Genetically altered animals through the lens of Directive 2010/63/EU

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Legal requirements are never fun

But…….Ignorance of the law does not exempt from compliance

The lens has 2 sides……

Points I want to address

- Why such an special focus on GAA
- General concepts regarding GAA in the Directive
  - Creation of a GA model
  - Establishment of a GA line
  - Maintenance of a GA line
- Specific matters when working with GAA within the European Union
  - Project application: what to ask for and why
  - Statistical report: what to report and when

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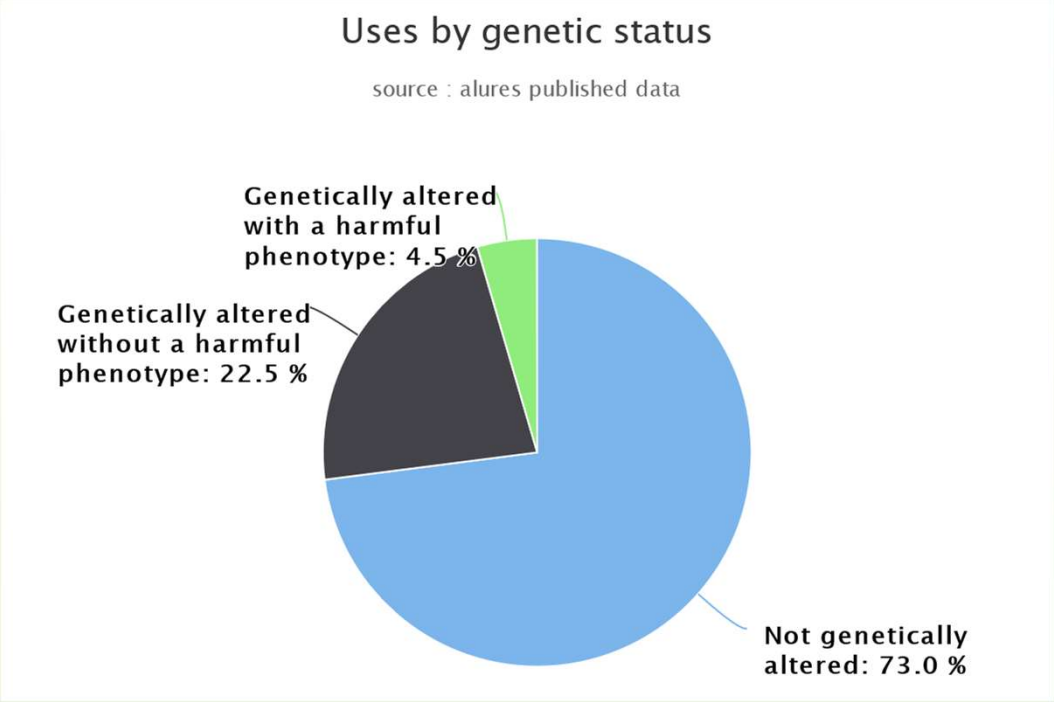
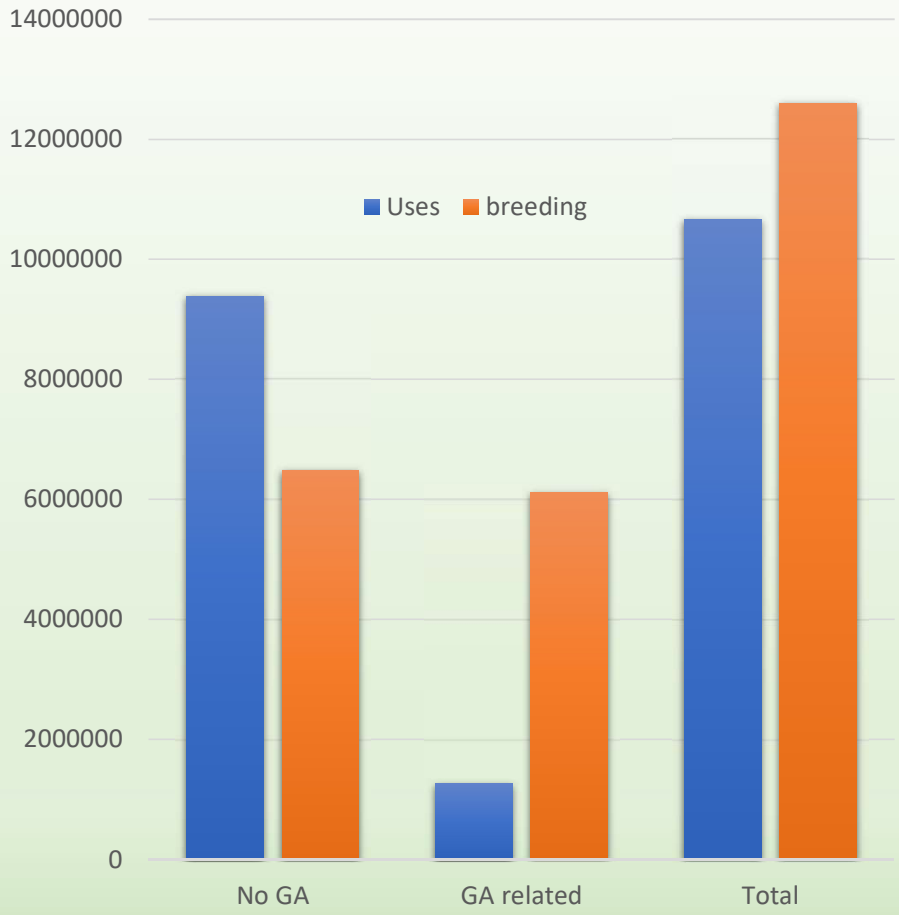
Statistics of GAA in the context of animal experimentation



9.388.162 uses of animals reported in 2017

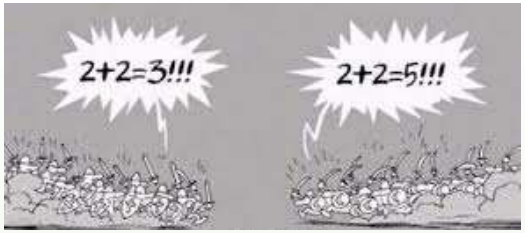


Reported data of 2017

Impact of Directive 2010/63 on GA models:

- General impact: they are animals used for research purposes
- Specific impact due to the possibility of genetic alterations affecting animal welfare.
- The umbrella of special protection is extended also to spontaneous and induced mutations. They are now considered GA models.

Where to look for implementation



- **ec.europa.eu/animals-in-science**.

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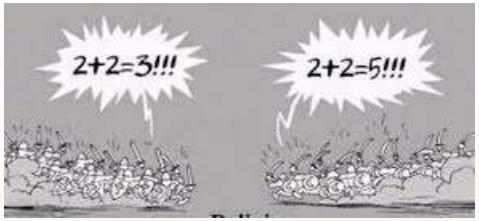
See the contact details for the National Committees.

Guidance documents and posters

It is important that the Directive on the protection of animals used for scientific purposes is implemented correctly. Therefore guidance material has been developed to help all stakeholders apply the principle of the Directive correctly. These publications aim to significantly improve animal welfare and science by enhancing a common understanding of the Directive's provisions across all Member States of the EU.

Guidance documents

- Animal Welfare Bodies and National Committees
- Education and Training Framework
- Genetically Altered Animals
- Inspections and Enforcement
- Non-technical Project Summaries
- Project Evaluation and Retrospective Assessment
- Severity Assessment Framework



Publication detail

Publication detail › Framework for the genetically altered animals …

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Framework for the genetically altered animals under Directive 2010/63/EU on the protection of animals used for scientific purposes

The following is intended as guidance to assist the Member States and others affected by Directive 2010/63/EU on the protection of animals used for scientific purposes (as amended by Regulation (EU) 2019/1010 of the European Parliament and of the Council) to arrive at a common understanding of the provisions contained in the Directive and to facilitate its implementation.

EU publications

How to cite

Download and languages

https://ec.europa.eu/environment/chemicals/lab_animals/interpretation_en.htm

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When is mandatory to have an authorized Project in the specific context of GA animals?

- When I intend to "**create**" a new genetically altered line
  - The Project authorization should cover all animals needed to generate the founder/s and all animals carrying the mutation till the line is "**established**"
- When I intend to "**maintain**" an established GA line with a phenotype that may compromise animal welfare, regardless if this adverse phenotype is shown or not

What is considered “creation”

- Any genetic alteration induced in any way that results in germline transmission
  - Mutations induced by chemical, physical or viral agents
    - Additive transgenesis: constitutive and inducible models (tet-on, tet-off)
    - ES cell based mutant models (KO, KI, Conditionals)
    - Any model obtained through genome edition (CRISPR/Cas technology)
  - Spontaneous mutations when detected
  - Crossing two GA lines even if those are already “established” *

The Directive includes and protects not only genetically modified animals, but all kind of genetically altered animals

What is considered “established line”

- A line that
  - Has a predictable transmission to progeny
    - Mendelian?
    - Sex-linked?
  - Has a preliminary welfare assessment done to detect potential harmful phenotype

But…… what is an initial welfare assesment?

Key Elements of a GA Rodent Welfare Assessment Scheme I

- Include animals of representative age groups:
  - soon after birth,
  - around weaning and again
  - following sexual maturity*
  - 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
- 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.

Key Elements of a GA Rodent Welfare Assessment Scheme II

- Overall appearance
- Size, conformation and growth
- Coat condition
- Behaviour: posture, gait, activity and interaction with environment
- Clinical signs
- Relative size
- Numbers: deaths, fertility records…..

Key Elements of a GA Rodent Welfare Assessment Scheme and III

For neonatal animals:

- Colour
- Activity
- Milk spot
- Litter sizes and homogeneity

As a result of the preliminary welfare assessment…

- We will have:
  - GA lines showing harmful phenotype that will request a project authorization for maintaining them
  - GA lines without harmful phenotype that will not request a project authorization anymore to maintain them, as any wt animal.

What is considered “maintenance”

- Breeding of a genetically altered lines with harmful phenotype to produce animals involved in other studies for other possible purposes and to renew breeding parental.
- Animals reported under this purpose will not be used for any other function besides breeding.

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Project application: creation

- Creating a new line always require a project authorization*
  - Purpose: in line to the reason that makes the creation necessary
  - Harm-benefit analysis
  - Compliance with 3 Rs
    - Is the line available in any repository?
    - Technique/species of choice
    - Experience in generation of founders?
    - Can harmful phenotype be avoided with a conditional/inducible approach?

Project application: creation

- Creating a new line always require a project authorization*
  - Numbers to be requested in the project application:
    - All animals we expect to obtain till the line is established carrying the mutation
    - All animals we expect to use in the generation of founders if this is made indoors
    - WT Animals used to breed the founders or their progeny to obtain the desired genetic information

  - **AND I strongly recommend to include those animals needed to "establish" the line according to the Directive**

* Some situations previously understood as "creation", may not require project authorization

- Congenics: moving a gene from one background to other, unless the new background represents a risk for animal welfare
- Crossing of two different established GA lines without harmful phenotype may not require project authorization if the expected resulting phenotype is non harmful (reporter genes)

In both situations a justification report by the animal welfare body has to be provided, and the competent authority has to recognize (agree) with this interpretation

Project application: maintenance

- Maintaining a GA line with harmful phenotype **always** require a project authorization

    - It doesn’t matter if housing conditions avoid any impact on animal welfare
    - It doesn’t matter if breeding schemes avoid appearance of harmful phenotype
    - It doesn´t matter if animals are killed before the harmful phenotype appears

Some frequent arguments against the need to ask for a project authorization:

- “My GA line is immunodeficient, but it is maintained in a SPF facility”
- “My GA line shows harmful phenotype in homozygosis but not as heterozygous and I keep it always breeding Het x WT”
- “My GA line shows welfare issues at 10 months old, and I never allow breeding animals older that 6 months”

The Directive clearly states:

- Article 1(2)

The elimination of pain suffering distress or lasting harm by a successful use of anesthesia, analgesia or other methods **shall not exclude the use of an animal in procedures** for the scope of this Directive

Hence, the 3 situations require a project authorization

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Reporting of "creation"

- All animals carrying the genetic alteration should be reported during the creation of a new line.
- In addition, those used for superovulation, vasectomy, embryo implantation **should equally be reported** (these may or may not be genetically altered themselves).
- Genetically normal animals (wild type offspring) produced as a result of creation of a new genetically altered line should not be reported ***as used*** in a project unless invasive genotyping. They are reported every 5 years
- Genetically normal animals used to breed founders or GA animals to expand the line during the creation, should be reported **as used** in a project regardless they are wt and they where used for natural mating

With the observed severity, not the one proposed in the project

Reporting of “maintenance”

- Animals carrying the mutation that show harmful phenotype
- All animals carrying the genetic alteration if submitted to an invasive genotyping method
- Genetically altered animals **NOT** showing harmful phenotype **should not be reported as USED**, they will be reported in the 5-years report.

  Those are the main source of the group of “bred but not used”

With the observed severity, not the proposed in the project

Summary of reporting criteria

	GA lines expressing harmful phenotype	GA lines Not expressing harmful phenotype	Wild type animals
Creation of a new strain or line	Report animals under the primary purpose intended for the line		Report animals used to generate founders or quimeras: donors recipients…
Maintenance of a established GA line not used in other procedures	Report under "maintenance of colonies"	**NOT reported annually unless invasive genotyping (yes in 5 years report)**	NOT reported unless invasive genotyping (Yes in 5 year report)
Animals from GA lines used in other procedures	Report under primary purpose of the procedure		
Animals from GA lines used to COLLECT tissues	Report under primary purpose of use	NOT reported anually yes every 5 years	NOT reported unless invasive genotyping

Thanks for your attention

Now is your turn for questions and clarifications