest level of severity experienced during the course of the procedure by the animal (*excluding* the level of severity related to the death).

1.2. **<u>Related</u>**: proceed to question 2.

2. Can an <u>informed decision</u> be made about the events leading to the death? Factors such as frequency of monitoring, use of analgesia, etc. will need to be given due consideration.

1.2.1 **<u>Yes</u>**, for example:

- Animal failing to fully recover consciousness in post-operative period, but under appropriate analgesic regime throughout;
- No clinical abnormalities recorded throughout the procedure, nor anticipated, but found dead a few hours after a clinical examination.

> ➢ The actual reported severity should reflect the severity as the result of the assumed events leading to death.

1.2.2. **<u>No</u>**

> ➢ The actual severity should be reported as "severe".

3. Considerations regarding animals taken from the wild

Article 10 requires that animals belonging to the species listed in Annex I may only be used in procedures where these animals have been bred for use in procedures. Authorities may give exemption to this requirement based on scientific justification.

Typical exemptions include the use of farm animals obtained from commercial farms and wild animals captured from nature (the natural environment).

Identified problems

There are differing views on whether or not capture from the wild is considered as part of a procedure, and whether or not harms during capture/transport should be considered for the reporting of actual severity.

Should capture/transport of wild animals be considered as part of the procedure (when the capture/transport itself is not carried out for a specific scientific purpose) this will create confusion as well as have direct influence on the actual reported severity:

For example:-

100 fish are captured for one specific project. Two fish die during the capture process due to injury in nets. These are reported as severe even if the adverse effects of the procedure carried out under the project would have otherwise been assessed as "mild".

100 fish are captured and transported to lab where they are then allocated to several projects over a period of time. Two fish die during capture process due to injury in nets. Any suffering due to capture could not be assigned to a project as not known to which, if any, project they would have been assigned.

Analysis

- Procedure is defined in Article 3(1) as:

  "*'procedure' means any use, invasive or non-invasive, of an animal for experimental or other scientific purposes, with known or unknown outcome, or educational purposes, which may cause the animal a level of 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.";*

- Project is defined in Article 3(2):

  "'*Project' means a programme of work having a defined scientific objective and involving one or more procedures";*

- Article 15 requires that

  "..all procedures are classified as 'non-recovery', mild', 'moderate' or 'severe'...";

- Finally, Article 54(2) requires actual severity to be reported on *the "..**use** of animals in **procedures**.."*

In summary, the Directive states that the severity classification concerns "procedures" (the *use* of an animal for a scientific purpose) and the reporting of actual severity is limited to the "*use* of animals in *procedures*".

Obtaining animals from farms or a supplier equally involves taking of the animals, their preparation for the transport and transport – as is the case when obtaining animals from the wild. These activities are not necessary for obtaining data to meet the scientific objectives.

By contrast, the capture and transport could be performed for a scientific purpose for example when the objective of the project is to compare different capturing methods or transport conditions.

Therefore, if the capture and transport are not carried out for a specific, or a component of the scientific objective (i.e. factors to be studied in the project), irrespective of the type of animals (purpose bred, farmed, wild), consequently these activities do not form part of the scientific procedure.

Articles 9, 10 and 11 covering different origins (types) of animals further confirm this logic:

- Article 9(1)states that "*animals **taken** from the wild **shall not be used in procedures*** **"** implying that the 'taking' of the animal does not yet form a part of the procedure
- Article 10(1) states that "*Member States shall ensure that animals belonging to the species listed in Annex I **may only be used in procedures** where those animals **have been bred** for use in procedures…."*
- Article 11(1) states that "***Stray and feral animals** of domestic species **shall not be used in procedures**"*

The explicit wordings above differentiate the obtaining/origin of the animals from their *use* in a procedure.

- The actual severity should **only** relate to the **effects of the scientific "procedure" carried out on that animal.**
- **Capture and transport (***unless* these are the specific, or a component of the, objective of the scientific procedure) should therefore ***not*** **be taken into account in the reporting of the actual severity.**

The purpose of Article 54(2) is to collect statistical data *inter alia* on the severities **caused by the procedures**. If data from capture/transport were taken into account, it would no longer be possible to obtain information on the actual severity of a *particular procedure* since it would be affected by the means by which animals are obtained.

Furthermore, the data reported would vary according to the type of animals (e.g. purpose bred, farmed, wild) resulting in non-uniform inclusion/exclusion of impacts from capture and transport.

Finally, the data from each Member States needs to be comparable in order to prepare a meaningful summary report at a European level as required by Article 57(2). If Member States approach this differently, this objective would not be met and the usefulness of the information undermined.

Ensuring the appropriate welfare during capture and transport under the Directive

It is important to remember that the scope of the Directive is significantly wider than that of the definition of a 'procedure'. Consequently, the protection of animals undergoing a procedure forms only a part of the welfare cover provided by the Directive. The Directive contains a number of other obligations to ensure the appropriate welfare measures are in place, even when an animal is outside of scope of the specific definition of a procedure.

See for example Articles 9(3) and 33(1)(e), Annex III, Section A (3.2).

- In addition to the general requirements to ensure no unnecessary pain, suffering, distress or lasting harms are imposed on animals the Directive regulates specifically the **capture** of wild animals under its Article 9(3):

  i. *The capture is carried out by*
     - ***competent persons***
     - *using* ***methods which do not cause avoidable pain, suffering, distress or lasting harm***.
  ii. *Any animal found, at or after capture, to be injured or in poor health shall be examined by a veterinarian or another competent person and action shall be taken to minimise suffering. Competent authorities may grant exemptions from the requirement of taking action to minimise the suffering of the animal if there is scientific justification.*

- Concerning the transport, Article 33(1) stated that *"(e) animals are transported under appropriate conditions"*.

- Annex III, Section A(3.2) provides that:

" *3.2. Animals taken from the wild*

Transport containers and means of transport adapted to the species concerned shall be available at capture sites, in case animals need to be moved for examination or treatment.

Special consideration shall be given and appropriate measures taken for the acclimatisation, quarantine, housing, husbandry, care of animals taken from the wild and, as appropriate, provisions for setting them free at the end of procedures."

Life-time experience and reuse

It is important to note that the life-time experience of the animal should be taken into account when considering reuse of animals in a procedure in line with Article 16. Consequently, any harms experienced in capture/transport should be taken into consideration as part of that life-time experience of the animal.

In all circumstances, adverse welfare consequences, whether during capture, transport or during the course of procedures within a project should be assessed and measures taken to minimise these as far as possible (in the case of projects, consistent with the scientific objectives), and action taken to avoid recurrence.

4. Clarification for miscellaneous reporting questions

- **Translational and applied research for dentistry:** to be reported under **"musculoskeletal system".** To facilitate easy identification of animals used for the purposes of dentistry, it is suggested to request users to add in addition the word "**dentistry**" in the "specify other"-field.

- **Genotyping (tissue sampling/genetic characterisation) using an invasive method** (such as tail tipping) requires to be reported (unless the tissue is obtained as a by-product from identification e.g. ear clipping)**:** the reporting (whether reported as part of a continued use or as first use) depends on the purpose of the genotyping:
    - If the genotype is required be confirmed to that particular animal **as a prerequisite for the further procedure to be carried out**, the genotyping of that animal would be considered the first step in a "continued use" and the purposes under which the animal is reported should reflect the purpose of the subsequent use. The actual severity for the animal should reflect the highest level of severity experienced by the animal whether during the genotyping or the subsequent (continued) use.
      N.B. If the genotyping confirms that the animal is **not suitable** for the purpose, the animal should still be reported under the intended purpose, and the actual severity according to the adverse effect as a result of genotyping.
    - If the genotyping is done to the animal as part of the routine verification in a breeding colony of an established line to confirm that the genotype has not varied from the intended genetic background and that animal later is used in another procedure(/s), the latter use is considered re-use and all such events should be reported separately in the statistics, i.e.;
        - first use under 'maintenance of the established GA line' with the severity related to the actual severity experienced by the animal as the result of the invasive genotyping, and
        - as re-use under the specific purpose(/s) animal is used for.

- **Tolerance-studies** with target species or **combined tolerance-efficacy studies** are carried out "**with a view** to satisfying legislative requirements" and therefore these should be reported under "Regulatory use and routine production" under **"Other efficacy and tolerance testing".**

- Non-regulatory toxicology under translational and applied research covers **discovery toxicology and investigations** to prepare for the regulatory submission and method development.

- Studies required for regulatory submissions such as preliminary studies and MTD (Maximum Tolerated Dose –studies should be reported under Regulatory use and routine production" under **"Other efficacy and tolerance testing".**

- **Dose-range-finding (DRF) studies,** when carried out "with a view to" satisfying legislative requirements, should be reported under "Regulatory use and routine production" under **"Other efficacy and tolerance testing".**

Disclaimer:

This document is intended as guidance to assist the Member States and others affected by this Directive to arrive at a common understanding of the provisions contained in the Directive. All comments should be considered within the context of Directive 2010/63/EU on the protection of animals used for scientific purposes and the related Commission Implementing Decision 2012/707/EU. Only the Court of Justice of the European Union is entitled to interpret EU law with legally binding authority.

References

Directive 2010/63/EU
http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32010L0063

Commission Implementing Decision 2012/707/EU
http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02012D0707-20140115

Working document on a Severity Assessment Framework
http://ec.europa.eu/environment/chemicals/lab_animals/pdf/guidance/severity/en.pdf