GUIDANCE FOR STATISTICAL REPORTING

on the use of animals for scientific purposes – PART 2

COMMISSION BRUXELLOISE DE L'EXPÉRIMENTATION ANIMALE

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INTRODUCTION

Since 2014, all EU member states are required to submit information on scientific procedures to the European Commission (EC) in a common format. Each member state is responsible for the collection and quality assurance of their data. Following the European Directive 2010/63/EU being transposed into Belgian law in May 2013, the 2014 collection underwent substantial changes. As a result, some inconsistencies were expected in the reporting of the 2014 information as data suppliers became familiar with the new reporting requirements and data collection format. However, it was noticed that, several years after the legislation was amended, users still encountered difficulties in submitting the statistical data.

This guideline has been developed in response to the difficulties that were reported 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.

The guidance does not provide information on HOW the data providers should submit their statistical returns in accordance with Article 54 of Directive 2010/63/EU. Statistical data must be entered in a form which automatically populates an Excel file which has been created by the European Commission specifically for this purpose. The form itself has a help sheet for guidance on its completion. However, a guidance document (Guidance for statistical reporting on the use of animals for scientific purposes – Part I) has already been established to help the data providers complete the form: https://leefmilieu.brussels/themas/dierenwelzijn/dierproeven-een-strikt-omlijnde-praktijk/brusselse-commissie-voor-dierproeven . The data should be entered on each use of an animal. When entering data for an animal, only one option within a category can be selected. The mandatory fields for the European Commission are marked with an asterisk (*) at the end of the title so these must not be left blank. The form will not validate if any of these are left blank. The fields 'id1, id2 and id3' are not required by the European Commission but are required by Leefmilieu Brussel / Bruxelles Environnement, so these must also be filled out. The use of "other" category requires a compulsory entry in the narratives to provide further details.

LEGISLATION

1. DIRECTIVE 2010/63/EU

The protection and welfare of animals is an area covered by a wide range of EU legislation. This includes the protection of wildlife, zoo animals, farm animals, animals in transport and animals used for scientific purposes. Animal studies, whether for the development or production of new medicines, for physiological studies, for studying environmental effects or for the testing of chemicals or new food additives, has to be carried out **in compliance with EU legislation**.

Since 1986, the EU has had in place specific legislation covering the use of animals for scientific purposes. On 22 September 2010 the EU adopted **Directive 2010/63/EU** (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32010L0063) which updated and replaced the 1986 Directive 86/609/EEC on the protection of animals used for scientific purposes. The aim of the new Directive was to strengthen legislation, and improve the welfare of those animals still needed to be used, as well as to firmly anchor the principle of the Three R's, to Replace, Reduce and Refine the use of animals, in EU legislation. The scope is now wider and includes fetuses of mammalian species in their last trimester of development and cephalopods, as well as animals used for the purposes of basic research, higher education and training. It lays down minimum standards for housing and care, regulates the use of animals through a systematic project evaluation requiring inter alia assessment of pain, suffering, distress and lasting harm caused to the animals. It requires regular risk-based inspections and improves transparency through measures such as publication of non-technical project summaries and retrospective assessment. The development, validation and implementation of alternative methods is promoted through measures such as establishment of a Union reference laboratory for the validation of alternative methods supported by laboratories within Member States and requiring Member States to promote alternative methods at national level. Directive 2010/63/EU took full effect on 1 January 2013.

2. REGULATION (EU) 2019/1010

On 5 June 2019 the European Parliament and the Council adopted **Regulation (EU) 2019/1010** (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2019.170.01.0115.01.ENG&toc=OJ:L:2019:170:TOC) on the alignment of reporting obligations in the field of legislation related to the environment amending inter alia Directive 2010/63/EU. The Regulation aims at *improving the evidence base for EU policies, increasing transparency for the public and simplifying reporting to reduce administrative burden*. Besides Directive 2010/63/EU it covers nine other pieces of environmental legislation.

In relation to Directive 2010/63/EU, the Regulation **amends articles 43 and 54 of the Directive**. **Article 54 of Directive 2010/63/EU lays down the provisions concerning the annual and 5-yearly statistics.** This article provides that :

1. Member States shall by 10 November 2023, and every five years thereafter, send the information on the implementation of this Directive and in particular of Article 10(1) and Articles 26, 28, 34, 38, 39, 43 and 46 to the Commission. Member States shall submit and publish that data, by electronic transfer in a format established by the Commission in accordance with paragraph 4. No later than six months after the submission by Member States of the data referred to in the second subparagraph, the Commission services shall publish and regularly update a Union-wide overview on the basis of that data.

2. Member States shall collect and make publicly available, on an annual basis, statistical information on the use of animals in procedures, including information on the actual severity of the procedures and on the origin and species of non-human primates used in procedures. Member States shall submit that statistical information to the Commission, at the latest by 10 November of the following year, by electronic transfer, in a non-summarized format established by the Commission in accordance with paragraph 4. The Commission shall establish and maintain a searchable, open access database containing that statistical information. On an annual basis, the Commission services shall make publicly available the statistical information submitted by the Member States in accordance with this paragraph and a summary report thereof.

3. Member States shall submit to the Commission, on annual basis, detailed information on exemptions granted under Article 6(4)(a).

4. The Commission shall, by means of implementing acts, establish a common format and information content for submitting the information referred to in paragraphs 1, 2 and 3 of this Article. Those implementing acts shall be adopted in accordance with the examination procedure referred to in Article 56(3).

Member State annual statistical data on animal use will be made publicly available through an **open access, searchable EU database** that should be operational **from January 2021**. Centrally available data provides an opportunity for EU-wide analyses of current animal use to inform policy as well as to identify areas in need of alternative strategies to help Replace, Reduce, and Refine animal use in areas with the highest impact.

3. COMMISSION IMPLEMENTING DECISION 2020/569/EU

This Decision sets out a common format for submitting information on the use of animals for scientific purposes as referred to in paragraphs 1, 2 and 3 of Article 54 of Directive 2010/63/EU. This system will allow the Commission to assess effectiveness of the implementation of the legislation and help ensure consistency in its application. The first data under the new statistical reporting format will be collected on the 1st of January, 2021 and reported in2022.

Commission Implementing Decision 2020/569/EU: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2020.129.01.0016.01.ENG&toc=OJ:L:2020:129:TOC

Annex III of Commission Implementing Decision 2020/569/EU describes in Part A the detailed data categories (see Annex I pg. 42) and in Part B the detailed instructions for the provision of statistical data on the use of animals for scientific purposes under article 54 (2).

4. RECOMMENDATION 2007/526/EC

The Recommendation (adopted on 18 June 2007) (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32007H0526) introduces guidelines for the accommodation and care of animals used for experimental and other scientific purposes. This Recommendation compliments Annex III of Directive 2010/63/EU, which is based on provisions found therein, and sets down firm rules on requirements for accommodation and care of experimental animals. It also aligns EC legislation with the revised Council of Europe guidelines (Appendix A of Convention ETS 123), on accommodation and care of laboratory animals.

KEY PRINCIPLES AND TERMS

1. DEFINITIONS FOR A PROCEDURE AND A PROJECT

1.1. Project

According to the Directive, a project means a program of work having a **defined scientific objective** and involving **one or more procedures**.

Projects can vary in size and complexity, for example, from the work of a single scientist consisting of a single blood harvest procedure in a single species, to multiple complex procedures and a wide range of species. It is up to the ethical committee to determine which size and which complexity of a project is allowed.

1.2. Procedure

According to the Directive, "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 according to good veterinary practice. This includes any course of action intended, or liable, to result in the birth or hatching of an animal (for example caesarean section) or the creation and maintenance of a genetically modified animal line in any such condition, but excludes the killing of animals **solely** for the use of their organs or tissues. Procedures may be carried out **only** within the framework of a project (I.e. requiring a project authorization and are carried out in a user's establishment = LA1230xxx). The use of animals killed solely for the use of their organs or tissues does not require a project authorization (exemptions see pg. 13) but still has to be reported to the ethical committee to which the establishment is affiliated. The breeding of an animal is a regulated procedure if the animal is bred from, or is the descendant of, an animal whose genes have mutated or been modified.

Within a project, procedures will be performed to meet a defined scientific purpose. Procedures may be simple or complex depending on the purpose.

The purpose may be achieved by using a single step procedure (for example withdrawal of blood), but much more commonly requires a number of steps used in combination to achieve a single outcome, and which requires the use of the same animal (for example antibody production would generally require a number of antigen injections to stimulate antibody production, and a number of blood samples to achieve the desired outcome).

Examples of Procedures

A single subcutaneous injection of a test substance may be given in a pharmaceutical project to attain the objective of understanding the drug distribution within the body tissues. The animal is then killed by a method listed in Annex IV of Directive 2010/63/EU. This project comprises of one procedure (the injection of the test substance) which may cause the animal pain, suffering, distress or lasting harm.

In contrast, a procedure to assess the effect of the test substance on blood pressure using telemetry would require a number of separate technical steps to be carried out to meet this single scientific purpose (multi – step procedure). The animal would need to be anaesthetized, blood pressure transducer implanted and, following a suitable recovery period, administered the test substance by subcutaneous injection. The animal is then killed by a method listed in Annex IV of Directive 2010/63/EU. In this example three steps namely anesthesia, surgical implantation of blood pressure transducer and injection of the test substance) need to be used in combination to meet the single scientific purpose of understanding the effects of the substance on blood pressure[1]. All the steps need to be made in the correct sequence and using the same

[1] In this example, each step, **if used in isolation to meet a single scientific purpose**, would be considered a "procedure" as each may cause the animal 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.

animal to achieve the objective of the study – neither omitting a step nor using a different animal at any stage would allow the objective to be met.

2. REQUIREMENTS FOR A PROJECT AUTHORIZATION

See also :
https://ec.europa.eu/environment/chemicals/lab_animals/pdf/posters/DG_ENVI_A1_poster_laboratory_animal_Vertical_190524_PRINT_HD_GAA.pdf

2.1. Creation

Creation: a creation of a new genetically altered line requires a project AUTHORIZATION until such time when the line is "**established**". Creation includes the crossing of different lines to create a new genetically altered line where the phenotype of the new line cannot be determined *prospectively* as non-harmful.

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 an initial welfare assessment completed (see Annex II and III, pg. 46-47).

<table>
<tr><th colspan="3">CREATION of GAA lines requiring project authorization</th></tr>
<tr><td colspan="3">Introduction of genome engineering reagents into embryos and germ cells</td></tr>
<tr><td>E.g. gene editing tools, DNA, ES cells
Embryo implantation, vasectomy</td><td>Mating 2 GA lines to create a new line</td><td>Chemical mutagenesis</td></tr>
<tr><td colspan="3">Birth of offspring
Genotyping
Confirmation that genetic alteration is present</td></tr>
<tr><td colspan="3">Breeding until stable transmission of the desired genetic alteration
Characterization of phenotype</td></tr>
<tr><td colspan="3">Welfare Assessment
- With subsequent decision on the classification of the line</td></tr>
</table>

2.2. Maintenance

Maintenance: the use of animals for the maintenance of colonies of genetically altered established lines, **with** a likely harmful phenotype, requires a project AUTHORIZATION. However, this could be considered under multiple generic authorization (Article 40.4 of the Directive).

The use of animals for the maintenance of colonies of genetically altered established lines **without** a likely harmful phenotype is not considered a procedure and thus does not require a project authorization.

Genetically altered lines requiring a specific, intentional (non-accidental) intervention to induce gene expression (e.g. chemical induction, mating of Cre with appropriate Lox animals) can be considered as having a non-harmful phenotype until deliberate induction of gene expression. Therefore, their breeding does not require project authorization.

Genetically altered lines which retain a risk of the development of a harmful phenotype (e.g. age onset of disease or tumors; risk of infection due to compromised immune system) regardless of the applied refinement (e.g. barrier conditions, culling at early age), in line with Article 1(2), their breeding requires project AUTHORIZATION as the application of refinement does not eliminate the risk.

If welfare issues are later identified, these should be reviewed to consider whether the welfare problems may be attributed to the genetic alteration. If so, these should be re-classified as "harmful phenotypes" and brought back under project AUTHORIZATION.

<table>
<tr><th colspan="3">MAINTENANCE</th></tr>
<tr><th colspan="2">Non-Harmful Phenotype</th><th rowspan="3">Harmful Phenotype</th></tr>
<tr><td colspan="2">Tissue sampling method for genotyping</td></tr>
<tr><td>below threshold (non-invasive) tissue sampling</td><td>above threshold tissue sampling</td></tr>
<tr><td>NO PROJECT AUTORIZATION
Breeding as conventional animals</td><td>PROJECT AUTORIZATION
For above threshold sampling methods, when not linked to marking of the animal</td><td rowspan="2">PROJECT AUTORIZATION
Breeding of harmful phenotype, including above threshold tissue sampling, all under project AUTHORIZATION</td></tr>
<tr><td colspan="2">If unexpected problems arise and animals from a non-harmful line are considered harmful after re-evaluation / welfare assessment then line to be moved and maintained under Project Authorization. →</td></tr>
</table>

2.3. Use

Use: use of (genetically altered) animals in a procedure requires a project AUTHORIZATION. These animals may or may not exhibit a harmful phenotype.

3. USE AND RE-USE

3.1. General

Each use of the animal shall be reported at the end of each procedure.

Information on the place of birth and for non-human primates also the generation and information on whether the animal was obtained from a self-sustaining colony shall only be reported for naïve animals, that is to say animals used for the first time. For reused animals, this information is therefore not recorded.

Any subsequent categories shall show the number of uses of animals in procedures. These numbers cannot be cross referenced with the total numbers of naïve animals.

The actual suffering of the animal in the procedure shall be reported. In some cases this could be influenced by a previous use. However, the severity will not always increase in a subsequent use and in some cases may even decrease as a result (habituation). Therefore, the actual severity to be reported shall always be determined on a case-by-case basis taking into account any impact from previous uses.

3.2. Re-use versus continued use

"Re-use" is a term to indicate the subsequent use of an animal which has already completed a procedure (or series of procedures/techniques) for a particular scientific purpose; *when another, naïve, animal could have been used instead of that animal.*

For the purposes of determining whether there is a 'reuse', the following shall apply:

- A single use is the use of one animal for a single scientific/experimental/ educational/training purpose. A single use extends **from the time when the first technique is applied to the animal until the completion** of data

collection, observations or achievement of educational objective. This is usually a single experiment, test or training of a technique.

- A single use **may contain a number of steps (techniques)** all necessarily related to achieve a single outcome and which require the use of the same animal.
- Examples of preparation for the purposes of continued use include:
  (a) surgical techniques (such as cannulation, implantation of telemetry, ovariectomy, castration, hypophysectomy);
  (b) non-surgical techniques (such as feeding modified diets, induction of diabetes, induction of transgene expression);
  (c) breeding of genetically altered animals of harmful phenotype;
  (d) genetic characterization using an invasive method (which was not carried out for the purposes of identification/marking of the animal) and where an animal of that genotype is required for the next step.
- When the prepared animal is used in the procedure intended for it, **the entire procedure**, including any preparation (regardless of the location this has taken place) is reported at the end by the end-user taking into account the severity associated with the preparation. For example, for the breeding of a genetically altered animal and its end use, the reporting shall take into account the severity associated with all the steps (for example, the effect of the phenotype, if expressed; genetic characterization, if performed; and end use).
- The use of an animal is **only reported once** by the end-user at the end of the complete procedure including where the preparatory steps described above and the end use have been carried out under separate projects.
- Where a prepared animal is not subsequently used for a scientific purpose, the establishment in which the animal is killed shall **report the preparation** as an independent use in the statistics as per the intended purpose, provided that the preparation of the animal has been above the threshold of minimum pain, suffering, distress and lasting harm. However, if this preparation concerns maintenance of a genetically altered animal line, the criteria by which animals are reported are provided for in section 12. Purposes : Maintenance of colonies of established genetically altered animals, not used in other procedures , pg. 34.
- If the animal has been genotyped (genetic characterization/tissue sampling) as part of a routine verification in a genetically altered breeding colony of an established line to confirm that the genotype has not varied from the intended genetic background and that animal is later used in another procedure, not requiring that particular genotype, that use is considered reuse and all such uses shall be reported separately in the statistics, that is to say:
  (a) first use under 'maintenance of the established genetically altered line' with the severity related to the actual severity experienced by the animal as the result of the invasive genotyping, and
  (b) as reuse under the specific purpose the animal is used for.

The circumstances under which an animal may be re-used can be found in Article 16 of Directive 2010/63/EU : the actual severity of the previous procedures was 'mild' or 'moderate'; it is demonstrated that the animal's general state of health and well-being has been fully restored; the further procedure is classified as 'mild', 'moderate' or 'non-recovery'; and it is in accordance with veterinary advice, taking into account the lifetime experience of the animal.

Examples of Use and Re-use

Example 1 - Re-use

Purpose 1: to obtain sheep blood to make diagnostic plates for bacteriology.
Purpose 2: to determine the effect on blood parameters in sheep of a test dietary supplement, which may have adverse effects.

First use

A sheep is used on the first procedure to obtain a blood sample for use in preparation of diagnostic plates.

Second use

The same animal is then used on a second unrelated project to study the metabolism of a dietary supplement, blood samples are taken for analysis and other non-invasive measurements are made.

The two studies are not related. Any naive sheep could have been used for the second study. Use of the sheep for the dietary study would constitute re-use of that animal.

Example 2 – Re-use

Purpose: The use of a sheep on more than one occasion to provide blood samples for use in preparation of diagnostic plates.
The first blood sample obtained by jugular venipuncture constitutes the first use – the purpose is attained.
As a different animal could be used on each occasion, the second sample (procedure) and each subsequent sample (procedure) is classified as re-use.
There is no scientific need to take multiple samples from the same animal.

Example 3 - Use and continued use

Purpose: To determine the effects of genetic defect X in mice by measuring changes in blood parameters with age and undertaking a histological analysis of adult brain structure.
Use
This could be undertaken within a single study.
Step 1 - Production and genotyping of GM mouse
Step 2 - Blood sampling

or
Continued use
Split between a procedure (on the same or different project) under which the mouse with the genetic defect was produced and genotyped (Step 1), with the use continued under a second procedure (Step 2), possibly on a different project, on which the sampling and final preparation for histology are carried out.

In this example Step 1 may be on a project at an establishment that specializes in breeding mice with genetic alterations (under a generic project authorization) and Step 2 on the project of the scientist studying that particular genetic defect.

Example 4 - More complicated situations

Depending on the scientist's intention and the specific design of the study, it may not be immediately clear whether some studies should be regarded as re-use or continued use.
Example:
If the metabolism of a series of drugs is studied in an individual animal this will constitute continued use if serial-data from individual animals which had been used for each preceding study is needed to interpret each subsequent study (a within animal design) and data from a different animal would not have satisfied the scientific objective.

However, if each study is to be interpreted independently of the others and without reference to earlier findings (and therefore any animal could have been used) this will constitute re-use. What actually happens to the animal is the same in each series of studies, but the way in which data are analyzed determines whether the subsequent use is regarded as re-use or continued use.

REPORTING

The annual statistics publication predominately focuses on experimental procedures and procedures counted under the creation and breeding of genetically altered (GA) animals. Experimental procedures include all animals used in basic research, regulatory use, translational/applied research, protection of the natural environment, higher education and training, preservation of species and forensic enquiries. The creation and breeding of GA animals includes some animals that were bred with the intention of producing GA animals.

All end-users should report their animals **at the end of the procedure**. For any animals which are bred or used in a project which finishes within a given calendar year, the statistical information relating to that project should be submitted to LB / BE **at the start of the following year** upon request. For any projects having a duration of more than one year, and which are not due to be completed within that calendar year, at the end of each calendar year the project manager should collate details only on **any animals which have died, been killed or removed from study** within that year; this information should be at the start of the following year at the request of LB / BE. Information should not be provided on animals which are subject to continued use as part of that project authorization.

Information on animals which have not undergone any procedures but which have been killed for tissues or organs or because they are deemed to be surplus to needs should be provided at the start of the following year at the request of LB / BE.

In 2-step / multi-step procedures it is the last 'user' who should report at the end of all steps (e.g. when a genetically altered animal is genotyped at the breeder (step 1) and subsequently used in a user establishment (step 2), the user should report the animal at the end of the procedure and includes the severity from the genotyping (step 1)).
If genetically altered animals that were **genotyped** at the breeder (step 1) are killed **as surplus** at that breeder than the breeder should report that animal with the related actual severity.

When an animal is **re-used** (see definition under 3.2.) this re-use shall be reported, under the specific purpose(/s) the animal is used for, at the end of each procedure. Information on the place of birth, and for non-human primates also the generation and information on whether the animal was obtained from a self-sustaining colony, shall not be recorded (this was already reported when the animal was used for the first time). The actual suffering of the animal in the procedure shall be reported. In some cases this could be influenced by a previous use. However, the severity will not always increase in a subsequent use and in some cases may even decrease as a result (habituation). Therefore, the actual severity to be reported shall always be determined on a case-by-case basis taking into account any impact from previous uses.

PRACTICES UNDER THE SCOPE OF THE DIRECTIVE

1. ANIMALS KILLED FOR ORGANS AND TISSUES / SENTINELS

Animals killed for organs and tissues, as well as **sentinels**, are excluded from the provision of annual statistical data, *unless* any of the following applies:

a) the killing is performed under a project authorization using a method not included in Annex IV of Directive 2010/63/EU;
b) the animal has gone through a previous intervention, which has been above the threshold of minimum pain, suffering, distress and lasting harm prior to being killed;
c) the animal is from a genetically altered animal line with an intended harmful phenotype and which has expressed the harmful phenotype before being killed for organs and tissues.

Other animals killed for organs and tissues (**those not reported in the annual statistics**) are reported as part of the five-year implementation report (under the category: Number of animals bred, killed and not used in procedures).

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. ANIMALS THAT ARE BRED AND KILLED WITHOUT BEING USED IN A PROCEDURE

Animals that are bred and killed without being used in a procedure shall not be included in the annual statistical data *apart from* the following animals:

a) **genetically altered animals with an intended and exhibited harmful phenotype;**
b) those animals that have been **genotyped (genetic characterization / tissue sampling) using an invasive method**, which was not carried out for the purposes of identification/marking of the animal.

An invasive method shall be a method which may cause the animal 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.

The animals that are bred and killed without being used in a procedure (not reported in annual statistics) shall be reported as part of the five-year implementation report.

If animals are used as refinement to provide compatible group housing for animals in procedures (and which would otherwise be left alone), these are considered 'stock' animals (provided no procedures are carried out on them). When these animal (= stock animals providing social groups) are killed, these are reported as part of the implementation report, required once every five years.

3. GENETICALLY NORMAL ANIMALS

Genetically normal animals (wild type offspring) **born during the creation of a new genetic line** shall be excluded from the provision of annual statistical data and shall instead be reported as part of the five-year implementation report, *unless* such animals have been genotyped using an invasive method.

4. LARVAL, FOETAL AND EMBRYONIC FORMS (INCLUDING CEPHALOPODS AND FISH)

Larval forms of animals shall be included once they become capable of independent feeding.

Foetal and embryonic forms of mammalian species shall be excluded from the provision of annual statistical data. Only animals that are **born**, including by Caesarean section, **and live** are to be counted. When studies involve both mother and offspring, the mother shall be reported when she has been subject to a procedure above the threshold of minimum pain, suffering, distress and lasting harm. Offspring shall be reported when they are an integral part of the procedure.

Fish shall be reported from the stage of independent feeding when the gut is open end to end and the fish would normally take food. The time at which fish feed independently is different for each species and in many cases dependent on the temperature at which they are kept. Temperature should be set to maintain optimal welfare, as determined by the person responsible for the welfare and care of the animals and for species specific information in coordination with the designated veterinarian. Zebrafish larvae, which are kept at approximately +28C shall be reported 5 days post fertilisation. Due to the small size of some fish and cephalopod species, the count may be done on the basis of estimation.

All **cephalopod species** shall be reported under the heading 'cephalopod' from the stage at which the animal becomes capable of independent feeding, that is to say immediately after hatching.

5. GENETICALLY ALTERED ANIMALS

See also :
https://ec.europa.eu/environment/chemicals/lab_animals/pdf/posters/DG_ENVI_A1_poster_laboratory_animal_Vertical_190524_PRINT_HD_GAA.pdf

For the purposes of statistical reporting, "genetically altered animals" refer to either of the following:

- genetically modified (such as transgenic, knock-out and other forms of genetic alteration) and induced mutant animals (irrespective of the type of mutation) and
- animals with spontaneous deleterious mutations maintained for research for that specific genotype.

Genetically altered animals shall be reported in any of the following cases:

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 (detailed criteria on page 20)[2]; or

[2] This category also includes genetically altered animals during maintenance of an established line, irrespective of whether the line is of non-harmful or harmful phenotype, and
- for which the genotype has been confirmed using an invasive method, which was not carried out for the purposes of identification/marking of the animal, and the animal is killed without further use;
- that are of unsuitable genotype, confirmed using an invasive method, which was not carried out for the purposes of identification/marking of the animal.

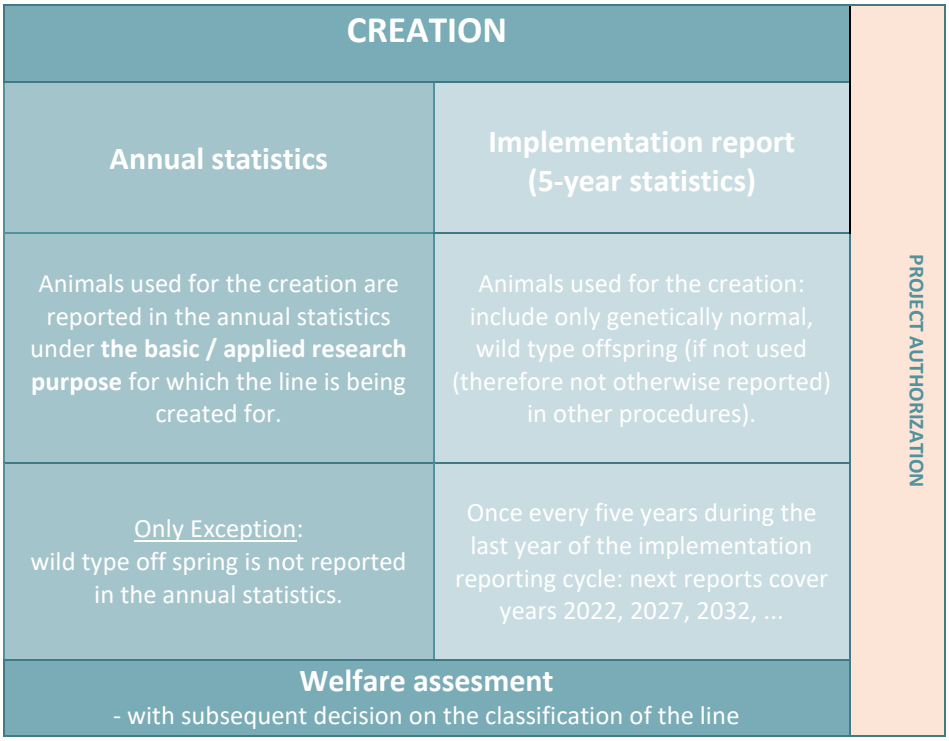
c) when used in procedures other than maintenance of a line.

All animals *carrying the genetic alteration* shall be reported during the **creation of a new line**. In addition, those used for superovulation, vasectomy, embryo implantation shall 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 shall not be reported in annual statistics, *unless* the animal has been genotyped (genetic characterization/tissue sampling) using an invasive method, which was not carried out for the purposes of identification / marking of the animal. Genetically normal animals (wild type offspring) not reported in annual statistics are covered in the five-year implementation report.

In the category 'Purposes', the animals used for the *creation* of a new genetically altered line shall be reported in the *respective category for which the line is being created for* (generally expected to be 'basic research' or 'translational and applied research').

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 (see Annex II and III, pg.46 – 47). 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. Such animals include, amongst others, those that require a specific bio-secure environment (for example, special housing arrangements to protect animals that are particularly sensitive to infection as a consequence of the gene alteration), or additional care beyond that required for conventional animals to maintain their health and well-being.

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. Such animals include, amongst others, inducible and cre-lox lines, which require an active intervention for the harmful phenotype to be expressed.



[2] This category also includes re-derivation when it is done solely for scientific purposes (i.e. not to benefit health/welfare of colony), and animals used for embryo transfer and vasectomy during maintenance of an established line

'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. This category also includes genetically altered animals during maintenance of an established line, irrespective of whether the line is of intended non-harmful or harmful phenotype, that have been subject to invasive genotyping (genetic characterization/tissue sampling).

<table>
<tr><th colspan="3">MAINTENANCE</th></tr>
<tr><th colspan="2">Non-Harmful Phenotype</th><th rowspan="3">Harmful Phenotype</th></tr>
<tr><th colspan="2">Tissue sampling method for genotyping</th></tr>
<tr><th>below threshold (non-invasive) tissue sampling</th><th>above threshold tissue sampling</th></tr>
<tr><td>NO PROJECT AUTHORIZATION</td><td>PROJECT AUTHORIZATION</td><td>PROJECT AUTHORIZATION</td></tr>
<tr><td rowspan="2"><u>Implementation report:</u>
All unused animals that were killed and not genotyped using invasive method.</td><td rowspan="2"><u>Annual statistics</u> under 'Maintenance of colonies….':
All unused animals that were killed and were genotyped using invasive method (not carried out for marking).</td><td><u>Annual statistics</u> under 'Maintenance of colonies…': All unused animals that were killed and that had exhibited harmful phenotype and/or were genotyped using invasive method (not carried out for marking).</td></tr>
<tr><td><u>Implementation report</u>: all unused animals that were killed without having exhibited harmful phenotype and were not genotyped using invasive method.</td></tr>
<tr><td colspan="3">Animals that are not killed and continue to be used in procedures</td></tr>
</table>

All genetically altered animals which are used in other procedures (not for the creation or maintenance of a genetically altered line) shall 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.

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

<table>
<tr><th>USE</th></tr>
<tr><th>PROJECT AUTHORIZATION</th></tr>
<tr><td><u>Annual statistics</u>: All animals that are used in procedures are reported in the annual statistics after completion of the procedure and for the purpose for which that procedure was carried out.</td></tr>
</table>

PRACTICES THAT ARE EXEMPTED FROM THE SCOPE OF THE DIRECTIVE

1. BACKGROUND

Directive 2010/63/EU establishes measures for the protection of animals used for scientific or educational purposes.

Article 1 paragraph 5 of this Directive lists a number of **practices which are exempted** from the requirements of the Directive. These are as follows:

(a) non-experimental agricultural practices;
(b) non-experimental clinical veterinary practices;
(c) veterinary clinical trials required for the marketing authorization of a veterinary medicinal product;
(d) practices undertaken for the purposes of recognized animal husbandry;
(e) practices undertaken for the primary purpose of identification of an animal;
(f) practices not likely to cause pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle according to good veterinary practice.

In determining whether or not an investigation falls within the scope of the Directive the key question to address is: *Is the study to be undertaken for a scientific or educational purpose involving live vertebrates (including foetal forms of mammals in the last third of development and independently feeding larval forms) or live cephalopods?*

If the answer is yes, then the clauses in Paragraph 5 (see list above) needs to be reviewed to determine whether or not there is an exemption from the scope of the Directive.

If the practices are being undertaken as part of routine agricultural, animal husbandry or veterinary practice to manage health, welfare and care practices, are being applied for the primary purpose of identification, or are unlikely to cause pain, suffering, distress or lasting harm, these do not fall within the scope of Directive 2010/63/EU.

2. NON-EXPERIMENTAL AGRICULTURAL PRACTICES

Agriculture can be defined as the **production of food, feed, fiber and other goods** by the systematic raising of domesticated plants and animals. Agriculture covers all activities essential to food/feed/fiber production, including all techniques for raising and "processing" livestock. Agriculture includes agronomy, animal husbandry, and aquaculture. Agriculture practices are simply practices used in agriculture to facilitate farming.

A number of Council Directives set requirements for the protection of animals kept for farming practices [for example general standards are contained in 98/58/EC, and minimum standards set for calves in 2008/119/EC, pigs in 2001/88/EC and 2001/93/EC, laying hens in 1999/74/EC, and chickens kept for meat production in 2007/43/EC].

Examples of agricultural practices include disbudding/dehorning of cattle, castration of lambs, pigs and cattle, debeaking in poultry, nutritional manipulation of weight in broiler replacement breeders, rearing and weaning practices in dairy and veal calves, restraint around parturition e.g. pigs and advanced breeding techniques for agricultural purposes, such as embryo transfer and vasectomy to, for example, improve health or genetics of the flock or herd.

Simple observational studies of commercial agricultural practices which do not include any additional practices/interventions which may cause pain, suffering, distress or lasting harm do not fall within the scope of Directive 2010/63/EU.

For example, a study to compare the effects of intensive and extensive rearing systems on production and behavioral indices in growing pigs. Study consists of simple observations on commercial farms using different rearing systems (which comply with national/EU legislation), and subsequent comparison of behavior and production records.

3. NON-EXPERIMENTAL CLINICAL VETERINARY PRACTICES

Clinical veterinary practice can be defined as procedures and techniques performed by veterinary surgeons **in the course of their professional duties** which ensure the health and welfare of animals committed to their care.

Examples include :

- taking blood samples from an animal, or animals within a herd, to assist in clinical management e.g. disease diagnosis, metabolic/biochemical profile.
- taking a series of biopsies from an animal for diagnosis and monitoring the efficacy of treatment
- imaging to assist in diagnosis and monitoring of treatment
- giving veterinary treatment, including to animals undergoing scientific procedures when treatment is for the animal's benefit and not part of a scientific procedure

4. VETERINARY CLINICAL TRIALS REQUIRED FOR THE MARKETING AUTHORIZATION OF A VETERINARY MEDICINAL PRODUCT

During the development of veterinary medicines, a great deal of work will have been performed on animals authorized under 2010/63/EU.

There usually comes a point when it is necessary to test the efficacy and safety of new preparations in the target species **under field conditions**. Pharmaceutical companies need to generate these data in order to support an application for a marketing authorization.

Directive 2001/82/EC details the requirements for veterinary clinical trials. Animals used in such trials are under veterinary care and appropriate clinical care, including alternative treatments, is provided should the test product prove ineffective or animal welfare is compromised.

5. PRACTICES UNDERTAKEN FOR THE PURPOSES OF RECOGNISED ANIMAL HUSBANDRY

Animal husbandry may be defined as the system of taking care of domestic animals, including those held and used in laboratories.

This definition encompasses **all husbandry and care practices** *including housing conditions and colony management, monitoring reproductive, growth and health indices*.

For example:

- Single housing of males may be necessary to minimize aggression;
- Vaginal swabbing (mice/dogs) or blood sampling (dogs) to determine stage of oestrus and optimum time for mating;
- Single housing on grid floors to check for mating plugs (rats & mice);
- Weighing fish under general anesthesia to monitor growth to facilitate dietary and stocking management;
- Management of diet (composition, quantity and availability) to meet requirements of animals – e.g. managing obesity in older animals; infrequent feeding of snakes to mimic biological needs.

Simple observational comparisons of different animal husbandry practices, which do not include any additional practices/interventions which may cause pain, suffering, distress or lasting harm, do not fall within the scope of Directive 2010/63/EU. For example, a study to compare the effects of cage changing frequency on growth rates and behavior in mice.

6. PRACTICES UNDERTAKEN FOR THE PRIMARY PURPOSE OF IDENTIFICATION OF AN ANIMAL

Animals are identified for a number of reasons, for example - to facilitate identification of individual animals held in groups, to facilitate routine stock and breeding management and to facilitate tracing of animals for health and disease control.

Practices undertaken **for the primary purpose** of identification are not within the scope of the Directive.

This should not be confused with the requirements of Article 32. For animals used within the scope of the Directive, there is a requirement that each dog, cat and non-human primate shall be provided with a permanent individual identification mark in the least painful manner possible (Article 32).

For reasons of good welfare, consideration should be given to the method chosen for identification, and should be the most refined technique appropriate to the need. For example, where it is only necessary to identify an animal for a short period of time, the use of a non-toxic dye or fur clipping would not cause any pain or discomfort.

Alternatively, there may be a **legal requirement** in farm species for two separate methods to be employed, for example an ear tag and a transponder.

7. PRACTICES NOT LIKELY TO CAUSE PAIN, SUFFERING, DISTRESS OR LASTING HARM EQUIVALENT TO, OR HIGHER THAN, THAT CAUSED BY THE INTRODUCTION OF A NEEDLE ACCORDING TO GOOD VETERINARY PRACTICE

Practices undertaken for a scientific or educational purpose which do not reach a "threshold" of pain, suffering, distress or lasting harm equivalent to or higher than that caused by the introduction of a needle according to good veterinary practice do not fall within the scope of the Directive.

Annex VIII of the Directive provides some examples of equivalent "thresholds" for other classes of procedure, such as **dietary manipulations** and **psychological stress**:

- assessing body composition by non-invasive measures and minimal restraint;
- monitoring ECG with non-invasive techniques with minimal or no restraint of habituated animals;
- application of external telemetry devices that are expected to cause no impairment to socially adapted animals and do not interfere with normal activity and behavior;
- breeding genetically altered animals (maintenance) which are expected to have no clinically detectable adverse phenotype;[3]
- adding inert markers in the diet to follow passage of digesta;
- withdrawal of food for <24h in adult rats;
- open field testing.

In addition it is important to note that **a series or combination of "below threshold" techniques** together may have the effect of causing an animal **pain, suffering, distress or lasting harm**. Using the food withdrawal as an example, if this was repeated frequently, it is likely that there would be adverse welfare consequences for the animal. Similarly, multiple or cumulative minor changes to an animal's environment may cause sufficient disturbance to the animal to be considered a mild procedure.

[3] NOTE: Genotyping with a tissue sampling method which is above threshold (invasive) is always considered a procedure (and requires a project approval).

DIFFICULTIES IN REPORTING WHICH ARE LIKELY TO RESULT IN NON-UNIFORM REPORTING OF STATISTICAL DATA

1. REPORTING OF GENETICALLY ALTERED (GA) ANIMALS

An animal with a *harmful phenotype* for the purposes of this Directive and in context of genetically altered animals is to be understood as an animal who is likely to experience, as a consequence of the genetic alteration pain, distress, suffering or lasting harm equivalent to, or higher than that caused by the introduction of a needle in accordance with good veterinary practice.

Commission Implementing Decision 2020/569//EU states the following in Annex III, Part B, point A:

1. *For the purposes of statistical reporting, "genetically altered animals" refer to either of the following:*
   - *genetically modified (such as transgenic, knock-out and other forms of genetic alteration) and induced mutant animals (irrespective of the type of mutation) and*
   - *animals with spontaneous deleterious mutations maintained for research for that specific genotype.*
2. *Genetically altered animals shall be 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 (detailed criteria in section 12. Purposes, Maintenance of colonies of established genetically altered animals, not used in other procedures, pg. 34); or*
   c) *when used in procedures other than maintenance of a line*

<u>Reporting animals during creation of new line</u>

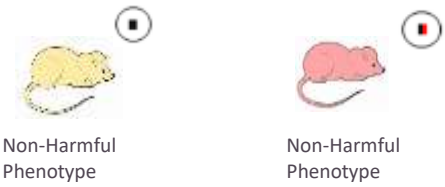
3. *All animals carrying the genetic alteration shall be reported during the creation of a new line. In addition, those used for superovulation, vasectomy, embryo implantation shall be reported (these may or may not be genetically altered themselves). Genetically normal animals (wild type offspring) produced as a result of creation of a new genetically altered line shall not be reported in annual statistics, unless the animal has been genotyped (genetic characterization/tissue sampling) using an invasive method, which was not carried out for the purposes of identification/marking of the animal. Genetically normal animals (wild type offspring) not reported in annual statistics are covered in the five-year implementation report.*
4. *In the category 'Purposes', the animals used for the **creation** of a new genetically altered line shall be reported in the **respective category for which the line is being created for** (generally expected to be 'basic research' or 'translational and applied research').*

Creation of new GA line

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

Example 1

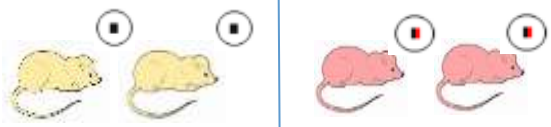
Non-GA parent +
GA parent



Non-Harmful Phenotype

Non-Harmful Phenotype

Non-GA offspring +
GA offspring



Non-Harmful Phenotype

Non-Harmful Phenotype

Non-Harmful Phenotype

Non-Harmful Phenotype

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"

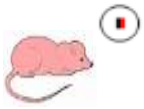
* unless the animal has been genotyped (genetic characterization/tissue sampling) using an invasive method, which was not carried out for the purposes of marking of the animal or was used in other procedures.

Creation of new GA line

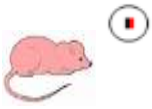
Project approval required (severity unknown, to be established within this project)

Example 2

GA parents
(recessive Harmful phenotype)



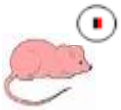
Non-Harmful Phenotype



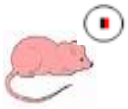
Non-Harmful Phenotype

GA offspring (recessive harmful Phenotype) + wt offspring

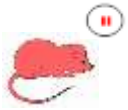
Non-Harmful Phenotype



Non-Harmful Phenotype



Non-Harmful Phenotype



Harmful Phenotype

5-year report:
1 Wt offspring *

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

* unless the animal has been genotyped (genetic characterization/tissue sampling) using an invasive method, which was not carried out for the purposes of marking of the animal or was used in other procedures.

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. Such animals include, amongst others, those that require a specific bio-secure environment (for example special housing arrangements to protect animals that are particularly sensitive to infection as a consequence of the gene alteration), or additional care beyond that required for conventional animals to maintain their health and well-being. 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. Such animals include, amongst others, inducible and cre-lox lines, which require an active intervention for the harmful phenotype to be expressed.*
*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.*

According to the above point 5,in order to be able to consider a new strain or line to be established, such a welfare assessment is needed.

A practical problem found is that on the basis of the above and in the absence of an animal welfare assessment in some occasions animals are reported under "creation", even if in practice they are being used for "maintenance" or in other procedures. This has significant impacts on the accuracy of statistical reporting:

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

The wording of point 6 above reflects that animals are used in procedures or for maintenance once the line is established ("… ***from this time 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" and the welfare assessment completed.

Not carrying out the welfare assessment necessary for the establishment of the line cannot be used as an argument not to report the animals according to their real use. Doing so will undermine the objectives of the statistical reporting and the purpose legislation intends to achieve, and could thus be considered a failure in implementation.

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 authorization. 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 authorization for a **creation** of a new genetically altered line, there would be significant impacts on the accuracy of statistical reporting:
Equally, breeding of animals from a line with ***non****-harmful* phenotype does not require project authorization, and subsequently no reporting under annual statistics. Exception: the animal has been genotyped (genetic characterization/tissue sampling) using an invasive method, which was not carried out for the purposes of marking of the animal.

Maintenance of an established GA line

Maintenance breeding: severity known and documented

Example 1 : project approval not required if non-harmful phenotype is known and documented *

Non-GA parent +
GA parent

Non-Harmful Phenotype

Non-Harmful Phenotype

Non-GA offspring +
GA offspring

Non-Harmful Phenotype

Non-Harmful Phenotype

Non-Harmful Phenotype

Non-Harmful Phenotype

5-year report:
All unused animals that were killed and not genotyped using invasive method

Annual statistics:
Under **"Maintenance of colonies…"** : all unused animals that were killed and were genotyped using invasive method (not carried out for marking).

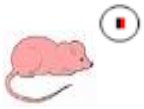
* unless the animal will be genotyped (genetic characterization/tissue sampling) using an invasive method, which was not carried out for the purposes of marking of the animal (e.g. genotyping is a procedure).

Maintenance of an established GA line

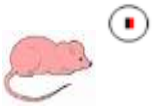
Maintenance breeding: severity known and documented

Example 2: project approval required if harmful phenotype is known and documented

GA parents
(recessive Harmful phenotype)



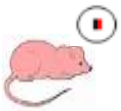
Non-Harmful Phenotype



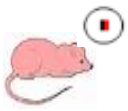
Non-Harmful Phenotype

GA offspring (recessive harmful Phenotype) + wt offspring

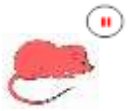
Non-Harmful Phenotype



Non-Harmful Phenotype



Non-Harmful Phenotype



Harmful Phenotype

5-year report:

All unused animals that were killed without having exhibited harmful phenotype and were not genotyped using invasive method

1 Wt offspring *
4 animals GA (parents + offspring),
non-harmful phenotype,
severity = "mild up to and including"

Annual statistics:

Under **"Maintenance of colonies…"** : all unused animals that were killed and that had exhibited harmful phenotype and / or were genotyped using invasive method (not carried out for marking).

1 GA animal, harmful phenotype, severity as observed

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. GENOTYPING

If the tissue for the genotyping is not obtained as a by-product from identification, such as ear-marking, nor was obtained using a non-invasive method all these should be covered by a project AUTHORIZATION and reported in the annual statistics.

Genotyping (tissue sampling/genetic characterization) **using an invasive method** (such as tail tipping, fin clipping, …) 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. The reporting is done only by the user at the end of the procedure.

  If the genotyping confirms that the animal is not suitable for the purpose and, as a consequence, is not used in a subsequent procedure, the animal should still be reported by the establishment where the animal is killed, under the intended purpose, with the actual severity related to the 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.

Genotyping (tissue sampling/genetic characterization) **using a non - invasive method** (e.g. hair sampling) does not require reporting.

3. ASSIGNING ACTUAL SEVERITY IF ANIMALS ARE FOUND DEAD

Severity of animals found dead shall be determined by reference to whether the death is the result of factors related to the procedure that the animal was under-going. If not related (such as in the case of death due to deficiencies in equipment or environmental controls; inappropriate husbandry practices; unrelated disease and infections), the actual reported severity shall reflect the most severe effects experienced by that animal during the course of the procedure (excluding the experience preceding the death).

If the death is *related* to the procedure, the actual reported severity shall be 'severe' unless an informed decision can be made that the severity can be assigned a lesser category.

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 procedure** 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 unrelated or related to the procedure the animal was under-going?

1.1 **Unrelated**

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;
- Ageing animals: deaths in animals on long-term studies should be evaluated to **clearly differentiate** deaths as a **result of the procedure** (related) from those as a **consequence of the natural ageing process** (unrelated). 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 GA breeding of an established line, 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 program 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. **Related**: proceed to question 2.

2. Can an informed decision 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 **Yes**, 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. **No**

The actual severity should be reported as "severe".

<table>
<tr><th colspan="3">ASSIGNING ACTUAL SEVERITY IF ANIMALS ARE FOUND DEAD</th></tr>
<tr><th colspan="2">Death related to the procedure</th><th>Death NOT related to the procedure</th></tr>
<tr><td colspan="2">Can an informed decision 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.</td><td rowspan="2">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).</td></tr>
<tr><td>YES.
The actual reported severity should reflect the severity as the result of the assumed events leading to death. The highest reached severity should be reported (death or otherwise).</td><td>NO.
The actual severity should be reported as "severe".</td></tr>
</table>

4. CONSIDERATIONS REGARDING ANIMALS TAKEN FROM THE WILD

The actual severity shall 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 procedures) shall therefore not be taken into account in the reporting of actual severity, including if the animal dies during capture or transport.

Article 10 of the Directive 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) of the Directive 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) of the Directive:

Project means a program of work having a defined scientific objective and involving one or more procedure.

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 scientific purpose, irrespective of the type of animals (purpose bred, farmed, wild), and 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*** " implies 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 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 minimize suffering. Competent authorities may grant exemptions from the requirement of taking action to minimize 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 acclimatization, 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

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 minimize these as far as possible (in the case of projects, consistent with the scientific objectives), and action taken to avoid recurrence.

5. PLACE OF BIRTH

Animals born at an authorized breeder in the Union
Animals born in the Union but not at an authorized breeder
Animals born in rest of Europe
Animals born elsewhere

Origin is based on the place of birth that is to say "born in" and not according to where the animal is supplied from.

- Animals born at an authorized breeder in the Union (for example LA2230…) refers to animals born at breeders authorized and registered under Article 20 of Directive 2010/63/EU.

Animals born in the Union but **not at an authorized breeder** includes, amongst others, wild animals, farm animals (unless the breeder is authorized under Article 20 of Directive 2010/63/EU), as well as any exemptions granted under Article 10(3) of Directive 2010/63/EU.

- Animals born **in rest of Europe** includes, amongst others, animals born in Switzerland, Turkey, Russia and Israel, and groups together all animals, irrespective of whether they have been bred in registered breeding establishments or other establishments, and includes, amongst others, animals that have been captured in the wild.

- Animals born **elsewhere** groups together all animals irrespective of whether they have been bred in registered breeding establishments or other establishments, and includes, amongst others, animals that have been captured in the wild.

6. NON-HUMAN PRIMATE (NHP) – PLACE OF BIRTH

NHP born at an authorized breeder in the Union
NHP born in the Union but not at an authorized breeder, and NHP born in rest of Europe
NHP born in Asia
NHP born in America
NHP born in Africa
NHP born elsewhere

Origin is based on the place of birth, that is to say **'born in'** and not the place where the animal is supplied from.

For the purposes of this reporting:

- 'NHP born at an authorized breeder in the Union' (and Norway) refers to NHP born at breeders as authorized and registered under Article 20 of Directive 2010/63/EU.
- 'NHP born in the Union but not at an authorized breeder, and NHP born in rest of Europe' includes, amongst others, animals born in Switzerland, Turkey, Russia and Israel.
- 'NHP born in Asia' includes, amongst others animals born in China.
- 'NHP born in America' refers to animals born in the North, Central and South America.
- 'NHP born in Africa' includes also animals born in Mauritius.
- 'NHP born elsewhere' includes also animals born in Australasia. The origins of NHP born elsewhere shall be reported.

7. NON-HUMAN PRIMATE –COLONY TYPE

'Self-sustaining colony' covers non-human primates obtained from colonies in which animals are bred only within the colony or sourced from other self-sustaining colonies but not taken from the wild, and where the animals are kept in a way that ensures that they are accustomed to humans.

8. NON-HUMAN PRIMATE - GENERATION

F0
F1
F2 or greater

- F0 refers to animals that are captured from the wild.
- F1 refers to animals that are born in captivity from one, or two parents, that were captured from the wild.
- F2 or greater refers to animals that are born in captivity to parents both of which were themselves born in captivity.

9. GENETIC STATUS

Not genetically altered
Genetically altered *without* a harmful phenotype
Genetically altered *with* a harmful phenotype

Not genetically altered refers to all animals that have not been genetically altered, including also genetically normal parent animals used for the creation of a new genetically altered animal line/strain.

Genetically altered without a harmful phenotype refers to

a) animals used for the **creation of a new line,** carrying the genetic alteration but exhibiting no harmful phenotype ;
b) genetically altered animals **used** in other procedures (not for creation or maintenance) but exhibiting no harmful phenotype.

Genetically altered with a harmful phenotype refers to:

a) animals used for the **creation of a new line** and exhibiting a harmful phenotype;
b) those used for **maintaining an established line** with an intended harmful phenotype and exhibiting a harmful phenotype; and
c) genetically altered animals **used** in other procedures (not for creation or maintenance) and exhibiting a harmful phenotype.

10. CREATION OF A NEW GENETICALLY ALTERED LINE

Animals used for the creation of a new genetically altered line/strain identifies animals which are *used for the creation* of a new genetically altered line/strain, separating from other animals used for the purposes of 'basic research' or 'translational and applied research'. This includes the crossing of different lines to create a new genetically altered line where the phenotype of the new line cannot be determined prospectively as non-harmful.

11. SEVERITY

Actual severity shall be *reported for each animal individually* by reference to the most severe effects experienced by that animal during the course of the entire procedure. Those effects can occur during any of the steps (not necessarily the last) of a multi-step procedure. Actual severity may be higher or lower than the classification predicted prospectively. Cumulative suffering shall also be considered when assigning actual severity.

Non-recovery – Animals which have undergone a procedure that has been performed entirely under general anesthesia and from which the animals have not recovered consciousness shall be reported as 'non-recovery'. This also includes the situation where animals have failed to recover consciousness from anesthesia during the first step of a planned recovery procedure (nothing is done to a conscious animal. All interventions on an unconscious animal under a general anesthesia, and the animal is killed without gaining consciousness.) In general, non-recovery procedures are always planned multisystemic. An exemption to the rule is a procedure in which animal accidentally dies under a general anaesthesia, provided no prior intervention has taken place. This should also be recorded as non-recovery.

Mild (up to and including) - Animals which have undergone a procedure as a result of which the animals have experienced short-term mild pain, suffering or distress shall be reported as 'Mild'. This includes situations where there has been no significant impairment of the well-being or general condition of the animals. This category shall also include animals used in an authorized project, but which have ultimately not been observed to have experienced a level of pain, suffering, distress or lasting harm equivalent to that caused by the introduction of a needle in accordance with good veterinary practice with the exception of animals required for the *maintenance* of colonies of genetically altered animals of established lines *with an intended harmful phenotype* and *which have not exhibited* pain, suffering, distress or lasting harm as a consequence of the harmful genotype.

Moderate - Animals which have undergone a procedure as a result of which the animals have experienced short-term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress as well as procedures that cause moderate impairment of the well-being or general condition of the animals shall be reported as 'Moderate'.

Severe - Animals which have undergone a procedure as a result of which the animals have experienced severe pain, suffering or distress, or long-lasting moderate pain, suffering or distress as well as procedures, that have caused severe impairment of the well-being or general condition of the animals shall be reported as 'Severe'.
Where the use of an animal in a procedure results in severe pain, suffering or distress that is long-lasting and cannot be ameliorated, whether pre-authorized or not, the animal and their use shall be reported under the 'severe' category. Commentary shall be added in the Member State narrative in Section C. In such cases, the following shall be reported: species, numbers, whether prior exemption was authorized, details of the use and reasons why the 'severe' classification was exceeded.

12. PURPOSES

The purpose of studies needs to be carefully established. Only the main purpose shall be reported.

Basic research
Translational and applied research
Regulatory use and routine production
Protection of the natural environment in the interests of the health or welfare of human beings or animals
Preservation of species
Higher education
Training for the acquisition, maintenance or improvement of vocational skills
Forensic enquiries
Maintenance of colonies of established genetically altered animals, not used in other procedures

Basic research

Basic research refers to studies of a fundamental nature including physiology. Studies that are designed to add knowledge about normal and abnormal structure, functioning and behavior of living organisms and environment, this includes also fundamental studies in toxicology. Investigation and analysis focused on a better or fuller understanding of a subject, phenomenon, or a basic law of nature instead of on a specific practical application of the results.
The animals used for the creation of a new genetically altered animal line (including crossing of two lines) *intended to be used for the* ***purposes of basic research*** (for example, developmental biology, immunology) shall be reported ***according to the purpose category they are being created for***. In addition, they are reported in "Creation of a new genetic line – Animals used for the creation of a new genetically altered line/strain".
All animals carrying the genetic alteration shall be reported during the creation of a new line. Also animals used in creation, such as for superovulation, vasectomy and embryo implantation, are reported here. The reporting shall exclude non-genetically altered (wild type) offspring, unless that animal has been genotyped (genetic characterization/tissue sampling) using an invasive method, which was not carried out for the purposes of identification/marking of the animal.
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.

Translational and applied research

Translational and applied research refers to animals used for purposes as described in Article 5(b) and (c) of the Directive excluding any regulatory use of animals.

This also includes discovery toxicology and investigations to prepare for the regulatory submission and method development. This does not include studies required for regulatory submissions.
The animals used for the *creation* of a new genetically altered animal line *intended to be used for the* ***purposes of translational or applied research*** (for example, cancer research, vaccine development) shall be recorded *according to the purpose* they are being created for. In addition, they shall be reported in "Creation of a new genetic line – Animals used for the creation of a new genetically altered line/strain".

All animals carrying the genetic alteration shall be reported during the creation of a new line. Also animals used in creation, such as for superovulation, vasectomy and embryo implantation, shall be reported here. The reporting shall exclude non-genetically altered (wild type) offspring.

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.

Regulatory use and routine production

'Regulatory use' covers the use of animals in procedures with a view to satisfying regulatory requirements that is to say for producing, placing and maintaining products/substances on the market, including safety and risk assessment for food and feed. This includes tests carried out in respect of products/substances for which a regulatory submission was foreseen but ultimately not made, for instance because they were deemed unsuitable for the market by the developer and thus fail to reach the end of the development process. This also includes animals used in the manufacturing process of products such as antibodies and blood based products, for example, animals used in the manufacturing of serum-based medicinal products shall be included within this category).

The efficacy testing during the development of new medicinal products is excluded and shall be reported under category "Translational and applied research".

Protection of the natural environment in the interests of the health or welfare of human beings or animals

This refers to studies aimed at investigating and understanding phenomena such as environmental pollution, loss of biodiversity, and epidemiology studies in wild animals.

This excludes any regulatory use of animals for ecotoxicology purposes.

Higher education

This refers to animals used for delivering theoretical knowledge within a higher education program.

Training for the acquisition, maintenance or improvement of vocational skills

This refers to animals used for training to acquire and maintain practical, vocational skills such as animals used in training of medical doctors.

Maintenance of colonies of established genetically altered animals, not used in other procedures

This contains 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 bred for is not recorded.

This category also includes genetically altered animals during maintenance of an established line, *irrespective of whether the line is of non-harmful or harmful phenotype*, and either of the following applies:

- the genotype has been *confirmed using an invasive method,* which was not carried out for the purposes of identification / marking of the animal, and the animal is killed without further use;

- the animals are of *unsuitable genotype*, *confirmed using an invasive method,* which was not carried out for the purposes of identification / marking of the animal.

The method used for genotyping shall be indicated in the comments (Comment 2).

This category also includes to re-derivation when it is done solely for scientific purposes (that is to say not to benefit health / welfare of the colony) during maintenance of an established line, and animals used for embryo transfer and vasectomy.

This excludes all animals needed for the *creation* of a new genetically altered line and those used *in other procedures* (other than creation/maintenance).

Animal of a *harmful phenotype* having experienced adverse effect, and not genotyped using an invasive method, before being killed
Animal of a *harmful phenotype* having experienced adverse effect, and genotyped using an invasive method, before being killed
Animal of a confirmed genotype using an invasive method, and the animal is killed without further use *(irrespective of whether the animal is from a non-harmful or harmful phenotype line)*
Animal of an unsuitable genotype established using an invasive method *(irrespective of whether the animal is from a non-harmful or harmful phenotype line)*
Other Maintenance

13. BASIC RESEARCH STUDIES

Oncology
Cardiovascular Blood and Lymphatic System
Nervous System
Respiratory System
Gastrointestinal System including Liver
Musculoskeletal System
Immune System
Urogenital/Reproductive System
Sensory Organs (skin, eyes and ears)
Endocrine System/Metabolism
Developmental Biology
Multisystemic
Ethology / Animal Behaviour /Animal Biology
Other Basic Research

Oncology
Any research studying oncology shall be included here regardless of the target system.

Nervous system
This category includes, amongst others, neuroscience, peripheral or central nervous system, psychology.

Musculoskeletal System
This category includes, amongst others, dentistry.

Sensory Organs (skin, eyes and ears)
Studies on nose shall be reported under 'Respiratory System' and those on tongue shall be reported under 'Gastrointestinal System including Liver'

Developmental Biology
Developmental Biology covers studies of changes associated with an organism from embryogenesis (when not carried out as part of reproductive toxicity study), to growth, aging and death, and includes, amongst others, cell differentiation, tissue differentiation and organogenesis.

Multisystemic
This shall only include research where more than one system is the primary interest, such as on some infectious diseases, and excluding oncology. Otherwise, the reporting should be done under the main target organ.

Ethology / Animal Behavior /Animal Biology category covers both animals in the wild and in captivity with the primary goal of learning more about that specific species.

Other Basic Research
Research that is not related to an organ/system listed above or is not organ/system specific. Particular attention needs to be paid before using category 'other' to ensure that none of the pre-defined categories could be used.

Remarks

Animals used for the production and maintenance of infectious agents, vectors (for example, arthropod feeding) and neoplasms, animals used for other biological material and animals used for the production of antibodies for the purposes of research, but excluding the growth of hybridoma cells by ascites method in the production of monoclonal antibodies (which is covered under category "Regulatory use and routine production by product type") shall be reported in the respective categories under "Basic research studies". Where more than one category applies to the purpose of the animal use, only the main purpose shall be reported.

14. TRANSLATIONAL AND APPLIED RESEARCH

Human Cancer
Human Infectious Disorders
Human Cardiovascular Disorders
Human Nervous and Mental Disorders
Human Respiratory Disorders
Human Gastrointestinal Disorders including Liver
Human Musculoskeletal Disorders
Human Immune Disorders
Human Urogenital/Reproductive Disorders
Human Sensory Organ Disorders (skin, eyes and ears)
Human Endocrine/Metabolism Disorders
Other Human Disorders
Animal Diseases and Disorders
Animal Nutrition
Animal Welfare
Diagnosis of diseases
Plant diseases
Non-regulatory toxicology and ecotoxicology

Any applied research on *human cancer* shall be included in category 'Human cancer' regardless of the target system.

Any applied research on human infectious disorders shall be included in 'Human Infectious Disorders' regardless of the target system.

Any regulatory use of animals, such as regulatory carcinogenicity studies, shall be excluded from category 'Translational and applied research' and reported under category 'Regulatory use and routine production'.

Studies on disorders of the nose shall be reported under 'Human Respiratory Disorders' and those of the tongue shall be reported under 'Human Gastrointestinal Disorders including Liver'.

Particular attention shall be paid before using category 'Other Human Disorders' to ensure that none of the pre-defined categories should be used instead.

Diagnosis of diseases includes, amongst others, animals used in direct diagnosis of diseases such as rabies, botulism, but excluding those covered under regulatory use.

Non-regulatory toxicology and Ecotoxicology refers to discovery toxicology and investigations to prepare for the regulatory submission and method development. This category does not include studies required for regulatory submissions such as preliminary studies and MTD (Maximum Tolerated Dose –studies which shall 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, are also excluded and covered in 'Regulatory use and routine production' under 'Other efficacy and tolerance testing'.

Animal welfare refers to studies as per Article 5(b)(iii) of Directive 2010/63/EU.

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.

Remarks

Animals used for the production and maintenance of infectious agents, vectors (for example arthropod feeding) and neoplasms, animals used for other biological material and animals used for the production of antibodies for the purposes of translational and applied research, but excluding the growth of hybridoma cells by ascites method in the production of monoclonal antibodies (which is covered under category "Regulatory use and routine production by type") shall be reported in the respective categories under "Translational and applied research". Where more than one category applies to the purpose of the animal use, only the main purpose shall be reported.

15. REGULATORY USE AND ROUTINE PRODUCTION

Use of animals in procedures carried out with a view to satisfying regulatory requirements i.e., for producing, placing and maintaining products/substances on the market, including safety and risk assessment for food and feed.

This includes tests carried out on products/substances for which no regulatory submission is made (i.e., tests performed on those products/substances (for which a regulatory submission was foreseen) that are ultimately deemed unsuitable for the market by the developer, and thus fail to reach the end of the development process).

This category also includes animals used in the manufacturing process of products if that manufacturing process requires regulatory approval (e.g., animals used in the manufacturing of serum-based medicinal products should be included within this category).

16. REGULATORY USE AND ROUTINE PRODUCTION BY TYPE

Quality control (including batch safety and potency testing)
Other efficacy and tolerance testing
Toxicity and other safety testing including pharmacology
Routine production by product type

Efficacy testing during the development of new medicinal product is excluded and shall be reported under category "Translational and Applied research".

Quality control refers to animals used in the testing of purity, stability, efficacy, potency and other quality control parameters of the final product and its constituents and any controls carried out during the manufacturing process for registration purposes, to satisfy any other national or international regulatory requirements or to satisfy the in-house policy of the manufacturer. This includes, amongst others, pyrogenicity testing.

Other efficacy and tolerance testing
Efficacy testing of biocides and pesticides is covered under this category as well as the tolerance testing of additives in animal nutrition. This covers also dose-range-finding studies, when carried out with a view to satisfying legislative requirements.

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".

Toxicity and other safety testing (including safety evaluation of products and devices for human medicine and dentistry and veterinary medicine) covers studies carried out on any product or substance to determine its potential to cause any dangerous or undesirable effects in humans or animals as a result of its intended or abnormal use, manufacture or as a potential or actual contaminant in the environment. Where studies involve both mother and offspring, the mother shall be reported if she has been subject to a procedure above the threshold of minimum pain, suffering, distress and lasting harm. Offspring shall be reported if they are an integral part of the procedure such as in the case of end-points for reproduction.

Routine production by product type covers the production of antibodies and blood products by established methods. This excludes immunization of animals for subsequent hybridoma production carried out for the purposes of basic or applied and translational research within a given project, which shall be captured under basic or applied research under the appropriate category. The use of animals for antibody production for commercial purposes, including immunization for the subsequent hybridoma production, shall be reported under 'Routine production'/'Monoclonal and polyclonal antibodies (excluding ascites method)'. All use of the ascites method for the culture of monoclonal antibodies shall be reported under 'Routine production'/'Monoclonal antibodies by ascites method only'.

17. TYPE OF LEGISLATION

Legislation on medicinal products for human use
Legislation on medicinal products for veterinary use and their residues
Medical devices legislation
Industrial chemicals legislation
Plant protection product legislation
Biocides legislation
Food legislation including food contact material
Feed legislation including legislation for the safety of target animals, workers and environment
Cosmetics legislation
Other legislation

Type of legislation shall not be reported for animals whose use falls within the category Routine production.
The type of legislation shall be reported by reference to the *intended primary* use.
Testing of the quality of water, other than waste water, shall be reported under 'Food legislation'. Quality testing of waste water shall be reported under 'Other legislation'.

18. ORIGIN OF LEGISLATION

Legislation satisfying Union requirements
Legislation satisfying national requirements only (within Union)
Legislation satisfying Non-Union requirements only

The origin of legislation shall not reported for animals whose use falls within the category Routine production.

The use shall be reported in reference to the region for which the test is being carried out, not where it is carried out.

Where national legislation is derived from Union legislation, the use shall be reported under Legislation satisfying EU requirements.

Legislation satisfying Union requirements also includes any international requirement, which at the same time satisfies Union requirements (such as testing to ICH, VICH, OECD guidelines, European Pharmacopoeia monographs).

Where the test is carried out to satisfy the legislation of one or more Member States (not necessarily the one in which the test is being carried out), and the requirement is not derived from Union law, the use shall be reported under Legislation satisfying national requirements only (within Union).

Legislation satisfying Non-Union requirements is to be chosen only when there is no equivalent requirement to carry out the test to satisfy Union legislation .

19. ROUTINE PRODUCTION BY PRODUCT TYPE

Blood based products
Monoclonal antibodies by ascites method only
Monoclonal and polyclonal antibodies (excluding ascites method)
Other products

Routine production by product type covers the production of antibodies and blood products using established methods. This excludes immunization of animals for subsequent hybridoma production when carried out for the purposes of basic or applied research within a given project. That immunization shall be captured under basic or applied research under the appropriate category.

All use of the ascites method for the culture of monoclonal antibodies shall be reported under 'Monoclonal antibodies by ascites method only'.

The use of animals for antibody production for commercial purposes, including immunization for the subsequent hybridoma production, shall be reported under 'Monoclonal and polyclonal antibodies (excluding ascites method)'.

20. QUALITY CONTROL (INCLUDING BATCH SAFETY AND POTENCY TESTING)

Batch safety testing
Pyrogenicity testing
Batch potency testing
Other quality controls

Batch safety testing excludes pyrogenicity testing which shall be reported separately under Pyrogenicity testing.

21. TOXICITY AND OTHER SAFETY TESTING BY TEST TYPE

Acute (single dose) toxicity testing methods (including limit test)
Skin irritation/corrosion
Skin sensitization
Eye irritation/corrosion
Repeated dose toxicity
Carcinogenicity
Genotoxicity
Reproductive toxicity
Developmental toxicity
Neurotoxicity

Kinetics (pharmacokinetics, toxicokinetics, residue depletion)
Pharmaco-dynamics (including safety pharmacology)
Phototoxicity
Ecotoxicity
Safety testing in food and feed area
Target animal safety
Combined end-points
Other toxicity or safety testing

Repeated dose toxicity includes also immunotoxicological studies.

Reproductive toxicity includes, amongst others, extended one-generation reproductive toxicity studies, also when including cohorts for developmental neuro- and immunotoxicity.

Developmental toxicity includes also developmental neurotoxicity studies. Extended one-generation reproductive toxicity studies including cohort for developmental neurotoxicity shall be reported under reproductive toxicity.

Neurotoxicity includes, amongst others, acute delayed effects (for example delayed neurotoxicity of organophosphorus substances following acute exposure) and repeated dose studies for the purposes of neurotoxicity, but excludes developmental neurotoxicity. Extended one-generation reproductive toxicity studies including cohort for developmental neurotoxicity shall be reported under reproductive toxicity.

Kinetics refers to pharmacokinetics, toxicokinetics and residue depletion. However, if testing for toxicokinetics is performed as part of the regulatory repeated dose toxicity study, it shall be reported under repeated dose toxicity.

Safety testing in the food and feed area includes also testing of drinking water (including target animal safety testing).

Target animal safety testing ensures that a product for a specific animal can be used safely on that species (excluding batch safety testing which is covered under quality control).

Combined end-points include, amongst others, combination of carcinogenicity and chronic toxicity study, screening studies combining reproductive toxicity and repeated dose toxicity.

21.1. Acute toxicity testing methods

LD50, LC50
Other lethal methods
Non-lethal methods

The sub-category shall be reported on the basis of the type of method used and not on the basis of the level of severity experienced by the animal as a result of that method.

'LD50, LC50' refer only to test methods that provide a point estimate for LD50/LC50 such as OECD test guidelines 203, 403 and 425.

'Other lethal methods' refers to those methods that categorize substances in a class, that is to say, methods involving assignment of a range in which LD50 would fall, such as fixed dose methods and acute toxic class methods. It is likely that a number of deaths will occur but not as many as those expected in LD50-type methods.

21.2. Repeated dose toxicity

28 days or less
29 - 90 days
more than 90 days

21.3. Ecotoxicity

Acute toxicity (ecotoxicity)
Chronic toxicity (ecotoxicity)
Reproductive toxicity (ecotoxicity)
Endocrine activity (ecotoxicity)
Bioaccumulation (ecotoxicity)
Other ecotoxicity

Ecotoxicity refers to toxicity relating to the aquatic and terrestrial environment.

Ecotoxicity studies addressing short-term toxicity to determine LC/LD50 shall be reported under 'acute toxicity (ecotoxicity)'.

Ecotoxicity studies addressing long-term toxicity, for example, early life cycle test or full life cycle tests, shall be reported under 'chronic toxicity (ecotoxicity)'.

Ecotoxicity studies carried out to primarily assess endocrine properties of substances and addressing, for example, amphibian metamorphosis, development and growth, fish sexual development and reproduction, shall be reported under 'endocrine activity (ecotoxicity)'.

ADDITIONAL SUGGESTIONS

More information at:

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

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ANNEXE I: FLOWCHART OF STATISTICAL DATA INPUT CATEGORIES UNDER ARTICLE 54(2) OF DIRECTIVE 2010/63/EU

Type of animal
Mice (*Mus musculus*)
Rats *(Rattus norvegicus*)
Guinea-Pigs *(Cavia porcellus*)
Hamsters (Syrian) *(Mesocricetus auratus*)
Hamsters (Chinese) (*Cricetulus griseus)*
Mongolian gerbil (*Meriones unguiculatus*)
Other rodents (other *Rodentia*)
Rabbits (*Oryctolagus cuniculus)*
Cats (*Felis catus*)
Dogs (*Canis familiaris*)
Ferrets (*Mustela putorius furo*)
Other carnivores (other *Carnivora*)
Horses, donkeys and cross-breeds (*Equidae*)
Pigs (*Sus scrofa domesticus*)
Goats (*Capra aegagrus hircus*)
Sheep (*Ovis aries*)
Cattle (*Bos taurus*)
Prosimians (*Prosimia*)
Marmoset and tamarins (eg. *Callithrix jacchus*)
Cynomolgus monkey (*Macaca fascicularis*)
Rhesus monkey *(Macaca mulatta)*
Vervets *(Chlorocebus spp.) (*usually either *pygerythrus* or *sabaeus)*
Baboons *(Papio spp.)*
Squirrel monkey (eg. *Saimiri sciureus*)
Other species of New World monkeys *(*other species of *Ceboidea*)
Other species of Old World monkeys *(*other species of *Cercopithecoidea*)
Apes *(Hominoidea)*
Other mammals (other *Mammalia*)
Domestic fowl (*Gallus gallus domesticus*)
Turkey *(Meleagris gallopavo)*
Other birds (other *Aves*)
Reptiles (*Reptilia*)
Rana (*Rana temporaria* and *Rana pipiens*)
Xenopus (*Xenopus laevis and Xenopus tropicalis*)
Other amphibians (other *Amphibia*)
Zebra fish (*Danio rerio*)
Sea bass *(spp. from families e.g. Serranidae, Moronidae)*
Salmon, trout, chars and graylings *(Salmonidae)*
Guppy, swordtail, molly, platy *(Poeciliidae)*
Other fish (other *Pisces*)
Cephalopods (*Cephalopoda*)

Reuse
Reuse (No/Yes)

YES **NO**

Non-human primate?
NO **YES**

Species other than non-human primate - Place of birth
Animals born at an authorised breeder in the Union
Animals born in the Union but not at an authorised breeder
Animals born in rest of Europe
Animals born elsewhere

Genetic status
Not genetically altered
Genetically altered *without* a harmful phenotype
Genetically altered *with* a harmful phenotype

Creation of a new genetically altered line
Animals used for the creation of a new genetically altered line/strain (No/Yes)

Severity
Non-recovery
Mild (up to and including)
Moderate
Severe

Purposes	
Basic research	
Translational and applied research	
Regulatory use and routine production	
Protection of the natural environment in the interests of the health or welfare of human beings or animals	**END**
Preservation of species	**END**
Higher education	**END**
Training for the acquisition, maintenance or improvement of vocational skills	**END**
Forensic enquiries	**END**
Maintenance of colonies of established genetically altered animals, not used in other procedures	**END**

Non-human primate (NHP) - Place of birth
NHP born at an authorised breeder in the Union
NHP born in the Union but not at an authorised breeder, and NHP born in rest of Europe
NHP born in Asia
NHP born in America
NHP born in Africa
NHP born elsewhere

Non-human primate - Colony type
Self-sustaining colony (No/Yes)

Non-human primate - Generation
F0
F1
F2 or greater

Basic research studies
Oncology
Cardiovascular Blood and Lymphatic System
Nervous System
Respiratory System
Gastrointestinal System including Liver
Musculoskeletal System
Immune System
Urogenital/Reproductive System
Sensory Organs (skin, eyes and ears)
Endocrine System/Metabolism
Developmental Biology
Multisystemic
Ethology / Animal Behaviour /Animal Biology
Other Basic Research

END

Translational and applied research
Human Cancer
Human Infectious Disorders
Human Cardiovascular Disorders
Human Nervous and Mental Disorders
Human Respiratory Disorders
Human Gastrointestinal Disorders including Liver
Human Musculoskeletal Disorders
Human Immune Disorders
Human Urogenital/Reproductive Disorders
Human Sensory Organ Disorders (skin, eyes and ears)
Human Endocrine/Metabolism Disorders
Other Human Disorders
Animal Diseases and Disorders
Animal Nutrition
Animal Welfare
Diagnosis of Diseases
Plant Diseases
Non-regulatory Toxicology and Ecotoxicology

END

Regulatory use and Routine production

Quality control (including batch safety and potency testing)
Batch safety testing
Pyrogenicity testing
Batch potency testing
Other quality controls

END

Routine production by product type
Blood based products
Monoclonal antibodies by ascites method only

Quality control (including batch safety and potency testing)
Other efficacy and tolerance testing
Toxicity and other safety testing including pharmacology
Routine production by product type

END

Monoclonal and polyclonal antibodies (excluding ascites method)
Other products

END

Type of legislation
Legislation on medicinal products for human use
Legislation on medicinal products for veterinary use and their residues
Medical devices legislation
Industrial chemicals legislation
Plant protection product legislation
Biocides legislation
Food legislation including food contact material
Feed legislation including legislation for the safety of target animals, workers and environment
Cosmetics legislation
Other legislation

Origin of legislation
Legislation satisfying Union requirements
Legislation satisfying national requirements only (within Union)
Legislation satisfying Non-Union requirements only

END

Acute-toxicity testing methods
LD50, LC50
Other lethal methods
Non lethal methods

END

Toxicity and other safety testing by test type	
Acute (single dose) toxicity testing methods (including limit test)	
Skin irritation/corrosion	END
Skin sensitisation	END
Eye irritation/corrosion	END
Repeated dose toxicity	
Carcinogenicity	END
Genotoxicity	END
Reproductive toxicity	END
Developmental toxicity	END
Neurotoxicity	END
Kinetics (pharmacokinetics, toxicokinetics, residue depletion)	END
Pharmaco-dynamics (including safety pharmacology)	END
Phototoxicity	END
Ecotoxicity	
Safety testing in food and feed area	END
Target animal safety	END
Combined end-points	END
Other toxicity or safety testing	END

Repeated dose toxicity
28 days or less
29 - 90 days
more than 90 days

END

Ecotoxicity
Acute toxicity (ecotoxicity)
Chronic toxicity (ecotoxicity)
Reproductive toxicity (ecotoxicity)
Endocrine activity (ecotoxicity)
Bioaccumulation (ecotoxicity)
Other ecotoxicity

END

ANNEXE II: KEY ELEMENTS OF A GA RODENT WELFARE ASSESMENT SCHEME

Include animals of representative age groups

- soon after birth, around weaning and again following sexual maturity[4]
- a minimum of 7 males and 7 females sampled from more than one litter
- data from a minimum of two breeding cycles (from F2 onwards)
- comparisons made wherever possible with similar non GA animals.

CRITERIA	WHAT TO LOOK FOR
Overall Appearance	Is the animal morphologically 'normal'? Are there any malformations or any other indicators that the phenotype has been affected? For example skeletal deformity or hydrocephalus.
Size, conformation and growth	Are there any deviations from expected size or growth curve?
Coat condition	Is there any piloerection, areas of fur loss, loss of whiskers, barbering? Is the skin / fur in good condition?
Behavior - Posture, gait, activity and interactions with the environment	Do they exhibit the full repertoire of behaviors appropriate for the strain/species, including social interactions, grooming, walking, running, digging, climbing? Are these normal? Is the animal hunched or reluctant to move? Is movement impaired or is there any difficulty with orientation? Any signs of rigidity or tremors? Any abnormal activity levels? Prolonged inactivity could indicate chronic stress or depression (anhedonia) and/or sickness/pain, particularly if linked with a hunched posture and/or rough or unkempt coat. Unusual activity, such as hyperactivity, could indicate stereotypy or other behavioral abnormality.
Clinical signs	For example - nasal or ocular discharge, swollen or closed eyes; increased respiratory rate; dyspnea; seizures/twitches/tremors; increased vocalization with handling; overgrown teeth; presence of tumors, neurological or musculoskeletal abnormalities. Is metabolism impaired, for example, increased or decreased food or water intake, excessive urination? Consistency of faeces.
Relative size	Any unusual changes in size of the animals should be noted, and comparisons made within the litter. It may be helpful to generate a growth curve for the line.
Numbers	Where death occurs, it is important to maintain accurate records such that any pre- or post-weaning losses can be investigated. Where appropriate (e.g. higher than anticipated mortality rate), post mortem examinations should be carried out to help determine the cause of death. A review of fertility can also be helpful in assessment of whether or not the modification is having an effect e.g. conception rates; abortions; stillbirths.

[4] *and at additional time points as considered appropriate by a prospective review of the potential impact of the gene alteration e.g. where there is an age dependent onset of disease

ANNEXE III: ADDITIONAL CONSIDERATIONS FOR ASSESMENT IN NEONATAL ANIMALS

CRITERIA	WHAT TO LOOK FOR
Color of pups (for neonate only)	Do any pups show evidence of abnormal skin color (e.g. anemia, poor circulation)
Activity of pups (for neonate only)	Any abnormal activity, e.g. reduced wriggling? Righting reflex intact?
Milk spot (for neonate only)	Do any pups fail to show presence of a milk spot? Any evidence of mis-mothering?
Litter	Litter sizes; litter homogeneity; development and growth of pups