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COMMISSION STAFF WORKING DOCUMENT

Summary Report on the statistics on the use of animals for scientific purposes in the Member States of the European Union and Norway in 2022

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Report of statistical information on the use of animals in procedures

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I. Abstract

Data reported in 2022 marks an important step in the publication of statistical data on the use of animals for scientific purposes. Indeed, this sixth report demonstrates clear positive impacts of the Directive in different areas reported by Member States, particularly in the use of animals for regulatory purposes.

In 2022, uses for regulatory procedures continued to decrease significantly with -16.2% compared to 2021 and -32% compared to 2018, which represents more than half a million uses of animals for this purpose – a trend that started already in 2015. This decrease is mainly due to continuous reductions in uses related to legislations on medicinal products for human use (-50.2% representing 519,088 less uses compared to 2018), food legislation including food contact material (-80.4% representing 33,500 less uses) and "Other legislations" (-47.7% representing 23,598 less uses).

Regulatory use purposes where alternative methods are or have become available that saw significant decreases over the past five years are: batch potency testing (-39.9% representing 300,259 less uses), batch safety testing (-40.6% representing 55,813 less uses), safety testing in food and feed area (-73.9% representing 30,433 less uses), pyrogenicity testing (-35.7% representing 10,647 less uses) and skin irritation/corrosion (-30.1% representing 1,205 less uses).

Looking at overall numbers, the total number of animals used for the first time in 2022 decreased back to 8.39 million (-10.9% compared to 2021) confirming the exceptional nature of the increase reported in 2021 and a longer-term decreasing trend (-5% compared to 2018). In particular, mammalian species used for the first time saw a decrease of -10.1% over the past five years (-25% for rats and -9% for mice).

Reuses of animals went below 100,000 reuses for the first time (92,448), confirming a continuous decrease of reuses since 2018 (minus 64.229 reuses).

With regard to areas that could be improved, in categories where large number of animals are used, first uses of zebra fish tend to increase over time (+39.7% compared to 2018), as well as birds (+18.5%) and rabbits (+12.1%). In reference to purposes, routine production uses saw an important increase (+34.8% compared to 2018). Since 2021, data is available separately on the use of mouse ascites method and other methods for the production of mono- and polyclonal antibodies, both of which should be monitored closely.

The data presented in this report can be consulted at both Union and Member State level using the open-access public ALURES Statistical EU database[1].

[1] https://environment.ec.europa.eu/topics/chemicals/animals-science/statistics-and-non-technical-project-summaries_en

II. Executive Summary

This report presents statistical data on the use of animals for scientific purposes in the Member States of the European Union (EU) and Norway during 2022 under Directive 2010/63/EU[2] ("the Directive") on the protection of animals used for scientific purposes. References to "EU" and "Union" data from here on in this report, are therefore to be understood to cover 27 EU Member States and Norway, unless otherwise specified.

Data from Norway was incorporated in these reports in 2018 and United Kingdom data was no longer reported after 2019. Therefore, to enable analysis of the evolution of the data, this report goes back to 2018 and excludes data reported by United Kingdom.

The presentation of data follows that of the previous reports distinguishing animals used directly in research, testing, routine production and for educational (including training) purposes ("research and testing" from here on), from those used for the creation and maintenance of genetically altered animals in support of the Union research needs. The content of the different sections considers the evolution of uses of animals over time, comparing the 2022 data with 2021 and using the year 2018 as reference to identify longer term trends.

The data presented in this report can be consulted at both Union and Member State level using the open-access public ALURES Statistical EU database[3].

II.1. Numbers and origins of animals

In 2022, the total number of animals used for the first time in research and testing covering the 28 countries (EU-27 and Norway) was about 8.39 million. This is 10.9% lower than in 2021 and 5% lower compared to 2018 (Table 1). This decrease confirms the exceptional increase of first time uses in 2021 due to three projects that accounted for over 1.3 million fish uses.

	2018	2019	2020	2021	2022	Change 2022 vs 2021	Change 2022 vs 2018
Total	8,822,404	8,579,439	7,938,064	9,406,233	**8,385,397**	**-10.9%**	**-5%**

Table 1: Total numbers of animals used for the first time between 2018 and 2022

Looking at species used (Figure 1), 2022 saw the proportion of fish used for the first time decrease by 6.3 percentage points to 30.3%, for the same reason. The proportion of mice was 47.8%, remaining the most used species, followed by salmon, trout chars and graylings (15.4%), rats (7.5%), domestic fowls (5%) and rabbits (4.4%).

[2] Directive 2010/63/EU OJ L276, 20.10.2010, p.33-79

[3] https://environment.ec.europa.eu/topics/chemicals/animals-science/statistics-and-non-technical-project-summaries_en

Information about the origin of animals is important. Animals bred outside the Union do not benefit from the accommodation and care standards provided by the Directive. Moreover, an increase in transport times may negatively impact their welfare. In 2022, the proportion of animals born outside of the Union increased, representing 6.1% of all animals used for the first time (excluding non-human primates) (Section IV.1.2). Also, the proportion of animals born in the Union but not at a registered breeder increased, representing 10% of the total, a category including animals from, for example, farms, and studies carried out using wild animals, especially wild fish.

The Directive provides additional protection for non-human primates due to their genetic proximity to human beings, their highly developed social skills and capacity to experience pain, suffering and distress.

In 2022, 5,784 non-human primates were used for the first time representing about 0.1% of all uses, mostly cynomolgus monkeys (Figure 1). In order to end the capturing of animals from the wild including for the purposes of breeding, the Directive requires moving towards using non-human primates that have been bred, ultimately, in self-sustaining colonies, from parents who themselves have been bred in captivity. In 2022, 74.4% of them came from self-sustaining colonies (Section IV.1.2). Exceptionally, 20 prosimians coming from the wild were reported as part of a study for the preservation of species. As explained in the French Member State narrative, they were not removed from their natural habitat and only subject to mild severity procedures.

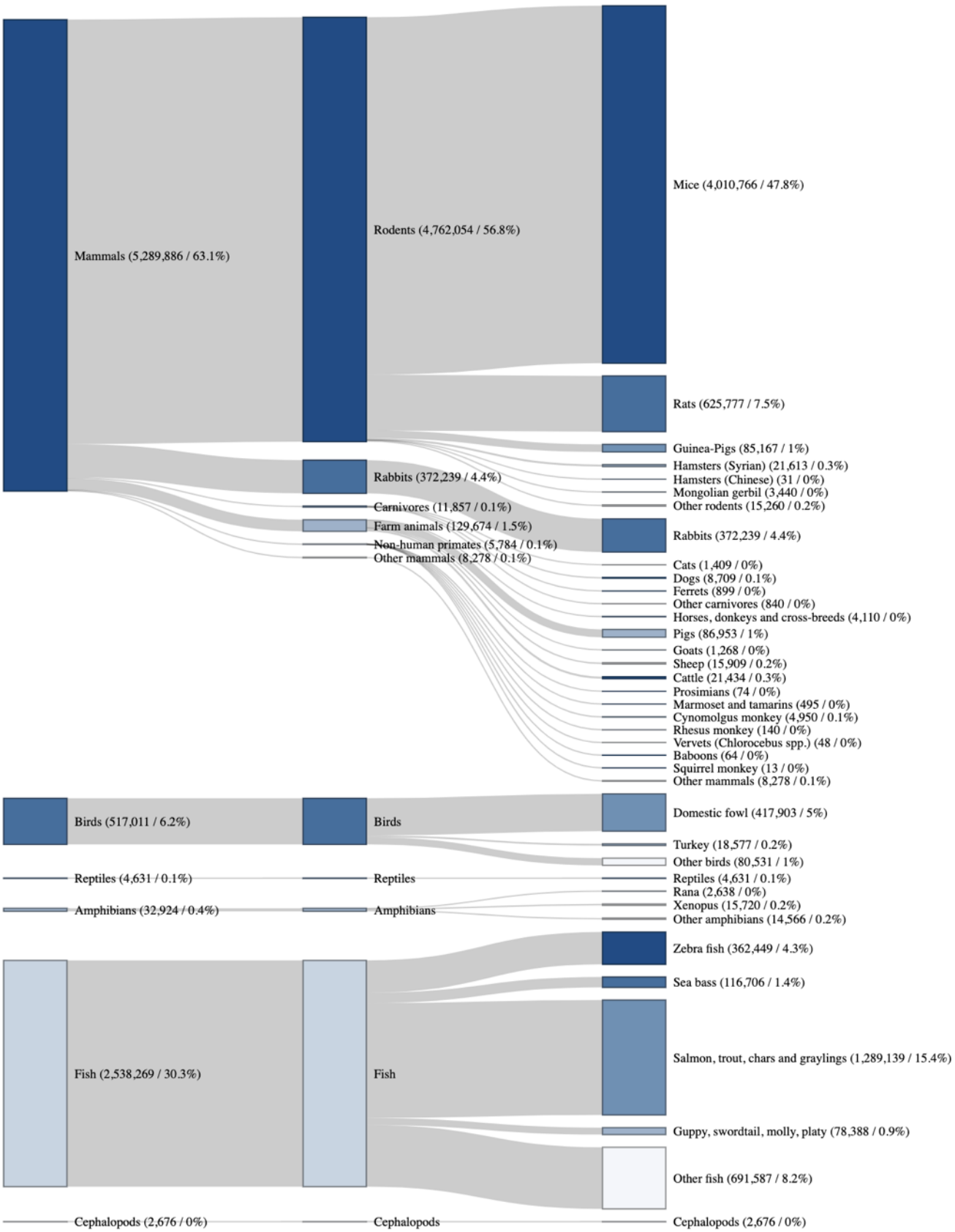


Figure 1: Numbers of animals used for the first time by main classes of species in 2022

II.2. Uses of animals in research and testing

In 2022, 8.48 million uses (first and any subsequent reuse) of animals for scientific purposes were reported (Figure 2). As in previous years, the main purpose was research (72%) with 37% for basic research and 35% for translational and applied research. A further 13% of animal uses were for regulatory uses to satisfy regulatory requirements, followed by other uses (9%) and routine production (6%).

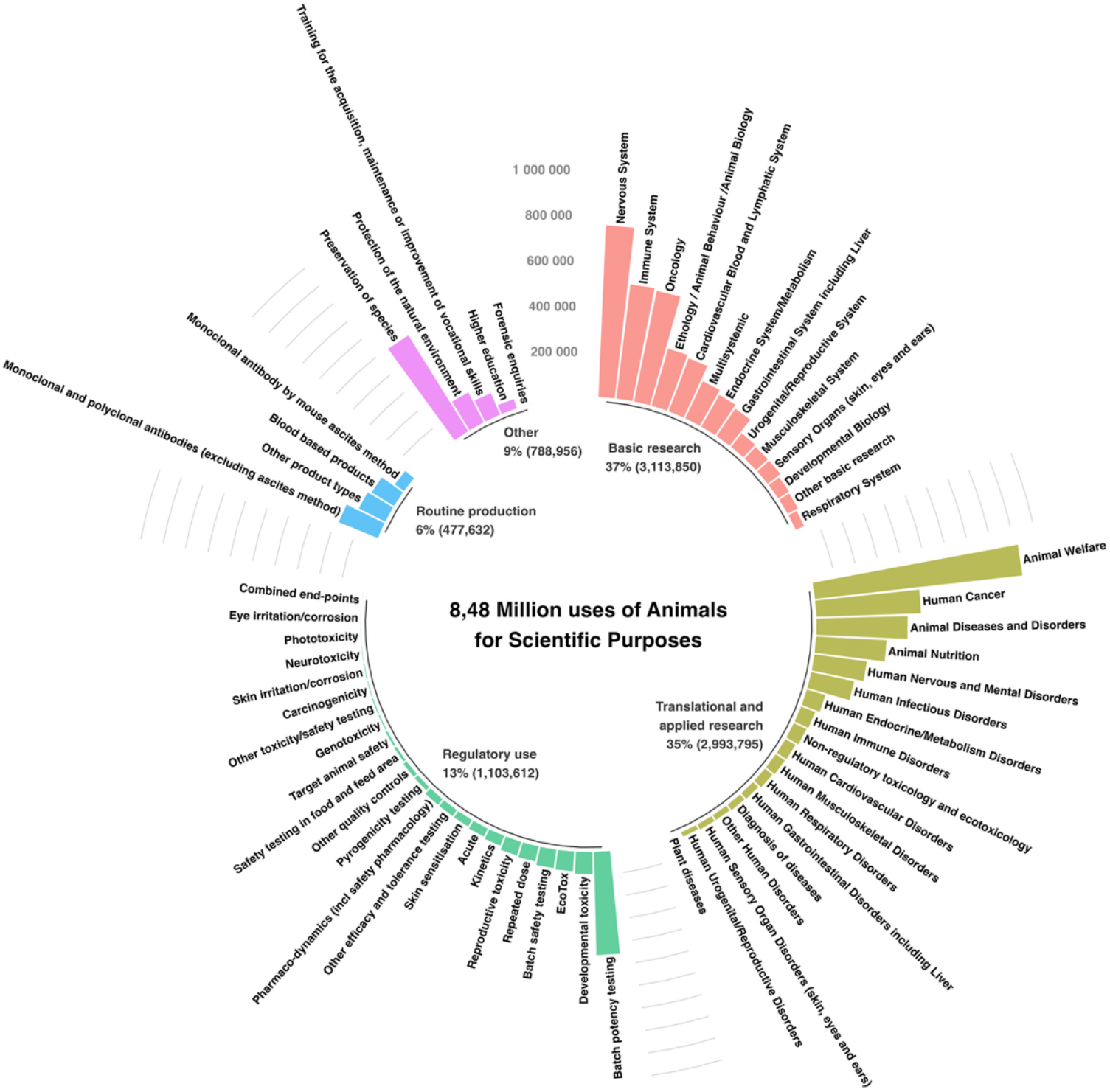


Figure 2: All uses of animals for research and testing in 2022

In terms of evolution, uses for translational and applied research decreased the most (-22.5%) compared to 2021 which can be explained by the fact that the three projects responsible for the 2021 increase of uses belonged to this category. Regulatory uses continued to decrease (-16.2%) as well as the "Other purposes" (-12.1%). Significant reductions over the last five years can be seen in batch potency testing (-39.9% representing 300,259 less uses), batch safety testing (-40.6% representing 55,813 less uses), safety testing in food and feed area (-73.9% representing 30,433 less uses), pyrogenicity testing (-35.7% representing 10,647 less uses) and skin irritation/corrosion (-30.1% representing 1,205 less uses).

Basic research remained almost stable (+3%), while routine production saw an increase of +15.7% (Section IV.2.1). Basic research remained almost stable (+3%), while routine production saw an increase of +15.7% (Section IV.2.1). Efforts should be made to reduce the use of animals for the routine production of mono- and polyclonal antibodies (+75.1%), and the use of mouse ascites method (+33.7%) which results in high severities and has alternative production methods available, should be discontinued (Section IV.2.2.3).

The proportion of 'severe' uses continued a downward trend, decreasing a further -0.5 percentage point.

	2018	2019	2020	2021	2022
Non-recovery	521,765 (5.8%)	494,368 (5.7%)	330,392 (4.1%)	336,332 (3.5%)	304,582 (3.6%)
Mild [up to and including]	4,311,312 (48%)	4,380,747 (50.3%)	3,921,024 (48.7%)	4,395,210 (46.2%)	3,789,476 (44.7%)
Moderate	3,169,559 (35.3%)	2,955,923 (33.9%)	3,006,764 (37.3%)	3,863,470 (40.6%)	3,599,739 (42.5%)
Severe	976,445 (10.9%)	884,186 (10.1%)	796,750 (9.9%)	918,202 (9.7%)	784,048 (9.2%)
Total	**8,979,081 (100%)**	**8,715,224 (100%)**	**8,054,930 (100%)**	**9,513,214 (100%)**	**8,477,845 (100%)**

Table 2: Severity of uses reported in 2022

The graphical presentation below shows the purpose areas with most severe uses. In 2022, most of these were conducted for research purposes while routine production was mostly mild (with the exception of production of monoclonal antibodies by mice ascites). In proportion, uses in translational and applied research tended to be more severe than those reported in basic research especially for the study of animal diseases and disorders (Figure 3).

When analysing all the sub-categories of purposes, animal disease and disorders is now the highest number of severe uses (about 124,982 uses) followed by batch potency testing (88,421). Looking at the proportion of severe uses within a sub-category: combined end-points[4] (84.3%) is the first, followed by production of monoclonal antibodies by mouse ascites (49.1%), animal diseases and disorders (31.2%) and acute toxicity (27.6%).

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

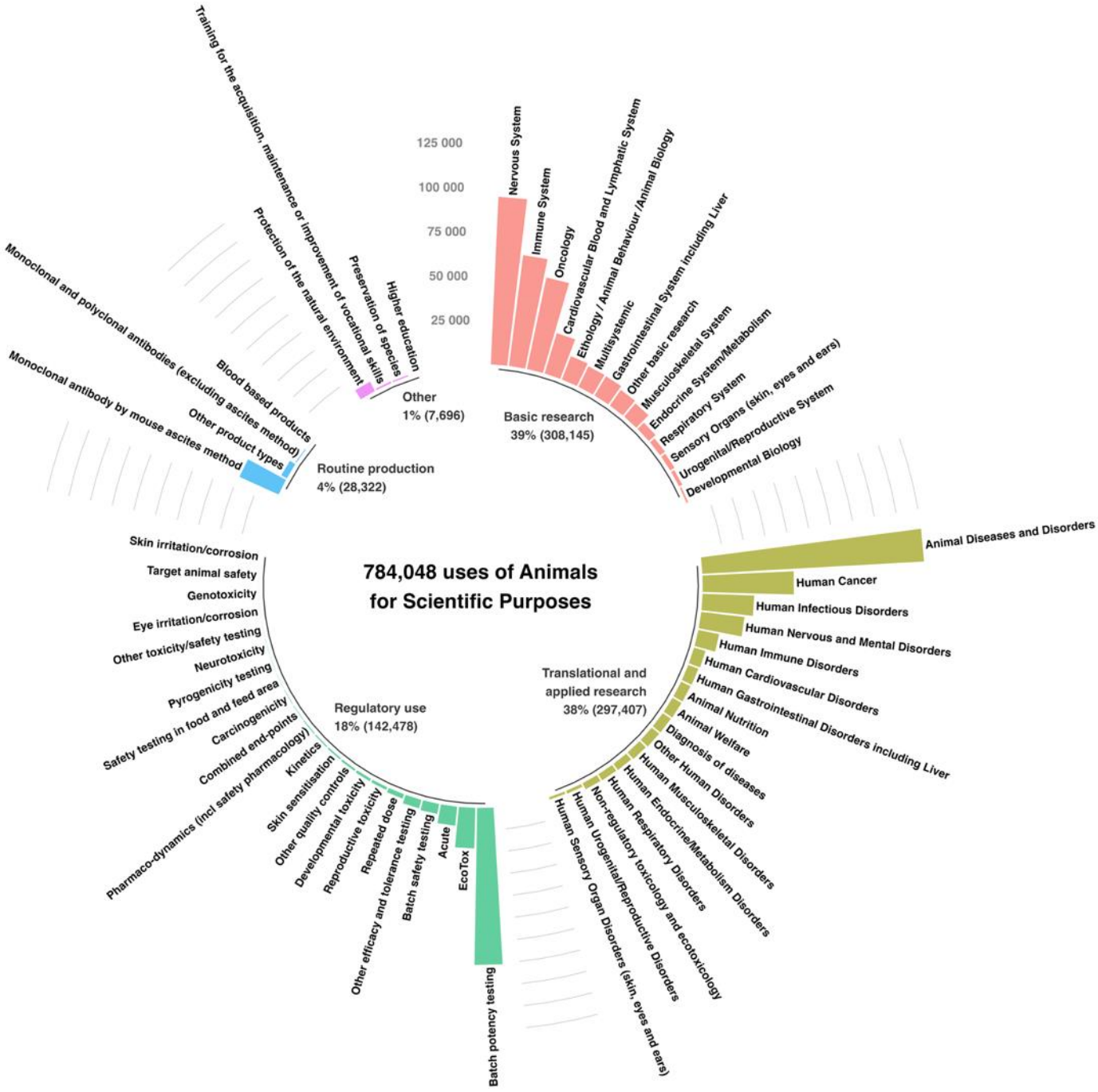


Figure 3: Severe uses of animals for research and testing in 2022

In 2022, the majority of uses to satisfy regulatory requirements of specific sector legislation occurred in relation to placing on the market of medicinal products for humans (46.7%), veterinary medicinal products (27.7%) and industrial chemicals (13.8%) (Figure 4).

Most of regulatory uses continue to be performed to comply with regulatory requirements originating from the Union legislation (95.8%). In terms of severity level resulting from uses to satisfy regulatory requirements, 12.9% of these uses were reported as severe, 21.1% as moderate, 65.3% mild (and up to mild) and 0.7% as non-recovery (Section IV.2.2.2.1) which improved compared to previous years.

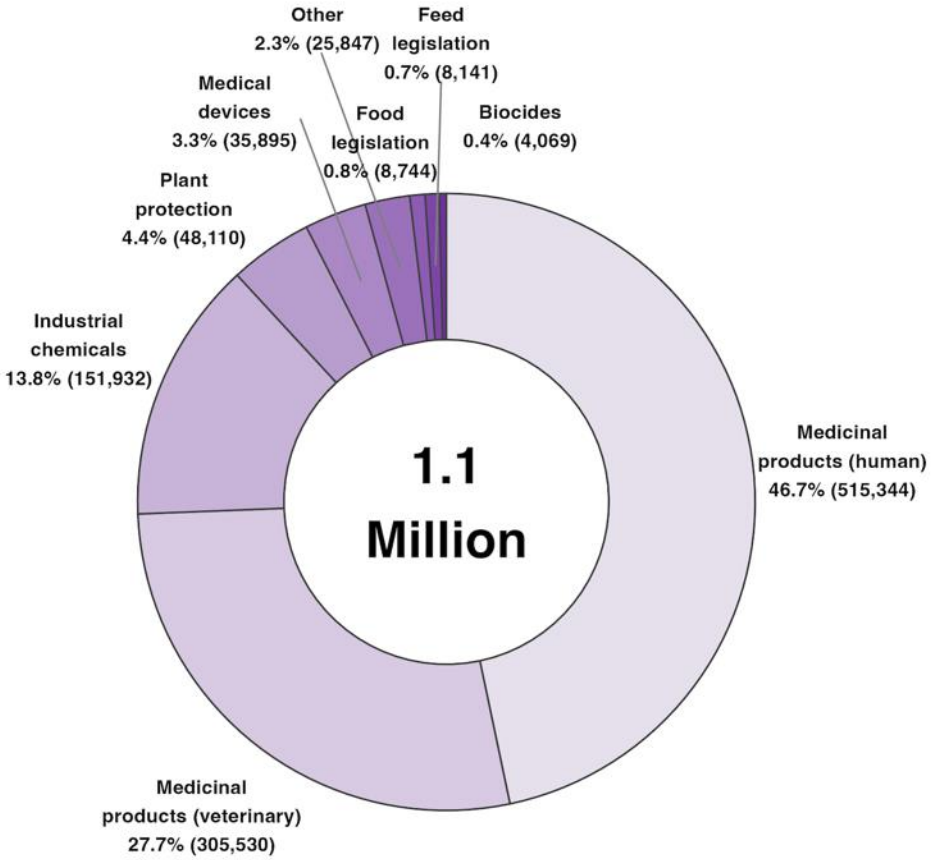


Figure 4: Regulatory uses by type of legislation in 2022

In line with the principle of the Three Rs[5], the total number of animals used in procedures can be reduced by performing more than one procedure on an animal, however, under strict conditions, taking into account the lifetime experience of the individual animal. The proportion of reuses decreases continuously since 2018 and is now around 1.1% of all uses (compared to 1.7% in 2018), for the first time below 100,000. Proportionally, large mammals are reused more often, such as farm animals, cats, dogs, and some species of non-human primates.

The proportion of genetically altered animals used in research and testing saw a recent increase compared to 2018 raising from 20% to 24.8%. In 2022, 2.1 million uses were carried out on animals that were genetically altered. 4.3% had a harmful phenotypic alteration and 20.5% a non-harmful phenotype (Figure 5). Zebra fish and mice continue to be the most genetically altered species with 51.3% and 45.9% of all uses respectively.

[5] Replacement, reduction and refinement as the guiding principle for more ethical use of animals in testing and scientific research.

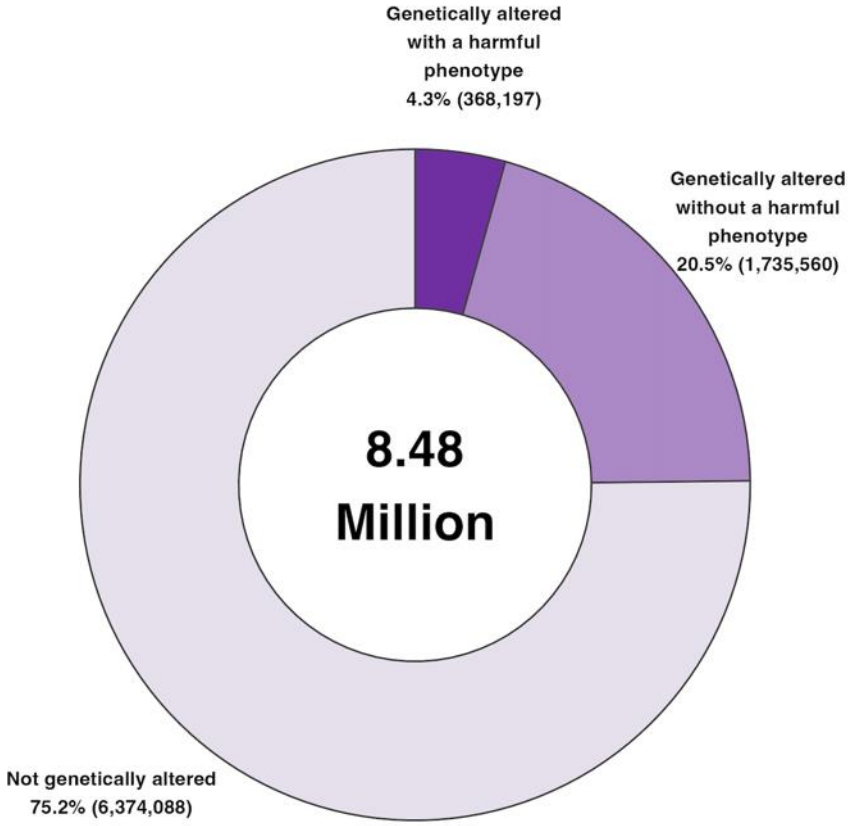


Figure 5: Genetic status of animals used in 2022

Genetically altered animals are used almost exclusively for research purposes with basic research accounting for 74.5% of uses of genetically altered animals (Section IV.2.3.2).

II.3. Creation and maintenance of genetically altered animal lines for research purposes

The number of animals used for the first time for the creation and maintenance of genetically altered (GA) animal lines to meet the research needs in the Union is 852,145. There was an overall increase of +28.1% compared to 2021, driven mainly by the maintenance of existing GA lines showing an increase of +65.7% while creation of new genetically altered animal lines decreased (-4.3%) (Table 3).

	2018	2019	2020	2021	2022	Change 2022 vs 2021	Change 2022 vs 2018
GA Creation	378,876	311,714	388,729	356,706	**341,233**	-4.3%	-9.9%
GA Maintenance	531,068	347,055	297,899	308,259	**510,912**	+65.7%	-3.8%
Total	**909,944**	**658,769**	**686,628**	**664,965**	**852,145**	**+28.1%**	**-6.4%**

Table 3: Total numbers of animals used for the creation and maintenance of genetically altered animal lines in 2022

In 2022, the number of animal uses for the creation of new genetically altered animal lines remained stable at 341,233 uses compared to previous years. The main species used for this purpose were, as before, mice and zebra fish, 73.4% and 24.2% respectively (Section IV.3).

In 2022, the category basic research covered 92.5% of all uses for the creation of new genetically altered animal lines. The purposes for which these were created are: nervous system (19.5%), multisystemic research (18.7%) and cardiovascular, blood and lymphatic system (11.1%).

Animals used for the first time for the 'maintenance of colonies of genetically altered animals of established lines' increased importantly compared to 2021 (+65.7%). This can be partly explained by the use of a recently disseminated guidance document on genetically altered animals under the Directive[6] that was endorsed by Member States National Contact Points responsible for the implementation of the Directive at their meeting in November 2021. The guidance document addresses *inter alia* previously noted incoherences in the reporting of the maintenance of genetically altered animals. It is hoped that the use of the guidance document will continue to improve harmonisation and accuracy of the reporting of genetically altered animals.

6 https://op.europa.eu/en/publication-detail/-/publication/7ff424e1-eb8f-11ec-a534-01aa75ed71a1/language-en/format-PDF/source-282386407

BACKGROUND

The objective of the Commission Staff Working Document is to present statistical information on the use of animals in procedures in the European Union and Norway under Directive 2010/63/EU[7] of 22 September 2010 on the protection of animals used for scientific purposes. The obligation to collect statistical data is covered by Article 54(2) of the Directive. The data format for the 2022 data was laid out in Commission Implementing Decision 2020/5697/EU[8].

This statistical report contains the results of the data collected by all 27 Member States and Norway in 2022. References to "EU" and "Union" data from here on in this report, are therefore to be understood to cover 27 EU Member States and Norway, unless otherwise specified.

III. DATA SUBMITTED AND GENERAL ASSESSMENT

III.1. Data Submitted

The data were collected according to the Commission Implementing Decision 2020/569/EU establishing a format for the submission of the information pursuant to Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes.

All submitted data is available at open access ALURES Statistical EU Database[9] and can be analysed at both Union and Member State level from 2021 data onwards.

III.2. General Considerations

This report aims at providing a comprehensive overview on the use of animals in procedures in the European Union and Norway in 2022.

In this report, data are presented either in the form of figures or summary tables providing information on a specific aspect of the Directive. Overall numbers are given for the year 2022. Numbers from previous years (2018-2020) have been recalculated by excluding data from the United Kingdom. A systematic trend analysis provides information on the evolution of the Directive's objectives, this is done by comparing 2022 data with the previous year to account for short term changes and 2018 for longer term changes. The year 2018 was chosen a reference as it was the first year including data from Norway and already the fourth year for other Member State under Directive 2010/63/EU, making a comparison possible on more robust data.

Key findings are presented in the form of tables and graphics. However, in some cases, further information in the text may have been drawn from Member State narratives (see Part C of this Staff Working Document). Member State narratives have been helpful in providing information such as for the content of 'other' categories (for example, "Other rodents", "Other basic research") or for understanding the origin of projects using particularly high numbers of animals.

[7] Directive 2010/63/EU OJ L276, 20.10.2010, p.33-79

[8] OJ L 129, 24.4.2020, p. 16–50

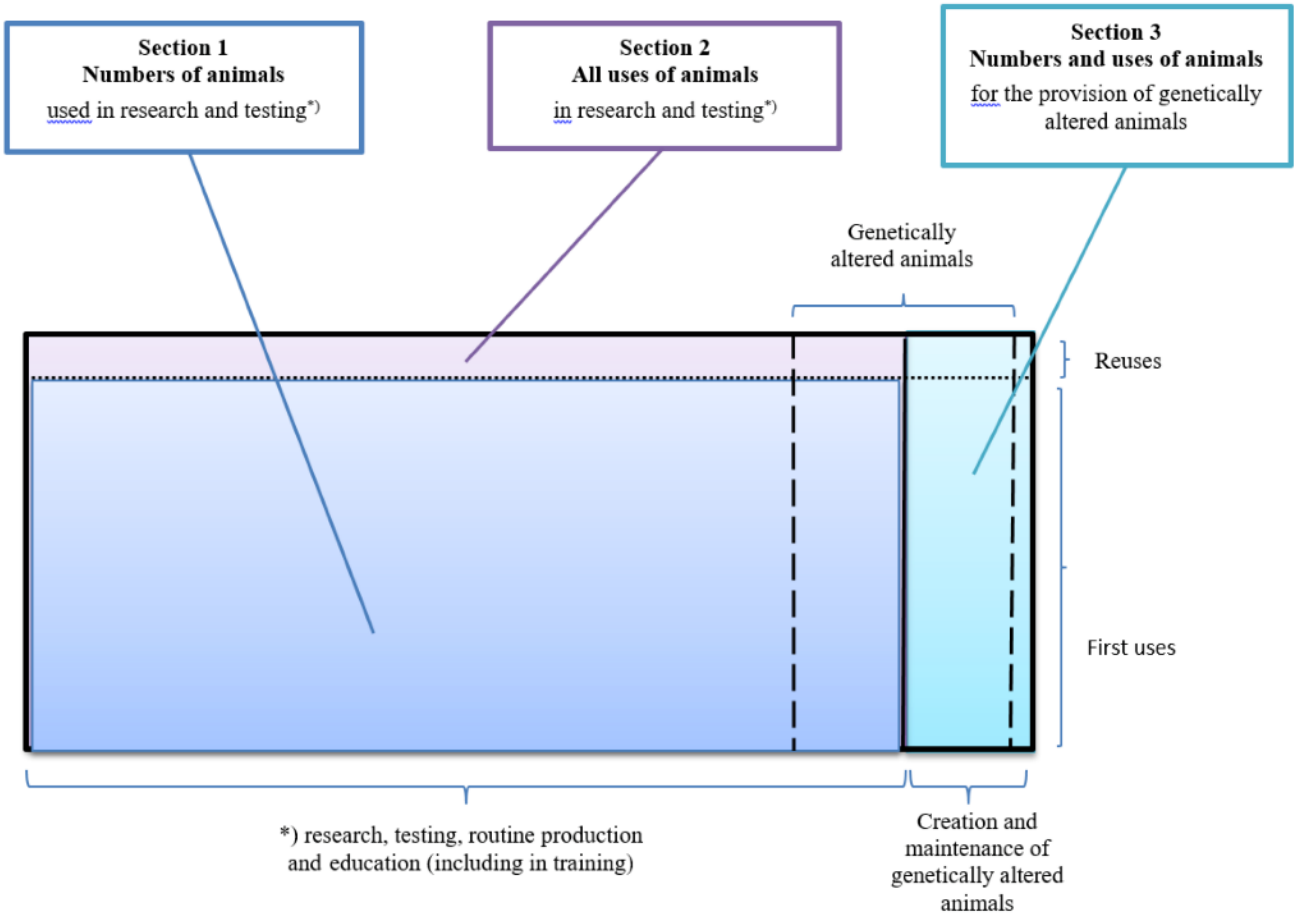
[9] https://environment.ec.europa.eu/topics/chemicals/animals-science/statistics-and-non-technical-project-summaries_en

The Commission and Member States continue to work together to address issues and questions arising from reporting obligations to ensure uniform understanding of the reporting requirements, such as the reporting of actual severity, animals used for the maintenance of genetically altered animal lines and accurate reporting under different purpose categories.

III.3. Report structure

The objective of this report is to present all these data structured in a manner that allows for an improved understanding of when and how animals are still used in science today. It is hoped that, in line with the Directive aims, this way of reporting will better facilitate the identification of animal use areas on which efforts for the development and validation of alternative approaches can be focused.

Therefore, Part A of the report is composed of three sections as illustrated in the picture below:



Numbers of animals used for research, testing, routine production and educational purposes[10] in the EU – Section 1 (IV.1)

The first section focuses on the *numbers of animals* used, for the first time, for the purposes of research, testing, routine production and education (the term 'education' in the context of this report also includes animals used for the purposes of training). These animals can be either conventional animals or those that have been genetically altered. This part reports on their numbers and origins. It excludes animals that have been used for the creation of a new genetically altered animal line, or maintenance of an existing genetically altered animal line. These are covered in part three below.

Details of all uses of animals for research, testing, routine production and educational purposes in the EU – Section 2 (IV.2)

The second section focuses on the way in which animals are used in these scientific procedures, *covering all uses, both the first and any subsequent reuse.* This serves to draw an overall picture of all uses of animals for the purposes of research, testing, routine production and education in the Union. This part takes into account the nature of the procedures, their legislative context, reuse of animals, the genetic status of the animals, and the severities experienced by the animals.

Numbers and uses of animals for the creation and maintenance of genetically altered animals in the EU – Section 3 (IV.3)

The third section focuses on the provision of *genetically altered animals* needed to support scientific research in the Union. It reports, on one hand, animals used in procedures for *the creation* of new genetically altered animal lines and, on the other, animals used for *the maintenance* of colonies of existing genetically altered animals. As in part one of this report, it provides the actual numbers of animals, used for the first time, as well as more detailed information taking into account all uses (first, and any subsequent reuse) for the purposes of creation and maintenance of genetically altered animal lines. It also provides further information on the type of research for which new genetically altered animal lines are being created. These animals have not been used in other scientific procedures, in other words the data are separate from those covered in parts one and two of this report.

Part B of this report contains Union-level data that have been used as the basis for conclusions in Part A of the report. Part C of this report provides data from the Member States together with their respective narratives.

Information outside of the scope of the statistical report

What remains outside of the scope of annual statistical reporting – even if covered by the scope and provisions of the Directive, are:

- Foetal forms of mammals;
- Animals killed solely for organs and tissues, and sentinels, unless the killing is performed under a project authorisation using a method not included in Annex IV of Directive 2010/63/EU;

[10] In this context 'Research' means basic, applied and translational research, animals used for the purposes of protection of the natural environment in the interests of the health or welfare of human beings or animals, preservation of the species and forensic enquiries; 'testing' refers to regulatory use of animals and 'education' includes animals used for training purposes. Glossary in IV.4. provides further information on some of the categories of scientific use purposes.

- Animals bred and killed without being used, apart from genetically altered animals with intended and exhibited harmful phenotypes, and those having been genotyped with an invasive method before being killed.

Additional information on animals bred and killed without being used will be reported in the five-year report on the implementation of the Directive in line with Article 54(1) of the Directive.

III.4. Glossary of terms

Generations and origins of non-human primates

- F0 - an animal born in the wild
- F1 - first generation captive bred animal
- F2 or greater - second or higher generation captive-bred animal
- SSC - animal sourced from a self-sustaining colony where no further animals are obtained from the wild

Genetically altered animals

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);
- animals with spontaneous deleterious mutations maintained for research for that specific genotype.

Genetically altered animals are reported either:

a) When used for the creation of a new animal line;
b) When used for the maintenance of an established line with an intended ***and*** exhibited harmful phenotype; 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 characterisation/tissue sampling);
c) When used in other (scientific) procedures (i.e. not for the creation or the maintenance of a line).

The reporting of genetically altered animals is summarised in the diagram below.

<u>Creation</u>

All animals *carrying a genetic alteration* are reported during the creation of a new line. Also, those used for superovulation, vasectomy and embryo implantation are reported (these may or may not be genetically altered).

Genetically normal animals (*wild-type offspring*) produced as a result of the creation of a new genetically altered line are not reported, unless these have been subjected to a procedure, for example an invasive method for the sole purposes of genotyping.

Establishment and maintenance of breeding colonies

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. This marks the transition from "creation" to "breeding".

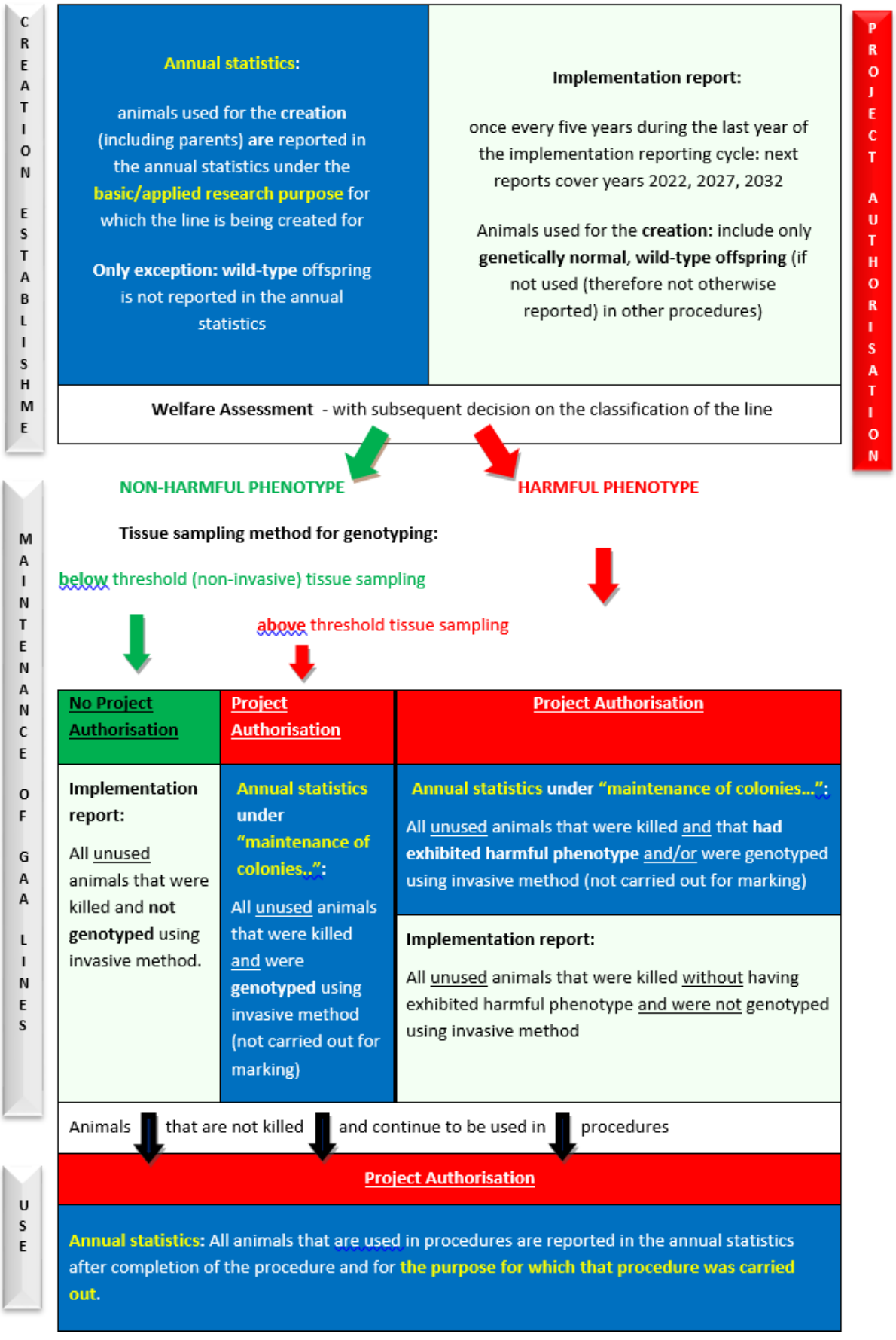
The welfare assessment determines if the newly established line is expected to have an *intended harmful phenotype (characteristic/trait)* i.e., an effect of genetic alteration that impacts negatively on an animal's health or welfare, such as muscle weakness, diabetes, or tumor development.

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 is not reported in the annual statistics.

If the welfare assessment concludes that the line *is* expected to have a harmful phenotype, its breeding falls within the scope of a procedure. If this is the case, and if the animal is not used in other procedures and it has exhibited, before being killed, pain, suffering, distress of lasting harm as a result of the harmful phenotype, it is reported under the category *Maintenance of colonies of established genetically altered animals, not used in other procedures.*

Use in procedures (other than creation or maintenance of a genetically altered line)
All genetically altered animals which are used in procedures (not for the creation or maintenance of a genetically altered line) are reported under their respective purposes they were used for. These animals may or may not exhibit a harmful phenotype.

Diagram for the reporting of the creation, maintenance and use of genetically altered animals


CREATION ESTABLISHME
Annual statistics:
animals used for the creation (including parents) are reported in the annual statistics under the basic/applied research purpose for which the line is being created for
Only exception: wild-type offspring is not reported in the annual statistics
Implementation report:
once every five years during the last year of the implementation reporting cycle: next reports cover years 2022, 2027, 2032
Animals used for the creation: include only genetically normal, wild-type offspring (if not used (therefore not otherwise reported) in other procedures)
PROJECT AUTHORISATION
Welfare Assessment - with subsequent decision on the classification of the line
NON-HARMFUL PHENOTYPE
HARMFUL PHENOTYPE
Tissue sampling method for genotyping:
below threshold (non-invasive) tissue sampling
above threshold tissue sampling
MAINTENANCE OF GAA LINES
No Project Authorisation
Project Authorisation
Project Authorisation
Implementation 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)
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)
Implementation report:
All unused animals that were killed without having exhibited harmful phenotype and were not genotyped using invasive method
Animals that are not killed and continue to be used in procedures
USE
Project Authorisation
Annual statistics: 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.

Procedure

"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.
This includes any course of action intended, or liable, to result in the birth or hatching of an animal 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.

Purposes - Main categories of purposes of uses for research, testing, routine production and education (including training)

Basic research

Basic research includes studies of a fundamental nature including physiology. Studies that are designed to add knowledge about normal and abnormal structure, functioning and behaviour of living organisms and environment, this includes 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 a specific practical application of the results.

Translational and applied research

Translational and applied research includes animals used for purposes as described in Article 5(b) and (c) of the Directive, that is to say,

"(b) translational or applied research with any of the following aims:

(i) the avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality or their effects in human beings, animals or plants;

(ii) the assessment, detection, regulation or modification of physiological conditions in human beings, animals or plants; or

(iii) the welfare of animals and the improvement of the production conditions for animals reared for agricultural purposes;

(c) for any of the aims in point (b) in the development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feed-stuffs and other substances or products;"
This category also includes discovery toxicology and investigations to *prepare* for the regulatory submission and method development. This does not include studies *required* for regulatory submissions.

Regulatory use

Regulatory uses cover 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. It also includes tests carried out in respect of products/substances for which a regulatory submission was foreseen but ultimately not made, for instance because these were deemed unsuitable for the market by the developer and thus fail to reach the end of the development process.

Routine production

Routine production includes animals used in the manufacturing process of products such as antibodies and blood products including polyclonal antisera by established methods.

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

This category includes 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.

Preservation of species

Studies aimed at conserving species, often those at risk of extinction, for example to investigate improved breeding strategies or preservation of habitats.

Higher education

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

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 the training of medical doctors or veterinarians.

Forensic enquiries

Studies to assist the investigation of forensic enquiries.

Severities experienced by the animals

The impact on animal welfare is reported by assigning an animal's experience to a 'severity' category – "mild", "moderate" or "severe". There is a further category termed "non-recovery" which relates to where animals are placed under general anesthesia before they are used and are killed afterwards before regaining consciousness.

The reported severity reflects the highest degree of pain, suffering, distress or lasting harm observed to be actually experienced by the animal during the course of its use. Further guidance on severity assessment can be found at http://ec.europa.eu/environment/chemicals/lab_animals/pdf/Endorsed_Severity_Assessment.pdf.

i. **Non-recovery -** Animals which have undergone a procedure that has been performed entirely under general anaesthesia from which the animal has not recovered consciousness shall be reported as Non-recovery.

ii. **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, as well as when there has been no significant impairment of the well-being or general condition of the animals shall be reported as Mild.

This category also includes any animals used in an authorised project, but which have ultimately *not* been observed to have experienced a level of pain, suffering, distress or lasting harm above the minimum threshold (equivalent to that caused by the introduction of a needle in accordance with good veterinary practice) for example untreated control animals ("up to mild"). However, 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 are not reported in annual statistics.

iii. **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 have caused moderate impairment of the well-being or general condition of the animals shall be reported as Moderate.

iv. **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.

In the exceptional circumstances where, under the safeguard clause, the Severe classification is exceeded these animals and their use will be reported under Severe. Should this occur, further explanation on the circumstances of this use is provided in the respective Member State narrative.

Species of animals

The Directive applies to live non-human vertebrate animals, including independently feeding larval forms and fetal forms of mammals as from the last third of their normal development, and live cephalopods.
Larval forms and cephalopods are reported in the statistics when they become capable of independent feeding. Due to the small size of many larval forms of fish and cephalopod species, the count for these animals may be done on the basis of estimation.

The Three Rs

Replacement, reduction and refinement of the use of animals for scientific purposes.

Use and reuse

The "use" of an animal within a project extends from the time the procedure (or first procedure/technique in a series) is applied to it, to the time when the observations, or the collection of data (or other products) for a particular scientific purpose (usually a single experiment or test), are completed.
"Reuse" is a term to indicate any subsequent use of an animal, which has already completed a procedure (or series of procedures/techniques) for a particular scientific purpose. Article 16 of the Directive on reuse defines it as a use when a different animal on which no procedure has previously been carried out could also be used. Article 16 also defines the conditions under which an animal may be reused.

IV. COMPILATION AND OVERVIEW OF THE EU DATA BETWEEN 2018 AND 2022

IV.1 Numbers of animals used for research, testing, routine production and educational purposes in the EU

This part focuses on the numbers of animals used *for the first time* in procedures for the purposes of research, testing, routine production and education. Therefore, it excludes all reuses of animals that are considered in the second part which reports on all uses of animals. It also excludes animals that are used either for the creation of new genetic altered lines or the maintenance of colonies of established genetically altered animal lines. However, animals used for research, testing, routine production and educational purposes can be conventional or genetically altered.

In addition to the numbers of animals, this part also provides information on the species in relation to their origin, and for non-human primates, information on progress to purpose-bred animals, by recording generation and the type of colony non-human primate is sourced from.

IV.1.1. Numbers of animals used for the first time

In 2022, the number of animals used for the first time in the Union was 8.39 million.

	2018	2019	2020	2021	2022	Change 2022 vs 2021	Change 2022 vs 2018
Mammals	5,885,013 (66.7%)	5,751,005 (67%)	5,195,063 (65.4%)	5,430,721 (57.7%)	**5,289,886 (63.1%)**	-2.6%	-10.1%
Birds	436,316 (4.9%)	498,218 (5.8%)	510,108 (6.4%)	500,478 (5.3%)	**517,011 (6.2%)**	+3.3%	+18.5%
Reptiles	1,544 (0%)	1,972 (0%)	2,072 (0%)	2,433 (0%)	**4,631 (0.1%)**	+90.3%	+199.9%
Amphibians	24,412 (0.3%)	43,499 (0.5%)	37,821 (0.5%)	39,887 (0.4%)	**32,924 (0.4%)**	-17.5%	+34.9%
Fish	2,470,851 (28%)	2,267,777 (26.4%)	2,191,367 (27.6%)	3,429,630 (36.5%)	**2,538,269 (30.3%)**	-26%	+2.7%
Cephalopods	4,268 (0%)	16,968 (0.2%)	1,633 (0%)	3,084 (0%)	**2,676 (0%)**	-13.2%	-37.3%
Total	**8,822,404 (100%)**	**8,579,439 (100%)**	**7,938,064 (100%)**	**9,406,233 (100%)**	**8,385,397 (100%)**	**-10.9%**	**-5%**

Table 4: Numbers of animals used for the first time by main types of species between 2018 and 2022

Table 4 above shows a decrease of -10.9% compared to 2021, and an overall decrease of -5% compared to 2018 data. This seems to confirm the temporary increase in 2021 as a result of the three large fish studies. Looking at the main categories of species, the general decreasing tendency of mammals used for the first time is confirmed (-10.1% compared to 2018), while birds (+18.5% compared to 2018) and reptiles (+200%) first uses see an increase.

The proportional distribution of species came back to a situation closer to the 2020 numbers, confirming here as well the exceptional temporary increase of fish used for the first time in 2021. In proportion, mammals represent 63.1% of all animals used for the first time (in decrease, -3.6 points compared to 2018), fish represent 30.2% (in increase, +2.3 points compared to 2018) and birds 6.2% (increase of 13 points).

Table 5 shows the breakdown of species used for the first time in 2022 by types of species. Mice are the most used species (47.8%), followed by salmon, trout, char and graylings (15.4%), rats (7.5%), domestic fowl (5%), rabbits (4.4%) and zebra fish (4.3%) (Figure 1).

	2018	2019	2020	2021	2022	Change 2021 vs 2020	Change 2021 vs 2018
Mammals							
Rodents							
Mice	4,410,737	4,318,913	3,879,691	4,087,398	**4,010,766**	-1.9%	-9.1%
Rats	829,906	792,744	665,155	682,758	**625,777**	-8.3%	-24.6%
Guinea-Pigs	123,486	111,652	111,172	108,827	**85,167**	-21.7%	-31%
Hamsters (Syrian)	9,397	10,427	17,355	27,666	**21,613**	-21.9%	+130%
Hamsters (Chinese)	20	17	149	96	**31**	-67.7%	+55%
Mongolian gerbil	4,269	3,672	2,978	3,703	**3,440**	-7.1%	-19.4%
Other rodents	19,534	28,273	28,186	17,802	**15,260**	-14.3%	-21.9%
Rabbits							
Rabbits	332,097	342,644	343,521	356,388	**372,239**	+4.4%	+12.1%
Carnivores							
Cats	1,517	2,140	2,464	2,021	**1,409**	-30.3%	-7.1%
Dogs	14,802	10,388	8,716	10,133	**8,709**	-14.1%	-41.2%
Ferrets	1,041	1,455	1,250	1,495	**899**	-39.9%	-13.6%
Other carnivores	4,267	4,325	6,867	2,186	**840**	-61.6%	-80.3%
Farm animals							
Horses, donkeys and cross-breeds	1,626	1,388	3,831	3,113	**4,110**	+32%	+152.8%
Pigs	79,699	77,424	73,509	78,192	**86,953**	+11.2%	+9.1%
Goats	1,443	1,124	998	1,086	**1,268**	+16.8%	-12.1%
Sheep	17,398	15,782	17,489	15,650	**15,909**	+1.7%	-8.6%
Cattle	22,580	19,059	22,175	22,068	**21,434**	-2.9%	-5.1%
Non-human primates							
Prosimians	170	194	54	59	**74**	+25.4%	-56.5%
Marmoset and tamarins	289	142	196	170	**495**	+191.2%	+71.3%
Cynomolgus monkey	5,349	4,741	4,220	4,747	**4,950**	+4.3%	-7.5%
Rhesus monkey	210	182	227	278	**140**	-49.6%	-33.3%
Vervets (Chlorocebus spp.)	16	25	34	3	**48**	+1500%	+200%
Baboons	30	33	53	44	**64**	+45.5%	+113.3%
Squirrel monkey	25	0	0	13	**13**	0%	-48%
Other species of Old World Monkeys (Cercopithecoidea)	22	2	0	0	**0**	NA	-100%
Other mammals							
Other mammals	5,083	4,259	4,773	4,825	**8,278**	+71.6%	+62.9%
Birds							
Domestic fowl	341,763	390,340	423,737	413,885	**417,903**	+1%	+22.3%
Turkey	0	0	0	17,234	**18,577**	+7.8%	NA
Other birds	94,553	107,878	86,371	69,359	**80,531**	+16.1%	-14.8%
Reptiles							
Reptiles	1,544	1,972	2,072	2,433	**4,631**	+90.3%	+199.9%
Amphibians							
Rana	3,563	6,169	1,722	2,792	**2,638**	-5.5%	-26%
Xenopus	14,074	17,386	17,806	20,523	**15,720**	-23.4%	+11.7%
Other amphibians	6,775	19,944	18,293	16,572	**14,566**	-12.1%	+115%
Fish							
Zebra fish	259,468	318,426	277,328	340,316	**362,449**	+6.5%	+39.7%
Sea bass	0	0	0	646,076	**116,706**	-81.9%	NA
Salmon, trout, chars and graylings	0	0	0	1,914,360	**1,289,139**	-32.7%	NA
Guppy, swordtail, molly, platy	0	0	0	88,787	**78,388**	-11.7%	NA
Other fish	2,211,383	1,949,351	1,914,039	440,091	**691,587**	+57.1%	-68.7%
Cephalopods							
Cephalopods	4,268	16,968	1,633	3,084	**2,676**	-13.2%	-37.3%
Totals							
Total	**8,822,404**	**8,579,439**	**7,938,064**	**9,406,233**	**8,385,397**	**-10.9%**	**-5%**

Table 5: Numbers of animals used for the first time by types of species between 2018 and 2022

The number of mammals used for the first time in 2022 decreased slightly (-2.6%) compared to 2021 which confirms the long-term decrease (-10.1% compared to 2018). This is particularly true for rats (-24.6%) and guineas-pigs (-31%). On the contrary, the first uses of rabbits tended to increase (+12.1%) and well as horses, donkeys and cross-breeds (+152.8%).

In 2022, the first uses of non-human primates increased for the second consecutive year (+8.8% compared to 2021) but still decreased slightly compared to 2018 (-5.4%). The most important increases amongst species with more than 50 animals used for the first time are marmosets and tamarins (+191.2% compared to 2021) followed by baboons (+45.5%) and prosimians (+25.4%). During the same period, the first uses of rhesus monkey decreased by -49.6%.

Fish used for the first time decreased by -26% compared to 2021, coming back to the levels reported in 2018 (+2.4%). Sea bass showed the most important decrease (-81.9% compared to 2021) which is explained by a study using 500,000 sea bass in 2021 (see previous report). Salmon, trout chars and graylings decreased by -32.7% for the same reason (two studies reporting 800,000 first uses in 2021) and guppy, swordtail, molly, platy by -11.7%. Only the first uses of zebra fish increased (+6.5%) confirming the long-term tendency (+39.7% compared to 2018).

Figure 6: Examples of "Other species" used for the first time in 2022

Three out of six “other” species categories decreased compared to 2021: “Other carnivores” (-61.6%), “Other rodents” (-14.3%) and “Other amphibians” (-12.1%) while the three others increased: “Other mammals” (+71.6%), “Other fish” (+57.1%) and “Other birds” (+16.1%).

Looking at the Member State narratives, figure 6 shows some examples of the main species reported under “Other” species categories.

IV.1.2. Origin of animals used for the first time

The origin (place of birth) of animals is divided into two categories depending on whether the species belongs to the category of non-human primates or not. For non-human primates, more detailed information is collected on their origin (continent of origin), their generation and the type of colony the animal is sourced from (see Part IV.1.2.2.2).

IV.1.2.1. Place of birth of animals (other than non-human primates)

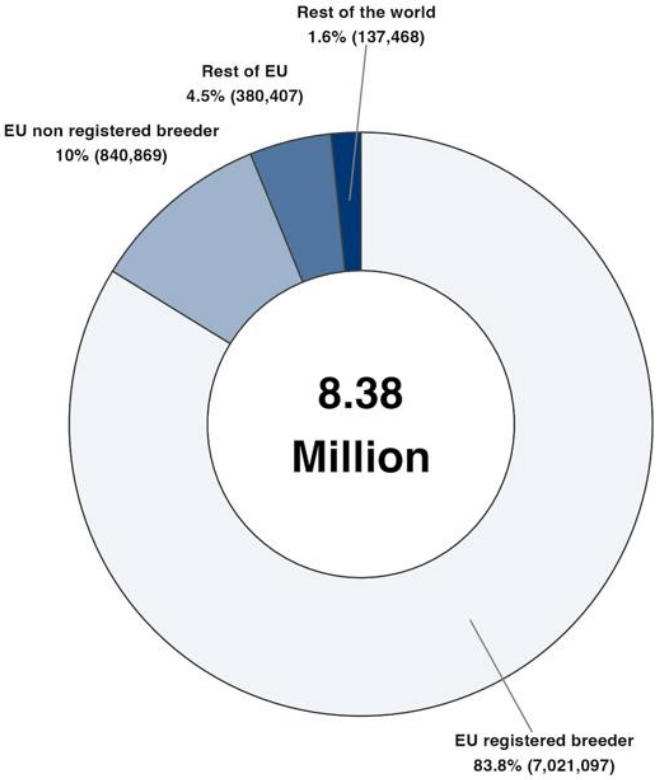


Figure 7: Place of birth of animals other than non-human primates in 2022

In 2022, 83.8% of the animals used for scientific purposes for the first time were born in the Union at registered breeders[11], 10% in the Union but not at registered breeders and 6.1% were born outside of the Union (either in the rest of Europe or outside of Europe). This represents a change compared to previous years as animals born outside of the Union were always below 5% (Table 6).

[11] This includes animals born at registered breeders in Norway authorised under the conditions of Directive 2010/63/EU.

	2018	2019	2020	2021	2022
Animals born in the EU at a registered breeder	87.8% (7,742,669)	84.9% (7,283,376)	84.1% (6,668,758)	80.8% (7,593,584)	**83.8% (7,020,925)**
Animals born in the EU but not at a registered breeder	8.9% (785,668)	13.5% (1,161,131)	14.3% (1,137,778)	17% (1,600,700)	**10% (840,813)**
Animals born in rest of Europe	2.4% (208,244)	0.8% (67,351)	0.6% (50,783)	1.2% (114,388)	**1.6% (137,468)**
Animals born in rest of world	0.9% (79,712)	0.7% (62,262)	1% (75,961)	1% (92,247)	**4.5% (380,407)**
Total	**100% (8,816,293)**	**100% (8,574,120)**	**100% (7,933,280)**	**100% (9,400,919)**	**100% (8,379,613)**

Table 6: Place of birth of animals other than non-human primates between 2018 and 2022

Looking at the species most bred in the EU but not at a registered breeder (table 7), they are mainly farm animals or "other species". Farm animals are horses, donkeys and cross-breeds (89.1% of horses, donkeys and cross-breeds used for the first time), sheep (71.4%), goats (69.2%), cattle (68.6%) and pigs (65.3%). Other species commonly coming from non-registered breeders in the EU are rana (63.3%) and reptiles (55.2%%).

Dogs (39.2%) and cats (50.9%) are also often coming from the EU but not from a registered breeder. The most common reason for using dogs and cats that came from non-registered breeders in the Union were procedures in pet dogs and cats, which had blood samples taken for studies of genetic disorders, or pet animals, which were involved in patient studies for better treatment methods.

	Percent	Number
Horses, donkeys and cross-breeds	**89.1%**	3,661
Other carnivores	**85.4%**	717
Sheep	**71.4%**	11,359
Goats	**69.2%**	878
Other rodents	**69.2%**	10,563
Cattle	**68.6%**	14,713
Pigs	**65.3%**	56,771
Rana	**63.6%**	1,678
Other birds	**57%**	45,921
Other amphibians	**55.5%**	8,090
Reptiles	**55.2%**	2,558
Cats	**50.9%**	717
Other mammals	**41.4%**	3,430
Dogs	**39.2%**	3,416
Domestic fowl	**38.1%**	159,402
Sea bass	**34%**	39,676
Other fish	**31.5%**	217,650
Xenopus	**15.7%**	2,472
Salmon, trout, chars and graylings	**15.1%**	194,347
Ferrets	**9.8%**	88

Table 7: Main species bred in in EU but not at a registered breeder 2022

Species coming from the rest of the Europe (table 8) are "other birds" (17.8%), "Other fish" (9.8%), Xenopus (8%) or "Other carnivores" (6.2%). These include animals coming from the wild or animals used for preservation of species or protection of the natural environment.

	Percent	Number
Other birds	**17.8%**	14,351
Other fish	**9.8%**	67,997

	Percent	Number
Xenopus	**8%**	1,256
Other carnivores	**6.2%**	52
Cats	**5.2%**	73
Ferrets	**4.4%**	40
Other mammals	**3.2%**	261
Salmon, trout, chars and graylings	**2.9%**	36,843
Other amphibians	**0.7%**	107
Cephalopods	**0.6%**	16

Table 8: Main species bred in the rest of Europe in 2022

Last, the category “other mammals” (52.5%, probably bat species) has most of animals coming from the rest of the world (Table 9). 2022 saw also an important increase of salmon, trout, chars and graylings and “Other fish” coming from the rest of the world that explains the increase of the proportion of animals used for the first time in this category.

	Percent	Number
Other mammals	**52.5%**	4,348
Dogs	**20.2%**	1,763
Ferrets	**18.9%**	170
Salmon, trout, chars and graylings	**18.2%**	233,996
Other birds	**16.5%**	13,291
Other fish	**12.4%**	86,071
Xenopus	**12.3%**	1,931
Reptiles	**11.4%**	526
Cats	**5.2%**	73
Hamsters (Syrian)	**4%**	860

Table 9: Main species bred in the rest of the world in 2022

IV.1.2.2. Origin of non-human primates

The Directive provides additional protection for non-human primates due to their genetic proximity to human beings, their highly developed social skills and capacity to experience pain, suffering and distress. Furthermore, the Directive recognises that the capture of non-human primates from the wild is highly stressful for the animals concerned and carries an elevated risk of injury and suffering during capture and transport. In order to end the capture of animals from the wild including for the purposes of breeding, the Directive introduced provisions with the objective of moving towards using non-human primates that have been bred, ultimately, in self-sustaining colonies, from parents who themselves have been bred in captivity (see Article 10 of the Directive).

In order to monitor progress, more detailed information is collected on both the origin and generation of non-human primates used in scientific procedures in the Union.

IV.1.2.2.1. Non-human primates - Source

In 2022, the three main sources of non-human primates were registered breeders in Africa, Asia and the EU. These sources represented 97.9% of non-human primates used for scientific purposes (Figure 8).

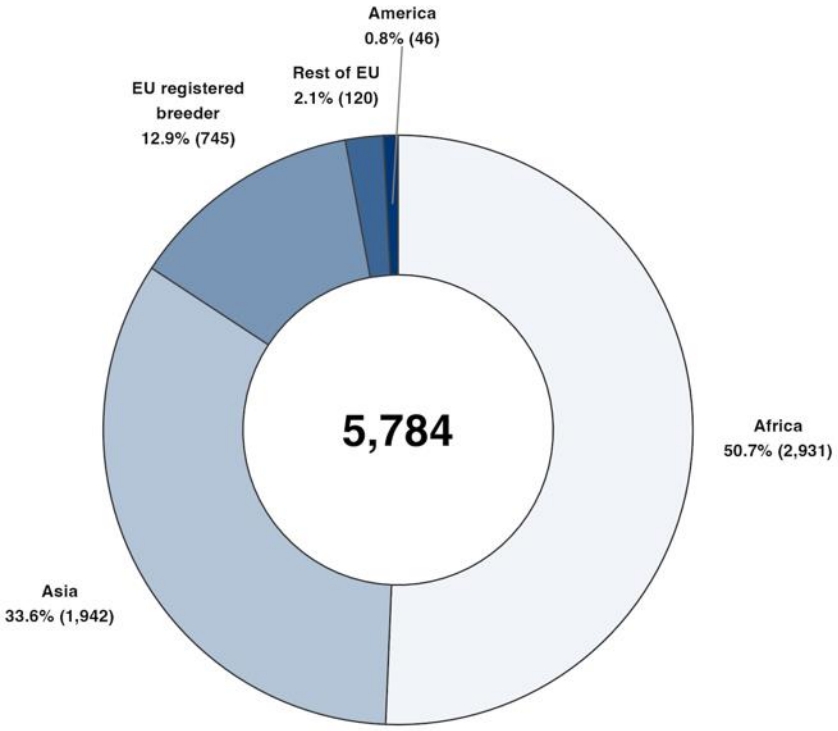


Figure 8: Source of non-human primates in 2022

Since 2018, the proportion of non-human primates born at an EU-registered breeder remain stable being set at 12.9% in 2022. The number of animals born in Asia continued to decrease slightly (33.6%), while the number of animals born in Africa increased to 50.7%. This can be explained by a decrease of the non-animal primates exported from Asian countries since 2019 and the Chinese export ban that was introduced in 2020. 120 non-human primates were born in the rest of Europe and none in the rest of the world in 2022. No clear trend emerges since 2018 with regard to the origin of non-human primates (table 10).

	2018	2019	2020	2021	2022	Change 2021 vs 2020	Change 2021 vs 2018
Animals born at a registered breeder within EU	842 (13.8%)	684 (12.9%)	643 (13.4%)	669 (12.6%)	**745 (12.9%)**	+11.4%	-11.5%
Animals born in rest of Europe	0 (0%)	0 (0%)	1 (0%)	0 (0%)	**120 (2.1%)**	NA	NA
Animals born in Asia	2,737 (44.8%)	2,418 (45.5%)	1,584 (33.1%)	2,034 (38.3%)	**1,942 (33.6%)**	-4.5%	-29%
Animals born in America	12 (0.2%)	18 (0.3%)	36 (0.8%)	24 (0.5%)	**46 (0.8%)**	+91.7%	+283.3%
Animals born in Africa	2,466 (40.4%)	1,917 (36%)	2,291 (47.9%)	2,587 (48.7%)	**2,931 (50.7%)**	+13.3%	+18.9%
Animals born elsewhere	54 (0.9%)	282 (5.3%)	229 (4.8%)	0 (0%)	**0 (0%)**	NA	-100%
Total	**6,111 (100%)**	**5,319 (100%)**	**4,784 (100%)**	**5,314 (100%)**	**5,784 (100%)**	**+8.8%**	**-5.4%**

Table 10: Evolution of non-human primates source between 2018 and 2022

In 2022, cynomolgus monkeys still represented the vast majority of non-human primates used for the first time (85.6%) and were sourced almost entirely from outside of the Union (Table 11). In contrast, other species of non-human primates were mainly sourced from EU-registered breeders except for Vervet (*Chlorocebus spp*).

	Animals born at a registered breeder within EU	Animals born in rest of Europe	Animals born in Asia	Animals born in America	Animals born in Africa
Prosimians	48 (64.9%)	26 (35.1%)	0 (0%)	0 (0%)	0 (0%)
Marmoset and tamarins	406 (82%)	89 (18%)	0 (0%)	0 (0%)	0 (0%)
Cynomolgus monkey	84 (1.7%)	0 (0%)	1,935 (39.1%)	0 (0%)	2,931 (59.2%)
Rhesus monkey	133 (95%)	0 (0%)	7 (5%)	0 (0%)	0 (0%)
Vervets (Chlorocebus spp.)	2 (4.2%)	0 (0%)	0 (0%)	46 (95.8%)	0 (0%)
Baboons	59 (92.2%)	5 (7.8%)	0 (0%)	0 (0%)	0 (0%)
Squirrel monkey	13 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	**745 (12.9%)**	**120 (2.1%)**	**1,942 (33.6%)**	**46 (0.8%)**	**2,931 (50.7%)**

Table 11: Source of non-human primates by species in 2022

IV.1.2.2.2. Non-human primates – Colonies and generation

From 2021 onwards, the reporting of whether a non-human primate is sourced from a self-sustaining colony is separated from the reporting of generation. In 2022, of the non-human primates bred in captivity, the majority came from self-sustained colonies (74.7%), an important increase compared to 2021 (+18.9 points) (table 12).

	Self-sustaining colony	Non self-sustaining colony
Prosimians	54 (73%)	20 (27%)
Marmoset and tamarins	315 (63.6%)	180 (36.4%)
Cynomolgus monkey	3,723 (75.2%)	1,227 (24.8%)
Rhesus monkey	123 (87.9%)	17 (12.1%)
Vervets (Chlorocebus spp.)	32 (66.7%)	16 (33.3%)
Baboons	64 (100%)	0 (0%)
Squirrel monkey	10 (76.9%)	3 (23.1%)
Total	**4,321 (74.7%)**	**1,463 (25.3%)**

Table 12: Non-human primates colony type by species in 2022

With regard to the source of non-human primates, animals born in Africa now coming mostly from a self-sustained colony, which was not the case in the previous years (table 13).

	Self-sustaining colony	Non self-sustaining colony
Animals born at a registered breeder within EU	524 (70.3%)	221 (29.7%)
Animals born in rest of Europe	100 (83.3%)	20 (16.7%)
Animals born in Asia	1,664 (85.7%)	278 (14.3%)
Animals born in America	32 (69.6%)	14 (30.4%)
Animals born in Africa	2,001 (68.3%)	930 (31.7%)
Total	**4,321 (74.7%)**	**1,463 (25.3%)**

Table 13: Non-human primates colony type by source in 2022

Most non-human primates are from the second generation or higher (75%). For non-human primates born at a registered breeder in the Union, in America or in Asia, less than 7% of non-human primates used for the first time were from the first generation. In Africa, first generation animals represented

45.7% in 2022 (Table 14). 20 of them came from the wild in 2022 (F0), which did not happen in previous years except in 2015. This is explained in the French Member State narrative by the use of 20 prosimians for the preservation of species (animals not removed from natural habitat, subject to mild procedure for blood sampling).

	F2 or greater	F1	F0
Animals born at a registered breeder within EU	723 (97%)	22 (3%)	0 (0%)
Animals born in rest of Europe	100 (83.3%)	0 (0%)	20 (16.7%)
Animals born in Asia	1,883 (97%)	59 (3%)	0 (0%)
Animals born in America	43 (93.5%)	3 (6.5%)	0 (0%)
Animals born in Africa	1,591 (54.3%)	1,340 (45.7%)	0 (0%)
Total	**4,340 (75%)**	**1,424 (24.6%)**	**20 (0.3%)**

Table 14: Generation of non-human primates by source in 2022

IV.2. Details of all uses of animals for research, testing, routine production and educational purposes in the EU

This part focuses on all uses of animals for the purposes of research, testing, routine production and education, including the first and any subsequent reuse. It provides detailed information on the reason for use (for example the specific research area, or type of testing) as well as additional information related to the actual severity experienced by the animals, their genetic status and reuse. In addition, information on the use of animals to satisfy regulatory requirements is collected.

IV.2.1. Overview of the main scientific purposes and the related severities

In 2022, the total number of all uses (first use and any subsequent reuse) for the purposes of research, testing, routine production and education is 8.48 million. This is a decrease of -10.9% compared to 2021 and of -5.6% compared to 2018 (Table 15).

	2018	2019	2020	2021	2022	Change 2022 vs 2021	Change 2022 vs 2018
Basic research	3,982,963 (44.4%)	3,668,904 (42.1%)	3,293,349 (40.9%)	3,022,541 (31.8%)	**3,113,850 (36.7%)**	+3%	-21.8%
Translational and applied research	2,650,730 (29.5%)	2,535,951 (29.1%)	2,510,499 (31.2%)	3,862,731 (40.6%)	**2,993,795 (35.3%)**	-22.5%	+12.9%
Regulatory use	1,622,816 (18.1%)	1,482,372 (17%)	1,404,240 (17.4%)	1,317,252 (13.8%)	**1,103,612 (13%)**	-16.2%	-32%
Routine production	354,209 (3.9%)	438,236 (5%)	406,860 (5.1%)	412,889 (4.3%)	**477,632 (5.6%)**	+15.7%	+34.8%
Other	368,363 (4.1%)	589,761 (6.8%)	439,982 (5.5%)	897,801 (9.4%)	**788,956 (9.3%)**	-12.1%	+114.2%
Total	**8,979,081 (100%)**	**8,715,224 (100%)**	**8,054,930 (100%)**	**9,513,214 (100%)**	**8,477,845 (100%)**	**-10.9%**	**-5.6%**

Table 15: Total number of uses of animals by main purpose categories between 2018 and 2022

IV.2.1.1. Main categories of scientific purposes

Most uses were conducted for research purposes (72%) with 36.7% of the uses being carried out for basic research and 35.3% for translational and applied research purposes. A further 13% of animal uses in procedures were carried out for regulatory use to satisfy regulatory requirements, while routine production concerned 5.6% of uses.

Other categories (9.3%) include the protection of the natural environment in the interest of the health or welfare of human beings or animals, the preservation of species, the higher education, the training for the acquisition, maintenance or improvement or vocational skills and forensic enquiries (Figure 9). Preservation of species particularly remained high compared to years before 2021 but decreased slightly (-15.3% compared to 2021).

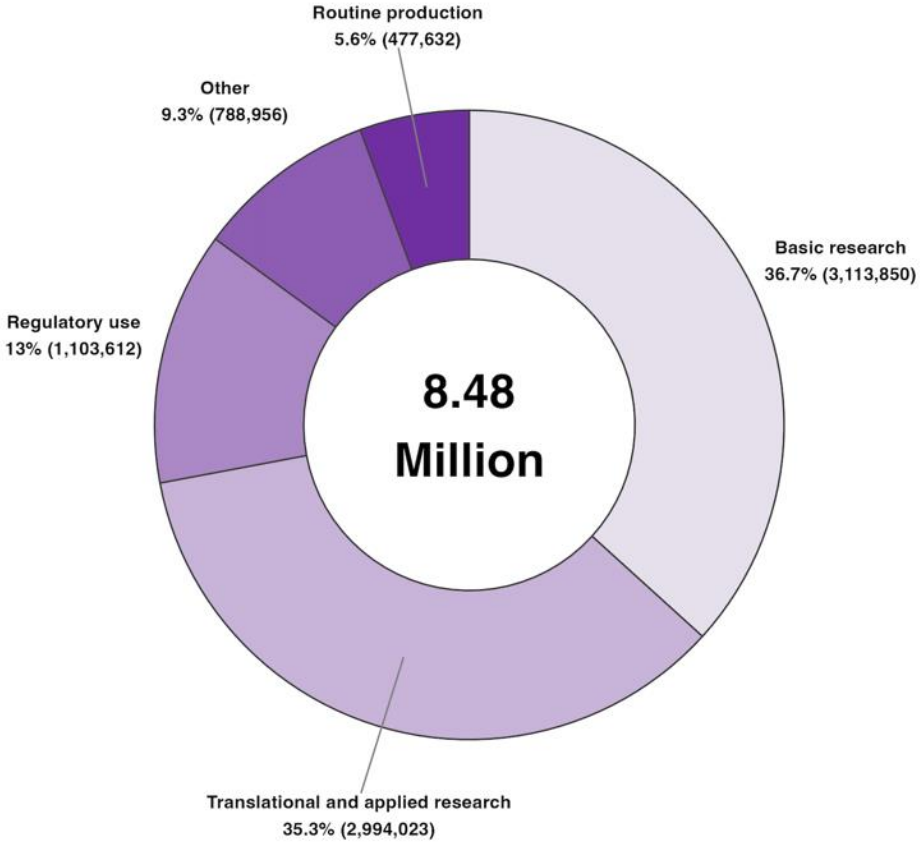


Figure 9: Uses of animals used for scientific purposes in 2022

Uses for translational and applied research decreased the most (-22.5%) compared to 2021. This decrease can be explained by the temporary increase in 2021 with three projects led by Member States on fish (two using salmon in Norway and one using larval form of sea bass in Spain as mentioned in their respective Member State narratives). Regulatory use also saw an important decrease compared to 2021 (-16.2%) which confirms the general trend (-32% compared to 2018).

Routine production, on the contrary, saw an increase of +15.7% compared to 2021 with an overall increase of +34.8% compared to 2018. Basic research and other categories remained almost stable (table 15).

IV.2.1.2. Severity of uses

Directive 2010/63/EU requires the reporting of the actual severity experienced by each animal when used for scientific purposes. In 2022, 44.7% of uses, were reported as 'mild' (up to and including), 42.5% as 'moderate', and 9.2% as 'severe' while 3.6% of uses were reported as 'non-recovery'. Compared to 2018, the number of uses reported as non-recovery decreased proportionally (-2.2% points) as well as the severe ones (-1.9% points) (Table 24).

Since the actual severities are linked to the type of uses, and the use patterns vary between Member States, it is not advisable to compare overall actual severities between Member States. As an example, a Member State with high proportion of animal use for the purposes of regulatory testing is likely to have higher proportion of severe uses compared to another Member State having mainly uses in the areas of education and training.

	2018	2019	2020	2021	2022
Non-recovery	521,765 (5.8%)	494,368 (5.7%)	330,392 (4.1%)	336,332 (3.5%)	**304,582 (3.6%)**
Mild [up to and including]	4,311,312 (48%)	4,380,747 (50.3%)	3,921,024 (48.7%)	4,395,210 (46.2%)	**3,789,476 (44.7%)**
Moderate	3,169,559 (35.3%)	2,955,923 (33.9%)	3,006,764 (37.3%)	3,863,470 (40.6%)	**3,599,739 (42.5%)**
Severe	976,445 (10.9%)	884,186 (10.1%)	796,750 (9.9%)	918,202 (9.7%)	**784,048 (9.2%)**
Total	**8,979,081 (100%)**	**8,715,224 (100%)**	**8,054,930 (100%)**	**9,513,214 (100%)**	**8,477,845 (100%)**

Table 16: Severity of uses reported in 2022

In 2022, when looking at specific purposes, most of the uses reported as severe were conducted in routine production for the production of monoclonal antibodies by mouse ascites (49.1%); regulatory testing for combined end-points (84.3%), acute toxicity testing (27.6%), ecotoxicity testing (26.5%) and batch potency testing for quality checks (19.5%); in basic research for "other basic research" (21%); in translational and applied research for animal diseases and disorders (31.2%) and "other human disorders" (21.5%).

In absolute numbers, the most important number of severe uses was reported for animal diseases and disorders (124.982), nervous system (94,447), batch potency testing (88,421), immune system (62,924), oncology (52,701) and human cancer (51.371).

On the contrary, the lowest number of severe uses for specific procedures were reported for forensic inquiries (0); for preservation of species (0.1%); for higher education (0.1%); for training for the acquisition, maintenance or improvement of vocational skills (0.9%); in routine production for monoclonal and polyclonal antibodies production (excluding mouse ascites method) (0.3%) and blood based products (0); in regulatory use for phototoxicity testing (0%), target animal safety (0.3%), pyrogenicity testing for quality checks (0.6%), genotoxicity testing (0.7%); and in translational and applied research for animal welfare (0.6%) (Figure 10).

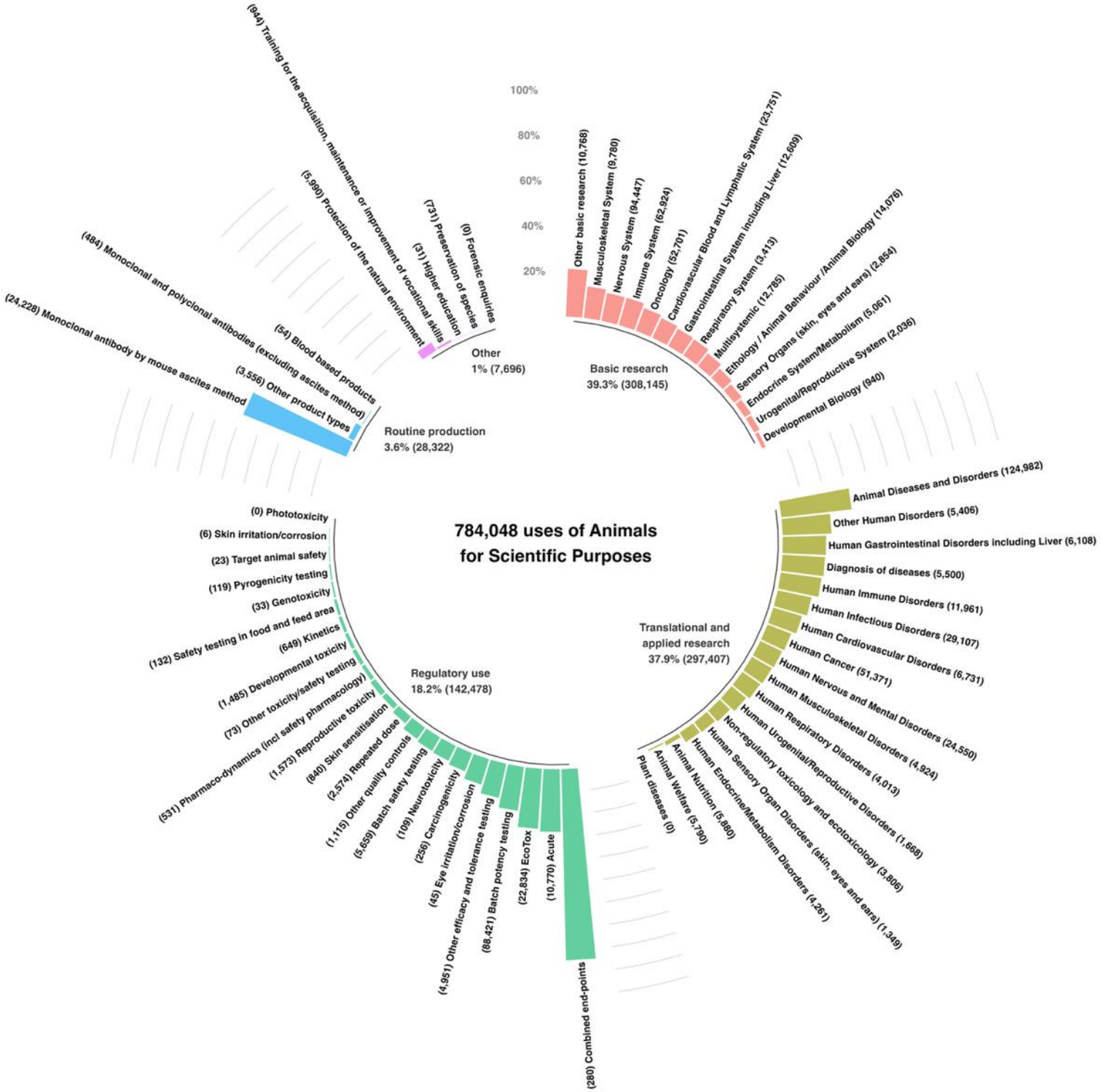


Figure 10: proportion of severe uses of animals by detailed purposes in 2022

IV.2.2. Detailed information on use purposes

	2018	2019	2020	2021	2022	Change 2022 vs 2021	Change 2022 vs 2018
Basic research							
Oncology	432,191	446,116	414,164	443,223	**495,488**	+11.8%	+14.6%
Cardiovascular Blood and Lymphatic System	254,931	240,688	213,763	241,922	**249,190**	+3%	-2.3%
Nervous System	687,420	738,363	640,405	722,137	**754,200**	+4.4%	+9.7%
Respiratory System	52,932	50,869	43,240	46,783	**40,030**	-14.4%	-24.4%
Gastrointestinal System including Liver	141,132	131,664	122,515	119,086	**145,692**	+22.3%	+3.2%
Musculoskeletal System	76,753	89,109	66,665	70,091	**70,123**	0%	-8.6%
Immune System	547,768	528,229	506,177	534,775	**505,356**	-5.5%	-7.7%
Urogenital/Reproductive System	83,859	84,852	84,777	104,037	**75,838**	-27.1%	-9.6%
Sensory Organs (skin, eyes and ears)	52,418	59,362	51,324	49,117	**70,040**	+42.6%	+33.6%
Endocrine System/Metabolism	187,421	180,441	152,863	175,901	**159,984**	-9%	-14.6%
Developmental Biology	0	0	0	52,320	**56,178**	+7.4%	NA
Multisystemic	309,585	175,765	236,967	160,247	**179,636**	+12.1%	-42%
Ethology / Animal Behaviour /Animal Biology	988,510	724,065	637,819	240,084	**260,765**	+8.6%	-73.6%
Other basic research	168,043	219,381	122,670	62,818	**51,330**	-18.3%	-69.5%
Translational and applied research							
Human Cancer	450,538	439,884	416,399	444,519	**459,214**	+3.3%	+1.9%
Human Infectious Disorders	190,731	224,597	192,464	195,281	**191,861**	-1.8%	+0.6%
Human Cardiovascular Disorders	66,479	60,283	55,682	51,854	**50,945**	-1.8%	-23.4%
Human Nervous and Mental Disorders	259,148	257,900	209,497	251,175	**231,878**	-7.7%	-10.5%
Human Respiratory Disorders	54,579	46,101	46,248	46,558	**42,054**	-9.7%	-22.9%
Human Gastrointestinal Disorders including Liver	37,868	41,399	35,462	32,676	**32,039**	-1.9%	-15.4%
Human Musculoskeletal Disorders	38,092	33,562	38,250	43,090	**48,648**	+12.9%	+27.7%
Human Immune Disorders	85,686	68,609	69,893	69,092	**66,235**	-4.1%	-22.7%
Human Urogenital/Reproductive Disorders	11,850	17,535	14,318	17,864	**20,236**	+13.3%	+70.8%
Human Sensory Organ Disorders (skin, eyes and ears)	35,175	38,339	30,289	28,989	**24,934**	-14%	-29.1%
Human Endocrine/Metabolism Disorders	115,102	128,928	94,337	85,898	**84,469**	-1.7%	-26.6%
Other Human Disorders	26,505	22,744	21,316	34,910	**25,155**	-27.9%	-5.1%
Animal Diseases and Disorders	937,444	850,073	505,383	976,663	**400,918**	-59%	-57.2%
Animal Nutrition	0	0	0	722,635	**309,716**	-57.1%	NA
Animal Welfare	137,426	205,137	688,564	735,313	**915,652**	+24.5%	+566.3%
Diagnosis of diseases	143,637	45,873	40,999	28,660	**29,212**	+1.9%	-79.7%
Plant diseases	38	108	106	22	**10**	-54.5%	-73.7%
Non-regulatory toxicology and ecotoxicology	60,432	54,879	51,292	97,532	**60,619**	-37.8%	+0.3%
Regulatory use							
Quality control (incl batch safety and potency testing)							
Batch safety testing	137,324	128,459	137,518	129,667	**81,511**	-37.1%	-40.6%
Pyrogenicity testing	29,815	30,687	24,139	23,695	**19,168**	-19.1%	-35.7%
Batch potency testing	752,958	643,369	577,534	533,527	**452,699**	-15.1%	-39.9%
Other quality controls	22,030	22,048	20,541	25,516	**18,202**	-28.7%	-17.4%
Toxicity and other safety testing including pharmacology							
Acute and sub-acute toxicity testing methods							
LD50, LC50	23,765	21,044	16,421	16,992	**18,560**	+9.2%	-21.9%
Other lethal methods	499	562	5,259	4,025	**6,011**	+49.3%	+1104.6%
Non lethal methods	21,947	20,702	14,952	15,100	**14,401**	-4.6%	-34.4%
Skin irritation/corrosion	3,998	2,945	4,070	3,824	**2,793**	-27%	-30.1%
Skin sensitisation	37,606	38,854	38,024	37,736	**33,029**	-12.5%	-12.2%
Eye irritation/corrosion	840	452	491	339	**383**	+13%	-54.4%

	2018	2019	2020	2021	2022	Change 2022 vs 2021	Change 2022 vs 2018
Repeated dose toxicity							
up to 28 days	43,484	37,190	37,777	36,775	**34,860**	-5.2%	-19.8%
29 - 90 days	22,938	23,141	22,503	21,720	**21,249**	-2.2%	-7.4%
> 90 days	12,888	8,827	7,174	9,159	**11,240**	+22.7%	-12.8%
Carcinogenicity	3,233	2,650	1,569	6,573	**2,970**	-54.8%	-8.1%
Genotoxicity	4,342	4,094	4,808	6,388	**4,587**	-28.2%	+5.6%
Reproductive toxicity	58,562	55,794	61,970	75,041	**62,606**	-16.6%	+6.9%
Developmental toxicity	63,271	68,330	68,022	94,523	**96,807**	+2.4%	+53%
Neurotoxicity	4,273	629	2,130	486	**1,489**	+206.4%	-65.2%
Kinetics	62,325	55,210	44,377	62,180	**46,250**	-25.6%	-25.8%
Pharmaco-dynamics (incl safety pharmacology)	78,934	72,909	56,047	31,435	**32,043**	+1.9%	-59.4%
Phototoxicity	519	414	114	525	**600**	+14.3%	+15.6%
Ecotoxicity							
Acute toxicity	54,115	51,523	51,860	43,916	**36,347**	-17.2%	-32.8%
Chronic toxicity	26,824	28,354	24,910	31,499	**37,086**	+17.7%	+38.3%
Reproductive ecotoxicity	240	224	2,653	735	**710**	-3.4%	+195.8%
Endocrine activity	790	8,784	13,679	11,457	**8,286**	-27.7%	+948.9%
Bioaccumulation	3,511	3,733	1,977	3,524	**2,027**	-42.5%	-42.3%
Other ecotoxicity	3,339	3,706	2,034	3,204	**1,850**	-42.3%	-44.6%
Safety testing in food and feed area	41,208	37,600	31,124	15,295	**10,775**	-29.6%	-73.9%
Target animal safety	6,290	4,979	27,686	10,225	**8,343**	-18.4%	+32.6%
Combined end-points	0	0	0	2,401	**332**	-86.2%	NA
Other toxicity/safety testing	4,145	16,922	14,305	5,236	**4,297**	-17.9%	+3.7%
Other efficacy and tolerance testing							
Other efficacy and tolerance testing	96,803	88,237	88,572	54,534	**32,101**	-41.1%	-66.8%
Routine production							
Blood based products	211,522	229,445	228,762	155,994	**113,959**	-26.9%	-46.1%
Monoclonal antibody by mouse ascites method	54,941	37,473	41,916	36,882	**49,309**	+33.7%	-10.3%
Monoclonal and polyclonal antibodies (excluding ascites method)	0	0	0	105,580	**184,830**	+75.1%	NA
Other product types	87,746	171,318	136,182	114,433	**129,534**	+13.2%	+47.6%
Other							
Protection of the natural environment in the interests of the health or welfare of human beings or animals	119,297	203,939	224,485	173,464	**147,460**	-15%	+23.6%
Preservation of species	83,683	224,921	106,058	585,531	**496,041**	-15.3%	+492.8%
Higher education	0	0	0	43,879	**42,439**	-3.3%	NA
Training for the acquisition, maintenance or improvement of vocational skills	0	0	0	93,945	**102,839**	+9.5%	NA
Higher education or training for the acquisition, maintenance or improvement of vocational skills	165,110	160,544	109,334	0	**0**	NA	-100%
Forensic enquiries	273	357	105	982	**177**	-82%	-35.2%
Total	**8,979,081**	**8,715,224**	**8,054,930**	**9,513,214**	**8,477,845**	**-10.9%**	**-5.6%**

Table 17: Total number of uses of animals by detailed purpose categories between 2018 and 2022

IV.2.2.1. Research related uses

Research-related uses are split between basic research on one side and translational and applied research on the other. The total number of uses for research purposes decreased by -11.3% between 2021 and 2022 (-29.4% decrease in translational and applied research and +1.4% increase in basic research).

Looking at the repartition of uses of animals for research and testing in 2022 (Table 17), the five most important categories of uses in 2022 were animal welfare, nervous system, immune system, oncology and human cancer which is in line with the previous years' reporting (showing 2021 as an exception).

Classifying the research purposes by research objectives centred on human, animal or other research[12], research centred on animals is subject to important variations between 2018 and 2022 while research focusing on humans remains stable (despite a slight decrease in 2020 due to the COVID-19 pandemic) (table 18).

	2018	2019	2020	2021	2022	Change 2021 vs 2020	Change 2021 vs 2018
Animal centred research	2,063,380 (31.1%)	1,779,275 (28.7%)	1,831,766 (31.6%)	2,674,695 (38.8%)	**1,887,051 (30.9%)**	-29.4%	-8.5%
Human centred research	4,341,800 (65.5%)	4,151,212 (66.9%)	3,798,014 (65.4%)	4,050,205 (58.8%)	**4,108,635 (67.3%)**	+1.4%	-5.4%
Other research	228,513 (3.4%)	274,368 (4.4%)	174,068 (3%)	160,372 (2.3%)	**111,959 (1.8%)**	-30.2%	-51%
Total	**6,633,693 (100%)**	**6,204,855 (100%)**	**5,803,848 (100%)**	**6,885,272 (100%)**	**6,107,645 (100%)**	**-11.3%**	**-7.9%**

Table 18: Total number of uses for research purposes between 2018 and 2022

Comparing the main species used in research centred on humans, mice (82.1%), fish (zebra fish in particular) (8.7%) and rats (6.7%), with the main species use in research focusing on animals are fish (75%), birds (15.4%) and other mammals (farm animals in particular) (5.3%), shows that the species used for these two purposes are extremely different (Figure 11).

12 Animal centred research (ethology/animal behaviour /animal biology; animal diseases and disorders; animal nutrition; animal welfare); other research (other basic research; plant diseases; non-regulatory toxicology and ecotoxicology); remaining categories considered as human centred research

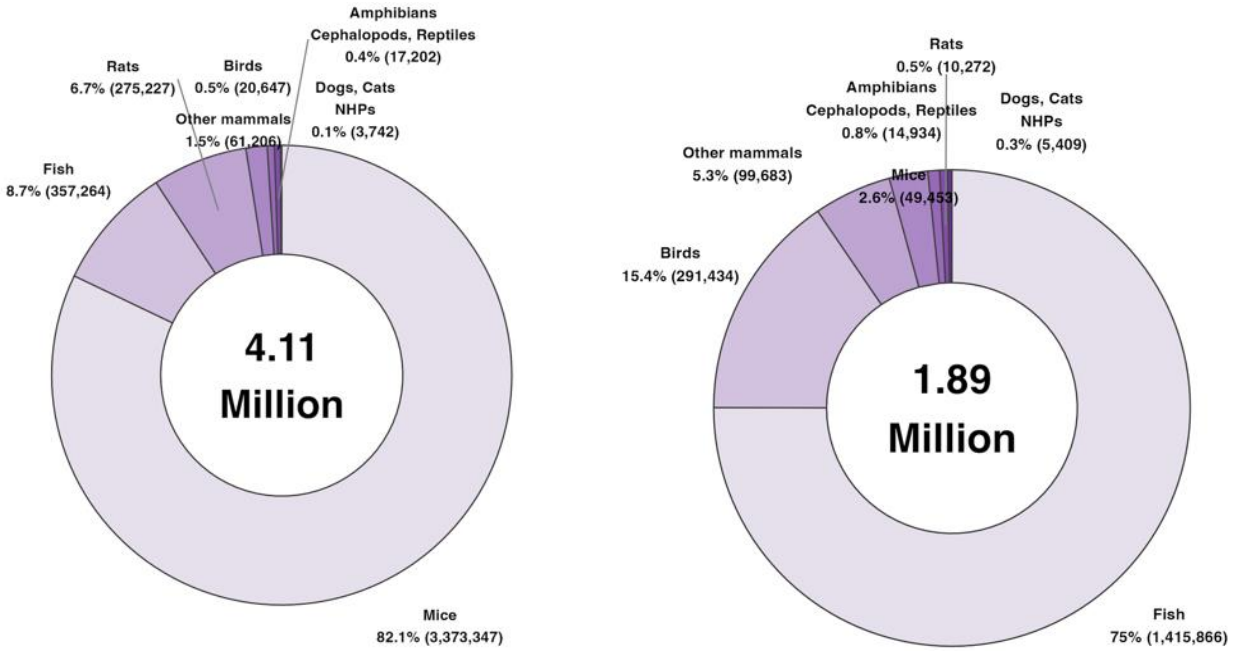


Figure 11: Main species used for human research (left) and animal research (right) in 2022

Translational and applied research uses for animal centred research may be looked at more carefully in the future to understand why research projects in these domains requires such important amounts of animals and why the number of animals used changes significantly between years.

IV.2.2.1.1 Basic research

Basic research was the first main area for which animals were used with more than three million uses in 2022 which is a small increase of +3% compared to 2021. The three main domains of basic research using most animals are nervous system, immune system and oncology that all together account for more than half of the uses in basic research (Figure 12).

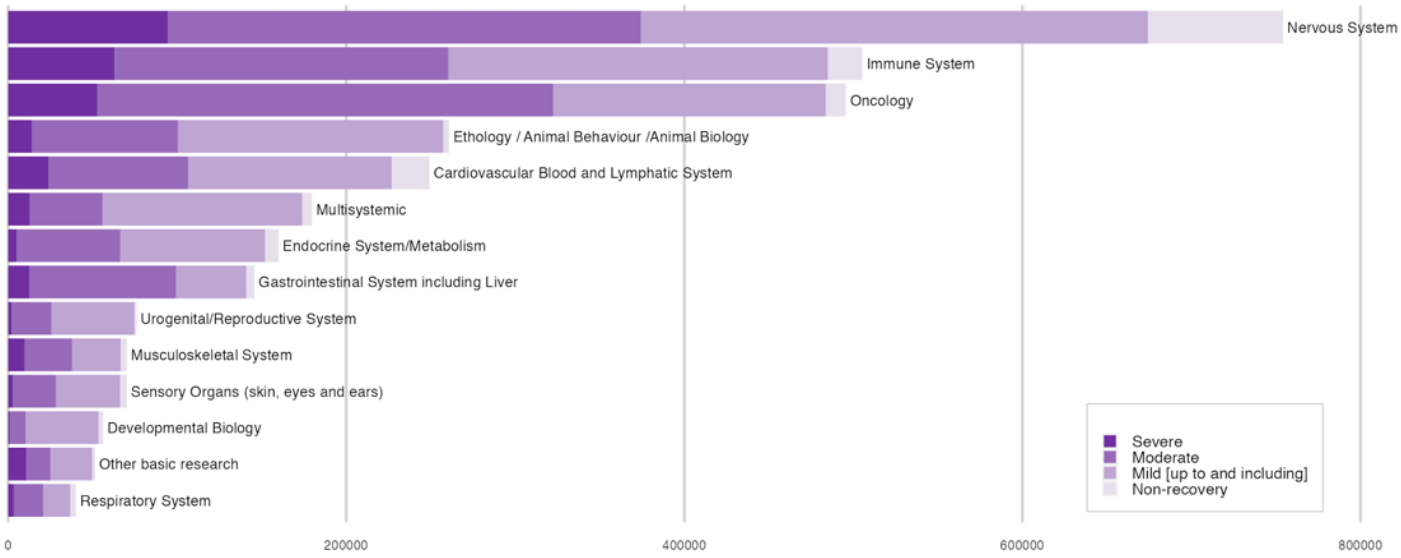


Figure 12: Basic research related uses by type of research and severity in 2022

In 2022, the main decreases happened for the areas urogenital/reproductive system (-27.1%) and respiratory system (-14.4%) compared to 2021. Sensory organs (skin, eyes and ears) (+42.6%) and gastrointestinal system including liver (+22.3%) increased the most (Table 17).

"Other basic research" decreased as well (-18.3%) confirming the general trend (-69.5% compared to 2018). It includes, for example basic research on environmental toxicology, parasitology or epigenetics.

IV.2.2.1.2. Translational and applied research

Translational and applied research accounted for 2.99 million uses of animals in 2022, an important decrease compared to 2021 (-22.5%) confirming the temporary nature of the 2021 increase.

Animal welfare, animal diseases and disorders and animal nutrition still represent more than half of the uses in 2022 (Figure 13). While uses for animal disease and disorders (-59%) and animal nutrition (-57.1%) decreased significantly, animal welfare uses continued to increase (+24.5%) compared to 2021. This last category saw an important increase as well compared to 2018 (+566.3%).

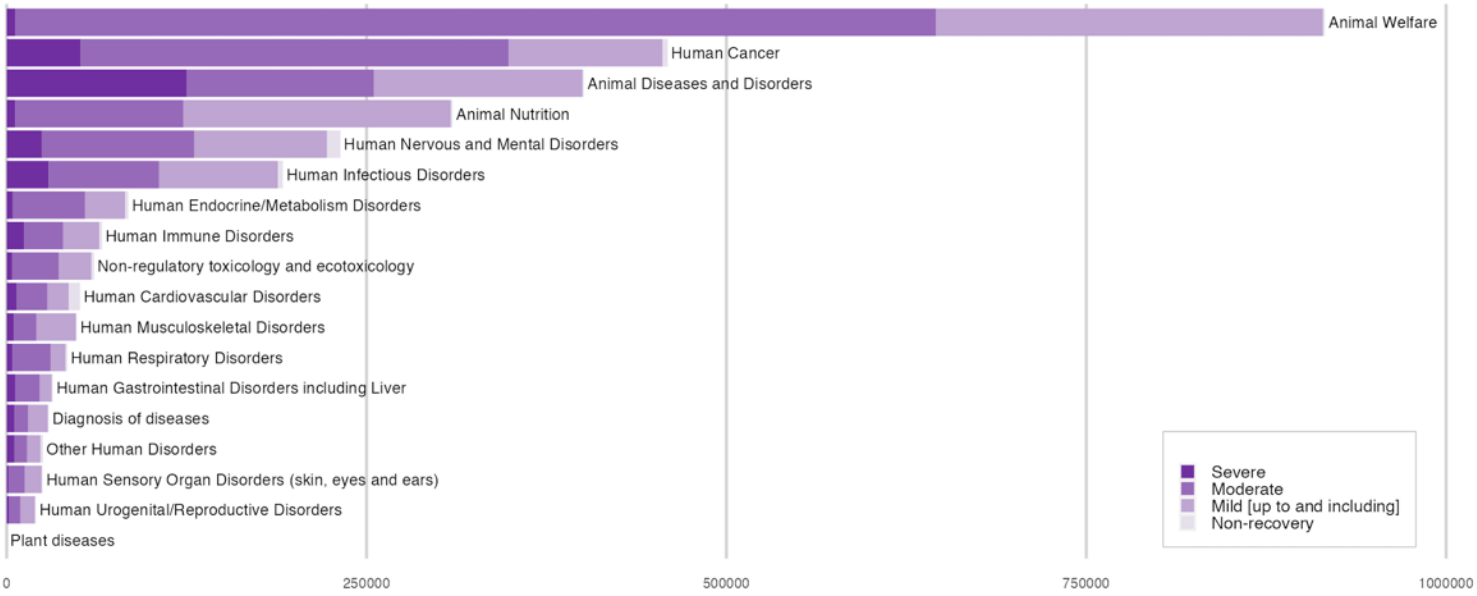


Figure 13: Translational and applied research related uses by type of research and severity in 2022

Compared to 2021, the categories related to the study of human disorders remained overall stable. There was an increase of uses for human urogenital/reproductive disorders (+13.3%) and human musculoskeletal disorders (+12.9%) confirming a longer- term trend with a respective increase of +70.8% and +27.7% compared to 2018. On the downward trend, 2022 confirmed the global decrease tendency compared to 2018 for diagnosis of diseases (-79.7%), human sensory organ disorders (skin, eyes and ears) (-29.1%), human cardiovascular disorders (-23.4%), human respiratory disorders (-22.9%), human immune disorders (-22.7%) (Table 17).

“Other Human Disorders” includes areas such as mitochondrial disfunctioning, studies related to the pharmacokinetics of compounds in drug development, radiation studies or rare diseases.

IV.2.2.2. Uses of animals for regulatory purposes

Regulatory uses cover 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. It also includes tests carried out on products/substances for which a regulatory submission was foreseen but ultimately not made, for instance because these were deemed unsuitable for the market by the developer and thus failed to reach the end of the development process.

In 2022, regulatory uses accounted for 1.1 million uses. The total number of uses for regulatory purposes decreased by -16.2% compared to 2021 and by a remarkable -32% compared to 2018. 51.8% of these uses were related to quality control (including batch safety and potency testing), 45.3% related to toxicity and other safety testing including pharmacology and the remainder (2.9%) were for other efficacy and tolerance testing. These three categories all continuously decreased since 2018 (table 19).

	2018	2019	2020	2021	2022	Change 2021 vs 2020	Change 2021 vs 2018
Quality control (incl batch safety and potency testing)	942,127 (58.1%)	824,563 (55.6%)	759,732 (54.1%)	712,405 (54.1%)	**571,580 (51.8%)**	-19.8%	-39.3%
Toxicity and other safety testing including pharmacology	583,886 (36%)	569,572 (38.4%)	555,936 (39.6%)	550,313 (41.8%)	**499,931 (45.3%)**	-9.2%	-14.4%
Other efficacy and tolerance testing	96,803 (6%)	88,237 (6%)	88,572 (6.3%)	54,534 (4.1%)	**32,101 (2.9%)**	-41.1%	-66.8%
Total	**1,622,816 (100%)**	**1,482,372 (100%)**	**1,404,240 (100%)**	**1,317,252 (100%)**	**1,103,612 (100%)**	**-16.2%**	**-32%**

Table 19: Regulatory uses by type of research between 2018 and 2022

The main species used for regulatory purposes are mice (33.6%), rats (26.9%), "Other mammals" (15.1%), birds (11.8%) and fish (11.1%) (Figure 14).

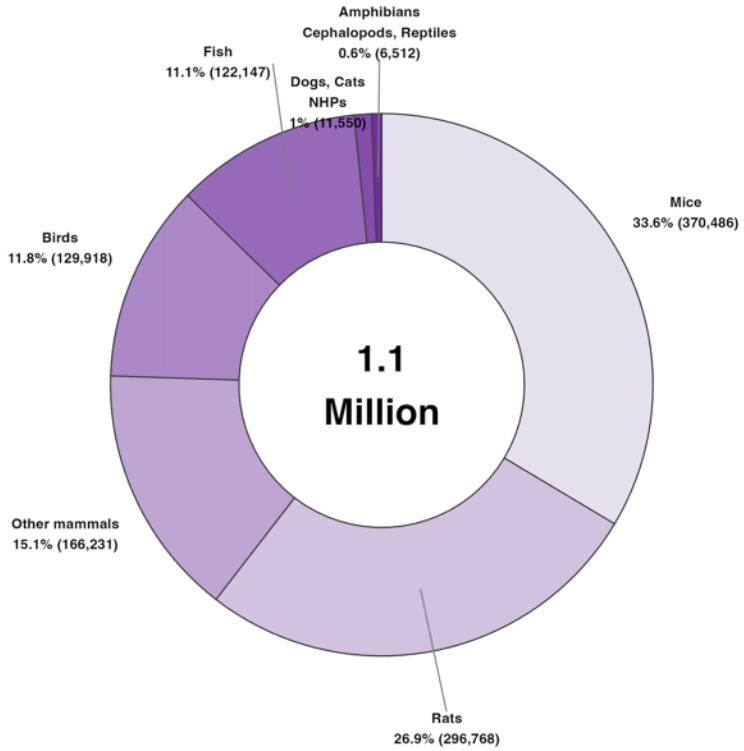


Figure 14: Main species used for regulatory purposes in 2022

The "Other efficacy and tolerance testing" category of regulatory use refers to uses that are neither linked to quality control nor to toxicity testing. These uses are related to, for example, efficacy (immunogenicity) of human and veterinary vaccines.

IV.2.2.2.1. Details of the regulatory use purposes

IV.2.2.2.1.1. Quality control related uses

Quality control includes uses of animals in the testing of purity, stability, efficacy, potency and other quality control parameters product (and its constituents) such as vaccines, 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.

Quality control related uses represented 571,580 uses in 2022. A large majority of these uses were related to batch potency testing purposes (79.1%), a severe procedure (88,421 uses in 2022) (figure 15). Nevertheless, this specific category is continuously decreasing (-39.3% compared to 2018) a trend that started since 2015 (see previous reports).

"Other quality controls" decreased in 2022 compared to 2021 (-28.7%) and are related for example to pathogenicity tests, product purity or potency.

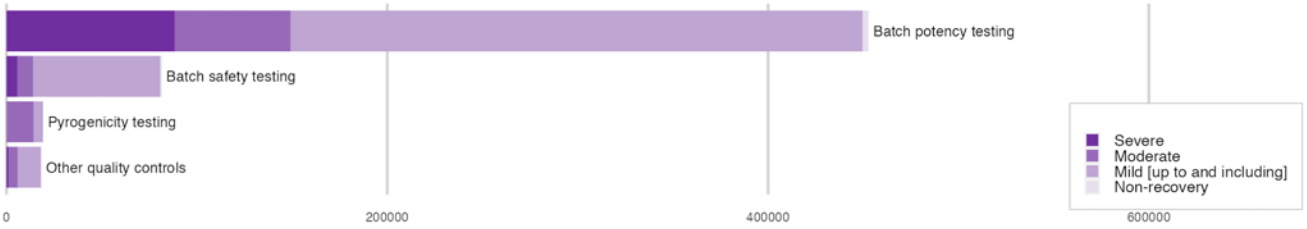


Figure 15: Quality control related uses by type of use and severity in 2022

In 2022, pyrogenicity testing, for which alternative methods (applicable to most use cases) are available, continued to decrease (-19.1% compared to 2021 and -35.7% compared to 2018). In 2022, these uses are reported mainly by six countries covering 98.5% of all pyrogenicity testing, namely France (37.3%) and Spain (33.5%). Bulgaria (9.4%), Italy (7.9%), Germany (5.6%) and Ireland (5%) (Table 20).

	2018	2019	2020	2021	2022	Change 2021 vs 2020	Change 2021 vs 2018
Austria	1,923 (6.4%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**	NA	-100%
Bulgaria	132 (0.4%)	300 (1%)	309 (1.3%)	3,100 (13.1%)	**1,800 (9.4%)**	-41.9%	+1263.6%
Czech Republic	53 (0.2%)	51 (0.2%)	41 (0.2%)	0 (0%)	**0 (0%)**	NA	-100%
France	9,578 (32.1%)	12,007 (39.1%)	10,325 (42.8%)	9,711 (41%)	**7,145 (37.3%)**	-26.4%	-25.4%
Germany	6,291 (21.1%)	6,457 (21%)	3,223 (13.4%)	1,371 (5.8%)	**1,078 (5.6%)**	-21.4%	-82.9%
Greece	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**12 (0.1%)**	NA	NA
Hungary	20 (0.1%)	37 (0.1%)	14 (0.1%)	0 (0%)	**0 (0%)**	NA	-100%
Ireland	145 (0.5%)	525 (1.7%)	943 (3.9%)	657 (2.8%)	**966 (5%)**	+47%	+566.2%
Italy	2,243 (7.5%)	2,177 (7.1%)	2,025 (8.4%)	1,531 (6.5%)	**1,518 (7.9%)**	-0.8%	-32.3%
Poland	192 (0.6%)	225 (0.7%)	186 (0.8%)	177 (0.7%)	**147 (0.8%)**	-16.9%	-23.4%
Romania	372 (1.2%)	30 (0.1%)	0 (0%)	25 (0.1%)	**0 (0%)**	-100%	-100%
Slovenia	64 (0.2%)	51 (0.2%)	68 (0.3%)	68 (0.3%)	**73 (0.4%)**	+7.4%	+14.1%
Spain	8,802 (29.5%)	8,827 (28.8%)	7,005 (29%)	7,055 (29.8%)	**6,429 (33.5%)**	-8.9%	-27%
Total	**29,815 (100%)**	**30,687 (100%)**	**24,139 (100%)**	**23,695 (100%)**	**19,168 (100%)**	**-19.1%**	**-35.7%**

Table 20: Number of uses for pyrogenicity testing per country between 2018 and 2022

IV.2.2.2.1.2. Toxicity and other safety testing including pharmacology

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.

Toxicity and other safety testing including pharmacology represented 499,931 uses of animals in 2022, which corresponds to 5.9% of all uses of animals. Most of the uses in this area were related to developmental toxicity, reproductive toxicity, kinetics, acute or chronic toxicity for ecotoxicity testing (Figure 16).

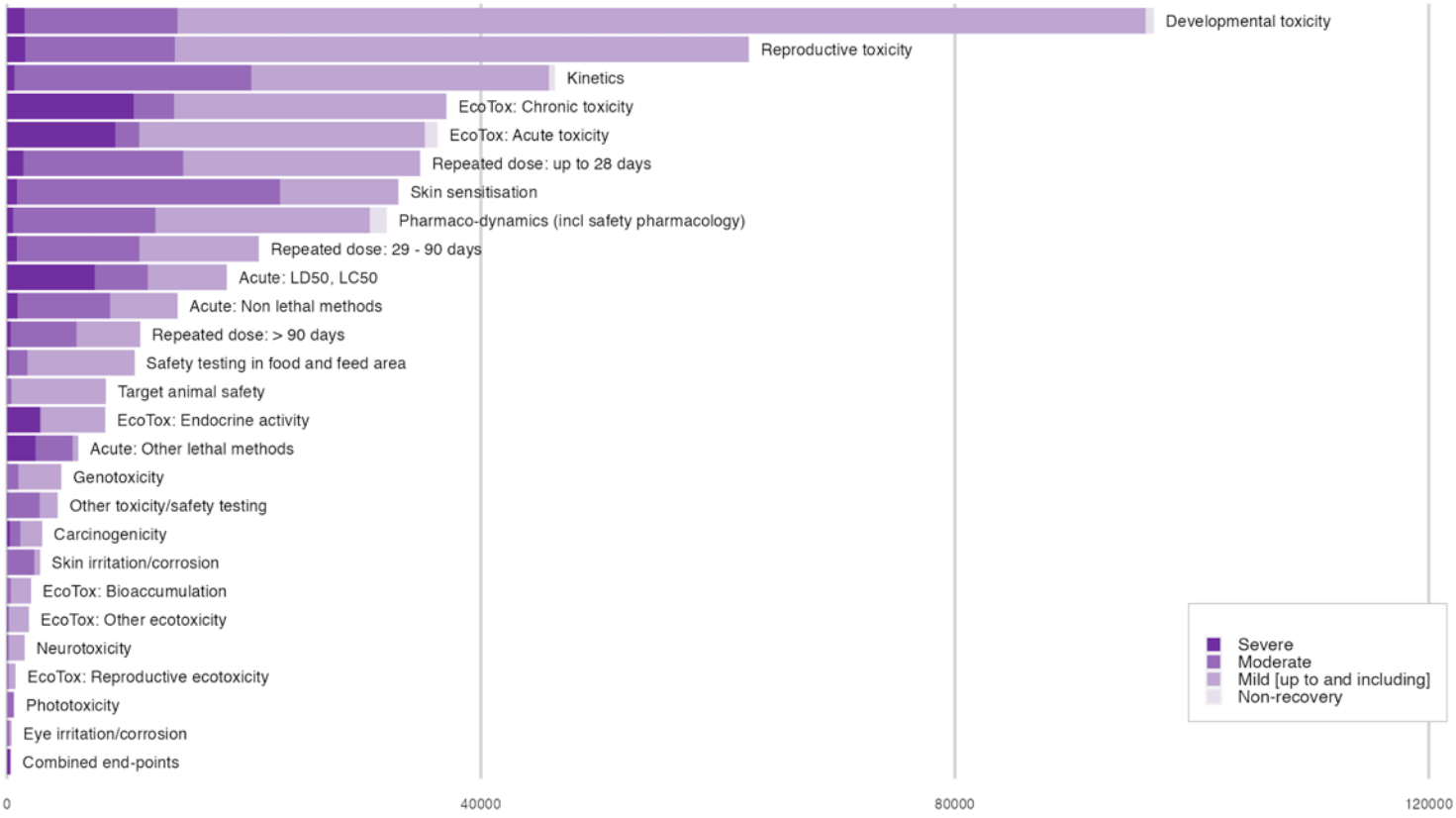


Figure 16: Toxicity and other safety testing including pharmacology by type of use and severity in 2022

Compared to the year 2021, the total number of uses for toxicity and other safety testing including pharmacology decreased more than previous years (-9.2%), a continuous trend since 2018 (-14.4%). The most important decreases were for combined endpoints (-86.2%), carcinogenicity (-54.8%), bioaccumulation (-42.5%), "Other ecotocixity" (-42.3%), safety testing in food and feed area (-29.6%) and genotoxicity (-28.2%). Only two categories showed a more important increase: neurotoxicity (+206.4%) and "Other lethal methods" for acute and sub-acute toxicity testing (+49.3%),

Some of these variations could be related to testing campaigns required by EU or national authorities, creating a temporary increase for specific endpoints during a year or two. Positive signs of long-term decrease can be observed though, comparing to 2018 for safety testing in food and feed area (-73.9%) (table 17).

"Other toxicity/safety testing" are related for example to the immunogenicity or efficacy of vaccines under development.

IV.2.2.2.2. Legislative aspects of regulatory uses

In 2022, the majority of uses to satisfy regulatory requirements of specific sector legislation occurred in relation to placing on the market of medicinal products for humans (46.7%), veterinary medicinal products (27.7%) and industrial chemicals (13.8%) (table 21).

	2018	2019	2020	2021	2022	Change 2021 vs 2020	Change 2021 vs 2018
Legislation on medicinal products for human use	1,034,432 (63.7%)	900,033 (60.7%)	758,902 (54%)	673,409 (51.1%)	**515,344 (46.7%)**	-23.5%	-50.2%
Legislation on medicinal products for veterinary use and their residues	265,949 (16.4%)	270,806 (18.3%)	319,853 (22.8%)	302,125 (22.9%)	**305,530 (27.7%)**	+1.1%	+14.9%
Medical devices legislation	52,335 (3.2%)	50,849 (3.4%)	50,427 (3.6%)	48,932 (3.7%)	**48,110 (4.4%)**	-1.7%	-8.1%
Industrial chemicals legislation	123,837 (7.6%)	117,867 (8%)	122,736 (8.7%)	165,086 (12.5%)	**151,932 (13.8%)**	-8%	+22.7%
Plant protection product legislation	46,189 (2.8%)	51,778 (3.5%)	67,174 (4.8%)	55,798 (4.2%)	**35,895 (3.3%)**	-35.7%	-22.3%
Biocides legislation	1,788 (0.1%)	1,985 (0.1%)	4,442 (0.3%)	2,578 (0.2%)	**4,069 (0.4%)**	+57.8%	+127.6%
Food legislation including food contact material	41,641 (2.6%)	34,252 (2.3%)	28,326 (2%)	16,079 (1.2%)	**8,141 (0.7%)**	-49.4%	-80.4%
Feed legislation including legislation for the safety of target animals, workers and environment	7,200 (0.4%)	10,512 (0.7%)	10,356 (0.7%)	13,780 (1%)	**8,744 (0.8%)**	-36.5%	+21.4%
Other legislation	49,445 (3%)	44,290 (3%)	42,024 (3%)	39,465 (3%)	**25,847 (2.3%)**	-34.5%	-47.7%
Total	**1,622,816 (100%)**	**1,482,372 (100%)**	**1,404,240 (100%)**	**1,317,252 (100%)**	**1,103,612 (100%)**	**-16.2%**	**-32%**

Table 21: Regulatory uses by type of legislation between 2018 and 2022

Between 2018 and 2022, regulatory uses saw a constant decrease (-32%) mainly due to the decrease of uses for legislation on medicinal products for human use (-50.2%). In the same period, food legislation including food contact material (-80.4%) and "Other legislation" uses decreased as well (-47.7%) (Table 21).

The significant decrease of uses for the legislation on medicinal products for human use can be explained partly by the efforts of regulators at European level, including the European Directorate for the

Quality of Medicines & HealthCare (EDQM) [13], as well as the European Commission and industry via the EPAA[14] such as:

- the Abnormal Toxicity Test (ATT) suppression from the European Pharmacopoeia (Ph. Eur.) in 2019;
- the rationalisation of toxicity testing requirements for toxoid vaccines (acellular pertussis vaccines, tetanus vaccines and diphtheria vaccines) 2020, 2021 and 2022 respectively[15];
- the requirements revision for extraneous agent testing of vaccines in 2018[16];
- the decision, in 2021, to completely replace the rabbit pyrogen test (RPT) in the Ph. Eur. within five years;

[13] https://www.edqm.eu/en/replacement-reduction-and-refinement-of-animal-testing-3rs-latest-achievements

[14] European Partnership for Alternative Approaches to Animal Testing - European Commission (europa.eu)

[15] For acellular pertussis vaccines, the Histamine sensitisation test (HIST) in mice was replaced with the CHO cell clustering assay. For tetanus vaccines, the Test for specific toxicity performed on the final lot and the Test for irreversibility of tetanus toxoid carried out on the bulk purified toxoid (all tests in guinea pigs) were deleted. For diphtheria vaccines the test for specific toxicity in guinea pigs that is performed on the final lot as part of the validation of the production process was removed.

[16] The changes included the deletion of the tests on adult mice and guinea pigs and restricting the use of the test on suckling mice and control eggs to cases in which the tests provide risk mitigation.

- the facilitation of the transition from *in vivo* to *in vitro* methods, through the concept of "substitution" of animal tests for the quality control of vaccines, introduced in 2018[17].

With regard to food legislation including food contact material the replacement of the use of mouse bioassay for the purposes of shellfish toxin testing by the so-called Lawrence method can explain the decrease, as provided by Regulation 2017/1980[18].

In 2022, uses for biocides legislation (+57.8%) is the only legislation type that saw a significant increase but concerning the lowest amount of uses (4,069).

In 2022, the majority of regulatory uses were performed to satisfy regulatory requirements originating from the Union (95.8%). Non-EU requirements accounted for 2.5% and national requirements for 1.5%, proportions that remained stable over the past five years (Table 22).

	2018	2019	2020	2021	2022
Legislation satisfying EU requirements	1,546,115 (95.3%)	1,397,499 (94.3%)	1,336,241 (95.2%)	1,266,926 (96.2%)	**1,057,456 (95.8%)**
Legislation satisfying national requirements only [within EU]	31,617 (1.9%)	38,358 (2.6%)	36,633 (2.6%)	16,951 (1.3%)	**18,467 (1.7%)**
Legislation satisfying non-EU requirements only	45,084 (2.8%)	46,515 (3.1%)	31,366 (2.2%)	33,375 (2.5%)	**27,689 (2.5%)**
Total	**1,622,816 (100%)**	**1,482,372 (100%)**	**1,404,240 (100%)**	**1,317,252 (100%)**	**1,103,612 (100%)**

Table 22: Regulatory uses by origin of regulatory requirement between 2018 and 2022

The sub-category on legislation satisfying EU-requirements also includes any requirements for which international harmonisation has been achieved, such as for testing to OECD, ICH19 and VICH20 standards. Harmonisation of testing requirements at a global level is of utmost importance when aiming to avoid unnecessary duplication of testing.

	Quality control (incl batch safety and potency testing)	Toxicity and other safety testing including pharmacology	Other efficacy and tolerance testing
Legislation on medicinal products for human use	303,768 (58.9%)	201,211 (39%)	10,365 (2%)
Legislation on medicinal products for veterinary use and their residues	264,819 (86.7%)	25,307 (8.3%)	15,404 (5%)
Medical devices legislation	2,572 (5.3%)	44,761 (93%)	777 (1.6%)
Industrial chemicals legislation	0 (0%)	151,932 (100%)	0 (0%)
Plant protection product legislation	0 (0%)	35,895 (100%)	0 (0%)
Biocides legislation	0 (0%)	3,769 (92.6%)	300 (7.4%)
Food legislation including food contact material	115 (1.4%)	8,026 (98.6%)	0 (0%)
Feed legislation including legislation for the safety of target animals, workers and environment	222 (2.5%)	3,683 (42.1%)	4,839 (55.3%)

17 The chapter 5.2.14 Substitution of in vivo method(s) by in vitro method(s) for the quality control of vaccines provides guidance on the validation of substitute methods where a direct head-to-head comparison with an existing in vivo method is not possible. The chapter provides examples of methodological frameworks for the substitution of in vivo tests for vaccine potency and safety.

18 OJ L 285, 1.11.2017, p. 8–9

19 The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use

20 The International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products

	Quality control (incl batch safety and potency testing)	Toxicity and other safety testing including pharmacology	Other efficacy and tolerance testing
Other legislation	84 (0.3%)	25,347 (98.1%)	416 (1.6%)
Total	**571,580 (51.8%)**	**499,931 (45.3%)**	**32,101 (2.9%)**

Table 23: Regulatory use by type of legislation in 2022

Legislation on medicinal products for human or veterinary uses is mainly related to quality controls, although the proportion of quality controls on medicinal products for human used is less and less important over time (it used to be 70.8% in 2018). Industrial chemicals legislation, medical devices legislation, food legislation including food contact material, plant protection products legislation and other legislation focus more specifically on toxicity testing. Feed legislation is mainly related to other efficacy and tolerance testing (table 23).

In terms of severity levels, in 2022, for regulatory uses, 12.9% were reported as severe (-2.3 percentage points compared to 2021), 22.9% as moderate, 65.3% mild (and up to mild) and 0.7% as non-recovery. Even if the total number of uses are not the most significant, the highest proportion of severe uses took place in the area of food legislation including legislation for the safety of target animals, workers and environment (29.3%) and biocides (30%) (Table 24).

	Non-recovery	Mild [up to and including]	Moderate	Severe
Legislation on medicinal products for human use	5,926 (1.1%)	320,361 (62.2%)	121,926 (23.7%)	67,131 (13%)
Legislation on medicinal products for veterinary use and their residues	44 (0%)	208,223 (68.2%)	50,230 (16.4%)	47,033 (15.4%)
Medical devices legislation	58 (0.1%)	17,162 (35.7%)	29,857 (62.1%)	1,033 (2.1%)
Industrial chemicals legislation	1,048 (0.7%)	111,599 (73.5%)	26,798 (17.6%)	12,487 (8.2%)
Plant protection product legislation	0 (0%)	29,115 (81.1%)	1,781 (5%)	4,999 (13.9%)
Biocides legislation	700 (17.2%)	2,059 (50.6%)	89 (2.2%)	1,221 (30%)
Food legislation including food contact material	0 (0%)	6,659 (81.8%)	1,304 (16%)	178 (2.2%)
Feed legislation including legislation for the safety of target animals, workers and environment	0 (0%)	5,842 (66.8%)	343 (3.9%)	2,559 (29.3%)
Other legislation	0 (0%)	19,747 (76.4%)	263 (1%)	5,837 (22.6%)
Total	**7,776 (0.7%)**	**720,767 (65.3%)**	**232,591 (21.1%)**	**142,478 (12.9%)**

Table 24: Severity of regulatory uses by type of legislation in 2022

IV.2.2.3. Routine production uses

Routine production includes the production of antibodies and blood products, including polyclonal antisera by established methods. The main species used for this purpose are rabbits (59.4%), rodents (28.2%) and birds (11%).

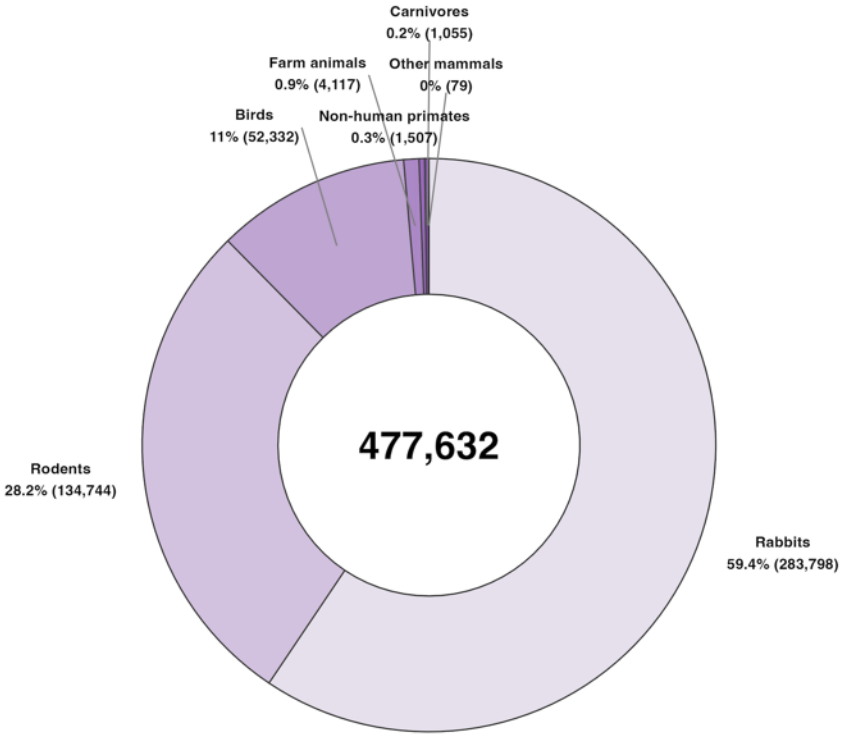


Figure 17: Main species used for routine production in 2022

In 2022, there were about 412,889 routine production uses, which represented 4.3% of all uses of animals in the Union which represents a slight increase compared to 2020 (+1.5%).

In 2022, the production of mono- and polyclonal antibodies (excluding mouse ascites method) and of monoclonal antibodies by mouse ascites method represented more than half of the uses for routine production. Those two categories increase significantly by +75.1% and +33.7% respectively, compared to 2021 while blood-based products uses decreased by -26.9% (Figure 18).

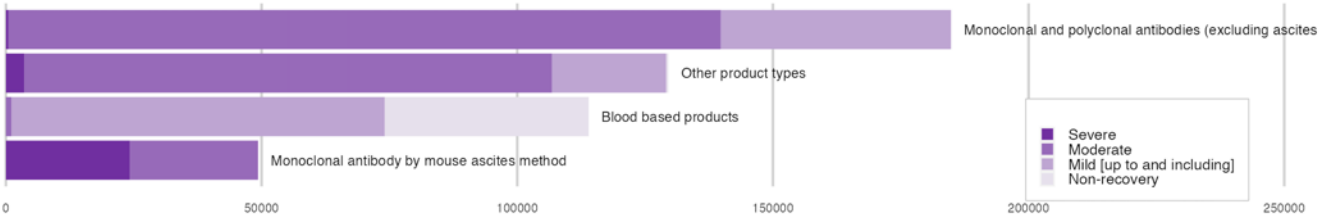


Figure 18: Routine production uses by product type and severity in 2022

While blood-based products involved only mild and moderate levels of severity, monoclonal antibody production by the mouse ascites method involved mostly severe uses (49.1%). This is one of the most severe procedures, still not showing a steady decrease since 2018 although it decreased by -10.3% compared to 2018. In total, only three Member States reported the use of the mouse ascites method for the production of monoclonal antibodies, of which one Member State (France) represents 99.5% of uses. In 2022, Germany stopped the use of animals for this purpose (Table 25).

	2018	2019	2020	2021	2022	Change 2021 vs 2020	Change 2021 vs 2018
Belgium	11 (0%)	17 (0%)	0 (0%)	0 (0%)	**0 (0%)**	NA	-100%
Czech Republic	93 (0.2%)	76 (0.2%)	125 (0.3%)	75 (0.2%)	**255 (0.5%)**	+240%	+174.2%
France	53,073 (96.6%)	35,801 (95.5%)	40,649 (97%)	36,664 (99.4%)	**49,038 (99.5%)**	+33.7%	-7.6%
Germany	1,565 (2.8%)	1,456 (3.9%)	1,056 (2.5%)	113 (0.3%)	**0 (0%)**	-100%	-100%
Hungary	134 (0.2%)	0 (0%)	66[21] (0.2%)	0 (0%)	**0 (0%)**	NA	-100%
Poland	0 (0%)	4 (0%)	0 (0%)	30 (0.1%)	**0 (0%)**	-100%	NA
Spain	65 (0.1%)	119 (0.3%)	20 (0%)	0 (0%)	**16 (0%)**	NA	-75.4%
Total	**54,941 (100%)**	**37,473 (100%)**	**41,916 (100%)**	**36,882 (100%)**	**49,309 (100%)**	**+33.7%**	**-10.3%**

Table 25: Number of uses for monoclonal antibody by mouse ascites method per country between 2018 and 2022

The production of mono- and polyclonal antibodies (excluding the mouse ascites method) was reported by 14 Member States in 2022 (against 17 in 2021). “Other product types” that represented 27.6% of the uses were mostly related to antigenic or protein production.

IV.2.2.4. Other types of uses

In 2022, the last five categories of uses reported as part of the Directive covered 788,956 uses, a decrease compared to 2021 (-12.1%) but remaining higher than before (+114.2% compared to 2018) due to the increase of uses for preservation of species (+323.6%).

Looking at the species used for these purposes, two categories emerge: on one side, protection of the natural environment in the interests of the health or welfare of human beings or animals and preservation of species uses mainly fish species (98.4% and 83.5%), while higher education and training for the acquisition, maintenance or improvement of vocational skills uses more mice (41.2% and 60.7%)and rats (25.2% and 21.5%) (Table 26).

	Protection of the natural environment	Preservation of species	Higher education	Training for vocational skills	Forensic enquiries
Mice	738 (0.5%)	272 (0.1%)	17,481 (41.2%)	62,428 (60.7%)	0 (0%)
Rats	205 (0.1%)	9 (0%)	10,678 (25.2%)	22,093 (21.5%)	0 (0%)
Other mammals	9,167 (6.2%)	1,053 (0.2%)	6,516 (15.4%)	13,813 (13.4%)	117 (66.1%)
Fish	123,186 (83.5%)	488,315 (98.4%)	3,848 (9.1%)	2,032 (2%)	0 (0%)
Birds	13,074 (8.9%)	4,267 (0.9%)	2,692 (6.3%)	1,443 (1.4%)	60 (33.9%)
Amphibians, Cephalopods, Reptiles	1,090 (0.7%)	2,105 (0.4%)	1,021 (2.4%)	91 (0.1%)	0 (0%)
Dogs, Cats, NHPs	0 (0%)	20 (0%)	203 (0.5%)	939 (0.9%)	0 (0%)
Total	**147,460 (100%)**	**496,041 (100%)**	**42,439 (100%)**	**102,839 (100%)**	**177 (100%)**

Table 26: Main species used for “Other types of uses” in 2022

[21] Hungary reported this number as resulting from a misclassification during their submission process.

In 2022, with 496,041 uses in 2022, preservation of species is the biggest category of the remaining purposes, representing more than half of the other uses. On the contrary, forensic inquiry uses are limited to about a thousand, a decrease of -82% compared to 2021. (Figure 19).

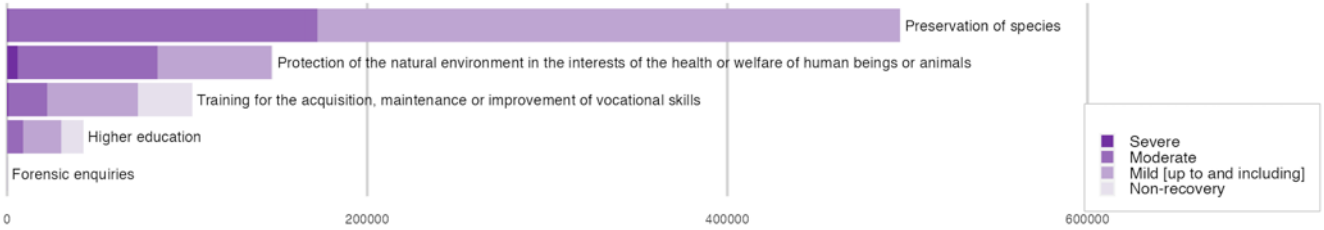


Figure 19: Other types of uses in 2022 including their severity

IV.2.3. Information on reuses and genetic status of animals

The Directive requires additional elements to be recorded related to the use of animals for scientific purposes, such as reuse and information on the genetic status of the animals.

IV.2.3.1. Reuses

In line with the principle of the Three Rs, the total number of animals used in procedures can be reduced by performing procedures on animals more than once. However, this should only take place when this does not result in poor animal welfare and is evaluated on a case-by-case basis. Under Directive 2010/63/EU, reuse of animals in procedures is permitted only under specific conditions related to the actual level of severity the animal has experienced in a previous procedure, and the health and well-being of the animal, taking into account the lifetime experience of the individual animal. A reuse cannot be authorised for a procedure, in which the animal may reach 'severe' level of pain, suffering or distress. Also, an animal may be reused following a severe procedure only in exceptional circumstances and after a veterinary examination of that animal.

In 2022, the proportion of reuses was at 1.1% which continues the decrease compared to 2018 both in absolute value (-41%) and proportion (-0.6 percentage point) (table 27).

	2018	2019	2020	2021	2022	Change 2021 vs 2020	Change 2021 vs 2018
No	8,822,404 (98.3%)	8,579,439 (98.4%)	7,938,064 (98.5%)	9,406,233 (98.9%)	**8,385,397 (98.9%)**	-10.9%	-5%
Yes	156,677 (1.7%)	135,785 (1.6%)	116,866 (1.5%)	106,981 (1.1%)	**92,448 (1.1%)**	-13.6%	-41%
Total	**8,979,081 (100%)**	**8,715,224 (100%)**	**8,054,930 (100%)**	**9,513,214 (100%)**	**8,477,845 (100%)**	**-10.9%**	**-5.6%**

Table 27: Reuses of animals used for research, testing, routine production and educational purposes

In proportion, procedures requiring the re-use of animals are mostly related to regulatory use: skin irritation/corrosion (52.4%), eye irritation/corrosion (23.5%), pyrogenicity testing (17.3%); non-regulatory toxicology and ecotoxicology (10.1%); higher education (9.7%); and training for the acquisition, maintenance or improvement of vocational skills (9.4%).

In absolute numbers, the main procedures requiring re-use of animals were batch potency testing (10,952), training for the acquisition, maintenance or improvement of vocational skills (9,685), animal nutrition (9,017), and non-regulatory toxicology and ecotoxicology (6,107) (Figure 20).

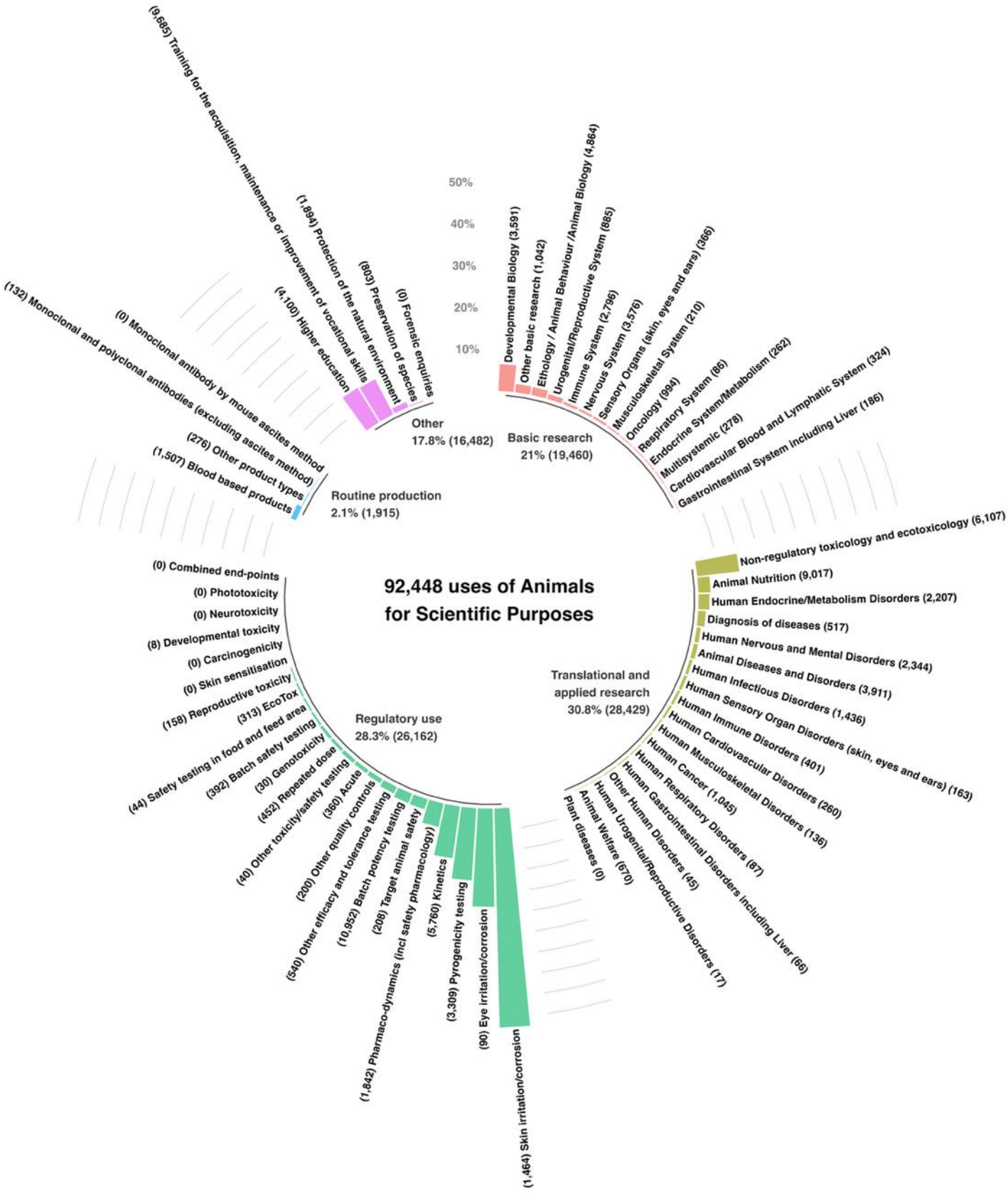


Figure 20: Reuses of animals used for research, testing, routine production and educational purposes

	Percent	Number
Rhesus monkey	58.5%	197
Cats	58.4%	1,974
Goats	52%	1,374
Dogs	39.4%	5,659
Squirrel monkey	27.8%	5
Cynomolgus monkey	24.5%	1,607
Baboons	22.9%	19
Reptiles	22%	1,306
Xenopus	20.8%	4,118
Horses, donkeys and cross-breeds	19.4%	988
Other carnivores	19.3%	201
Cattle	14.8%	3,721
Sheep	9.3%	1,627
Marmoset and tamarins	5.9%	31
Vervets (Chlorocebus spp.)	5.9%	3
Other mammals	5.4%	476
Prosimians	5.1%	4
Ferrets	4.5%	42
Pigs	2.7%	2,455
Rats	1.6%	10,158
Rabbits	1.5%	5,678
Zebra fish	1.3%	4,857
Guinea-Pigs	1.2%	1,025
Other fish	1%	6,638
Mice	0.8%	31,549
Other rodents	0.8%	126
Cephalopods	0.7%	18
Other birds	0.7%	549
Sea bass	0.6%	661
Guppy, swordtail, molly, platy	0.3%	254
Hamsters (Syrian)	0.3%	55
Salmon, trout, chars and graylings	0.3%	4,128
Domestic fowl	0.2%	920
Mongolian gerbil	0.1%	3
Other amphibians	0.1%	9
Turkey	0.1%	13
Hamsters (Chinese)	0%	0
Rana	0%	0

Table 28: Reuses by type of species in 2022

In proportion, non-human primates are reused more often than other species: rhesus monkeys (58.5%), squirrel monkey (27.8%), cynomolgus monkeys (24.5%) and baboons (22.9%); but in limited absolute numbers. Other animals frequently reused in proportion are large mammal species such as cats (58.4%), goats (52%), dogs (39.4%), horses, donkeys and cross-breeds (19.4%), cattle (14.86%) or sheep (9.3%); in larger numbers. Xenopus (20.8%) and reptiles (22%) are also often re-used.

In absolute numbers, the main species reused for scientific purposed in 2022 were mice (31,549), rats (10,158), rabbits (5,678) and dogs (5,659) (Table 28).

According to the Directive, reuse of an animal is not allowed in a procedure classified prospectively as severe. In 2022, most of the reuses, the actual reported severities were mild (68%) or moderate (28.2%) (Figure 21).

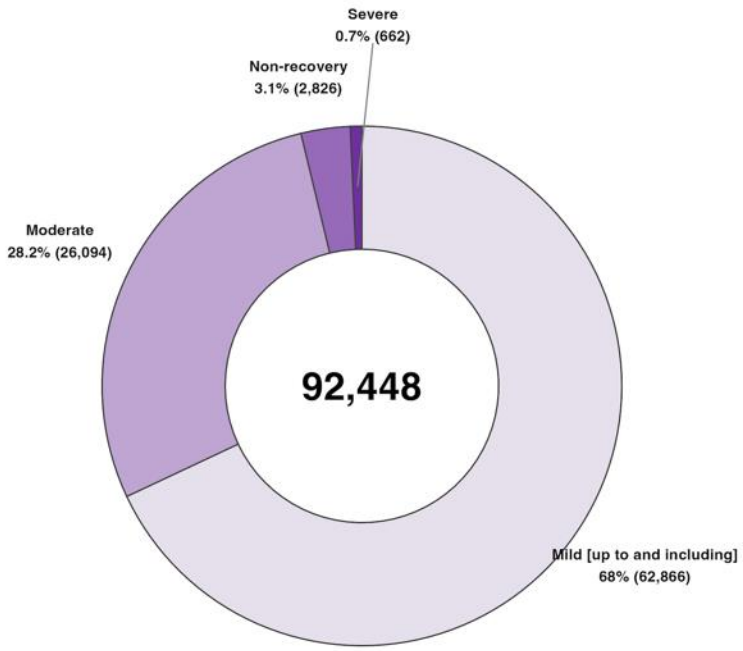


Figure 21: Reuses by severity in 2022

However, in some cases, even if the procedure is prospectively classified in a lower severity category, an individual animal may reach severity category "severe" due to unforeseen events occurring during the procedure. Only a very small number of such cases 0.7% was reported, with an increase to 662 such cases compared to 2020.

These 662 uses should be investigated by the authorities to eliminate any recurrence of any repetitive unforeseen adverse effects. Furthermore, these events, if recurring, may suggest a need for a revision of the prospective classification for future uses (Table 29).

	2018	2019	2020	2021	2022
Mild [up to and including]	107,781 (68.8%)	99,518 (73.3%)	83,574 (71.5%)	73,346 (68.6%)	**62,866 (68%)**
Moderate	36,053 (23%)	32,921 (24.2%)	30,946 (26.5%)	30,201 (28.2%)	**26,094 (28.2%)**
Non-recovery	12,762 (8.1%)	3,022 (2.2%)	1,733 (1.5%)	2,670 (2.5%)	**2,826 (3.1%)**
Severe	81 (0.1%)	324 (0.2%)	613 (0.5%)	764 (0.7%)	**662 (0.7%)**
Total	**156,677 (100%)**	**135,785 (100%)**	**116,866 (100%)**	**106,981 (100%)**	**92,448 (100%)**

Table 29: Severity classification of reuse procedures

IV.2.3.2. Use of genetically altered animals

Some of the animals used in procedures for purposes of research, testing, routine production and education are genetically altered. This section presents the types of genetic alteration reported. A welfare assessment is required to be performed on a newly created genetically altered animal line to establish whether the line is expected to have an intended non-harmful or harmful phenotype.

Intended non-harmful phenotypes include animal models where no adverse effects are noted during development, breeding and maintenance under conventional laboratory animal conditions. In addition,

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

Intended harmful phenotypes include animal models where gene alteration induces a specific genetic disorder or disease, or increases incidence of / susceptibility to for example tumour development. Other examples of harmful phenotype lines include 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.

In 2022, 2.1 million uses for the purposes of research were carried out on animals that were genetically altered. Of these, 17.5% were carrying a harmful phenotype (Figure 22).

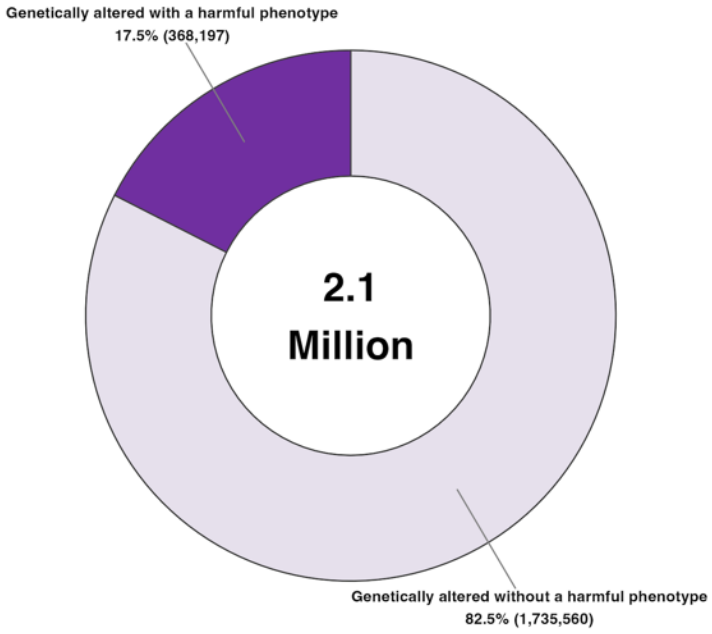


Figure 22: Uses of animals by type of genetic alteration in 2022

In 2022, the proportion of the uses of genetically altered animals for scientific purposes increased to 24.8% (+4.3 points compared to 2021). The percentage of the uses of such animals without a harmful phenotype increased 20.5% (+3.5 points), as well as the uses of such animals with a harmful phenotype at 4.3% (+0.8 points) (Table 30).

	2018	2019	2020	2021	2022	Change 2022 vs 2021	Change 2022 vs 2018
Genetically altered with a harmful phenotype	275,660 (3.1%)	326,789 (3.7%)	313,937 (3.9%)	328,660 (3.5%)	**368,197 (4.3%)**	+12%	+33.6%
Genetically altered without a harmful phenotype	1,518,800 (16.9%)	1,590,870 (18.3%)	1,485,665 (18.4%)	1,620,801 (17%)	**1,735,560 (20.5%)**	+7.1%	+14.3%
Not genetically altered	7,184,621 (80%)	6,797,565 (78%)	6,255,328 (77.7%)	7,563,753 (79.5%)	**6,374,088 (75.2%)**	-15.7%	-11.3%
Total	**8,979,081 (100%)**	**8,715,224 (100%)**	**8,054,930 (100%)**	**9,513,214 (100%)**	**8,477,845 (100%)**	**-10.9%**	**-5.6%**

Table 30: Genetic status of animals used in 2022

Amongst the species, which have been genetically altered, uses of mice accounted for the highest numbers, followed by zebra fish and rats. Even if mice account for the most animals being genetically altered, in proportion, 51.2% of zebra fish was genetically altered, followed by mice (45.9%), while only 4.3% of rats were genetically altered and used in procedures for purposes of research, testing, routine production in 2022 (Table 31).

In terms of trends in the evolution since 2018, the proportion of genetically altered mice is increasing (from 36.1% to 45.9%) while the proportion genetically altered rabbits (from 7.3% to 5%) is decreasing.

	2018	2019	2020	2021	2022
Mice	36.1%	38.9%	40.6%	41.7%	**45.9% (1,854,363)**
Rats	3.5%	3.2%	3.4%	3.7%	**4.3% (27,540)**
Hamsters (Syrian)	2.8%	5.3%	0%	0.8%	**2.5% (543)**
Other rodents	0%	0.1%	0%	0%	**0.2% (24)**
Rabbits	7.5%	6.2%	5.1%	5.4%	**5% (18,915)**
Dogs	0.4%	0.2%	0.1%	0.1%	**0.1% (12)**
Ferrets	0%	0.7%	3%	0%	**1.8% (17)**
Pigs	0.6%	0.8%	0.8%	1%	**1.1% (968)**
Sheep	0%	0%	0%	0.3%	**0% (5)**
Prosimians	0%	0%	0%	23.4%	**0% (0)**
Domestic fowl	0%	0%	0%	0.1%	**0.2% (804)**
Xenopus	20.2%	8.4%	7.4%	2.6%	**9.6% (1,903)**
Other amphibians	11.2%	18.2%	27.4%	32%	**20.4% (2972)**
Zebra fish	42.5%	49.1%	52.4%	49.2%	**51.3% (188,545)**
Other fish	0.2%	0.2%	0.3%	0.7%	**0.6% (3916)**

Table 31: Genetically altered species used between 2018 and 2022

Genetically altered animals are used almost exclusively for research purposes. In 2022, the proportion of genetically altered animals was the highest in basic research for sensory organs (70.1%), cardiovascular, blood and lymphatic system (67.3%), musculoskeletal system (65.3%), endocrine system/metabolism (59.6%), nervous system (57%), oncology (56.4%) and developmental biology (55.7%); in translational and applied research to a lower extent for other human disorders (45.4%), human musculoskeletal disorders (65.7%), human cancer (45.2%), human cardiovascular disorders (41.6%); and in training for the acquisition, maintenance or improvement of vocational skills (21.6%) (Figure 23).

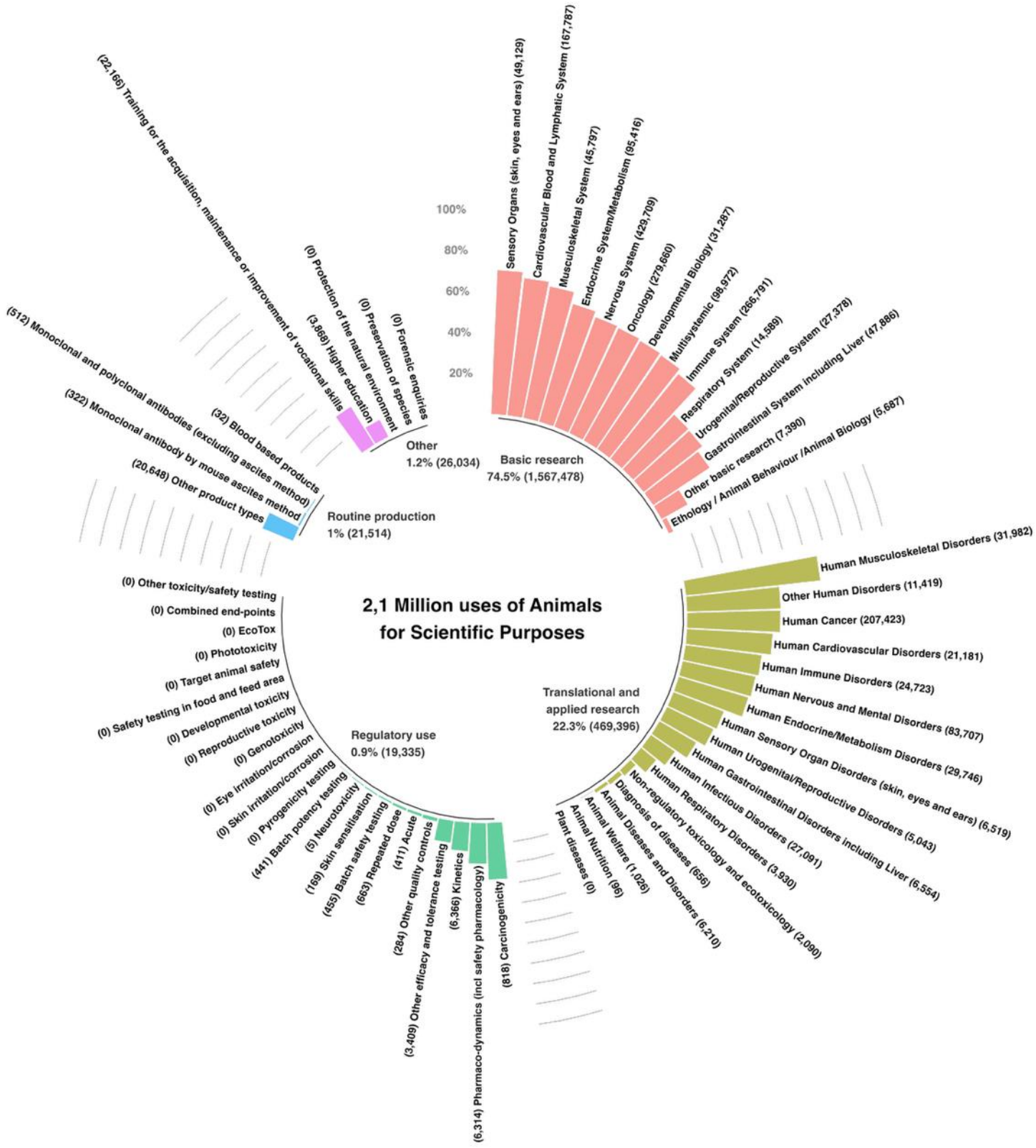


Figure 23: Proportion of genetically altered animals uses by purpose in 2022

IV.3. Numbers and uses of animals for the creation and maintenance of genetically altered animals in the EU

In the context of Directive 2010/63/EU, Member States are also required to report the animals used in procedures for the creation of new genetically altered animal lines and the maintenance of colonies of established genetically altered animal lines to support the research needs in the Union.

The diagram in part IV.3 provides further understanding of the reporting requirements for both creation and maintenance of genetically altered animal lines.

IV.3.1. Numbers of animals used for the creation and maintenance of genetically altered animals

In 2022, 852,145 animals were used for the provision of genetically altered animals for the purposes of scientific research.

This included 341,233 animals used for the first time for the creation of new genetically altered animal lines (table 32), which remains stable compared to the previous years.

510,912 animals were used for the first time for the maintenance of colonies of established genetically altered animal lines (table 32). In comparison to 2021, this represents an increase of +65% that is partly explained in the France Member State narrative mentioning that uses of invasive genotyping techniques on animal not expressing genetic alteration were included in the statistical data submission for the first time in 2022.

The reporting requirements for the maintenance of colonies of established genetically altered animal lines are particularly complex (more information is provided under section IV.3.3 below). The Commission, Member States and key stakeholder organisations developed a comprehensive guidance document on genetically altered animals which was endorsed by Member State National Contact Points responsible for the implementation of the Directive in their meeting in November 2021. The guidance is available in all Union languages and disseminated by the National Contact Points after its publication in 2022 to help address issues with *inter alia* coherent and correct reporting of genetically altered animals.

IV.3.2. All uses of animals for the creation of new genetic altered animal lines

The creation of a new genetic altered animal line is reported under the research purpose category for which the line is being created for. The reporting covers all animals carrying the genetic alteration. In addition, those used for superovulation, vasectomy and embryo implantation are equally 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 are not reported in the annual statistics. (see diagram in Part IV.4).

Counting all uses, the main species that were used for the creation of new genetic altered animal lines were mice and zebra fish, 71.6% and 23.7% respectively. Other species, although in small numbers, include other species of fish, rats, Salmon, trout, chars and graylings (table 32).

	2018	2019	2020	2021	2022	Change 2022 vs 2021	Change 2022 vs 2018
Mammals							
Rodents							
Mice	274,354	214,078	293,821	255,978	**250,551**	-2.1%	-8.7%
Rats	6,168	3,635	5,138	1,938	**1,910**	-1.4%	-69%
Guinea-Pigs	0	0	0	0	**0**	NA	NA
Hamsters (Syrian)	89	116	449	270	**120**	-55.6%	+34.8%
Other rodents	0	0	0	0	**0**	NA	NA
Rabbits							
Rabbits	324	305	277	392	**216**	-44.9%	-33.3%
Carnivores							
Dogs	0	0	18	0	**0**	NA	NA
Ferrets	4	0	0	0	**0**	NA	-100%
Other carnivores	0	0	6	0	**0**	NA	NA
Farm animals							
Horses, donkeys and cross-breeds	0	0	10	0	**0**	NA	NA
Pigs	62	124	131	385	**279**	-27.5%	+350%
Goats	0	0	0	0	**38**	NA	NA
Sheep	0	0	263	0	**6**	NA	NA
Cattle	0	1	28	0	**0**	NA	NA
Non-human primates							
Marmoset and tamarins	10	47	0	0	**8**	NA	-20%
Other mammals							
Other mammals	70	6	10	0	**6**	NA	-91.4%
Birds							
Domestic fowl	100	286	293	525	**612**	+16.6%	+512%
Other birds	0	10	183	36	**0**	-100%	NA
Reptiles							
Reptiles	0	0	42	0	**0**	NA	NA
Amphibians							
Xenopus	0	1,748	819	961	**4,160**	+332.9%	NA
Other amphibians	100	271	368	805	**1,324**	+64.5%	+1224%
Fish							
Zebra fish	84,996	89,392	89,787	94,186	**82,864**	-12%	-2.5%
Other fish	16,087	3,122	2,103	3,248	**3,780**	+16.4%	-76.5%
Salmon, trout, chars and graylings	0	0	0	1,754	**3,232**	+84.3%	NA
Totals							
Total	**382,364**	**313,141**	**393,746**	**360,478**	**349,106**	**-3.2%**	**-8.7%**

Table 32: Uses of animals for the creation of new genetically altered animal lines by species between 2018 and 2022

IV.3.2.1. Creation of new genetically altered animal lines by genetic status

Animals that are not genetically altered but reported under the category 'creation of a new genetically altered animal line' include, for example, genetically normal parent animals or a part of the offspring that does not carry the genetic alteration. Of those that were genetically altered, 68.6% were of a non-harmful phenotype.

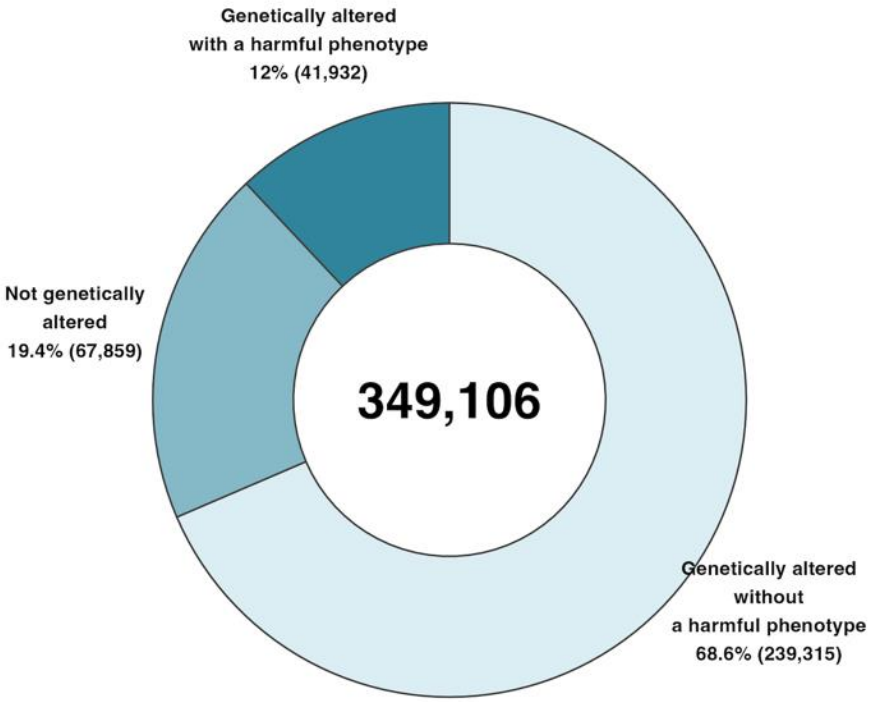


Figure 24: Creation of new genetically altered animal lines: genetic types of animals used in 2022

IV.3.2.2. Creation of new genetically altered animal lines by scientific purposes

The creation of new genetic lines is only carried out for research purposes. In 2022, 349,106 uses (first and any subsequent reuses) were reported for the purposes of creating new genetically altered animal lines (Figure 25).

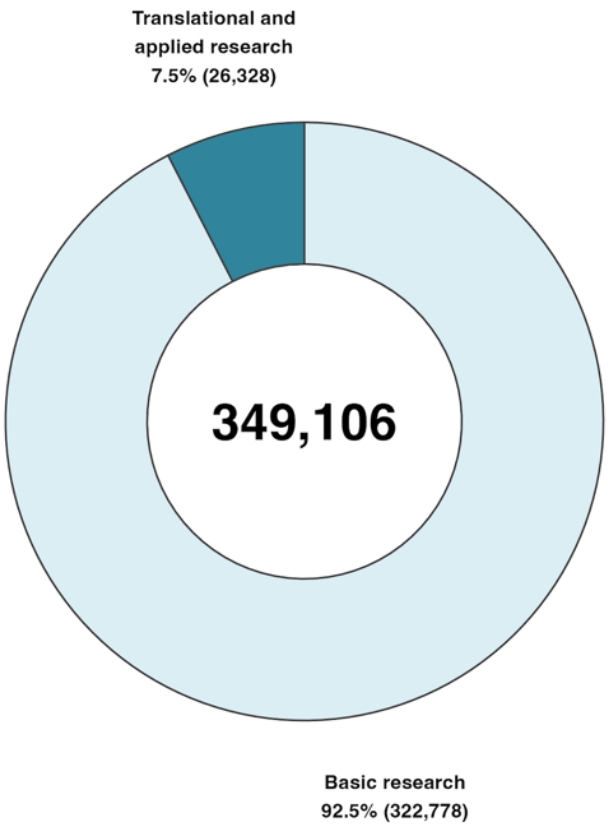


Figure 25: Creation of new genetically altered animal lines: uses for research purposes in 2022

92.5% of the new genetically altered lines were created for purposes covered under basic research (Figure 25). The table 33 below presents all sub-categories from both basic and translational and applied research together.

In 2022, basic research purposes concerned of uses for nervous system (19.5%), multisystemic (18.7%), and cardiovascular, blood and lymphatic system (11.1%%). The most important sub-category under translational and applied research for which new genetically altered animal lines were created was human infectious disorders (2.7%).

	2018	2019	2020	2021	2022	Change 2022 vs 2021	Change 2022 vs 2018
Basic research							
Oncology	50,180 (13.1%)	38,803 (12.4%)	43,817 (11.1%)	37,678 (10.5%)	**29,298 (8.4%)**	-22.2%	-41.6%
Cardiovascular Blood and Lymphatic System	42,560 (11.1%)	41,118 (13.1%)	43,422 (11%)	48,546 (13.5%)	**38,889 (11.1%)**	-19.9%	-8.6%
Nervous System	63,676 (16.7%)	53,772 (17.2%)	82,050 (20.8%)	48,432 (13.4%)	**68,193 (19.5%)**	+40.8%	+7.1%
Respiratory System	510 (0.1%)	200 (0.1%)	521 (0.1%)	489 (0.1%)	**669 (0.2%)**	+36.8%	+31.2%
Gastrointestinal System including Liver	9,283 (2.4%)	12,091 (3.9%)	13,739 (3.5%)	12,066 (3.3%)	**13,808 (4%)**	+14.4%	+48.7%
Musculoskeletal System	11,068 (2.9%)	6,259 (2%)	7,936 (2%)	5,784 (1.6%)	**6,621 (1.9%)**	+14.5%	-40.2%
Immune System	33,529 (8.8%)	26,794 (8.6%)	25,299 (6.4%)	34,146 (9.5%)	**30,405 (8.7%)**	-11%	-9.3%
Urogenital/Reproductive System	22,100 (5.8%)	20,606 (6.6%)	15,246 (3.9%)	10,040 (2.8%)	**16,908 (4.8%)**	+68.4%	-23.5%
Sensory Organs (skin, eyes and ears)	10,957 (2.9%)	8,172 (2.6%)	3,637 (0.9%)	4,815 (1.3%)	**2,501 (0.7%)**	-48.1%	-77.2%
Endocrine System/Metabolism	17,086 (4.5%)	11,787 (3.8%)	16,411 (4.2%)	18,219 (5.1%)	**28,009 (8%)**	+53.7%	+63.9%
Developmental Biology	0 (0%)	0 (0%)	0 (0%)	9,776 (2.7%)	**18,827 (5.4%)**	+92.6%	NA
Multisystemic	59,131 (15.5%)	55,062 (17.6%)	71,956 (18.3%)	106,912 (29.7%)	**65,270 (18.7%)**	-38.9%	+10.4%
Ethology / Animal Behaviour /Animal Biology	2,388 (0.6%)	1,410 (0.5%)	1,363 (0.3%)	1,558 (0.4%)	**100 (0%)**	-93.6%	-95.8%
Other basic research	23,830 (6.2%)	22,099 (7.1%)	13,257 (3.4%)	4,834 (1.3%)	**3,280 (0.9%)**	-32.1%	-86.2%
Translational and applied research							
Human Cancer	3,221 (0.8%)	2,562 (0.8%)	3,048 (0.8%)	2,189 (0.6%)	**1,265 (0.4%)**	-42.2%	-60.7%
Human Infectious Disorders	1,062 (0.3%)	84 (0%)	2,456 (0.6%)	1,883 (0.5%)	**9,339 (2.7%)**	+396%	+779.4%
Human Cardiovascular Disorders	2,298 (0.6%)	1,844 (0.6%)	1,619 (0.4%)	1,489 (0.4%)	**1,610 (0.5%)**	+8.1%	-29.9%
Human Nervous and Mental Disorders	3,156 (0.8%)	2,467 (0.8%)	4,036 (1%)	1,661 (0.5%)	**4,685 (1.3%)**	+182.1%	+48.4%
Human Respiratory Disorders	608 (0.2%)	35 (0%)	377 (0.1%)	178 (0%)	**457 (0.1%)**	+156.7%	-24.8%
Human Gastrointestinal Disorders including Liver	3,224 (0.8%)	344 (0.1%)	443 (0.1%)	664 (0.2%)	**876 (0.3%)**	+31.9%	-72.8%
Human Musculoskeletal Disorders	317 (0.1%)	217 (0.1%)	430 (0.1%)	431 (0.1%)	**2,290 (0.7%)**	+431.3%	+622.4%
Human Immune Disorders	429 (0.1%)	769 (0.2%)	491 (0.1%)	1,817 (0.5%)	**1,220 (0.3%)**	-32.9%	+184.4%
Human Urogenital/Reproductive Disorders	171 (0%)	122 (0%)	207 (0.1%)	954 (0.3%)	**299 (0.1%)**	-68.7%	+74.9%
Human Sensory Organ Disorders (skin, eyes and ears)	783 (0.2%)	729 (0.2%)	894 (0.2%)	862 (0.2%)	**3,088 (0.9%)**	+258.2%	+294.4%
Human Endocrine/Metabolism Disorders	10,551 (2.8%)	2,434 (0.8%)	1,726 (0.4%)	2,706 (0.8%)	**880 (0.3%)**	-67.5%	-91.7%
Other Human Disorders	820 (0.2%)	3,018 (1%)	38,953 (9.9%)	1,916 (0.5%)	**86 (0%)**	-95.5%	-89.5%
Animal Diseases and Disorders	9,169 (2.4%)	295 (0.1%)	157 (0%)	357 (0.1%)	**163 (0%)**	-54.3%	-98.2%
Animal Welfare	223 (0.1%)	0 (0%)	127 (0%)	0 (0%)	**0 (0%)**	NA	-100%
Diagnosis of diseases	0 (0%)	20 (0%)	87 (0%)	9 (0%)	**8 (0%)**	-11.1%	NA
Non-regulatory toxicology and ecotoxicology	34 (0%)	28 (0%)	41 (0%)	67 (0%)	**62 (0%)**	-7.5%	+82.4%
Total	**382,364 (100%)**	**313,141 (100%)**	**393,746 (100%)**	**360,478 (100%)**	**349,106 (100%)**	**-3.2%**	**-8.7%**

Table 33: Uses of animals for the creation of new genetically altered animal lines by type of research between 2018 and 2022

IV.3.2.3. Creation of new genetically altered animal lines by severity

Severities reported under the creation of new genetically altered animal lines include impacts from surgical techniques used during creation (embryo transfer; vasectomy), tissue sampling (using an invasive method for genotyping) and effects caused by the phenotype of the genetic alteration.

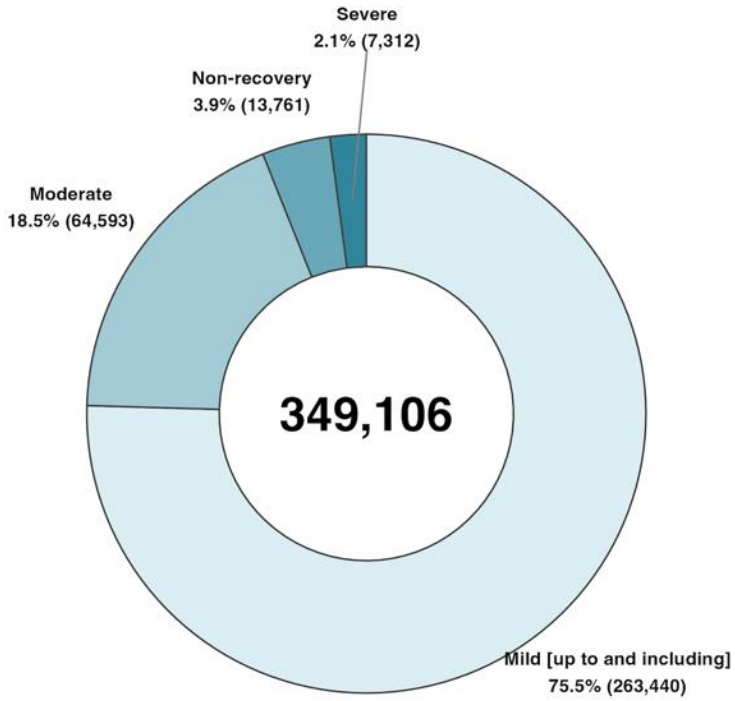


Figure 26: Uses of animals for the creation of new genetically altered animal lines by severities in 2022

IV.3.2.4. Reuses

In 2022, the number of reuses for the creation of new genetic lines was 2.3% (Table 34).

	Yes	No
Mice	0% (42)	100% (250,509)
Rats	0% (0)	100% (1,910)
Hamsters (Syrian)	0% (0)	100% (120)
Rabbits	0% (0)	100% (216)
Pigs	0.7% (2)	99.3% (277)
Goats	0% (0)	100% (38)
Sheep	0% (0)	100% (6)
Marmoset and tamarins	0% (0)	100% (8)
Other mammals	0% (0)	100% (6)
Domestic fowl	0% (0)	100% (612)
Xenopus	0% (0)	100% (4,160)
Other amphibians	0% (0)	100% (1,324)
Zebra fish	9.4% (7,829)	90.6% (75,035)
Other fish	0% (0)	100% (3,780)
Salmon, trout, chars and graylings	0% (0)	100% (3,232)
Total	**2.3% (7,873)**	**97.7% (341,233)**

Table 34: Reuse of animals used for the creation of new genetically altered animal lines in 2022

IV.3.3. All uses of animals for the maintenance of colonies of established genetically altered animal lines

Directive 2010/63/EU requires Member States to report animals used for the maintenance of colonies for genetically altered animals. This category 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* before being killed.

This category also includes genetically altered animals 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 (tissue sampling/ genotyping), which was not carried out for the purposes of 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 marking of the animal.

Given the complexity of the reporting obligations, errors in the reporting of uses under maintenance of colonies continued to be detected. The new guidance document on genetically altered animals under the Directive, finalised in November 2021[4], is expected to improve consistency and accuracy of reporting.

IV.3.3.1. Maintenance of colonies of established genetically altered animal lines by genetic status

In 2022, 315,828 uses were reported under the maintenance of colonies of established genetically altered animal lines. Amongst these uses, 82.4% were genetically altered without a harmful phenotype, 11.9% with a harmful phenotype and 5.8% without genetic alteration (Figure 27).

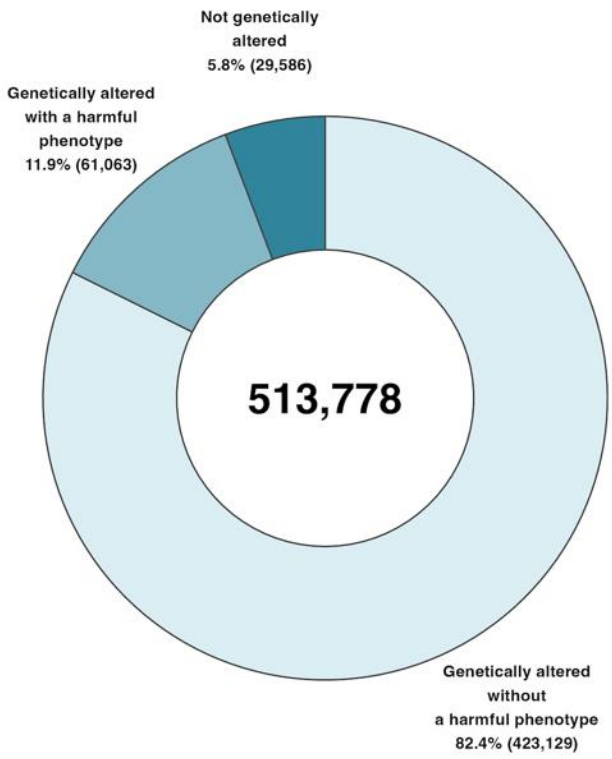


Figure 27: Genetic status of animals used for the maintenance of colonies of established genetically altered animal lines in 2022

IV.3.3.2. Maintenance of colonies of established genetically altered animal lines by severity

In 2022, 87% of the uses the severities remained at mild (and up to mild) level (Figure 28). Drawing from the previous figure 27 in which it was stated that 82.4% percent of animals were of non-harmful phenotype, the severities seem to relate to the effects of tissue sampling (invasive genotyping). For those classed as having a harmful phenotype, the severities can be linked to the phenotype and invasive tissue sampling. Where animals are found dead and no informed decision can be made on the cause and the events preceding death, this results in reporting these as ‘severe’.

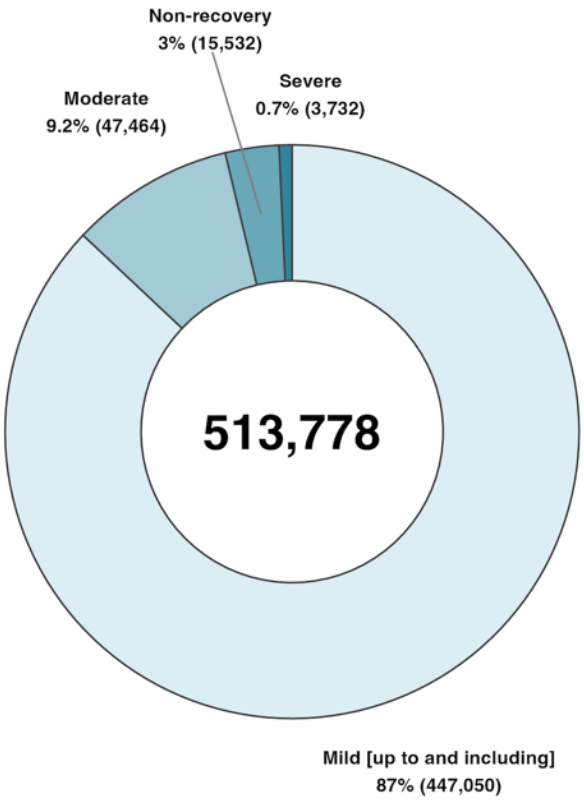


Figure 28: Uses of animals for the maintenance of colonies of genetically altered animal lines by severity in 2022

IV.3.3.2. Maintenance of colonies of established genetically altered animal lines by species

Mice and rats are the most common genetically altered animals used for scientific purposes and are therefore the main species also used for the maintenance of colonies.

	2018	2019	2020	2021	2022	Change 2022 vs 2021	Change 2022 vs 2018
Mammals							
Rodents							
Mice	518,180	336,427	292,513	279,911	**463,034**	+65.4%	-10.6%
Rats	3,827	3,935	8,214	10,193	**10,744**	+5.4%	+180.7%
Other rodents	0	0	0	0	**41**	NA	NA
Carnivores							
Dogs	5	10	14	7	**27**	+285.7%	+440%
Farm animals							
Pigs	0	0	92	0	**0**	NA	NA
Birds							
Domestic fowl	219	0	0	0	**0**	NA	-100%
Amphibians							
Xenopus	0	0	0	0	**19**	NA	NA
Fish							
Zebra fish	7,840	11,520	2,001	25,144	**39,189**	+55.9%	+399.9%
Other fish	1,262	911	1,794	573	**724**	+26.4%	-42.6%
Totals							

	2018	2019	2020	2021	2022	Change 2022 vs 2021	Change 2022 vs 2018
Total	**531,333**	**352,803**	**304,628**	**315,828**	**513,778**	**+62.7%**	**-3.3%**

Table 35: Uses of animals for the maintenance of colonies of established genetically altered animal lines by species between 2018 and 2022

IV.3.3.3. Reuses

These reuses involved mainly three types of species: mice, rats and zebra fish (table 36).

	Yes	No
Mice	0.1% (667)	99.9% (462,367)
Rats	3.1% (328)	96.9% (10,416)
Other rodents	0% (0)	100% (41)
Dogs	0% (0)	100% (27)
Xenopus	0% (0)	100% (19)
Zebra fish	4.8% (1,871)	95.2% (37,318)
Other fish	0% (0)	100% (724)
Total	**0.6% (2,866)**	**99.4% (510,912)**

Table 36: Reuses by species for the maintenance of colonies of established genetically altered animal lines in 2022

V. Member State narratives 2022

Introduction

Member States submitted 2022 statistical data to the Commission using the categorisation of data attributes provided in the Annex III of Commission Implementing Decision 2020/569/EU. The submissions include data from all 27 Member States of the EU in 2022, and Norway.

The Member State data is available through public ALURES Statistical EU Database[22]

It is important to know that some Member States may require additional data to be reported at national level; for example, statistics on the number of animals killed for organs and/or tissue. Therefore, national statistical publications sometimes differ from the data reported to the Commission and published in ALURES.

In addition, each Member State has provided a narrative for their data of 2022 which can be found in this section of the EU summary report.

22 https://environment.ec.europa.eu/topics/chemicals/animals-science/statistics-and-non-technical-project-summaries_en

Austria

(a) General information on any changes in trends observed since the previous reporting period:

In Austria, the total number of animals used for scientific purposes in 2022 is 211 338 (2021: 218 244) and is within the fluctuation range of the last 10 years (approximately -3.2 % or in absolute numbers -6 906 animals). The numbers include all uses (first and any subsequent reuse) of animals for the purposes of research, testing, routine production and education (including training) as well as uses of animals for the creation and maintenance of genetically altered animals.

186,026 animals were **used for the first time** for research, testing, routine production and education and training purposes which is an increase of 2 615 animals compared to 2021 (total number of animals used 183 411).

All uses of animals for research, testing, routine production and education and training purposes increased from 184 419 animals in 2021 to 186 698 animals, mainly mice and other fish, in 2022. Approx. 50 % were classified as mild (up to and including) procedures. Severe procedures reduced from approx. 12 % in 2021 to 8 % in 2022.

Numbers of animals used for the **creation of new genetically altered animal lines** for the first time decreased from 28 259 in 2021 to 18 648 animals, mainly mice and zebra fish, in 2022. Approx. 93 % were classified as mild (up to and including) procedures.

A total number of 5 992 animal uses (only mice) are reported in 2022 for the **maintenance of genetically altered animal lines**. Approx. 70 % were classified as mild (up to and including) and 30 % as moderate procedures.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

In AT significant annual fluctuations in different categories are observed which can be explained by the varying focus on different research areas and research activities:

In 2022 an increase in **all uses of animals** is observed in “Translational and applied research” (from 71 012 animals used in 2021 to 75 152 in 2022), mainly in the categories “Human Cancer” and “Diagnosis of Diseases” (from 25 animals used in 2021 to 1 437 in 2022), and “Preservation of species” (from 137 animals used in 2021 to 1 520 in 2022)

A decrease in all uses of animals is observed in “Basic Research” (from 94 513 animals used in 2021 to 93 482 in 2022), mainly in the category “Multisystemic” (from 10 601 animals used in 2021 to 3 812 in 2022), and “Regulatory use and Routine production” (from 13 918 animals in 2021 to 10 698 in 2022).

A decrease of animals used for the **creation of new genetically altered lines** was observed in “Basic research” (from 27 868 animals used in 2021 to 18 636 in 2022) uses, mainly in the categories

"Oncology" (from 6 701 animals used in 2021 to 1 756 in 2022) and "Multysystemic" (from 14 503 animals used in 2021 to 10 181 in 2022)

There is an increase observed in numbers of animals used for the **Maintenance of genetically altered animal lines** from 5 566 animals in 2021 to 5 992 animals in 2022.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

Approx. 50 % of **all uses of animals** for research, testing, routine production and education and training purposes were classified as mild (up to and including) procedures. Severe procedures reduced from approx. 12 % in 2021 to 8 % in 2022.

Approx. 93 % of uses for the **creation of new genetically altered animal lines** were classified as mild (up to and including) procedures (compared to 90 % in 2021).

Approx. 70 % of uses for the **maintenance of genetically altered animal lines** were classified as mild (up to and including) (78 % in 2021) and 30 % as moderate (22 % in 2021) procedures.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

The competent authorities promote the 3R principle at all steps of the authorization processes, in particular by putting emphasis on minimizing pain, suffering, distress and lasting harm by adequate humane endpoints. During inspections specific refinement methods and best practices in housing and care conditions for the animals are discussed and further implementation and dissemination is encouraged.

The Austrian Government promotes projects to further advance the development of alternative approaches which could reduce the number of animals used in procedures or which refine procedures or which replace the use of animals at all. In addition, the national 3Rs centre is supported to promote alternative approaches to animal testing and to disseminate information and implement 3R best practice. The 3R centre organises open-access seminar series addressing current 3R-relevant developments. Furthermore, the Austrian Government annually awards and honours outstanding scientific publications that have or could have major impacts on the 3Rs.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

"Other mammals" (total 113 animals) include i.a. Barbastella barbastellus (33), Lepus europaeus (15), Cervus elephalus (17), Myotis spp. (12), Pipistrellus spp. (43),
"Other birds" (total 229 animals) include i.a. Sylvia atricapilla (50), Falco spp. (31), Corvus spp. (33), Coturnix coturnix (18),

"Other fish" (total 13 611 animals) include i.a. Alburnus spp. (3 778), Squalius cephalus (1 200), Rutilius spp. (1 625), Chondrostoma nasus (1 600), Oryzias latipes (1 446), and

"Other amphibian" (total 3 759 animals) include mainly Ambystoma mexicanum (3 640).

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

n.a.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

n.a.

- **Species:**
- **Numbers of animals:**
- **Whether exceeding the 'severe' classification was pre-authorised or not:**
- **Details of the use:**
- **Reasons why the 'severe' classification was exceeded:**

Procedures involving severe pain, suffering or distress that is likely to be long-lasting and cannot be ameliorated, as referred to in Article 15(2) were not performed.

Belgium

(a) General information on any changes in trends observed since the previous reporting period:

The total number of animal procedures conducted in 2022 encompass:

- all uses of animals for research, testing, routine production, education, and training purposes (433 495);
- all uses of animals for the creation of new genetically altered animal lines (23 732) and
- all uses of animals for the maintenance of genetically altered animal lines (16 102).

In 2022, there were 473 329 animal procedures conducted, which marked a slight decrease compared to the 477 675 procedures performed in 2021. This reduction amounted to 4 346 fewer animal procedures, accounting for an approximate decline of 0.91%.

All uses of animals for research, testing, routine production, education, and training purposes (433495).

In this category, there was a slight decrease, with 434 327 uses in 2021 and 433 495 in 2022.

When looking at the species used for research, testing, routine production, education, and training, certain trends emerge. Procedures involving mice increased by around 5.9% from 2021 to 2022, while the use of rabbits showed a modest increase of around 2.9%. The use of domestic fowl shows a significant decrease of roughly 24.3%. This decrease can be partly explained by a reduction in the number of feed additive studies. In such studies, the primary parameter is often feed conversion, which can be evaluated at group level and requires multiple replicates of several animals to provide sufficient statistical power. The number of Zebra fish procedures decreased by around 4.7% and the number of rat procedures remained relatively stable with a slight decrease of about 0.4%. Less commonly used species show more pronounced variations. However, due to the small sample sizes, these fluctuations are not relevant when considering general trends.

Looking at the reuse of animals used for research, testing, routine production, education, and training, we see that that the number of reuses remains stable. In 2021, the reuse rate was 0.68%, and in 2022, it was 0.65%.

All uses of animals for the creation of new genetically altered animal lines (23732).

There is a decrease in the use of animals for the creation of new genetically altered lines (-17%). This decrease is mainly due to a decrease in the number of animals used to establish new lines in basic research on the immune system (2021: 5 801; 2022: 680), on the nervous system (2021: 7 048; 2022: 5 541) and oncology (2021: 7 746; 2022: 5 920). This does not seem to be related to the total number of animals used in these fields. There is, in fact, an increase in the use of animals for basic research on the nervous system and in oncology. However, there is a decrease in studies related to the immune system.

When analysing the use of animals for the creation of new genetically altered animal lines, some notable trends concerning the species used emerge. The number of procedures on mice shows a

significant decrease of about 16% from 23 790 in 2021 to 19 980 in 2022. This is related to the above-mentioned decrease in the number of animals used to establish new lines in basic research on the immune system, the nervous system, and oncology. Conversely, the number of procedures on rats increased from 133 in 2021 to 180 in 2022,an increase of about 35%. Of course, this involves a much smaller absolute number. In addition, goats were introduced into this category in 2022, with 32 procedures, marking a new trend in their use for genetic research. This occurred in an applied study in the field of Animal Diseases and Disorders. Xenopus, which had no recorded procedures since 2017, was reintroduced in 2022 with 84 procedures, indicating a new use for the creation genetically altered animal lines. This involves a specific user working with modified Xenopus lines in the field of basic research on the nervous system and basic research on oncology. Finally, Zebra fish shows a decrease in procedures of around 23%, from 4 395 in 2021 to 3 336 in 2022. This decrease is distributed across different domains where animals have been used to create genetically modified lines.

All uses of animals for the maintenance of genetically altered animal lines (16102).

In this category, use increased from 14 760 uses in 2021 to 16 102 uses in 2022. This represents an increase of about 9.08%.

Looking at the use of animals for the maintenance of genetically altered animal lines in 2022, we see that the number of procedures on mice increased slightly from 14 041 in 2021 to 15 906 in 2022, an increase of about 13.2%. In contrast, the number of procedures on rats decreased significantly, from 719 in 2021 to only 48 in 2022, indicating a significant decline. The use of Zebra fish, which had 120 procedures in 2020 and 0 procedures in 2021, saw an increase to 148 procedures in 2022.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

The number of animal procedures in the 'Basic research' category decreased between 2015 and 2020 but increased in 2021 and continued to increase in 2022. In 2022, the use in this category was close to the pre-pandemic level of 2019.

In particular, there was a significant increase in the number of uses in oncology. For example, there was an increase of 50.31% in 2022 (61 834 uses) compared to 2019 (41 138 uses). The main areas of research in the basic research category in 2022 were oncology (32.68%), immune system (17.77%) and nervous system (15.36%).

A decrease of 12.23% was observed in the ‘Translational and applied research’ category in 2022 (135 034 procedures) compared to 2021 (153 838 procedures). The temporary increase in the number of procedures in this category in 2021 (from 120 195 in 2020 to 153 838 in 2021, an increase of 28%) has now been reversed. In terms of the number of uses, it is back to a level comparable to 2019 (130 712 uses). Between 2021 and 2022, the largest decrease was observed in the categories of ‘Animal diseases and disorders’ (-11 863 uses, i.e. a decrease of 25%). This is due to several factors. On the one hand, there is a decrease in the number of studies on feed additives, as mentioned above. On the other hand, efforts have been made to refine experimental designs. For example, there has been a strong focus on better characterisation of pathogenic strains, including the validation of different animal models. This allows the desired effect to be assessed with a smaller number of animals.

The category ‘Higher education’ doubled in 2022 compared to 2021. The majority of species were rodents but birds account for a third of the species used in this category. These are practical sessions for bioengineering students learning to work with chickens as farm animals, with a particular focus on the hatching process.

The category ‘Training for the acquisition, maintenance or improvement of vocational skills’ increased by 40.69% in 2022 compared to 2021. Three-quarters of the animals used in this category were rodents but 15% were birds (domestic fowl and turkeys) and 10% were pigs. The use of domestic fowl and turkeys involves inducing certain conditions in the animals in order to educate and train poultry veterinarians and professionals working in the field to recognise injuries caused by these conditions and to intervene promptly. In the case of pigs, the main aim is to practise various surgical techniques using robotic surgery. Training on live animals is the final training step required to closely mimic working on live humans.

The category ‘Protection of the natural environment in the interests of the health or welfare of human beings or animals’ increased dramatically in 2022 compared to 2021. In 2022, a Zebra fish project (200 uses) focused on improving the assessment and identification of endocrine-disrupting substances, including resorcinol, to protect human health and the environment. Cattle (39 uses) were also involved in research on environmental aspects of agriculture. In addition, amphibians (70 uses) were used to improve control measures for invasive American bullfrog populations using sterile individuals.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

There has been a downward trend in the severe category in recent years. However, since 2020, the share of severe procedures has remained stable at around 11% of total animal use.

Despite what appeared to be a trend within the mild (downward trend) and moderate (upward trend) categories in recent years, this does not continue in 2022. Instead, in 2022 there is an increase of 8.9% within the mild category and a decrease of 10.07% within the moderate category.

We observe a significant increase in the mild category in ‘Basic research’ (from 88 193 mild uses in 2021 to 99 435 mild uses in 2022) and in the 'Regulatory use and routine production' category (from 68 593 mild uses in 2021 to 92 207 mild uses in 2022). The latter is partly explained by a shift in severity from moderate to mild within the 'Blood products' category.

For moderate procedures, there is an increase in ‘Basic research’ (from 67 632 moderate uses in 2021 to 72 461 moderate uses in 2022). As mentioned above, there is a decrease in the number of moderate uses in the 'Regulatory use and routine production' category. There was also a decrease in the number of moderate uses in the 'Translational and applied research' category (from 71 502 moderate uses in 2021 to 52 507 moderate uses in 2022). This is related to the overall decrease in the 'Translational and applied research' category in 2022.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

In addition to efforts at the user level, the regions responsible for promoting the 3Rs (Replacement, Reduction, Refinement) have taken the following initiatives:

- In the Brussels-Capital Region and the Flanders Region, continuation of the RE-Place project (database pooling expertise on alternative methods to animal testing) and funding of several specific 3R research projects.
- In the Brussels-Capital Region and the Walloon Region, continued funding of the project that wants to ensure the pooling of equipment and skills (around the implementation of alternative methods to animal experimentation) of the entire French-speaking scientific community in Belgium.
- In the Brussels-Capital Region, continued funding for the creation of a Brussels Platform for 3R Alternatives (IC-3Rs). This platform will provide the Brussels-Capital Region with a competent centre for the promotion of animal welfare and the application of the 3Rs in order to have a significant impact, ranging from the increased adoption of innovative techniques and 3R alternatives to the development of policy and regulatory measures.
- In the Flemish Region, drawing up of an action plan in collaboration with researchers and organisations to reduce the number of animal procedures in the region and beyond.

Although efforts have been made to promote the 3Rs for a number of years, it is not possible to make a clear statement about their impact on the statistics.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

With regard to the use of fish, the category 'Other fish' accounts for 17.05% in 2022. Within this category, it consists mainly of *Oreochromis niloticus* (3 351 uses, or 39%), *Rutilus rutilus* (1 290 uses, or 15%) and *Anguilla anguilla* (1 216 uses, or 14%). These species are only used in the category of basic research, of ethology, animal behaviour and animal biology and for species preservation.

Regarding the use of amphibians, the use of 'Other amphibians' accounts for 20.22% in 2022. In this category, it is mainly the use of *Lithobates catesbeianus* (70 uses, or 93.33%) for the protection of the natural environment in the interest of human or animal health or welfare.

At the purpose level, within the category 'Regulatory uses – quality control', the category 'Other quality control' accounts for 14.41% (or 4 420 uses) of the total. These 4 420 mice were used for EPO potency determination. More mice were used for this purpose in recent years compared to 2019 due to the need to validate new standards for erythropoietin. In addition, there was a higher use in 2022 due to a shortage of EPO raw material in 2021, which required more batches to be tested to make up the shortfall.

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

169 animals were used in the category 'Monoclonal and polyclonal antibodies (excluding ascites)', including 115 mice, 53 rats and 1 *Llama Glama*. In general, users prefer in vitro methods such as the phage display method, but sometimes it appears that synthetic monoclonal antibodies are slightly different from animal antibodies and are more difficult to produce. Animals are still used for the production of polyclonal antibodies and no alternative method has been validated. Users report that it would be possible to produce polyclonal antibodies in vitro by mixing monoclonal antibodies, but these mixtures never fully reproduce the performance of the in vivo polyclonal antibody, especially for complex antigens (e.g. cells). In addition, some products require marketing authorisation and cannot be easily changed. These products are used to make a life-saving drug for which there is currently no alternative.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

As in previous years, there were no cases where the 'severe' classification was exceeded.

- **Species: /**
- **Numbers of animals: /**
- **Whether exceeding the 'severe' classification was pre-authorised or not: /**
- **Details of the use: /**
- **Reasons why the 'severe' classification was exceeded: /**

Bulgaria

(a) General information on any changes in trends observed since the previous reporting period:

The total number of animals, used for 2022, compared to 2021 is reduced. This is due to the fact, that our country is trying to implement the 3Rs and on the other hand, due to the fact, that some of the sessions in the universities were performed online and/or some of the tests were filmed. The used animals are only animals born in the EU at register breeder. The number of used rana, other amphibians and pigs are increased, the number of used rats, mice and rabbits are reduced.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

The number of used for 2022 animals, compared to 2021 is reduced. In some cases, depending on the project type, the Ethic Commission made recommendations for reduction of the used animals. The project authorization is not given of projects, who do not consider with the recommendation. It was asked, some of the tests to be filmed. On the other hand, this is due to the online sessions in the universities.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

The procedures with non-recovery severity are increased.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

The number of re-used animals is decreased. Often, the Ethic Commission made recommendations for reduction of the used animals.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

The high percentage of basic research reported by Bulgaria for 2022 is due to:

- the large number of requested and permitted projects to defend dissertation works related to animal nutrition;

- the increased interest in researching the impact of new food additives that can be included in the composition of animal feed, respectively and researching their impact on the quality of production in farm animals.

The increased number of used "other" categories, due to the fact, that for training purposes **"*rana ridibunda*"** is used.

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

There are no such animals used.

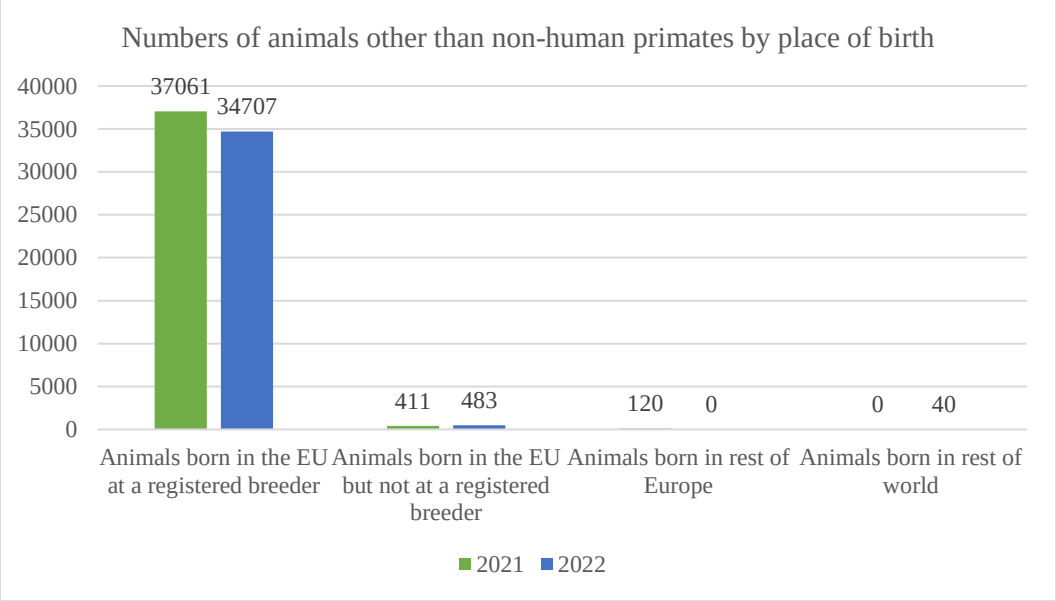
(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

There are no such cases.

- **Species:**
- **Numbers of animals:**
- **Whether exceeding the 'severe' classification was pre-authorised or not:**
- **Details of the use:**
- **Reasons why the 'severe' classification was exceeded:**

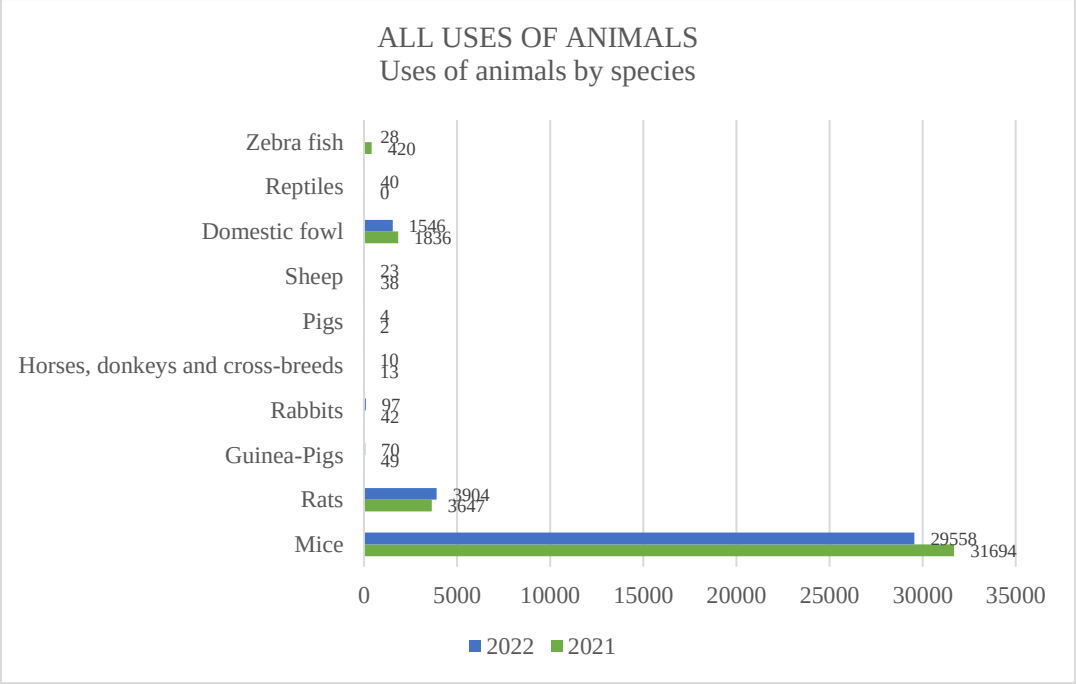
Croatia

(a) General information on any changes in trends observed since the previous reporting period:



Main changes observed in relation to the origin of animals are:

- Animals born in the EU at a registered breeder decreased by 2,354
- Animals born in the EU but not at a registered breeder increased by 72
- Unlike the previous year 2021, no animals were born in 2022 in the rest of Europe
- The introduction of 40 animals born in the rest of world between 2021 and 2022.



The main changes observed in relation to the use of animals per species between 2021 and 2022 were:

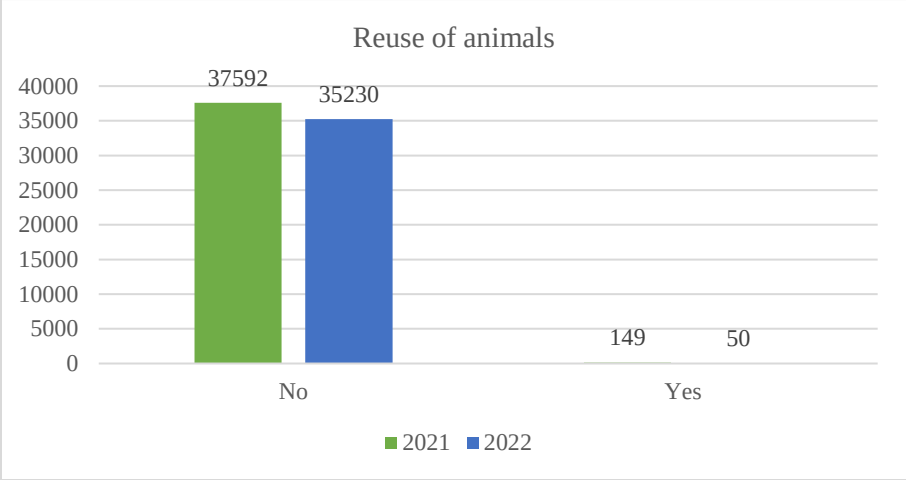
- Decrease in the use of mice (-2,136), sheep (-15), domestic fowl (-290) and zebra fish (-392)
- Increase in the use of rats (+257), guinea-pigs (+21), rabbits (+55)
- Pigs saw a slight increase (+2) and horses, donkeys and cross-breeds saw a slight decrease (-3)
- 40 reptiles were used in 2022 compared to none in 2021.

When categorize the total use of animals at cold-blooded vertebrates and warm-blooded vertebrates, the following changes were observed between 2021 and 2022:

- A decrease of 352 Cold-blooded vertebrates (fishes and reptiles)
- Warm-blooded vertebrates also saw a decrease by 2,109 (birds and mammals).

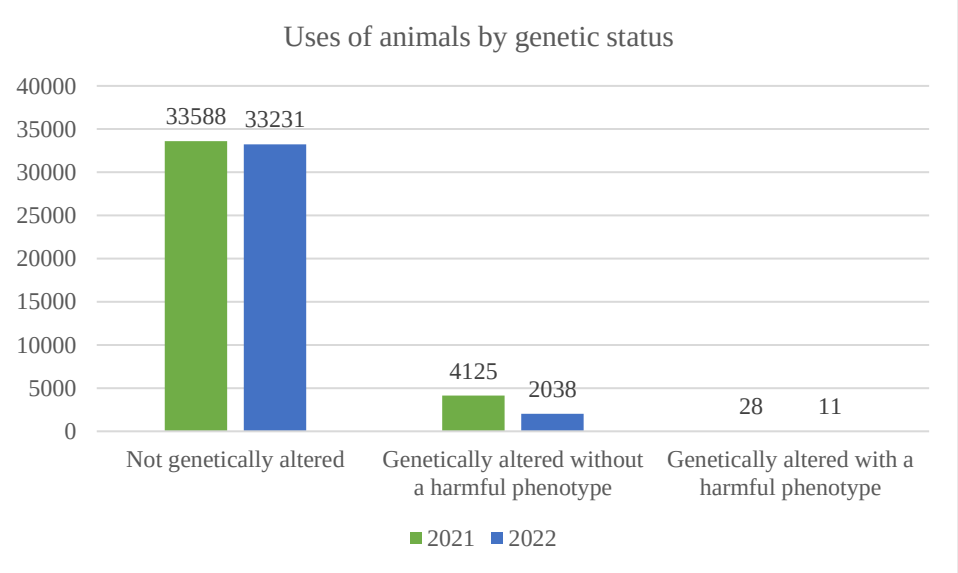
Regarding the categorization of animal species, mammals were the most common species used for scientific purposes in 2022, but saw an significant decrease; 1,819 fewer mammals were used.

No changes occurred from previous period regarding use of primates, dogs and cats while those species were not used for scientific purposes in Croatia.



In 2022, a decrease of 2,362 animals that were not reused in scientific purposes was observed, but also a decrease of 99 animals that were reused for the aforementioned purposes.

Out of a total of 35,280 animals used in 2022, 35,230 were used for the first time, and 50 of them were re-used again.



Regarding difference in the genetic status of animals between 2021 and 2022, the following was observed:

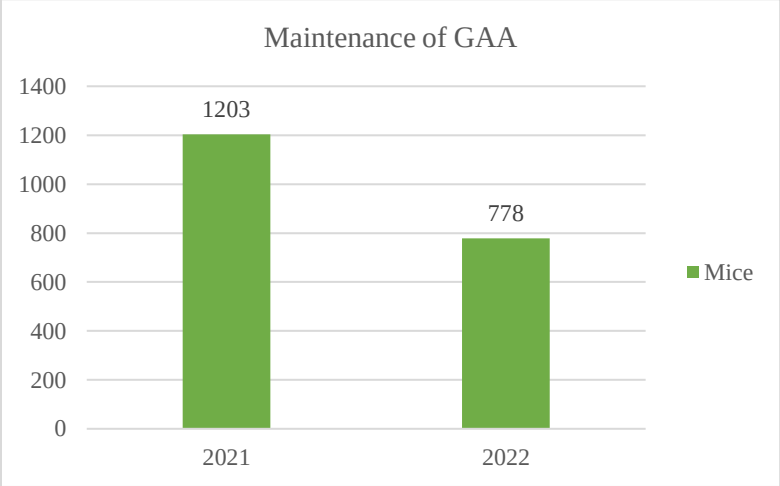
- No of not genetically altered animals decreased by 357 animals
- No of genetically altered animals without a harmful phenotype significantly decreased by 2,087
- No of genetically altered animals with a harmful phenotype decreased by 17 animals.

When changes were summed up overall decrease of 2,461 in the difference in genetic status in animals between 2021 and 2022 occured.

In 2022, 62 zebra fish were used for the creation of new genetically altered animal lines, which is 5 less than in the previous year.

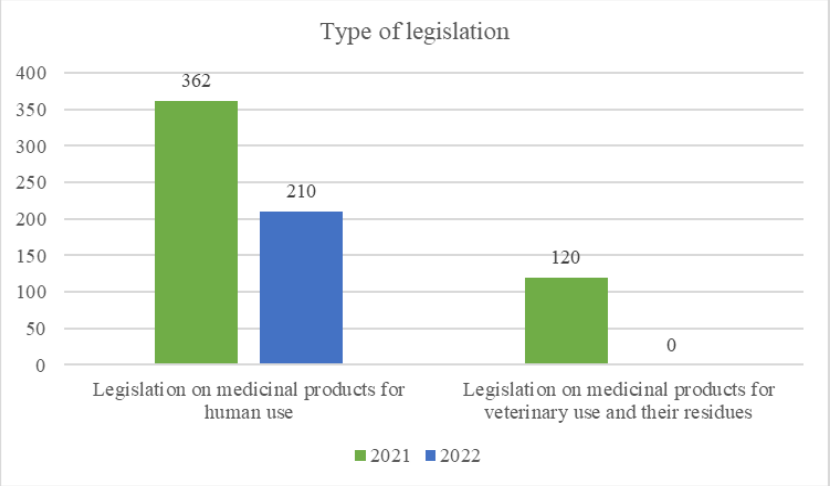
The same number (62 of them) was re-used procedures whose severity was “mild (up to and including)” for translational and appplied research uses – non-regulatory toxicology and ecotoxicology.

As for the genetic status, they were genetically altered without a harmful phenotype.



425 fewer mice were used in 2022 to maintain the GAA. Also, all 425 mice were used for the first time and all were genetically altered without a harmful phenotype.

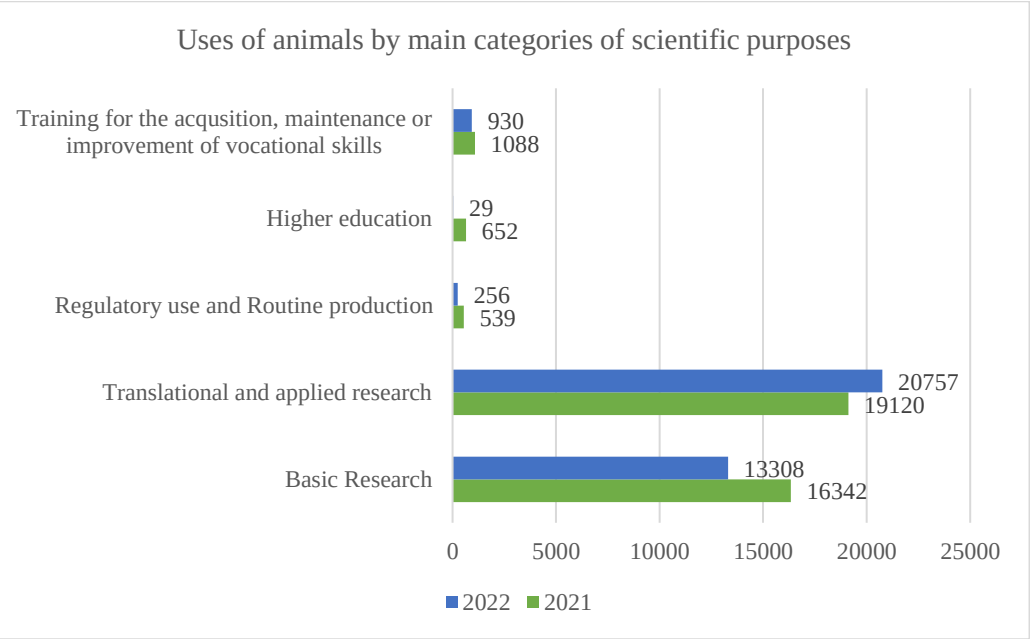
Severity of the procedures was “mild (up to and including)”.



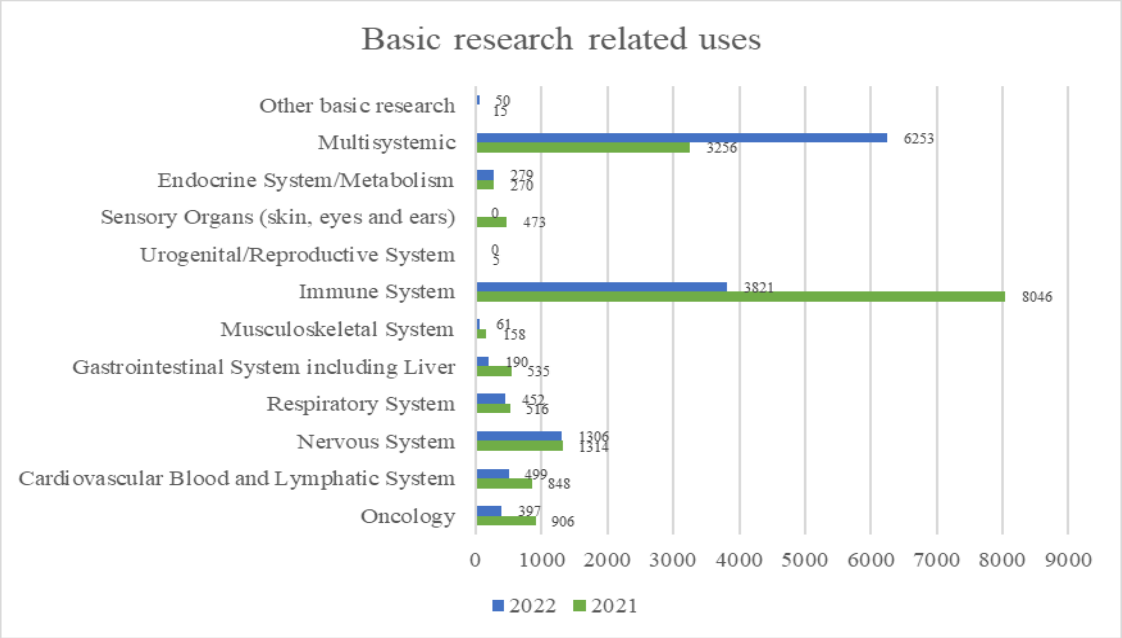
Between 2021 and 2022 there was a drop when it comes to legislative actions related to medicinal product for human use (by 152) which meet EU requirements.

None of the legislative actions were related to medicinal products for veterinary use and their residues.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:



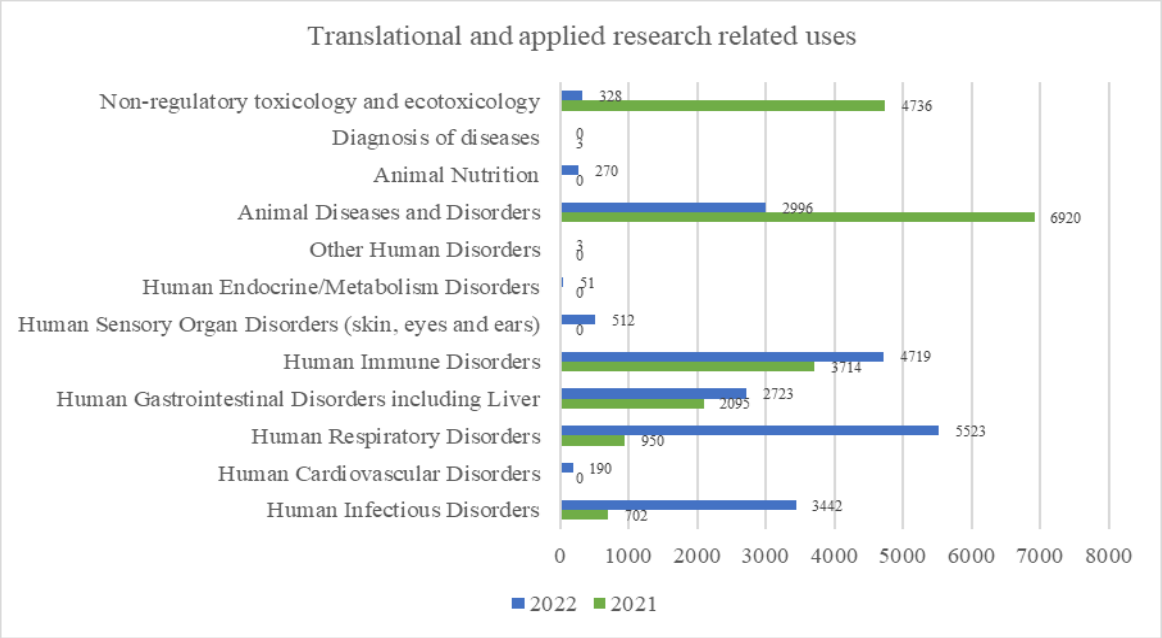
- *Basic Research:* 3,034 fewer animals used for basic research in 2022
- *Translational and Applied Research:* 1,637 more animals used for translational and applied research in 2022.
- *Regulatory Use and Routine Production:* 283 fewer animals used for regulatory use and routine production in 2022.
- *Higher Education:* 623 fewer animals used for higher education in 2022.
- *Training for the Acquisition, Maintenance, or Improvement of Vocational Skills:* 158 fewer animals used for training purposes in 2022.



A significant decrease was recorded in the following purposes:

- *Oncology:*509 fewer animals used in 2022
- *Cardiovascular Blood and Lymphatic System:* 349 fewer animals used in 2022
- *Gastrointestinal System including Liver:*345 fewer animals used in 2022
- *Immune System:* 4,225 fewer animals used in 2022
- *Sensory Organs (skin, eyes, and ears):* 473 fewer animals used in 2022

Only significant increase was recorded in *Multisystemic purposes:* 2,997 more animals were used in 2022.



A significant increase was recorded in the following purposes:

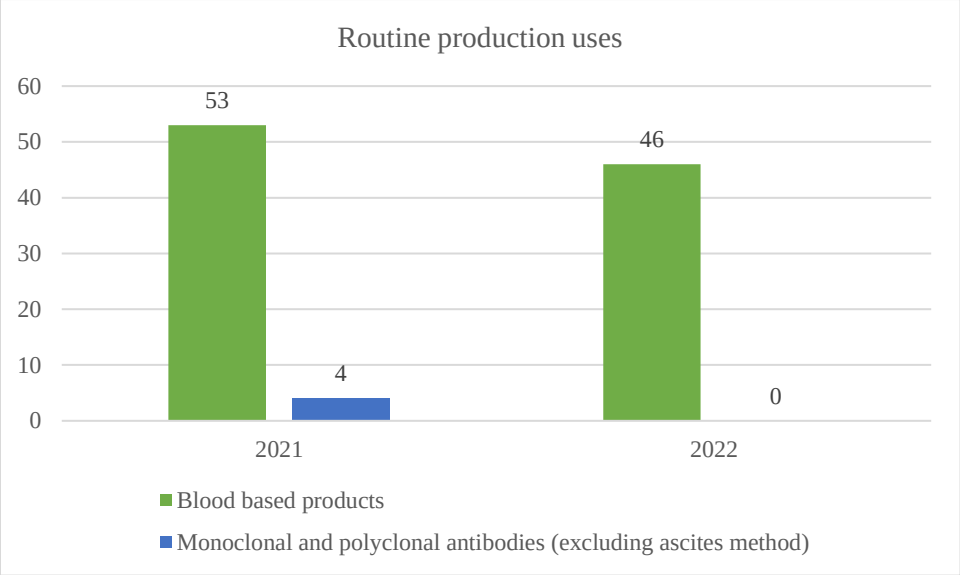
- *Human Infectious Disorders:* 2,740 more animals used in 2022
- *Human Respiratory Disorders:* 4,573 more animals used in 2022.
- *Human Gastrointestinal Disorders including Liver:* 628 more animals used in 2022
- *Human Immune Disorders:* 1,005 more animals used in 2022
- *Human Sensory Organ Disorders (skin, eyes, and ears):* 512 more animals used in 2022.

Significant decrease was recorded in the following purposes:

- *Animal Diseases and Disorders:* 3924 fewer animals used in 2022
- *Non-regulatory Toxicology and Ecotoxicology:* 4408 fewer animals used in 2022.

The difference in basic research related uses between 2021 and 2022 is 3,034 fewer uses.
The difference in translational and applied research related uses between 2021 and 2022 is 1,637 more uses.

So, the difference between the two categories for the given years is the contrast in the magnitude of changes: there were fewer uses in basic research in 2022 compared to 2021, while there were more uses in translational and applied research in 2022 compared to 2021.

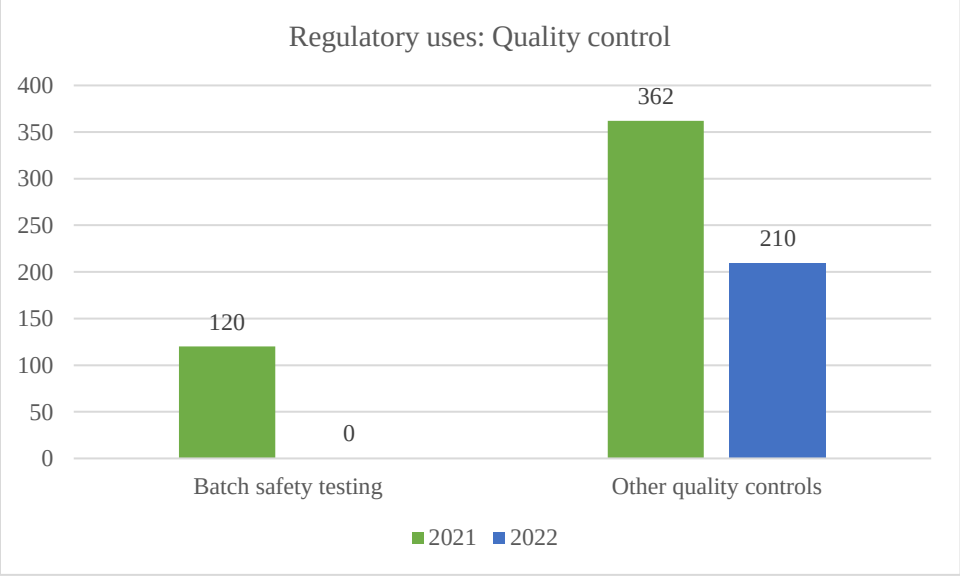


Blood-based products:

- an increase of 49 animals used for the production of blood-based products between 2021 and 2022.

Monoclonal and polyclonal antibodies (excluding ascites method):

- none of animals were used for the production of monoclonal and polyclonal antibodies (excluding ascites method) in 2022.



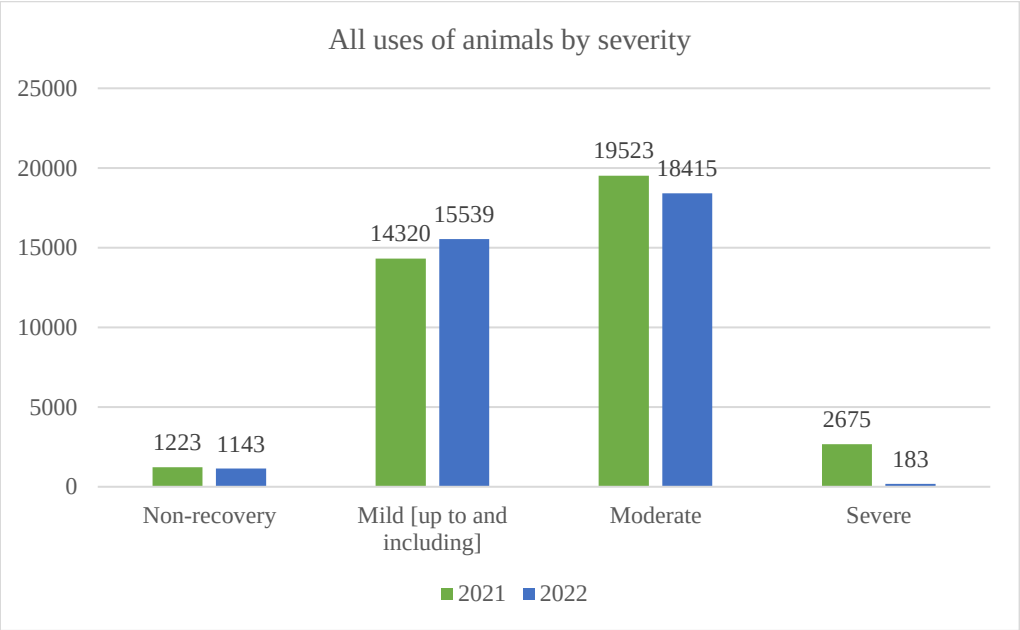
Batch safety testing:

- none of the animals were used for batch safety testing in 2022.

Other quality controls:

- a decrease of 152 animals used for other quality control purposes between 2021 and 2022.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:



Non-recovery:

- In 2022, this number decreased by 80 animals.

Mild [up to and including]:

- In 2022, this number increased by 1,219 animals.

Moderate:

- In 2022, this number decreased by 1,108 animals.

Severe:

- In 2022, this category showed the largest decrease; 2,492 fewer animals underwent procedures classified as severe.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

The national ethics committee pays special attention to the 3R principles during each evaluation of a particular project, and if they consider that the 3R principles can be improved, they act as an advisory body to each project applicant with instructions on how to improve them.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

In Basic research in category "other", 17 animals were used for the purpose of aging, one animal was used for the purpose of environmental impact on genome mutation and 32 animals were used for R&D of survival rations.

In category “Other quality controls” in accordance with regulations on medicinal products for human use, 210 mice were used for the purpose of potency testing of animal blood derivates for human use.

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

In 2021, no such uses reported.

(g) Details on cases where the ‘severe’ classification is exceeded, whether pre-authorised or not:

No such cases reported.

- **Species:**
- **Numbers of animals:**
- **Whether exceeding the ‘severe’ classification was pre-authorised or not:**
- **Details of the use:**
- **Reasons why the ‘severe’ classification was exceeded:**

Cyprus

(a) General information on any changes in trends observed since the previous reporting period:

A small change was observed in the number of projects that animals were used in comparison to 2021, specifically in 2022 the number of projects where animals were used was 23 whilst in 2021 was 29. Comparing to 2021, the number of animals used in 2022 fell slightly. As in previous years, the greater proportion of animals used in 2022, concerns mice. Also, a small number of dogs, rabbits and frogs were used. In addition, 60 frogs were reused-females frogs reused for ovulation in 2 projects.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

There were no significant changes in the use of animals in any specific areas.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

A decrease in the number of animals used under severe severity procedures is observed, comparing to 2021 and the animals used, concern the same project for both years.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

The National Committee for the Welfare of Animals used for Scientific Purposes, ensures the 3Rs implementation at the Project evaluation during the procedure for licencing.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

Not applicable for 2022.

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

There were no such cases for the year 2022.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

There were no such cases for the year 2022.

Czechia

(a) General information on any changes in trends observed since the previous reporting period:

The statistical data has been collected since 1993 in the Czech Republic. Overall numbers were decreased between 2021 and 2022. There were significant decrease numbers of animals in category *"Other legislation*". There were significant decrease numbers of animals in category "*other fish*". Opposite to this there is a significant increase of numbers of specie domestic fowl. There were Re-Use of 6 Rhesus monkey. Overall, there are increases in numbers Re-Use of animals. There is continual decrease in Creation of New GAA lines.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

Continual significant decrease numbers of animals in category "*Other legislation*" for purposes of waste legislation and toxicity of waste is due to changes of waste legislation.

Significant increase of numbers of domestic fowl in category "*Routine Production*" for purposes of acquisition of live attenuated lines of selected species of chicken coccidian for vaccine production is due to new user establishment and increase in production.

Significant decrease of numbers of animals in category "*Other Fish*" because of less experiments in category "*Animal nutrition*", "*Ethology*" and "*Animal Welfare*".

There were less uses of cattle and goats in category "*Higher education*", *"Training for the acquisition", "Maintenance or improvement of vocational skills", "Translational and applied research".*

The user establishment disappeared here so there is a less uses of sheep.

There is continual increase in category "*Basic research Multisystemic*". There were mainly experiments which included creation of new GAA lines which are not connected with research of one organ in organism and rederivation for "recovery" of animals breed for different purposes.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

There is significant increase of moderate uses between 2021 and 2022. This increase is due to increase of numbers of domestic fowl in category "*Routine Production*", "*Other product types*" for purposes of acquisition of live attenuated lines of selected species of chicken coccidian for vaccine production.

Opposite to this there is decrease of severe uses in 2022. There is also decrease of mild uses between 2020 and 2021. After continual decreases of non-recovery uses is in 2022 increase in this category of severity.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

Continual significant decrease numbers of animals in category "*Other legislation*" for purposes of waste legislation and toxicity of waste is due to changes of waste legislation. Waste legislation is going to require procedures without animals.

There is also continual decrease in sever category of severity.

We are expecting more impacts in subsequent years.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

In category "*Routine production*", "*Other product types*" 91.34 % of experiments were for purposes of acquisition of live attenuated lines of selected species of chicken coccidian for vaccine production and 8.66 % of experiments were for production of antigen of Rabbit haemorrhagic disease.

In category "*Testing by Legislation*", "*Other Legislation*" included mainly experiments for the purposes of waste legislation and toxicity of waste.

In category "*Other basic research*" is main part experiments in parasitology and toxicology with different species. For purposes of toxicology were often use species Cyprinus carpio.

In category "Other Fish", more than 43% are of specie Cyprinus carpio and 17% are of species Perca fluviatilis the most uses in category "Basic Research". Other species are Abramis brama, Acipenser baerii, Acipenser ruthenus, Alburnus alburnus, Ameirus nebulosus, Ameiurus nebulosus, Anguilla Anguilla, Aspius aspius, Barbatula barbatula, Barbus barbus, Blicca bjoerkna, Carassius auratus, Carassius Carassius, Carassius gibelio, Chondrostoma nasus, Clarias gariepinus, Cottus gobio, Cottus poecilopus, Ctenopharyngodon Idella, Cyprinus carpio (43%), Esox Lucius, Gobio gobio, Gymnocephalus cernuus, Hypophthalmichthys nobilis, Lepomis gibbosus, Leuciscus idus, Leuciscus leuciscus, Nothobranchius furzeri, Pseudorasbora parva, Rutilus rutilus, Sander lucioperca, Scardinus erythrophthalmus, Silurus glanis, Squalius cephalus, Tinca tinca, Vimba vimba.

Animals in category "Other birds" are often use for purposes Ethology / Animal Behaviour /Animal Biology, more than 31% are of species Hirundo rustica and 12% are of species Sylvia atricapilla and 10% are of species Phylloscopus collybita. Other species are Acrocephalus arundinaceus, Acrocephalus palustris, Acrocephalus schoenobaenus, Acrocephalus scirpaceus, Aegithalos caudatus, Aegolius funereus, Anas domesticus, Anas platyrhynchos, Anas platyrhynchos f. domestica, Anser domesticus, Anthus trivialis, Cairina moschata f. domestica, Carduelis carduelis, Certhia familiaris, Chloris chloris, Cinclus cinclus, Coccothraustes coccothraustes, Columba livia f. domestica, Columba livia f. domestica, Corvus frugilegus, Cyaniste caeruleus, Cyanistes caeruleus, Emberiza citronella, Emberiza schoeniclus, Erithacus rubecula, Ficedula albicollis, Fringilla coelebs, Glaucidium passerinum, Hippolais icterina, Lanius collurio, Linaria cannabina, Locustella fluviatilis, Locustella luscinioides, Melopsittacus undulatus, Motacilla alba, Numida meleagris f. domestica, Parus major, Passer montanus, Phasianus colchicus, Phylloscopus sibilatrix, Poecile palustris, Prunella modularis, Serinus canaria, Serinus serinus, Sitta europaea, Strix aluco, Sturnus vulgaris, Sylvia borin, Sylvia communis, Sylvia curruca, Turdus merula, Turdus philomelos, Turdus viscivorus.

"Other rodents" were use in different categories. Other species are Apodemus agrarius, Apodemus flavicollis, Apodemus sp., Apodemus sylvaticus, Clethrionomys glareolus, Fukomys anselli, Fukomys mechowii, Fukomys micklemi, Heterocephalus glaber, Lagurus lagurus, Mastomys coucha, Microtis arvalis, Microtus agrestis, Microtus arvalis, Microtus sp., Mus musculus, Myodes glareolus, Rattus norvegicus, Rattus rattus, Spalax ehrenbergi, Spermophilus citellus

In category "*Other amphibians*", more then 96 % are of specie Bufo bufo use in category Ethology / Animal Behaviour /Animal Biology.

In category "*Other mammals*", more than 43 % are of species Castor fiber use in category "*Protection of the natural environment in the interests of the health or welfare of human beings or animals*".

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

In category "*routine production*" were 0.68 % from "*routine production*" uses for "*production of monoclonal antibodies by mouse ascites method"* and 0.07% from "*routine production*" were uses (rabbits) for "*production of monoclonal and polyclonal antibodies (excluding ascites method)"*.

This Antibodies are a critical part of medical diagnostics to detect infections, allergies, tumours, hormones, or many other biological markers. Replacement of this specific antibodies from animals is not possible in this time due to quality of this antibodies.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

Classification "severe" was not exceeded in 2022.

- **Species:**
- **Numbers of animals:**
- **Whether exceeding the 'severe' classification was pre-authorised or not:**
- **Details of the use:**
- **Reasons why the 'severe' classification was exceeded:**

Denmark

This narrative has been drawn up solely on the basis of information submitted by users of laboratory animals in Denmark. This means that we have not gathered additional information from the facilities.

(a) General information on any changes in trends observed since the previous reporting period:

The number of laboratory animals in 2022 (222 837) is similar to the number in 2021 (220 611).

The most commonly used laboratory animals are:

- Mice (64.7%)
- Rats (11.1%)
- Pigs (10.8%)
- Salmon, trout, char, grayling (6.0%)
- Zebrafish (3.0%)
- Rabbits (1.4%)
- Other (3.0%)

Even if the number of laboratory animals in 2022 is very similar to that of 2021, there are nevertheless both increases and decreases in specific species that merit comment.

In the area of GMOs, there has been a steep drop from 2021 to 2022 in the number of mice (7 503 to 2 855), bringing the number back to the usual level from the period 2015-2020. More zebrafish were used in 2022 than in 2021 (904 vs 554). In some cases, the zebrafish is a better model animal than the mouse, as zebrasfish have a faster generational renewal and have been shown to be suitable for regeneration experiments.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

For the first time in many years, the use of non-human primates was recorded in Denmark (baboons (5) and prosimians (6)). Authorisation for this was granted to a zoo that used the primates in studies to improve anaesthetisation methods for specific ape species. The studies were carried out immediately prior to the planned killing of the animals.

The number of rabbits used has decreased by around 30% from 2021 (3 029 vs 4 363). A private company producing antibodies used around 1 000 fewer animals in 2022 than in 2021. This is not

necessarily attributable to the use of alternative methods ('replacement'), but more likely to supply and demand in the field of antibodies.

The number of dogs used has decreased by around 50% from 2021 (165 vs 328). One particular contract research laboratory used 150 fewer dogs in 2022 than in 2021, but we do not know the reason for this.

It is also worth noting that the number of pigs used in 2022 is the highest since the entry into force of the Directive (2010/63/EU). A total of 23 641 pigs were used, which is an increase of 38% on 2021. The increase can be explained by increased research into more environmentally friendly production conditions and animal welfare (for example, umbilical hernias and piglet mortality) in conventional farming.

The number of animals reported in the 'Other mammals' category has also increased significantly since 2021 (110 vs 26). Of these 110 animals, 50 are hedgehogs, used in a research project to investigate the impact of robot lawnmowers on wild hedgehogs.

A further 55 animals were used in relation to the EU Habitats Directive (25 grey seals, 25 harbour seals and 5 harbour porpoises). The two seal species and the porpoises are all included under the Directive, and protection of these animals is therefore required. Movements and preferred habitats of wild harbour porpoises and seals cannot be studied by any means other than marking the animals. For example, this is necessary to answer specific questions about their vulnerability to changes in their habitat caused by fishing, construction works, underwater noise and climate change.

The remaining 5 of the 110 animals are 5 white rhinoceroses, on which blood tests were carried out by a zoo during pharmacokinetic studies.

It is also notable that in the category 'domestic fowl', 77% fewer animals were reported in 2022 than in 2021 (355 vs 1 534). Animals in this category are mainly used in connection with the development and testing of vaccines for animals of relevance to agriculture. In addition, animals in this category have been used in studies to provide knowledge to improve poultry production (laying hens and broilers) in order to reduce nutrient release to the environment (for example nitrogen and phosphorus) and to improve animal health and welfare. The reason for the 77% drop in this category has not been determined.

The number of sea bass also decreased sharply between 2021 and 2022 (2 762 vs 660), a fall of 76%. The fall can be attributed to a completed research project on the optimisation of housing conditions at fish farms.

The salmonids category (salmon, trout, char, grayling) increased by 50% from 2021 to 2022 (8 761 vs 13 113). The increase is likely to be due largely to the initiation of infection pathology studies linked to the following reference laboratories: the national reference laboratory for fish, crustacean and bivalve mollusc diseases, the EU reference laboratory for fish and crustacean diseases, and the OIE reference laboratory for the fish disease VHS (viral haemorrhagic septicaemia).

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

The proportion of laboratory animal uses in 2022 that were categorised as ‘severe suffering’ is 1.9%.

In Denmark, the use of animals in the highest severity category can be divided into two sub-groups: disease models for conditions causing particular suffering in humans, and studies required by law with an unknown end point.

The Danish 3R-Center and the Danish National Committee for the Protection of Animals used for Scientific Purposes have focused for a number of years on those laboratory animals which are subject to experiments in the highest severity category in order to gain up-to-date knowledge in the field and to discuss ways of reducing the impact on the animals with researchers.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

Considerable efforts are being made in Denmark by both the Danish 3R-Center and the Danish National Committee for the Protection of Animals used for Scientific Purposes. For instance, they organise an annual international 3R symposium for researchers in the field, an annual meeting of the country’s animal welfare bodies, cooperation with 3R organisations internationally, the distribution of research funding for 3R research, and extensive communication activities on two websites (3rcenter.dk/natud.dk). It is not known what impact the work of the 3R Center and the Committee has had on the number of research animals or on severity levels.

(e) Further breakdown on the use of ‘other’ categories if a significant proportion of animal use is reported under this category:

There were 149 laboratory animals reported in the category ‘other amphibians’, the vast majority of which (95%) are axolotls. They were used to gain an understanding of the processes that allow the axolotl to regenerate tissue.

There were 411 laboratory animals reported in the category ‘other birds’. The three most frequently used species in this category are zebra finches (28%), redstarts (19%) and willow warblers (11%), which together represent 58% of the birds used in this category. The remaining 42% comprises 22 other species.

Zebra finches, for example, are used in experiments to study how the ear develops in that species. Another experiment explored the possibility of developing the use of birds as a new model organism to identify more effective physiological mechanisms for oxygen transport in tissue. This research field is important to understanding how oxygen supply to tissue has limited the evolution of sensory systems, but also addresses issues that are highly relevant to many human circulatory diseases.

One research project used redstarts and willow warblers to learn about the spread of disease in birds with a focus on zoonoses. Over recent decades, there has been an increased focus on wild animals and birds in connection with the spread of disease because a number of emerging diseases originate or spread from animals – such as influenza, avian schistosomes, borrelia and West Nile virus. Some diseases, such as West Nile virus, have the potential to spread with migratory birds from southern

regions. Since 2011 blood samples have been collected to monitor West Nile virus in migratory birds.

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

Denmark has not recorded cases involving these categories.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

- **Species:**
- **Numbers of animals:**
- **Whether exceeding the 'severe' classification was pre-authorised or not:**
- **Details of the use:**
- **Reasons why the 'severe' classification was exceeded:**

Denmark has not exceeded the 'severe' classification so there is nothing to note here.

Estonia

(a) General information on any changes in trends observed since the previous reporting period:

3 324 animals were used for scientific or educational purposes during 2022. Compared to 2021 (3 886 uses), there has been a slight decrease in the use of animals. No animals were reused in 2022. No significant change compared to previous year in the use of cattle, pigs and rabbits. Use of domestic fowl and other birds has increased along with use of mice and rats. Decrease can be detected in the use of all fish.

	2021		2022	
Species	**No of animals**	**% of animals**	**No of animals**	**% of animals**
Cattle	254	6.54%	243	7.31%
Domestic fowl	74	1.90%	139	4.18%
Mice	1 563	40.22%	1 838	55.29%
Other birds	302	7.77%	376	11.31%
Other fish	1 185	30.49%	161	4.84%
Other rodents			1	0.03%
Pigs	4	0.10%	4	0.12%
Rabbits	48	1.24%	51	1.53%
Rats	375	9.65%	511	15.37%
Salmon, trout, chars and graylings	81	2.08%		
Grand Total	**3 886**	**100.00%**	**3 324**	**100.00%**

All animals used in 2022 are born in the EU. Numbers of animals born in the EU at a registered breeder have increased. This is mostly due to reduced use of animals born in the wild in 2022 compared to previous years.

	2021		2022	
Place of birth	**No of animals**	**% of animals**	**No of animals**	**% of animals**
Animals born in the EU at a registered breeder	1 994	51.31%	2 400	72.20%
Animals born in the EU but not at a registered breeder	1 892	48.69%	924	27.80%
Grand Total	**3 886**	**100.00%**	**3 324**	**100.00%**

There has been a steady decrease in use of GA animals during the last years. All GA animals, that were used, were without a harmful phenotype.

	2021		2022	
Genetic status	**No of animals**	**% of animals**	**No of animals**	**% of animals**
Genetically altered without a harmful phenotype	592	15.23%	320	9.63%
Not genetically altered	3 294	84.77%	3 004	90.37%
Grand Total	**3 886**	**100.00%**	**3 324**	**100.00%**

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

	2021		2022	
Purpose	**No of animals**	**% of animals**	**No of animals**	**% of animals**
Basic Research	2 736	70.41%	2 083	62.67%
Protection of the natural environment in the interests of the health or welfare of human beings or animals	539	13.87%	111	3.34%
Regulatory use and Routine production	126	3.24%	190	5.72%
Training for the acquisition, maintenance or improvement of vocational skills	4	0.10%	35	1.05%
Translational and applied research	481	12.38%	905	27.23%
Grand Total	**3 886**	**100.00%**	**3 324**	**100.00%**

Changes compared to previous year are mostly due to research fields that scientists choose to pursue, the funding they get, and this reflects in the types of projects authorised. Decrease can be seen in uses for the protection of the natural environment. A project for this purpose was mainly conducted during 2021 (most animals authorised to be used, were used that year) and finalised in 2022. There is increase in animals used for translational research and it could be attributed to a slight increase in the number of authorised projects classified as such during the past couple of years. Most animals in this purpose category are used for human cancer research. But as in previous years, even though a decrease can be observed, in 2022 most animals were used for basic research where most animals were used for oncology research.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

Change can be seen in an increase of mild and decrease of moderate severities. Although numbers of animals used in mild procedures has not necessarily increased that much (by 64 animals) in 2022, the number of moderate uses has decreased significantly (by 543 animals). This comes from a substantial amount mice and fish being used in less severe procedures compared to previous years.

	2021		2022	
Severity	**No of animals**	**% of animals**	**No of animals**	**% of animals**
Mild [up to and including]	2 052	52.80%	2 116	63.66%
Moderate	1 623	41.77%	1 080	32.49%
Non-recovery	117	3.01%	123	3.70%
Severe	94	2.42%	5	0.15%
Grand Total	**3 886**	**100.00%**	**3 324**	**100.00%**

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

Laboratory Animal Science courses take place. Attention is paid to how the 3Rs are approached in project applications. During evaluation process all justifications are reviewed to make sure 3Rs are appropriately considered prior to authorisation.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

16.18% of all animals used in 2022 were categorised as "other" (significant drop compared to 38.26% in 2021). This includes 10 barn swallows (*Hirundo rustica*), 10 common reed warblers (*Acrocephalus scirpaceus*), 10 sedge warblers (*Acrocephalus schoenobaenus*), 192 common gulls (*Larus canus*), 154 pied flycatchers (*Ficedula hypoleuca*), 38 Eurasian perch (*Perca fluviatilis*), 111 European flounder (*Platichthys flesus*), 12 pikeperch (*Sander lucioperca*) and 1 (one) Siberian flying squirrel (*Pteromys volans*). Purposes of their use was for Basic Research, more specifically Ethology / Animal Behaviour / Animal Biology, Endocrine System/Metabolism and Protection of the natural environment in the interests of the health or welfare of animals.

Species list	2021	2022
Other birds	302	376
Other fish	1 185	161
Other rodents		1
Grand Total	1 487	538

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

Animals have been used for the purpose of routine production of monoclonal and polyclonal antibodies, excluding ascites method. 190 animals (139 domestic fowl, 51 rabbits) used in 2022 for this purpose. In the case of phage display and other methods not using live animals, the main drawback is that the antibodies that are isolated are less specific and often lack the necessary biological activity. Therefore, live animals are used to obtain biologically active and/or highly specific (polyclonal) antibodies that could be used for both therapeutic and *in vitro* diagnostic purposes.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

- **Species:** N/A
- **Numbers of animals:** N/A
- **Whether exceeding the 'severe' classification was pre-authorised or not:** N/A
- **Details of the use:** N/A
- **Reasons why the 'severe' classification was exceeded:** N/A

No such cases in 2022.

Finland

(a) General information on any changes in trends observed since the previous reporting period:

The overall picture changed little from the previous years. Procedures totalled 93 650 animals (compared to 104 886 in 2021). Mice were the main species used in different research areas, as well as in the creation of genetically altered animals. The creation of GM mice stayed at a similar level as the previous year. The use of rats, rabbits and dogs has decreased gradually over the years.

The number of procedures with dogs and cats remained at previous years' levels due to research projects in which disease genes or metabolic parameters were studied (182 cats and 1 209 dogs). In these projects, single blood samples were taken from pet animals.

(b) Information on a significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

The changes of procedures in different research areas can mostly be considered as a normal annual variation in animal research. There was a decrease in animal use in basic research (–12 611 procedures). However, the most important research areas (Oncology, Cardiovascular circulatory and lymphatic system, Nervous system, Immune system) continued with similar animal use as during previous years. The human nervous system and mental disorders was the main research area in the translational research. The use of animals for this purpose has shown a decreasing trend in recent years, still being 13 714 procedures in 2022.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

Severe procedures (3 401) were decreased as compared to the years 2020 (4 763) and 2021 (4 687). The main purpose for the use of animals (mice and rats) in severe procedures was the research of the human nervous system disorders and mental disorders (2 228). In the past years, there has been a reduction in severe procedures, which is due to effective use of 3Rs and more careful assessment of the actual severity of procedures for each animal. Moreover, testing of new medicinal candidates in animal models as a service depends on the needs of customers. This kind of animal use may so vary greatly in different years.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

FIN3R – the Finnish 3R Centre https://fin3r.fi/en organizes education and training on 3R issues for researchers. The activities started in 2022 with 4 webinar events. The centre also actively disseminates information about the events organized by other 3R centers. Sharing of knowledge about 3R possibilities is important to improve the quality of research and the welfare of animals.
The Project Evaluation Board has produced and published new guidelines with current references including issues with project application, experimental techniques, surgical operation, anaesthesia and analgesia:
https://avi.fi/en/services/individuals/licences-notices-and-applications/animals/laboratory-animals

(e) Further breakdown on the use of the 'other' categories if a significant proportion of animal use is reported under this category:

Other rodents used were bank voles (Myodes glareolus). Other carnivores were minks (Neovison vison) and foxes (Vulpes lagopus, Vulpes vulpes).

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

In routine production, polyclonal antibodies were produced in swine, sheep and rabbits for diagnostic purposes, altogether 446 procedures. The necessity to produce antibodies in animals was discussed with the producers during their project license process. The reason for the use of animals were difficulties in finding non-animal methods with enough reliability and effectiveness to produce antibodies in large volumes. The possibilities for starting non-animal production are supervised in inspections and when project licenses are processed.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

Severe classification was not exceeded in any of the procedures.

France

(a) General information on any changes in trends observed since the previous reporting period:

The 2022 survey comprises responses from all of the establishments approved for the use of animals for scientific purposes (user establishments). As a result of mergers or closures, the number of establishments has fallen from 589 in 2021 to 575. Of these 575 establishments, 40 stated that they had no uses to report for 2022.

In 2022 the Commission updated the guidance on genetically altered animals, which extended the scope of the survey (inclusion of data on farm animals genotyped by techniques not allowing identification but not used in an experimental procedure). As a result of this change of method, the total number of animal uses declared in 2022 is **2 128 058**, compared to 1 893 897 in 2021. If the same parameters are applied as the previous year, the number of uses for 2022 has decreased to 1 802 025, a fall of 4.9% on 2021. The steady downward trend observed in recent years continues.

Species used

The 2022 figures confirm the dominance of the mouse model in experimental procedures (**66%** of uses). Rabbits (**9%**) and rats (**7.8%**) remain the two next most popular species. Fish account for **8.7%** of uses, all species combined.

The number of uses of cats remains stable at **1 127**, compared to 1 018 in 2021 (a [10.]7% increase). These uses are largely for regulatory tolerance tests for veterinary drugs (39%) or studies regarding nutrition or veterinary medicine (39%). Of these uses, **88%** are classified as 'mild'.

Uses of dogs decreased by 9.6%, with **3 961** uses (4 383 uses in 2021). The proportion of reuses is **44%**. The tests carried out mainly concern regulatory toxicology in human or veterinary medicine (**84%**). Of these uses, **63%** are classified as mild.

Uses of primates increased from 3 593 to **4 147** in 2022, an increase of 13%. Of these uses, 45% concern regulatory testing in human medicine and 30% concern the production of biological fluids (mainly blood products), compared to 17% in 2021, or 629 additional uses.

The increase in the use of primates is attributable solely to an increase in production of blood products. These products are used by laboratories in France, but two thirds of them are also exported to other European countries and third countries.

Primates used for the first time are from parents born in captivity in **68%** of cases (F2 generation or higher; 76% in 2021). This fall was largely due to high global use of primates justified by a significant increase in activity, in particular linked to the COVID-19 pandemic, which led to supply difficulties. Primates from colonies without input of wild-caught animals (self-sustaining colonies) represented 39.4% of uses (27% in 2021).

The proportion of primates being reused was **27%**, compared to 22% in 2021. This increase could be due to the work carried out to explain the definition of reuse to user establishments.

A group of 20 F0 generation primates was declared in 2022. In fact, this declaration concerns a project for the conservation of the species carried out on non-captive wild animals. It is a conservation project for the common brown lemur (*Eulemur fulvus*) carried out in a French overseas department. After two blood samples are taken, the animals are released without being held in a laboratory.

Salmonids such as salmon (56 019 uses; an increase of 30% on 2021) and moronidae such as sea bass (23 660 uses; an increase of 40% on 2021) account for 44% of uses of fish (compared to 34% in 2021). The research projects involving these two categories of fish concern in particular population studies in relation to environmental changes such as climate change, which may affect the behaviour of polychlorinated biphenyls (PCBs) by making them more bioavailable. Other projects concern the optimisation of livestock by genetic selection or the development of probiotics with a view to pathogen resistance and growth improvement.

The number of uses of birds has decreased by 20% compared to 2021. There were 15 084 uses of turkeys (*Meleagris*), of which 14 863 relate to the production of a vaccine strain for this species (compared to 11 781 in 2021).

Certain species for which uses had increased in 2021 as a result of COVID-19-related studies returned to a number of uses close to that of 2020 and 2019. The golden hamster had 10 109 uses, a decrease of 17% compared to 2021; the ferret had 49 uses, down by 81%. This is also the case for guinea pigs, for which uses decreased by 35%.

Reuses

All species combined, the number of reuses was **20 032** in 2022 compared to**24 584** in 2021. This corresponds to an overall reuse rate of 0.94%. This rate is higher for large species with longer lifespans such as carnivores, livestock or primates, as indicated above.

Genetically altered animals

The proportion of uses of genetically altered animals was **38.5%**, as compared to **26%** in 2021. This increase is due to the expansion of the scope of the survey in 2022, which now includes the breeding of genetically altered animals more broadly. Using the same parameters as in 2021, the proportion would be 23%.

As in 2021, the vast majority of these animals were mice (**93%**). The proportion of phenotypes identified as harmful has remained broadly stable in recent years, with **5.1%** in 2022 compared to 4.4% in 2021.

(b) Information on a significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

The vast majority of animal uses fall into three main areas:

- basic research;
- translational or applied research;
- regulatory uses or production of biological products for therapeutic use.

These areas together account for **86%** of uses (92% in 2021). Taking into account the expanded scope of data collection to include the maintenance of colonies, the proportions for all the other areas have seen a corresponding decrease.

The share of animal uses for basic research increased from 38% in 2021 to **39%** in 2022. Once again, the field of neuroscience used the most animals, with 258 037 uses, followed by immunology and oncology with 132 390 and 122 337 respectively.

The increase in the number of uses in the field of basic research concerns two species in particular – mice (+ 24%) and zebrafish (+ 50%). One project alone, which used a zebrafish larval model to differentiate endocrine disruptors involved in metabolic liver diseases, used 21 469 animals.

Toxicological and regulatory research for the development, production or quality and safety testing of medicines or foodstuffs follows, with **24%** of uses (28% in 2021). In this area, there is an increase in the number of rabbit uses, with 179 252 uses (+ 12% compared to 2021), which is directly linked to the production of a drug for transplant rejection in humans. This drug, which is mainly produced in France, is distributed worldwide with growing demand due to proven efficacy. Overall, there has been a significant drop in animal uses in toxicological and regulatory research.

Translational or applied research decreased from 26% in 2021 to **23%** in 2022. Among the human diseases studied, a large share was attributable to research on cancer (27.4%) and neurological and psychiatric disorders (6%). Uses in the areas of animal welfare or animal diseases (23%) mainly concern productive livestock and species for human consumption.

Other scientific fields remain considerably less represented.

- Maintenance of colonies of genetically altered animals appears to be on the increase as a result of the change of method, with **11%** of uses (using the same parameters as 2021, it would be 4.8%).
- Species conservation remains fairly stable, with 2% of uses (compared to 2.6% in 2021). It is notable that **99.6%** of these uses concern fish (85% in 2021), with a total of 18 projects. For example, a project to monitor migratory fish populations required the use of 23 480 animals, and a project to evaluate estuary restocking required the use of 7 388 animals. These uses concern multiple wild species including eel, mullet, meagre, sea bass, flounder, whiting, sole, sturgeon, lamprey, conger, minnow, roach and bullhead.
- The area of education and vocational training remains very stable at **1.7%** of uses (1.8% in 2021).

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

As in previous years, the vast majority of experimental procedures were of mild or moderate severity (**84%**). Uses falling under the category 'non-recovery' are stable, comprising **4.7%**, in line with the figures from previous years.

Uses classified as 'severe' have fallen, with **12%** as compared to 14% in 2021. This decrease is due to the broadening of the scope of data collection, which added 326 033 uses in the area of animal

husbandry for which no 'severe' classifications were recorded. Using the same parameters as in 2021, the proportion of uses in the 'severe' category is comparable to 2021 at 14%.

It is notable that fish other than zebrafish account for 22% of the severe uses. Work has begun to refine the assessment of severity classification for these species.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

France continues to actively promote the principles of replacement, reduction and refinement (the 'three Rs'). To this end, a national centre dedicated to the 3Rs principle – the FC3R – was established in 2021, managed by the National Institute for Health and Medical Research.

The centre's mission is to fund research on the development of alternative methods, to develop and disseminate training on the application of the 3Rs, to support research projects by providing engineering in strict compliance with the 3Rs, to encourage the deposition of animal lines, to make unpublished data or negative results accessible in order to avoid unnecessary duplication of projects, and to ensure transparent communication on the use of animals for scientific purposes. The centre, in close coordination with the National Committee for the Protection of Animals used for Scientific Purposes (CNEA) and the National Committee for Ethics in Animal Research (CNREEA), contributes to a constructive national dialogue on this societal concern.

The FC3R promotes synergies and collaboration between existing bodies involved in the use of animals for scientific purposes. It works closely with the French authorities as well as with private partners. The centre is attentive to all stakeholders, in particular the charity sector.

After 2 years of operation, the FC3R centre now has a high profile nationally. A website with a large amount of information and resources was launched in June 2022: https://www.fc3r.com/

Two calls for projects have already been carried out. A total of 213 applications were submitted and 26 projects were funded. A third call is in progress and will focus on 'Digital Approaches'. Alongside this, FC3R is preparing an inventory of the existing alternative methods in France.

The FC3R is now a member of the management committee for the COST-IMPROVE initiative '3Rs concepts to improve the quality of biomedical science', which will make it possible to publicise national and international training courses run by networks of 3R centres. The steering and reflection board is considering setting up working groups on the following topics: training and education, frames of reference, indicators, severity of procedures.

France's commitment to the promotion and development of the 3Rs is also continuing through the ongoing activity of the National Committee for the Protection of Animals used for Scientific Purposes (CNEA), which issues recommendations and opinions. The latest reference document published by the CNEA is a glossary giving definitions of scientific terms directly related to the use of animals for scientific purposes (National Committee for the Protection of Animals used for Scientific Purposes).

National activities also include the work of the National Committee for Ethics in Animal Research (CNREEA), which draws up recommendations, the most recent of which concerns the use or

development of alternative methods to replace animal models in the field of antibody production (National Committee for Ethics in Animal Research).

Professional associations for animal research and national networks of ethics committees and animal welfare monitoring structures are also important players in the field.

One specific example demonstrating France's commitment is the work to reduce the number of animal uses in the production of monoclonal antibodies by ascites. The user establishments concerned have been contacted individually and project authorisations are being reviewed. Restrictions on the duration of authorisations are planned, or authorisations may be repealed for establishments which do not demonstrate the need to continue production using animals owing to a lack of available alternatives.

(e) Further breakdown on the use of the 'other' categories if a significant proportion of animal use is reported under this category:

Declarations in the category 'other' are subject to thorough checks in order to minimise its use. In basic research, the 'other' category accounted for **0.17%** of uses. Almost one third of these uses concerned a study on ageing.

For regulatory uses/routine production, the 'other' category accounts for **4.2%** of uses (4.7% in 2021). This is due in particular to the production of toxoplasm for toxoplasmosis testing kits, the production of a vaccine strain for veterinary use and the production of a protein for therapeutic use. These three projects, which address public health issues and which do not fall into the pre-defined categories of the statistical survey, alone account for 84 340 uses (84 728 in 2021).

As in 2021, the 'other fish' category remains sizeable, accounting for **2.8%** of uses (59 798). This is due to numerous environmental studies, including on issues relating to climate change. This category includes very diverse species such as sole, eel, sturgeon and three-spined stickleback, to name but the main ones. Half of these uses concern behavioural studies (15 576) or species conservation studies (16 921).

The 'other birds' category accounted for **0.4%** of uses (7 958). This category includes domestic species such as ducks, as well as wild species such as tits and penguins. Most concern behavioural or animal welfare studies.

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

The category PR52 (production of monoclonal antibodies by ascites method) comprises **49 038** uses (compared to 36 664 in 2021 and 40 649 in 2020), This increase is mainly linked to an increase in production of a test used in France and several other countries for the clinical follow-up of patients with long COVID and the production of control batches necessary for validation of transition plans for antibody production by non-animal methods (bioequivalence and stability studies).

Regular monitoring of existing authorisations and extensive dialogue with relevant user establishments continued during 2022. This helps to support the transition plans of the authorisation

holders. These plans must allow for a transition to non-animal production (*in vitro*) as quickly as possible and not be limited to a transfer of activities to countries with a lower level of animal protection than the European Union. Many of these monoclonal antibodies are used by hospitals and laboratories in France and abroad for biomedical diagnostics. The need to maintain continuity of care provision has led to authorisations being renewed for a limited period. The establishments have all initiated efforts to develop alternative methods (*in vitro*) but are still encountering difficulties with analytical quality, insufficient quantities, robustness of tests or time needed for validation by the authorities of the countries concerned.

With a view to the withdrawal of the 'rabbit pyrogen test' from the European Pharmacopoeia in 2025, a similar approach has been taken with establishments that hold this type of authorisation.

Work to reduce the number of animals used in areas such as skin and eye irritation continued in 2022. There is a significant decrease of 15% compared to 2021 in uses for skin irritation, 8.6% for skin sensitisation and 38% for eye irritation. Many of these uses are still required by law for the validation of medical devices; changes in these regulations will help to further reduce the use of animals.

In November 2021 standard ISO 10993-13:2010 regarding skin irritation and sensitisation tests to assess the safety of medical devices, which initially rejected *in vitro* tests to assess the safety of medical devices, was reviewed. A new standard (ISO 10993-23:2021) provides for replacement by an *in vitro* test for skin irritation only. For other irritations (eye, mouth, vaginal, rectal, penile, etc.), *in vitro* models have not yet been validated to assess the safety of medical devices.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

No authorisations were issued in 2022 for applications exceeding the 'severe' classification with intense pain which is likely to be long-lasting and cannot be ameliorated.

Germany

(a) General information on any changes in trends observed since the previous reporting period:

In 2022, approximately 1.73 million vertebrates and cephalopods were used in animal testing in Germany under Section 7(2) of the Animal Welfare Act (*Tierschutzgesetz*). Section 7(2) of the Act defines the term 'animal test'. The figures were around 7% lower than in the previous year (2021). This continued the downward trend of previous years.

Around 80% of the animals used for testing are rodents, mainly mice and rats. Mice accounted for about 73% of the animals used and rats around 6%. Some 12% of the animals were fish, around 4% were rabbits and around 2% were birds. These proportions were more or less the same as in the previous year. Furthermore, when compared over the long term, there were no substantial changes in the distribution of animals used. For example, the figure for mice increased by 1% compared to the previous year (around 72% in 2021), continuing the gradual increase since 2019 (around 71% in 2020 and around 67% in 2019). The proportion of fish used (12% in both 2020 and 2021) was the same as in the previous years. The use of birds and rabbits increased by around 1% compared with the 2021 reporting year, which constituted a return to 2020 levels. The proportion of other animals used for testing fell again compared to the previous two years, to around 3% (around 4% in 2021 and around 5% in 2020) but was not as low as in 2019 or 2018 (around 2% in 2019 and around 1% in 2018).

Going beyond the requirements of the EU Laboratory Animals Directive, Germany also includes animals killed for scientific purposes without first having undergone procedures or treatments, for instance in order to use their organs or cell material for scientific purposes. Some 712 000 animals were used for this purpose in 2022. This represents a significant increase of around 11% compared to 2021 (some 644 000 animals). Details of these animals, killed under Section 4(3) of the Animal Welfare Act, will be sent separately to the European Commission for the 2022 reporting year as part of the notification under Article 54(1) of Directive 2010/63/EU.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

<u>Genetically modified animals</u>
The number of genetically modified animals used decreased compared to the previous year: around 918 000, as compared to some 953 000 in 2021. Despite this decrease, the proportion of genetically modified animals out of the total number of animals used continued to increase. The proportion was just over 53% in 2022, continuing the upward trend of recent years (ca 51% in 2021, ca 48% in 2020 and ca 43% in 2019). This concerned in particular mice (85%) and fish (14%). In 2022, 468 rats were also used for the production of new genetically modified lines; in 2021 the number had fallen to just 29. There was also a further increase in the number of genetically modified domestic chickens (990 animals) and pigs (884 animals), the number and proportion of which have steadily increased since reporting year 2018, although the total of such genetically modified species still accounted for considerably less than 1% of genetically modified animals in 2022.

It should be noted that the number and proportion of animals bred, subject to authorisation, in order to conserve colonies of genetically modified animals but not subsequently used in any further

procedure, increased by around 21% compared to 2021. This marked the end of a three-year downward trend in the number of these animals. The latest figures were 2% lower (around 190 000 animals in 2022, around 157 000 in 2021 and around 187 000 in 2020). As in 2021, a significant number of zebrafish was reported in this category, making up 12% (around 11% in 2021).

Primates

Following a decrease in the number of animal tests with primates since 2018, the number increased to 2 204 in 2022. This was 318 more than in 2021, when the figure was 1 886: an increase of 17%. Around 89% of these were for regulatory purposes or routine production. In 2022, a total of 1 978 primates were used for the first time, while the remaining 226 animals (around 10%) were used again. Of the animals used for the first time, around 98% came from self-sustaining colonies, of which around 14% were from Europe, some 39% from Africa and some 47% from Asia. Thus there was essentially no change in the origin and stock of the animals compared with the previous year.

As in 2021, it was mainly long-tailed macaques that were used in 2022 (around 86% of uses in 2022 and 91% in 2021).

Dogs and cats

In the case of dogs and cats, used in particular for statutory testing, the routine production of blood-based products and research on animal diseases, there was only a slight change in the number of uses: 2 874 (dogs) and 538 (cats) (2 657 and 862 respectively in 2021). Compared to 2021, the number of uses of dogs increased by around 8%, but for cats the number decreased by around 38%, offsetting the sharp increase in the use of cats in 2021.

Scientific purposes

Although many scientific questions can be answered nowadays through the use of cell cultures, computer-assisted procedures and other alternative methods, it is not yet possible to do without the use of animals for scientific purposes, including biomedical research. Specifically, approximately 55% of the animals used in animal testing under Section 7(2) of the Animal Welfare Act were used for basic research and approximately 14% were used for translational research, e.g. researching human and animal diseases. Around 16% of the animals were used for regulatory purposes and routine production, such as the production and quality control of medical products and reagents or for toxicological safety tests. Some 11% of the animals used were needed to maintain colonies of established genetically modified animals and could not be used in other procedures. Around 4% were needed for other purposes, such as training or further education, protecting the natural environment or species protection. There were no major changes compared to 2021.

The proportion of tests in the field of basic and translational research and regulatory use changed by no more than 1% in each case compared to the previous year. Only the proportion of animals used to maintain colonies of genetically modified animals used for testing increased comparatively sharply, by around 3% compared to 2021.

- **Basic research**

  In 2022, as in previous years, important areas of basic research included, in particular, research into the nervous system and the immune system (21% and 19%), and the cardiovascular system (ca 13%). The figure for developmental biology research, which was included for the first time in 2021, increased from just over 2% in 2021 to just under 4% in 2022. The share corresponding to the category ‘other basic research’ decreased to less than 1% of all uses in the ‘basic research’

category compared to the previous year (around 2% in 2021). This reduction may be due largely to the efforts of the competent authorities to critically question, on the advice of the European Commission, any uses of 'other' categories.

- **Human and animal diseases**
  As in previous years, there was an emphasis on human cancers in the area of research into human and animal diseases. Some 42% of the animals used for testing in this area were used for this purpose. The share corresponding to animal nutrition, an area of research first included in 2021, fell from just over 5% of reported animal tests in translational research in 2021 to less than 2% of uses in 2022.

- **Regulatory use and routine production**
  As in previous years, the focus in regulatory uses was on toxicity and safety testing, including pharmacology. Almost 70% of notifications about regulatory uses in 2022 concerned this area, an increase of around 9% compared to 2021. At the same time, the percentage of quality control (batch safety and potency testing) decreased from around 27% in 2021 to just 19% in 2022. However, the routine production proportion remained stable, at around 9%. Around 63% of routine production could be ascribed to the production of antibodies (excluding the ascites method), a category which was first included in 2021. This figure was around 3% higher than in 2021. Unlike most recently in 2021, no production of antibodies using the ascites method was reported in the 2022 reporting year.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

The severity of tests under Section 7(2) of the Animal Welfare Act was predominantly 'mild' (approximately 66% of uses). Around 25% of the tests were classified as 'moderate' and 4% were classified as 'severe'. The proportion of mild tests increased by around 3% compared to 2021 (63%), reversing the trend in 2022 after the most recent fall in 2021. The proportion of moderate tests fell by around 1% compared to 2021. The proportion of severe tests was below 4%, the lowest level since 2018. The proportion of tests on animals carried out entirely under general anaesthesia and from which the animal never regained consciousness was around 5%, also the lowest level since 2018. Overall, however, there were few reported changes, and these constituted normal variations in the distribution of severity from year to year.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

Germany is endeavouring to reduce the number of animals used in tests. This involves launching and supporting various projects aimed at replacing animal testing with alternative methods as quickly as possible, such as setting up and operating the German Centre for the Protection of Laboratory Animals (*Deutsches Zentrum zum Schutz von Versuchstieren*, Bf3R) by the Federal Ministry of Food and Agriculture (*Bundesministerium für Ernährung und Landwirtschaft*, BMEL), research funding by the Bf3R, supporting the Foundation for the promotion of substitute and complementary methods to reduce animal testing (*Stiftung zur Förderung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen*) and annually awarding the BMEL's Animal Welfare Research Prize. The Federal Ministry of Education and Research (*Bundesministerium für Bildung und*

Forschung, BMBF) also funds research and development on alternative methods of animal testing on an annual basis.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

'Other species' category

Overall, around 3% of all animals used were declared as 'other species' in 2022. The largest groups in this category were 'other fish' (around 56% of 'other' animals), 'other birds' (around 24%), 'other rodents' (around 13%) and 'other mammals' (around 4%).

In the 'other mammals' sub-category, mainly native wild animals, in particular bats, but also larger wild animals such as wild boar, deer and roe deer, for which there is no separate category, were reported. The animals are used mainly in basic ethological research and research for species conservation.

In the 'other birds' category, native wild bird species (e.g. tits, raptors and migratory birds) in particular were used. These species were used mainly in basic ethological research. This may have partially concerned animals caught in the wild that were re-released after the test was finished. In addition, around 13% of the 'other birds' reported were zebra finches, used mainly for basic ethological research but also for research into the nervous system.

In the category of 'other fish', the fathead minnow (*Pimephales promelas*) was increasingly reported as being used for regulatory purposes, in particular ecotoxicity. This species accounted for almost 18% of 'other fish'. In addition, around 9% of this category were reported as turquoise killifish (*Nothobranchius furzeri*), which do not belong to an existing category but are widely used in basic research and are also being genetically modified. Finally, the category of 'other' fish also includes native wild fish (e.g. whitefish, carp, perch and European eel), which were used mainly for basic ethological research. This may have mainly concerned fish caught in the wild that were re-released after the test was finished. The new category of 'sea bass (spp. from families e.g. *Serranidae*, *Moronidae*)', added in 2021, was insufficiently used by those carrying out the reporting due to the narrow taxonomic scope. Only 840 fish were included in this category in 2022, with many perch-like fish still being reported as 'other fish'.

Field mice, bank voles, root voles and wood mice in particular were reported as 'other rodents'. In particular, field mice (69% of 'other rodents') were used in authorisation procedures for plant protection products.

'Other uses' category

Overall, less than 1% of all animal tests were reported under 'other uses' in 2022. Around 43% of these were in the 'other basic research' and around 36% in the 'other regulatory purposes' categories.

In the context of other basic research, there was particular emphasis on the following areas:

- studies in age research (ca 31%)
- epigenetics (ca 30%)
- molecular biology (ca 8%)

- testing of various new methods for marker, blood and biopsy sampling with the aim of refining these methods

The sharp decrease in the use of the 'other basic research' category may be largely due to the active efforts of the competent authorities to critically question the reasons for classification under 'other' purposes.

In the area of 'other regulatory uses' the emphasis was mainly on the following topics:

- efficacy tests that could not be assigned to an existing category (ca 62%)
- pharmacometrics (ca 15%)

'Other legislation' category

The following other legislation was of particular relevance in this category:

- testing of substances hazardous to water pursuant to the Administrative Regulation on the Water Resources Act (*Verwaltungsvorschrift zum Wasserhaushaltsgesetz*)
- testing of products under the Infection Protection Act (*Infektionsschutzgesetz*)

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

In the 2022 reporting year, 23 cases were reported of pyrogenicity tests and eye or skin irritation tests under authorisation procedures for medicinal products and chemicals. They involved 1 294 rabbits. There may already be authorised substitute methods for these uses.

The Federal Institute for Risk Assessment (*Bundesinstitut für Risikobewertung*, BfR) has had each of these notifications verified by the competent authorities and received the following feedback:

- in the case of 1 063 uses (approx. 82%), a substitute method was not or not yet recognised;
- in the case of 187 uses (approx. 14%), the tests were necessary due to international requirements;
- in the case of 3 uses, the substitute method was not applicable due to the solubility of the substance;
- in the case of 41 uses, the competent authority merely reported that the use had been plausible in individual cases.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

In 2022, no tests were reported or authorised in Germany where the 'severe' classification was exceeded.

Greece

(a) General information on any changes in trends observed since the previous reporting period.

A decreased use of animals is reported in Greece for 2022 by 7.17 %. This is attributed to the diminished number of animals used by user establishments in Greece in relation to the projects authorized by competent authorities and completed in the year.

A decreased use of animals born in the EU but not by a registered breeder by 55.22 % has been reported compared to 2021. This refers mainly to the lower number of fish of domestic species (other fish) that have been used.

(b) Information on significant increase or decrease in use animals in any of the specific areas and analysis of the reasons thereof

A) An increase in the number of mice used in 2022 is note compared to 2021 by 13.5%. This is attributed to the fact that more mice were used for creation of new GA line for basic and translational research projects from an establishment that didn't execute any protocols last year (17 times more mice for this purpose compared to 2021).

Regarding rats, their number used is slightly increased to the number used in 2021 by 3.57%. This depends on the protocols authorised and completed for 2022.

A significant increase in the number of rabbits used in 2022 compared to previous years. This is due to the fact that some establishments execute protocols during some years and they don't during some others (see point 2E).

A significant increase in the use of pigs is noted by 86.84 % due to an increase in higher education training programs that are performed with the use of live animals in Greece and the authorisation of a new user establishment dealing with this species. Face-to-face attendance increased in 2022 following the COVID-19 pandemic.

A 74.22% decrease in the use of domestic fowls is noted compared to 2021 due to less number of them required in authorised protocols which were used for "animal nutrition" purposes (a significant decrease is noted for this particular purpose).

A significant decrease by 91.86% is also noted on animals used for "animal welfare" purposes compared to the previous year (2021) due to less animals used from the establishment executing these particular protocols.

A significant increase by 127% in the use of zebra fish for the first time is noted due to an increased number required for basic research.

B) Approximately the same proportions of genetically altered mice with a harmful phenotype is noted in Greek statistical data in 2022 compared to 2021 while not genetically altered mice are significantly less (43.92% less compared to 2021) and there is an increase in the number of genetically altered mice without a harmful phenotype. Furthermore a significant increase by 66.68% is noted in basic research. This is attributed to the different type of protocols performed in Greek establishments for

2022 as well as more zebra fish used for basic research and mice were used for basic research and creation of new GA lines. The same applies to slight differences for all other categories of protocols.

C) A significant use of fish is still depicted in Greek statistical data in 2022 again compared to other MS for 2021 but lowered by 25.69% compared to 2021. This is due to the fact that Greece is a Mediterranean country and has a number of user establishments dealing with studies on fish biology, behaviour/ethology and protection of natural environment. *Sea bass and zebra fish* are the main species used. In user establishments, fish are maintained under similar commercial production conditions, and most of the projects consist of variations in the rearing parameters (temperature, photoperiod, dissolved oxygen, tank size, feed type and frequency, rearing density, etc.) that may cause stress to the animals and are classified as "mild".

D) No cephalopods were used in 2022.

E) It has to be noted that the use of various species differs among each year according to the protocols authorised and funding received by user establishments. Minor changing trends can be recorded as noted above.

F) It has to be noted that some user establishments do not perform protocols with the use of animals every year. Data from these establishments are only presented when appropriate.

G) It has to be noted that the purpose of animals used for scientific purposes differs among each year according to the protocols authorised and funding received by user establishments. Minor changing trends can be recorded. For example in 2022 around 21.05% of protocols addressed Immune system basic research studies, 20.57% addressed musculoskeletal system basic research studies (significant increase compared to 2021 related to the increase of zebra fish in basic research) and 25.61% nervous system basic research studies, 31.54% addressed human immune disorder protocols for translational and applied research related issues (significant increase 59.5% compared to 2021 due to more animals required from authorised protocols) and 20.03 % addressed human nervous and mental disorder protocols for translational and applied research related issues.

H) It has to be noted that the use of animals for various systems either for basic or translational and applied research varies among each year according to the nature of protocols chosen by researchers.

I) A continuous increase in the use of genetically altered animals is constantly noted during the last years, due to the type of projects authorised and the research trends of recent years globally. Their number remains stable between 2021 and 2022 with the exception of genetically altered animals without a harmful phenotype where there is an increase in their number due to an establishment not performing protocols in 2021 but performing in 2022. Furthermore, there is a significant increase in the use of genetically altered animals used for the first time for various purposes (e.g. nervous system basic research studies, immune system basic research studies, human nervous and mental disorders for translational and applied research uses etc.).

J) A continuous decrease use of dogs and cats is noted during the years, according to the relevant protocols authorised. Their number has diminished between 2021 and 2022.

K) Animals used for non-regulatory toxicology and ecotoxicology purposes were triple-used compared to 2021, reaching, thus, almost the levels of 2015 due to the fact that two more establishments executed this kind of protocols this year compared to 2021.

L) A significant decrease 95% in Toxicity and other safety testing including pharmacology was noted due to just one establishment executing this kind of protocols in 2022.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof

A) Animals with "Non recovery" severity seem to be increased by 63.79 % due to an increase in the protocols authorized for higher education purposes.

B) Animals with "moderate" severity seem to have decreased by 11.77%. This can be attributed to the training of project evaluation committees that took place in Greece in 2019 by the Greek National Committee for the protection of animals used for scientific purposes which has led to the implementation of better criteria for the assessment of severity and, thus, better enforcement of legislation.

C) The severity of procedures is decreased by 26.07% compared to 2021 as less severe protocols were authorized in 2022.

(d) Particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any

Laboratory animal science training courses are organised annually in Greece. No impacts on statistics are noted.

(e) Further breakdown on the use of "other" categories if a significant proportion of animal use is reported under this category

A) A significant proportion of other fish is constantly reported in Greece for 2022 although with an increasing trend compared to 2021 due to more establishments executing protocols.

Greece remains a leading country in Mediterranean fish production and significant research is carried out in this field compared to other MS. *Sparus aurata* and *Dicentrarchus labrax* are the leading species, with *Oreochromis mozambicus, Nile tilapia*, *Seriola dumerili* and *Argyrosomous regius* to follow in general. Procedures on fish include behavioural studies or drug testing, which cause stress to the animals and are classified as "mild". "Severe" use where applicable has already been presented above.

B) Also see point no. 6

C) 35 animals were used for an AL amyloeidosis project categorized as mild severity.

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

Rabbits were used from one establishment for pyrogen testing. However, rabbit pyrogen testing is about to be completely replaced within 5 years time approximately, according to a decision taken from the European Pharmacopoeia (Ph. Eur.) Commission on its 170th session in June 2021.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not, covering the species, numbers, whether prior exemption was authorised, the details of the use and the reasons why 'severe' classification was exceeded

There was no case where “severe” classification was exceeded for 2022.

Hungary

(a) General information on any changes in trends observed since the previous reporting period:

The total number of animals used for experimental and other scientific purposes in 2022 was 119.517, which represents 21,74 % decrease compared to 2021. The reason is for the decreased use that there was less experiment in 2022, with less animal uses for scientific purposes. The proportion of re-used animals was 1.02 % which 37.83 % increase compared to 2021. The decrease in the number of animals is due less animal use in translational and applied research, regulatory use and routine production, and basic research.

The number of genetically altered animal used without a harmful phenotype in 2022 was 8.725, which represent about 5,43% decrease compared with the previous year. In 2022 the percentage of use is 7.3 % from total uses. This shows increase compare with 2021.

The number of genetically altered animal used with a harmful phenotype in 2022 was 273, which represent 53.25% decrease compared with 2021. In 2022 the percentage of use is 0.22% from total uses. This also shows decrease compare with 2021.

There is a significant change on the data of origin of animals compare with the previous year. In 2022 the number of animals born in the EU at a registered breeder shows 24.57 % decrease, and the number of animals born in the EU but not at a registered breeder shows 11.21% increase compare with 2021. There was not used any animal born in rest of Europe in 2022, but the used of number of animals born in rest of world shows 392.3% increase.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

The vast majority (88.27%) of used animals were warm-blooded vertebrates. The number of fish used in experiments remained in high level (13.215). The reason is a large-scale ecotoxicological testing on fish. There was significant increase in case of amphibians from 0 to 300 and reptiles from 0 to 500 in 2022.

In case of mice there was a 36.02% decrease and in number of rats also decreased 22.47%.

When analysed by the purposes of the use of animals, the following categories shows significant increase: Protection of the natural environment in the interests of the health or welfare of human beings or animals (from 478 to 875), Preservation of species (from 0 to 800) and Training for acquisition, maintenance or improvement of vocational skills (from 490 to 617). Compare with the data of 2021, the following categories shows significant decrease: Basic research (from 40491 to 35050), Translational and applied research (from 39758 to 25856), Regulatory use and Routine production (from 70386 to 55352) and Higher education (from 1106 to 967).

In the basic research related uses, the used for following purposes increased significant: Gastrointestinal System including Liver (from 2030 to 2576), Sensory Organs (skin, eyes and ears) (from 96 to 252), Endocrine System/Metabolism (from 1587 to 2279), Developmental Biology (from

100 to 1163), Ethology/Animal Behaviour/Animal Biology (from 692 to 2650), Other basic research (from 375 to 8449).

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

The number of uses of animals by severity in all types decreased. Non-recovery from 44490 to 3030, Mild from 76445 to 67719, Moderate from 38223 to 28411 and Severe 33551 to 20357.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

Due to the stringent national measures, the use of non-human primates for scientific purposes has been replaced by other methods where possible and their proportion is very low in Hungary. The use of non-human primates occurs only if there is not any alternative method.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

Percentage of other birds was 7,56% total was 2616 from 37582. These were Ficedula albicollis, goose, mulard duck, zebra finch.

Percentage of other fish (1418) was 11.29% from the total 12554. These were European perch, Silurus glanis, Poecilia reticulata, Macropodus opercularis, Cyprinus carpio, Squalius cephalus, Esox Lucius and Sander lucioperca.

Percentage of other basic research was 24.1 % from the total 35050. These were mainly basic research in environmental toxicology (92.31%).

The percentage of other product types were 6.66% from the total 60 routine production uses.

The percentage of other efficacy and tolerance testing was 3.62% from the total 55929 regulatory uses. These were Acute oral toxicity OECD 431.

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

Based on the results from an in vitro study (guideline: OECD TG 439), it was not clear whether the test substance was irritant or not. An in vivo study was required to clarify the result according to the data requirements of the registration dossier.

In other case number of animals is required and regulated by Ph. Eur in force. Animals are used in quality control batch release tests.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not: There was none in 2022.

Ireland

(a) General information on any changes in trends observed since the previous reporting period:

- There was an 23% decrease in the total number of uses of animals for research, testing, routine production and education and training purposes reported for 2022 versus 2021, with 93 825 total uses of animals in 2022 compared to 122 383 total uses of animals in 2021. A similar decrease of 24% was noted in the numbers of animals used for first time in 2022 versus 2021 (92 939 animals used for the first time in 2022 compared to 121 558 animals in 2021).

- The total percentage of animals reused in 2022 mirrored the percentage figure for 2021 at <1% of total uses. Cattle are the species most commonly reused in Ireland. The reuse of sheep increased as a result of a project using sheep for research into human cardiovascular disease. The reuse of cats decreased in 2022 versus 2021 (52 cats reported as reused in 2021 with 18 reported as reused in 2022).

- Mice remain the most commonly used species at 73% of all uses. There was a 26% decrease in the number of mice used, with 67 320 mice used in 2022 versus 91 497 in 2021. This was driven by a 29% reduction in the number of mice used for Regulatory testing (including batch safety and potency testing) noted in 2022.

- There was a 23% decrease in the numbers of rats used, with 13 082 rats used in 2022 versus 17 050 in 2021. This was primarily due to a reduction in the number of rats used for Regulatory testing (batch potency testing) of a human medicinal (biological) product.

- There was a 54% increase in the number of rabbits used, with 1 012 used in 2022 versus 657 during 2021. This increase was mainly as a result of sponsor companies requiring increased volumes of rabbit pyrogen testing as part of the process of investigating and validating non-animal alternative tests to detect pyrogenic contaminants, in preparation for the removal of the rabbit pyrogen test as a regulatory requirement over the coming years.

- There was a 36% increase in the number of dogs used for the first time in 2022 versus in 2021 (from 83 in 2021 to 113 in 2022). This was due to increased volumes of research and development and regulatory testing of canine veterinary medicinal products for the veterinary marketplace.

- There was a 39% decrease in reported uses of ferrets in 2022 versus 2021, with 219 ferrets used in 2022 compared to 358 in 2021. This was as a result of reduced volumes of regulatory testing of influenza vaccines in 2022 compared to 2021.

- The number of uses of pigs reported in 2022 increased by greater than threefold from 2021 as several large-scale studies on porcine nutrition and anti-microbial resistance were undertaken during 2022.

- There was a 31% increase in uses of sheep in 2022 versus 2021. This was driven by increased volumes of research into reducing the environmental footprint (greenhouse gas emissions) of the sheep production sector undertaken during 2022.

- There were no uses reported under the category of 'Other birds' for 2022 (92 birds had been reported under this species category in 2021), as two wildlife studies involving Great tits and European Storm Petrels had concluded during 2021.

- There was an almost threefold increase in the number of Xenopus used during 2022 versus 2021 (131 used in 2022 versus 53 during 2021). This was due to an increased requirement for Xenopus larvae to perform Translational and applied research into human sensory organs.

- There was a 73% decrease in the number of uses of fish under the species category 'salmon, trout, chars, and graylings (1 364 in 2022 versus 4 986 in 2021), as several wildlife conservation studies concluded during 2021.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

- The number of animals reported as being used for the purpose of 'Basic research' decreased by 18% from 2021. The reasons for this decrease are not known.

- There was a 28% decrease in the total number of animals used for 'Regulatory Use and Routine production' in 2022 (59 245 animals) versus 2021 (82 325 animals). This was driven by a significant reduction in the number of animals used for the secondary purpose 'Quality Control (including batch safety and potency testing)'. 23 003 fewer animals were used for 'Quality Control (including batch safety and potency testing)' in 2022 (59 224 animals) than in 2021 (82 227 animals), representing a 28% decrease.

- There was a 47% increase in the number of animals used for pyrogenicity testing (966 in 2022 versus 657 in 2021). This increase is as a result of sponsor companies requiring increased volumes of rabbit pyrogen testing as part of the process of investigating and validating non-animal alternative tests to detect pyrogenic contaminants, in preparation for the removal of the rabbit pyrogen test as a regulatory requirement over the coming years.

- There was a 54% decrease in the number of animals (cattle, sheep, and fish) used for the purpose 'Protection of the natural environment in the interests of the health or welfare of human beings or animals. This was due to two large-scale studies aimed at protection of the marine environment and conserving and managing the Irish salmonid population concluding during 2021.

- There was an almost fourfold increase in the number of animals used for the purpose 'Preservation of species' (773 animals in 2022 versus 217 animals in 2021). This increase was as a result of a large-scale conservation study using fish.

- There was a sevenfold increase in the number of zebrafish reported as used for the creation of new genetically altered animal lines. This increase was due to a project undertaken during 2022 to create new genetic lines of zebrafish for the purposes of generating genetically altered zebrafish larvae as a model for inherited forms of blindness.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

The distribution of animal numbers across the four severity categories in 2022 mirrors that of 2021, with no significant changes noted.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

- We have focussed significant efforts over the past number of years to ensure that there is a move to non-animal alternatives for regulatory testing, and this effort is reflected in the ongoing reduction in numbers of uses of animals for these types of tests noted for 2022. There is a positive trend of a decrease of 28% in the number of animals used for 'Regulatory Use and Routine production' in 2022 (59 245 animals) versus 2021 (82 325 animals).

- Compared with 2017 (the year in which total number of uses overall and uses of animals for regulatory testing peaked in Ireland), there has been an 70% reduction in the numbers of animals used for regulatory testing (59 245 animals used in 2022 versus 194 816 animals in 2017), or as an absolute value, 135 571 fewer animals were used for the purpose of regulatory testing in Ireland in 2022 than were used in 2017. The key driver of this welcome reduction is the 70% decrease in the numbers of animals used for the tertiary purpose 'Quality Control – batch potency testing' in 2022 (58 113 animals) versus 2017 (192 015 animals).

  Where it is necessary to perform batch potency testing using animals (for instance if there is no non-animal alternative available for a specific product), we have also mandated the implementation of humane endpoints for these tests. This has resulted in reductions in the actual severity experienced by the animals, which is particularly important in relation to reducing the numbers of animals experiencing severe severity.

- Efforts are ongoing to encourage sponsor companies to transition to non-animal alternative tests for the detection of pyrogenic contaminants, with multiple sponsor companies now in the process of validating non-animal alternative tests and gaining regulatory acceptance for these.

- Furthermore, throughout 2022 we shared regular communications with end-users (quarterly newsletter as well as direct correspondence with relevant stakeholders) on 3Rs topics and developments, including on the EURL ECVAM reviews of advanced non-animal models in biomedical research, useful Reduction resources such as the NC3Rs EDA, and a wide range of Refinement resources.

(e) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

With regards to species, only 68 animals in total were reported as 'other' species, accounting for just 0.07% of total animal use. This relates to wildlife/marine-life conservation studies using wild fish species (e.g., shark and tuna species) and squirrels.

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

- 966 rabbits were used for pyrogenicity testing in 2022. Alternative testing methods to determine the pyrogenicity of medicinal products are recognised under the legislation of the Union. However, the use of these methods for certain classes of medicinal products (e.g., some blood/protein-based medicines) poses challenges. Therefore, in order to meet the necessary regulatory safety requirements for release of certain medicinal products onto the market there is currently no alternative to the use of the rabbit pyrogen test. However, sponsor companies are engaged in validating non-animal alternative tests and gaining regulatory acceptance for these.

- Other types of regulatory testing using animals (such as batch potency determination e.g., of botulinum toxin medicinal products) are only authorised in circumstances where there is no non-animal alternative test validated and accepted by medicines regulators to be used for the specific product(s) in question.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

This was not exceeded during 2022.

Italy

The data for Italy for 2022 are from the Ministry of Health – Directorate-General for Animal Health and Veterinary Medicines – Office 6 – Animal Welfare.

They were collected via the National Electronic Database and, after suitability testing, were sent to the European Commission through the DECLARE platform.

(a) General information on any changes in trends observed since the previous reporting period:

There was a general decrease in the number of naïve animals used (from 548 933 in 2019 to 420 506 in 2022). The decrease can be explained, to a large extent, by restrictions resulting from the COVID-19 health emergency.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

There has been an increase in the number of naïve animals of the Danio *rerio* fish species, which were mostly used in basic research in the fields of oncology and the study of nervous diseases.

(c) Information on any changes in trends in actual severity and analysis of the reasons:

There has been a slight decrease in the animals used in procedures with a 'severe' level of suffering.

Researchers' ever more conscious use of tools such as score sheets for the clinical assessment of the severity of procedures contributes to a more appropriate classification of the level of suffering.

(d) Information on any changes in trends in actual severities and analysis of the reasons thereof:

We would point out the increased awareness on the part of the Animal Welfare Bodies in checking the application of the 3Rs principle in the context of the evaluation of research projects before issuing the reasoned opinion which is essential for the application for authorisation of a research project.
It should be stressed that the 3Rs principles are dealt with at various professional refresher events aimed at researchers and persons performing designated veterinarian and animal welfare officer duties.

Projects using only alternative methods entailing the complete replacement of the animal model have been financed from ministerial funds.
The above activities contribute, in part, to the decrease in the number of animals used for scientific purposes.

(e) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

Routine production by product type/Other product types (code PR53): **'Antigen production' and 'Immune serum'**

Regulatory use/Other efficacy and tolerance studies (code PR71): **'Efficacy and tolerability of food additives and medicinal products in poultry species'; 'Vaccine immunogenicity' in rabbits.**

Regulatory use/Ecotoxicity/Other ecotoxicity (code PR103): **'Ecotoxicity diagnostic tests on waste and materials'**

Types of legislation/Other standards (code LT10): **'Environmental rules', Regulation (EC) No 1272/2008**

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

Use of animals for pyrogenicity tests on rabbits for the release of batches of medicines for certain non-EU countries (USA and other countries) and for the release of medical devices.

Skin sensitisation and skin irritation tests to test medical devices as required by OECD 406 Guidelines or carried out in accordance with ISO 10993-10. ISO 10993-23 and the LLNA (Local Lymph Node Assay) test, as a sensitisation test required by ISO 10993 (Biological evaluation of medical devices) for the development of medical devices for implantation.

Eye irritation tests carried out in accordance with OECD No 405, the REACH Regulation (Annex VIII) and CLP classification (Regulation (EC) No 1272/2008) on medical devices.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

None

Latvia

(a) General information on any changes in trends observed since the previous reporting period:

The competent authority approved 12 pilot projects in 2020, 9 in 2021 and 10 in 2022. The total number of animals used for scientific purposes has declined in recent years – 4 002 were used in 2020, 3 741 in 2021 and 2 207 in 2022 –, but there has been an increase in the total number of active authorisations per calendar year. Since it generally takes more than one calendar year to design a pilot project, most of the pilot projects authorised in preceding years are implemented in a subsequent period. Thus, taking all authorisations issued in previous years into consideration, authorisations were active for 34 pilot projects in 2020, 35 in 2021 and 42 in 2022. As in previous years, for various reasons, animals were not used in all pilot projects for which authorisations had been issued, nor were all authorised pilot projects actively pursued. Most pilot projects involve several procedures, so often, depending on the design of the pilot project, some of the procedures may be implemented within a single year and some successively over the course of the entire project. This does not mean, however, that all procedures were carried out for all projects and that all projects were implemented as planned. In some cases, projects or procedures were temporarily suspended owing to a lack of funding or because no additional *in vivo* studies were carried out prior to clinical trials (studies) because reliable and acceptable data had already been obtained using new alternative methods not yet available when the pilot project was initially planned or because the first *in vivo* tests of newly synthesised substances demonstrated that the further use of animals would be superfluous because the new substances being investigated had proven to be ineffective and did not demonstrate the sought-after characteristics. Science is advancing rapidly from year to year, so researchers use *in vitro* methods as much as possible once new information in their field of research is obtained, leading to a reduction in the overall number of animals used. In most cases, especially where long-term projects (of five years' duration) are concerned, researchers use fewer animals than indicated in the project design.

A total of 109 genetically modified animals were used in 2019 and 281 in 2020. The number of genetically modified animals used in procedures continued to increase in 2021, rising to 491, declining slightly to 394 observed in 2022. The use of animals with harmful genotypes has also slightly declined (n=101 in 2022, n=104 in 2021). This trend is due to the fact that a project was completed in the previous year in which genetically modified animals were actively used. Although the total number of genetically modified animals varies (sometimes increasing, at others slightly decreasing), the percentage of genetically modified animals compared to the total number of animals used in procedures nevertheless shows an upward trend, with the respective annual figures being 2.6% in 2019, 7.3% in 2020, 13.1% in 2021 and 17.1% in 2022. The variations and decline in the number of genetically modified animals is related to the overall decline in the number of animals used. The increase in the percentage of genetically modified animals relative to the total number of animals used in procedures can be explained by the need for a pathological model to evaluate new substances with potential therapeutic effects and new therapeutic methods. Science is constantly evolving, but currently it is often less harmful to assess the effectiveness of a particular substance or treatment method using genetically modified animals than to establish a pathological model (e.g. use a specific diet or surgical method to induce a pathological condition). In most cases it is possible, using genetically modified animals, to create and use in research the early stages of a disease or pathological condition for which clinical manifestations cannot yet be observed, but which can be detected only using specific diagnostic methods, devices or analyses (tests) that cause animals much

less discomfort and harm than the full course of development of the disease or the induction of a pathological condition. Consequently animals are subjected to lighter and shorter procedures. Furthermore, in some cases where animals are used as models for research into vaccines and immunological cancer treatments, a model is needed which is very similar to a human being and fulfils specific conditions (e.g. has cell receptors which are identically expressed). In such cases, it is often possible to replace higher-sensitivity species with lower-sensitivity species (e.g. mice or rats may be used instead of primates).

This year (2022) there was no reuse of animals in procedures performed during *in vivo* studies.

Most of the animals used in procedures are of EU origin (86.3%) and the majority – 76.8% – are from registered test animal breeders. For various reasons (see Table 1) animals are also obtained from other parts of the world, including from unregistered test animal breeders.

In 2022 the number of animals used in procedures and not born at a registered test animal breeder in the EU declined significantly, from 17.0% in 2021 to 6.9% in 2022, whereas the number of animals used in procedures and born in other countries of the pan-European region, but not in the EU, increased slightly – from 8.0% in 2021 to 11.6% in 2022. The decline in the number of animals obtained from unregistered test animal breeders in the EU is related to the completion in the previous year of several pilot projects involving field studies and the use of farmed or wild animals. In some studies into wild species, however, it is difficult to determine from which country or exact part of the world migratory birds or bats come from, especially in cases where breeding sites are contiguous and do not have strict boundaries.

Table 1

Animals not born at a registered test animal breeder

Region	Species	Count	Explanation of need
America	mice	30	Special animal strains not available to breeders in European and EU countries.
	rats	15	
European Union countries	mice	142	Animals bred in a test animal user's undertaking for use in their specific procedure.
	pigs	10	Irreversible procedure (acquisition, maintenance or upgrading of higher education or professional skills)
	cats	119	Epidemiological and fundamental research
Pan-European region (non-EU) countries	wild birds	257	Field studies (fundamental research/ethology/animal biology)

(b) Information on significant increases or decreases in the use of animals in any of the specific areas and an analysis of the reasons therefor:

A comparison of data on animals used in procedures over the last five years shows that the total number of mice used decreased from 2 975 in 2018 to 1 407 in 2022. As Figure 1 shows, fewer mice are being used every year. The main reason for this is constant scientific progress and ever more careful planning of procedures, with a progressive increase in the use of *in vitro* methods.

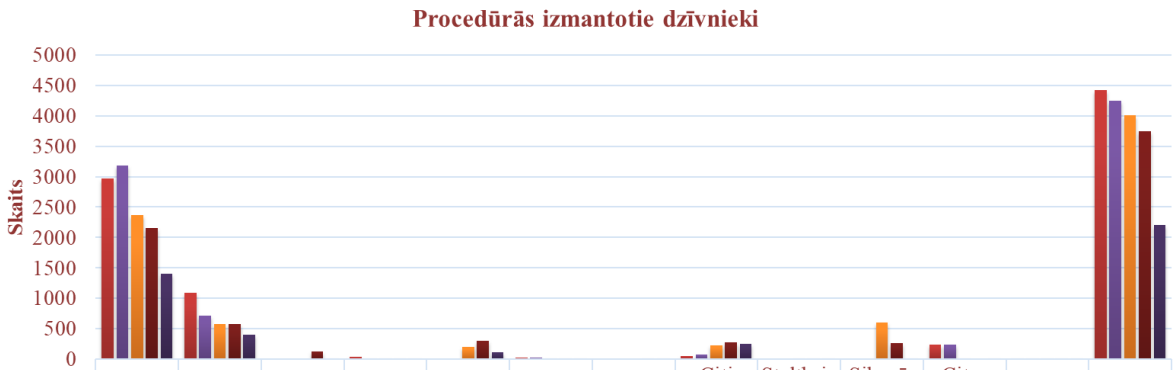
The total number of rats used in experiments is also tending to decline (see Figure 1). Sometimes, in isolated studies (mainly those involving the testing of new substances), rats are chosen rather than mice because of their larger size. This characteristic enables researchers to obtain more biological matter (e.g. tissues, blood samples or tumour cells) for further *in vitro* testing. This makes it possible to obtain all the necessary information and carry out the study while at the same time using fewer animals in procedures.

The number of pigs used increased slightly this year, from 7 in 2021 to 10 in 2022. Since these animals are used in only one procedure (for the acquisition of higher education or the acquisition, maintenance or upgrading of professional skills), the increase is mainly due to the specificities of project implementation in the year in question.

This year there was also a slight decrease in the use of birds of other species which, as before, can be explained by the specificities of project implementation in the year in question, including affordability. The total number of animals planned for use in each project and procedure was not exceeded. No other mammal species not referred to in the report classification have been used in procedures in the year in question; the reason for this being that the relevant pilot projects were completed in the previous year.

In 2022 there was a decrease in the number of cats used in procedures; these were subjected to light procedures, mainly as part of epidemiological studies (coronavirus and COVID-19-related studies) and ocular physiological studies. The harm done to these animals during the procedures was associated with the removal of blood, nasal discharge and tear samples. After sampling, all these animals were immediately returned to their owners. In most cases, the owners themselves were present when the animals were subjected to the procedures, and often procedures were carried out in housing to reduce stress and discomfort for the animals.

Figure 1



	Peles	Žurkas	Kāmji	Truši	Kaķi	Cūkas	Liellopi	Citi putni	Staltbriedis	Sikspārņi	Citas zivis	Aitas	Kopā
2018	2975	1093		36		24		49			240	0	4417
2019	3178	717		6		24		80			240	0	4245
2020	2368	578		2	198	10		232	4	610	0	0	4002
2021	2151	584	132	12	299	7	2	284	4	266		0	3741
2022	1407	410	0	0	119	10	0	257	0	0	0	4	2207

Upon the completion of procedures in a number of pilot projects in which the relevant animal species were the only ones used in Latvia for the pilot projects in question, those species were no longer used in 2022, e.g. hamsters, rabbits, red deer, bats and fish (see Figure 1). Mention should also be made of cases where procedures are lengthy and animals are used continuously to make various observations for several years in a row. In accordance with legislation, reports on such projects are submitted only in one of the calendar years during which the pilot project is running (e.g. the pilot project involving bovine animals).

(c) Information on change in trends in actual severities and analysis of the reasons therefor:

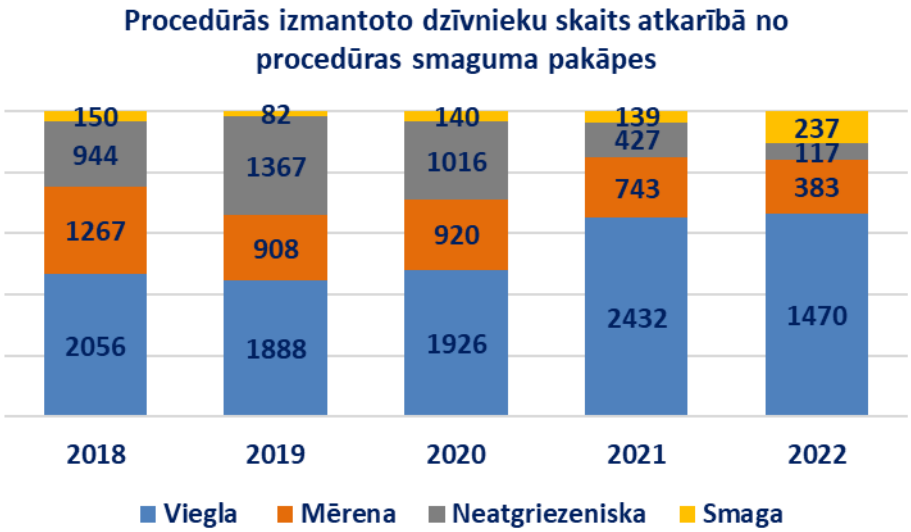
The vast majority (66.6%, n = 1 470) of all test animals were subject to **light procedures** in 2022 (see Figures 2 and 3) – of these, 1 462 were actually used in planned light procedures. Eight mice (objective of the pilot project – fundamental research: musculoskeletal system) were subjected to a light rather than a moderate procedure, as these animals were used solely for mating purposes to obtain the requisite modified genotype.

In 2022, a total of 237 animals (10.7%) were subjected to **severe procedures**; of these, 166 mice and 4 sheep were subjected to a planned severe procedure, whereas the rest endured severe suffering due to unforeseen circumstances.

1. During a planned moderate procedure (objective of the pilot project – fundamental research: musculoskeletal system), a single genetically altered mouse with a homozygous genotype and a harmful phenotype had a fatal outcome at an early (neonatal) stage of birth.

2. During a planned moderate procedure (objective of the pilot project – fundamental research: endocrine system/metabolism), 31 mice were found to have lost more than 20% of their body mass following the application of tamoxifen. Because of this, they were humanely killed in order not to expose them to further severe suffering.

Figure 2



In 2022, 383 animals (17.3%) were used in **moderately severe procedures**; of these, 286 mice and 97 rats were subjected to planned moderately severe procedures.

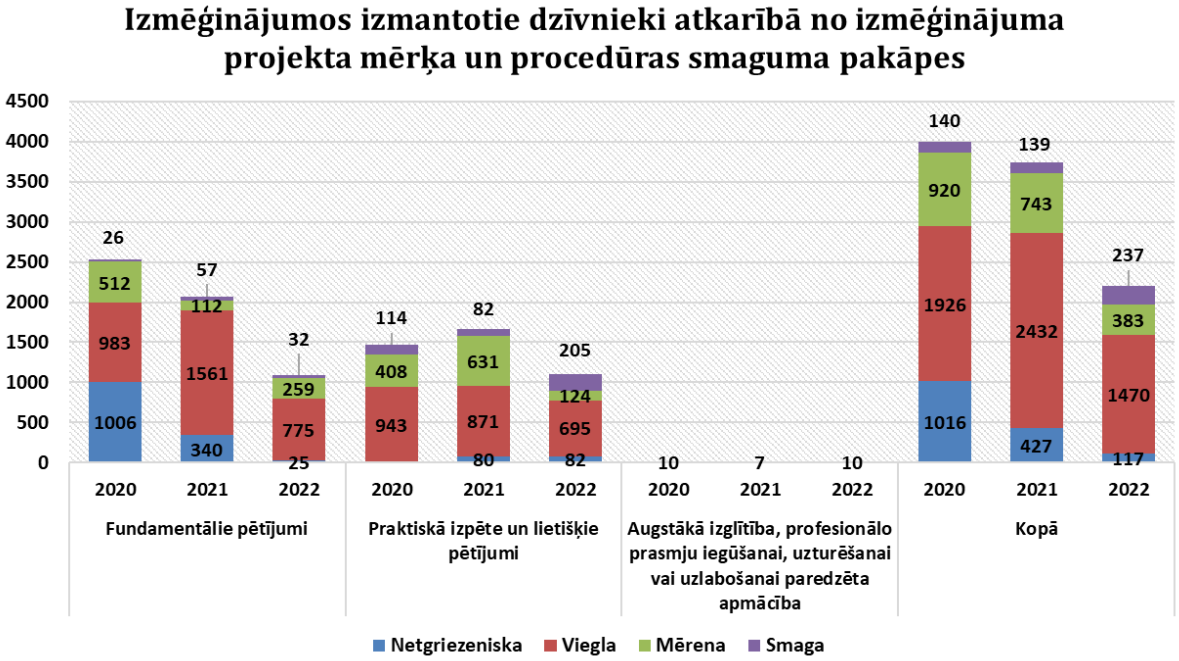
In 2022, 117 (5.3%) animals were used in **irreversible procedures**; of these, 82 rats and 10 pigs were used in planned irreversible procedures, while a small proportion of the animals was subjected to irreversible procedures because of unforeseen circumstances. During a planned moderate procedure, four mice (objective of the pilot project – fundamental research: nervous system) and 16 mice (objective of the pilot project – fundamental research: musculoskeletal system) experienced a rupture of the meninges due to unforeseen circumstances during surgical interventions. Because of this, the animals were humanely killed by giving them an overdose of anaesthetic. During these procedures five mice also died during surgical interventions in the brain cavity without recovering consciousness after anaesthesia.

A comparison of the number of animals used in procedures shows an increase in the number of animals used in severe procedures in 2022 in comparison to the previous year (up from 139 (3.7% of the total number of animals used in procedures) to 237 (10.7% of the total number of animals used in procedures)). This can be explained by the fact that pilot projects involving severe procedures which had been authorised in previous years were, for various reasons, not actively pursued. This resulted in procedures which had been scheduled for implementation in previous years being implemented additionally in 2022. The objectives of the severe procedures were mainly ‘Translational and applied research: human cancer, human cardiovascular disorders and human nervous and mental disorders.

There has been a decline in the number of animals used in moderate and irreversible procedures in comparison to the previous year. A total of 427 animals were used in irreversible procedures in 2021 (11.4% of the total number of animals used in procedures) and 117 (5.3%) in 2022, whereas 743 animals were used in moderate procedures in 2021 (19.8% of the total number of animals used in procedures), and 383 (17.3 % of the total number of animals used in procedures) in 2022. By contrast, the number of animals used in light procedures has declined, but slightly increased in percentage terms. A total of 2 432 animals were used in 2021 (65.0% of the total number of animals used in

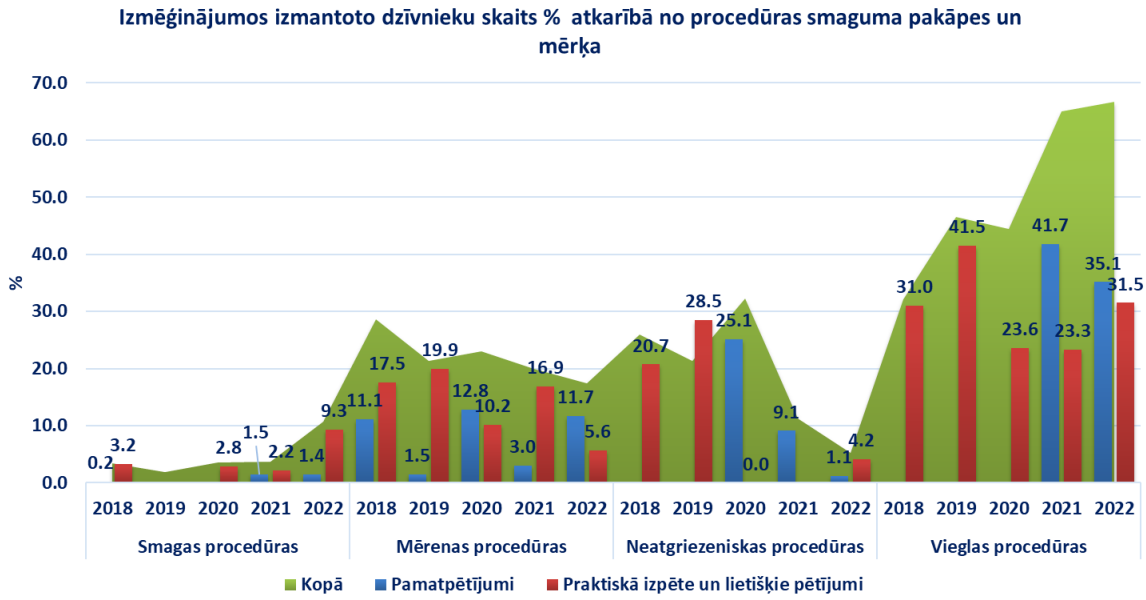
procedures) and 1 470 animals in 2022 (66.75% of the total number of animals used in procedures). The decline in the number of animals subjected to light procedures can be explained by the overall decline in the number of animals used in procedures and the fact that a number of so-called 'field studies' were completed in previous years when research was conducted into the behaviour and infectious and invasive diseases of wildlife. The completion of these projects furthermore coincides with a period in which, as mentioned above, pilot projects involving more severe procedures which had been scheduled for implementation in previous years but were postponed for various reasons until 2022 (funding, impact of the COVID-19 pandemic) are being actively pursued.

Figure 3



The main research objectives are fundamental research and translational and applied research. This is due to the tendency for researchers to focus on research into vaccines or new pharmaceuticals with therapeutic effects, currently common infections and pathological conditions caused by various injuries, and non-communicable diseases (stroke, cancer, metabolic disorders, metabolic syndrome and diabetes). Research into these pathologies also takes place at genetic level.

Over the course of the previous year the 'fundamental research' strand has tended to diminish and the 'translational and applied research' strand to grow (see figures 3 and 4). Since 2020, the percentage of fundamental research studies has changed, declining from 63.1% in 2020 to 49.4% in 2022. The proportion of studies falling within the translational and applied research strand has changed accordingly, from 41.5% in 2021 to 50.1% in 2022. In the **fundamental research strand** in 2022, animals were most used in the following sub-strands: cardiovascular blood and lymphatic system (n=263), ethology/animal behaviour/animal biology (n=257), musculoskeletal system (n=227) and endocrine system/metabolism (n=132). In the translational and applied research strand in 2022, animals were most used in the following sub-strands: human cardiovascular disorders (n=420), human nervous and mental disorders (n=224), human cancer (n=193) and animal diseases and disorders (n=85).



When carrying out a detailed assessment of the pilot projects, experts representing the relevant scientific fields perform a detailed assessment of the methodology used in those projects and pay close attention to the reasons for choosing alternative methods and the *in vitro* studies carried out prior to *in vivo* testing. The rationale for choosing the number of animals and species is also assessed, and testers are increasingly being encouraged to assess not only the need to use animals in studies, but also the need for each manipulation and the possibility of minimising the suffering of animals during procedures. Therefore, as a result of systematic and careful work, there is an increasing downward trend in the number of animals used for experimental purposes year-on-year. By contrast, the number of authorisations issued for pilot projects is increasing.

Similarly, in their ongoing scientific work, researchers are looking for new alternative methods and ways to minimise the use of animals in procedures, and the recommendations of the project evaluation committee with regard to the 3RS principles are also being taken into account. Project authors are also seeking to increase the use of *in vitro*, *in silico* and *ex vivo* methods (e.g. use isolated organs, cells or organelles instead of live animals), in particular in first-stage toxicity and efficacy tests. Scientists also use organs and tissues from animals that were used in other light procedures as control group animals.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impact on statistics, if any:

Authors of scientific projects are seeking to use *in silico*, *in vitro* and *ex vivo* methods in substance testing to determine the most efficacious test component before resorting to the use of animals. A literature review is also carried out and cooperation undertaken with other scientists conducting similar studies. Additionally other equivalent research is used for data comparison and analysis in order to avoid, as far as possible, duplication of research and to reduce the use of animals in procedures. When assessing a pilot project, the competent authority and experts ensure and verify the scientific feasibility and benefits of the project, analyse the possibility of replacing animals with alternative methods and assess the number of animals required for procedures, specific research methods and tests. The competent authority and experts also verify whether it is possible to attain the

project's objectives as set out in the project plan. If it is found that animal suffering or the total number of animals used in procedures can be reduced, applicants are instructed to make the relevant changes to the pilot project before authorisation is granted. Furthermore, when inspections are carried out at test animal user undertakings, each project is checked for compliance with its approved methodology.

(e) Further breakdown of the category 'Other', where a significant proportion of animal use is reported in this category:

In 2022, 257 common starlings (*Sturnus vulgaris*) were used in procedures involving 'other animals' in the 'ethology/animal behaviour/animal biology' substrand of the 'fundamental research' strand. In 2022, animal species such as bats, red deer and blackcaps were no longer used in procedures, because the practical activities and procedures involving these animals had been completed the previous year. Investigation and analysis of collected samples continued during the remaining period of authorisation of some of the above projects in 2022, and data were statistically processed to enable research data to be successfully published.

(f) Information on the use of animals in categories for which the method or testing strategy to obtain the results sought, not entailing the use of live animals, is recognised under Union legislation.

No precedents for the use of animals in such categories have been identified.

(g) Details of cases where a 'severe' classification has been exceeded, whether previously approved or not:

Species:

- **Number of animals:** 0

- **Was the 'strict' classification previously allowed or not:**
  No permits were issued for pilot projects involving procedures that might exceed the 'severe' classification threshold. No information was received from users in 2022 that a 'severe' classification had been exceeded in any procedure.
- **More detailed information on use:**

- **Reasons why the 'severe' classification was exceeded:**
  No such cases were detected in 2022.

Lithuania

(a) General information on any changes in trends observed since the previous reporting period:

In 2022, there were 6174 laboratory animals used for scientific or educational purposes in Lithuania. In comparison to the previous year 372 (were 5802) more animals were used in the projects.

The number of users:

YEAR	THE NUMBER OF USERS
2013	8
2015	12
2017	14
2020	15
2021	16
2022	15

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

The most common primary purpose for using animals Basic Research (~ 35 % increase, 3723 animals was 2740); Trans/Appl Research (~ 49 % increase, 1776 animals was 1185), Higher education 70 % decrease / 560 animals was 1877).

Basic Research	[PB9] (Basic Research) Sensory Organs (skin, eyes and ears) - 2264 [PB7] (Basic Research) Immune System - 328 [PB3] (Basic Research) Nervous System - 334 [PB2] (Basic Research) Cardiovascular Blood and Lymphatic System - 10 [PB13] (Basic Research) Other - 126 [PB10] (Basic Research) Endocrine System/Metabolism - 411 [PB1] (Basic Research) Oncology - 250	3723
Trans/Appl Research	[PT37] (Trans/Appl Research) Non-regulatory toxicology and ecotoxicology - 55 [PT32] (Trans/Appl Research) Other Human Disorders - 43 [PT31] (Trans/Appl Research) Human Endocrine/Metabolism Disorders - 88 [PT30] (Trans/Appl Research) Human Sensory Organ Disorders (skin, eyes and ears) - 241 [PT29] (Trans/Appl Research) Human Urogenital/Reproductive Disorders - 105 [PT28] (Trans/Appl Research) Human Immune Disorders - 186 [PT27] (Trans/Appl Research) Human Musculoskeletal Disorders - 26 [PT24] (Trans/Appl Research) Human Nervous and Mental Disorders - 276 [PT23] (Trans/Appl Research) Human Cardiovascular Disorders - 143 [PT22] (Trans/Appl Research) Human Infectious Disorders - 379 [PT21] (Trans/Appl Research) Human Cancer -234	1776
Higher education	[PE42-1] Higher education – 560	560
Regulatory use/Toxicity and	[PR85] (Regulatory use/Toxicity and..) Skin sensitisation - 15 [PR81] (Regulatory use/Toxicity and../Acute toxicity) LD50, LC50 -– 100	115

The increase of the animals was used in the Basic Research is the use of animals in project of Sensory Organs. Some approved establishments did not perform any projects in 2022 and other started or continued new projects in the end of the previous year.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

Most part of the animals (~69 %) were used for the procedures classified as mild [up to and including] severity, moderate (~ 26 %), non-recovery (~ 4 %), severe ~ 1 %.

Animals for the procedures classified as mild [up to and including during year 2022 was used 4285, moderate 1598, non-recovery 249, and severe 42 animals.

The 'severe' classification reported in 2022 consists of 42 animals (Mice (Mus musculus) ~1 % of the total number of used animals. The animals were killed or found dead after anaesthesia.

Projects:

1. "Investigations of ecotoxicological potential of chemical, physical and biological factors and their complex in fish" – 2 ([A35] Other fish (other Pisces) Salmo trutta fario).

2. "Study of the effectiveness of new generation functional food components with mice for scientific and educational purposes" – 11 ([A1] Mice (Mus musculus)).

3. "Study of the efficacy, pharmacokinetics and pharmacodynamics of new pharmaceuticals in mice and rat's models of ocular diseases – 3 ([A1] Mice (Mus musculus).

4. "Studies on the toxicity, pharmacokinetics and pharmacodynamics of the latest medicinal products in various models of eye disease in mice and rats that have been developed" – mice 4 ([A1] Mice (Mus musculus), rats – 1 ([A2] Rats (Rattus norvegicus).

5. "Application of mesenchymal cells and their secreted exosomes for the treatment of COVID"19 – mice 18 ([A1] Mice (Mus musculus).

6. "The evaluation of the treatment efficiency of electromagnetoporation combined with chemicals and dendritic cell vaccines for the treatment of mouse tumours" – mice 3 ([A1] Mice (Mus musculus).

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

Activities undertaken under Article 47 of Directive 2010/63/EU on the protection of animals used for scientific purposes to contribute to the development, validation and promotion of alternative approaches and dissemination of information thereon at the national level for the period 2013–2015 are publically available on the webpage of the European Commission http://ec.europa.eu/environment/chemicals/lab_animals/3r/pdf/Article_47_LT.pdf

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

As regards the category "Other", other fish (Oncorhynchus mykiss (fish 340), Salmo trutta fario 118, Salmo trutta fario 2 were used during the reporting in 2022.

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

No testing such testing has taken place.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

No such cases reported.

Luxembourg

(a) General information on any changes in trends observed since the previous reporting period:

In Luxembourg we could observe a general decrease in the total number of animals used in procedures from 25 293 animal in 2017, 13 751 animals in 2018, 11 117 animals in 2019, 5 341 animals in 2020, 4 967 animals in 2021 to 3 601 animals in 2022. This decrease may be a consequence of the reduction of the animal research activity and the application of the replacement and reduction.

Concerning the distribution in the species, the main reduction is due to a decreased use in mice in procedures, notably in 2022: 3201 mice were used compared to 4 114 mice used in 2021.

Regarding the purpose of the animal uses, no trends ware observed during the last year. The main category is basic research, followed by translational and applied research and training.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

In Luxembourg, a constant decrease in the total number of uses and in the total number of uses in mice could be observed in the last years. This decrease may be a consequence of the reduction of the animal research activity and the application of the replacement and reduction.

Furthermore, in Luxembourg two main institutions are involved in animal testing. Both modernised. Due to the small number of the parties involved in animal experiments (3 facilities in total), the development of the animal facilities has a strong impact on the total number of animals used.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

Comparing the actual severities during the last years, no trend has been observed.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

The particular efforts taken to promote the principle of the Three Rs have been:

- The competent authority organised a second edition of a 3 R symposium in 2022, where all the members of the animal welfare bodies and users participated focusing on the replacement, reduction and refinement.

- Refinement of the housing and care of the animals is ensured, inter alia, by modernisation of the animal facilities and by a new animal facility. Another point is the environment enrichment of the cages or aquariums, in particular, providing animals with appropriate housing that allows the expression of species-specific behaviours, such as nesting opportunities for mice.

- During the inspection attention is put on points such as that the staff follows the project protocol and in particularly that the humane endpoints are respected and the score sheets are reviewed. When procedures are conducted which involve pain or invasive procedures, it is verified that these procedures are carried out under appropriate general or local anaesthesia and that appropriate analgesia or another method is used to ensure that pain, suffering and distress are kept to a minimum.

- Additional care is taken during the project evaluation , inter alia, a review of the referenced literatures, a check of the most up to date references have been considered, a check whether there are alternative methods in place and the statistical calculation is reviewed. Regarding the alternative methods, it is checked if all measures are taken to reduce pain, suffering or lasting harms, if the humane endpoints are appropriate, if the housing, health checks of the animals are appropriate etc.

- Regarding the Reduction the national research institutes are collaborating with other research groups and are sharing data and resources (animals, tissue, organs and equipment) between research groups. Furthermore, one institute owns an IRM, which enables longitudinal studies in the same animals and which is put at the disposal of other institutes.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

The category "other" was not reported.

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

No uses of animals took place in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, which are recognised under the legislation of the Union.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

- **Species: /**
- **Numbers of animals:/**
- **Whether exceeding the 'severe' classification was pre-authorised or not: /**
- **Details of the use: /**
- **Reasons why the 'severe' classification was exceeded:**

In 2022, there was no case where the severe-classification has been exceeded.

Malta

Not provided

Netherlands

(a) General information on any changes in trends observed since the previous reporting period:

In 2022 a total of all uses, excluding GAA creation, of 426 960 was registered (versus 2021: 408 396). In 2022, the numbers and uses of animals for the creation and maintenance of genetically altered animals registered was 1 509 (versus 2021: 3 808). Totalling to 428 469 uses in 2022, this rise of around 3.8% is consistent with the numbers of recent years but follows an upward trend. Possible explanation for the upward trend are an increased capacity by some institutions, partially related to the results of Brexit and concentration of certain activities within the EU.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

By category of scientific purpose the numbers have remained fairly stable. Noteworthy are the categories *preservation of species*, from 33 024 to 58 925 uses related to predominantly (sea)fish and bird preservation (see **e**). Also, the category *basic research* increased from 108 744 to 119 560 uses, with a stark increase in the subcategory *Ethology / Animal Behaviour /Animal Biology* from 26 917 to 41 092 uses. The category *translational and applied research* dropped from 100 175 to 87 971 uses mostly related to a drop in subcategories *Animal nutrition* (-6 044 uses) and *Human Infectious Disorders* (-4 586 uses).

Per species the largest increase can be witnessed in the categories: *other fish* (+33 214), *other birds* (+6 346) and *Zebra fish* (+5 169) uses. The biggest decreases are for the categories: *mice* (-7 795), *domestic fowl* (-5 094) and *rats* (-3 00) uses. Noteworthy are the decrease in research with *Syrian hamsters* (-1 577) often used in Covid19 research. For *reptiles*, *seabass* and *other amphibians* the amount of uses dropped to 0 in 2022.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

The trends in severities is relatively stable, a small change has occurred the amount of *mild* has dropped form 56.5% to 51.5%, *moderate* has gun up from 36.7% to 40.1%. .

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

In the Netherlands, continuous efforts have been taken to promote the principles of the 3R's.
However, it is not possible to trace back these efforts to specific items in the statistics.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

For 2022 two categories need further breakdown, *other birds* (33.9% or 18 794 uses) and *other fish* (81.2% or 90 401 uses).

Other birds are mainly: *Parus major* (21.1% or 3 959 uses), *Cyanistes caeruleus* (6.7% or 1 183 uses) and *Turdus merula* (5.7% or 1 068 uses). *Other fish* are mainly *Anguilla anguilla* (74.7% or 67 519 uses), *Pimephales promelas* (7% or 6 331 uses) and *Gasterosteus aculeatus* (3.9% or 34 821 uses).

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

In total 41 records, with a total of 654 uses, have been registered. As explanation for the use of animal testing the majority of entries is a result of *in vitro* test being positive or inconclusive and required an *in vivo* test.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

In 2022 exceedance of the severity classification 'severe' has not been reported and no exemption was authorised.

Poland

(a) General information on any changes in trends observed since the previous reporting period:

For years, Poland has seen a slight overall downward trend in the number of animals used. This is due to greater awareness among those carrying out experiments (thanks to ongoing training) as well as the work of ethics committees and their unique composition in Europe (scientists, humanists and representatives of organisations with the statutory objective of animal protection). The number of animals used in 2020 and 2021 decreased dramatically due to the COVID-19 pandemic which saw a lot of testing suspended. In 2022, there was a slight increase, indicating a return to normal working patterns.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

The fluctuation observed in the number of animals used of certain species seems to be a natural consequence of the end of one type of experiment and the start of others, connected to the receipt of research grants linked to an increase in the popularity of a given field of research or orders from external parties. For example, in 2022 a significant increase could be seen in the number of dogs used, including dog patients, as a result of a specific experiment which started at one centre. The only constant trend appears to be a steady increase in interest in using zebrafish for research on the part of those carrying out experiments.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

As a result of informing users that information must be provided on the actual severity of procedures on animals and not the severity described in experiment approvals, there appears to be a downward trend in the number of animals being used in severe procedures, with an increase in mild and moderate procedures. This trend confirms the possible overestimation of the severity of procedures in experiment approvals. Animal protection organisations are well represented within the Polish competent authorities, i.e. the local ethics committees on animal experiments, and observations show that the representatives of these organisations often propose increasing the severity category.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

The statutory tasks of the National Ethics Committee on Animal Experiments (KKE) are to pursue the three Rs and promote alternative research. The KKE supports training courses for persons planning or carrying out experiments in programmes that incorporate this topic. Such information is also provided to local ethics committees on animal experiments (during training courses, via the KKE's website and through direct contact). Organisations' welfare teams also use the KKE's website, advice and recommendations. When issuing authorisation for experiments to be carried out, ethics committees are required to take into account the existence of alternative methods and the application of the three Rs in the specific experiment concerned. To this end, the model application form for authorisation contains a specific field in which the user must enter the method of applying the three

Rs in the experiment concerned. In 2018 an additional obligation was added to the application form, as a reminder that procedures must not be carried out, or must be terminated immediately, if alternative methods to the procedures set out in the application are approved in the European Union during the period in which the committee's authorisation is valid. In 2017, the KKE also took the initiative to set up a cooperation network between organisations and authorities involved in the application of alternative methods. Furthermore, welfare teams monitor how the three Rs principle is applied.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

In 2022, as in previous years, animal species reported in the 'other' field were mainly wild animal species. In Poland, there is a relatively large group of researchers who conduct experiments on these species. For example, in 2022, more than 43% of testing on birds, 15% of testing on fish and 100% of testing on amphibians used wild species.

In 2022, a large-scale experiment was carried out in the natural environment on American mink (*Neogale vison*), which is an invasive species in Poland and an increasing problem.

There is also one user which, under procedures required by law as part of routine production, employs tests not included in the list provided in the report (APIs). Those tests are therefore reported in the 'other' category. This user tests herbal medicinal products and in 2022 its activities in Poland accounted for nearly 90% of tests under the category 'legally required/routine production'.

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

In 2022, such cases were identified in 10 instances. Explanations point to inconsistencies with other legislation or the specific nature of the tested product.

Examples of problems encountered and explanations:

1. In accordance with OECD Guideline No 203 'Fish, Acute Toxicity Testing', one of the species recommended for testing is rainbow trout (*Oncorhynchus mykiss*).
2. In all possible cases, we have replaced pyrogen testing with the LAL test. The presence of non-endotoxin pyrogens can be tested by alternative methods. However, this is recommended for new products. With the consent of the competent authority, alternative methods of analysis may be used for control purposes, provided that the methods used enable a clear decision on whether compliance with monograph standards would be achieved using official methods. In case of doubt or dispute, only analysis methods under the Pharmacopoeia (in this case rabbit pyrogen testing) are authoritative.
3. The research indicated still requires animals to be used for testing even though validated methods have been developed:
    a. European Pharmacopoeia (Ph. Eur.) test for pyrogens (General Chapter 2.6.8). At its 170th session in June 2021, the European Pharmacopoeia Commission decided to engage

on a path that should ultimately lead to the complete replacement of the rabbit pyrogen test (RPT) in the Ph. Eur. within approximately 5 years.

b. The ISO 10993-11:2017 group of standards concerns testing of medical devices. 'Biological evaluation of medical devices – Part 11: Tests for systemic toxicity (point 4.1. General, Annex B, Dosage volume, B.1 General, Annex G Information on material-mediated pyrogens)'. PN EN ISO 10993-1: 'Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process' (Annex A Biological evaluation tests). PN EN ISO 10993-2: 'Biological evaluation of medical devices – Part 2: Animal welfare requirements'.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

- **Species:**
- **Numbers of animals:**
- **Whether exceeding the 'severe' classification was pre-authorised or not:**
- **Details of the use:**
- **Reasons why the 'severe' classification was exceeded:**

In 2022, no such cases were found.

Portugal

(a) General information on any changes in trends observed since the previous reporting period:

In 2022, there was an increase in animal use compared to 2021. The total number of animals used in 2022 was 91 287, which constitutes a 10.78% increase in animal use, compared to 2021 (total uses: 82 406).

This figure includes the number of animals used for the creation of genetically altered lines, 7 789, which represent 8.53% of all animal uses. From this total number, 1 939 of these animals were used for the creation of the new genetically altered animal lines for the first time, and the remaining 5 850 animals (all zebrafish) were used before in this field.

The figure also includes the number of 2 569 animals used for the maintenance of genetically altered lines which represent 2.81% of all animal uses.

In the uses of animals for research, testing, routine production and education and training purposes (total uses: 80 929), mice continue to be the most used animal species (60.1%), followed by other fish category (17.8%), Sea bass (8.7%), Zebra fish (7.4%) and Rats (6.0%).

There are increases in the use of Fish (total uses: 25 777), of birds (total uses: 320), and od cephalopods (total uses: 12). Simultaneous there is a decrease of the use of Mammals (total uses: 52 911) and of amphibians (total use: 1).

There was a decrease on the reuse of animals for research, testing, routine production and education and training purpose, with a total reuses of 1 908 animals, only 2.36% of the total number, compared to the previous year with a total reuse of 2 783 animals, 3.58% of the total number.

Basic research (65.7 %) continues to be the most frequent purpose for which animals were used to, followed by translational and applied research (33.3%), and by Training for the acquisition, maintenance or improvement of vocational skills (0.8%) and Higher education (0.2%).

The use of animals for Regulatory and Routine production only represents 0.1% of all uses reported.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

There was a significant increase in studies related with animal nutrition of fish species used in human food chain, from 4 089 to 15 423 animals and in animal welfare research (16 animals used in 2021 and 456 animals in 2022). Also in human cancer studies, there was an increase from 502 animals used in 2021, to 2 101 in 2022, as well as in Human Musculoskeletal Disorders from 792 to 1 021 in 2022.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

There was a change in the trends of actual severities. In 2022 there was a reduction of the severe and moderate categories percentage and there was an increase of the up to mild category, as well as an increase in the non-recovery procedures:

- Mild: 50.1% (2021- 39.1%)
- Moderate: 27.8% (2021- 37.2%)
- Severe: 14.5% (2021- 18.35%)
- Non-recovery: 7.6% (2021- 5.4%)

The number of animals experiencing Moderate and Severe actual severities are the reflection of procedures performed for studies in the areas of immune and nervous system, oncology, and infectious and cardiovascular disorders, which is a trend that has occurred in the latest years.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

There is no particular effort worthy of specification, only the application of the principles of 3Rs when planning and when the evaluation of projects submitted for authorisation to the competent authority, in addition to those that are applied and considered case-by-case in each of the establishments where animals were lodged during their involvement in procedures/projects.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

There was a significant increase in the use of Other fish in 2022 (total uses: 14 332), compared to 2021 (total uses: 1 535) specially in the species used for human consumption.

In the entry of Other fish, and among the several species that were reported of having been used, "linguado" *(Solea spp)*, "pregado" *(Psetta spp)*, *Sparus aurata,* Sea Bass *(Dicentrarchus labrax), Diplodus sargus* were the most used animals species, representing, respectively, 53.38%, 22.29%, 6.91%, 5.30% e 4.19% of all uses of this entry.

There was a decrease in the use of Other mammals (total uses: 50), compared to the previous year (73), belonging to studies of Animal biology with the following animal species:

- *Globicephala macrorhynchus*
- *Steno bredanensis*
- *Tursiops truncates*

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

No information to report in this regard.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

- **Species:**
- **Numbers of animals:**
- **Whether exceeding the 'severe' classification was pre-authorised or not:**
- **Details of the use:**
- **Reasons why the 'severe' classification was exceeded:**

These cases have not occurred in Portugal.

Romania

(a) General information on any changes in trends observed since the previous reporting period:

Since the previous reporting year, there has been a slight increase in the number of animals used for scientific purposes, from 15382 in 2021 to 16446 in 2022.

(b) Information on significant increase or decrease in use animals in any of the specific areas and analysis of the reasons thereof:

The number of guinea pigs and domestic fowls used for batch safety testing increased compared to the previous reporting year.

As regards the use of animals by main categories of scientific purposes, there have been a significant drop in basic research while the proportion of animals used for regulatory use/routine production purposes increased, mainly in the categories of Batch safety testing and Batch potency testing (whereas in the previous reporting year, no batch potency studies had been carried out). A significant number of animals were used for diagnosis of diseases.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

The proportion of non-recovery uses continued to be lower that in previous reporting years (2017-2020) mainly due to a better assessment of the degree of severity of the procedures (e.g. for diagnostic tests for diseases).

Moreover, the proportion of uses classified as severe decreased in favour of the mild and moderate uses due to a drop in projects involving chronic pain, debilitating diseases and uses of new medicines.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

Selecting animals that are suitable for the projects; appropriate management of animals bred for scientific purposes so as to avoid the production of excess animals; implementation of early humane endpoints.

(e) Further breakdown on the use of "other" categories if a significant proportion of animal use is reported under this category:

A small number of animals has been reported under “other” category.

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

No animal use has been reported for purposes for which alternative methods are recognised under the legislation of the European Union.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

There were not cases where "severe" classification was exceeded.

Slovakia

(a) General information on any changes in trends observed since the previous reporting period:

When compared to previous reporting year, we observed decrease in the overall use of animals in research. This decrease is mainly attributed to the restrictions imposed by COVID 19 pandemic, where several research groups postponed projects, and many of these were finished only in 2022. Despite new project authorisations in 2022, though fewer compared to previous year, several research groups did not use the animals, mainly due to finalizing older projects that were postponed for the reasons above. Moreover the consequence of energy crisis resulted in overall increase of running costs of animal facilities, which led to reduction of animal breeding, buying and ultimately use.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

After a span of 3 years, we report the use of pigs by one establishment within a newly approved project focusing on protection of the natural environment in the interests of the health or welfare of human beings or animals. In the category of regulatory use and routine production, increase of animal used is observed. This rise is mainly attributed to the higher number of generic projects approved in 2021, with most animals used and reported this operational year. Moreover, many establishments have financial difficulties (energy prices, maintenance), and testing of compounds for commercial sponsors is one of the alternates to compensate this limitation. In comparison to last year, there was an increase in the number of animals used in nervous system research, which was also linked to the higher number of GA animals used this year, given that the establishment that utilized the reported GAA works in the field of neuroscience. The increase reflects the pre-COVID-19 trend in the use of the animals in this particular field.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

Compared to previous year, increase of the severe severity is observed. This is mainly attributed to the increase in the regulatory studies as mentioned above, where quantitatively or qualitatively harmful compounds were tested. Another reason for the increase in the severity can be linked to Translational and applied research in the field of oncology compared to previous year where no such research was conducted (0 vs 54 animals).

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

In Slovak Republic, our law stipulates the compliance with the 3R principles stipulated by the SVFA, conforming to international standards. The projects are approved only after proper scrutiny for adherence to these principles, and monitored during the project duration. PARERE guidelines for improving the quality of the projects in regard to 3Rs is promoted as part of educational programme for the training and re-training of personnel in order to obtain the certificate of competence. Annually, national committee organizes meetings where members of animal welfare bodies, experts and researchers working with animals gather to discuss animal practices, as well as, novel regulations and new trends in the field. Promotion of these principles is also provided by Slovak National

Platform for 3Rs, including co-organizing the recent event "21st International Congress ESTIV" in Barcelona 2022.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

In the category of "other birds", compared to previous year, reduced numbers of animals, namely *Coturnix japonica, Teaniopygia guttata, Teaniopygia guttata* and *Lonchura striata* were used. This was due to general decrease of breeding / buying of animals in the establishment due to the reasons mentioned in point a). In the category of regulatory uses: Toxicity: Acute and sub-acute, animals reported were tested using OECD 423 guideline, in the generic projects of acute oral toxicity.

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

Animals were not used in such categories. On the other hand, few projects were rejected during evaluation process due to not adhering to the replacement, where the goals of the projects could be achieved by recognised alternative method.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

- **Species:** N/A
- **Numbers of animals:** N/A
- **Whether exceeding the 'severe' classification was pre-authorised or not:** N/A
- **Details of the use:** N/A
- **Reasons why the 'severe' classification was exceeded:** N/A

There were no cases of exceeding the severe classification.

Slovenia

(a) General information on any changes in trends observed since the previous reporting period:

In 2022 the number of uses in animals slightly increased compared to the previous year. Beside rodents and rabbits, who still represent the highest percentage (more than 96%) of all used animals in Slovenia, higher percentage of fish (14.26%) was used compared to previous year. Fish (trout) were used in basic research for the purpose of gastrointestinal system including liver and for higher education purposes. There was also a slight increase of the use in domestic fowl, where most of the animals (98%) were re-used. Animals were used for translational and applied research related uses for the purpose of animal nutrition (98%) and diagnosis of diseases (2%).

No cats, dogs and non-human primates were used for scientific purposes in 2022.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

More than 96% of all animals used are genetically not altered. Compared to previous 2 years where no animal with harmful phenotype was used, genetically altered animals with harmful phenotype were used in 2022. However, the percentage of genetically altered animals with harmful phenotype is below 1% compared to all animals used in 2022.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

Most procedures performed in Slovenia are classified as mild. Compared to 2021, there was a slight increase in the number of “non-recovery” and “severe” procedures. The species used in procedures classified as “severe” in 2022 were mice and fish (trout). The higher number could be the result of the studies for cancer and diagnosis of diseases and due to the project where trout were used. Half of the used fish were subjected to restrictive feeding and some animals probably didn’t receive enough feed and died. However, compared to all animals used in procedures in 2022, “severe” procedures still present less than 1%.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

We try to promote reduction, replacement and refinement principle during education and training courses for persons working with laboratory animals, through regular meetings with animal welfare officers, organization of different workshops, promoting the use of ETPLAS and e-modules, etc.

Example of reduction: re-use of animals whenever it is possible and justified, careful planning and designing projects.

Examples of refinement: refined animal housing, handling and measurements, gradual adaptation of animals to the handling and procedure, rehoming after the end of use, lowering the number of surplus animals with appropriate planning, mating schemes, communication among facility personnel and scientists, etc.

Example of replacement: in a project devoted to COVID vaccines, two vaccine candidates developed in previous studies were selected and tested in the golden hamster model using four different vaccination protocols. Replacement was conducted by using a virus neutralisation test on cell cultures prior to infecting and killing the animals.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

No significant proportion of animal use was reported under category "other".

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

Regulatory organs still require performing rabbit pyrogen test when there is LER (low endotoxins recovery) effect in BET method suspected or confirmed, to show if the rabbits can detect masked endotoxins. It is also required in registration process to show absence of pyrogenic substances with non-endotoxin origin. In-vivo testing of endotoxins/pyrogenic substances (like rabbit pyrogenicity testing) represents very low number of all tests (in-vitro and in-vivo) that detect endotoxins. There is ongoing plan to implement MAT test.

Rabbits are still used in one pharmaceutical company where they apply refinement procedures, e.g., re-use of animals, gradual adaptation of animals to the handling and procedure, rehoming after the end of use.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

"Severe" classification was never exceeded.

Spain

(a) General information on any changes in trends observed since the previous reporting period:

The use of animals decreased in 2022 by just over 166 000 with respect to the previous year.

When assessing this decrease it is necessary to consider the current situation. On the one hand, fewer projects were authorised in 2022 than in 2021 and, on the other, as has become custom, at the very early stages of fish development, there is a very high number of uses concentrated in a small number of procedures.

The use of animals to create a new line of genetically modified animals remains at similar levels as those seen over recent years (around 4 % of the total use of mice, for example). The species used are: mice (87 % of uses for the creation of a new line), rabbits (0.1 %), swine (0.2 %), zebrafish (9.3 %) and mendaka (3.4 %).

The evolution of the use of animals to maintain already existing lines is continuing the upward trend that we have seen over recent years. In this regard, in 2022 the main animals used were mice (91 %), zebrafish (8.5 %), rats (1 %) and xenopus (0.5 %).

Invasive techniques for genetic characterisation continue to be replaced, in particular distal tail docking through the use of excess tissue from the tagging of the animals.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

The species most widely used in 2022 was once again mice, followed by the group 'other fish' despite this category being split into subdivisions in 2021 in order to assign sea bass and salmonids, *inter alia*, their own entries. In third place was the use of the hen variety *Gallus domesticus*.

The biggest percentage increase in the number of uses has occurred in the group 'other fish', due to the use of meagre and bream in animal nutrition and animal welfare tests on individual animals in the early stages of their development, linked to production-driven research, as well as to research into diseases affecting these animal species, and their treatment and prophylaxis.

There has been an increase in the reported use of poultry (hens). Of note are uses in nutrition-related studies aimed at improving the growth, health and welfare of these animals due to their importance as production animals, tests for regulatory quality control purposes (batch potency testing in particular) in studies related to the development of new vaccines.

There has been an increase in the use of dogs compared to the previous year, notably those used in studies for regulatory purposes (pharmacokinetics, batch safety, quality control, toxicity), followed by studies in translational research where dogs are the target species, especially in studies related to nutrition, welfare and diseases, in particular leptospirosis, parvovirosis and respiratory diseases.

Swine, whose uses have also increased, are mostly used in translational research, both in studies as a species of livestock production and in relation to nutrition and ways to improve welfare. The reuse of animals in this type of study has steadily increased. Their use has also increased in studies of diseases where it is the target species, particularly those related to African swine fever, a disease of great interest because of the large economic impact it has on swine production.

The use of swine has also increased in studies related to various human pathologies; they are frequently selected because they are the best animal model for this type of study, especially those related to cardiovascular diseases.

There has been a significant increase in uses for the purpose of training to acquire and enhance professional skills (surgical techniques, specialists in cardiac electrophysiology for ventricular tachycardia ablation, etc).

It should also be noted that of the total reported uses of NHP (non-human primates) in this year, almost half (43 %) are reuses.

With regard to the purposes, under the 'regulatory heading', tests are reported in which this is the animal species of choice as there is no human cross-reactivity, for example in the case of biotechnological products or human diseases for which there is only equivalence with NHP (e.g. treatment for human haemophilia and there is only equivalence with NHP; treatment with an antimicrobial used in immunotherapy and only NHP are bioequivalent to humans; monoclonal antibody for treatment of human osteoporosis and there is only NHP equivalence).

As regards the origin of the animals (excluding primates), the number of animals born in the European Union in a place that is not a registered centre continues to grow, mainly due to the use of ‘production’ species such as poultry, swine, turkey, sheep, cattle, goat and aquaculture species (sea bass, salmon, bream, meagre). Although the centres where they are bred are registered for health purposes, they are not registered as centres for the breeding of animals used for scientific purposes.

Of note is the increase in studies related to animal nutrition with the use of 'target' species: such as 'production' species (poultry, swine, cattle) aquaculture species (salmon, sea bream, sea bass), as well as species traditionally considered as pets, such as cats and dogs.

With regard to the significant declines, the fall in the use of European sea bass once a project has been completed is well known, including the feeding of sea bass during the early stages, which took place in 2021.

Moreover, the use of rabbits has also decreased, with their main use being tests for the detection of pyrogens, followed by batch potency testing, feed and food safety tests and animal nutrition, with the decline in uses in the latter category being the most significant (in 2022, uses amounted to one fifth of uses in 2021).

The decrease in the use of the Syrian hamster has occurred mainly in translational research, in uses relating to research into human diseases, in particular cancer, infectious diseases and diseases caused by metal exposure and those affecting the nervous system.

The number of uses of cattle has fallen to 2018 levels. Uses have decreased mainly in the field of animal disease research, although given the health situation of cattle, this figure is expected to rise in 2023.

In other cases where the number of uses of a species or of uses relating to a given purpose is small, variations which, as a percentage, may appear to be very significant in relative terms, are actually often the result of the development or completion of a one-off project.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

In general, the downward trend in the reporting of 'non-recovery' uses continues, which can be attributed to the fact that the true meaning of the 'without recovery' severity classification continues to be misinterpreted.

The significant decrease in the number of 'up to mild' severity classifications is linked to the completion of a project on the nutrition of European sea bass that was initiated in 2021 and accounted for half a million uses at the very early stages of development.

The rise in severe uses is mainly the result of several challenge tests carried out in the early stages of sea bass, meagre and sea bream.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

The approaches used to respond to the 3Rs principle are varied, and in 2022 the Spanish State Research Agency included among the thematic priorities for projects the line 'Development of new alternative methods to reduce testing on animals', which has resulted in funding of almost EUR 5 800 000 for projects under this line[23].
In addition, the Animal Rights Awards were launched in 2023 to find the most innovative alternative to testing[24], with prize money totalling EUR 100 000.
The activities of the competent authorities for the assessment and authorisation of projects, as well as the animal rooms, continue to focus on the lines already identified in previous financial years and which have been supported and reinforced during the inspection of the centres:

[23] https://www.aei.gob.es/ayudas-concedidas/buscador-ayudas-concedidas?year=2022&convocatory=All&code=PLEC&area=&subarea=&title=&keywords=&cif=&name=&ccaa=All&province=All&granted%5Bmin%5D=&granted%5Bmax%5D=&summary=

[24] https://www.boe.es/eli/es/o/2022/12/23/dsa1360

- Optimisation of staff training, including activities to demonstrate that their skills are up to date.
- Establishment and promotion of a culture of taking care in establishments as an essential element in the application of the three Rs.
- Progressive increase in in silico and in vitro prior studies.
- Maintenance and updating of information pages on alternative strategies available to interested parties.
- In genotyping of genetically modified animals, maintenance of homozygosis lines, incremental use of excess tagging tissue for tissue characterisation.
- Monitoring and discussion of results in cases where options for improved implementation of the three Rs are identified.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

The following can be reported with regard to **'other animal species'**:

The use of other animal species has risen to 27 % of the total uses carried out in Spain in 2022, largely due to the use of juvenile forms of fish (mainly sea bass and meagre) in tests simulating commercial conditions and thus involving a large number of animals.

- **Other rodents**, which accounted for 0.02 % of the uses of rodents: almost all of the uses made were mild, and the only case reported as severe (a *Mus domesticus*) was qualified as such because the animal was found dead and it was not possible to determine the cause of death. Almost one third of these rodents are field mice (*Apodemus sylvaticus*). They were used in two projects, one to characterise this species (research on behaviour, ethology and biology of the animal in a well-defined geographical area), carried out in the natural environment, and the other to study their behaviour regarding their role in spreading seeds.

- **Other mammals.** These account for just 0.04 % of the uses of mammals. Of note are:

  - Greater noctule bat (Nyctalus lasiopterus), almost 50 % of uses, used to increase basic knowledge of its biology and behaviour as a first step in ensuring its preservation.
  - Greater white-toothed shrew (24 % of uses of other mammals) in basic research for the biology, behaviour and ethology of these animals.

- **Other birds**, which accounted for 5.34 % of the uses of birds:
  Almost all uses have been for the purpose of studying their biology, behaviour and ethology in the field of basic research, with a mild severity classification.
  Under this heading, uses involving wild species are reported. These are often protected species, for example eagles (imperial, osprey), common buzzards, seagulls and kites.
  There is a project ongoing using sparrows as a target species for a study on bird flu.

- **Other fish**, which accounted for 69.86 % of the uses of fish in 2022. Most notable are uses of aquaculture fish, such as sea bream (more than 40 % of all uses of fish) and meagre and sole (around 12 % each).
  In these fish the main purposes are to study the diseases of these animals, their nutrition, biology and behaviour, animal welfare and the development of vaccines and medication.
- **Other amphibians**, which accounted for most uses in amphibians, around 85.47 %, all classified as mild, carried out in centres specialising in wildlife work, with the aim of increasing understanding of their biology, behaviour and ethology. Of note are the uses of *Bufo spinosus* and *Pelophylax perezi*. *Pelophylax perezi* has been used occasionally in studies on non-regulatory toxicology and ecotoxocology, while *Pelobates cultripes* is reused to increase understanding of developmental biology.

As regards animals **'used for other purposes'** the following is noted:

- **Other basic research** accounted for 1.67 % of basic research uses.
  - Almost half of the uses were for food chain bioassays on turbot, classified as mild severity.
  - Around 23 % of uses were for the study of reproductive procedures in mice.
  - Studies have also been carried out in relation to parasitology, palatability of veterinary products, knowledge of pain mechanisms or adaptation and improvement of imaging techniques.

- **Applied research on other human diseases**
  This accounts for less than 1 % of all animal uses in applied research, and almost all uses have been for the study of diseases linked to mitochondrial dysfunction, using genetically modified mice without a harmful phenotype.

- **Other uses in routine production**
  These account for over half of uses intended for routine production. All of these uses were performed on *Gallus gallus,* classified as mild severity, and the aim was to produce antisera.
- **Other efficacy and tolerance testing**
  The vast majority of uses (83 %) have focused on effectiveness controls in the development of salmonid vaccines or challenge tests (14 %), also on salmonids.
- **Other quality controls** (3 % of quality controls):
  Almost all uses in other quality controls (more than 95 %) have been aimed at determining the absence of foreign agents.
- **Other toxicity and security testing**
  These account for 0.22 % of the uses to verify the toxicity and safety of the products, and the aim of these is to ascertain intracutaneous reactivity (38 % of uses) or rectal or vaginal irritation (38 % of uses).

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

In 2022, rabbits were used in Spain for the detection of pyrogens, in tests for ocular and dermal irritation or corrosion, and mice were used to obtain monoclonal antibodies by ascitis method.

Tests for the detection of pyrogens in rabbits

In 2022, the use of rabbits for the detection of pyrogens decreased from 7 055 uses in 2021 to 6 429 uses, representing a drop of more than 7 %. Most uses are moderate (more than 98 %) and the proportion of severe uses continues to decrease, from 3.15 % of severe uses in 2017 to 1.32 % this year.

In 2022 there were three authorised projects, at three different sites.

- Project 1: 23 uses. In 2022, the pyrogen test was carried out on eight batches, as the corresponding validation was being completed and was then submitted to the authorities with a view to acquiring authorisation for this test on rabbits to be replaced or removed from the Register of marketing authorisation for the pharmaceutical product.
- Project 2: 33 uses, which accounted for less than half of uses in 2021 (76 uses). Rabbits are used because they detect all pyrogenic agents, and not just endoxins.
- Project 3: 6 429 uses compared to 6 950 uses in 2021, as part of the control of blood products produced in the company. The bacterial endotoxin test cannot be considered as a substitute equivalent to the in vivo pyrogen test as it only allows the detection of pyrogenic substances generated by the presence of gram-negative bacteria and not from any other source. Following the strategy proposed by the European Directorate for the Quality of Medicines Healthcare (EDQM) of the Council of Europe for the replacement of the pyrogen test on rabbits, the company will develop and validate the Monocyte Activation Test (MAT) in those products for which the pyrogen test is currently required in the monographs of the European Pharmacopoeia. While the MAT has not been definitively developed and validated, the company maintains the test on rabbits as a safety criterion in the administration of blood products to patients and also when required by regulatory bodies and compendial references other than the European Pharmacopoeia and/or provided in the marketing licences.

Eye irritation/corrosion test in rabbits

In 2022, the uses made for this purpose increased from 9 in 2021 to 27. The uses have been made in two projects, developed in two user centres:

- Project 1: there have been 24 uses. The in vitro test is only validated for pure chemical products, not for medical and pharmaceutical extracts. ISO 10993-10 specifies that for medical devices the in vitro test is performed first, if the product is not corrosive, the in vivo test result should be confirmed. The in vitro test is also not valid for repeated dose irritation tests.

- There have been three uses in Project 2, performed in accordance with OECD TG 405. There are alternative in vitro tests that are used at preliminary stages, but there are currently no methods validated by the regulatory agencies to replace this test.

Skin irritation/corrosion test in rabbits

In 2022, there were 39 uses compared to the 131 uses carried out for this purpose in 2021, included in three projects. The total number of uses have been of minor severity.

- Project 1: There have been three uses, all in accordance with OECD TG 404. There are alternative in vitro tests that are used at preliminary stages, but there are currently no methods validated by the regulatory agencies to replace this test.
- Project 2: **There have been 33 uses.** It is a test for the assessment of the biocompatibility of medical devices, indicated as necessary for all medical devices in Annex A (Endpoints to be accepted in biological risk assessment) of ISO Standard 10993-1 (Biological evaluation of medical devices). The test is performed according to Standard: ISO 10993-10:2010, Biological evaluation of medical devices. Part 10: Skin irritation and sensitisation testing. 6.3 Animal irritation testing Different methods to determine 'In Vitro' dermal irritation for pure chemicals (EpiDermTM, EPISKIN) have been validated but these methods are not validated for extracts or solid materials (tissues, silicone sheets, etc.) of medical devices, which is the case for the products applied in this project.
- Project 3: There were three uses in 2022 (compared to 42 the previous year) The reason for these uses, as referred to in the 2021 report, is that the in vitro test is validated only for pure chemicals, whereas for medical and pharmaceutical extracts it is not validated. ISO 10993-10 specifies that for medical devices the in vitro test is performed first, if the product is not corrosive, the in vivo test result should be confirmed. The in vitro test is also not valid for repeated dose irritation tests.

Monoclonal antibody production in mice by ascites

There were 16 severe uses on mice. Use has been made of the animal model at certain times, given the impossibility of meeting production demand due to the non-functionality of the antibodies obtained on the in vitro platforms and the poor performance of those platforms. In parallel, in vitro production continues to be optimised.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

There are no records of this scenario having occurred.

Sweden

(a) General information on any changes in trends observed since the previous reporting period:

Total number of uses
There was a decrease in the total number of uses (reuse included) reported for 2022 (243 761) compared to both 2021 (264 301) and 2020 (274 076).
There is a tendency towards lower figures in the total use. This is likely due to a combination of factors such as tougher economic times, better planned projects, and an increased transition to animal-free methods.

Re-use
In 2022, 1 330 animals were reused (0.5%). This is less than to previous years; 2021: 2 044 (0.8%) and 2020: 3 094 (1.1%).

Creation of new genetically altered line
Only zebrafish and mice were used to create new genetically altered lines. There was a decrease in the use of animals in the creation of new genetically altered lines in 2022 (7 926, 3%) compared to 2021 (19 740, 7%) as well as to 2020 (14 406, 5%). As in 2021, zebrafish dominated over mice with 5 467 uses (2021: 14 864 uses, 2020: 6 735 uses). The use of mice was clearly lower in 2022 (2 459) compared to both 2021 (4 876) and 2020 (7 671).

Genetic status
The previous pattern with around fifty percent of the uses reported as not genetically altered, around forty percent of the uses reported as genetically altered without a harmful phenotype and the remaining reported as genetically altered with a harmful phenotype is repeated in 2022. The use of genetically altered animals with a harmful phenotype decreased slightly in 2022 (13 815, 6%) compared to 2021 (22 142, 8%), but was similar to 2020 (14 419, 5%). The use of genetically altered animals without a harmful phenotype increased in 2022 (102 450, 42%) compared to 2021: (95 059, 36%), but was proportionally similar to 2020 (113 621, 41%), albeit fewer in actual uses. The remaining uses, that is the not genetically altered animals, were 127 496, (52%) in 2022; 147 100, (56%) in 2021; and 146 036, (53%) in 2020.

Maintenance of genetically altered lines
There was a decrease in the uses for maintenance of genetically altered lines, 860 uses of mice compared to 1 225 in 2021 and 1 071 in 2020. Only mice were used. The Swedish 3Rs Center has received information from users on decreases in the use of animals in breeding following improved breeding programs and increased use of cryopreservation. This mainly concerns breeding that falls outside of this scope, however, it may have had an effect on these figures as well. For more information, see section (d).

Place of birth
192 601 animals were born in the Union at an authorised breeder in 2022 compared to 202 205 in 2021 and 214 381 in 2020. The number of animals born in the Union, but not at an authorised breeder was lower (47 748) in 2022 compared to both 2021 (58 353) and 2020 (55 003). 80 animals were reported as born in the rest of Europe, which is less compared to both 2021 (160) and 2020 (200). In

2022, 1 902 animals were born in the rest of the world, an increase compared to 1 536 in 2021 and 1 398 in 2020.

Non-human Primate Source

In 2022, all 36 of the non-human primates were re-uses, 21 uses of crab-eating macaque (*Macaca fascicularis*) aka cynomolgus monkey, and 15 uses of rhesus macaque (*Macaca mulatta*), thus neither source nor generation is listed. However, the user has informed that the animals were of F2 generation or greater and came from a self-sustaining colony.

In 2021, 53 of the non-human primates in 2021 were re-used, thus no source was noted. 6 were first time use and were sourced from Asia. Those animals were of F2 generation or greater and came from a self-sustaining colony. In 2020, all of the 23 non-human primates were re-used, thus no source was noted.

Increases

Hamsters

64 uses of Syrian hamsters were reported in 2022. 30 uses are reported under *Regulatory use and Routine production, Toxicity and other safety testing including pharmacology, Acute and sub-acute, Non lethal methods*, and 34 under *Translational and applied research, Human Cancer*. 51 uses of Syrian hamsters were reported in 2021 as compared to zero in 2020. All uses were within *Basic Research, Respiratory System*.

Rabbits

The uses on rabbits increased in 2022 (3 282) compared to 2021 (2 798), but was similar to 2020 (3 181). Most uses were within *Regulatory use and Routine production* (2 262), mainly in *Monoclonal and polyclonal antibodies (excluding ascites method)* (2 214), but also in *Blood based products* (48); followed by *Basic research* (1 003), more specifically *Cardiovascular Blood and Lymphatic System* (612), *Respiratory System* (139), *Immune system* (128), *Nervous system* (109) and *Other basic research* (15). Seven uses were reported under *Translational and applied research, Diagnosis of diseases.*

Most uses in 2021 were within *Regulatory use and Routine production* (2 444) followed by *Basic research* (317), more specifically *Respiratory System* (168), *Nervous System* (108) and *Other basic research* (41). In 2020, uses were mostly reported as *Basic Research*, the majority divided between *Other basic research* (2 057) *Respiratory System* (579), *Nervous System* (55), and *Cardiovascular Blood and Lymphatic System* (318). 18 uses were reported under *Translational and applied research*, all of them *Human Musculoskeletal Disorders,* as in 2020 (18).

Cats

92 uses of cats were reported in 2022, about half within *Translational and applied research, Diseases and Disorders (48),* and half within *Basic Research* (44), the majority in *Urogenital/Reproductive system* (40) and the remaining in *Multisystemic* (4).

This is an increase compared to 2021 (65), but a decrease compared to 147 uses in 2020. All uses in 2021 were reported under *Basic research*, 40 under *Oncology*, 5 under *Multisystemic* and 20 under *Other basic research*, more specifically development of laboratory analyses for veterinary use. Most uses of cats in 2020 were reported as *Translational and applied research* and *Animal diseases and disorders* (112 uses).

Other mammals
420 uses of *Other mammals* were reported in 2022, constituting, as in 2021 and 2020, less than 1% of all mammals. Most uses were within *Ethology/Animal Behaviour/Animal Biology* (311), followed by *Translational and applied research, Animal Nutrition* (108), and *Regulatory use and Routine production, Blood based products* (1).

In 2021, 324 uses were reported, divided between *Basic Research, Ethology/Animal Behaviour/Animal Biology* (160), *Protection of the natural environment in the interests of the health or welfare of human beings or animals* (143), *Basic research, Other basic research* (111) and *Regulatory use and Routine production, Routine production, Blood based products* (10). In 2020, 382 uses were reported, divided between *Basic Research, Ethology/Animal Behaviour/Animal Biology* (301), *Basic research, Other basic research, Virology* (76) and *Regulatory use and Routine production, Routine production, Blood based products* (5).

Turkey
In 2022, 124 uses were reported, all within *Translational and applied research, Animal Welfare* with the aim of improving animal welfare at the slaughter of turkeys. Zero uses were reported in 2021. This category did not exist in 2020, turkeys would then have been reported under *Other birds.*

Other amphibians
In 2022, 3 004 uses of *Other amphibians* were reported, all within *Basic Research* as in 2021 and 2020. 2022 shows a significant increase compared to 2021 (1 871) and 2020 (1 256), due to 1 454 uses reported in new studies within *Ethology/Animal Behaviour/Animal Biology*. Like previous years, uses were reported in *Cardiovascular Blood and Lymphatic system* (501), *Nervous System* (905), and *Basic research/Immune system (88).* In 2021 and 2020, most uses were reported under *Nervous System* (1 264 and 1 014) and 607 uses were reported under *Cardiovascular Blood and Lymphatic System* (607 and 242).

Zebra fish
33 269 uses of zebra fish were reported in 2022 showing a significant increase compared to 2021 (22 924) and 2020 (18 285). The zebra fish is becoming an increasingly used species in different research areas.

As in 2021 and 2020, most uses were reported in *Basic research* (24 471, 22 453 and 16 733), mainly within *Multisystemic* (10 317), *Cardiovascular Blood and Lymphatic System* (5 370), *Nervous System* (3 853) and *Musculoskeletal System* (2 128). In 2021 and 2020, the main uses within *Basic Research* were in subcategories *Cardiovascular Blood and Lymphatic System* (14 404, 4 644), *Nervous System* (2 162, 3 617), *Multisystemic* (2 017, 2 663) and *Developmental biology* (1 672, zero).

The uses in *Translational and applied research* are significantly higher compared to the previous years (3 183, 471 and 1 552). 3 063 within *Human Nervous and Mental Disorders* (251 and 1 432 for 2021 and 2020, respectively) and 120 in *Non-regulatory toxicology and ecotoxicology*, as compared to 220 in 2021 and zero in 2020.

Decreases

Mice
144 868 uses of mice were reported in 2022, showing a continuous decrease compared to 2021 (157 790) and 2020 (176 073). The main uses in 2022 were, as for 2021 and 2020, in Basic Research (97 917, 118 796, 136 071), but significantly lower. Mice are used for many different purposes, of which only the purposes with the highest usage are listed here.

Immune System: 23 629 (23 484 in 2021, 32 046 in 2020), *Oncology*: 18 099 (21 491 in 2021 and 20 116 in 2020), *Nervous System*: 16 697 (27 098 in 2021 and 28 048 in 2020), *Cardiovascular Blood and Lymphatic System*: 15 703 (23 274 in 2021 and 22 584 in 2020), *Endocrine System/Metabolism*: 10 171 (7 621 in 2021 and 11 603 in 2020) and *Other Basic Research:* 4 056 (2 079 in 2021 and 4 935 in 2020).

39 375 uses were reported within *Translational and applied research*, an increase compared to 2021 and 2020 (32 791 and 34 532). The main uses were within *Human Cancer*, 14 258 (9 415 and 9 926 in 2021 and 2020, respectively), *Human Endocrine/Metabolism Disorders*, 7 329 (8 165 and 8 287 in 2021 and 2020), *Human Nervous and Mental Disorders*, 5 789, a clear increase compared to 2021 (1 581) and 2020 (2 278), *Human Respiratory Disorders*, 3 498, a decrease from 2021 (5 636) and 2020 (4 479), *Human Immune Disorders*, 2 365, an increase compared to 2021 (1 012) and 2020 (1 106) and *Human Cardiovascular Disorders*, 1 869, a decrease compared to 2021 (2 063) and 2020 (4 722).

There was a decrease in the use within *Higher education*, 42, compared to 2021 (865), as for the use in *Training for the acquisition, maintenance or improvement of vocational skills*, 1 936, compared to 2021 (2 072). The 2022 uses are less than the 2 415 uses in 2020 under *Higher education or training for the acquisition, maintenance or improvement of vocational skills.*

There has been an increase in the uses within *Regulatory use*, 2 279, compared to 1 865 in 2021 and 1 462 in 2020. The main difference is in *Toxicity and other safety testing including pharmacology*, 1 697 uses in 2022, 1 492 in 2021 and 889 in 2020.

Rats
11 180 uses of rats were reported in 2022 showing a continuous decrease compared to 2021 (15 439) and 2020 (18 385). The use of rats continues to decrease as it has done for the past decades. Most uses (7 414) were reported in *Basic Research*, less than in 2021 (10 125) and 2020 (12 520), mainly in *Nervous System* (2 790), also less than in 2021 (3 610) and 2020 (4 997). Another large use (1 684) in *Basic research* was the purpose *Cardiovascular Blood and Lymphatic System*, similar to 2021 (1 713), but higher than 2020 (845).

2 877 uses were reported in *Translational and applied research*, a decrease compared to both 2021 (3 793) and 2020 (4 975), main use was in *Human Nervous and Mental Disorders*, 1 114, which was similar to 2021 (1 197) and 2020 (1 327), followed by *Human Respiratory Disorders*, 562, which was similar to 2021 (513), but significantly lower compared to 2020 (1 713).

394 uses were reported under *Toxicity and other safety testing including pharmacology*, a decrease compared to both 2021 (819) and 2020 (439); mainly (173) in *Acute and subacute* (zero in 2021, but 330 in 2020), followed by 114 in *Kinetics* (207 in 2021 and 109 in 2020).
462 uses were reported under *Training for the acquisition, maintenance or improvement of vocational skills*, compared to 414 in 2021; and 33 uses were reported in *Higher education*, compared to 288 in

2021. This is comparable to the 451 uses in 2020 under *Higher education or training for the acquisition, maintenance or improvement of vocational skills.*

Other rodents

Zero uses of other rodents were reported in 2022. In 2021, 56 uses of other rodents were reported, a decrease compared to 82 in 2020. All uses in 2021 were within *Basic Research, Immune System.* In 2020, the uses were also within *Basic Research*; *Immune System* (79), and in *Musculoskeletal System* (3).

Dogs

430 uses of dogs were reported in 2022, a decrease compared to both 2021 (566) and 2020 (494). The highest use was reported in *Translational research (304),* an increase compared to 2021 (32), but less than 2020 (392). The uses were mostly within *Animal Diseases and disorders* (291), but also in *Human cardiovascular disorders* (7) and *Human Gastrointestinal Disorders including Liver* (6). 90 uses were reported in *Basic research* (50 in *Musculoskeletal system*, 20 in both *Endocrine System/Metabolism* and *Gastrointestinal System including Liver*), significantly less than in 2021 (481), but similar to 2020 (102), and 36 in *Training for the acquisition, maintenance or improvement of vocational skills* (48 in 2021). For 2020, the uses in *Translational research* were mostly within *Animal Diseases and disorders* (282). The 102 uses in *Basic research* in 2020 were all but two within the *Musculoskeletal System*.

Ferrets

Zero uses were reported for ferrets in 2022. 2 uses of ferrets were reported in 2021 and 18 in 2020. All uses for 2021 were within *Basic research, Other basic research*, specifically development of laboratory analyses for veterinary use, whereas the uses in 2020 were within *Translational and applied research, Human Infectious Disorders*.

Other carnivores

109 of the uses of carnivores were recorded as other carnivores (17%), a decrease compared to 2021 (215) but similar to 2020 (119). Individual species are brown bear (*Ursus arctos*, 76), raccoon dog (*Nyctereutes procyonoides*, 18), wolverine (*Gulo gulo*, 5), lynx (*Lynx lynx*, 7), and wolf (*Canis lupus*, 3). All uses of bears, raccoon dogs, lynxes and wolverines were in *Preservation of Species*. The wolves were used in *Basic Research* with subcategory *Ethology/Animal Behaviour/Animal Biology*. In 2021, the uses were within *Preservation of Species* (88), *Protection of the natural environment in the interests of the health or welfare of human beings or animals* (29), *Basic Research, Nervous System* (26) and *Translational and applied research, Human Infectious Disorders* (72). All 119 uses in 2020 were reported as *Basic Research, Ethology/Animal Behaviour/Animal Biology*.

Horses, donkeys and cross-breeds

288 uses were reported in 2022, a decrease compared to 2021 (416) and 2020 (570). The highest use is in *Translational and applied research* (129), with 93 in *Animal Diseases and Disorders* and 36 in *Diagnosis of Diseases.* 69 uses are reported in *Routine production, Blood Based Products*. 55 uses are reported in *Basic Research*; 20 in *Other Basic Research,* 17 in *Multisystemic,* 15 in *Immune System,* and 3 in *Oncology*. Also, 35 uses were reported in *Training for the acquisition, maintenance or improvement of vocational skill,* a clear decrease compared to 2021 (111). Since there now are two headlines under which to report animal use for educational purposes, the headlines will not match between 2021 and the previous years. However, the overall use is similar with 122 uses in 2020 under *Higher Education or Training for the Acquisition, Maintenance or Improvement of Vocational Skills*.

In 2021, most uses were in *Basic research* (183), mostly in *Musculoskeletal System* (134) and *Multisystemic* (34). In 2020, 198 uses were reported in *Basic Research*, mostly within Multisystemic (177). Within *Translational and applied research* 54 uses were reported in 2021, a clear decrease compared to 250 in 2020.

Pigs

There was a clear decrease in the use of pigs in 2022 (963) compared to both 2021 (1 403) and 2020 (1 325). Most uses were within *Training for the acquisition, maintenance or improvement of vocational skills* (346; 759 in 2021 and 689 in 2020) and within *Translational and applied research (346);* mostly in *Human Cardiovascular Disorders* (146), *Human Infectious Disorders* (60) and *Diagnosis of Diseases* (40). In 2021 and 2020, 221 and 263 uses were reported under *Translational and applied research*. Most uses were in *Animal Nutrition* (80) and *Human Cardiovascular Disorders* (59).

235 uses were reported in *Basic Research; Immune system* (94), *Multisystemic* (52) and *Cardiovascular Blood and Lymphatic System* (29). In 2021 and 2020, 423 and 373 uses respectively were reported, with most uses under *Ethology/Animal Behaviour/Animal Biology* in 2021 (228) and in *Cardiovascular Blood and Lymphatic System* in 2020 (118).
36 uses were reported in *Higher education* in 2022.

Goats

The use of goats (45) is similar to 2021 (51) and 2020 (59), but still a decrease. The main use was within *Translational and applied research, Animal Welfare* (22), followed by 16 uses in *Training for the acquisition, maintenance or improvement of vocational skills*, very similar to 17 in 2021 and 16 uses in 2020, then reported under *Higher education or training for the acquisition, maintenance or improvement of vocational skills*. Remaining uses were reported in *Regulatory use and Routine Production, Blood Based products* (7). The main use in 2021 was in *Regulatory use and Routine production, Blood based products*, 34, comparable to 2020 (25). In 2021, no uses were reported under *Translational and applied research* (13 in 2020).

Sheep

The uses on sheep decreased in 2022 (193) compared to 232 in 2021 (232), but showed an increase compared to 2020 (167). 96 uses were reported within *Regulatory use and Routine production, Routine production, Blood based products,* 46 in *Training for the acquisition, maintenance or improvement of vocational skills*, 45 in *Translational and applied research, Animal Welfare* and 6 in *Basic Research, Urogenital/Reproductive System.*

In 2021, 98 uses were reported in *Basic Research*, mainly *Ethology/Animal Behaviour/Animal Biology* (90) and 90 in *Regulatory use and Routine production, Blood based products.* 44 uses were reported under *Training for the acquisition, maintenance or improvement of vocational skills*, an increase compared to 2020 (20), then recorded under *Higher education or training for the acquisition, maintenance or improvement of vocational skills*. The majority of uses in 2020 were reported in *Translational and applied research* (95), but 52 uses were reported under *Basic Research, Cardiovascular Blood and Lymphatic System* (40) and *Urogenital/Reproductive System (12).*

Cattle

808 uses were reported in 2022, a clear decrease from 2021 (3 383) and 2020 (2 908). Most uses

(545) were in *Training for the acquisition, maintenance or improvement of vocational skills* as in 2021 (1 430) with corresponding figures in 2020 (1 397), then recorded under *Higher education or training for the acquisition, maintenance or improvement of vocational skills.* 252 uses were reported in *Translational and applied research;* 238 in *Animal Diseases and Disorders*, and 14 in *Animal Welfare. In 2021,* 1 115 uses were reported under *Translational and applied research*, a slight decrease compared to 2020 (1 359). The major part was in *Animal Diseases and Disorders* (852) as it was for 2020 (1 116). 11 uses in 2022 were reported under *Routine production, Blood based products*, similar to 2021 (12) but higher than 2020 (zero).

No uses were reported under *Basic research* in 2022, which is a decrease compared to 2021 (1 281) and to 2020 (152). Most uses under *Basic research* in 2021 were under *Other Basic research* (896), specifically under development of feeding or husbandry systems (816) and genetic analysis of blood tests to determine the breeding values of meat breeds (80), and *Ethology/Animal Behaviour/Animal Biology* (370). In 2020, 68 uses were reported under *Ethology/Animal Behaviour/Animal Biology* and 84 under *Other Basic research*, more specifically within development of feeding or husbandry systems and tests of milking machines.

Monkeys, Cynomolgus

21 uses of Cynomolgus monkeys were reported in 2022, all within *Translational and Applied Research, Human Nervous and Mental Disorders*. 28 uses of Cynomolgus monkeys were reported in 2021, compared to 4 uses in 2020. Most uses in 2021 (22) were reported in *Basic Research, Nervous System*, whereas the remaining uses (6) were reported as *Translational and Applied Research, Human Immune Disorders.* In 2020, all uses were in *Basic Research*; *Cardiovascular Blood and Lymphatic System*.

Monkeys, Rhesus

15 uses of Rhesus monkeys were reported in 2022, compared with 31 uses in 2021 and 19 uses in 2020. All uses in 2022 and 2021 were reported as *Translational and Applied Research, Human Immune Disorders*, whereas all uses in 2020 were reported as *Translational and Applied Research, Human Infectious Disorders*.

Domestic fowl

The use of domestic fowl, 80, is significantly lower than in 2021 (1 182) and in 2020 (1 988). The main use (59) was within *Regulatory use and Routine production, Blood based products* (72 in 2021 and 21 in 2020). Remaining uses (21) were within *Translational and applied research* were reported under *Animal Diseases and Disorders*, a clear decrease from 2021 (555) and 2020 (146). Zero uses were reported under Basic Research, a decrease compared to 2021 (555) and to 2020 (1 777). Main use under *Basic research* in 2021 and 2020 was within *Ethology/Animal Behaviour/Animal Biology*, 416 and 880.

Other birds

5 179 uses of Other birds were reported, a slight decrease compared to 2021 (5 526), but higher than in 2020 (3 906). All uses were reported under *Basic research, Ethology/Animal Behaviour/Animal Biology*. The major uses in 2021 and 2020 were also in *Ethology/Animal Behaviour/Animal Biology* (5 123 and 3 524), followed by *Endocrine System/Metabolism* (167 and 52) and *Cardiovascular Blood and Lymphatic System* (8 and 16).

In 2021 and 2020, use was reported in *Protection of the natural environment in the interests of the health or welfare of human beings or animals* (221 and 314) and in 2020 under *Preservation of Species* (7).

Reptiles
No reptiles were reported in 2022 nor in 2021. In 2020, all 34 uses were reported as *Basic Research, Ethology/Animal Behaviour/Animal Biology*.

Frogs, Rana
No uses of the frogs *Rana temporaria* and *Rana pipiens* were reported in neither 2022, 2021 nor 2020.

Frogs, Xenopus
All 45 uses of Xenopus in 2022 were within *Basic Research*, 33 in *Developmental biology*, and 12 in *Nervous system*. It is a decrease compared to 2021 (93) and especially to 2020 (1,761). In 2021, the majority (69) were reported as *Protection of the natural environment in the interests of the health or welfare of human beings or animals* (2020: 1 679), and the remaining were uses within *Basic research, Other basic research.* In 2020, a smaller portion was reported as *Basic Research, Nervous System* (58) and *Ethology/Animal behaviour/Animal Biology* (24).

Salmon, trout, chars and graylings
6 824 uses were reported in 2022 c**o**mpared to 7 083 in 2021. 2021 was the first year these species were reported separately from *Other fish.* The main use was within *Protection of the natural environment in the interests of the health or welfare of human beings or animals* (4 117) as in 2021 (7 266). Uses in *Basic Research* (1 294, 1 007 in 2021) were within *Ethology/Animal Behaviour/Animal Biology* (1 223), less than 2021 (593), and *Cardiovascular Blood and Lymphatic System* (71), less than 2021 (184). 972 uses were reported in *Preservation of species* and 217 in *Higher Education* (114 in 2021). 224 uses were reported in *Translational and applied research*, all within *Non-regulatory toxicology and ecotoxicology* (270 in 2021) In 2021, 504 uses were within *Animal Nutrition*.

Guppy, swordtail, molly, platy
Only 1 use was reported, and this was in *Basic Research*, *Ethology/Animal Behaviour/Animal Biology*, zero uses were reported in 2021, which was the first year these species were reported separately from *Other fish*.

Other fish
32 273 uses were reported in 2022, a decrease compared to 2021 (41 630) and 2020 (42 504). The major use (16 959) of *Other fish* is reported under *Protection of the natural environment in the interests of the health or welfare of human beings or animals*, a decrease compared to 2021 (32 807), but an increase compared to 2020 (8 208). This is followed by 7 900 uses in *Basic Research*, mainly in *Ethology/Animal Behaviour/Animal Biology* (7 629), an increase compared to 2021 (2 026), but significantly lower than in 2020 (25 005). The total use in *Basic Research* was higher in 2022 than in 2021 (3 193), but significantly lower than in 2020 (25 709). 5 547 uses were reported in *Translational and Applied Research,* less than in 2021 (5 930), and in 2020 (5 883), all uses were in *Non-regulatory toxicology and ecotoxicology* these years. The remaining uses in 2022 were 996 in *Higher education*, (747 in 2021) and 871 in *Preservation of species* (126 in 2021).

Guinea-pigs
184 uses were recorded in 2022, a decrease compared to 2021 (687) and 2020 (409).

The highest use was in *Basic research* (148), within *Respiratory System* (124) and *Sensory organs* (24). In 2021 and 2020, the highest use was in *Basic research* (589, 295), within *Sensory organs* (346, 192) and in *Respiratory System* (243, 103). Uses in *Translational and applied research* decreased to 28 from 86 in 2021 and 113 in 2020. All uses were reported under *Human Respiratory Disorders*, except for 13 uses in *Diagnosis of Diseases* in 2020. 8 uses were reported in *Regulatory use and Routine Production, Blood Based Products.*

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

Overall, the uses are relatively steady, however, some differences can be seen.

In proportion to the total number of uses, the uses in *Basic research* have been equal between 2022 and 2021, with 156 986 uses (64%) and 166 707 uses (63%), respectively, while it represents a higher proportion in 2020 (202 547, 74%).

Most uses in *Basic Research* for 2022 were reported under *Nervous System* (29 146), a decrease compared to both 2021 (34 322) and 2020 (38 876). The second largest use was reported in *Cardiovascular Blood and Lymphatic System*; 25 416, a decrease compared to both 2021 (40 190) and 2020 (28 987). 25 395 uses were reported under *Immune System*, similar to 2021 (24 737), but lower than 2020 (32 975). Following that was the use in *Oncology* (18 962), lower than both 2021 (22 112) and 2020 (20 806).

An increase is seen in *Ethology/Animal Behaviour/Animal Biology;* 16 014, compared to 2021 (9 838), but it is still only about half of the use in 2020 (30 081). *Musculoskeletal System* increased (5 299) compared to 2021 (4 798 uses), but remained lower than in 2020 (7 048). The use in *Multisystemic* increased (12 448) compared to 2021 (7 153) and 2020 (11 308). 1 814 uses were reported in *Developmental Biology*, about half of the uses (3 360) reported in 2021, when this category was introduced. An increase is visible in *Other basic research* in 2022 (5 487) compared to 2021 (5 010), but not compared to 2020 (10 193). Similarly, an increase was shown in *Gastrointestinal System including Liver* in 2020 (2 220) compared to 2021 (1 827), but not in comparison with 2020 (4 341). 1 102 uses were reported in the use for *Urogenital/Reproductive System*, similar to 2021 (1 067) but less than in 2020 (1 289). An increase is seen for uses in *Endocrine System/Metabolism* (11 520) compared to 2021 (9 085), but still lower than 2020 (14 163). The use for *Respiratory System* is lower in 2022 (1 695) than in both 2021 (2 361) and 2020 (2 597). There was a decrease in the use for *Sensory Organs (skin, eyes and ears)*, 267 in 2020, compared to 1 245 in 2021 and 833 in 2020.

In proportion to the total number of uses, the uses in 2022 reported as *Translational and Applied Research*, 52 710 (21%) is similar to 2021 (46 643, 18%), and 2020 (50 357, 18%).

The largest use is within *Human Cancer*, 14 450, an increase to both 2021 (9 092) and 2020 (10 046). The second largest group of use is *Human Nervous and Mental Disorders*, 9 987 uses, a large increase compared to both 2021 (3 035) and 2020 (5 037).

The third largest group of use is *Human Endocrine/Metabolism Disorders*, 7 432, where similar uses were reported for 2021 (8 540) and 2020 (8 575). *Non-regulatory toxicology and ecotoxicology*, follows with 6 330 uses, similar to 2021 (6 622) and 2020 (6 462), followed by *Human Respiratory Disorders*, 4 104, a decrease compared to 2021 (6 279) and 2020 (6 395). The use in *Human Immune Disorders*, is higher in 2022 (2 543) than in 2021 (1 182) and 2020 (1 106). The use in *Human Cardiovascular Disorders* continues to decrease, 2 383 (2 954 in 2021, 5 613 in 2020). Also, the uses in *Other Human Disorders*, 1 276, was lower than in both 2021 (2 618) and 2020 (1 616).

There have been slightly more uses in *Human Infectious Disorders* 1 246, compared to 2021 (1 151), but lower than in 2020 (1 527). A sharp increase is seen in *Human Urogenital/Reproductive Disorders*, 1 098, compared to previous years, 467 in 2021 and 157 in 2020. The use in *Animal Diseases and Disorders* was lower, 766, than in both 2021 (1 442) and 2020 (1 826). An increase is seen in *Animal Welfare*, 371, compared to 2021 (267) and 2020 (247). A decrease was seen in *Human Gastrointestinal Disorders including Liver* with 202 uses compared to 678 in 2021, but it was higher compared to 2020 (78). *Human Musculoskeletal Disorders* increased (154) in 2022 compared to 2021 (101), but was still lower compared to 2020 (273).
The use in *Diagnosis of Diseases*, 128 was higher than in 2021 (15), but lower than in 2020 (421). There was a tenfold decrease in the use under *Human Sensory Organ Disorders (skin, eyes and ears)* 124, compared to 2021 (1 199) and 2020 (971). The use in *Animal Nutrition* has decreased to 116 compared to 806 uses in 2021, which was the first year uses could be recorded in that category.

There is a more than doubled increase in *Regulatory use and Routine production*, 5 216 uses in 2022 and 5 426 uses for 2021, compared to 2020 (2 080). The main difference is found in in *Routine production* with 310 uses in *Blood based products* (391 in 2021) and 2 270 uses in *Monoclonal and polyclonal antibodies (excluding ascites)* (2 329 in 2021) compared to 2020 when 137 uses were reported in *Blood based products*. An increase was seen in *Quality control (incl. batch safety and potency testing);* 515, compared to 313 in 2020, but not to 2020 (547), all used in *Batch potency testing*. A decrease was seen in *Toxicity and other safety testing including pharmacology* (2 121) compared to 2021 (2 366), but it was still higher than in 2020 (1 353). The main part of this was 1 034 within *Acute and sub-acute* (zero use in 2021 and 1 189 in 2020), followed by 817 uses in *Repeated dose toxicity* (zero in both 2021 and 2020). Zero uses were reported in *Pharmacodynamics*, where 1 448 were reported in 2020 (zero in 2020) and zero uses were also reported in *Carcinogenicity*, where 575 uses were reported in 2021 (zero uses in 2020).

The use in *Protection of the natural environment in the interests of the health or welfare of human beings or animals*, 21 076, has decreased to about half of the use in 2021 (40 540) but is still higher compared to 2020 (10 201).

The category *Preservation of species* has increased in 2022 (1 949), compared to 2021 (221) but is similar to 1 834 in 2020.

There was a decrease in both *Higher education* (1 432) and *Training for the acquisition, maintenance or improvement of vocational skills* (3 432) compared to 2021 (2 014 and 4 914 respectively.) 2021 was the first year that the educational purpose was divided into two categories instead of being reported under the same category *Higher education or training for the acquisition, maintenance or improvement of vocational skills*, as it was in 2020 (5 986).

Maintenance of colonies of established genetically altered animals, not used in other procedures had 860 uses in 2022, lower than both 2021 (1 225) and 2020 (1 071).

There is an increase in the use in *Legislation on medicinal products for veterinary use and their residues*, 515, compared to 2021 (313), reaching almost the same level as in 2020 (547). There is, on the other hand, a decrease in the category *Legislation on medicinal products for human use*, 2 121 compared to 2020 (2 366) but higher than 2020 (1 396). No uses were reported under *Medical devices legislation* (27 in 2021 and zero in 2020).

In many cases, the uses under the different purposes go up and down between the years. It is not clear what the changes depend on, but there are always natural fluctuations in the number of projects and in the research topics, and there might still be an effect of Covid-19 on the research performed.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

The previous pattern with around thirty percent of the uses reported as mild, around sixty percent of the uses reported as moderate, close to ten percent reported as severe and the remaining reported as non-recovery is repeated in 2022.

Mild severity
Between 25 and 32% of the total use in the past three years falls into the Mild severity category (2022: 66 137, 27%; 2021: 84 425, 32%; and 2020: 69 065, 25%). Compared to the entire use, mild severity constitutes between a fourth and a third of the uses. The figures show a decrease between 2022 and 2021, an increase between 2021 and 2020, and a slight increase between 2022 and 2020.

Moderate severity
Between 57 and 63% of the total use in the past three years falls into the Moderate severity category (2022: 149 570, 61%; 2021: 149 398, 57%; and 2020: 172 068, 63%). The figures show a slight increase between 2022 and 2021, a larger decrease between 2021 and 2020, and a small decrease between 2022 and 2020.

Severe
Between 7 and 9% of the total use in the past three years falls into the Severe severity category (2022: 17 819, 7%; 2021: 21 088, 8%; and 2020: 23 768, 9%). This is to be considered stable.

Non-recovery
Non-recovery severity is reported in 3 to 4% of the total use in the past three years (2022: 10 135, 4%; 2021: 9 390, 4%; and 2020: 9 175, 3%). This is to be considered very stable.

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

The Swedish National Committee for the Protection of Animals Used for Scientific Purposes drives the Swedish national work with the 3Rs, with the Swedish 3Rs Center as its executive body. The main task for the 3Rs Center is to carry out the projects decided by the National Committee and to

support 3Rs work nationally. Below follow some activities that National Committee and Swedish 3Rs Center has had during the year.

Webinars, workshops, meetings

The National Committee and the 3Rs Center has, following the rapid move into digital meetings due to the pandemic years, continued to promote the 3Rs through webinars and digital workshops or other meetings. However, since we once again can meet face-to-face, on-site-meetings have also taken place.

During the year, the 3Rs Center arranged three webinars on non-animal-based research. Topics such as the Swedish Research Council's 3Rs project grants as well as evaluation and formal validation of new test methods has been on the program.

A practical workshop with focus on modern in vitro methods was arranged together with a university. The workshop aimed to give the participants a practical understanding of current in vitro methods and techniques. The university showed a sustainable cell culture lab where disposable materials are limited and where various substances are, to the greatest extent possible, completely animal-free. The attendees received information about and demonstrations of various in vitro methods on site as well as opportunities to try some methods themselves.

A digital meeting for veterinarians was held in the spring of 2022. The program included a presentation on the training of laboratory animals, which was given by a person with many years of experience in handling and training mice and rats for experiments. One veterinarian talked about how they have managed to ban tail handling in their facilities and another veterinarian talked about their work to refine ways to tag and biopsy mice and rats. The participants had the opportunity to prepare questions to address, which led to good discussions throughout the group.

The 3Rs Center has arranged five workshops on refinement for mice and rats, in English as per request from the target groups, together with RISE, Research Institutes of Sweden, and the Swedish University of Agricultural Sciences. The workshops contained lectures about how housing and handling research animals has improved over the years, but also how we can handle and train mice and rats to reduce their stress. Feedback from the participants include stories about inspiration to change their way of handling the animals, to create activation for the animals or to start training the animals before an experiment.

The 3Rs Center has also organized a series of webinars about voluntary intake of pain relief and other drugs to research animals, zebrafish welfare and care, less traditional animals in research, a template tool to assess ill health in rodents, enrichment for animals in research, and how to reduce the number of animals in research by using both sexes.

The 3Rs Center and the National Committee participate in events organised by others, for example the FELASA congress, the EUSAAT conference and the Danish 3Rs symposium. On several occasions they have had the opportunity to present their work – as part of the program or as a poster.

Networking and collaboration

The 3Rs Center works together with five other 3Rs centres in Europe (Denmark, United Kingdom, Germany, Switzerland and the Netherlands) and during 2022, they have met regularly to share ongoing national work, but also to plan a joint webinar series on Culture of care in 2023.

To encourage collaboration and exchange of best practice between Animal Welfare Bodies in Sweden, the 3Rs Center has organised meetings, both nationally and locally. Together with Swedish animal technicians and animal caretakers, the 3Rs Center has also instated an informal network, to share insights and learnings on a more regular basis. Further, the continuous work with a national replace network has proceeded.

The 3Rs Center also continues its collaboration with Swedish government agencies to support their 3Rs work, organising seminars and other meetings together. The 3Rs Center supports several international groups and projects together with other agencies, for example the PEPPER platform.

Activities for students and teachers

To reach tomorrow's researchers – today's students – the 3Rs Center has produced a wide range of educational material specifically aimed at the upper secondary school. A large part of the work has been launched in 2022.

Another activity is to participate together with the non-profit organisation Public & Science in Researchers' Night. This event poses an opportunity for students in upper secondary school to meet with researchers and discuss research, animal experimentation and non-animal methods, with the aim of increasing the general knowledge on the subject.

Materials and resources

An important step for the visibility and dissemination of information about the 3Rs in Sweden, is the digital letter *Focus on the 3Rs*. During 2022, four editions were published: *Validation of new methods*, *Education and training*, *Research and practice*, and *5 years with the 3Rs Center*.
In addition to these focus letters, the 3Rs Center publishes interviews and other material where researchers and animal welfare bodies tell about their 3Rs research and good examples of practice and work.

In order to increase awareness and knowledge of non-animal methods, the 3Rs Center has produced several films explaining different replacement concepts in a simple and pedagogic way. Early autumn 2022, two animated short films were released, one about reducing and replacing animal experimentation in general; how to do it and why it is important, and one on cell models and the increasingly advanced technology.

The 3Rs Center is working on two more films in collaboration with researchers, funders, government agencies and other stakeholders. The films will show concrete examples of how researchers have succeeded in replacing animal models in their studies and will also highlight different perspectives on what is required in order to replace more animal experiments in Sweden. These films will be published in 2023.

Ongoing projects

The work with compiling a strategy to replace animal experiments has progressed during the year. The aim of this strategy is mainly to steer the work of the National Committee in replacement related issues, but it will also be used to inspire other stakeholders. One cornerstone in the work is to have an open dialogue with relevant stakeholders to ensure that the content is well anchored in advance. In 2021, the 3Rs Center collected material for the strategy during an open workshop bringing together researchers, representatives from national regulators, research funders and animal welfare organisations. During 2022, the work to anchor the material with target groups began by having a

dialogue with the Central Ethics Committee on Animal Experiments and by presenting it to research funders. The work will continue in 2023.

It is difficult to measure whether the actions that the 3Rs Center performs have reduced the number of animals used in Swedish research. However, the National Committee and the 3Rs Center are continuously striving towards informing and inspiring researchers to find new ways to replace, reduce and refine animal use.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

No significant share of the use of the 'other' categories under the 'Purposes' category has been reported in 2022.

Other carnivores
109 of the uses of carnivores were recorded as *Other carnivores* (17%), a decrease compared to 2021 (215), but similar to 2020 (119). Individual species are brown bear (*Ursus arctos*, 76), raccoon dog (*Nyctereutes procyonoides*, 18), wolverine (*Gulo gulo*, 5), lynx (*Lynx lynx*, 7), and wolf (*Canis lupus*, 3). All uses of bears, raccoon dogs, lynxes and wolverines were in *Preservation of Species*. The wolves were used in *Basic Research* with subcategory *Ethology/Animal Behaviour/Animal Biology*.

In 2021, uses were reported for brown bear (74), American mink (*Neovison vison*, 72), raccoon dog (29), European polecat (*Mustela putorius*, 26), wolverine (11), lynx (2), and wolf (1). All uses of bears, wolverine, lynx and wolf were in *Preservation of Species*. The raccoon dogs were used in *Protection of the natural environment in the interests of the health or welfare of human beings or animals*, the polecats in *Basic Research, Nervous System* and the minks in *Translational and applied research, Human Infectious Disorders*.

In 2020, uses were reported for brown bear (66), raccoon dog (35), wolf (2), wolverine (8) and lynx (8). All uses were reported as *Basic Research* with subcategory *Ethology/Animal Behaviour/Animal Biology*.

Other birds
5 179 uses (86 %) of the birds were reported as Other birds in 2022. The most common species was the collared flycatcher (*Ficedula albicollis,* 2 431), followed by Eurasian blue tit (*Cyanistes caeruleus*, 1 334), European pied flycatcher (*Ficedula hypoleuca*, 572), great tit (*Parus major*, 377), common blackbird (*Turdus merula*, 283), Siberian jay (*Perisoreus* infaustus, 174), European pied flycatcher x collared flycatcher (hybrid) (6) and Montagu's harrier (*Circus pygargus*, 2). All uses were within *Basic research: Ethology/Animal Behaviour/Animal Biology.*

In 2021, 82% of the uses (5 526) of the birds were reported as *Other birds*, an increase compared with 202 (3 906, 66%). The largest uses were then found in great tit (1 300), European robin (*Erithacus rubecula*, 433), zebra finch (*Taeniopygia guttata*, 372), European pied flycatcher (397), willow warbler (*Phylloscopus trochilus*, 363), Eurasian blue tit (292), great reed warbler (*Acrocephalus arundinaceus*, 221), common blackbird (158), Japanese quail (*Coturnix japonica*, 92) and song thrush (*Turdus philomelos*, 78). In 2020, main uses were with European pied flycatcher (489), great tit (486), marsh tit (*Poecile palustris*, 461), willow warbler (363), zebra finch (340),

Eurasian blue tit (265), common blackbird (235) and the great reed warbler (211). Main uses were within *Basic research: Ethology/Animal Behaviour/Animal Biology* (5 123 in 2021 and 3 524 in 2020), some in *Endocrine System/Metabolism* (167 in 2021 and 72 in 2020), *Cardiovascular Blood and Lymphatic System* (16 in 2020), *Protection of the natural environment in the interests of the health or welfare of human beings or animals* (221 in 2021 and 314 in 2020) and finally 7 uses in *Preservation of species* (2021).

Other fish

32 273 (45%) of the reported uses of fish constitutes of *Other fish*, a decrease proportionally speaking compared to 2021 with 4 ,803 uses (57%), as well as to 2020 (42 504, 70%). In 2022, most uses are reported of roach (*Rutilus rutilus*, 6 230), Atlantic mackerel (*Scomber scombrus*, 5 000), European perch (*Perca fluviatilis*, 4 538), Atlantic herring (*Clupea harengus*, 2 865), common bleak (*Alburnus alburnus*, 2 805), gobies (*Pomatoschistus*, 2 072), minnow (*Phoxinus phoxinus*, 1 481), Pygmy halfbeak (*Dermogenys collettei*, 1 204), pike (*Esox lucius*, 1 105), white bream (*Blicca bjoerkna)* x common bream (*Abramis brama*) (hybrid) (590), Atlantic cod (*Gadus morhua*, 546), three-spined stickleback (*Gasterosteus aculeatus*, 466).

In 2021, most Other fish are reported as roach (10 396), European perch (6 026), three-spined stickleback (5 770), common bleak (5 136), Atlantic herring (3 664), minnow (2 386) and white bream (*Blicca bjoerkna*, 2 338).
In 2020, the main uses were with Atlantic salmon (*Salmo salar*, 20 491), brown trout (*Salmo trutta*, 2 938), European perch (2 624), Atlantic herring (2 500), corkwing wrasse (*Symphodus melops*, 1 928), ninespine stickleback (*Pungitius pungitius*, 1 606) and guppy (*Poecilia reticulata*, 1 586). Following the introduction of a separate category for *Salmonidae*, the species recorded under *Other fish* has changed since 2021.

Other amphibians

99 % of the amphibian use in 2022 were reported as Other amphibians, 3 004, all used in *Basic research: Ethology/Animal Behaviour/Animal Biology* (1 454), *Nervous System* (961), *Cardiovascular Blood and Lymphatic System* (501) and *Immune System* (88). The most frequently used species in 2022 was the Iberian ribbed newt (*Pleurodeles waltl*, 1,550), followed by moor frog (*Rana arvalis*, 946), common toad (*Bufo bufo*, 324), and edible frog (*Pelophylax kl. esculentus*, 184).

In 2021, most uses were reported of Iberian ribbed newt (1 846) and the remaining 25 were eastern newt (*Notophthalmus viridescens).* All uses were reported in *Basic research*; more specifically in *Nervous System* (1 264) and *Cardiovascular Blood and Lymphatic System* (607). In 2020, 1 230 uses of Iberian ribbed newt and 26 uses of eastern newt were reported. All uses were, as in 2021, reported in *Basic research*; *Nervous System* (1 014) and *Cardiovascular Blood and Lymphatic System* (242).

Other mammals

420 uses of *Other mammals* were reported in 2022, constituting, as previous years, a very small amount of the mammal uses. They were 222 wild boar (*Sus scrofa*), 108 reindeer (*Rangifer tarandus tarandus*), 52 elk (*Alces alces*), 37 roe deer (*Capreolus capreolus*), and 1 alpaca (*Vicugna pacos*). The reindeer were used in *Translational and applied research, Animal Nutrition*, the alpaca was used in *Regulatory use and Routine production, Blood based products* and all other uses were within *Ethology/Animal Behaviour/Animal Biology*.

In 2021, 324 uses of *Other mammals* were reported, less than 1% of the mammal use. They were 164 wild boar (*Sus scrofa*), 74 elk (*Alces alces*), 65 roe deer (*Capreolus capreolus*), 10 alpaca (*Vicugna pacos*), 6 northern bat (*Eptesicus nilsonii*) and 5 Nathusius' pipistrelle (*Pipistrellus nathusii*). In 2020, 382 uses (less than 1%) of Other mammals were reported, 145 wild boar, 118 elk, 38 roe deer, 34 Daubenton's bat (*Myotis daubentonii*), 33 soprano pipistrelles (*Pipistrellus pygmaeus*), 8 Brandt's bat (*Myotis brandtii*), 5 alpaca, and 1 whiskered bat (*Myotis mystacinus*).

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

There are no reports on any such cases in SE up to this date.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

- **Species:**
- **Numbers of animals:**
- **Whether exceeding the 'severe' classification was pre-authorised or not:**
- **Details of the use:**
- **Reasons why the 'severe' classification was exceeded:**

There are no reports on any such cases in SE up to this date.

Norway

(a) General information on any changes in trends observed since the previous reporting period:

In some of the earlier years, the numbers of fish have been very high, at the most 11,5 mill in 2016, where 10,6 mill were used in two experiments during the development om non-medicinal methods for removal of salmon lice. We believe that our increased focus on reduction in presentations in meetings and during consideration of applications may have contributed to lower total numbers of used fish over the last years.

(b) Information on significant increase or decrease in use of animals in any of the specific areas and analysis of the reasons thereof:

- In 2022 fish species comprised 94% of the animals used. The number of animals used decreased from 2 to 1,4 mill, and the number of "other fish" decreased by 31%. from 2021 to 2022. The decreased numbers of fish is a result of fewer experiments with a high number of fish in 2022 than in 2021. The number of fish is highly influenced by the number om experiments with a high number of fish in the particular year.

- The number of mice used in procedures went from 52 554 in 2021 to 53 817 in 2022.

- The number of rats went from 4 498 in 2021 to 3 397 in 2022. There is not much variation in the number of approved projects using rats, but high numbers of animals were reported in 2021 from three specific projects.

- The use of guinea pigs increased from 120 in 2021 to 274 in 2022 due to one single vaccination project with mild severity.

- The use of dogs increased from 41 in 2021 to 190 in 2022. These 190 dogs were used in 8 different projects. Four dogs were used in a terminal project, the rest were used in projects with mild severity, like blood or biopsy sampling, feeding projects etc.

- The use of horses increased from 92 in 2021 to 281 in 2022. The horses were used in 8 different projects with mild severity, only involving blood sampling or reproductive sampling.

- The use of goats increased from 404 in 2021 to 602 in 2022. The goats were used in two different projects of mild severity with only blood and milk sampling.

- The use of cattle increased from 294 in 2021 to 578 in 2022. The animals were used in eight different projects of mild severity like educational and feeding projects.

- In 2021 there was a decrease in the use of domestic fowl due to relatively high numbers reported from specific, large projects in previous years. In 2022 the number has again increased, from 867 to 1456, probably by the same reason. In Norway a field project can only last for two years.

(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

The majority of animals, 55,9%, were reported in the "moderate" category, demonstrating an increase by 2% compared to 2021. The "mild" category represented 37,6% of the animals, with a decrease by 3,5% from 2021. More animals in the moderate category than in the mild over the last two years is a result of many salmon used in a few experiments testing new delicing methods. The % of animals in the "severe" category have been relatively low and stable over the last years (2022: 5,3%, 2021:5,25%, 2020: 5,4%) as has the "non-recovery" category (2022: 1,5%, 2021:0,26%, 2020: 0,63%).

(d) Information on particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

- We continue to give lectures on the 3Rs to researchers and user establishments.
- We are in continuous contact with the lab performing tests of food samples and patient serum for botulinum toxin in Norway with the aim of replacing the use of mice with a chemical method.
- The lab has in 2023 been granted funding from the research fund of The Norwegian Animal Protection Alliance (NAPA) to develop and validate a chemical method.
- We continue our practice with mandatory use of multimodal analgesia for all surgical procedures on rats and mice. (General anaesthesia, local anaesthesia, preemptive and postoperative analgesia, preferrably using both NSAIDs and opioids). Removal of one or more components must be scientifically justified.
- Stressful behavioural tests with mice and rats where the results are difficult to interpret, for example Morris water maze and Tail suspension test, have been replaced with less stressful tests.
- We have an extensive dialogue with the applicant while evaluating the application, often resulting in refinements and sometimes also reductions.

(e) Further breakdown on the use of 'other' categories if a significant proportion of animal use is reported under this category:

In the category “other fish” 1.312.835 fish were used and this category comprised 92,8% of the total number of the animals used. In this category 30 species were used and the vast majority, i.e. 1.091.533 individuals and 83%, was Atlantic salmon, followed by trout comprising 54.188 individuals (4,1% of “other fish”).

(f) Information on the uses of animals in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, is recognised under the legislation of the Union:

For vaccines the use of alternative methods for batch testing is recognized under the legislation. For batch testing of fish vaccines IPNV is the only component tested not using live animals, i.e. by measuring the amount of virus.

(g) Details on cases where the ‘severe’ classification is exceeded, whether pre-authorised or not:

- **Species:**
- **Numbers of animals:**
- **Whether exceeding the ‘severe’ classification was pre-authorised or not:**
- **Details of the use:**
- **Reasons why the ‘severe’ classification was exceeded:**

We have no cases where 'severe' classification is exceeded.

VI. MEMBER STATE COMPARATIVE TABLES FOR 2022

Introduction

Four Member State comparative tables are provided for 2022 covering:

- **Numbers of animals**, by species, used for purposes of research, testing, routine production and education (including training)
- **Numbers of all uses** (first and any subsequent reuse) of animals, by species, for the purposes of research, testing, routine production and education (including training)
- **Numbers and uses** of animals, by species, for the **creation of genetically altered animals**
- **Numbers and uses** of animals, by species, for the **maintenance of genetically altered animals**

Table 1: Numbers of animals used for the first time for research, testing, routine production and educational purposes by species and Member State (Part 1)

		Austria	Belgium	Bulgaria	Croatia	Cyprus	Czech Republic	Denmark	Estonia	Finland	France	Germany	Greece	Hungary	Ireland	Italy
Mammals																
	Rodents															
	Mice	151,168	241,774	2,002	29,558	2,181	68,571	141,762	1,838	50,207	1,146,205	947,948	13,515	38,621	67,320	251,345
	Rats	3,565	13,292	1,395	3,904	0	13,625	24,324	511	8,966	153,989	103,263	2,436	20,773	13,082	82,064
	Guinea-Pigs	96	6,432	0	50	0	1,929	1,429	0	0	32,160	6,430	0	2,664	741	11,226
	Hamsters (Syrian)	588	3,613	0	0	0	118	163	0	274	10,084	1,975	0	14	42	442
	Hamsters (Chinese)	0	0	0	0	0	25	0	0	0	0	0	0	0	0	6
	Mongolian gerbil	18	17	0	0	0	0	0	0	0	717	2,566	0	0	0	0
	Other rodents	52	167	0	0	0	1,829	0	1	1,972	1,117	6,159	0	0	23	383
	Rabbits															
	Rabbits	997	62,829	12	97	3	4,577	3,029	51	89	188,986	66,805	120	591	1,012	8,980
	Carnivores															
	Cats	14	213	0	0	0	17	0	0	182	363	214	0	9	32	0
	Dogs	89	331	0	0	4	206	165	0	1,189	2,192	1,440	0	174	113	653
	Ferrets	0	0	0	0	0	3	0	0	0	49	105	0	0	219	46
	Other carnivores	0	0	0	0	0	0	1	0	428	12	115	0	0	0	0
	Farm animals															
	Horses, donkeys and cross-breeds	33	80	0	0	0	59	25	0	45	76	2,056	0	47	259	0
	Pigs	1,839	7,712	11	4	0	1,524	23,641	4	385	13,829	11,627	222	1,463	2,460	1,945
	Goats	2	9	0	0	0	28	21	0	0	166	378	0	20	19	23
	Sheep	121	563	0	3	0	95	48	0	343	3,473	3,262	1	10	3,544	54
	Cattle	1,555	3,323	0	0	0	1,445	1,464	243	148	1,088	3,648	0	1	2,427	2,495
	Non-human primates															
	Prosimians	0	0	0	0	0	0	6	0	0	65	3	0	0	0	0
	Marmoset and tamarins	0	0	0	0	0	0	0	0	0	268	206	0	0	0	16
	Cynomolgus monkey	0	0	0	0	0	0	0	0	0	2,560	1,711	0	0	0	266
	Rhesus monkey	0	2	0	0	0	0	0	0	0	16	42	0	0	0	10
	Vervets (Chlorocebus spp.)	0	0	0	0	0	0	0	0	0	48	0	0	0	0	0
	Baboons	0	0	0	0	0	0	5	0	0	54	5	0	0	0	0
	Squirrel monkey	0	0	0	0	0	0	0	0	0	10	3	0	0	0	0
	Other mammals															
	Other mammals	113	43	0	0	0	44	108	0	66	4,158	1,682	0	0	0	26
Birds																
	Domestic fowl	879	41,308	0	1,546	0	42,488	355	139	2,383	58,565	14,525	540	37,111	102	33,456
	Turkey	0	285	0	0	0	177	0	0	1	15,084	1,437	0	414	4	89
	Other birds	229	2,073	0	0	0	4,221	411	376	485	7,931	11,361	0	2,616	0	289
Reptiles																
	Reptiles	0	0	0	40	0	862	86	0	0	2,065	177	0	500	0	0
Amphibians																
	Rana	0	0	550	0	0	0	0	0	0	0	1,912	0	0	0	0
	Xenopus	826	150	0	0	0	0	180	0	0	4,579	6,637	0	0	131	149
	Other amphibians	3,080	75	296	0	0	124	147	0	157	624	2,212	0	300	0	0
Fish																
	Zebra fish	7,332	36,901	0	28	0	7,412	6,548	0	8,668	40,652	105,082	5,291	10,368	0	17,420
	Sea bass	0	0	0	0	0	0	660	0	0	23,660	840	5,789	0	0	451
	Salmon, trout, chars and graylings	1,265	760	0	0	0	6,211	13,113	0	4,172	56,019	11,893	0	0	1,364	1,735
	Guppy, swordtail, molly, platy	0	336	0	0	0	75,402	0	0	0	0	261	0	100	0	866
	Other fish	12,165	8,368	0	0	0	4,148	1,397	161	1,330	58,283	24,369	487	984	45	438
Cephalopods																
	Cephalopods	0	15	0	0	0	0	0	0	0	680	55	0	0	0	25
Totals																
	Total	**186,026**	**430,671**	**4,266**	**35,230**	**2,188**	**235,140**	**219,088**	**3,324**	**81,490**	**1,829,827**	**1,342,404**	**28,401**	**116,780**	**92,939**	**414,898**
	%	**2.2**	**5.1**	**0.1**	**0.4**	**0**	**2.8**	**2.6**	**0**	**1**	**21.8**	**16**	**0.3**	**1.4**	**1.1**	**4.9**

Table 1: Numbers of animals used for the first time for research, testing, routine production and educational purposes by species and Member State (Part2)

		Latvia	Lithuania	Luxembourg	Malta	Netherlands	Norway	Poland	Portugal	Romania	Slovakia	Slovenia	Spain	Sweden	Total	%
Mammals																
	Rodents															
	Mice	1,314	4,079	3,201	0	126,789	47,369	64,400	47,524	6,238	4,111	4,021	406,855	140,850	**4,010,766**	**47.8**
	Rats	410	1,206	0	0	89,366	3,377	14,881	4,775	3,167	4,000	81	48,251	11,074	**625,777**	**7.5**
	Guinea-Pigs	0	65	0	0	4,229	274	5,056	0	3,080	256	0	8,866	184	**85,167**	**1**
	Hamsters (Syrian)	0	0	0	0	2,730	16	12	0	0	0	38	1,440	64	**21,613**	**0.3**
	Hamsters (Chinese)	0	0	0	0	0	0	0	0	0	0	0	0	0	**31**	**0**
	Mongolian gerbil	0	0	0	0	60	0	0	0	0	18	0	44	0	**3,440**	**0**
	Other rodents	0	0	0	0	741	140	2,452	130	0	0	0	94	0	**15,260**	**0.2**
	Rabbits															
	Rabbits	0	216	0	0	17,748	12	745	60	38	75	0	11,885	3,282	**372,239**	**4.4**
	Carnivores															
	Cats	119	0	0	0	12	2	0	0	0	22	0	118	92	**1,409**	**0**
	Dogs	0	0	0	0	676	157	97	0	40	0	0	822	361	**8,709**	**0.1**
	Ferrets	0	0	0	0	356	0	0	0	0	0	0	121	0	**899**	**0**
	Other carnivores	0	0	0	0	24	63	144	0	0	0	0	0	53	**840**	**0**
	Farm animals															
	Horses, donkeys and cross-breeds	0	0	0	0	403	281	139	0	0	0	2	398	207	**4,110**	**0**
	Pigs	10	67	0	0	6,715	737	1,250	300	91	24	9	10,139	945	**86,953**	**1**
	Goats	0	0	0	0	25	2	58	0	0	0	0	494	23	**1,268**	**0**
	Sheep	4	2	0	0	1,538	371	285	44	103	0	0	1,852	193	**15,909**	**0.2**
	Cattle	0	10	0	0	1,144	428	311	28	0	0	0	869	807	**21,434**	**0.3**
	Non-human primates															
	Prosimians	0	0	0	0	0	0	0	0	0	0	0	0	0	**74**	**0**
	Marmoset and tamarins	0	0	0	0	5	0	0	0	0	0	0	0	0	**495**	**0**
	Cynomolgus monkey	0	0	0	0	18	0	0	0	0	0	0	395	0	**4,950**	**0.1**
	Rhesus monkey	0	0	0	0	68	0	0	0	0	0	0	2	0	**140**	**0**
	Vervets (Chlorocebus spp.)	0	0	0	0	0	0	0	0	0	0	0	0	0	**48**	**0**
	Baboons	0	0	0	0	0	0	0	0	0	0	0	0	0	**64**	**0**
	Squirrel monkey	0	0	0	0	0	0	0	0	0	0	0	0	0	**13**	**0**
	Other mammals															
	Other mammals	0	0	0	0	578	733	314	50	0	0	0	188	175	**8,278**	**0.1**
Birds																
	Domestic fowl	0	0	0	0	39,790	1,456	6,472	320	3,452	191	8	132,737	80	**417,903**	**5**
	Turkey	0	0	0	0	84	0	838	0	0	0	0	40	124	**18,577**	**0.2**
	Other birds	257	0	0	0	18,661	13,487	5,560	0	20	65	0	7,310	5,179	**80,531**	**1**
Reptiles																
	Reptiles	0	0	0	0	0	0	117	0	0	0	0	784	0	**4,631**	**0.1**
Amphibians																
	Rana	0	0	0	0	0	0	0	0	0	0	0	176	0	**2,638**	**0**
	Xenopus	0	0	0	0	2,611	0	0	1	0	0	0	423	33	**15,720**	**0.2**
	Other amphibians	0	0	0	0	0	740	72	0	0	0	0	3,735	3,004	**14,566**	**0.2**
Fish																
	Zebra fish	0	0	400	0	18,012	12,124	14,530	5,313	0	0	0	38,606	27,762	**362,449**	**4.3**
	Sea bass	0	0	0	11,970	0	0	0	6,358	0	0	0	66,978	0	**116,706**	**1.4**
	Salmon, trout, chars and graylings	0	0	0	7,653	1,231	1,161,969	692	8	0	0	930	13,312	6,812	**1,289,139**	**15.4**
	Guppy, swordtail, molly, platy	0	0	0	0	1,422	0	0	0	0	0	0	0	1	**78,388**	**0.9**
	Other fish	0	510	0	5,696	90,281	145,410	2,781	14,098	0	0	0	288,508	32,128	**691,587**	**8.2**
Cephalopods																
	Cephalopods	0	0	0	0	98	0	0	12	0	0	0	1,791	0	**2,676**	**0**
Totals																
	Total	**2,114**	**6,155**	**3,601**	**25,319**	**425,415**	**1,389,148**	**121,206**	**79,021**	**16,229**	**8,762**	**5,089**	**1,047,233**	**233,433**	**8,385,397**	**100**
	%	**0**	**0.1**	**0**	**0.3**	**5.1**	**16.6**	**1.4**	**0.9**	**0.2**	**0.1**	**0.1**	**12.5**	**2.8**	**100**	

Table 2: All uses (first use and all subsequent reuses) of animals for research, testing, routine production and educational purposes by species and Member State (Part 1)

		Austria	Belgium	Bulgaria	Croatia	Cyprus	Czech Republic	Denmark	Estonia	Finland	France	Germany	Greece	Hungary	Ireland	Italy
Mammals																
	Rodents															
	Mice	151,588	242,805	2,006	29,558	2,181	68,800	142,779	1,838	50,238	1,151,921	963,398	13,582	38,917	67,320	254,471
	Rats	3,617	13,632	1,475	3,904	0	13,657	24,696	511	8,966	156,593	106,646	2,436	21,000	13,082	83,947
	Guinea-Pigs	96	6,440	0	70	0	1,957	1,434	0	0	32,538	6,540	0	2,786	741	11,289
	Hamsters (Syrian)	588	3,613	0	0	0	118	163	0	274	10,109	2,000	0	14	42	442
	Hamsters (Chinese)	0	0	0	0	0	25	0	0	0	0	0	0	0	0	6
	Mongolian gerbil	18	17	0	0	0	0	0	0	0	717	2,569	0	0	0	0
	Other rodents	52	167	0	0	0	1,829	0	1	1,972	1,117	6,195	0	0	23	383
	Rabbits															
	Rabbits	1,053	62,872	282	97	3	4,603	3,029	51	89	190,469	67,125	132	644	1,012	9,472
	Carnivores															
	Cats	14	213	0	0	0	129	0	0	182	1,127	538	6	9	50	0
	Dogs	109	979	0	0	4	485	237	0	1,209	3,934	2,873	8	209	264	759
	Ferrets	0	0	0	0	0	3	0	0	0	49	122	0	0	219	46
	Other carnivores	0	0	0	0	0	0	1	0	493	12	115	0	0	0	0
	Farm animals															
	Horses, donkeys and cross-breeds	69	98	0	10	0	74	41	0	101	464	2,149	0	115	269	0
	Pigs	1,846	7,848	11	4	0	1,574	23,698	4	385	14,008	11,946	224	1,571	2,460	1,974
	Goats	2	10	0	0	0	37	21	0	0	588	397	0	20	19	23
	Sheep	121	586	0	23	0	402	48	0	564	3,545	3,390	1	13	3,691	110
	Cattle	1,588	3,475	0	0	0	1,482	1,476	243	399	1,534	3,906	0	1	2,987	2,495
	Non-human primates															
	Prosimians	0	0	0	0	0	0	6	0	0	65	7	0	0	0	0
	Marmoset and tamarins	0	0	0	0	0	0	0	0	0	297	207	0	0	0	17
	Cynomolgus monkey	0	0	0	0	0	0	0	0	0	3,603	1,905	0	0	0	295
	Rhesus monkey	0	36	0	0	0	6	0	0	0	43	69	2	5	0	14
	Vervets (Chlorocebus spp.)	0	0	0	0	0	0	0	0	0	51	0	0	0	0	0
	Baboons	0	0	0	0	0	0	5	0	0	73	5	0	0	0	0
	Squirrel monkey	0	0	0	0	0	0	0	0	0	15	3	0	0	0	0
	Other mammals															
	Other mammals	113	87	0	0	0	44	115	0	169	4,169	1,700	0	0	0	26
Birds																
	Domestic fowl	879	41,317	0	1,546	0	42,536	355	139	2,393	58,565	14,676	540	37,168	102	33,467
	Turkey	0	285	0	0	0	179	0	0	1	15,084	1,437	0	414	4	89
	Other birds	229	2,073	0	0	0	4,261	411	376	485	7,958	11,433	0	2,616	0	343
Reptiles																
	Reptiles	0	39	0	40	0	862	86	0	0	3,193	177	0	500	0	0
Amphibians																
	Rana	0	0	550	0	0	0	0	0	0	0	1,912	0	0	0	0
	Xenopus	874	212	0	0	60	720	480	0	0	6,468	7,345	0	0	131	224
	Other amphibians	3,080	75	296	0	0	124	149	0	157	624	2,212	0	300	0	0
Fish																
	Zebra fish	7,332	36,901	0	28	0	7,742	7,678	0	8,668	40,952	105,670	5,471	11,697	0	17,420
	Sea bass	0	0	0	0	0	0	660	0	0	23,660	840	5,799	0	0	451
	Salmon, trout, chars and graylings	1,265	824	0	0	0	7,677	13,483	0	4,172	56,019	11,893	0	0	1,364	1,735
	Guppy, swordtail, molly, platy	0	336	0	0	0	75,559	0	0	0	0	358	0	100	0	866
	Other fish	12,165	8,540	0	0	0	6,895	1,567	161	1,339	59,615	24,493	947	1,418	45	961
Cephalopods																
	Cephalopods	0	15	0	0	0	0	0	0	0	680	55	0	0	0	25
Totals																
	Total	**186,698**	**433,495**	**4,620**	**35,280**	**2,248**	**241,780**	**222,618**	**3,324**	**82,256**	**1,849,859**	**1,366,306**	**29,148**	**119,517**	**93,825**	**421,350**
	%	**2.2**	**5.1**	**0.1**	**0.4**	**0**	**2.9**	**2.6**	**0**	**1**	**21.8**	**16.1**	**0.3**	**1.4**	**1.1**	**5**

Table 2: All uses (first use and all subsequent reuses) of animals for research, testing, routine production and educational purposes by species and Member State (Part2)

	Latvia	Lithuania	Luxembourg	Malta	Netherlands	Norway	Poland	Portugal	Romania	Slovakia	Slovenia	Spain	Sweden	Total	%
Mammals															
Rodents															
Mice	1,314	4,098	3,201	0	128,201	47,381	64,400	47,610	6,238	4,111	5,005	407,977	141,377	**4,042,315**	**47.7**
Rats	410	1,206	0	0	90,170	3,397	14,923	4,849	3,167	4,000	81	48,390	11,180	**635,935**	**7.5**
Guinea-Pigs	0	65	0	0	4,233	274	5,056	0	3,080	256	0	9,153	184	**86,192**	**1**
Hamsters (Syrian)	0	0	0	0	2,735	16	12	0	0	0	38	1,440	64	**21,668**	**0.3**
Hamsters (Chinese)	0	0	0	0	0	0	0	0	0	0	0	0	0	**31**	**0**
Mongolian gerbil	0	0	0	0	60	0	0	0	0	18	0	44	0	**3,443**	**0**
Other rodents	0	0	0	0	741	140	2,542	130	0	0	0	94	0	**15,386**	**0.2**
Rabbits															
Rabbits	0	216	0	0	17,787	12	1,003	60	38	75	73	14,438	3,282	**377,917**	**4.5**
Carnivores															
Cats	119	0	0	0	141	2	0	0	0	22	0	739	92	**3,383**	**0**
Dogs	0	0	0	0	1,008	190	97	22	40	0	0	1,511	430	**14,368**	**0.2**
Ferrets	0	0	0	0	381	0	0	0	0	0	0	121	0	**941**	**0**
Other carnivores	0	0	0	0	24	143	144	0	0	0	0	0	109	**1,041**	**0**
Farm animals															
Horses, donkeys and cross-breeds	0	0	0	0	474	281	160	59	0	0	2	444	288	**5,098**	**0.1**
Pigs	10	67	0	0	7,071	737	1,250	301	91	24	9	11,332	963	**89,408**	**1.1**
Goats	0	0	0	0	224	602	58	64	0	0	0	532	45	**2,642**	**0**
Sheep	4	2	0	0	1,662	475	390	59	108	0	2	2,147	193	**17,536**	**0.2**
Cattle	0	10	0	0	2,236	578	311	66	0	0	0	1,560	808	**25,155**	**0.3**
Non-human primates															
Prosimians	0	0	0	0	0	0	0	0	0	0	0	0	0	**78**	**0**
Marmoset and tamarins	0	0	0	0	5	0	0	0	0	0	0	0	0	**526**	**0**
Cynomolgus monkey	0	0	0	0	32	0	0	0	0	0	0	701	21	**6,557**	**0.1**
Rhesus monkey	0	0	0	0	145	0	0	0	0	0	0	2	15	**337**	**0**
Vervets (Chlorocebus spp.)	0	0	0	0	0	0	0	0	0	0	0	0	0	**51**	**0**
Baboons	0	0	0	0	0	0	0	0	0	0	0	0	0	**83**	**0**
Squirrel monkey	0	0	0	0	0	0	0	0	0	0	0	0	0	**18**	**0**
Other mammals															
Other mammals	0	0	0	0	578	742	314	50	0	0	0	227	420	**8,754**	**0.1**
Birds															
Domestic fowl	0	0	0	0	39,894	1,456	6,490	320	3,484	207	383	132,826	80	**418,823**	**4.9**
Turkey	0	0	0	0	95	0	838	0	0	0	0	40	124	**18,590**	**0.2**
Other birds	257	0	0	0	18,794	13,487	5,594	0	20	65	0	7,499	5,179	**81,080**	**1**
Reptiles															
Reptiles	0	0	0	0	0	0	131	0	15	0	0	894	0	**5,937**	**0.1**
Amphibians															
Rana	0	0	0	0	0	0	0	0	0	0	0	176	0	**2,638**	**0**
Xenopus	0	0	0	0	2,837	0	0	1	0	0	0	441	45	**19,838**	**0.2**
Other amphibians	0	0	0	0	0	740	72	0	0	0	0	3,742	3,004	**14,575**	**0.2**
Fish															
Zebra fish	0	0	400	0	18,012	12,424	14,530	5,963	0	0	0	38,656	27,762	**367,306**	**4.3**
Sea bass	0	0	0	11,970	0	0	0	7,009	0	0	0	66,978	0	**117,367**	**1.4**
Salmon, trout, chars and graylings	0	0	0	7,653	1,231	1,164,185	692	8	0	0	930	13,312	6,824	**1,293,267**	**15.3**
Guppy, swordtail, molly, platy	0	0	0	0	1,422	0	0	0	0	0	0	0	1	**78,642**	**0.9**
Other fish	0	510	0	5,696	90,401	145,418	2,781	14,328	0	0	0	288,672	32,273	**698,225**	**8.2**
Cephalopods															
Cephalopods	0	0	0	0	98	0	0	30	0	0	0	1,791	0	**2,694**	**0**
Totals															
Total	**2,114**	**6,174**	**3,601**	**25,319**	**430,692**	**1,392,680**	**121,788**	**80,929**	**16,281**	**8,778**	**6,523**	**1,055,879**	**234,763**	**8,477,845**	**100**
%	**0**	**0.1**	**0**	**0.3**	**5.1**	**16.4**	**1.4**	**1**	**0.2**	**0.1**	**0.1**	**12.5**	**2.8**	**100**	

Table 3: Uses of animals for the creation of new genetically altered animal lines in basic translational and applied research by species, reuse and Member State

	Reuse	Austria	Belgium	Croatia	Czech Republic	Denmark	Finland	France	Germany	Greece	Hungary	Ireland	Italy	Latvia	Luxembourg	Netherlands	Norway	Poland	Portugal	Spain	Sweden	Total	%
Mice	No	6,836	19,975	0	2,815	2,855	10,450	44,403	121,335	8,670	1,791	42	3,484	93	0	1,509	2,196	692	1,798	19,106	2,459	**250,509**	**100.0**
Mice	Yes	0	5	0	0	0	0	0	15	0	0	0	22	0	0	0	0	0	0	0	0	**42**	**0.0**
Mice	**Total**	**6,836**	**19,980**	**0**	**2,815**	**2,855**	**10,450**	**44,403**	**121,350**	**8,670**	**1,791**	**42**	**3,506**	**93**	**0**	**1,509**	**2,196**	**692**	**1,798**	**19,106**	**2,459**	250,551	**100.0**
Rats	No	0	180	0	23	0	0	1,143	468	0	0	0	96	0	0	0	0	0	0	0	0	**1,910**	**100.0**
Rats	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Rats	**Total**	**0**	**180**	**0**	**23**	**0**	**0**	**1,143**	**468**	**0**	**0**	**0**	**96**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1,910**	**100.0**
Hamsters (Syrian)	No	0	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**120**	**100.0**
Hamsters (Syrian)	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Hamsters (Syrian)	**Total**	**0**	**120**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**120**	**100.0**
Rabbits	No	0	0	0	0	0	0	160	0	0	36	0	0	0	0	0	0	0	0	20	0	**216**	**100.0**
Rabbits	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Rabbits	**Total**	**0**	**0**	**0**	**0**	**0**	**0**	**160**	**0**	**0**	**36**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**20**	**0**	**216**	**100.0**
Pigs	No	0	0	0	29	0	0	0	185	0	0	0	17	0	0	0	0	0	0	46	0	**277**	**99.3**
Pigs	Yes	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**2**	**0.7**
Pigs	**Total**	**0**	**0**	**0**	**31**	**0**	**0**	**0**	**185**	**0**	**0**	**0**	**17**	**0**	**0**	**0**	**0**	**0**	**0**	**46**	**0**	279	**100.0**
Goats	No	0	32	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	**38**	**100.0**
Goats	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Goats	**Total**	**0**	**32**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	38	**100.0**
Sheep	No	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	**6**	**100.0**
Sheep	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Sheep	**Total**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	6	**100.0**
Marmoset and tamarins	No	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	**8**	**100.0**
Marmoset and tamarins	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Marmoset and tamarins	**Total**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	8	**100.0**
Other mammals	No	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	**6**	**100.0**
Other mammals	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Other mammals	**Total**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**0**	6	**100.0**
Domestic fowl	No	0	0	0	0	0	0	83	529	0	0	0	0	0	0	0	0	0	0	0	0	**612**	**100.0**
Domestic fowl	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Domestic fowl	**Total**	**0**	**0**	**0**	**0**	**0**	**0**	**83**	**529**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	612	**100.0**
Xenopus	No	688	84	0	0	0	0	3,005	383	0	0	0	0	0	0	0	0	0	0	0	0	**4,160**	**100.0**
Xenopus	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Xenopus	**Total**	**688**	**84**	**0**	**0**	**0**	**0**	**3,005**	**383**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	4,160	**100.0**
Other amphibians	No	679	0	0	0	0	0	0	645	0	0	0	0	0	0	0	0	0	0	0	0	**1,324**	**100.0**
Other amphibians	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Other amphibians	**Total**	**679**	**0**	**0**	**0**	**0**	**0**	**0**	**645**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	1,324	**100.0**
Zebra fish	No	8,999	3,336	0	0	904	735	3,778	43,105	0	282	3,085	1,092	0	1,087	0	1,036	0	137	2,052	5,407	**75,035**	**90.6**
Zebra fish	Yes	0	0	62	0	0	0	0	1,042	0	475	0	0	0	0	0	400	0	5,850	0	0	**7,829**	**9.4**
Zebra fish	**Total**	**8,999**	**3,336**	**62**	**0**	**904**	**735**	**3,778**	**44,147**	**0**	**757**	**3,085**	**1,092**	**0**	**1,087**	**0**	**1,436**	**0**	**5,987**	**2,052**	**5,407**	82,864	**100.0**
Salmon, trout, chars and graylings	No	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3,232	0	0	0	0	**3,232**	**100.0**
Salmon, trout, chars and graylings	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Salmon, trout, chars and graylings	**Total**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3,232**	**0**	**0**	**0**	**0**	3,232	**100.0**
Other fish	No	1,446	0	0	0	0	0	180	1,414	0	0	0	0	0	0	0	0	0	4	736	0	**3,780**	**100.0**
Other fish	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Other fish	**Total**	**1,446**	**0**	**0**	**0**	**0**	**0**	**180**	**1,414**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**736**	**0**	3,780	**100.0**
All Species	**No**	**18,648**	**23,727**	**0**	**2,867**	**3,759**	**11,185**	**52,764**	**168,072**	**8,670**	**2,109**	**3,127**	**4,689**	**93**	**1,087**	**1,509**	**6,464**	**698**	**1,939**	**21,960**	**7,866**	**341,233**	**97.7**
All Species	**Yes**	**0**	**5**	**62**	**2**	**0**	**0**	**0**	**1,057**	**0**	**475**	**0**	**22**	**0**	**0**	**0**	**400**	**0**	**5,850**	**0**	**0**	**7,873**	**2.3**
All Species	**Total**	**18,648**	**23,732**	**62**	**2,869**	**3,759**	**11,185**	**52,764**	**169,129**	**8,670**	**2,584**	**3,127**	**4,711**	**93**	**1,087**	**1,509**	**6,864**	**698**	**7,789**	**21,960**	**7,866**	349,106	**100.0**

Table 4: Uses of animals for the maintenance of colonies of established genetically altered animal lines by species, reuse and Member State

	Reuse	Austria	Belgium	Croatia	Denmark	Finland	France	Germany	Ireland	Italy	Netherlands	Norway	Poland	Portugal	Romania	Slovakia	Spain	Sweden	Total	%
Mice	No	5,992	15,701	778	688	209	216,809	163,996	486	878	4,626	4,240	2,892	2,563	165	0	41,484	860	**462,367**	**99.9**
Mice	Yes	0	205	0	0	0	0	46	0	0	0	0	0	0	0	416	0	0	**667**	**0.1**
Mice	**Total**	**5,992**	**15,906**	**778**	**688**	**209**	**216,809**	**164,042**	**486**	**878**	**4,626**	**4,240**	**2,892**	**2,563**	**165**	**416**	**41,484**	**860**	**463,034**	**100.0**
Rats	No	0	48	0	0	0	7,495	2,822	0	0	0	0	0	6	0	0	45	0	**10,416**	**96.9**
Rats	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	328	0	0	**328**	**3.1**
Rats	**Total**	**0**	**48**	**0**	**0**	**0**	**7,495**	**2,822**	**0**	**0**	**0**	**0**	**0**	**6**	**0**	**328**	**45**	**0**	**10,744**	**100.0**
Other rodents	No	0	0	0	0	0	0	0	0	41	0	0	0	0	0	0	0	0	**41**	**100.0**
Other rodents	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Other rodents	**Total**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**41**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**41**	**100.0**
Dogs	No	0	0	0	0	0	27	0	0	0	0	0	0	0	0	0	0	0	**27**	**100.0**
Dogs	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Dogs	**Total**	**0**	**0**	**0**	**0**	**0**	**27**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**27**	**100.0**
Xenopus	No	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	0	**19**	**100.0**
Xenopus	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Xenopus	**Total**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**19**	**0**	**19**	**100.0**
Zebra fish	No	0	0	0	0	0	1,101	22,835	61	0	233	10,953	0	0	0	0	2,135	0	**37,318**	**95.2**
Zebra fish	Yes	0	148	0	0	0	0	0	0	0	0	0	0	0	0	0	1,723	0	**1,871**	**4.8**
Zebra fish	**Total**	**0**	**148**	**0**	**0**	**0**	**1,101**	**22,835**	**61**	**0**	**233**	**10,953**	**0**	**0**	**0**	**0**	**3,858**	**0**	**39,189**	**100.0**
Other fish	No	0	0	0	0	0	3	721	0	0	0	0	0	0	0	0	0	0	**724**	**100.0**
Other fish	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Other fish	**Total**	**0**	**0**	**0**	**0**	**0**	**3**	**721**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**724**	**100.0**
All Species	**No**	**5,992**	**15,749**	**778**	**688**	**209**	**225,435**	**190,374**	**547**	**919**	**4,859**	**15,193**	**2,892**	**2,569**	**165**	**0**	**43,683**	**860**	**510,912**	**99.4**
All Species	**Yes**	**0**	**353**	**0**	**0**	**0**	**0**	**46**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**744**	**1,723**	**0**	**2,866**	**0.6**
All Species	**Total**	**5,992**	**16,102**	**778**	**688**	**209**	**225,435**	**190,420**	**547**	**919**	**4,859**	**15,193**	**2,892**	**2,569**	**165**	**744**	**45,406**	**860**	**513,778**	**100.0**